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Cyanobacterial evolution during the Precambrian

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Abstract: Life on Earth has existed for at least 3.5 billion years. Yet, relatively little is known of its evolution during the first two billion years, due to the scarceness and generally poor preservation of fossilized biological material. Cyanobacteria, formerly known as blue green algae were among the first crown Eubacteria to evolve and for more than 2.5 billion years they have strongly influenced Earth's biosphere. Being the only organism where oxygenic photosynthesis has originated, they have oxygenated Earth's atmosphere and hydrosphere, triggered the evolution of plants –being ancestral to chloroplasts– and enabled the evolution of complex life based on aerobic respiration. Having such a strong impact on early life, one might expect that the evolutionary success of this group may also have triggered further biosphere changes during early Earth history. However, very little is known about the early evolution of this phylum and ongoing debates about cyanobacterial fossils, biomarkers and molecular clock analyses highlight the difficulties in this field of research. Although phylogenomic analyses have provided promising glimpses into the early evolution of cyanobacteria, estimated divergence ages are often very uncertain, because of vague and insufficient tree-calibrations. Results of molecular clock analyses are intrinsically tied to these prior calibration points, hence improving calibrations will enable more precise divergence time estimations. Here we provide a review of previously described Precambrian microfossils, biomarkers and geochemical markers that inform upon the early evolution of cyanobacteria. Future research in micropalaeontology will require novel analyses and imaging techniques to improve taxonomic affiliation of many Precambrian microfossils. Consequently, a better understanding of early cyanobacterial evolution will not only allow for a more specific calibration of cyanobacterial and eubacterial phylogenies, but also provide new dates for the tree of life.

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Introduction

Throughout the history of Earth cyanobacteria have triggered key evolutionary events, due to their ability (i) to produce oxygen, (ii) to exist endosymbiotically and (iii) to fix free nitrogen and CO_2. They allowed for the origin of complex life based on aerobic respiration, after they initiated a global oxygenation of the planet's atmosphere and hydrosphere during the Great Oxidation Event (GOE), more than 2.45 billion years (Ga) ago (Holland 1984, 2002; Bekker *et al.* 2004). Following an ancient endosymbiosis, cyanobacteria were fundamentally involved in the origin of plants during the Proterozoic (Sagan 1967). Even today cyanobacteria have an essential impact on carbon and nitrogen cycles within Earth's biosphere. Nevertheless, relatively little is known about their origin and evolutionary history.

Fossil evidence supports the presence of bacteria long before the origin of eukaryotes (Knoll *et al.* 2006; Schopf 2006; Wacey *et al.* 2011; Sugitani *et al.* 2013). However, morphological characteristics to taxonomically identify bacteria are rather few and, in addition, frequently lost during the fossilization process. Yet, although classification of microbial fossils is difficult, additional lines of evidence can be considered and may help to reconstruct the early evolution of life. Geochemical evidence can provide valuable insights into the appearance or importance of different bacterial groups, such as cyanobacteria (Lyons *et al.* 2014; Satkoski *et al.* 2015). Cyanobacteria are the only organism where oxygenic photosynthesis has evolved. There is strong support for the presence of appreciable amounts ($\sim 3 \times 10^{-4}$ present atmospheric levels (PAL)) of free oxygen around 3.0–3.2 Ga from chromium, iron, molybdenum (Mo) and carbon isotopes (Nisbet *et al.* 2007; Crowe *et al.* 2013; Lyons *et al.* 2014; Planavsky *et al.* 2014; Satkoski *et al.* 2015). Morphological analysis of stromatolites and other microbially induced sedimentary structures (MISS) support an origin of cyanobacteria by 3.2–2.7 Ga (Flannery & Walter 2012; Homann *et al.* 2015) and perhaps even by 3.4–3.5 Ga (Hofmann *et al.* 1999; Van Kranendonk 2006). Moreover results of phylogenomic analyses point towards the presence of cyanobacteria in the Archean, well before the rise of atmospheric oxygen (Schirrmeister *et al.* 2013; Schirrmeister *et al.*

2015). Phylogenomic studies further indicate that an ancient transition to multicellularity in cyanobacteria possibly provided adaptive advantages (Schirrmeister *et al.* 2015), such as motility within bacterial mats to position themselves optimally (Stal 1995), or improved surface attachment during mat formation (Young 2006). These, in combination with adaptation to higher salinities and the ability to form laminated mats, would have helped cyanobacteria to spread and diversify at the end of the Archean (Blank & Sanchez-Baracaldo 2010; Schirrmeister *et al.* 2013). However, the identification of fossil cyanobacteria remains a challenge and divergence times may be improved by new fossil discoveries. Increased quantity and quality of calibration dates will be an essential step to create future trees of life (Benton *et al.* 2009). Such calibration points for phylogenetic reconstruction could stem from fossil-, geochemical- or biogeographical data (e.g. speciation events associated with island formation). This review provides a summary of the early evolution of life as seen in the fossil record, and evaluates the evidence for the presence of cyanobacteria.

Evidence for Earth's earliest life

Three main lines of evidence can be distinguished that inform our understanding of the earliest life forms on Earth (Wacey 2009): (i) morphological evidence (e.g. microfossils and trace fossils); (ii) chemical evidence (e.g. isotopic and elemental ratios, biomarkers); and (iii) sedimentary structures (e.g. stromatolites and MISS). Each of these come with inherent difficulties in interpretation and evidence for early life is frequently inconclusive or controversial.

Difficulties in interpreting morphological evidence

The major difficulty in interpreting microfossil or trace fossil evidence for early life is that these microstructures frequently comprise very simple shapes (e.g. spheres, rods, filaments and tubes) that may be difficult to differentiate from natural geological phenomena (e.g. mineral crystals, volcanic microtextures). Post-depositional contamination provides an additional complication, since biological material can colonize, or be carried into cavities in the rock long after the initial deposition of the sediment (see Wacey 2009 for a summary).

Such difficulties have been highlighted by the debate regarding filamentous microstructures from a hydrothermal chert vein in the 3.46 Ga Apex Basalt, Pilbara Craton, Western Australia. These filaments were originally classified as 11 different taxa of bacteria (Schopf & Packer 1987; Schopf 1993) and were even compared with extant cyanobacteria (Schopf 1993). A subsequent re-examination of the geological and petrographic context of the filaments led to the hypothesis that the filaments were non-biological pseudofossils, formed by movement of carbon around recrystallizing quartz grains (Brasier *et al.* 2002, 2005). Renewed geochemical and morphological analysis of the carbon then provided additional evidence consistent with a biological formation mechanism (Schopf 2006; Schopf *et al.* 2007; Schopf & Kudryavtsev 2009, 2012). However, most recently it has been shown that, when examined at the nano-scale, the distribution of carbon in and around these filaments bears no resemblance to *bona fide* prokaryotic micro-organisms (Brasier *et al.* 2015, Wacey *et al.* 2015). Instead, the filaments are now interpreted as chains of hydrothermally-altered minerals onto which later carbon was adsorbed (Brasier *et al.* 2015; Wacey *et al.* 2015). This debate highlights how non-biological mineral growths may superficially mimic fossil organisms (see also Garcia-Ruiz *et al.* 2003) and how multiple high-spatial resolution analytical techniques are required to differentiate true microfossils from these so-called biomorphs. Of particular relevance here is the similarity of these mineral artefacts to multicellular cyanobacteria, since the stacks of mineral grains coated with carbon mimic the chains of cells of a cyanobacterial trichome.

Difficulties in interpreting chemical evidence

Due to the favoured usage of light ^{12}C over heavier ^{13}C isotopes during biological carbon fixation, the ratio of naturally occurring carbon isotopes will be significantly changed by biological activity, so that organic material in ancient rocks having negative (<c. −10‰) $\delta^{13}C$ values provides evidence *consistent* with biological processing (e.g. Schidlowski 1988). However, carbon isotope evidence alone is rarely sufficient to prove biogenicity, as demonstrated by the debate surrounding the very earliest possible evidence for life on Earth from Greenland. Negative $\delta^{13}C$ values from the ∼3.7–3.8 Ga Isua Supracrustal Belt (mean of −30‰, Mojzsis *et al.* 1996; mean of −19‰, Rosing 1999), and ∼3.85 Ga Akilia Island (mean of −37‰; Mojzsis *et al.* 1996) of Southwestern Greenland have been interpreted as the earliest evidence for life on Earth. However, these data are very controversial (Whitehouse & Fedo 2007). Both the age and the sedimentary nature of the Akilia Island rocks have been questioned (Fedo & Whitehouse 2002). Furthermore, the Greenland rocks are highly metamorphosed (at least up to amphibolite facies) and the graphite containing the light $\delta^{13}C$ signal may have originated from later metasomatic (hot fluid) reactions via the disproportionation of iron carbonates (Van Zuilen *et al.* 2002). The possibility of both meteoritic and recent contamination (Schoenberg *et al.* 2002) has also been raised. Finally, carbon isotope data alone cannot prove a biological origin for carbonaceous material because various abiotic processes also fractionate carbon isotopes to a similar extent (Horita & Berndt 1999; Sherwood Lollar *et al.* 2002; van Zuilen 2003).

More complex chemical organic compounds derived from cell membrane lipids, known as 'molecular fossils' or 'biomarkers', also experience problems with interpretation in Precambrian rocks. For example, lipid biomarkers characteristic of cyanobacteria (2α-methylhopanes) and eukaryotes (steranes) were reported from 2.7 Ga rocks of the Pilbara Craton, Australia (Brocks *et al.* 1999; Summons *et al.* 1999). It has also been suggested that the presence of 2α-methylhopanes might correlate with specific habitats (Ricci *et al.* 2014). However, several studies have questioned these findings, either reinterpreting the organic material as later contamination (Rasmussen *et al.* 2008) or showing that the 2α-methylhopane biomarkers are not unique to cyanobacteria (e.g. they are also known to be found in anoxic

photosynthesizers; Rashby *et al.* 2007; Welander *et al.* 2010). Most recently, it has been shown that these biomarkers are not indigenous to the Archean rocks in which they are found; instead, they are a product of modern contamination, likely caused by non-ideal drill-core sampling procedures (French *et al.* 2015). This study goes on to suggest that such biomarkers are not likely to be preserved in currently known Archean rocks, due to the thermal history of these deposits (French *et al.* 2015).

Difficulties in interpreting sedimentary structures

Macroscopic sedimentary structures such as stromatolites are frequently cited as some of the earliest evidence for life on Earth (e.g. Hofmann *et al.* 1999; Allwood *et al.* 2006; Van Kranendonk 2006). The biogenicity of stromatolites at first glance appears assured because all modern examples involve a contribution from microorganisms (e.g. Reid *et al.* 2000). In ancient rocks, however, the biogenicity of a stromatolite cannot be assumed because evidence of the microorganisms implicated in their genesis are almost never found (Riding 1992), and near identical macroscopic structures have been produced in the laboratory without the aid of biology (McLoughlin *et al.* 2008). That said, some specific types of stromatolites (e.g. coniform and tufted varieties) have not yet been replicated in either computer models or laboratory experiments without the influence of biology, so hold greater promise for decoding early life.

Such ongoing difficulties and debates emphasize the importance for novel approaches and techniques to elucidate the early evolution of the biosphere. Single lines of evidence, especially from such old deposits, have now largely been shown to be insufficient to stand up to robust critical examination, and so we must search for multiple lines of evidence to allow for more convincing interpretations of the early history of life on Earth.

Phylogenetic history of cyanobacteria

In recent years an increasing amount of newly sequenced genome data has accumulated. At the same time phylogenetic methods have been refined, enabling better estimations of the evolutionary relationships of biological groups. Combined with fossil data, molecular clocks provide a powerful tool to date the evolution of life, if calibrations and methods have been applied accordingly. Studies have questioned the bifurcating history of the tree of life, due to genetic exchange via lateral gene transfer (LGT; Ochman *et al.* 2000; Kunin *et al.* 2005). Yet, even though LGT can occur, as shown for example at threonyl tRNA synthease (Zhaxybayeva *et al.* 2006), ribosomal genes seem to be rather conserved (Schirrmeister *et al.* 2012) and large-scale multi-gene phylogenetic analyses have improved our understanding of cyanobacteria and reconstructed the phylum's history with increased statistical support for deep-branching (Shih *et al.* 2013; Bombar *et al.* 2014; Sanchez-Baracaldo *et al.* 2014; Schirrmeister *et al.* 2015).

Differences in results of phylogenetic and -genomic studies relate to, (i) taxon sampling, (ii) sequence data used, (iii) phylogenetic methods applied and (iv) calibration points applied. Different cyanobacterial morphotypes of varying phenotypical complexity (Castenholz 2001) did not evolve in a monophyletic nature (Fig. 1). Today independently evolved unicellular and multicellular lineages exist, which seem to originally have descended from ancient multicellular cyanobacteria (Schirrmeister *et al.* 2011). Results of phylogenomic studies including molecular clocks, have suggested an Archean origin of cyanobacteria, possibly in freshwater, followed by an early diversification at the beginning of the Proterozoic (Blank & Sanchez-Baracaldo 2010; Schirrmeister *et al.* 2015). However, in order to date the evolutionary history of the biosphere on the scale of single phyla or even the tree of life, informative and accurate calibrations are essential. Currently, confidence intervals for the reconstructed node ages are quite large and vary strongly between studies, due to few calibration points and frequently wide time ranges between minimum and maximum ages. Additionally, taxonomic sampling has a strong effect on the reconstructed phylogenies and hence, divergence events deduced from the tree. A combination of different data, including both fossil and geochemical, together with an increased number of calibrations should become the state of the art to calibrate phylogenetic trees.

Preservational quality of Precambrian bacterial fossils

The Precambrian fossil record of bacteria is extremely patchy. This is partly, due to the fact that most Precambrian rocks habitable for life have been subducted back into the Earth, or have been heavily metamorphosed by igneous activity or collisional mountain building. It is also partly due to differential taphonomic processes that affect the preservation quality of bacterial remains. In general, Precambrian fossil preservation quality is poor which hinders the taxonomic interpretation of such remains, but this can be interspersed with deposits exhibiting exceptional preservation (e.g. phosphates of the 1 Ga Torridon Formation of Scotland (Wacey *et al.* 2014), or cherts of the 0.85 Ga Bitter Springs Formation of Australia (Schopf 1968), where direct morphological comparisons can be made to extant bacteria).

Taxonomic bias in microfossil preservation

Attempts to infer the taxonomic affinity of body fossils generally include analyses of bacterial sizes. Experiments have shown that sizes of cyanobacterial cells may decrease during fossilization under higher temperatures (>>100°C) (Oehler 1976), yet, even when this is considered, size analyses could still provide helpful information when undertaking comparisons with living microbes. Rapid mineralization following death (or even causing death) is crucial for the successful preservation of any organism. In many cyanobacteria, preservation may be enhanced by the presence of sheaths surrounding the cells formed by exopolymeric substances, which may delay cellular decay and, hence raise the possibility for mineralization and fossilization (Knoll 1985). Sheaths have also been shown to contain functional groups (e.g. carboxyl) that help mediate rapid mineralization (Konhauser 2007). Anoxic environments

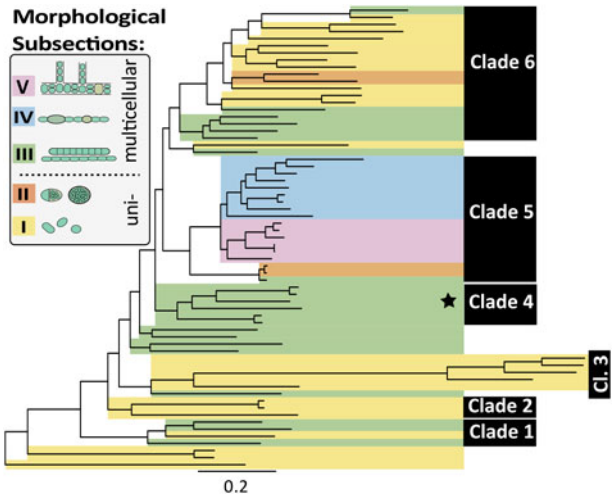

Fig. 1. Schematic for the phylogenomic tree reconstructed for cyanobacteria. Schematic of the Maximum Likelihood tree reconstructed by Schirrmeister *et al.* (2015) based on 756 concatenated genes. Cyanobacterial subsections are displayed in colours, where yellow and orange refer to unicelluar taxa and green, blue and purple describe multicellular taxa. Most multicellular and unicellular lineages existing today appear to have descended from an ancient multicellular lineage. Species from the genus *Oscillatoria* (star) may reach filament widths of >100 μm as shown in Fig. 2.

are also beneficial for cellular preservation since many pathways of decay are arrested in the absence of oxygen (Canfield 1994); in such situations, fossils may then be preserved in pyrite (iron sulphide) due to the activity of anoxygenic heterotrophs such as sulphate-reducing bacteria, for example, as observed in some microenvironments of the 1.9 Ga Gunflint chert (Wacey *et al.* 2013).

Taphonomic bias in microfossil preservation

The chemistry of the immediate environment around decaying organisms also affects the quality of preservation. Most Precambrian fossils are preserved as kerogenous carbon within a fine-grained silica (chert) matrix (Golubic & Seong-Joo 1999). The fine grain size of chert means that morphological details of an organism can sometimes be preserved without too much modification by mineral growth, while the hardness of chert means that it is resistant to later weathering. The same has recently been found for preservation of kerogenous carbon in a phosphate (apatite) matrix (Strother *et al.* 2011), while the combination of clay minerals and phosphate may be even more beneficial for cellular preservation (Wacey *et al.* 2014). Many younger cyanobacteria are calcified, with variable amounts of original organic material preserved (Riding 1992; Golubic & Seong-Joo 1999); indeed calcification of some cyanobacteria may already occur during their lifetime, following carbonate precipitation during carbon fixation, as has been suggested to occur in microbialites from Lake Alchichica, Mexico (Couradeau *et al.* 2013). Calcification, however, generally leads to poorer preservation of morphological details due to neomorphic growth of carbonate grains, and is thought to be responsible for the lack of microfossil preservation in most Precambrian stromatolites (Riding 1992; Schopf *et al.* 2007). In some cases organic material may be completely replaced by minerals, as seen in some putative microfossils from ~3.4 Ga rocks in the Barberton Greenstone Belt (Westall *et al.* 2001b), although in such cases the biogenicity of the microfossils then becomes questionable.

Insights from trace fossils and stromatolites

Trace fossils are non-body remains that record the activity of an organism or biological community in the rock record. These may be dwellings, feeding tracks or indicators of movement. In relation to ancient bacteria, they may include (i) microbial borings, such as claimed from 3.35 to 3.5 Ga pillow lavas from Australia and South Africa (Furnes *et al.* 2004) or (ii) MISS, such as found in numerous Archean rock units including the 3.48 Ga Dresser Formation, Western Australia (Noffke 2010; Noffke *et al.* 2013). MISS depict responses of a microbial mat community to sedimentary processes, such as erosion, deposition and latency. MISS have yet to be reproduced in the laboratory in the absence of biology, and generally occur as a suite of macroscopic and microscopic morphological features, all of which require biological mediation.

Stromatolites are also often cited as evidence for early life on Earth (e.g. Allwood *et al.* 2006; Van Kranendonk 2006, 2011). Stromatolites are similar to MISS, in that they record the growth of a microbial community and its interaction with sediment but have traditionally been classified separately from MISS because they dominantly occur in carbonate settings rather than the siliciclastic settings of MISS (Noffke 2010). Some simple stromatolite morphotypes can be replicated in the absence of biology (e.g. McLoughlin *et al.* 2008) so great care must be taken when interpreting ancient examples. However, tufted or coniform stromatolites (Flannery & Walter 2012) appear to be uniquely biological, and have been cited as evidence for the presence of photoautotrophic bacterial communities growing upwards towards a source of light (i.e. phototaxis). In addition, since the tufts of modern microbial mats are almost exclusively composed of vertically aligned clumps of cyanobacteria, tufted structures in the Archean, at least as far back as 3.2–2.7 Ga (Flannery & Walter 2012; Homann *et al.* 2015), have been suggested to indicate the presence of cyanobacteria at that time. Coniform stromatolites have been reported from even older rocks (~3.4–3.5 Ga; Hofmann *et al.* 1999; Allwood *et al.* 2006; Van Kranendonk 2006); these forms were also likely heavily influenced by phototactic growth of microorganisms but these authors stop short of confidently ascribing them to cyanobacterial activity.

The Precambrian fossil record

As noted previously, Precambrian fossil deposits are rather sporadic in nature and vary in their preservational quality. Nonetheless, as shown in Fig. 2, there is now a fairly coherent record of microfossils from almost 3.5 Ga, right through to definitive examples of cyanobacteria in Palaeo- to Mid-Proterozoic rocks (e.g. Barghoorn & Tyler 1965;

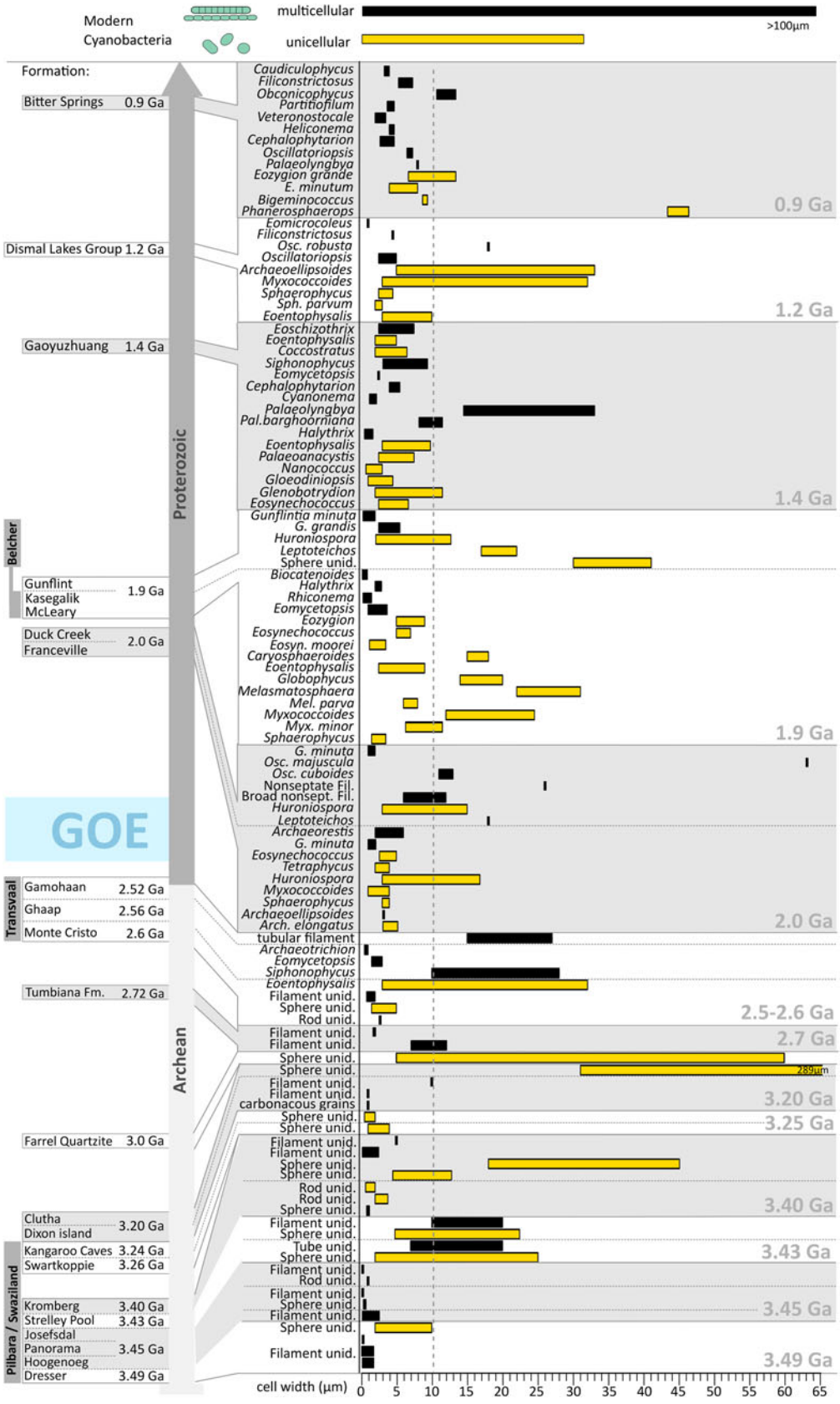

Fig. 2. Distribution of cell widths across Precambrian deposits. Timeline on which cell widths of Precambrian microfossils are summarized based on previous studies. In the Proterozoic only a subset of known deposits is shown. On the top cell widths of modern multicellular and unicellular cyanobacteria are shown. Cell widths of unicellular (yellow) and multicellular (black) microfossils of Precambrian sites correspond to values shown in Table 1. Most modern bacteria are significantly smaller than 10 µm (dashed line), with exception of some cyanobacterial and proteobacterial species. Throughout the Proterozoic several fossils strongly resemble modern cyanobacteria from subsections I, II and IV. Microfossils from the Archean have been compared with cyanobacteria in some studies, but not proven beyond doubt. Large filamentous fossils from 2.7 to 2.6 Ga resemble *Lyngbya* type cyanobacteria in cell width. Several large Archean fossils including the very large 3.2 Ga spheres are of unknown affinity.

Table 1. *Precambrian deposits described in this study*

Age in Ga	Rock unit	Reference	Microfossils	Morphotype	Size in µm Min.	Size in µm Max.
0.90	Bitter Springs Formation	Schopf & Blacic (1971)	*Caudiculophycus acuminatus* n.sp.*	Filament	3.3	4
			Filiconstrictosus majuculus n.sp.*	Filament	5.3	7.3
			Obconicophycus amadeus n.sp.*	Filament	10.7	13.4
			Partitiofilum n.sp.*	Filament	3.7	4.7
			Veteronostocale amoenum n.sp.*	Filament	2	3.5
			Heliconema funiculum n.sp.*	Filament	4	4.7
			Cephalophytarion variabie n.sp.*	Filament	2.7	4.7
			Oscillatoriopsis breviconvexa n.sp.*	Filament	6.5	7.3
			Palaeolyngbya minor n.sp.*	Filament	7.9	8.1
			Eozygion grande n.sp.*	Sphere	6.7	13.4
			Eozygion minutum n.sp.*	Sphere	4	8
			Bigeminococcus lamellosus n.sp.*	Sphere	8.7	9.4
			Phanerosphaerops capitaneus n.sp.*	Sphere	43.3	46.3
1.20	Dismal Lakes Group, Greenhorn Formation	Horodyski & Donaldson (1980)	*Eomicrocoleus crassus* n.sp.*	Filament	1	1
			Filiconstrictosus sp.	Filament	4.5	4.5
			*Oscillatoriopsis robusta**	Filament	18	18
			Oscillatoriopsis curta n.sp.*	Filament	2.5	5
			Archaeoellipsoides grandis n.sp.*	Rod	5	33
			Myxococcoides grandis n.sp.*	Sphere	3	32
			*Sphaerophycus medium**	Sphere	2.5	4.5
			*Sphaerophycus parvum**	Sphere	2	3
			Eoentophysalis dismallakesensis n.sp.*	Sphere	3	10
1.40	Jixian Group, Gaoyuzhuang Formation	Seong-Joo & Golubic (1999)	*Siphonophycus inornatum**	Filament	2	8
			*Eoschizothrix composita**	Filament	2.5	7.5
			*Eontophysalis belcherensis**	Sphere	2	5
			Coccostratus dispergens n.sp.*	Sphere	2	6.5
		Yun (1981)	*Siphonophycus inornatum* n.sp.*	Filament	3.1	9.4
			*Eomycetopsis filiformis**	Filament	2.5	2.5
			Cephalophytarion taenia n.sp.*	Filament	4	5.5
			Cyanonema ligamen n.sp.*	Filament	1.2	2.2
			Palaeolyngbya maxima n.sp.*	Filament	14.5	33
			*Palaeolyngbya barghoorniana**	Filament	8.2	11.5
			Halythrix sp.*	Filament	0.5	1.7
			*Eontophysalis belcherensis**	Sphere	3	9.8
			*Palaeoanacystis vulgaris**	Sphere	2.5	7.5
			Coniunctiophycus gaoyuzhuangense n.sp.*	Sphere	2.5	6.5
			Coniunctiophycus conglobatum n.sp.*	Sphere	0.8	2
			*Nanococcus vulgaris**	Sphere	0.7	3
			Gloeodiniopsis pangjapuensis n.sp.*	Sphere	0.8	3
			Gloeodiniopsis hebeiensis n.sp.*	Sphere	1	4.5
			Glenobotrydion varioforme n.sp.*	Sphere	2	11.5
			Eosynechococcus?	Sphere	2.5	6.7
1.90	Animikie Group, Gunflint Formation	Lanier (1989)	*Gunflintia minuta**	Filament	0.3	2
			*Gunflintia grandis**	Filament	2.5	5.5
			Huroniospora spp.*	Sphere	2.1	12.7
			Leptoteichos glolubicii	Sphere	17	22
			Sphere unidentified	Sphere	30	41
			Biocatenoides sphaerula?	Filament	0.2	0.9
1.90	Belcher Supergoup, Kasegalik and McLeary Formation	Hofmann (1976)	*Halythrix* sp.*	Filament	2	2.9
			Rhiconema antiquum n.sp.?	Filament	0.3	1.5
			Eomycetopsis filiform is?	Filament	1	3.7
			*Eozygion minutum**	Sphere	5	9
			Eosynechococcus grandis n.sp.*	Rod	5	7
			Eosynechococcus medius n.sp.*	Rod	3	4
			Eosynechococcus moorei n.sp.*	Rod	1.2	3.5
			Caryosphaeroides sp.*	Sphere	15	18
			Eoentophysalis n.gen.*	Sphere	2.5	9
			Globophycus sp.*	Sphere	14	20
			Melasmatosphaera magna?	Sphere	22	31
			Melasmatosphaera media?	Sphere	11	21
			Melasmatosphaera parva?	Sphere	6	8
			Myxococcoides sp.*	Sphere	12	24.5
			*Myxococcoides inornata**	Sphere	10	13

			*Myxococcoides minor**	Sphere	6.3	11.5
			*Sphaerophycus parvum**	Sphere	1.5	3.5
2.00	Wyloo Group, Duck Creek Formation	Knoll *et al.* (1988)	*Gunflintia minuta*	Filament	1	2
			Oscillatoriopsis majuscula sp. nov.*	Filament	63	63
			Oscillatoriopsis cuboides sp. nov.*	Filament	11	13
			Nonseptate large filaments	Filament	26	26
			Broad nonseptate filaments	Filament	6	12
			Huroniospora spp.	Sphere	3	15
			Leptoteichos golubicii	Sphere	18	18
2.00	Franceville Group, Formation C	Amard & Bertrand Sarfati (1997)	*Archaeorestis sp.*	Filament	2	6
			Gunflintia minuta?	Filament	1	2.1
			*Eosynechococcus medius**	Rod	2.6	5
			*Tetraphycus major**	Sphere	2	4
			Huroniospora sp.?	Sphere	3	16.8
			Myxococcoides minuta?	Sphere	1	4
			*Sphaerophycus parvum**	Sphere	3	4
			*Archaeoellipsoides dolichos**	Rod	3.2	3.2
			*Archaeoellipsoides elongatus**	Rod	3.1	5.2
2.52	Transvaal Supergroup, Gamohaan Formation	Klein *et al.* (1987)	*Siphonophycus transvaalensis**	Filament	15	27
2.56	Transvaal Supergroup, Ghaap Formation	Altermann & Schopf (1995)	*Archaeotrichion* sp.	Filament	0.5	1
			*Eomycetopsis cf. filiformis**	Filament	1.5	3
			*Siphonophycus transvaalensis**	Filament	10	28
			Eoentophysalis sp.*	Sphere	3	32
2.60	Transvaal Supergroup, Monte Cristo Formation	Lanier (1986)	Unbranched, segmented filaments	Filament	0.8	2
			Solitary and clustered coccoids	Sphere	1.5	5
			Spheroid to ovoid or ellipsoidal	Rod	2.7	2.7
2.72	Fortescue Group, Tumbiana Formation	Schopf (2006)	Narrow, unbranched, septate filaments	Filament	2	2
			Broad unbranched sheath-enclosed filaments*	Filament	7	12
3.00	Gorge Creek Group, Farrel quartzite	Sugitani *et al.* (2009)	Spheroidal microstructures (hollow/ subspherical)	Sphere	5	60
3.20	Moodies Group, Clutha Formation	Javaux *et al.* (2010)	Carbonacous spheroidal microstructures	Sphere	31	289
			Uniseriate, unbranched trichomes	Filament	10	10
3.20	Pilbara Supergroup, Dixon island Formation	Kiyokawa *et al.* (2006)	Dendritically stalked filaments	Filament	1	1
			Carbonaceous grains	Sphere	1	1
3.24	Pilbara Supergroup, Kangaroo Caves Formation	Rasmussen (2000)	Threadlike, unbranched and uniform filaments	Filament	0.5	2
3.26	Swaziland Supergroup, Swartkoppie Formation	Knoll & Barghoorn (1977)	Microspheroids with smooth organic walls	Sphere	1	4
3.40	Swaziland Supergroup, Kromberg Formation	Walsh (1992)	Hollow filament (unid.)	Filament	5	5
			Non-septate filaments	Filament	0.2	2.5
			Ellipsoidal (unid.)	Sphere	18	45
			Spheroids	Sphere	4.5	12.8
3.40	Swaziland Supergroup, Kromberg Formation	Westall *et al.* (2001a)	Rod-shaped (like 'rice grains')	Rod	0.65	2
			Sausage shaped rods	Rod	2	3.8
			Spherules	Sphere	0.8	1.2
3.43	Pilbara Supergroup, Strelley Pool Formation	Sugitani *et al.* (2013)	Hollow tubular carbonaceous filaments	Filament	10	20
			Hollow spheroids	Sphere	4.8	22.4
3.43	Pilbara Supergroup, Strelley Pool Formation	Wacey *et al.* (2011)	Hollow cylindrical tubes	Filament	7	20
			Hollow spheroids and ellipsoids	Sphere	2	25
3.45	Pilbara Supergroup, Panorama Formation	Westall *et al.* (2006b)	Filament (unidentified)	Filament	0.25	0.25
			Coccoidal structures	Sphere	0.35	0.7
			Rod/vibroid-shaped (unid.)	Rod	0.4	0.4
3.45	Swaziland Supergroup, Josefsdal Formation	Westall *et al.* (2006a)	Parallel filaments	Filament	0.25	0.25
			Rods/vibroids	Rod	1	1
3.45	Swaziland Supergroup, Hoogenoeg Formation	Walsh & Lowe (1985)	Hollow filament (unid.)	Filament	0.2	2.6
3.49	Pilbara Supergroup, Dresser Formation	Glikson *et al.* (2008)	Carbonacous matter aggregates	Sphere	2	10
3.49	Pilbara Supergroup, Dresser Formation	Ueno *et al.* (2001)	Branched filaments	Filament	0.2	0.4
			Other filaments	Filament	0.1	1.8
			Spiral filaments	Filament	0.2	1.8

Sizes of microfossils remains shown in Fig. 2 as previously described for Precambrian deposits. A star (*) indicates that those fossils have been compared with cyanobacteria. A (?) indicates that the authors were uncertain whether these fossils could be associated with cyanobacteria. For older deposits, due to loss of taxonomic characteristics, it becomes harder to classify these microstructures.

Hofmann 1976; Schopf & Walter 1983; Altermann & Schopf 1995; Javaux *et al.* 2010; Wacey *et al.* 2011; Sugitani *et al.* 2013).

In Fig. 2 and in the discussion that follows we include all putative microfossils previously reported in the literature that adhere to (or with further work could adhere to) accepted antiquity and biogenicity criteria. These criteria have been developed and refined by a number of authors (e.g. Schopf & Walter 1983; Buick 1990; Brasier *et al.* 2004; Sugitani *et al.* 2007) and a summary can be found in Wacey (2009). We acknowledge that many of the putative microfossils included here, particularly small spheroids and those older than 3.2 Ga, fall into the category of 'further work needed to confirm their biogenicity', but we wish to be as inclusive as possible for this review article. 'Microfossils' not included in this discussion are those that have subsequently been reinvestigated and shown to fail one or more of the biogenicity or antiquity criteria. These include objects whose source rocks cannot be relocated (e.g. Awramik *et al.* 1983), objects shown to be more recent contaminants (e.g. Nagy 1974), and objects whose morphology has been shown to be incompatible with that of a biological organism (e.g. Schopf 1993).

Almost all of these microfossils are preserved by rapid, early permineralization by silica, although rare examples of non-silicified material also exist (Javaux *et al.* 2010). The fossiliferous assemblages comprise filamentous/tubular (multicellular) and spherical (unicellular) organic fossils of varying sizes, which have mostly been deposited in either shallow marine or intertidal environments. Preservation of individual cells within fossilized filaments is rare in Precambrian deposits, especially in the Archean and often only the surrounding sheaths in the form of tubes are preserved, as demonstrated for some of the very oldest body fossils in the ~3.4 Ga Strelley Pool Formation, Pilbara Craton of Western Australia (Wacey *et al.* 2011). Fossil taxa disparity increases in the Proterozoic, in terms of both size and distinguishable morphotypes. Though, this might be a result of increased preservation quality, the question arises, whether this also may be a result of increased adaptability following intensified competition. Although bacterial sizes have been demonstrated to decrease slightly during permineralization by silica at high temperatures (Oehler 1976), relatively large spheres, up to 300 μm in diameter (and comparable in size with some of the largest extant bacteria), have nonetheless been described in Archean rocks (e.g. 3.0 Ga Farrel Quartzite of Western Australia, Sugitani *et al.* 2009; 3.2 Ga Moodies Group of South Africa, Javaux *et al.* 2010; 3.4 Ga Strelley Pool Formation of Western Australia, Sugitani *et al.* 2010, 2013).

Precambrian cyanobacterial microfossil record

The earliest fossiliferous deposits are found in the East Pilbara Granite-greenstone Terrane of the Pilbara Craton, Western Australia (Van Kranendonk 2006; Wacey 2012) and the Barberton Greenstone Belt, South Africa (Walsh 1992; Westall *et al.* 2006b). Both sites contain rocks of Palaeoarchean age with metamorphosed approaching 3.5 Ga containing the first indications for life (body fossils). The 3.52–2.97 Ga Pilbara Supergroup contains potential microfossils at a number of stratigraphic intervals, including the ~3.48 Ga Dresser Formation, ~3.45 Ga Panorama Formation, ~3.43 Ga Strelley Pool Formation, ~3.24 Ga Kangaroo Caves Formation and ~3.0 Ga Farrel Quartzite. In these deposits, spherical fossils (Fig. 2) have a wide range in diameters from <1 μm to around 100 μm, whereas filaments/tubes have a much more restricted range of widths from <1 to 20 μm and do not appear to preserve individual cells. Of particular note is the Strelley Pool Formation, which not only contains the largest spheres and filaments, but also spindle-like structures that can have diameters well in excess of 50 μm (Sugitani *et al.* 2010; Wacey *et al.* 2011). Fewer fossils have been documented from the Swaziland Supergroup of the Barberton Greenstone Belt, although spheres, filaments and spindles are all represented, along with putative rods and sausage-shaped cells (Knoll & Barghoorn 1977; Walsh & Lowe 1985; Walsh 1992; Westall *et al.* 2001a; Tice & Lowe 2004; Westall *et al.* 2006b). Of particular note in the South African deposits are very large spheres (30–300 μm) discovered within siliciclastic sediments of the 3.2 Ga Moodies Group (Javaux *et al.* 2010). Nevertheless, poor preservation of these structures does not allow for a comparison with cyanobacteria. Towards younger Precambrian deposits morphological characteristics are preserved with increasing details allowing for direct comparisons with modern bacterial phyla, including cyanobacteria (Fig. 3).

Between ~3.0 and ~2.6 Ga there is somewhat of a gap in the body fossil record with only rare filaments (Fig. 2) described from the 2.7 Ga Tumbiana Formation of Western Australia (Schopf & Walter 1983); these are up to 12 μm in diameter and superficially resemble cyanobacteria, such as genus *Lyngbya*. The first large filaments (up to ~30 μm in width) comparable with extant cyanobacteria have been reported from younger deposits towards the end of the Archean, such as "*Siphonophycus*" from 2.5 to 2.6 Ga Gamohaan Formation and Ghaap Dolomite of the Transvaal Supergroup, South Africa (Klein 1987; Altermann & Schopf 1995). From this time onwards, throughout the Proterozoic, fossiliferous deposits often contain various distinguishable morphotypes, including larger spheres and filaments that have been unambiguously classified as cyanobacteria (e.g. *Eoentophysalis* and at least 10 other morphotypes from the ~1.9 Ga Belcher Supergroup of Canada; Hofmann 1976). Finally, microfossils from the 0.85 Ga Bitter Springs Formation of central Australia (Schopf 1968) are worthy of particular note as they are among the best preserved Precambrian remains and have been compared with various cyanobacterial forms from subsections I – IV (see below for explanation of subsections), identifying families such as *Chroococcales, Oscillatoriales* and *Nostocales*.

Size comparison of fossil and modern eubacteria

Several characteristics have been described to distinguish cyanobacterial taxa based on structural and developmental differences (Rippka *et al.* 1979; Castenholz 2001). Living cyanobacterial taxa have been categorized into five different subsections, where subsections I and II comprise unicellular

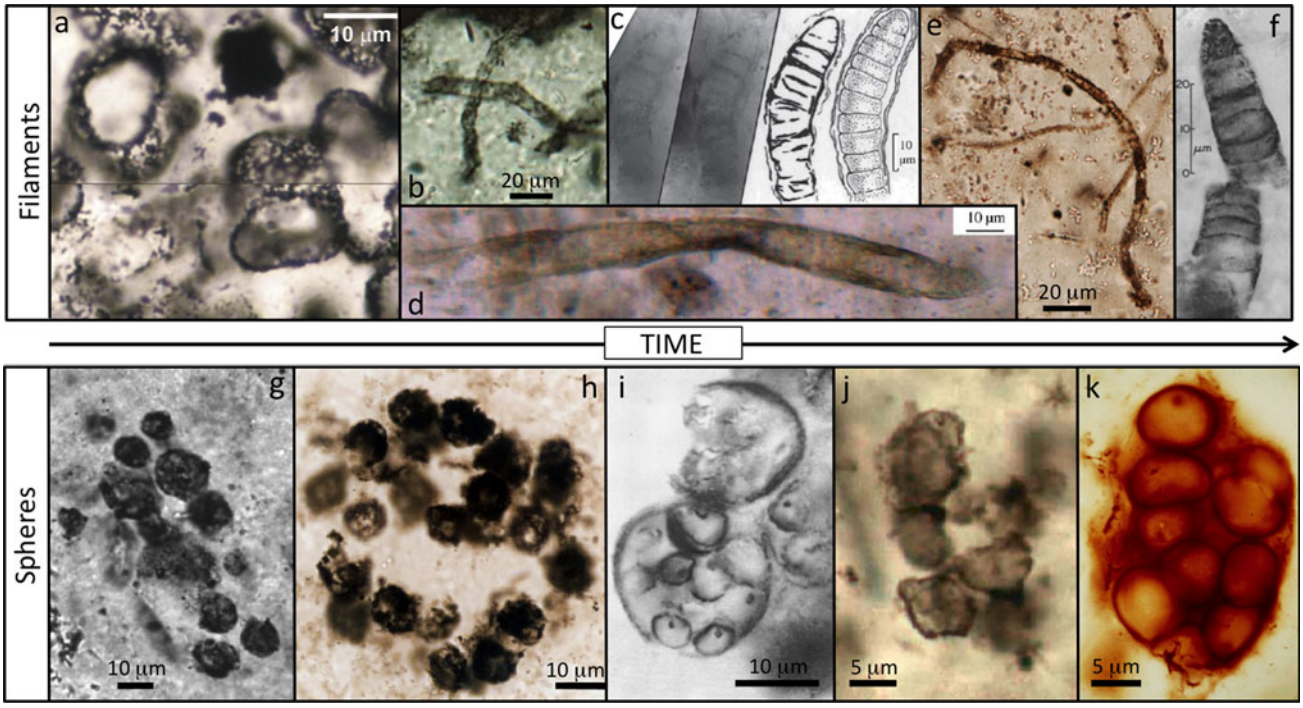

Fig. 3. Microfossils from Precambrian units. Shown are representative filamentous (a–f) and spheroidal (g–k) microfossils from Precambrian units. While older microfossils have lost most characteristics for identification, younger fossils show remarkable similarity to living cyanobacterial morphotypes. (a) Unidentified tubular filaments from the 3.43 Ga Strelley Pool Formation. (b) Unidentified tubular filament from the 3.2 Ga Dixon Island Formation (reproduced with permission from Kiyokawa *et al.* 2006). (c) Segmented filament plus interpretative sketches (cf. *Lyngbya*) from the 2.73 Ga Tumbiana Formation (reproduced with permission from Schopf 2006). (d) Non-segmented filament identified as *Siphonophycus transvaalense* from the 2.5 Ga Gamohaan Formation (reproduced with permission from Schopf 2006). (e) Filament identified as *Gunflintia grandis* from the 1.88 Ga Gunflint Formation. (f) Segmented filament identified as *Obconicophycus amadeus* from the 0.85 Ga Bitter Springs Formation (reproduced with permission from Schopf & Blacic 1971). (g) Cluster of unidentified spheres from the 3.43 Ga Strelley Pool Formation (reproduced with permission from Sugitani *et al.* 2013). (h) Cluster of unidentified spheres from the 3.0 Ga Farrel Quartzite (reproduced with permission from Sugitani *et al.* 2009). (i) Spheres identified as *Eoentophysalis belcherensis* from the 1.9 Ga Belcher Group (reproduced with permission from Hofmann 1976). (j) Cluster of unidentified spheres from the 1.878 Ga Gunflint Formation. (k) Spheres identified as *Myxococcoides minor* from the 0.85 Ga Bitter Springs Formation (credit, ucmp.berkely.edu).

taxa differing in their mode of cell division and subsections III to V contain multicellular taxa. Cell differentiation into heterocysts for nitrogen fixation or akinetes, as resting cells have been described only for subsections IV to V. However, many characteristics may be lost during the early stage of decay and fossilization or later during the multitude of geological events that have affected Earth's oldest rocks. Putative akinetes have been described from the Paleoproterozoic 2.0 Ga Franceville Group (Amard & Bertrand-Sarfati 1997), while putative heterocysts and akinetes have both been described from the 1.9 Ga Gunflint Formation (Licari & Cloud 1968). The quality of these images is insufficient, however, to determine if these are true primary biological features or perhaps taphonomic artefacts. Often cell sizes, particularly cell width, plus general structure (uni- versus multicellular), are the only characteristics that remain. Most modern bacteria are significantly smaller (<2 μm) in cell widths than eukaryotic cells. Exceptions are found among cyanobacteria and proteobacteria, where taxa such as *Beggiatoa* and *Thiomargarita* show cell widths larger than 10 μm (Castenholz 2001; Garrity *et al.* 2005). Therefore, although microfossils with sizes below 10 μm are not very informative for assigning a specific eubacterial affiliation, sizes exceeding 10 μm may provide valuable information. Large proteobacteria mostly belong to one order, the *Thiotrichales*, which include *Beggiatoa, Thiomargerita* and *Thioploca*, whereas large cyanobacterial species occur in several separately evolved form-genera, such as *Chroococcus, Oscillatoria, Lyngbya* and *Staniera* (Fig. 4). In the fossil record microfossils that exceed sizes of 10 μm (dashed line in Fig. 2) occur already well before the end of the Archean (Altermann & Schopf 1995; Javaux *et al.* 2010; Sugitani *et al.* 2010; Wacey *et al.* 2011), long before the appearance of eukaryotes, around 1.6 Ga (Javaux 2011; Knoll 2014). Such large fossils are consistent with the presence of cyanobacterial or proteobacterial taxa, perhaps even in the early Archean. Unfortunately, little is currently known about the evolution of multicellular proteobacteria, so pinpointing the first appearance of cyanobacteria by use of fossils alone is difficult.

Organic carbon fractionation in Precambrian deposits

Chemical fossils include fractionations of the stable isotopes of certain elements (e.g. carbon) that can be related to a biological

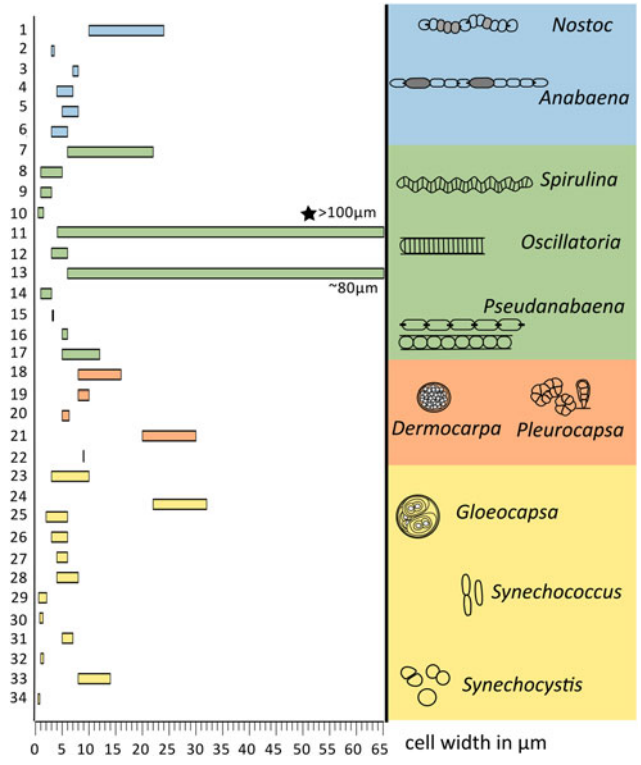

Fig. 4. Cell widths of modern cyanobacterial genera. Cell widths of modern cyanobacterial form-genera as described in Bergey's Manual of Systematic Bacteriology (Castenholz 2001). Modern unicellular cyanobacteria from subsections I and II are presented in yellow and orange. Extant multicellular cyanobacteria are shown in green (subsection III) and blue, if they are capable of forming akinetes and heterocysts (subsections IV). Cell widths within trichomes of cyanobacteria from subsection V vary greatly (Castenholz 2001) and are therefore not included in the size comparison. Among the largest cyanobacterial taxa belong to the genera *Oscillatoria* (star) and *Lyngbya*. Numbers refer to taxon names in Table 2.

metabolism, plus chemical compounds (biomarkers) that may be related to certain types of organism. Although it has recently been suggested that biomarkers are unlikely to be found in Archean shales (French *et al.* 2015), carbon isotopes may still be of some use for reconstructing the early evolutionary history of cyanobacteria. Significant carbon isotope fractionation takes place during photosynthesis by living organisms, such as plants or phototrophic bacteria, which prefer the lighter ^{12}C isotopes, leaving an increased amount of ^{13}C in the inorganic reservoir (Van Der Merwe 1982). Carbon isotope fractionation may be useful to distinguish between different metabolic pathways. For example, oxygenic phototrophs today show organic carbon isotope values ($\delta^{13}C$) between −30 and −25‰, a signature of the RuBisCO I enzyme operating during carbon fixation, whereas $\delta^{13}C$ values for methanogens range from −45 to −35‰ due to the activity of RuBisCO III in those organisms (Fig. 5) (Schidlowski 2001; Nisbet *et al.* 2007). Unfortunately, cyanobacteria do not possess a unique range of carbon isotope values, so these data alone cannot be used to pinpoint their presence in the rock record. Carbon isotope fractionation during biological carbon fixation appears to have occurred throughout the geological record (Schidlowski 2001) and, although the very earliest $\delta^{13}C$ data is rather controversial (Mojzsis *et al.* 1996; Rosing 1999), persistent fractionation between the organic carbon ($\delta^{13}C \sim -25‰$) and inorganic carbonate ($\delta^{13}C \sim 0‰$) reservoirs seem to indicate the presence of life since at least 3.5 billion years ago (Schidlowski 1988). Comparison of $\delta^{13}C$ values observed in different organisms today (Schidlowski 1992) with values from Precambrian deposits show that most fossil data partially overlap with values found for living cyanobacteria (Fig. 5). However, most fossil $\delta^{13}C$ values are also consistent with those of modern anoxic photosynthesizers. Strongly negative $\delta^{13}C$ values (<−40‰) in hydrothermal veins beneath the ~3.5 Ga Dresser Formation, Western Australia (Ueno *et al.* 2001), in the ~2.7 Ga Tumbiana Formation of the Fortescue Group, Western Australia (Strauss & Moore 1992), in the ~2.5 GaTransvaal Supergroup, South Africa (Fischer *et al.* 2009), and in the ~2.0 Ga Franceville Group, Gabon (Gauthier-Lafaye & Weber 2003) may indicate the presence of methanotrophic or methanogenic bacteria (Table 3). Interpreting these data, however, can be rather difficult. Significant differences in $\delta^{13}C$ values have been reported for different fossils within the same deposit (House *et al.* 2000) and for the same deposit across different studies (Barghoorn & Tyler 1965; Barghoorn *et al.* 1977b; Strauss & Moore 1992; House *et al.* 2000; Williford *et al.* 2013). While the latest high spatial resolution isotopic work shows great promise for discriminating between metabolisms (Williford *et al.* 2013), negative $\delta^{13}C$ values between about −20 and −30‰ in Precambrian deposits currently can neither confirm, nor reject the presence of cyanobacteria.

Geochemical evolution of the Precambrian

Increased sampling and advancements in geochemical proxies have revealed a complex picture of evolving ocean geochemistry during the Precambrian (e.g. Lyons *et al.* 2014), in which oceans were mostly anoxic and ferruginous with localized euxinic conditions (e.g. anoxic conditions with hydrogen sulphide) prior to the GOE beginning around 2.45 Ga (Fig. 6; Holland 1984; Bekker *et al.* 2004). However, there is now increasing evidence for at least low levels of oxygen (~3×10^{-4}PAL), at least periodically, prior to the GOE. For example, elevated levels of Mo and rhenium (Re) in ~2.5 Ga sediments have provided evidence for localized oxidative weathering (also known as 'whiffs of oxygen') before the GOE (Anbar *et al.* 2007; Wille *et al.* 2007), while evidence from Mo isotopes from the 2.9 Ga Sinqeni Formation, Pongola Supergroup, South Africa, indicate the presence of manganese oxidation, a process that would require significant free oxygen (Planavsky *et al.* 2014). Recent evidence for atmospheric oxygen, based on chromium isotopes from the 3 Ga Pongola Supergroup of South Africa (Crowe *et al.* 2013), suggests that oxygenic photosynthesis might have appeared at least for a short period 3 billion years ago. Lastly, evidence from Fe isotopes and U concentrations shows that there was

Table 2. *Sizes of modern Cyanobacteria*

Modern Cyanobacteria (Castenholz 2001)					
			Cell width in μm		
	Form-genus	Morphotype	Min	Max	
1	Calothrix	Filament	10	24	Subsection IV
2	Nostoc	Filament	3	3.5	
3	Nodularia	Filament	7	8	
4	Cyanospira	Filament	4	7	
5	Anabaenopsis	Filament	5	8	
6	Anabaena	Filament	3	6	
7	Trichodesmium	Filament	6	22	Subsection III
8	Spirulina	Filament	1	5	
9	Pseudanabaena	Filament	1	3	
10	Prochlorothrix	Filament	0.5	1.5	
11	Oscillatoria	Filament	4	100	
12	Microcoleus	Filament	3	6	
13	Lyngbya	Filament	6	80	
14	Limnothrix	Filament	1	3	
15	Leptolyngbya	Filament	3	3	
16	Geitlerinema	Filament	5	6	
17	Arthrospira	Filament	5	12	
18	Pleurocapsa	Sphere	8	16	Subsection II
19	Myxosarcina	Sphere	8	10	
20	Chroococcidiopsis	Sphere	5	6.3	
21	Staniera	Sphere	20	30	
22	Dermocarpa/Cyanocystis	Apical basal	9	9	
23	Gloeocapsa	Sphere	3	10	Subsection I
24	Chroococcus	Sphere	22	32	
25	Synechocystis	Sphere	2	6	
26	Microcystis	Sphere	3	6	
27	Gloeothece	Rod	4	6	
28	Dactylococcopsis	Spindle	4	8	
29	Synechococcus	Oval	0.6	2.1	
30	Cyanobium	Oval	0.8	1.4	
31	Chamaesiphon	Ellipsoidal	5	7	
32	Gloeobacter	Oval	1	1.5	
33	Prochloon	Sphere	8	14	
34	Prochlorococcus	Rod	0.5	0.8	

Sizes of modern cyanobacterial form-genera as described in Bergey's Manual of Systematic Bacteriology (Castenholz 2001). In living cyanobacteria five subsections can be morphologically distinguished. Subsections I and II comprise unicellular taxa, subsections III–V contain multicellular taxa. Sizes are shown for subsections I–IV, where numbers refer to taxa shown in Fig. 4. Subsection V cyanobacteria show strongly variable cell widths and are not described here.

a redox boundary in the 3.2 Ga ocean, with the shallow ocean containing relatively enriched O_2 contents (Satkoski *et al.* 2015) suggesting oxygenic photosynthesis may have appeared prior to 3.2 Ga.

During the GOE at the beginning of the Proterozoic (2.45–2.32 Ga) atmospheric O_2 increased globally, although concentrations are thought to have remained rather low (<0.1–0.001% PAL) for the rest of the Precambrian, (Berner & Canfield 1989; Lyons *et al.* 2014; Planavsky *et al.* 2014). Around this time Earth also experienced the Huronian glaciation, which has been suggested to have been caused by the disappearance of the methane-driven greenhouse effect, in turn resulting from increased pulses of oxygen associated with the GOE (Kasting 2005). Following the GOE at ~2.21–2.06, a global carbon isotopic excursion (Lomagundi) was recorded in marine and terrestrial carbonates, driven by the enhanced burial of organic carbon into sediments (Bekker *et al.* 2006), while there was also significant deposition of manganese just after atmospheric oxygenation (Kirschvink *et al.* 2000) and the first worldwide accumulation of phosphorites at ~2.0 Ga (Melezhik *et al.* 2005). After the global cessation of banded iron formation deposition at ~1.8 Ga, mildly oxygenated surface ocean conditions were often underlain by wedges of euxinic conditions in continental margin/slope settings, while the deep ocean remained anoxic and ferruginous (Fig. 6; Planavsky *et al.* 2011). During the Neoproterozoic, further extreme changes in biogeochemical cycles occurred, followed by the emergence and diversification of marine planktonic nitrogen-fixing cyanobacteria (Sanchez-Baracaldo *et al.* 2014) and metazoan (Erwin *et al.* 2011; Yuan *et al.* 2011).

The oxygenation of Earth's atmosphere has been linked to various processes, including a reduction in volcanic degassing leading to reduced sinks for O_2 (Van Kranendonk *et al.* 2012), and changes in nutrient availability during the Precambrian

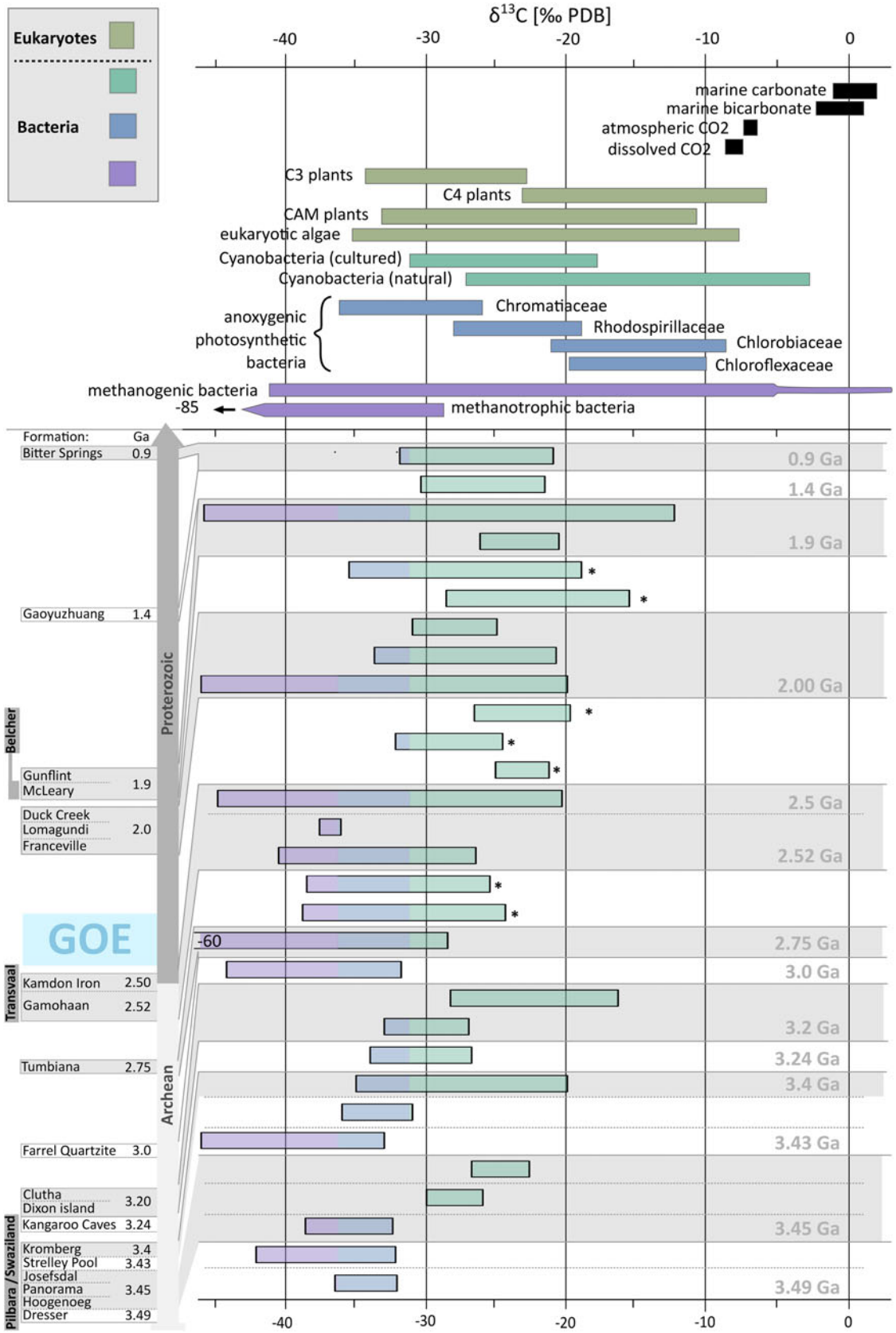

Fig. 5. Organic carbon isotope fractioning during the Precambrian. Shown on top are $\delta^{13}C$ values for living organisms (Schidlowski 1992; Schidlowski 2001). Below are plotted organic $\delta^{13}C$ values from different fossil Precambrian deposits that have been described in the literature and listed in Table 2. $\delta^{13}C$ values correspond to formations shown on the timeline in the figure. Values marked by a star (*) are not shown on the time line and (from top) refer to: 1.90 Ga Great Salve Supergroup, 1.98 Ga Earaheedy Group, 2.22 Ga Pretoria Group, 2.34 Ga Huronian Supergroup, 2.42 Ga Itabira Supergroup, 2.54 Ga Mt Silva and Mt McRae Fms., 2.55 Ga Malmani/Campbellrand Subgroup, (Karhu & Holland 1996). Deposits from all time periods show $\delta^{13}C$ values that could indicate a presence of cyanobacteria.

Table 3. *Carbon isotope fractionations in different Precambrian deposits*

Age in Ga	Rock unit	Reference	Organic $\delta^{13}C$ Min	Organic $\delta^{13}C$ Max
0.90	Bitter Springs Formation	House *et al.* (2000)	−31.9	−21
1.40	Jixian Group, Gaoyuzhuang Formation	Gou *et al.* (2013)	−30.4	−21.6
1.90	Animikie Group, Gunflint Formation	House *et al.* (2000), Barghoorn *et al.* (1977a, b)	−45.8	−12.4
1.90	Belcher Supergroup, McLeary Formation	Strauss & Moore (1992)	−26.2	−20.6
1.90	Great Slave Supergroup	Karhu & Holland (1996)	−35.5	−19
1.98	Earaheedy Group	Karhu & Holland (1996)	−28.6	−15.6
2.00	Wyloo Group, Duck Creek Formation	Schopf (1983), Wilson *et al.* (2010)	−31	−25
2.07	Lomagundi Group	Bekker *et al.* (2008)	−33.7	−20.8
2.03	Franceville Group	Gauthier-Lafaye & Weber (2003)	−46	−20
2.22	Pretoria Group	Karhu & Holland (1996)	−26.6	−19.8
2.34	Huronian Supergroup	Karhu & Holland (1996)	−32.2	−24.6
2.42	Itabira Group	Karhu & Holland (1996)	−25.1	−21.3
2.50	Transvaal Supergroup, Kamden Iron Formation	Fischer *et al.* (2009)	−44.83	−20.38
2.52	Transvaal Supergroup, Gamohaan Formation	Klein *et al.* (1987)	−37.6	−36.08
2.52	Transvaal Supergroup, Gamohaan Formation	Strauss & Moore (1992)	−40.5	−26.5
2.54	Mt Silvia and Mt McRae Formations	Karhu & Holland (1996)	−38.5	−25.5
2.55	Malmani/Campbellrand Subgroup	Karhu & Holland (1996)	−38.8	−24.4
2.75	Fortescue Group, Tumbiana Formation	Strauss & Moore (1992)	−60.9	−28.5
3.00	Gorge Creek Group, Farell Quartzite	House *et al.* (2013)	−44.2	−31.8
3.20	Moodies Group, Clutha Formation	Javaux *et al.* (2010)	−28.3	−16.4
3.20	Pilbara Supergroup, Dixon island Formation	Kiyokawa *et al.* (2006)	−33	−27
3.24	Pilbara Supergroup, Kangaroo Caves Formation	Duck *et al.* (2007)	−34	−26.8
3.42	Swaziland Supergroup, Kromberg Formation	Tice & Lowe (2006)	−35	−20
3.43	Pilbara Supergroup, Strelley Pool Formation	Sugitani *et al.* (2010)	−36	−31
3.43	Pilbara Supergroup, Strelley Pool Formation	Wacey *et al.* (2011)	−46	−33
3.45	Swaziland Supergroup, Josefsdal Formation	Westall *et al.* (2006a, b)	−26.8	−22.7
3.45	Pilbara Supergroup, Panorama Formation	Westall *et al.* (2006a, b)	−30	−26
3.45	Swaziland Supergroup, Hoogenoeg Formation	Strauss & Moore (1992)	−38.6	−32.4
3.49	Pilbara Supergroup, Dresser Formation	Ueno *et al.* (2001)	−42.1	−32.2
3.49	Pilbara Supergroup, Dresser Formation	Glikson *et al.* (2008)	−36.5	−32.1

Organic $\delta^{13}C$ values are shown in Fig. 5 as previously described for Precambrian deposits.

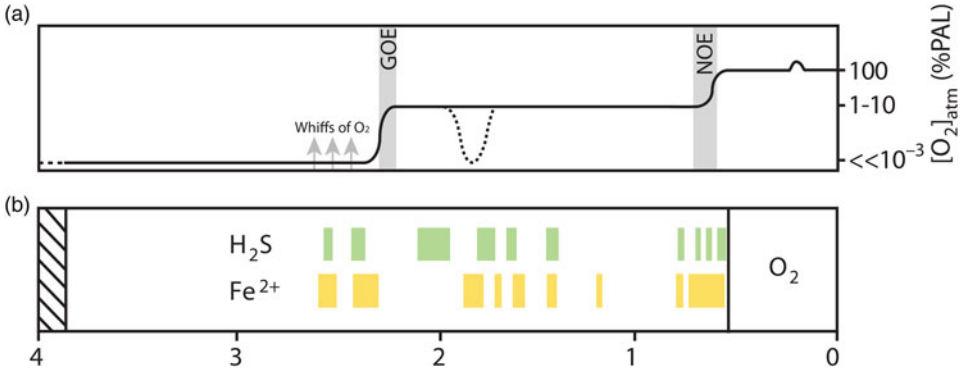

Fig. 6. Ocean geochemistry in the Precambrian. (a) Estimates of atmospheric oxygen compared with present atmospheric level (PAL). (b) Observations of the marine redox state based on the shale record showing the distribution of euxinic and ferruginous deep waters. The figure shown is a modification of Fig. 2 by Planavsky *et al.* (2011).

(Anbar & Knoll 2002; Lyons *et al.* 2014). Undoubtedly nutrient availability would have determined in which habitats cyanobacteria could have first evolved (e.g. manganese availability and the origin of oxygenic photosynthesis; Sousa *et al.* 2013) and consequently diversified (e.g. increased Mo and diversification of planktonic N-fixing cyanobacteria; Sanchez-Baracaldo *et al.* 2014). Nitrogen, phosphorous, as well as, some micronutrients exert major controls on primary productivity in the open-ocean (Jones *et al.* 2015) and for this reason geochemical models have focused on understanding how feedbacks might have restricted organic carbon burial and hence oxygen production (Lyons *et al.* 2014). Key trace metals essential for N-fixation, such as Mo (Zerkle *et al.* 2006), would have been depleted (Anbar & Knoll 2002) in the sulfidic conditions of early- to mid-Proterozoic open ocean (as opposed to coastal) habitats, which likely provided

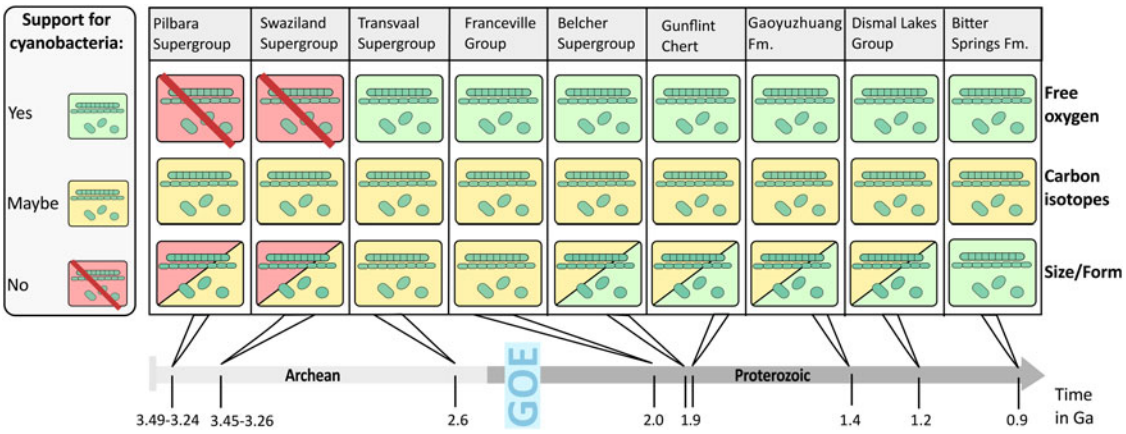

Fig. 7. Evaluation of the evidence for cyanobacteria throughout the Precambrian. The likelihood of a cyanobacterial presence in different Precambrian deposits is evaluated on the basis of three lines of evidence: (1) presence of free oxygen, (2) organic $\delta^{13}C$ values and (3) form and size of microfossils. Carbon isotopes do not offer a possibility to exclude the presence of cyanobacteria in any of the mentioned deposits. Evidence of free oxygen supports a presence of cyanobacteria from 2.9 Ga on. The presence of microfossils larger than 10 μm in rocks prior 3.0 Ga does not provide enough evidence alone for the presence of cyanobacteria.

a challenging environment (Scott *et al.* 2008; Planavsky *et al.* 2011; Lyons *et al.* 2014) for planktonic cyanobacteria to proliferate into. This is consistent with recent phylogenomic analyses of marine planktonic cyanobacteria (both nitrogen and non-nitrogen fixers) indicating that their widespread emergence in the Neoproterozoic would have significantly strengthened the biological pump in the ocean. Higher primary productivity towards the end of the Precambrian would have contributed to the major disruption of the carbon cycle, a further increase in oxygenation of the Earth's surface, oxygenation of the deep ocean and the extreme glaciation events recorded during the Cryogenian (850–635 Ma) (Fairchild & Kennedy 2007; Sanchez-Baracaldo *et al.* 2014). This is also consistent with significant changes in ocean geochemistry recorded towards the end of the Precambrian, with increasing Mo enrichment in 550 Ma open ocean black shales indicating a major rise in atmospheric oxygen concentrations (Scott *et al.* 2008; Sahoo *et al.* 2012; Reinhard *et al.* 2013).

One of the fundamental questions that remains unclear is why it took so long for atmospheric oxygen levels to rise around 2.4 Ga, if oxygenic photosynthesis did indeed evolve during the Archean. Previous suggestions of a terrestrial/freshwater origin of cyanobacteria (Blank & Sanchez-Baracaldo 2010) would be consistent with extremely low levels of primary productivity predicted by modelled benthic microbial ecosystems during the Archean and early Palaeoproterozoic (Lalonde & Konhauser 2015). A limited ecological habitat for cyanobacteria would also have kept oxygen and organic fluxes modest resulting in 'whiffs of oxygen' as recorded in the geological record (cf. Anbar *et al.* 2007), while pulses of Fe(II) associated with mantle plume events may have proved toxic to cyanobacteria (Swanner *et al.* 2015). The lack of oxidized minerals in shallow marine environments before 2.4 Ga also suggests that any continental oxygen production was not reflected in iron oxidation in the deep oceans (Blank & Sanchez-Baracaldo 2010).

Conclusion

Taxonomical classification of Precambrian fossils can prove challenging, if not impossible in some instances. Nevertheless, combined evidence of fossil and geochemical data may provide the possibility to exclude the presence of some phyla from certain fossil deposits. Focusing on cyanobacteria, size and shape, as well as the presence of free oxygen, in local oases before the GOE, do not fully exclude the possibility of cyanobacteria being part of the preserved microbial communities of the Precambrian deposits presented here (Fig. 7). In the early the Proterozoic preservation of taxonomic characteristics increases, allowing for a comparison of microfossils with living eubacterial phyla including cyanobacterial affinity. Although, organic $\delta^{13}C$ data allow for a presence of cyanobacteria in almost every deposit, similar negative values could also be a result of anoxic photosynthesis. Among living bacteria large filamentous taxa belong exclusively to cyano- and proteobacteria. Therefore, fossils exhibiting sizes larger than 10 μm strongly suggest a presence of one, or both of those phyla. Yet, little is known about the origin of multicellularity in Proteobacteria, nor about their preservation. Investigating the evolution of Proteobacteria could provide valuable information for the classification of large microfossils, particularly of filamentous forms. Novel techniques, such as three-dimensional imaging using tomographic microscopy, would enhance visualization of such Archean microbial communities and may help to identify additional characteristics to elucidate taxonomic affinities of microbes preserved within those deposits.

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