Proterozoic Photosynthesis – a critical review

N. J. Butterfield

Department of Earth Sciences,

University of Cambridge

Cambridge, UK CB2 3EQ

Email: njb1005@cam.ac.uk

Abstract

Chlorophyll-based photosynthesis has fuelled the biosphere since at least early Archean, but it was the ecological takeover of oxygenic cyanobacteria in the early Palaeoproterozoic, and of photosynthetic eukaryotes in the late Neoproterozoic, that gave rise to a recognizably modern ocean-atmosphere system. The fossil record offers a unique view of photosynthesis in deep time, but is deeply compromised by differential preservation and non-diagnostic morphologies. The pervasively polyphyletic expression of modern cyanobacterial phenotypes means that few Proterozoic fossils are likely to be members of extant clades; rather than billion-year stasis, their similarity to modern counterparts is better interpreted as a combination of serial convergence and extinction, facilitated by high levels of horizontal gene transfer. There are few grounds for identifying cyanobacterial akinetes or crown-group Nostocales in the Proterozoic record. Such recognition undermines the results of various Ancestral State Reconstruction analyses, as well as molecular clock estimates calibrated against demonstrably problematic Proterozoic fossils. Eukaryotic organisms are likely to have acquired their (stem-group nostoclean) photoendosymbionts/plastids by at least the Palaeoproterozoic, but remained ecologically marginalized by incumbent cyanobacteria until the late Neoproterozoic appearance of suspension feeding animals.

Key words: cyanobacteria, microfossils, biomarkers, eukaryotes, evolution

Introduction

With trivial exceptions, all of the carbon chemistry driving the modern biosphere originates from the photosynthetic reduction of CO₂. Other sources exist but none of these is large

enough to have left a measurable signature in the geological record (Rosing et al. 2006). In light of a continuous record of carbon isotope discrimination from at least 3.5 Ga (Schidlowski 1988, [Krissansen-Totton et al. 2015](#)), it is clear that photosynthesis was well established by at least the early Archean, and has played a central role in planetary function ever since.

It is equally apparent, however, that the form, distribution and geobiological impact of photosynthetic organisms have changed dramatically over time. On the modern Earth, roughly half of all primary productivity (and essentially all of its standing biomass) is generated by large, developmentally sophisticated embryophytes on land (Field et al. 1998), a state of affairs that extends back no further than the mid-Palaeozoic. The other half of global productivity is marine and, apart from a peripheral photic-zone ring, exclusively planktic. In the modern oceans, phytoplankton is dominated by three clades of chlorophyll *c*-containing protists (diatoms, coccolithophores and dinoflagellates), along with a background of cyanobacteria. None of these protistan groups has an unambiguous pre-Mesozoic fossil record, although sterane biomarkers point to a marine biological pump dominated by eukaryotes through the whole of the Phanerozoic (Summons et al. 1999).

By any sort of measure the expression of photosynthetic life in the preceding Proterozoic Eon (541–2500 Ma) is deeply non-uniformitarian. Not only are there no terrestrial embryophytes, the contribution of eukaryotes attenuates rapidly with time, such that pre-Ediacaran/Cryogenian systems appear to have been all but monopolized by default cyanobacteria (Butterfield 2015). At a deeper level even this may not hold: in the context of extensively stratified Proterozoic oceans there is a case for recognizing significantly enhanced input from anoxygenic photosynthesizers (Johnston et al. 2009) – and there was a time, of course, when oxygenic photosynthesis had yet even to evolve.

Phototrophy and Photosynthesis

As far as is known, biology has come up with just two mechanisms for converting light energy into chemical energy, and only one of these is genuinely photosynthetic. In the case of rhodopsins (e.g., the bacteriorhodopsin of phototrophic halobacteria and proteorhodopsin of proteobacteria) light energy drives a trans-membrane proton pump with the resulting proton-motive force used in ATP synthesis, cell motility and/or solute transport (Bryant and Frigaard 2006). Even so, there are no known instances of rhodopsin-based phototrophy mediating the electron transfer reactions required for carbon fixation, leaving such forms dependent on external sources for reduced carbon; in other words, they are photo-heterotrophs.

At least among extant organisms, true photosynthetic autotrophy is limited to the Bacteria (and a subset of recipient eukaryotes), and in all cases is based on the cyclic photo-oxidation of chlorophyll/bacteriochlorophyll (Chl/BChl) reaction centres followed by a reduction of CO₂ using the generated electrons. There are just four known clades of photosynthetic organisms: Chloroflexi (green non-sulphur bacteria), Chlorobi (green sulphur bacteria), photosynthetic Proteobacteria (purple sulphur and purple non-sulphur bacteria), and Cyanobacteria. Three minor BChl-containing groups – heliobacteria within the Firmicutes *Chloracidobacterium* within the Acidobacteria (Garcia Costas et al. 2012) and an isolated strain within the Gemmatimonadetes (Zeng et al. 2014) – are merely photo-heterotrophic.

Photochemical Reaction Centres lie at the core of BChl/Chl-based photosynthesis, with a primary purpose of transferring electrons from external donors to internal acceptors (Hohmann-Marriott & Blankenship 2011). ‘Type 1’ Reaction Centres use iron-sulphur clusters as electron acceptors and are found in Chlorobi, heliobacteria and *Chloracidobacterium* – versus the ‘Type 2’ quinone-based electron acceptors of Chloroflexi,

photosynthetic Proteobacteria and Gemmatimonadetes. Uniquely, cyanobacteria have both types of Reaction Centres, where they are known as Photosystem I (PS I) and Photosystem II (PS II). In concert with a Mn_4Ca 'Water Oxidizing Complex' connected to PS II, this arrangement of paired photosystems has the (thermodynamically remarkable) capacity to extract electrons from water, leaving diatomic oxygen as a waste product. By contrast, anoxygenic Chlorobi, Chloroflexi and Proteobacteria acquire their electrons from less challenging (more electro-negative) sources such as H_2 , H_2S and Fe^{2+} . Cyanobacteria are also unique in constructing their Reaction Centres from chlorophyll rather than the BChl used by other groups, though the phylogenetic polarity of the respective biosynthetic pathways remains unresolved.

For carbon fixation – the so-called dark reactions of photosynthesis – there are a number of alternative biosynthetic pathways. Whereas cyanobacteria and proteobacteria use the Rubisco-based Calvin-Benson cycle, Chlorobi use the reductive (reverse) citric acid/TCA cycle, and Chloroflexi the hydroxypropionate/reductive acetyl-CoA cycle (Hohmann-Marriott & Blankenship 2011).

Photosynthesis in Deep Time

In the absence of any alternative source of abundant free oxygen, it is clear that oxygenic photosynthesis – based on the water-oxidizing capacity of crown-group cyanobacteria – has dominated global export productivity since at least the ~ 2.4 Ga Great Oxidation Event (GOE). The reason for its long term success derives not only from the universal availability of water, but also the poisoning effects of waste oxygen on competing photosynthesizers and their electron donors. Chlorobi, for example, are obligate anaerobic photoautotrophs dependent on an external source of reduced sulphur species, ferrous iron and/or H_2 , while

facultatively aerobic/microaerophilic Chloroflexi require anoxia for the full development of their photosynthetic apparatus (Bryant and Frigaard 2006). Collectively, the photosynthetic proteobacteria tolerate a considerably broader range of redox conditions, from anoxic to fully aerobic, but are nonetheless marginalized under an oxygenated atmosphere due to the (abiotic) oxidization of their electron donors.

Prior to the build-up of atmospheric oxygen, cyanobacteria would have competed much more directly with anoxygenic photosynthesizers, potentially exploiting a similar range of reduced electron donors via suppression of PSII (cf., Cohen et al. 1986), but with the added – if energetically more costly – capacity to draw on water as other sources became locally exhausted. In the Fe²⁺-charged oceans of the early Proterozoic, ecological advantage presumably lay with specialized iron-oxidizing photosynthetic Proteobacteria and/or Chlorobi, with the oxidised fallout potentially responsible for contemporaneous Banded Iron Formation (e.g., Kappler et al. 2005). Even so, geochemical proxies point increasingly to the presence of water-oxidizing cyanobacteria well back into the Archean (Canfield et al. 2006, Rosing et al. 2006, Buick 2008, Crowe et al. 2013, Mukhopadhyay et al. 2014, Planavsky et al. 2014, Stüeken et al. 2015), with geological sinks and/or phosphate limitation (Jones et al. 2015) preventing significant accumulation of oxygen in the pre-GOE atmosphere.

A more direct account of palaeo-productivity can potentially be resolved from fossil biomarker molecules, though the early record is complicated by non-uniformitarian taphonomies, accumulative degradation and secondary contamination (Pawlowska et al. 2013, French et al. 2015a). The earliest well-preserved biomarker data – from the 1640 Ma Barney Creek Formation (BCF) of northern Australia – includes a wide range of C₄₀ carotenoid derivatives, several of which are considered diagnostic of particular photosynthetic groups (Brocks et al. 2005, Brocks and Schaeffer 2008); e.g., okenane (purple sulphur bacteria), chlorobactane and isorenieratane (Chlorobi), and β-carotane

(Cyanobacteria). The predominance of biomarkers derived from anaerobic photosynthesizers in the BCF is consistent with low levels of atmospheric oxygen in the late Palaeoproterozoic (e.g., Johnston et al. 2009), though it is notable that these same signatures of photic zone anoxia/euxinia are equally common through the Phanerozoic (French et al. 2015b).

Proterozoic microbialites and microfossils

Evidence of early photosynthesis can also be recognized in sedimentary structures formed by benthic microbial mats (stromatolites/microbialites), along with the more cryptic record of body fossils. Like all palaeontological data, however, these are compromised by major ecological and taphonomic biases, typically offering little in the way of precise physiological or taxonomic resolution. It is likely, for example, that many/most Proterozoic stromatolites were constructed by cyanobacteria-dominated mats, though there is no fundamental reason for ruling out anoxygenic photosynthesizers – especially before the GOE, or in anoxia-prone shelf settings such as late Palaeoproterozoic granular iron formations (Planavsky et al. 2009). The case for cyanobacterial involvement is stronger for larger-scale carbonate-facies stromatolites, with convincing examples extending back to the mid-Archean (Nisbet et al. 2007, Sim et al. 2012).

Most of the simple spheroids and filaments that dominate Proterozoic microfossil assemblages are also likely to be the remains of cyanobacteria, based on broadly comparable habit and habitat to extant forms. Supporting arguments include relatively large cell-size (compared to those of other Bacteria and Archaea), robust/multiple extracellular envelopes, distinctive cell-division patterns, phototactic orientation, and the unlikelihood of local electron donors apart from water (see Golubic and Hofmann 1976, Bartley 1996, Knoll and Golubic 1992, Knoll 2008, Buick 2008, Schopf et al. 2015). Unfortunately none of these

properties is uniquely cyanobacterial, leaving open a range of alternative interpretations – from anoxygenic phototrophs to heterotrophic/chemoautotrophic sulphur bacteria, protistan-grade eukaryotes, fungi and any number of extinct stem-group forms.

Despite the general loss of identity, there is a modest subset of Proterozoic microfossils that exhibit sufficiently distinctive similarity with living cyanobacteria to be convincingly recognized as such. Extant cyanobacteria have traditionally been classified into five orders based on morphological criteria: 1) Chroococcales (unicellular to simple pluricellular/colonial forms); 2) Pleurocapsales (unicellular to simple pluricellular forms producing palintomically reduced endospores/baeocytes); 3) Oscillatoriales (simple non-branching and undifferentiated filaments); 4) Nostocales (non-branching and false-branching filaments that differentiate N-fixing heterocysts and aestivating akinetes); 5) Stigonematales (true-branching/ramified filaments that differentiate heterocysts and akinetes). More recent molecular analyses, however, have identified pervasive polyphyly in all but the differentiated Nostocales and Stigonematales, leading to the introduction of corresponding form-taxonomic groupings, ‘subsections’ I–V (Rippka et al. 1979) (Fig. 1).

Unicellular/pluricellular microfossils comparable to subsection-I cyanobacteria are abundant in the Proterozoic record. By far the oldest and most convincingly cyanobacterial of these is *Eoentophysalis belcherensis* Hofmann, 1976, from the middle Palaeoproterozoic (~ 2 Ga) Belcher Island Supergroup, southeastern Hudson Bay (Fig. 2). In addition to their construction of continuous, phototactically oriented mats in intertidal/supratidal settings, these fossils preserve direct ontogenetic and taphonomic counterparts to modern entophysalid cyanobacteria living in comparable environments (Hofmann 1976, Golubic and Hofmann 1976). Whether they should be classified specifically within extant Entophysalidaceae – or indeed whether this taxon represents a clade rather than a grade of organization – depends on the degree to which such morphology is prone to evolutionary convergence. One obvious

test would be molecular interrogation of extant forms: if modern entophysalids proved to be polyphyletic (as found to be the case for most other morphologically simple cyanobacteria), there would be no a priori grounds for linking the Belcher fossils directly with any extant lineage, despite their convincing cyanobacterial credentials. By contrast, the more generic morphologies of co-occurring *Eosynochococcus*, *Sphaerophycus*, *Myxococcoides* and other simple spheroids cannot be unambiguously classified even as cyanobacteria.

The baeocyte-forming habit of extant subsection-II cyanobacteria has also proven to be polyphyletic, exhibiting sister-group relationships with both subsection-I unicells and subsection-III filaments (Ishida et al. 2001). Although baeocytes have yet to be identified in the fossil record, Green et al. (1988) demonstrated marked morphological similarities between middle Neoproterozoic *Eohyella rectoclada* (Fig. 3C) and the vegetative components of a modern hyellacean (subsection-II) cyanobacterium. A broadly comparable pseudo-filamentous habit is observed in ~1700 Ma *Eohyella campbellii* Zhang and Golubic, 1987 (Fig. 3B), though lack of any particular hyellacean synapomorphies in this population – along with its billion-year separation from *E. rectoclada* – makes its phylogenetic interpretation that much more problematic (Green et al. 1988, Knoll and Golubic 1992). Late Mesoproterozoic to middle Neoproterozoic *Polybessurus bipartitus* (Fig. 3A) has also been classified as a subsection-II cyanobacterium based on morphological comparison with an extant (but substantially smaller) marine pleurocapsalean (Green et al. 1987). This same type of stalked construction, however, is also found in freshwater subsection-I species (*Cyanostylon*), as well as in soil-dwelling chlorophytes (*Hormotilopsis*) and freshwater dinoflagellates (*Rufusiella*), underscoring the challenge of identifying subsection-II cyanobacteria without direct evidence of baeocytes or exact modern counterparts.

Simple subsection-III filaments are readily distinguishable from other cyanobacteria on morphological grounds but, once again, represent a polyphyletic grade of organization

rather than a phylogenetically coherent clade (Schirrmeister et al. 2011, 2013; Shih et al. 2013). Certainly, there is no homology between the multicellularity of subsection-III cyanobacteria and morphologically indistinguishable forms in the Chloroflexi, photosynthetic proteobacteria, or sulphur-oxidizing (beggiatocean) proteobacteria (cf., Schopf et al. 2015). That said, accessory morphological, 'behavioural,' environmental and taphonomic features often provide strong, if not conclusive, evidence for interpreting many/most simple Proterozoic filaments as subsection-III cyanobacteria (e.g., Schopf 1968, Hoffman 1976, Knoll and Golubic 1992, Seong-Joo and Golubic 1998, Butterfield et al. 1994) (Fig. 4). The oldest fossils for which this can be reasonably be invoked are relatively large (15–25 μm diameter) mat-forming filamentous sheaths (*Siphonophycus transvaalensis*) from the 2516 ± 4 Ma Gamohaan Fm. of South Africa (Fig. 5) (Klein et al. 1987; but see Czaja et al. [2014] for an alternative view).

The conspicuously polyphyletic distribution of simple filaments among extant cyanobacteria precludes the assignment of fossil counterparts to any particular clade, even in cases where phylum-level identification can be reasonably inferred. Helically coiled filaments from the early Neoproterozoic Wynnatt Fm. of NW Canada (Fig. 6; Butterfield and Rainbird 1998), for example, are closely comparable to extant *Arthrospira/Spirulina* cyanobacteria, but differ from any living species in expressing a robust extracellular sheath (see Nübel et al. 2000). Placement in the immediate stem-group would seem to be the most parsimonious interpretation, but is confounded by the deeply polyphyletic nature of the modern '*Spirulina*' (Nübel et al. 2000). As such, these fossils might just as well represent an entirely independent lineage of coiled filamentous cyanobacteria (or possibly even non-cyanobacteria; see Schmaljohann et al. 2001, fig. 3L). Likewise, exceptionally large coiled filamentous fossils assigned to *Obruchevella magna* (see Knoll 1992), or even larger, fully macroscopic *Grypania* (see Sharma and Shukla 2009; Butterfield 2009), could conceivably

be interpreted as independent experiments in assembling type-III cyanobacteria from (now extinct) lineages of exceptionally large unicells – analogous, perhaps, to the giant vacuolated unicells and filaments of modern *Thiomargarita* [see Schulz-Vogt 2007]).

Nostocales (subsection-IV) and Stigonematales (subsection V) cyanobacteria are notable for being both morphologically complex and phylogenetically legitimate, documenting a single, monophyletic origin of cellular differentiation (Nostocales + Stigonematales) and three-dimensional growth (Stigonematales) (Rippka et al. 1979, Shih et al. 2013, Dagan et al. 2013). Putatively heterocystous filaments from the early fossil record, however, have not stood up to scrutiny, while other habits such as false branching, intercellular constrictions, terminal hairs and sheath fabrics are not exclusive to these clades (Golubic et al. 1995). The oldest convincing occurrence of fossil heterocysts – and associated akinetes – occur in terrestrial hot-spring stigonemataleans from the early Devonian Rhynie Chert (Croft and George 1959), consistent with the predominately non-marine distribution of extant Nostocales/Stigonematales.

The only Proterozoic fossils currently interpreted as nostocalean cyanobacteria are isolated rod-shaped vesicles assigned to the form-genus *Archaeoellipsoides* Horodyski and Donaldson, 1980 (Fig. 7A, B). Widely reported from silicified peritidal mat biotas of Mesoproterozoic age, they have been shown to exhibit a size-frequency distribution similar to that of extant nostocalean akinetes, hence their interpretation as the aestivating fallout of *Nodularia/Aphanizomenon*-type plankton blooms (Golubic et al. 1995). Further, because all modern akinete-bearing cyanobacteria also differentiate nitrogen-fixing heterocysts, the interpretation of *Archaeoellipsoides* as akinetes has been used to infer that the host organism was also heterocystous, with important implications for understanding the early history of both nitrogen fixation and atmospheric oxygen (Tomitani et al. 2006).

It is certainly possible that fossil *Archaeoellipsoides* represents the akinetes of nostocalean cyanobacteria but, in my opinion, the supporting evidence and arguments remain less than compelling. Rod-shaped cells with comparable dimensions are found in other large Bacteria (Schulz-Vogt et al. 2007), as well as among extant protists (e.g., *Stichococcus* and *Spirotaenia* green algae) and marine microfossil assemblages (e.g., *Navifusa*; see Fatka and Brocke 2008). Moreover, there is no evidence of cellular trichomes or extracellular sheaths directly associated with any *Archaeoellipsoides* specimens, despite the co-occurrence of other well preserved fossils (e.g., Horodyski and Donaldson 1980, Sergeev et al. 1995), and their conspicuously intertidal/supratidal distribution undermines arguments for differential degradation in the plankton (cf. Golubic et al. 1995). In cases where morphologically similar cells are known to be constituents of multicellular filaments it is clear from associated features that these are not akinetes, or even prokaryotic (e.g., Fig. 7F; Butterfield 2004). For what it is worth, there is no evidence of akinetes in Meso-Neoproterozoic non-marine assemblages (Strother et al. 2011), where such fundamentally non-marine cyanobacteria might have been expected.

Even if *Archaeoellipsoides* does include specimens of cyanobacterial akinetes, it is a mistake to assume that the host organism was also capable of differentiating heterocysts (cf., Golubic et al. 1995, Tomitani et al. 2006, Knoll 2008). Although the two differentiated cell-types are demonstrably linked in crown-group Nostocales/Stigonematales, such typological reasoning cannot be automatically applied to fossil counterparts, particularly when they precede the oldest direct evidence of co-occurring akinetes and heterocysts by some 800–1000 million years. Given the relatively derived nature of this clade, and the possibility that heterocysts are evolutionary derivatives of akinetes (Adams and Duggan 1999), a significant portion of the underlying stem-group is expected to have borne akinetes alone (see Fig. 1).

And whatever the interpretation of Mesoproterozoic *Archaeoellipsoides*, there are vanishingly few grounds for extending the record of crown-group Nostocales another 800–1000 million years – to the 2.1 Ga Franceville Group of Gabon – based on the assignment of four or five indifferently preserved specimens to the same form-genus (Amard and Bertrand-Sarfati 1997, Tomitani et al. 2006) (Fig. 7E). Ranging from just 2.6 to 5.2 micrometres in diameter, these early rod-shaped microfossils might just as readily have been assigned to, e.g., *Eosynechococcus grandis* (Fig. 7C, D), which would carry fundamentally different physiological and phylogenetic implications.

The absence of compelling evidence for subsection-II or nostocalean/stigonematalean cyanobacteria in the Proterozoic record does not, of course, mean that they were not present – only that the available evidence fails to meet what might be considered minimal levels of diagnostic criteria (relative to the morphological convergence/polyphyly exhibited by their extant and fossil counterparts). Certainly there are accumulative taphonomic losses with time, but this is likely to be conflated with genuine character absence in early stem-groups, including pre-baeocyte phases in type-II cyanobacteria, and pre-heterocystous phases in type-IV cyanobacteria. At the same time, identification of positive morphological features offers only limited taxonomic constraints due to the pervasive polyphyly of all but the most differentiated cyanobacteria (none of which have an unambiguous Proterozoic record). Given the enormous amounts of phylogenetic territory underlying extant forms (Fig. 1), there is little basis for shoehorning early fossil cyanobacteria into extant clades (see Shih and Matzke 2013). Rather than representing billion-year evolutionary stasis, most of these recurrent morphologies are much more likely to be a product of serial convergence (Dvořák 2014) – comparable to that of other ‘living fossils’ such as cycads and bryophytes, where modern diversity has proven to have conspicuously shallower roots than initially suggested by the fossil record (Nagalingum et al. 2011, Laenen et al. 2014)).

More mechanically, there are major environmental and temporal biases accompanying almost all of the relevant taphonomic windows onto early cyanobacteria (Butterfield 2003). Most of the early microfossil, microbialite and biomarker records, for example, derive from shallow-water to supratidal facies, representing a trivial component of global productivity (Field et al. 1998). To a first approximation, there is no direct record of marine phytoplankton through the Proterozoic, partly because of the inherent buoyancy of planktic cyanobacteria (Reynolds et al. 1987), partly because of the limited opportunities for repackaging and vertical transport in the absence of suspension-feeding animals (Logan et al. 1995, Butterfield 2011), partly because of the sealing effects of benthic microbial mats (Pawlowska et al. 2013). Indeed, the taphonomic biases are of such a degree that the fossil record offers few useful constraints on the origin of modern cyanobacterial groups. At the coarsest level – oxygenic photosynthesis itself – the issues are better resolved by MIF (mass independent fractionation of sulfur), BIF (banded iron formation) and other proxies of ancient redox conditions.

Molecular approaches

Given the shortcomings of the fossil record, the only phylogenetically resolvable account of early photosynthesis lies in the analysis of extant lineages. Molecular signatures clearly distinguish each of the seven extant lineages of Chl/BChl-containing Bacteria and allow their individual phylogenies to be broadly interrogated (Raymond et al. 2002, Hohmann-Marriott & Blankenship 2011), even if much of the detail has been obscured by Horizontal Gene Transfer (HGT), Homologous Recombination (HR; see Dvořák et al. 2014), morphological convergence and intervening extinction.

Cyanobacteria are unquestionably the key players in terms of early Proterozoic photosynthesis. Among extant taxa there are two deep-branching lineages, one represented by *Gloeobacter*, a cosmopolitan, terrestrial, wet-rock cyanobacterium distinguished phenotypically by its lack of thylakoid membranes (Mareš et al. 2013); and the other by all remaining forms (Fig. 1). Within the non-*Gloeobacter* lineage, molecular analysis has corroborated the monophyly of heterocystous Nostocales+Stigonematales, and identified a genetically streamlined clade of *Synechococcus* and *Prochlorococcus* species (“SynPro”) that, at least numerically, dominates modern marine phytoplankton (Urbach et al. 1992, Roco et al. 2003). Other large-scale clades inevitably emerge from such analyses (e.g., Ishida et al. 2001, Tomitani et al. 2006, Gupta and Mathews 2010, Blank and Sánchez-Baracaldo 2010, Schirrmeyer et al. 2013, Shih et al. 2013), though few exhibit any phenotypic coherence – presumably due to the rapid rates of evolutionary turnover facilitated by HGT and HR. In order to account for their current gene distributions, for example, some 66% of cyanobacterial protein families must have experienced at least one HGT event (Dagan et al. 2013) – even as a core of vertically transmitted genes resolve their broader treelike phylogeny. The fundamental plasticity of cyanobacterial phenotype is reflected in their conspicuously divergent and convergent evolutionary responses to temperature (Hongmei et al. 2005), salinity (Garcia-Pichel et al. 1998, Moisaner et al. 2002), redox chemistry (Cohen et al. 1986), light intensity (Roco et al. 2003), N-limitation/diazotrophy (Zehr and Kudela 2011), multicellularity (Schirrmeyer et al. 2011, 2013), substrate stability (Garcia-Pichel and Wojciechowski 2009), and eukaryotic symbioses (Thompson et al. 2012, Hilton et al. 2013), the latter including a complete loss of photosynthesis within the past ~ 12 myr (Nakayama et al. 2014).

On the assumption that the phylogenetic relationships of extant cyanobacteria can be accurately reconstructed from molecular data, there is a further potential for inferring the

phenotype of extinct forms via Ancestral State Reconstruction (ASR). By applying such techniques, Schirmer et al. (2011) have inferred a multicellular constitution for the ancestry of all but the most basal cyanobacteria, and Blank and Sánchez-Baracaldo (2010) have argued that Archean cyanobacteria were uniformly small, unicellular, non-marine and devoid of robust extracellular sheaths. Under this latter model, cyanobacteria only colonized the oceans and acquired their conspicuous microbialite-building habits in relatively derived lineages, possibly linked to the GOE.

These are intriguing results, but they hang on a number of problematic assumptions. In addition to requiring a correct phylogeny, ASR is only viable when the vertically inherited component is closely tracked by organism phenotype, and in the absence of significant trends or changes in character state over time (Schluter et al. 1997). Given the high levels of HGT and pervasive convergent/divergent evolution in cyanobacteria, neither of these prerequisites is likely to hold (Dvořák et al. 2014), especially for divergences measured in billions of years. By the same token, it is a mistake to interpret the non-marine habit of *Gloeobacter* and other ‘basal’ lineages as somehow reflecting an ancestral condition simply because of their early cladogenetic isolation and tree asymmetry: the extant members of a species-poor lineage are no more or less likely to represent the ancestral state than their relatively diversified sister group (see Crisp and Cook 2005). Experimental investigation has also shown ASR to be unreliable in all but the most slowly evolving characters (Oakley and Cunningham 2000), while the addition of palaeontological data typically undermines or reverses results based exclusively on living forms – even in much younger eukaryotic clades constrained to vertical inheritance (e.g., Finarelli & Flynn 2006, Betancur-R et al. 2015). Given the revolutionary changes in ecosystem structure at both the beginning and end of the Proterozoic (Butterfield 2011, 2015), there is little likelihood of uniformitarian continuity in cyanobacterial evolution through deep time – or of ASR analysis reliably identifying extinct phenotypes.

A correct molecular phylogeny also allows molecular clock estimates of last common ancestry to be calculated – with a further potential for linking evolutionary innovations to major events in Earth history. Apart from the theoretical issues accompanying clock analysis of prokaryotic clades (e.g., Kuo and Ochman 2009), any such results inevitably rest on the reliability of palaeontological calibration points. At the broadest level, it is safe to assume that BChl/Chl-based photosynthesis had evolved by at least the early Archean (~3.5 Ga), and that oxygenic photosynthesis was established by at least the GOE (~2.4 Ga). But beyond this the constraints for early photosynthesis become much more problematic. As discussed above, the 2.1 Ga Franceville “*Archaeoellipsoides*” (Fig. 7E) cannot be credibly interpreted as crown-group nostocaleans, undermining the various clock analyses that have embraced it for their primary calibration (Blank and Sánchez-Baracaldo 2010, Schirrmeister et al. 2013, Dvořák et al. 2014, Sánchez-Baracaldo et al. 2014); strictly speaking, the first positive identification of heterocystous cyanobacteria post-dates the Franceville fossils by more than a billion and a half years. Blank and Sánchez-Baracaldo (2010) also use 1.7 Ga *Eohyella campbellii* (Fig. 3B) as a minimum age constraint for subsection-II cyanobacteria, though its lack of diagnostic morphology, and the polyphyly of “Pleurocapsales” in general (see above), make it a poor candidate for calibration. Schirrmeister et al.’s (2013) use of ca. 2 Ga *Gunflintia/Halythix* as a minimum age for sub-section III cyanobacteria is less problematic, but not because these fossils exhibit any diagnostic morphology (see Fig. 4A, B): this is simply the default interpretation for all featureless post-GOE filaments in shallow-water settings (and, as a generic argument, is less convincing than the larger mat-forming filaments in the considerably older Gamohaan Fm [Fig. 5]; Klein et al. 1987). By contrast, Blank and Sánchez-Baracaldo’s (2010) *maximum* age constraint of 2.45 Ga for Nostocales and “Pleurocapsales” has little basis beyond an asserted absence of ‘large cell diameters’ in the earlier record (undermined by documentation of relatively large diameter Archean fossils

[e.g., Klein et al. 1987, Sugitani et al. 2015]), along with a more reasonable assumption that akinete-bearing nostocaleans are unlikely to pre-date the GOE.

Whatever the applicability of ASR or molecular clocks to reconstructing early cyanobacterial evolution, the idea that oxygenic photosynthesis may have originated in non-marine environments – and remained largely restricted to them prior to the Proterozoic – has attracted considerable follow-up attention (e.g., Strother et al. 2011, Dagan et al. 2013, Sánchez-Baracaldo et al. 2014, Wellman and Strother 2015). Certainly there are good grounds for recognizing the derived nature of most extant groups of marine cyanobacteria, including the SynPro clade and various N-fixing forms (Sánchez-Baracaldo et al. 2014), but this should not be equated with absence of previous incumbents – particularly given the alacrity with which cyanobacteria adapt to novel circumstances. Oxygenic photosynthesis is the only type of photo-autotrophy that could be perennially sustained at the surface of a post-GOE ocean, and the long-term continuity of $\delta^{13}\text{C}$ signatures through the Proterozoic (see Schidlowski 1988, Krissansen-Totton et al. 2015) rules out the possibility of this habitat remaining fallow for any measurable length of time.

In the case of ray-finned fishes, it is clear from the fossil record that ASR-based claims for non-marine origins are simply an artefact of differential migration and extinction of marine forms (Betancur-R et al. 2015). Is it possible that such a process has similarly obscured the early history of cyanobacteria? Indeed, is it reasonable to invoke bacterial extinction at all given the astronomically large population sizes of most microorganisms (see discussion in Lane 2011)? Yes, absolutely. The fossil record is strewn with extinctions of once super-abundant marine plankton, and loss of intermediate stem-groups offers the only realistic explanation for the phylogenetic gappiness of extant diversity, both eukaryotic and prokaryotic (Butterfield 2015) (see Fig. 1). It is also worth noting the number of early cyanobacteria-like fossils that lack exact modern counterparts, including *Polybessurus* (Fig.

3A), various *Spirulina*-like filaments (Fig. 6; Knoll 1992), and *Grypania* (Sharma and Shukla 2009). None of this rules out the possibility that cyanobacteria may have originated in non-marine environments, but it is unlikely that such distinctions are relevant on geological time scales. The capacity of cyanobacteria to rapidly adapt, and re-adapt, to novel circumstances ensures multiple independent ventures into all but the most extreme photic-zone settings, punctuated by extinction and evolutionary turnover as new ecological circumstances arise.

Photosynthetic eukaryotes

At some point after the Archean evolution of crown-group cyanobacteria – and after the Palaeoproterozoic evolution of crown-group eukaryotes – a subsection of oxygenic photosynthesis was endosymbiotically repackaged as a eukaryotic phenomenon. In the process it exchanged HGT for a system of primarily vertical inheritance, and acquired a fundamentally new grade of developmental, evolutionary and ecological sophistication.

The precursor to the eukaryotic chloroplast was clearly an engulfed cyanobacterium, and there is a strong, if often oversimplified (Howe et al. 2008, Stiller 2014) argument for recognizing just a single primary endosymbiosis for all living photosynthetic eukaryotes (with the exception of *Paulinella chromatophora*; Nowack and Grossman 2012). Subsequent diversification gave rise to the three extant lineages with primary plastids comprising the eukaryotic super-group Plantae/Archaeplastida: Glaucophyta, Rhodophyta and Viridiplantae (Fig. 8; Keeling 2013). Although all of the primitively non-photosynthetic members of this clade have been lost to extinction (i.e., stem-group Plantae), analysis of the residual chloroplast genome can potentially resolve the phylogenetic affiliations of the ancestral cyanobacterial endosymbiont. Conclusions vary, but attention has fallen increasingly on extant clades of N₂-fixing cyanobacteria, especially heterocyst-bearing Nostocales (Dagan et

al. 2013, Ochoa de Alda et al. 2014). Taken literally, this would imply a fresh-water, multicellular and heterocystous origin of chloroplasts – analogous, perhaps, to the symbiotic relationship between modern *Richelia/Calothrix* and various marine diatoms (see Hilton et al. 2013) – followed by a loss of heterocysts, multicellularity and, at some point, freshwater habit. But this is to ignore the distinction between crown-group forms, which carry the full complement of characters that define an extant clade, and members of the underlying stem-groups (which do not). Certainly stem-group Nostocales (see Fig. 1) will have included forms that primitively lacked heterocysts, true and false branching, and even multicellularity, offering a much broader range of ‘ancestral states’ from which to draw the ancestral chloroplast. By the same token, there is no reason to assume that the nostocalean stem-group was exclusively or primitively non-marine, countering arguments for a freshwater origin of photosynthetic eukaryotes (Dagan et al. 2013, Wellman and Strother 2015). The only necessary implication of chloroplasts being most closely related to extant nostocaleans is that *total-group* Nostocales was established prior the first appearance of crown-group Plantae.

The oldest unambiguously photosynthetic eukaryote in the fossil record is late Mesoproterozoic (~ 1200 Ma) *Bangiomorpha*, identified on the basis of its large size, complex multicellular development and vertically oriented turf-forming habit in peritidal environments (Fig. 9A). More specifically, it exhibits direct morphological and developmental correspondence with the extant red alga *Bangia* (Rhodophyta, Bangiophyceae), pointing to both its crown-group phylogenetic affiliations and associated photosynthetic machinery (including primary red plastids). As ever, it is important to consider the possibility of morphological convergence, but in this case the combination of features is sufficiently diverse and derived to offer a convincing family-level diagnosis (Butterfield 2000, 2009, 2015). Broadly comparable levels of cellular and ontogenetic detail

in the much younger Doushantuo biota document the appearance of florideophycean red algae by at least the end of the Proterozoic (Xiao et al. 2004).

Early Neoproterozoic (~820 Ma) *Proterocladus* (Fig. 9B–D) and *Palaeastrum* (Fig. 9E) are also demonstrably eukaryotic (by virtue of their size and multicellular complexity) and have close morphological analogues among extant siphonocladalean and hydrodictyalean green algae (Viridiplantae) (Butterfield et al. 1994). As such, they are likely to have contained green primary plastids, though the relative simplicity of their multicellular expression presents a non-trivial possibility of convergence by other extant or extinct eukaryotes, including colourless osmotrophs, non-green plant protists, and/or unrelated lineages of green algae.

Given their unicellular to simple pluricellular nature, it is hardly surprising that glaucophytes have yet to be identified in the fossil record, despite their early evolutionary divergence (Keeling 2013). Their habit of adding additional mucilaginous layers with each cycle of cell division (Kies 1989) suggests that candidates could potentially be found among microfossils assigned to (morphologically indistinguishable) subsection-I cyanobacteria (e.g., *Gloeodiniopsis*, *Eogloeocapsa*). Somewhat more distinctive is the conspicuously concentric arrangement of cellulose microfibrils comprising the glaucophyte cell wall (Willison and Brown, 1978), intriguingly reminiscent of that seen in the long-ranging Proterozoic acritarch *Valeria lophostriata* (Hofmann 1999).

Proterozoic acritarchs (vesicular organic-walled microfossils of unknown affiliation) undoubtedly include a diverse range of photosynthetic protists, even if these will be impossible to resolve in any particular instance: most sphaeromorphic acritarchs are not demonstrably eukaryotic, and demonstrably eukaryotic forms are not necessarily photosynthetic. That said, the conspicuously limited metabolic repertoire of crown-group eukaryotes, combined with the limited preservation potential of most heterotrophic protists

(e.g., unwallied amoeboids, ciliates, flagellates; see Butterfield 2003, 2015), makes photosynthesis the most likely metabolism for most eukaryotic acritarchs – particularly if they show evidence of vegetative growth rather than the more regular habit of loricas, tests or aestivating spores/cysts. The oldest unambiguously eukaryotes in the fossil record are acanthomorphic acritarchs – *Tappania* – from the late Palaeoproterozoic of China and India (see Butterfield 2015), which are characterized by both conspicuously variable vegetative growth and relatively shallow-water (photic-zone) palaeoenvironments. Although fungus-like osmotrophy cannot be categorically ruled out as a mode of life (cf. Butterfield 2005), these fossils offer at least circumstantial evidence for the early appearance of photosynthetic eukaryotes.

Whether or not *Tappania* belongs to crown-group Plantae is, of course, another matter. Given the disparate range of photosymbiotic relationships practiced by extant eukaryotes (Dorrell and Howe 2012), including the independent acquisition of ‘primary plastids by the extant rhizarian *Paulinella chromatophora* (Nowack and Grossman 2012), it is unlikely that their Palaeoproterozoic counterparts remained exclusively heterotrophic over time. But, by the same token, these early ‘plant protists’ could represent any number of other, ultimately failed, experiments in plastid acquisition, not least one or more of the stem-group endosymbioses that appear to have preceded the nominal last common ancestor of extant chloroplasts and Plantae (Fig. 8) – the ‘shopping bag’ model of Howe et al. (2008). In this light, crown-group eukaryotes are likely to have exploited the ecological opportunities of mixotrophy and photosynthesis from the outset, taking ‘eukaryotic-grade’ photosynthesis back to at least the late Palaeoproterozoic.

Secondary plastids.

Unlike the singular occurrence of primary cyanobacterial plastids, it is clear that photosynthetic eukaryotes have become incorporated as secondary/tertiary chloroplasts on multiple occasions (Dorrell and Howe 2012, Keeling 2013) (Fig. 8). Secondary ‘green’ chloroplasts (derived from green-algal endosymbionts) have appeared independently in euglenids, chlorarachniophytes and a (secondarily heterotrophic) dinoflagellate, while other such dinoflagellates have acquired tertiary chloroplasts from diatoms, cryptomonads and haptophytes. Even so, it is the major clades of secondary ‘red’ plant protists – diatoms, haptophytes, dinoflagellates and cryptomonads – that ultimately revolutionized marine productivity. Can the early evolution of these Chl *c*-containing groups be detected in the Proterozoic record?

Cavalier-Smith (1999) argued forcefully for a single endosymbiotic acquisition of secondary ‘red’ chloroplasts, and the recognition of a monophyletic clade combining the traditional ‘chromistan’ algae (= photosynthetic stramenopiles/Ochrophyta, including diatoms, chrysophytes, phaeophytes, xanthophytes, pelagophytes, etc.) and alveolates (= dinoflagellates, ciliates and apicomplexans). Subsequent analyses appeared to support this ‘chromalveolate’ model, though clear links with Rhizaria pushed the host organism back to the base of SAR (the eukaryotic supergroup comprising Stramenopiles, Alveolates and Rhizaria), with the position of haptophytes and nucleomorph-bearing cryptomonads (the photosynthetic ‘hacrobians’) remaining unresolved (Fig. 8; Keeling 2013). Other approaches, however, have found evidence for chloroplast polyphyly in SAR (Baurain et al. 2010, Dorrell and Smith 2011), and more recent work makes a strong case for cryptomonads deriving from an independent acquisition of ‘red’ chloroplasts – with haptophytes emerging as a probable sister-group of SAR (Burki et al. 2012). It is still possible, of course, to invoke a single (secondary) endosymbiotic origin for SAR + haptophyte chloroplasts, but the original assumptions of the chromalveolate hypothesis are clearly in need of adjustment and

more rigorous testing; e.g., that a convincing complement of red algal genes is present in the genomes of ciliates, rhizarians and other pervasively non-photosynthetic lineages of SAR (Keeling 2013).

The phylogenetic topology of plastids in SAR has important implications for assessing the origin of Chl *c*-based photosynthesis, particularly in light of its conspicuously limited expression in the pre-Mesozoic fossil record. Under the chromalveolate model, for example, the presence of early Cambrian foraminifera/Rhizaria (Culver 1991), and (putative) Cryogenian ciliates/Alveolata (Bosak et al. 2011), would push the acquisition of secondary plastids well back into the Proterozoic (see Fig. 8). By contrast, a scenario allowing plastid polyphyly within SAR is more readily reconciled with a Phanerozoic – possibly even a Mesozoic – origin of at least some secondary ‘red’ plastids (Knoll et al. 2007).

There have been a number of claims for Chl *c*-containing algae in the early fossil record, including putative vaucheriacean xanthophytes in the Meso-Neoproterozoic (Hermann 1982, Butterfield 2004), putative phaeophytes from the Ediacaran (Xiao et al. 1998), and putative pelagophyte biomarkers (24-*n*-propylcholestane) from the Cryogenian (Raven 2012). Such interpretations, however, clash conspicuously with molecular clock analyses pointing to a post-Palaeozoic appearance of xanthophytes, phaeophytes and pelagophytes, and a late Neoproterozoic appearance for photosynthetic stramenopiles as a whole (Brown and Sorhannus 2010). Recently reported specimens of Mesoproterozoic *Palaeovaucheria* also reveal features that fall fundamentally outside the range of its modern namesake (Butterfield 2015), while putative Neoproterozoic counterparts (Fig. 7F; Butterfield 2004) fail to exhibit minimally diagnostic levels of vaucheriacean complexity. In the absence of other candidates, the fossil record offers few constraints on ochrophytan evolution before the early Cretaceous appearance of diatoms – or on ‘hacrobian’ evolution prior to the late Triassic appearance of coccolithophores.

The other principal group of extant Chl *c*-containing algae are the dinoflagellates. By all appearances the last common ancestor of extant dinoflagellates had already acquired its secondary ‘red’ plastid (Slamovits and Keeling 2008) which, in chromalveolate models, necessarily expands to include the underlying stem group. Recent discovery of a ‘red’ photosynthetic apicomplexan (*Chromera*) supports a single origin of dinoflagellate and apicomplexan plastids, but evidence of photosynthetic ancestry has yet to be demonstrated in their ciliate sister group (Keeling 2013). Outside the chromalveolate model, there is little evidence for invoking a pre-Phanerozoic, or even a pre-Mesozoic, origin of photosynthetic dinoflagellates. Earlier occurrences of dinocyst-like acritarchs (Butterfield and Rainbird 1998) and dinosteroid biomarkers (Talyzina et al. 2000) could conceivably represent stem-group forms but, by the same token, might predate acquisition of the dinoflagellate plastid itself. Apart from issues of secondary contamination or convergence, the vanishingly small concentrations of Proterozoic dinosteroid biomarkers (typically <1 ppb; see Summons et al. 1992) could reflect the more limited expression of primitively non-photosynthetic stem-dinoflagellates, secular changes in biomarker taphonomy (Pawlowska et al. 2013), or simply the general marginalization of eukaryotic productivity in early cyanobacteria-dominated oceans (Butterfield 2009, 2011, Lenton et al. 2014).

Discussion

Despite the deep gaps and biases of the historical record, it is clear that the Proterozoic witnessed two of the planet’s most revolutionary innovations in photosynthesis: first the takeover of planetary productivity by cyanobacteria near its beginning, and then the upgrade to a primarily eukaryotic platform that marks its end. Although oxygenic photosynthesis itself appears to have been a much earlier invention, the ‘age of cyanobacteria’ only emerged

as contemporary environments were progressively polluted by accumulating free oxygen. The tipping point would see incumbent anoxygenic photosynthesizers dramatically overrun by oxygen-tolerant cyanobacteria, alongside the various biogeochemical and climatic perturbations of the GOE – a bi-stable (and effectively irreversible) regime shift brought on by the positive feedback effects of a revolutionary new technology (Scheffer and Carpenter 2003, Goldblatt et al. 2006).

This early Palaeoproterozoic interval of planetary hysteresis is famously mirrored by events of the late Neoproterozoic. Like cyanobacteria with respect to the GOE, eukaryotes were ecologically marginalized through much of their early evolutionary history, but rose rapidly to dominance during an interval of pronounced biogeochemical and climatic change. As ever, models have focussed primarily on physical/chemical drivers, especially oxygen, to explain the Cryogenian-Cambrian regime shift. In the case of Rubisco-based photosynthesizers, however, rising oxygen concentrations are if anything likely to have curbed evolutionary expansion (via photorespiration feedbacks), so much of the discussion has shifted to the biological availability of nitrogen in stratified Proterozoic oceans (Anbar & Knoll 2002, Fennel et al. 2005, Stüeken 2013). By extension, the ability of many prokaryotes to fix their own nitrogen could account for the extended incumbency of cyanobacteria. But this is to ignore the alternative means by which photosynthetic eukaryotes acquire their requisite N, including heterotrophy/mixotrophy (Mitra et al. 2014), enhanced motility and recurring symbioses with diazotrophic cyanobacteria (e.g., Thompson et al. 2013, Hilton et al. 2013, Nakayama et al. 2014). Certainly eukaryotes dominate export productivity in the modern oceans without fixing their own nitrogen, while oxygen minimum zones are increasingly recognized as a net source of bioavailable N – rather than a denitrification sink – due to the diversity/abundance of heterotrophic diazotrophs (Zehr and Kudela 2011, Dos

Santos et al. 2012, Halm et al. 2012, Rahav et al. 2015). Such contributions may well have been substantially greater in stratified Proterozoic oceans.

If not oxygen or nitrogen limitation, what then accounts for the billion-year marginalization of photosynthetic eukaryotes? Certainly there is more to the ecology of prokaryotic vs. eukaryotic productivity than ambient chemistry, not least the ‘master trait’ of cell size and its myriad effects on light harvesting, buoyancy, predator avoidance and overall life history (Litchman and Klausmeier 2008). Although the predominance of cyanobacterial picoplankton in the modern mid-Pacific gyres is clearly related to the accompanying super-oligotrophic conditions, broadly comparable forms also dominate in eutrophic to mesotrophic systems, due in part to a positive feedback loop in which planktic cyanobacteria thrive in the stratified, nutrient-rich, turbid water conditions created by their inherent buoyancy (Reynolds et al. 1987, Scheffer and Carpenter 2003, Butterfield 2009, Paerl and Otten 2013).

Interestingly, cyanobacterial diazotrophy contributes little to the nitrogen budget in most eutrophic lakes and inland seas, even when the principle bloom-forming plankton are nostocaleans (Ferber et al. 2004). If anything, a more limited availability of N in Proterozoic shelf environments might have played to the strengths of eukaryotic phytoplankton, which thrive in clearer-water mesotrophic to oligotrophic conditions.

In modern aquatic ecosystems, the alternative clear-water state is typically engineered by suspension-feeding animals. By actively extracting suspended C_{org} , such forms transfer biological oxygen demand from the water column to the benthos, while at the same time reducing nutrient recycling, enhancing light penetration, and expanding opportunities for benthic colonization (Logan 1995; Butterfield 2011). In the relatively simple ecosystems characteristic of lakes, estuaries and restricted marine embayments, top-down control of phytoplankton populations is a primary driver of major regime shifts, often delivered by trophic cascade (Butterfield 1997, Kemp et al. 2005, Petersen et al. 2008, Brönmark et al.

2010). When it comes to clearing out the Proterozoic oceans, however, the key players would have been early sponge-grade metazoans (Sperling et al. 2007, Erwin and Tweedt 2011, Lenton et al. 2014). In addition to introducing an entirely novel means of processing water (turbulent-flow suspension-feeding), their selective extraction of picoplankton and DOC would have presented a powerful new selective pressure in favour of larger, more export prone, eukaryotic phytoplankton. Sponges are a key factor in suppressing light-attenuating plankton blooms in at least some modern shelf systems (e.g., Peterson et al. 2006), and are likely to have played a similar role in engineering the late Neoproterozoic oceans. For what it is worth, molecular clock estimates for the appearance of sponges coincide with the first biomarker evidence of cyanobacteria giving way to eukaryotic productivity – in the mid-Neoproterozoic (Summons et al. 1999, Erwin et al. 2011, Butterfield 2015). Shifts in plankton composition will also have had cascading effects on the illumination and oxygenation of shallow-water environments, with critical feedback effects on both benthic and planktic ecology (Butterfield 2011, Lenton et al. 2014).

Evolutionary singularities and convergence

Photosynthesis drives all but a trivial component of the modern biosphere, and has done so for at least the past 3.5 billion years. Given the complexity and universality of the underlying chlorophyll-based Reactions Centres it is also clear that it derives from a single last common ancestor – it is an evolutionary singularity. Even so, it is important to recognize that other routes were potentially available. Although no living rhodopsin-based phototrophs are truly photosynthetic, there appears to be no fundamental reason why their light-driven proton-pumping couldn't have been linked to a carbon fixation pathway (Bryant and Frigaard 2006), and might well have done so in the absence of a BChl/Chl incumbent. The same is true for

photosynthetic eukaryotes: although all plastids can be traced back to a single last common primary endosymbiosis, it is clear from the diverse range of extant more or less obligate photoendosymbioses (e.g., Dorrell and Howe 2012, Thompson et al. 2012, Nakayama 2014) that alternative routes would have been routinely available. When it comes to oxygenic photosynthesis, however, there are no obvious alternatives to the Mn_4Ca Water Oxidizing Complex, if only because of the enormous thermodynamic challenges associated with splitting water and sequentially capturing the generated electrons (Dismukes et al. 2001). To this extent, the evolution of oxygenic photosynthesis represents a more exclusive and contingent type of evolutionary singularity.

Once discovered, there was little to prevent oxygenic photosynthesis from eventually bringing about planetary oxygenation, hence the GOE. Such biogeochemical inevitability, however, does not extend to the takeover of planetary productivity by eukaryotes. Apart from the necessary – and conspicuously singular – evolution of the eukaryotic cell itself (Butterfield 2015, Booth and Doolittle 2015), ecological displacement of cyanobacteria remained contingent on two further evolutionary singularities: complex multicellular animals and complex multicellular plants. Taken together, there appear to have been just four unique and necessary innovations leading to modern photosynthetic diversity: 1) evolution of oxygenic cyanobacteria in the Archean, 2) evolution of crown-group eukaryotes in the Palaeoproterozoic, 3) evolution of suspension-feeding metazoans in the Neoproterozoic, and 4) evolution of terrestrial embryophytes in the Palaeozoic. All of the rest is demonstrably subject to evolutionary convergence and comes more or less for free (Conway Morris 2003). Interestingly, the two key Proterozoic contributions to photosynthesis occurred in non-photosynthetic lineages.

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Figure Captions

Fig. 1. Phylogenetic relationships and phenotypic expression of extant cyanobacteria, redrafted from Shih et al. (2013) to emphasize the distinction between extant crown-group clades and the (enormously greater) phylogenetic territory represented by stem-group forms. The grey lines schematically document the pervasive horizontal gene transfer (HGT) typical of prokaryotes, as well as the diversity of extinct stem-group forms. Apart from *Gloeobacter*, Nostocales and Stigonematales, phenotype is conspicuously polyphyletic, a pattern that can be reasonably extrapolated to extinct forms. The nostocalean affiliation of the eukaryotic primary plastid (Dagan et al. 2013) includes stem-group representatives, some of which are likely to have been unicellular, non-diazotrophic and marine. LCA = Last Common Ancestor; N = nitrogen fixation (diazotrophy), S = helically coiled *Spirulina*/*Arthrospira*-type filaments.

Fig. 2. The subsection-I cyanobacterium *Eoentophysalis belcherensis*; from the Palaeoproterozoic Kasegalik Formation, Belcher Group, Hudson Bay, Canada (Hofmann 1976). A, continuous pustulose mat (GSC 43590). B, localized pustule of cells showing multiple extracellular envelopes associated with cell division (GSC 43590). C, type specimen of *E. belcherensis* (GSC 42770). D, localized colonies (GSC 42770). E, continuous mat with embedded colonies (GSC 43587). F, localized colony of cells showing multiple extracellular envelopes (GSC 42769). GSC = Geological Survey of Canada (catalogue numbers). Scale bar in A = 50µm for A; 22 µm for B–F.

Fig. 3. Putative subsection-II fossil cyanobacteria. A, *Polybessurus bipartitus*, a stalked unicellular fossil broadly comparable to a modern baeocyte-forming marine

cyanobacterium, but also freshwater non-baeocyte-forming *Cyanostolon*; from the late Mesoproterozoic Hunting Formation, arctic Canada (Butterfield 2001). B, *Eohyella campbellii* (type specimen), an endolithic colony with pseudofilaments broadly comparable to those of extant hyellacean cyanobacteria; from the late Palaeoproterozoic Dahongyu Formation, Changcheng Group, north China (Zhang and Golubic 1987); photograph courtesy of J. Yao. C, *Eohyella rectoclada* (type specimen), an endolithic colony with pseudofilaments closely comparable to those of extant hyellacean cyanobacteria; from the early-middle Neoproterozoic Limestone-Dolomite Series, Eleonore Bay Group, East Greenland (Green et al. 1988); photograph courtesy of A. H. Knoll. Scale bar in C = 50 μm for A and C; = 17 μm for B.

Fig. 4. Filamentous microfossils of probable cyanobacteria (subsection-III). A, B, silicified *Halythrix* sp.; from the Palaeoproterozoic Kasegalik Formation, Belcher Supergroup, Canada (Hofmann 1976) (GSC 42769). C, silicified *Siphonophycus* sp. exhibiting the alternating vertical/horizontal orientation typical of mat-building photosynthetic cyanobacteria; from the early-middle Neoproterozoic Limestone-Dolomite Series, upper Eleonore Bay Group, East Greenland; photograph courtesy of A. H. Knoll. D, *Siphonophycus* sp. showing entangled mat-like habit in two dimensions (acid-isolated from shale); from the early Neoproterozoic Wynniatt Formation, Shaler Supergroup, NW Canada. E, false branching filament; from the early Neoproterozoic Svanbergfjellet Formation, Akademikerbreen Group, Spitsbergen (Butterfield 2009); false branching is most commonly expressed in nostocalean (subsection-IV) cyanobacteria, but is also encountered in subsection-III forms (cf., Taton et al. 2011). Scale bar in E = 15 μm for A and B; = 45 for C and D; = 90 μm for E.

Fig. 5. Mat-forming filamentous microfossils (*Siphonophycus transvaalensis*); from the latest Archean Gamohaan Formation, Transvaal Supergroup, South Africa (Klein et al. 1987). The combination of large diameter, robust external sheath, evidence of alternating vertical/horizontal orientation, and the stratigraphically adjacent occurrence of stromatolitic facies support the interpretation of these fossils as filamentous (subsection-III) cyanobacteria, notably some 100 million years prior to the GOE. Scale bar = 100 μm .

Fig. 6. Helically coiled filamentous microfossils comparable to extant *Spirulina*/*Arthrospira*, except for the presence of a robust extracellular sheath; acid-isolated from shales of the early Neoproterozoic Wynniatt Formation, Shaler Supergroup, NW Canada (Butterfield and Rainbird 1998). Scale bar = 25 μm .

Fig. 7. Rod-shaped microfossils morphologically comparable to the akinetes of nostocalean cyanobacteria. A, *Archaeoellipsoides grandis* (type specimen, type species); from the Mesoproterozoic ‘Lower Laminated Dolostone,’ Dismal Lakes Group, arctic Canada (Horodyski and Donaldson 1980) (GSC 57988). B, *Archaeoellipsoides grandis*; from the Mesoproterozoic Kotuikan Formation, Billyakh Group, northern Siberia (Sergeev et al. 1995). C, D, *Eosynechococcus grandis*; from the Palaeoproterozoic McLeary Formation, Belcher Supergroup, Canada (Hofmann, 1976) (GSC 42771). E, “*Archaeoellipsoides* sp.”; from the Palaeoproterozoic Franceville Group (figured in Tomitani et al. 2006). F, *Jacutianema solubila*; from the early-mid Neoproterozoic Svanbergfjellet Formation, Akademikerbreen Group, Spitsbergen (Butterfield 2004; SM X.40996). Scale bar = 20 μm for all images

Fig. 8. Origin, evolution and secondary distribution of plastids in photosynthetic eukaryotes.

The schematic phylogeny emphasizes the distinction between extant higher order clades and their underlying stem-groups, while the arrows represent likely endosymbiotic pathways of primary and secondary plastids. The horizontal dashes on the stem-lineages of Plantae and (?)SAR represent extinct photosymbioses suggested by the ‘shopping bag’ model of Howe et al. (2008), and the independent establishment of ‘primary plastids’ in *Paulinella* (Nowak and Grossman 2012). Extant clades of chlorophyll *c*-containing algae are depicted in orange, with the red arrows representing various routes by which they may have acquired their secondary ‘red’ plastids: the ‘Chromalveolate hypothesis’ (solid red line) minimizes the number endosymbiotic events, but there increasing evidence for a more polyphyletic history (dashed red lines).

Fig. 9. Proterozoic eukaryotes exhibiting a sufficient level of morphological complexity to be identified as crown-group Plantae (eukaryotes with primary plastids). A, *Bangiomorpha pubescens*, a bangiacean rhodophyte; from the late Mesoproterozoic Hunting Formation, arctic Canada (Butterfield 2000, 2009, 2015). B–D, *Proterocladus* spp., probable siphonocladalean chlorophytes; from the early-mid Neoproterozoic Svanbergfjellet Formation, Spitsbergen (Butterfield et al. 1994). *Palaeastrum dyptocranum*, a probable hydrodictyacean chlorophyte; from the early-mid Neoproterozoic Svanbergfjellet Formation, Spitsbergen. Scale bar = 20 μm for A; = 50 μm for B–D; = 70 μm for E.