

MICROFOSSILS OF PROKARYOTES

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INTRODUCTION

Bacteria and archaea make up the paraphyletic group of prokaryotes, and together with eukaryotes they form the three major domains of life. One can easily envision a world without eukaryotes, but it is difficult to imagine a biosphere without prokaryotes. Today prokaryotes colonize virtually every corner of the surface Earth system, from human guts to oceanic gyres to hydrothermal vents. Earth is home to millions of prokaryote species (SCHLOSS & others, 2016), which amount to a staggering number of individuals (WHITMAN, COLEMAN, & WIEBE, 1998; FLEMMING & WUERTZ, 2019; LOCEY & LENNON, 2019) and account for ~14–50% of carbon in the biosphere (WHITMAN, COLEMAN, & WIEBE, 1998; BAR-ON, PHILLIPS, & MILO, 2018). In fact, the biochemical capability to fix carbon and to produce oxygen can be evolutionarily traced to prokaryotes (cyanobacteria to be exact), and nitrogen fixation in nature is exclusively carried out by prokaryotes. Thus, it is safe to say that there would not be a biosphere without prokaryotes.

There are no credible reasons to doubt that prokaryotes were as abundant and important in the geological past as they are today. Yet, the fossil record of prokaryotes is extremely poor. This poor record is largely related to the fact that most prokaryotes—with the prominent exception of magnetotactic bacteria (BAZYLINSKI & FRANKEL, 2003) and some cyanobacteria (BENZERARA & others, 2014)—do not perform biologically controlled mineralization. Thus, the preservation of prokaryotes as fossils requires specific taphonomic conditions. Furthermore, the microscopic size and simple morphology of prokaryotic fossils means that they are difficult to study because of potential problems related to contamination from younger microbes, conflation with abiotic

structures, and convergence with eukaryotic microbes. Despite these challenges, there have been many reports of fossil prokaryotes since the late nineteenth century. This chapter is an overview of fossil prokaryotes, with a focus on bacteria, particularly cyanobacteria, preserved in Precambrian rocks.

HISTORY OF THE STUDY OF BACTERIAL FOSSILS

More detailed accounts of the history of fossil prokaryote research can be found in FENTON (1946), BANKS and others (1967), SCHOPF (1992a), and TAYLOR, TAYLOR, and KRINGS (2009). Prokaryotic fossils had been reported in the literature by the late nineteenth century, although some were not originally identified as such, others may be eukaryotic, and still others were later proven abiotic. For example, the tubular microfossil *Girvanella* NICHOLSON & ETHERIDGE, 1878 was first described as a foraminifer from Ordovician strata but later understood as a cyanobacterium (WOOD, 1957; RIDING, 1991). RENAULT (1896) described coccoidal and rod-shaped microstructures preserved in Carboniferous–Permian plant fossils under the extant bacterial genera *Micrococcus* COHN, 1872 and *Bacillus* EHRENBERG, 1835. These structures were originally interpreted and subsequently accepted as bacterial fossils (PIA, 1927; BANKS & others, 1967), but many of them probably represent inorganic particles (TAYLOR & KRINGS, 2005). During the early twentieth century, definitively biogenic and possibly bacterial fossils were reported in the literature. Worth mentioning are *Gloeocapsomorpha* ZALESSKY, 1917 from Middle Ordovician kukersites of the Baltic Shale Basin in Estonia, as well as the middle Cambrian fossils *Morania* WALCOTT, 1919 and *Marpolia* WALCOTT, 1919 from the Burgess Shale in Canada. *Gloeocapsomorpha* was compared with extant chroococcalean

cyanobacteria such as *Gloeocapsa* KÜTZING, 1843 and *Entophysalis* KÜTZING, 1843 (FOSTER, REED, & WICANDER, 1989; STASIUK & OSADETZ, 1990), but a cyanobacterial interpretation remains uncertain (BLOKKER & others, 2001) and some authors have interpreted *Gloeocapsomorpha* as a eukaryotic organism (e.g., a green alga) on the basis of organic geochemical evidence (HOFFMANN & others, 1987; DERENNE & others, 1991). The interpretation of *Marpolia* is also uncertain. It is commonly regarded as a cyanobacterium (WALCOTT, 1919; STEINER & FATKA, 1996), although WALCOTT (1919) also compared it with modern green and red algae, and fossils described as *Marpolia* may belong to different taxa or indeed different domains (LODUCA & others, 2017). *Morania*, on the other hand, has been generally accepted as a colonial organism consisting of cyanobacterial filaments (WALCOTT, 1919).

In addition to marine prokaryotes mentioned above, terrestrial cyanobacterial fossils have also been known from Phanerozoic deposits since the twentieth century. Among these, the most famous examples are various coccoidal and filamentous bacterial fossils from the Devonian Rhynie chert (KIDSTON & LANG, 1921; see also CROFT & GEORGE, 1959; EDWARDS & LYON, 1983; KRINGS & others, 2007; KRINGS, 2019; KRINGS & HARPER, 2019).

By the first half of the twentieth century, alleged bacterial microfossils had been reported from Precambrian rocks (WALCOTT, 1914, 1915; MOORE, 1918; GRUNER, 1922, 1923, 1924, 1925; ASHLEY, 1937). Many of these were later confirmed to be pseudofossils. For example, tubular structures illustrated in GRUNER (1923) and possibly those in ASHLEY (1937) are likely ambient pyrite trails (TYLER & BARGHOORN, 1963; KNOLL & BARGHOORN, 1974). Such trails are common in cherts and phosphorites ranging from the Archean (WACEY & others, 2008) to the Ediacaran (XIAO & KNOLL, 1999; SHE & others, 2016), and they were likely produced by pyrite crystal movement related to local build-up of degradational gas and pressure

dissolution (KNOLL & BARGHOORN, 1974). However, some of these early reports likely included *bona fide* Precambrian microfossils from the Proterozoic Belcher Supergroup (MOORE, 1918, fig. 14), Gunflint Formation (GRUNER, 1922, pl. 7; GRUNER, 1924, pl. 11), and Belt Supergroup (WALCOTT, 1914, pl. 20,2–6). In particular, GRUNER's reports were from the same stratigraphic unit—the Gunflint Formation—where paradigm-shifting discoveries were reported three decades later (TYLER & BARGHOORN, 1954; BARGHOORN & TYLER, 1965; CLOUD, 1965). But these earlier reports did not spark much interest at the time, perhaps because the quality of photomicrographs was poor (indeed, some reports had only camera lucida drawings), the great antiquity of these fossils was not appreciated, and preservation of bacterial fossils was not expected, as pointed out by KNOLL, BARGHOORN, and AWRAMIK (1978).

During the second half of the twentieth century, the study of Precambrian prokaryotes opened a new chapter. This was initiated by several high-profile reports of silicified bacterial microfossils from the Paleoproterozoic (~1880 Ma) Gunflint chert in Canada (TYLER & BARGHOORN, 1954; BARGHOORN & TYLER, 1965; CLOUD, 1965). The Gunflint fossils include stromatolite-associated coccoidal and filamentous fossils (Fig. 9.1) (BARGHOORN & TYLER, 1965), as well as coccoidal planktonic microbes (KNOLL, BARGHOORN, & AWRAMIK, 1978). These fossils were compared with extant cyanobacteria, iron-oxidizing bacteria, and fungi (BARGHOORN & TYLER, 1965; CLOUD, 1965). Serving as a search image in the field and in the laboratory, Gunflint-type stromatolitic cherts and microfossils soon opened the floodgates to numerous discoveries of Precambrian microfossils. Within a decade, Precambrian microfossils had been reported from many Precambrian cherts in North America and Australia, including the Neoproterozoic Bitter Springs Formation in Australia (Fig. 9.7) (BARGHOORN & SCHOPF, 1965; SCHOPF, 1968;

SCHOPF & BLACIC, 1971), the Neoproterozoic Skillogalee Dolomite in South Australia (SCHOPF & BARGHOORN, 1969; KNOLL, BARGHOORN, & GOLUBIC, 1975), the Neoproterozoic Beck Springs Formation in eastern California (CLOUD & others, 1969), the Paleoproterozoic Belcher Supergroup in Canada (HOFMANN, 1974; HOFMANN, 1976), Archean strata in South Africa (SCHOPF & BARGHOORN, 1967; KNOLL & BARGHOORN, 1977), and many other units. These were followed by reports of silicified microfossils, many of which are interpreted as cyanobacteria, from Precambrian cherts around the world (see summary in SCHOPF, 1983; SCHOPF & KLEIN, 1992; SERGEEV, SHARMA, & SHUKLA, 2012). Among these, Paleoarchean microfossils from Western Australia are the most contentious (AWRAMIK, SCHOPF, & WALTER, 1983; BUICK, 1984; SCHOPF & PACKER, 1987; SCHOPF, 1993; BRASIER & others, 2002; SCHOPF & others, 2002). The combined geochemical, paleontological, and sedimentological data indicate the existence of a microbial ecosystem on Earth at ~3500 Ma or earlier (ROISING, 1999; SCHOPF, 2006a), perhaps with diverse microbial metabolic pathways (SCHOPF & others, 2018).

Since the 1960–1970s, paleontologists have also been investigating Precambrian organic-walled microfossils preserved in fine-grained siliciclastic rocks or shales using hydrofluoric acid maceration techniques (XING & LIU, 1973; TIMOFEEV, HERMANN, & MIKHAILOVA, 1976; VIDAL, 1976), and some of these are filamentous microfossils that are interpreted as cyanobacteria (HERMANN, 1974). This line of research opened a new taphonomic window onto the Precambrian microbial world (VIDAL, 1981; HOFMANN & JACKSON, 1994; GREY, 2005; TANG & others, 2013). Together, microfossils preserved in cherts and shales provide a broader view of the paleoecology and taphonomy of Precambrian microbes.

MODES OF PRESERVATION

Because most prokaryotic microfossils are preserved in cherts and shales, silicifi-

cation and carbonaceous compression are the main modes of preservation. However, prokaryotic microfossils can also be replicated by phosphate, pyrite, gypsum, and other minerals; and they have been reported from ambers. These taphonomic modes are briefly described below.

SILICIFICATION

As a major permineralization pathway, silicification is responsible for the preservation of the majority of prokaryotic microfossils (Fig. 9), including those preserved in cherts of the Gunflint Formation (Fig. 9.1–9.3) and Bitter Springs Group in Australia (Fig. 9.7). Generally understood as a taphonomic process through which organisms are replaced by diagenetic silica, silicification of microbes is neither molecule-by-molecule replacement of cellular structures by silica nor wholesale replacement of the entire organism by silica, as sometimes occurs in silicification of animal skeletons (BUTTS, 2014). Rather, at the microscopic level, silicification is fundamentally a casting and molding process, with silica precipitating on organic substrates, such as cell walls and laminae of cyanobacterial sheaths, through chemical bonds between organic functional groups and silicic acids (LEO & BARGHOORN, 1976) and perhaps assisted by the presence of metallic ions (FERRIS, FYFE, & BEVERIDGE, 1988), thus producing molds or casts of microbial cells and sheaths. Thus, the organic substrates are encased within the replicating silica and are subsequently degraded to various degrees. The taphonomic survival of the organic substrates, albeit in degraded forms and in trace amounts, aids the recognition and identification of these fossils in thin section microscopy and is regarded by some geologists as an indispensable criterion for affirmation of biogenicity (BUICK, 1990).

A number of taphonomic experiments have been carried out to understand the silicification process. Degradation experiments have demonstrated that cyanobacterial cells degrade over periods of days to months

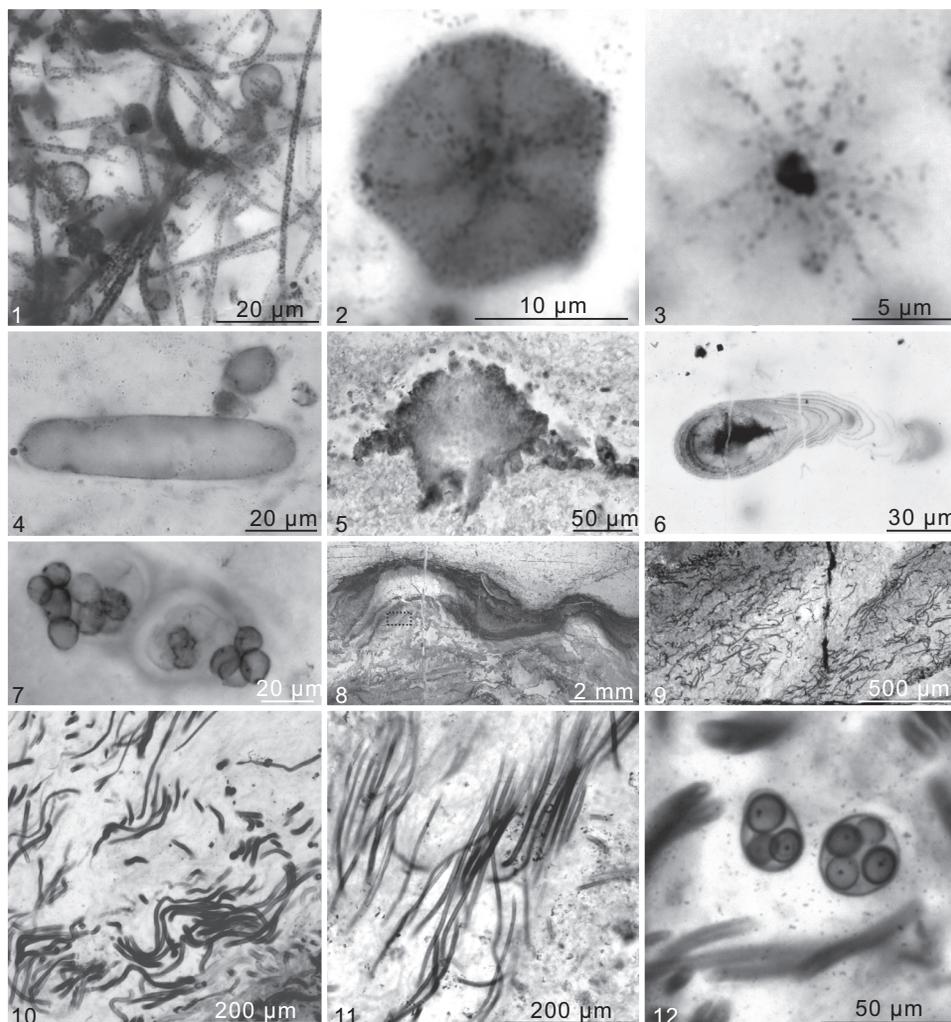


FIG. 9. Thin section photomicrographs of silicified prokaryotic microfossils from the ~1880 Ma Gunflint Formation in Canada (1–3), ~1400–1500 Ma Billyakh Group in Siberia (4–5), Tonian Draken Formation in Svalbard (6), Tonian Bitter Springs Group in Australia (7), and Tonian Jiudingshan Formation in North China (8–12). 1, Coccoidal specimens of *Huroniospora* BARGHOORN in BARGHOORN & TYLER, 1965 and filamentous specimens of *Gunflintia* BARGHOORN in BARGHOORN & TYLER, 1965. Although *Gunflintia* was described as a multicellular filament (BARGHOORN & TYLER, 1965), most specimens do not preserve trichome structure and may be identified as *Siphonophycus*; 2, *Kakabekia* BARGHOORN in BARGHOORN & TYLER, 1965; 3, possibly *Eoastrian* BARGHOORN in BARGHOORN & TYLER, 1965; 4, *Archaeoellipsoides* HORODYSKI & DONALDSON, 1980; 5, *Eoentophysalis* HOFMANN, 1976; 6, *Polybessurus* GREEN & others, 1987; 7, *Myxococcoides* SCHOPF, 1968; 8, stromatolites consisting of filamentous *Siphonophycus* SCHOPF, 1968; 9–11, close-up views of *Siphonophycus* filaments, 9 being a magnification of 8 (dotted line box); 12, *Caryosphaeroides* SCHOPF, 1968 in the center, with coccoidal cells arranged in tetrads and enclosed in a common envelope. Note intracellular inclusions that were interpreted as degraded nuclei (SCHOPF, 1968; but see KNOLL & BARGHOORN, 1975). Also note *Siphonophycus* filaments co-occurring with *Caryosphaeroides*. Fig. 1.1–1.3 and 1.7–1.12, new; Fig. 1.4–1.6 courtesy of Andrew H. Knoll, previously published as fig. 10, 2 and 17, 4 in Sergeev, Knoll, & Grotzinger, 1995, and fig. 12, 5 in Knoll, Swett, & Mark, 1991, respectively. Color version available in *Treatise Online* 160 (paleo.ku.edu/treatiseonline).

but cyanobacterial sheaths are much more resistant and can remain recognizable over longer time (GOLUBIC & BARGHOORN, 1977; BARTLEY, 1996). These experiments have been borne out by field observations showing the degraded but still recognizable cyanobacterial cells and sheaths in pigment-poor layers of modern microbial mats (GOMES & others, 2020), and they indicate that fossil mineralization must have occurred rapidly during early diagenesis in order to preserve cellular structures. Indeed, field observations of microbial silicification in modern hot spring sinters, which are widely regarded as modern taphonomic analogs of microbial silicification in Precambrian oceans, indicate that cyanobacterial and other microbes can be silicified shortly after death or even *in vivo* (RENAUT, JONES, & TIERCELIN, 1998), and that cyanobacterial sheaths are preferentially preserved through silica encrustation and permeation (RENAUT, JONES, & TIERCELIN, 1998; KONHAUSER & others, 2003). Mineralization experiments have also demonstrated that silica and clay minerals can coat on cyanobacterial sheaths, and silica can permeate cyanobacterial sheaths and cell walls, thus rapidly replicating cyanobacterial morphology in three dimensions (OEHLER & SCHOPF, 1971; WESTALL, BONI, & GUERZONI, 1995; TOPORSKI & others, 2002; NEWMAN & others, 2017). These encrustation and permeation processes may have been facilitated or accelerated by elevated silica concentrations in Precambrian seawaters and pore waters (MALIVA, KNOLL, & SIMONSON, 2005) and photosynthetic activity of cyanobacteria themselves (MOORE & others, 2020). Thus, it is not surprising that microbial silicification was common in Precambrian marine environments, but as biosilicification (e.g., in sponges, radiolarians, and diatoms) became more important and dissolved silica concentrations declined in Phanerozoic oceans (CONLEY & others, 2017), this taphonomic mode declined in and throughout the Phanerozoic, not only for bacterial silicification but for silicification in general (SCHUBERT, KIDDER, & ERWIN,

1997). Nor is it surprising that microbial silicification is common in hydrothermal settings (e.g., modern hot spring and Devonian Rhynie chert) where dissolved silica concentrations are high.

Yet silicification is not ubiquitous in all Precambrian marine environments. KNOLL (1985a) identified three sedimentary and geochemical factors that control microbial silicification: 1) sediment permeability, 2) silica availability in pore waters, and 3) local concentration of organic matter. It is possible that these factors can interact with each other to promote silicification. For example, the degradation of organic matter (and the partial degradation of organic substrates) can activate organic functional groups, thus facilitating the nucleation of silica. It can also drive down local pH values, thus promoting the precipitation of silica as the solubility of silica decreases with pH. These sedimentary and geochemical factors mean that silicification of microbes is environmentally restricted. Indeed, although there are notable exceptions (e.g., the Ediacaran Doushantuo Formation ZHANG & others, 1998; MUSCENTE, HAWKINS, & XIAO, 2015), most silicified microbial assemblages are preserved in either peritidal or hydrothermal environments (KNOLL, 1985a; KNOLL, 1985b; TREWIN, FAYERS, & KELMAN, 2003). As such, silicification provides a limited and probably biased view of the environmental and ecological ranges of prokaryotic microbes (KNOLL, 1985b; BUTTERFIELD & CHANDLER, 1992). Fortunately, this limitation is mitigated to some degree by other taphonomic modes, such as phosphatization and pyritization that are also known to preserve microbial fossils.

PHOSPHATIZATION

Although a different fossil mineralization process, phosphatization is mechanistically similar to silicification, and fossiliferous phosphorites tend to be siliceous (YAO & others, 2005; DONG & others, 2009; SERGEEV, SCHOPF, & KUDRYAVTSEV, 2020). Like silicification, phosphate encrustation

and impregnation of organic substrates are key processes that are responsible for the three-dimensional preservation of microbial cell morphology (XIAO, ZHANG, & KNOLL, 1998; XIAO & SCHIFFBAUER, 2009). Unlike silicification, however, the phosphatization is largely restricted to subtidal environments (ZHANG & others, 1998; MUSCENTE, HAWKINS, & XIAO, 2015) and occurs mostly in the Ediacaran and the Phanerozoic (SCHIFFBAUER, WALLACE, & others, 2014; MUSCENTE & others, 2017). Phosphatized cyanobacteria, for example, are best known from Ediacaran-Cambrian strata, including the Ediacaran Doushantuo Formation in the South China Craton (Fig. 10) (ZHANG & others, 1998; YUAN, XIAO, & TAYLOR, 2005), the early Cambrian (Terreneuvian) Yurtus Formation in the Tarim Basin of northwestern China (YAO & others, 2005; DONG & others, 2009) and equivalent strata in the South China Craton (WANG & others, 1984; DONG & others, 2009; GUO, LI, & SHU, 2010), and the middle Cambrian (Guzhuangian) Alum Shale Formation in Sweden (CASTELLANI & others, 2018). In addition, many Phanerozoic coprolites and cololites contain micrometer-sized spherical and rod-shaped structures interpreted as bacteria (LAMBOY & others, 1994; COSMIDIS & others, 2013; PESQUERO & others, 2014), although some of these spherical structures may be alternatively interpreted as phosphatic granules that may have been present in the digestive guts of some invertebrate animals (BUTTERFIELD, 2002; HAWKINS & others, 2018).

Relative to silicification, taphonomic experiments of phosphatization have been less successful and mostly focused on invertebrate degradation and mineralization (BRIGGS & McMAHON, 2016). Degradation experiments indicate that animal cells and tissues can be pseudomorphed by heterotrophic microbes and microbial biofilms (RAFF & others, 2008; RAFF & others, 2013; BUTLER & others, 2015), thus helping to stabilize anatomical details to be phosphatized during subsequent fossil mineralization. However, the giant sulfur

bacterium *Thiomargarita* SCHULZ & others, 1999 subjected to similar experiments did not seem to be pseudomorphed by microbial biofilms during degradation (CUNNINGHAM & others, 2012). Mineralization experiments thus far are limited and have only been able to partially phosphatize invertebrate animals (WILBY & BRIGGS, 1997; MARTIN, BRIGGS, & PARKES, 2003; HIPPLER & others, 2011). To our knowledge, no mineralization experiments have been carried out on prokaryotic organisms, and this represents a key gap in the study of prokaryote phosphatization and an area for future research.

Exceptional preservation of microbial fossils through silicification and phosphatization depends on a delicate balance between rapid mineralization and over-mineralization. Over-mineralization results in thick mineral coats that bias and disguise microbial morphologies, making it difficult to recognize mineralized microfossils in microscopy, particularly when organic substrates, such as cell walls and sheaths are completely obliterated. This has been observed in modern hot spring sinters (JONES, RENAUT, & ROSEN, 2001; PENG & JONES, 2012) as well as phosphatized microbes in the Ediacaran Doushantuo Formation in South China (XIAO & SCHIFFBAUER, 2009).

The inhibition of post-mineralization recrystallization is also an integral part of exceptional preservation through phosphatization (XIAO & HOCELLA, 2017). The successful fossilization of microscopic prokaryotic organisms, in particular, is critically dependent on the maintenance of fossilization minerals at micrometers or even nanometers in size; this is analogous to the achievement of the highest resolution in digital imaging by the smallest pixels. Exceptionally phosphatized microfossils from the Ediacaran Doushantuo Formation (Fig. 10), for example, are replicated by apatite minerals of tens to hundreds of nanometers in size (XIAO & SCHIFFBAUER, 2009). It is not completely understood why these apatite nanocrystals were prevented from dissolution and then recrystallization to become

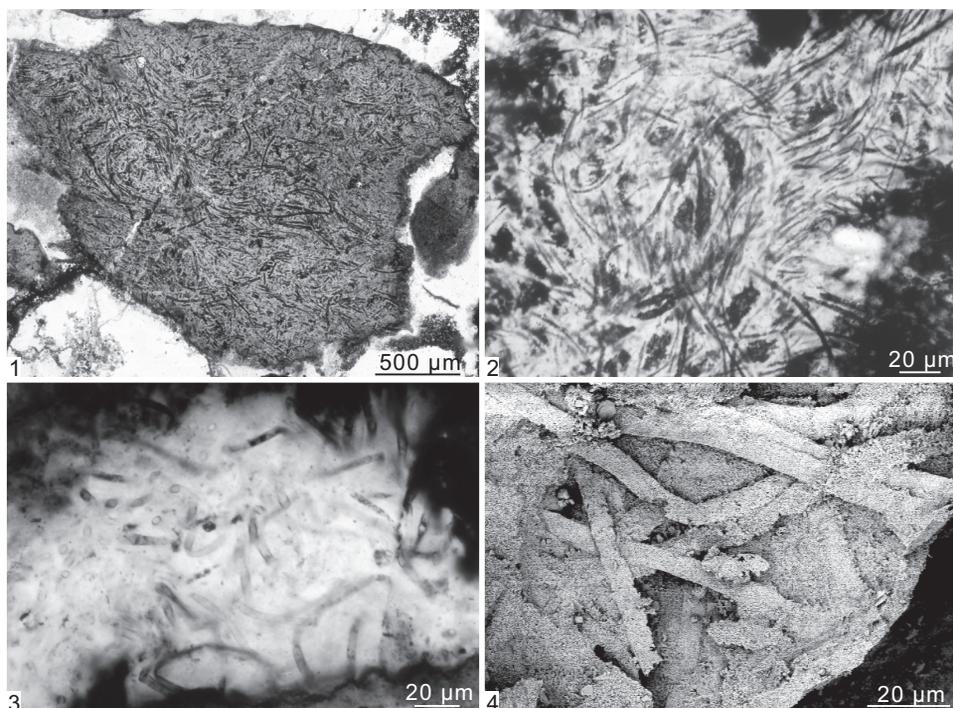


FIG. 10. Phosphatized *Siphonophycus* filaments from the Ediacaran Doushantuo Formation in South China. 1–3, thin section photomicrographs (new; photos taken by and courtesy of Lei Chen); 4, scanning electron microscopic (SEM) image (new; image by Shuhai Xiao). Color version of 1–3, *Treatise Online* 160 (paleo.ku.edu/treatiseonline).

larger crystals. However, it is possible that the dissolution of phosphate nanocrystals in the size range of tens to hundreds of nanometers is self-suppressed or self-inhibited by the limited formation and growth of dissolution pits, the size of which is constrained by the nanocrystal size (TANG, NANCOLLAS, & ORME, 2001). This may be a fruitful area for future exploration of phosphatization (XIAO & HOCELLA, 2017).

CALCIFICATION

Microbial calcification can occur as biologically controlled *in vivo* intracellular mineralization, biologically induced *in vivo* extracellular mineralization, or extrinsically induced *in vivo* or post-mortem extracellular mineralization. All three forms of mineralization can be found in cyanobacteria. Some cyanobacteria carry out biologically controlled mineralization and precipitate

intracellular carbonates (COURADEAU & others, 2012; BENZERARA & others, 2014), but thus far these cyano-bacterial biominerals are not known to be preserved and identified in the fossil record. More commonly, metabolic activities of cyanobacteria, particularly photosynthesis and carbon dioxide concentration mechanisms, promote an increase in local pH values and induce *in vivo* precipitation of calcium carbonate that impregnate the sheath (RIDING, 2006). This form of biologically induced mineralization results in extracellular sheath calcification and may be responsible for the preservation of the majority of calcified cyanobacterial fossils, such as *Girvanella* NICHOLSON & ETHERIDGE, 1878 (Fig. 11.1–11.2), *Epiphyton* BORNEMANN, 1886 (Fig. 11.3), and *Renalcis* VOLOGDIN, 1932. Finally, microbes can be entombed *in-vivo* or postmortem in carbonate deposits (Fig. 11.4–11.6) (KREMER & others,

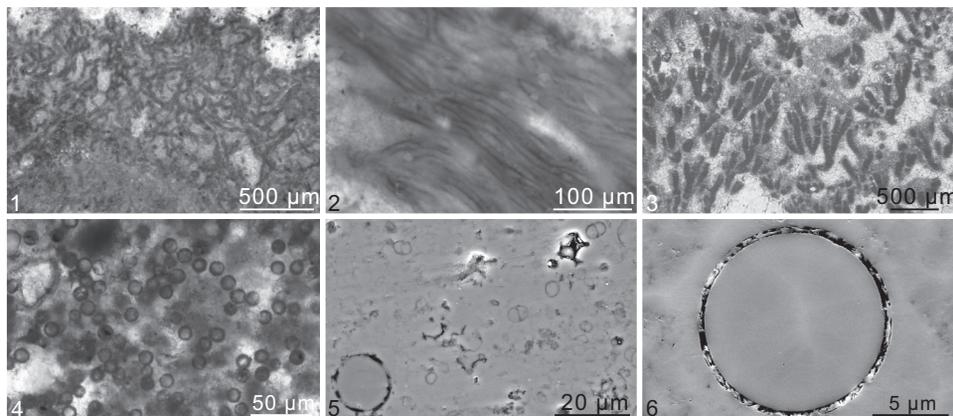


FIG. 11. Prokaryotic microfossils preserved in carbonate rocks. 1–2, *Girvanella* NICHOLSON & ETHERIDGE, 1878 from the Lower Ordovician Fenhshang Formation at the Liujiachang section, Songzi, Hubei Province, South China; 3, *Epiphyton* BORNEMANN, 1886 from Cambrian Stage 3, Zhangxia Formation in Laiwu, Shandong Province, North China. Both *Girvanella* and *Epiphyton* have been interpreted as calcified cyanobacteria (RIDING, 1991); 4–6, Coccoid microfossils, interpreted as methanogens on the basis of extremely high $\delta^{13}\text{C}_{\text{carb}}$ values up to 20‰ of the host dolomite concretions from the Middle Permian lacustrine deposits of the Lucaogou Formation in Xinjiang, northwestern China (SUN & others, 2020). Note two size classes in 5, representing two different taxa. 1–4 are thin section photomicrographs and 5–6 are SEM images. Images 1–3, new; photos taken by and courtesy of Jianbo Liu; 4–6, courtesy of Funing Sun and Wenxuan Hu, previously published as fig. 2D, 2G, and 2F, respectively, in Sun & others, 2020. Color version of 1–4 in *Treatise Online* 160 (paleo.ku.edu/treatiseonline).

2012; SUN & others, 2020)—including tufas, travertines, and speleothems whose precipitation is primarily driven by abiotic processes such as CO_2 degassing, although it is not always possible to determine whether biological processes also play a secondary role in facilitating calcification (JONES & PENG, 2012; LI & others, 2013; JONES & PENG, 2014).

Microbial calcification is not uniformly distributed across geological time, sedimentary environments, and taxonomic groups. As calcification is critically dependent on carbonate supersaturation levels, it is not surprising that microbial calcification tends to be focused on tropical shallow marine realms, for example evaporitic, peritidal, and reefal or mud mound environments. In addition, because various microbial metabolisms have different impacts on the precipitation and dissolution of carbonate minerals (CANFIELD & RAISWELL, 1991), it is anticipated that different groups of microbes have different propensities to induce calcification. As mentioned earlier, photosynthesis and

carbon dioxide concentration mechanisms of cyanobacteria facilitate fossilization through calcification (RIDING, 2006). But calcified cyanobacterial fossils have a non-uniform distribution in warm shallow marine environments across geological history. Although they range from the Meso-Neoproterozoic (KNOLL, FAIRCHILD, & SWETT, 1993; TURNER, NARBONNE, & JAMES, 1993; KAH & RIDING, 2007) to the Cenozoic (ARP, REIMER, & REITNER, 2001), they are mostly concentrated in the Paleozoic and early Mesozoic (ARP, REIMER, & REITNER, 2001). Geochemical, atmospheric, and biological factors have been implicated as controlling factors for the non-uniform distribution of calcified cyanobacterial microfossils in marine environments. For example, RIDING (2006) proposed that pCO_2 levels fell below $\sim 0.4\%$ (or $10\times$ present atmospheric level) at 750–700 Ma, driving the evolution of CO_2 -concentrating mechanisms and facilitating *in vivo* calcification of cyanobacterial sheaths in the Neoproterozoic and Paleozoic. ARP, REIMER, and REITNER (2001)

suggested that the Paleozoic abundance of cyanobacterial calcification may be related to high calcium concentrations in Paleozoic oceans. Biological factors were in play too. KNOLL, FAIRCHILD, and SWETT (1993), for example, suggested that, whereas the rarity of cyanobacterial calcification in the Precambrian may be attributed to the abundance of micrite (e.g., whiting) that outcompeted cyanobacterial sheaths as nucleation sites for calcite overgrowth in the sediment, the post-Mesozoic decline of cyanobacterial calcification was due to the ecological rise of calcareous phytoplankton.

PYRITIZATION AND RELATED PRESERVATION MODES

Bacteria and archaea are key players in the sulfur cycle (EHRlich & NEWMAN, 2009). Thus, it is not surprising that they play direct and indirect roles in the precipitation of sulfur-bearing minerals. Some sulfide-oxidizing bacteria (e.g., *Beggiatoa* TREVISAN, 1842, *Thiomargarita* SCHULZ & others, 1999, and *Thioploca* LAUTERBORN, 1907) produce intracellular sulfur granules (EHRlich & NEWMAN, 2009; BAILEY & others, 2013). Although such sulfur granules are not supposed to be stable in geological time scales, filamentous microfossils from the Ediacaran Doushantuo Formation in South China contain sulfur-rich granules that are interpreted as intracellular sulfur granules produced by sulfide-oxidizing bacteria (BAILEY & others, 2013). More commonly, microbial sulfate reduction promotes the precipitation of pyrite, which can replicate microbes in the fossil record through pyritization; often, it is the organisms that are degraded by sulfate reducing microbes, rather than the sulfate reducing microbes themselves, that are pyritized (SCHIFFBAUER, XIAO, & others, 2014). Pyritized microfossils are common in the geological record (SCHOPF & others, 1965; RASMUSSEN, 2000; MOORE & others, 2017). In some pyritized filamentous microfossils (e.g., those from the Ediacaran Krol Group

in India; Fig. 12), pyrite crystals seem to precipitate within a tubular sheath, thus outlining the filamentous morphology but not faithfully replicating the diameter of the filaments until a full internal mold is formed. Thus, pyritization seems to be initiated within partially degraded filamentous microbes (perhaps after the degradation of trichomes but before the complete destruction of the sheath), and can proceed to form pyritic internal mold of microbes. Finally, microbial fossils can be replicated by gypsum (VAI & LUCCHI, 1977; SCHOPF & others, 2012), the precipitation of which is primarily driven by abiotic processes such as evaporation.

PRESERVATION OF BIOMINERALS PRODUCED BY MAGNETOTACTIC BACTERIA

A number of iron bacteria can produce biologically controlled and biologically induced biominerals (BAZYLINSKI & FRANKEL, 2003; FRANKEL & BAZYLINSKI, 2003). Magnetotactic bacteria, for example, produce intracellular minerals such as magnetite (Fe_3O_4) and greigite (Fe_3S_4) that can have distinct morphologies and crystallographic features (Fig. 13) (BAZYLINSKI & FRANKEL, 2003; LI & others, 2013, 2020). These distinct crystals allow their identification in the fossil record, and indeed fossil magnetotactic bacteria have been reported in Mesozoic and Cenozoic sediments (CHANG & KIRSCHVINK, 1989; KOPP & KIRSCHVINK, 2008).

Some iron bacteria can also produce biologically induced biominerals with distinct morphologies. For example, the iron bacteria *Gallionella* EHRENBERG, 1838 and *Mariprofundus* EMERSON & others, 2007 can produce extracellular ferric-oxyhydroxide stalks that are twisted, branched, or organized into ribbon-like bands (FRANKEL & BAZYLINSKI, 2003; CHAN & others, 2011; KREPSKI & others, 2013). Morphologically similar stalks have also been identified in the fossil record and interpreted as evidence for iron bacteria

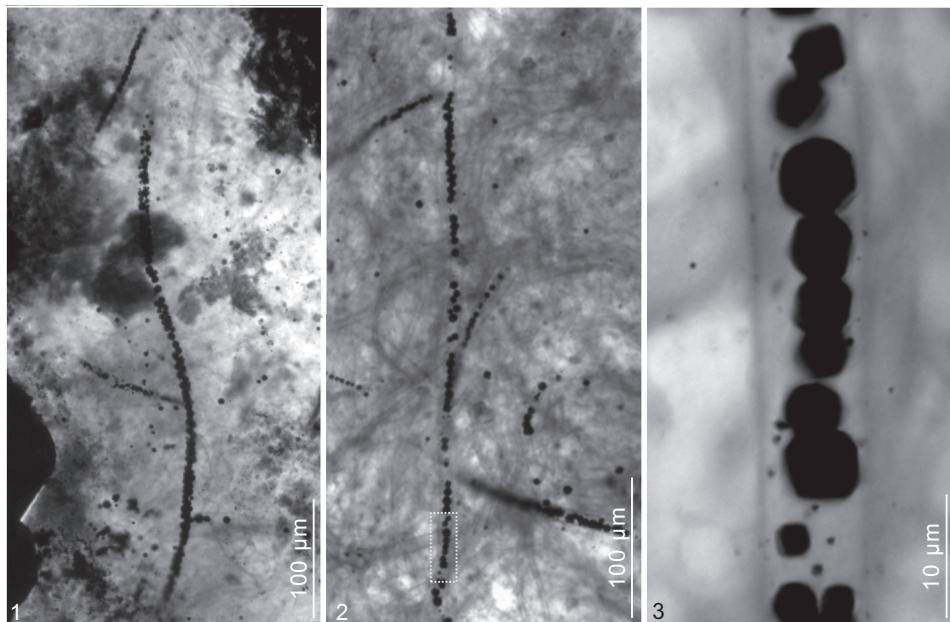


Fig. 12. Thin section photomicrographs of pyritized *Siphonophycus* filaments from the Ediacaran Krol Group in northern India. 3 is magnified view of 2 (yellow dotted-line box). Note that organic sheath is largely degraded in 1 and well preserved in 2–3. All images are new and were taken by Shuhai Xiao. Color version in *Treatise Online* 160.

(HOFMANN & others, 2008; KREPSKI & others, 2013; CROSBY, BAILEY, & SHARMA, 2014).

CARBONACEOUS PRESERVATION

Although traces of carbonaceous material are commonly found in mineralized prokaryotic fossils, they are typically impregnated or penetrated by replicating minerals such as microquartz and apatite, so that extraction of coherent organic-walled microfossils using hydrofluoric (HF) digestion method is difficult. In contrast, carbonaceous preservation of prokaryotic fossils in fine-grained siliciclastic rocks may manifest as compressed organic-walled structures with little mineral permeation or impregnation (XIAO & others, 2002; CALLOW & BRASIER, 2009), and these fossils can be extracted from the rock matrix using hydrofluoric acid digestion methods without compromising their structural integrity (Fig. 14) (TANG & others, 2013; TANG & others, 2015). In addition to carbonaceous compressions, structurally recognizable organic residues of prokaryotic microbes can also be preserved

in ambers (POINAR, WAGGONER, & BAUER, 1993; WAGGONER, 1994; DÖRFELT, SCHMIDT, & WUNDERLICH, 2000; SCHMIDT & SCHÄFER, 2005). Finally, carbonaceous coccoids, filaments, and sheets have been reported on the basis of scanning electron microscopic observation of fractured rock surface (sometimes after acid etching), and these have been interpreted as fossil microbes or as extracellular polymeric substances (WESTALL & FOLK, 2003; DAI, SONG, & SHEN, 2004; ROZANOV & ASTAFIEVA, 2009; LAN & others, 2020), although it is a significant challenge to demonstrate their syngenicity (ALTERMANN, 2001; EDWARDS & others, 2006).

TRACE FOSSILS

Some prokaryotic micro-organisms, particularly cyanobacteria, can bore into hard substrates and leave a trace fossil record (GOLUBIC, PERKINS, & LUKAS, 1975; COCKELL & HERRERA, 2008). Tunnels and galleries of tunnels interpreted as traces of euendolithic cyanobacteria have been reported from many phosphatic small shelly fossils from

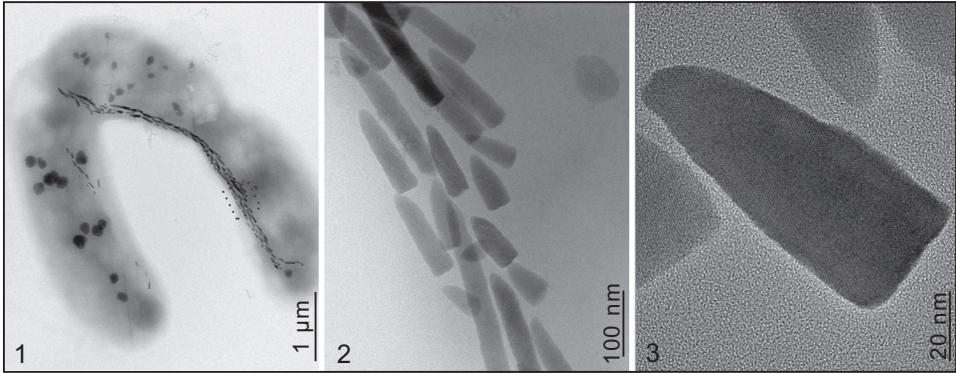


FIG. 13. Bright-field TEM (transmission electron microscopy) images (1–2) and high-resolution TEM image (3) of chains of straight bullet-shaped magnetite nanocrystals produced by extant magnetotactic deltaproteobacteria (strain WYHR-1) collected from Weiyang Lake, north of Xi'an city, Shaanxi Province, North China (Li & others, 2020). Images are new and courtesy of Jinhua Li.

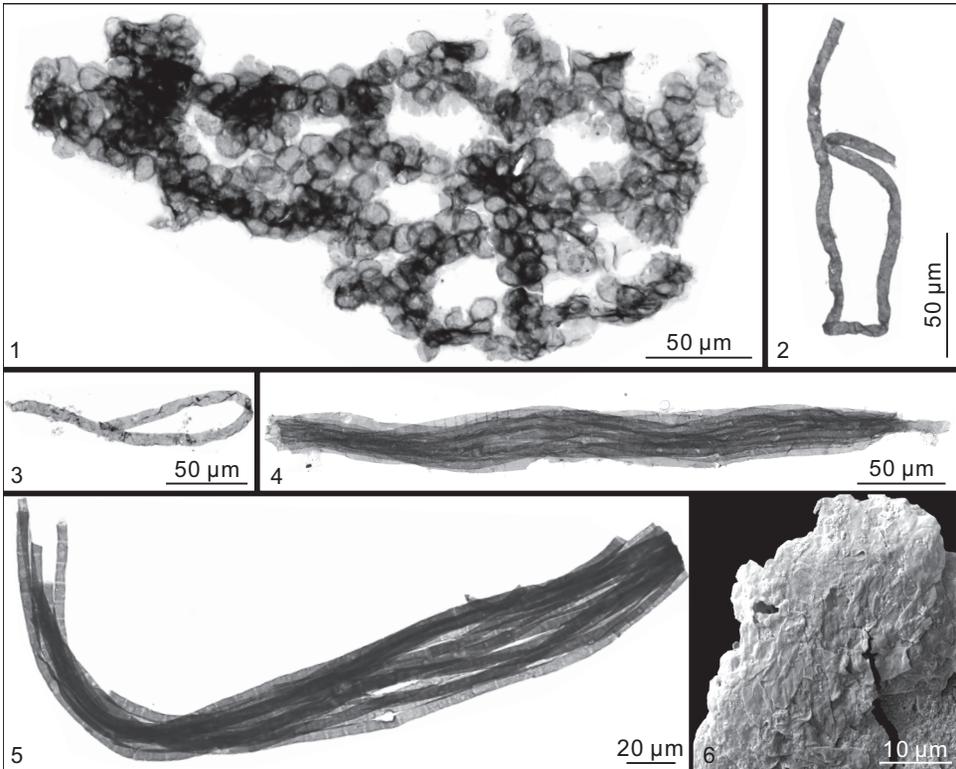


FIG. 14. Prokaryotic microfossils preserved as carbonaceous compressions in fine-grained sediments. 1, *Ostiana microcystis* HERMANN in TIMOFEEV, HERMANN, & MIKHAILOVA, 1976, a possible cyanobacterium (BUTTERFIELD, KNOLL, & SWETT, 1994); 2–3, *Siphonophycus typicum* (HERMANN, 1974; transferred to the genus *Siphonophycus* by BUTTERFIELD in BUTTERFIELD, KNOLL, & SWETT, 1994); 4–5, *Polytrichoides lineatus* HERMANN, 1974; 6, ellipsoidal cells of *Eosynechococcus moorei* HOFMANN, 1976. All specimens were extracted from shale samples using hydrofluoric acid digestion method. 1–4 are from the Tonian Liulaobei Formation in the North China Craton (TANG & others, 2013, fig. 5G, 13C, 13D, and 14A, respectively), and 5–6 are from the Tonian Gouhou Formation in the North China Craton (TANG & others, 2015, fig. 19E and 5B, respectively). Fig. 1–5 are transmitted light photomicrographs; 6 is an SEM image.

the Cambrian Period (RUNNEGAR, 1985; LI, 1997). These tunnels typically have smooth walls and a constant diameter along their length, but they are otherwise simple in morphology, and the distinction between cyanobacterial, fungal, and green algal borings can be difficult (GOLUBIĆ, PERKINS, & LUKAS, 1975). However, they can be easily differentiated from ambient pyrite trails in phosphorites and cherts, which are characterized by striated walls and commonly terminated by a pyrite grain (XIAO & KNOLL, 1999; SHE & others, 2016; YANG & others, 2017). They can also be easily differentiated from tubular structures in Paleoproterozoic pillow basalts that were controversially interpreted as putative bioerosional structures of early microbes (FURNES & others, 2004; STAUDIGEL & others, 2006).

CHALLENGES IN THE INTERPRETATION OF PROKARYOTIC MICROFOSSILS

To unambiguously demonstrate the syngenicity, biogenicity, and affinity of purported prokaryotic microfossils is a significant challenge, particularly in the study of Precambrian micropaleontology because of the poor age constraints, difficulty in stratigraphic correlation, and simple (and sometimes exotic) morphologies of ancient microorganisms. This challenge is highlighted in the debate on the earliest traces of microbial life on Earth (BUICK, 1990; BRASIER & others, 2005; BRASIER & others, 2006; JAVAUX, 2019). Below, indigeneity, syngenicity, biogenicity, and affinity are discussed separately for clarity purpose, although these are often intimately related.

INDIGENICITY AND SYNGENICITY

Syngenicity refers to the provenance of the purported microfossils. Syngenetic microfossils must be indigenous; they should be demonstrated to be enclosed within and thus have the same age of the host rock, rather than later contaminants. Contami-

nants can be introduced in the geological past, in the field, or in the laboratory (CLOUD & MORRISON, 1979). In early studies of Precambrian microfossils, there were numerous cases of contamination. Such examples included modern chasmoliths or extracellular polysaccharide strands, seemingly indigenous as they pass beneath mineral grains in sediment (CLOUD & MORRISON, 1979). Other examples involved modern fungal spores and hyphae that were introduced in the field and laboratory, particularly when samples were processed using acid digestion methods. MENDELSON and SCHOPF (1992) provided a comprehensive assessment of these contaminants.

An accepted criterion for indigeneity is to demonstrate—typically through petrographic observation of thin sections cut from freshly collected rock samples—that the purported microfossils are encased in rock matrix. In order to confirm syngenicity in thin sections, care must be taken to distinguish whether the purported microfossils were buried in the rock matrix at the time of deposition or are embedded in secondary cements/crystals that fill voids, fractures, veins, dikes, or volcanic vesicles (i.e., amygdaloids). In the latter case, the secondary cements/crystals should be independently dated because they can be markedly younger than the host rock. This can be achieved through relative dating using cement stratigraphy and cross-cutting relationships (ZHOU & others, 2015; GAN & others, 2021), analysis of mineral assemblages tied to dated metamorphic events (BENGTSON & others, 2017), or (when carbonaceous material is available) Raman spectroscopic analysis of carbonaceous material to determine maximum metamorphic temperatures (SCHOPF & others, 2005; SCHIFFBAUER & others, 2007; JAVAUX, MARSHALL, & BEKKER, 2010).

BIOGENICITY

Biogenicity refers to the biological origin of the purported microfossils. It should be

emphasized that, to prove biogenicity, the morphologies of the microfossils must be shown to be biological in origin. This is a distinction between morphological and chemical biosignatures. For example, a pyrite concretion may preserve chemical biosignatures because its sulfur isotopic composition indicates the involvement of microbial sulfate reduction, but this by itself does not offer evidence for a biological origin of the pyrite concretion.

CLOUD (1965, p. 27) argued that the null hypothesis in Precambrian micropaleontology should be that purported microfossils be initially regarded as abiotic in origin. He wrote, "... in considering what we may accept as unequivocal Precambrian fossils, the crucial point is not whether materials observed might conceivably be of vital origin, but whether they could have been produced by non-vital processes; and, if not, whether they are sure endemic to authentic Precambrian rocks." Only after an abiotic origin can be ruled out and syngenicity is confirmed can Precambrian microfossils be accepted. This restrictive approach is necessary because of the possibility of biomorphs that are abiotic in origin but morphologically mimic microfossils (GARCÍA-RUIZ & others, 2003; JAVAUX, 2019) and also because of the profound ramifications of false positives in the study of Precambrian (particularly Archean) microfossils.

In early debates on putative microfossils from the Paleoproterozoic Warrawoona Group in Western Australia, BUICK (1990) proposed a seven-point test to assess their syngenicity and biogenicity. He argued that *bona-fide* microfossils should be observed in petrographic thin sections, preserved in sedimentary rocks or low-grade metasediments, no smaller than the smallest extant modern microbes (i.e., $>0.01 \mu\text{m}^3$), comprised of kerogen, part of a larger population of similar morphologies, hollow structures, and show cellular elaborations. Subsequently, a number of authors proposed additional criteria to assess the morphology, ontogeny, metabolism, behavior, tapho-

nomy, chemistry, and geological context of purported microfossils (SCHOPF & others, 2010; BRASIER & WACEY, 2012; ROUILLARD & others, 2018; JAVAUX, 2019; ROUILLARD & others, 2021). For example, *bona fide* microfossils should have a stable species-specific morphology with a unimodal size distribution and would exhibit evidence of development (e.g., cell division and development of branching filaments), distinct cell wall ultrastructures, taphonomic degradation (e.g., degradation of cytoplasm, deflation of cell vesicles, and deformation of cell walls and sheaths), ecological interactions (e.g., aggregations and attachment to substrates), and metabolic activities (e.g., organic C and N isotope signatures, trace metal enrichment) (LEPOT, 2020).

Recent exploration of ancient microfossils have pushed the envelope beyond the preservation of organic-walled structures in sedimentary rocks as stipulated by BUICK (1990). Coccolidal, rod-shaped, and filamentous structures preserved in igneous rocks, sometimes with no traces of organic walls, may represent evidence for ancient life, including both prokaryotes and eukaryotes (Fig. 15.1) (BENGTSON & others, 2017; IVARSSON & others, 2020). More controversial are micrometer-sized titanite filaments or microtextures in altered volcanic glass of Paleoproterozoic pillow basalts that have been interpreted as bioerosional structures or trace fossils produced by chasmoendolithic and euendolithic microbes (Fig. 15.2) (FURNES & others, 2004; STAUDIGEL & others, 2006) and micrometer-sized hematitic tubular structures from >3.77 Ga ferruginous sedimentary rocks in the Nuvvuagittuq supracrustal belt in Canada that are regarded as putative microfossils, possibly representing iron-oxidizing bacteria (Fig. 15.3) (DODD & others, 2017). Given that inorganic and morphologically simple tubes and spheres can be produced abiotically (GARCÍA-RUIZ & others, 2003; GARCÍA-RUIZ & others, 2017; MCMAHON, 2019), extra efforts must be made to affirm the biogenicity of these purported microfossils, and alternative

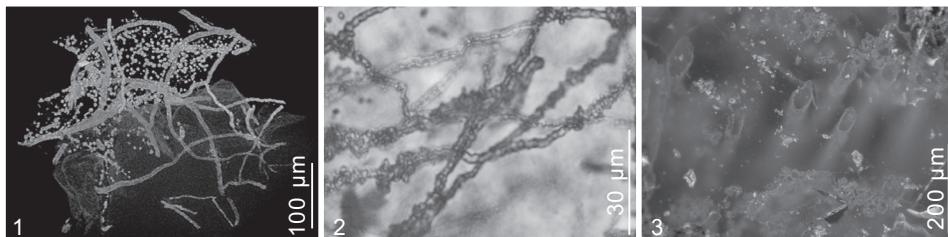


FIG. 15. Coccoidal, filamentous, and tubular structures with no preservation of organic walls. 1, synchrotron-based X-ray tomographic rendition of coccoidal structures (interpreted as unicellular prokaryotes) suspended in filamentous cobweb-like structures (interpreted as fungal hyphae) from Koko Seamount (Ivarsson & others, 2020, fig. 4C); 2, titanite microtextures from the ~3350 Ma Euro Basalt in Western Australia (see McLoughlin & others, 2020) (new; image by Nicola McLoughlin); 3, hematitic tubes in chert from jasper banded iron formation in hydrothermal vent deposits of the Nuvvuagittuq Supracrustal Belt (NSB) in Québec, Canada, constrained between ~3750 and ~4280 Ma (Dodd & others, 2017, fig. 2e). Photographed in a one-cm-thick polished slab under dark-field reflected light. 1 is courtesy of Magnus Ivarsson and Stefan Bengtson; 2 courtesy of Nicola McLoughlin; and 3 courtesy of Matthew Dodd and Dominic Papineau. Color version available in *Treatise Online* 160 (paleo.ku.edu/treatiseonline).

abiogenic origins must be ruled out before they can be considered evidence for ancient life (STAUDIGEL & others, 2008; GROSCH & McLoughlin, 2014; McMAHON, 2019; McLoughlin & others, 2020). Controversies notwithstanding, igneous rocks and inorganic preservation may represent underexplored archives of microbes in deep time and deep Earth (IVARSSON & others, 2020).

AFFINITY

With syngenicity and biogenicity established, the next challenge is to assess the affinity of the microfossils—whether they are prokaryotes or eukaryotes, and which group of prokaryotes they belong to. The most common microfossils are filaments, bacilloids, and coccoids, but these morphotypes occur in both eukaryotes and prokaryotes. To complicate interpretations further, subcellular structures such as melanosomes can be superficially similar in size and shape to bacilloidal and coccoidal bacteria (MOYER & others, 2014; VINTHER, 2015), although they are less relevant in the study of Precambrian microfossils. Eukaryotic cells are typically larger than prokaryotic cells, but there is a significant overlap (SCHOPF, 1992b; PANG & others, 2018). Thus, cell size is a suggestive but inconclusive criterion. Other morphological features, such as branching filaments, fused filaments, anastomosed

filaments, coccoidal diads and tetrads, cell differentiation, and cell wall ornaments can be useful in distinguishing eukaryotic from prokaryotic microfossils. Typically, eukaryotic cells are morphologically more complex than prokaryotic cells. However, many of the features listed above may occur in bacterial cells. For example, actinobacteria can develop branching filaments and some of them (e.g., *Streptomyces* WAKSMAN & HENRICI, 1943) have been reported to form anastomosis of network (ERIKSON, 1949; GREGORY, 1956). A number of cyanobacteria can develop branching filaments (e.g., *Fischerella* GOMONT, 1895), coccoidal diads and tetrads (e.g., *Chroococcus* NÄGELI, 1849 and *Gloeocapsa* KÜTZING, 1843), and morphologically and functionally differentiated cells (e.g., heterocysts and akinetes in *Anabaena* BORY *ex* BORNET & FLAHAULT, 1886a) (CASTENHOLZ, 2001). Thus, these features are not exclusively eukaryotic, and only more complex features such as spinose cell wall ornaments, differentiated holdfast, apical meristem, and parenchymatous thallus are regarded diagnostic characters for eukaryotes (KNOLL & others, 2006). Cell wall ultrastructures can also be useful. For example, the trilaminar structure with two electron-dense layers around a thicker electron-tenuous layer is said to be characteristic of eukaryotic cell walls (JAVAUX,

KNOLL, & WALTER, 2004; MOCZYDŁOWSKA, SCHOPF, & WILLMAN, 2010), although cell wall ultrastructures of modern eukaryotes and prokaryotes have not been thoroughly surveyed. Geochemical evidence can also be used to infer the prokaryotic versus eukaryotic affinities of microfossils. For example, combined micro-FTIR (Fourier-transform infrared spectroscopy) and Raman spectroscopic data—that is, FTIR CH₃/CH₂ absorbance ratio and Raman I-1350/I-1600 ratio of carbonaceous material—may be useful in distinguishing prokaryotic from eukaryotic microfossils (IGISU & others, 2009; QU & others, 2015; QU & others, 2018; BONNEVILLE & others, 2020), although diagenetic and thermal alteration of these parameters has not been completely understood (IGISU & others, 2018). As another example, methanogenic archaea can generate large carbon isotope fractionations that can be preserved in the geological record (STUEKEN & others, 2017; LEPOT, 2020). The assignment of prokaryotic microfossils to the various phylogenetic and physiological groups is another major challenge; but ecological, morphological, and chemical comparison with modern prokaryotic groups can provide some insights. This is discussed below for selected groups of prokaryotic microfossils.

SELECTED GROUPS OF PROKARYOTIC MICROFOSSILS CYANOBACTERIA

Modern cyanobacteria consist of five morphological groups (CASTENHOLZ, 2001). Subsection I includes unicellular/colonial cyanobacteria that reproduce by binary fission (e.g., *Prochlorococcus* CHISHOLM & others, 1992, *Synechococcus* NÄGELI, 1849, *Gloeocapsa*, *Entophysalis* KÜTZING, 1843, *Chroococcus*). Subsection II includes unicellular/colonial cyanobacteria that reproduce by internal multiple fissions and formation baeocytes (e.g., *Pleurocapsa* THURET in HAUCK, 1885, *Hyella* BORNET & FLAHAULT, 1888). Subsection III (e.g., *Lyngbya* AGARDH

ex GOMONT, 1892b, *Microcoleus* DESMAZIÈRES ex GOMONT, 1892a, *Oscillatoria* VAUCHER ex GOMONT, 1892b, *Spirulina* TURPIN ex GOMONT, 1892b, *Trichodesmium* EHRENBERG ex GOMONT, 1892b) and Subsection IV (e.g., *Anabaena*, *Nostoc* VAUCHER ex BORNET & FLAHAULT, 1886a, *Calothrix* AGARDH ex BORNET & FLAHAULT, 1886b) are both characterized by uniseriate and unbranched trichomes produced by binary fission in one plane, but the latter have differentiated cells (e.g., specialized N₂-fixing heterocysts and resting akinetes). Subsection V is characterized by multiserial or branching trichomes produced by binary fission in more than one plane, with some members having differentiated heterocysts (e.g., *Stigonema* AGARDH ex BORNET & FLAHAULT 1886c, *Fischerella*). Recent Phylogenetic analyses indicate that Subsections IV and V are monophyletic groups, whereas the other three are paraphyletic (SÁNCHEZ-BARACALDO, 2015; SCHIRMEISTER, GUGGER, & DONOGHUE, 2015).

Cyanobacteria play a major role in modern ecosystems and in the global carbon and oxygen cycles. The cyanobacteria *Prochlorococcus* and *Synechococcus* are the most abundant photosynthetic organisms in modern oceans, accounting for about 10% of the total ocean picoplankton cells in the euphotic zone and responsible for as much as 25% of ocean net primary productivity (FLOMBAUM & others, 2013). A single cyanobacterial genus, *Trichodesmium*, is responsible for nearly 50% of global marine N₂ fixation (SOHM, WEBB, & CAPONE, 2011; BERGMAN & OTHERS, 2013). Benthic cyanobacteria are also important sedimentary agents. They build microbial mats and stromatolites (STAL, 2012), stabilize sediments (NOFFKE, 2010), and perform bioerosion and biodegradation (GOLUBIC, PIETRINI, & RICCI, 2015). Cyanobacteria also played a transformative role in Earth history. The origin of oxygenic photosynthesis in a common ancestor of cyanobacteria is the geobiological foundation of the Great Oxidation Event and the origin of photosynthetic eukaryotes (KNOLL, 2008). Thus, it is expected that

cyanobacteria should be richly archived in the geological record. Indeed, they are the most common and widespread prokaryotic microfossils in the geological record, and some of the Precambrian microfossils first reported in the literature were compared and identified with cyanobacteria (BARGHOORN & TYLER, 1965; CLOUD, 1965).

A number of researchers have reviewed Precambrian cyanobacterial microfossils from different perspectives (KNOLL & GOLUBIC, 1992; GOLUBIC & LEE, 1999; SCHOPF, 2012; SERGEEV, SHARMA, & SHUKLA, 2012; KNOLL, 2016; SCHIRRMEISTER, SANCHEZ-BARACALDO, & WACEY, 2016; DEMOULIN & others, 2019). The identification of cyanobacterial microfossils is based on their combined morphologic, taphonomic, paleoecological, paleoenvironmental, and behavioral features that are considered with modern counterparts (KNOLL & GOLUBIC, 1992; GOLUBIC & LEE, 1999). Relative to other bacteria, cyanobacteria are typically larger in size and more complex in morphologies, some have sheaths, many are associated with stromatolites, and they commonly live in the photic zone or shallow marine environments where silicification occurs, although there are aspects of morphological and ecological convergences between cyanobacteria and some mat-forming sulfide-oxidizing bacteria. Some purported cyanobacterial fossils are morphologically simple. Examples include micrometer-sized coccoids such as *Myxococoides* SCHOPF, 1968 (Fig. 9.7) and tubular filaments such as *Siphonophycus* SCHOPF, 1968 (Fig. 9.9–9.12; Fig. 10.4). Their cyanobacterial interpretation is primarily based on their preservation, sometimes in life position (Fig. 9.8–9.9) in stromatolitic laminae (GOLUBIC & LEE, 1999; CAO, YUAN, & XIAO, 2001). It is assumed that these stromatolites were likely constructed by cyanobacteria. Others have a combination of morphologies and ecologies that support a cyanobacterial interpretation. These include *Eoentophysalis* HOFMANN, 1976 with colonial coccoidal cells forming microbial crusts (Fig. 9.5); *Eohyella* ZHANG

& GOLUBIC, 1987 being euendolithic and psuedofilamentous; and *Polybessurus* GREEN & others, 1987, with a stalk consisting of stacked cup-like gelatinous material (Fig. 9.6). Still others are character-rich and have distinctive, if not diagnostic, cyanobacterial features such as fossilized akinetes. The co-occurrence of *Archaeoellipsoides* HORODYSKI & DONALDSON, 1980 and *Filiconstrictosus* SCHOPF & BLACIC, 1971—which are interpreted as akinetes and short-trichome germlings, respectively—from the Mesoproterozoic Billyakh Group in Siberia provides a plausible case for fossil akinetes (GOLUBIC, SERGEEV, & KNOLL, 1995; SERGEEV, KNOLL, & GROTZINGER, 1995). Akinetes also occur in the Tonian fossil *Anhuithrix* PANG & others, 2018, and both akinetes and heterocysts have been reported in the Devonian microfossils *Langiella* CROFT & GEORGE, 1959 and *Kidstoniella* CROFT & GEORGE, 1959. These features facilitate morphological comparisons with modern cyanobacteria, where akinetes and heterocysts occur only in Subsections IV–V (CASTENHOLZ, 2001; UYEDA, HARMON, & BLANK, 2016, fig. S7). Various ecological and morphological comparisons have been proposed for a number of well-known cyanobacterial fossils (Table 1, p. 48–49), many of which were named after their modern counterparts (SCHOPF, 1994; KNOLL, 2016). Accepting the interpretations presented in Table 1, all five cyanobacterial subdivisions are represented in the fossil record.

When did cyanobacteria first evolve? This question can be addressed from the perspectives of molecular clocks, geochemical signatures, and fossils, but currently available data do not provide a tight constraint on this important evolutionary event. Molecular clocks give divergent results, with the estimated divergence time of crown-group cyanobacteria ranging widely from more than 3600 Ma to less than 2000 Ma, with very large error bars (SCHIRRMEISTER, GUGGER, & DONOGHUE, 2015; SHIH & others, 2017; see summary in DEMOULIN & others, 2019; GARCIA-PICHEL & others,

2019). Stable carbon isotope signatures of Archean organic carbon are consistent with but are not uniquely diagnostic of cyanobacterial metabolism (DEMOULIN & others, 2019), although LYONS, REINHARD, AND PLANAVSKY (2014) argue that the total organic carbon content in Archean shales presents strong evidence for oxygenic photosynthesis (and perhaps cyanobacteria) before the Great Oxidation Event at 2320–2450 Ma (BEKKER & others, 2004; HOLLAND, 2006; LUO & others, 2016). The report of 2-methylhopanoids—which were regarded as a biomarker of cyanobacteria—from the ~2700 Ma Jeerinah Formation in Western Australia (BROCKS & others, 1999) was later shown to be compromised by contaminations (RASMUSSEN & others, 2008; FRENCH & others, 2015), leaving the 1.64 Barney Creek Formation in Western Australia as the oldest known unit to contain appreciable amount of 2-methylhopanoids (SUMMONS & others, 1999; BROCKS & others, 2005). More recent studies, however, have brought uncertainty to the interpretation of 2-methylhopanoids as a cyanobacterial biomarker; it seems that 2-methylhopanoids can also be produced by diverse alphaproteobacteria, including the anoxygenic purple nonsulfur phototroph *Rhodospseudomonas palustris* (RASHBY & others, 2007) and the nitrifying bacterium *Nitrobacter vulgaris* (ELLING & others, 2020). Thus, it is possible that the biochemical capability to synthesize 2-methylhopanoids may have a broader phylogenetic distribution and a deeper evolutionary history than cyanobacteria. More convincing biomarker evidence for cyanobacteria comes from fossil porphyrins, coupled with compound-specific nitrogen isotope data, from the ~1100 Ma El Mreïti Group in the Taoudeni Basin of Mauritania in northwestern Africa (GUENELI & others, 2018).

The Archean micropaleontological record is sparse and intensely debated. Various microfossils have been reported from the ~3400–3500 Ma Warrawoona Group and Strelley Pool Formation in Western Australia (SCHOPF, 2006a; SCHOPF, 2006b; SUGI-

TANIA & others, 2013), and some have been compared with and interpreted as cyanobacteria (AWRAMIK, SCHOPF, & WALTER, 1983; SCHOPF & PACKER, 1987; SCHOPF, 1993), although their biogenicity is a continual debate (BUICK, 1984; BRASIER & others, 2002; WACEY, EILOART, & SAUNDERS, 2019). More convincing Archean and early Paleoproterozoic filamentous microfossils have been known from ~3235 Ma volcanogenic massive sulfide deposit in Sulfur Spring Group (RASMUSSEN, 2000) and the 2450–2210 Ma Kazput Formation of the Turee Creek Group in Western Australia (SCHOPF & others, 2015; FADEL & others, 2017; BARLOW & KRANENDONK, 2018), but none of these have been interpreted as cyanobacterial filaments. Filamentous microfossils described as *Siphonophycus transvaalensis* BEUKES, KLEIN, & SCHOPF in KLEIN, BEUKES, & SCHOPF, 1987 from the ~2500 Ma Gamoha Formation and the ~2600 Ma Campbellrand Group of the Transvaal Supergroup in South Africa are among the oldest microfossils that have been interpreted as cyanobacteria (KLEIN, BEUKES, & SCHOPF, 1987; ALTERMANN & SCHOPF, 1995), but the simple morphology of *Siphonophycus* (see Fig. 9.9–9.12, Fig. 10, Fig. 12, Fig. 14.2–14.3) means that this interpretation is open to scrutiny. Indeed, among the genera listed in Table 1, only *Eoentophysalis* (Fig. 9.5), *Eohyella*, and *Polybessurus* (Fig. 9.6) are regarded as uncontested cyanobacteria (DEMOULIN & others, 2019), although several others are likely or probable cyanobacteria when additional paleoenvironmental and taphonomic conditions are considered together with morphological features (KNOLL, 2016). As such, *Eoentophysalis belcherensis* HOFMANN, 1976 from the 2015–2018 Ma Belcher Supergroup in Canada (HOFMANN, 1976; HODGSKISS & others, 2019) represents the oldest unequivocal cyanobacterial fossil and provides a minimum age constraint on cyanobacterial divergence (Fig. 16).

Stromatolites have been reported from a number of Archean successions. Putative stromatolites are known from the

Table 1. Selected microfossils that have been interpreted as cyanobacteria. With the exception of *Anhuithrix* PANG & others, 2018, most are a few to a few tens of micrometers in cell/trichome diameter/width. See DEMOULIN & others (2019) for a more complete list of occurrences.

Fossil genus	Proposed cyanobacterial features	Oldest occurrence	Proposed modern analogs	Cyanobacteria?
<i>Eosynechococcus</i> Hofmann, 1976 (Fig. 6.6)	Rod-shaped cells, no sheath, sometimes two cells attached end-to-end, indicating symmetrical transverse binary fission in a single plane	2015–2018 Ma Belcher Supergroup, Canada (Hofmann, 1976; Hodgskiss & others, 2019)	<i>Synechococcus</i> , Subsection I	Probable
<i>Gloeocapsomorpha</i> Zalessky, 1917	Nested planar cell aggregates surrounded by multilaminated sheaths	Middle Ordovician oil shale, Baltic Shale Basin, Estonia (Zalessky, 1917; Foster, Reed, & Wicander, 1989)	<i>Gloeocapsa</i> & <i>Entophysalis</i> , Subsection I	Possible
<i>Eoentophysalis</i> Hofmann, 1976	Layers or crusts consisting of solitary cells, paired cells, planar tetrads, or irregular clusters of cells embedded in multilaminated sheaths	2015–2018 Ma Belcher Supergroup, Canada (Golubic & Hofmann, 1976; Hodgskiss & others, 2019)	<i>Entophysalis</i> , Subsection I	Likely
<i>Palaeopleurocapsa</i> Knoll, Barghoorn, & Golubic, 1975.	Sheathed pseudofilamentous cell packets	~800 Ma Skilloogalee Dolomite, Adelaide Geosyncline, southern Australia (Knoll, Barghoorn, & Golubic, 1975).	<i>Pleurocapsa</i> , Subsection II	Probable
<i>Eohyella</i> Zhang & Golubic, 1987	euendolithic pseudofilamentous cyanobacterium	~1625 Ma Dahongyu Formation, North China (Zhang & Golubic, 1987)	<i>Hyella</i> , Subsection II	Likely
<i>Polybessurus</i> Green & others, 1987 (Fig. 1.6)	Spherical cell subtended by a cylindrical stalk consisting of stacked cup-like envelopes and may have reproduced by baeocytes	~1200 Ma Avzyan Formation, Ural Mountains, Russia (Sergeev, 1994); ~1050 Ma Uluksan Group (Kah & Knoll, 1996; Gibson & others, 2018); Tonian Eleanor Bay Supergroup in eastern Greenland (Green & others, 1987); Tonian Draken Formation in Svalbard (Knoll, Swett, & Mark, 1991)	<i>Cyanostylon</i> , Subsection II	Likely
<i>Palaeolyngbya</i> Schopf, 1968	Cellular trichome singularly enclosed in sheath	Tonian (~825 Ma) Bitter Springs Group, Australia (Schopf, 1968; Normington & others, 2019)	<i>Lynngbya</i> , Subsection III	Probable
<i>Oscillatoriopsis</i> Schopf, 1968	Unsheathed uniseriate trichome, cells wider than long, slightly differentiated apical cells	Tonian (~825 Ma) Bitter Springs Group, Australia (Schopf, 1968; Normington & others, 2019)	<i>Oscillatoria</i> , Subsection III	Probable
<i>Obruchevella</i> Reitlinger, 1948	Helical tubular filaments	~1560 Ma Gaoyuzhuang Formation, North China (Shi & others, 2017)	<i>Spirulina</i> , Subsection III	Possible

Table 1 continued on next page

Fossil genus	Proposed cyanobacterial features	Oldest occurrence	Proposed modern analogs	Cyanobacteria?
<i>Siphonophycus</i> Schopf, 1968 (Fig. 1.9–1.12, 2.4)	Tubular filament interpreted as cyanobacterial sheaths; form genus	~2600 Ma Campbellrand Group (Altermann & Schopf, 1995) and ~2500 Ma Gamohaana Formation (Klein, Beukes, & Schopf, 1987), both of Transvaal Supergroup, South Africa	Tubular sheath of Subsection III filaments	Probable
<i>Eoschizothrix</i>	Sheathed multi-trichomous filaments	~1560 Ma Gaoyuzhuang Formation, North China Craton (Lee & Golubic, 1998)	<i>Microcoleus</i> & <i>Schizothrix</i> Subsection III	Probable
<i>Archaeoellipsoides</i> Horodyski & Donaldson, 1980 (Fig. 1.4)	Large (~100 µm) elongate sausage-shaped vesicles interpreted as isolated akinetes, sometimes co-occurring with short trichomes interpreted as germlings (Sergeev, Knoll, & Grotzinger, 1995)	(?) ~2100–2040 Ma Francevillian Group (Amard & Beertrand-Sarfati, 1997); ~1560 Ma Gaoyuzhuang Formation, North China Craton (Shi & others, 2017); 1653–1647 Ma McArthur Group, Australia (Tomtani & others, 2006); 1400–1500 Ma Billyakh Group, Siberia (Golubic, Sergeev, & Knoll, 1995; Sergeev, Knoll, & Grotzinger, 1995; Gorokhov & others, 2019); ~1400 Ma Dismal Lake Group, Canada (Horodyski & Donaldson, 1980)	Akinetes of Member IV cyanobacteria	Likely
<i>Veteronostocale</i> Schopf & Blacic, 1971	Unsheathed uniseriate trichome with rounded cells, no apical attenuation	Tonian (~825 Ma) Bitter Springs Group, Australia (Schopf & Blacic, 1971; Normington & others, 2019)	<i>Nostoc</i> , Subsection IV according to Schopf & Blacic (1971)	Probable
<i>Anhuithrix</i> Pang & others, 2018	Unbranched, uniseriate trichomes with sheathed vegetative cells and akinetes	Tonian Liulaobei Formation, North China (Pang & others, 2018)	<i>Anabaena</i> & <i>Nostoc</i> , Subsection IV	Likely
<i>Langiella</i> Croft & George, 1959 & <i>Kidstoniella</i> Croft & George, 1959	Branching trichomes with sheathed cells as well as differentiated heterocysts and (in <i>Langiella</i>) akinetes	Early Devonian (~400–412 Ma) Rhynie Chert, Scotland (Croft & George, 1959)	<i>Stigonema</i> , Subsection V	Likely

~3470 Ma Dresser Formation in Western Australia (Fig. 17.1) (BUICK, DUNLOP, & GROVES, 1981). Conical stromatolites from the ~3430 Ma Strelley Pool Formation in Western Australia (Fig. 17.2) are regarded as biosedimentary structures (HOFMANN & others, 1999; ALLWOOD & others, 2006), possibly related to cyanobacterial activities (SCHOPF, 2012). More convincing evidence for cyanobacterial metabolism comes from disrupted stromatolitic laminae due to bubble formation related to oxygenic photosynthesis (BOSAK & others, 2009);

such evidence first appears in stromatolites from the ~2700 Ma Tumbiana Formation in Western Australia (Fig. 17.3). Consistent with this inference, limited evidence for Fe and S cycling in strata hosting the Tumbiana stromatolites indicates photoautotrophy using water rather than iron or sulfur as electron donors (BUICK, 1992; STUEKEN & others, 2017). Overall, microfossils and stromatolites indicate that cyanobacteria may have diverged between 2700 Ma and 2000 Ma. If one accepts that the origin of cyanobacteria must predate the Great Oxidation Event (BEKKER

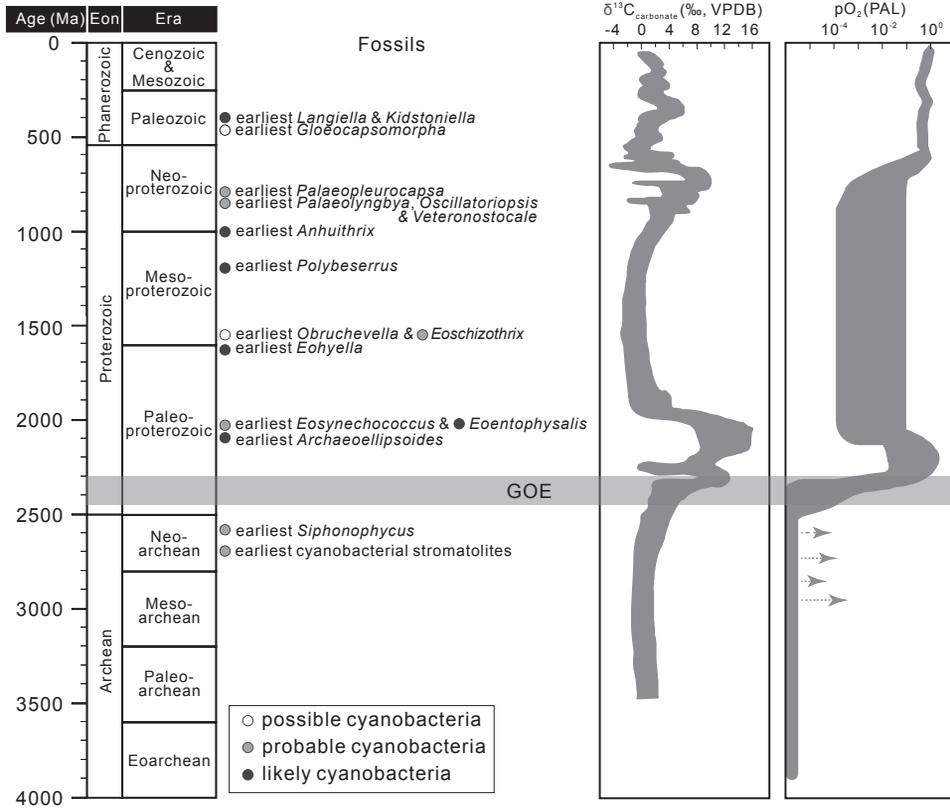


FIG. 16. Geological distribution of cyanobacterial microfossils. Hollow, gray, and solid circles represent the oldest known occurrence of possible, probable, and likely cyanobacterial microfossils. Purple bar represents the Great Oxidation Event (GOE) (adapted from Xiao & Tang, 2018 and Demoulin & others, 2019, and based on data in Table 1).

& others, 2004; HOLLAND, 2006; LUO & others, 2016), this window can be further narrowed to be 2700–2450 Ma (Fig. 16).

NON-CYANOBACTERIAL MICROBES

The identification of non-cyanobacterial microbes in the geological record is usually based only on geochemical data (e.g., carbon, iron, and sulfur isotopes) indicative of specific physiology or metabolism (e.g., STUEKEN & others, 2017; LEPOT, 2020). Thus, unlike cyanobacterial fossils, these inferred physiologies—because of their diverse phylogenetic distributions—do not define monophyletic groups. For example, iron oxidation (EMERSON, FLEMING, & MCBETH, 2010), dissimilatory iron reduction

(LOVLEY, 2013), dissimilatory sulfate/sulfur reduction (CANFIELD & RAISWELL, 1999), and methanotrophy (HANSON & HANSON, 1996; KNITTEL & others, 2005) occur in both bacteria and archaea. And methanogenesis occurs in multiple archaeal groups (LYU & LIU, 2018). Nonetheless, there are reports of body fossils of non-cyanobacterial prokaryotes, and their interpretations are sometimes based on characteristic morphological features and aided by geochemical data. These are briefly described below.

IRON-METABOLIZING MICROBES

Iron is involved in the metabolism of diverse bacteria and archaea, including dissimilatory Fe^{3+} reducing or Fe^{3+} respiring

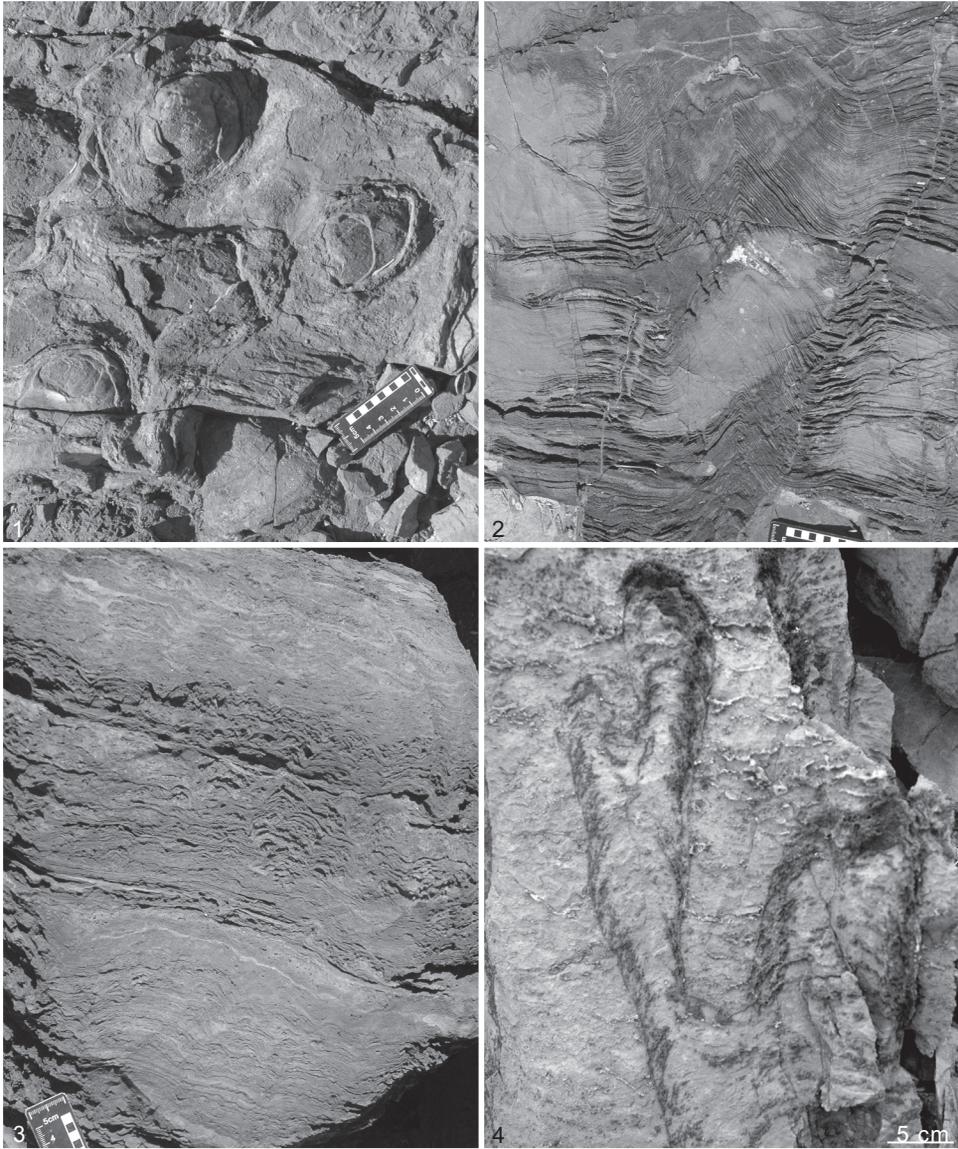


FIG. 17. Field photographs of representative Archean and Paleoproterozoic stromatolites. 1, possible coniform stromatolites (top view) from the ~3470 Ma Dresser Formation, North Pole, Western Australia (Buick, Dunlop, & Groves, 1981); 2, Conical stromatolite (vertical cross-sectional view) from the ~3430 Ma Strelley Pool Formation in Western Australia (Hofmann & others, 1999; Allwood & others, 2006); 3, microbial stromatolites (cross-sectional view) from the ~2700 Ma Tumbiana Formation of the Fortescue Group in Western Australia (AWRAMIK & BUCHHEIM, 2009); 4, branching stromatolites (cross-sectional view) from the ~2450–2210 Ma Kazput Formation of the Turee Creek Group in Western Australia (Martindale & others, 2015). All photos are new and by Shuhai Xiao. Color version available in *Treatise Online* 160.

bacteria (LOVLEY, 2013) such as *Geobacter* LOVLEY & others, 1993 and *Shewanella* MACDONELL & COLWELL, 1985, Fe²⁺ oxidizing bacteria (some of which are anoxygenic phototrophs) (BROCK & others, 1994), and magnetotactic bacteria (BAZYLINSKI & FRANKEL, 2003). There are a number of reports of iron-oxidizing microbial fossils. For example, *Frutexites*-like microstromatolites in Cenozoic basaltic seafloor are interpreted as structures produced by biofilms involving iron-oxidizing bacteria (HEIM & others, 2017; IVARSSON & others, 2020). Some filamentous microfossils from the Ediacaran Qigebulake Formation in China (ZHOU & others, 2015), the ~1880 Ma Gunflint Formation in Canada (BARGHOORN & TYLER, 1965; CLOUD, 1965), and the ~2450–2210 Ma Kazput Formation of the Turee Creek Group in northwestern Australia (FADEL & others, 2017) were compared with iron-oxidizing bacteria, but these microfossils do not seem to have diagnostic features uniquely characteristic of iron bacteria. Similarly, the Gunflint microfossil *Eoastrion* BARGHOORN in BARGHOORN & TYLER, 1965 (Fig. 9.3) has been compared with the extant Fe- and Mn-oxidizing bacterium *Metallogenium* PERFILEV & GABE, 1961 (CLOUD, 1965; ZAVARZIN, 1981), although the nature of *Metallogenium* remains enigmatic (KLAVENESS, 1999), and a recent study of *Eoastrion*-like structures from the ~2100 Ma FC Formation of the Francevillian in Gabon was unable to unequivocally confirm its biogenicity (LEKELE BAGHEKEMA & others, 2017). Additionally, tubular structures from the >3750 Ma Nuvvuagittuq supracrustal belt in Canada (Fig. 14.3) were tentatively compared with iron-oxidizing bacteria (DODD & others, 2017), but their biogenicity has been debated (MCMAHON, 2019). Some extant iron-oxidizing bacteria do produce morphologically distinct stalks (e.g., branching and twisted Fe-oxyhydroxide stalks in *Gallionella*) (CHAN & others, 2011) that can be preserved in the fossil record and thus offer promising diagnostic features

for this group of bacteria (JOHANNESSEN & others, 2020). Morphologically similar stalks have been reported from Jurassic hydrothermal deposits at ODP site 801 in the western Pacific Ocean (KREPSKI & others, 2013), Pennsylvanian coal beds in Ohio, USA (e.g., SCHOPF & others, 1965, fig. 12), the late Paleoproterozoic (~1700 Ma) Jhamarkotra Formation in India (CROSBY, BAILEY, & SHARMA, 2014), the late Paleoproterozoic Chuanlinggou Formation in the North China Craton (LIN & others, 2019), and late Paleoproterozoic (1.74 Ga) jasper in the lower Cleopatra Rhyolite in central Arizona, USA (LITTLE & others, 2021). These are intriguing and more convincing evidence for iron-oxidizing bacteria in the fossil record.

Both microaerophilic iron-oxidizing bacteria and anoxygenic photoferrotrophs have been implicated in the deposition of Precambrian banded iron formations (KAPPLER & others, 2005; KONHAUSER & others, 2002; CHI FRU & others, 2013; CHAN, EMERSON, & LUTHER, 2016). If so, then Archean and Paleoproterozoic banded iron formations can be regarded as indirect evidence for iron-oxidizing bacteria (HEIMANN, 2021, see p. 91–127). In fact, CHI FRU and others (2013) reported what appears to be anoxygenic photoferrotroph fossils from a Quaternary hydrothermal vent field on Milos Island, Greece.

Magnetotactic bacteria represent a special group of iron bacteria that can uptake complexed ferric iron and, through reduction and partial oxidation of Fe, precipitate intracellular magnetite (Fe₃O₄) or greigite (Fe₃S₄) nanocrystals in membranous magnetosomes (BAZYLINSKI & FRANKEL, 2003). Magnetite crystals produced by magnetotactic bacteria have distinct morphologies and crystallographic features that allow their identification in the fossil record (see Fig. 13) (BAZYLINSKI & FRANKEL, 2003; LI & others, 2020). Magnetofossils have been reported from Mesozoic and Cenozoic sediments (CHANG & KIRSCHVINK, 1989; KOPP

& KIRSCHVINK, 2008; ROBERTS & others, 2011) and even Precambrian stromatolites (CHANG & others, 1989).

SULFUR-METABOLIZING MICROBES

Sulfur cycling in the water column and sediments can be inferred from geochemical data. For example, sulfate reduction, sulfide oxidation, and sulfur disproportionation can be inferred from sulfur isotope data (CANFIELD & RAISWELL, 1999; SHEN & BUICK, 2004; JOHNSTON & others, 2005), and anoxygenic photosynthesizers such as green and purple sulfur bacteria can be inferred from biomarker data (BROCK & others, 2005). The body fossil record of sulfur-metabolizing microbes is scarce, primarily because they generally do not have diagnostic morphological features. Nonetheless, sulfur-metabolizing microbial fossils have been reported in the literature. For example, SCHOPF and others (2015) reported filamentous microbial communities from the Paleoproterozoic Turee Creek Group and Duck Creek Formation in Australia, and interpreted them as sulfureta in which sulfate/sulfur-reducing and sulfide-oxidizing microbes worked together to cycle sulfur species. This interpretation is based on inferred community ecology and the cobweb-like microbial fabrics that are often found in sulfureta. It is possible that these microbes also recycled iron species (FADEL & others, 2017). Additionally, BAILEY and others (2013) reported septate filamentous microfossils with sparse intracellular sulfur globules from the Ediacaran Doushantuo Formation and interpreted them as sulfide-oxidizing bacteria analogous to the extant *Beggiatoa*. Finally, BAILEY and others (2007) interpreted the animal embryo-like microfossil *Megasphaera* CHEN & LIU, 1986 from the Ediacaran Doushantuo Formation in the South China Craton as a giant sulfide-oxidizing bacterium analogous to the extant genus *Thiomargarita*, but this interpretation has been refuted (XIAO, ZHOU, & YUAN, 2007; CUNNINGHAM & others, 2012).

METHANOGENS AND METHANOTROPHS

Microbial activities of methanogens in the geological record are chiefly inferred from $\delta^{13}\text{C}$ data, because they produce a CH_4 pool extremely depleted in ^{13}C and correspondingly a CO_2 pool enriched in ^{13}C (LEPOT, 2020). This isotopic signal can be recorded as extremely high $\delta^{13}\text{C}_{\text{carb}}$ values of carbonate sourced from the CO_2 pool as long as CH_4 is effectively removed from the system (SUN & others, 2020) or as extremely negative $\delta^{13}\text{C}_{\text{carb}}$ values of carbonate related to anaerobic oxidation of methane (JIANG, KENNEDY, & CHRISTIE-BLICK, 2003; WANG & others, 2008), or as extremely negative $\delta^{13}\text{C}_{\text{org}}$ values of organic carbon produced by methanotrophs or methylotrophs in general (STUEKEN & others, 2017; XIAO & others, 2017). Thus, extremely negative $\delta^{13}\text{C}_{\text{org}}$ values (as low as -57%) from the ~ 2700 Ma Fortescue Group in Western Australia indicate that both methanogens and methanotrophs must have evolved by the Neoproterozoic. Body fossils of methanotrophs or methylotrophs, however, are extremely rare, although SUN and others (2020) recently reported micrometer-sized coccoidal methanogens from dolomite concretions in Permian lacustrine deposits of northwestern China. These coccoids are morphologically indistinct and their interpretation as fossil methanogens was largely based on the extremely positive $\delta^{13}\text{C}_{\text{carb}}$ values of the host dolomite concretions.

SUMMARY AND FUTURE PROSPECTS

Prokaryotes (bacteria and archaea) are ubiquitous, abundant, and physiologically diverse. They play essential roles in modern Earth systems and were likely as important in the geological past as they are today. Yet, their fossil record is rather sparse, and the prokaryote paleontology is a relatively young science. Since the 1950s, however, we have learned a great deal about prokaryotes in

the geological past and the field continues to grow rapidly. Prokaryotic microfossils are known in a number of taphonomic modes: silicification, phosphatization, calcification, pyritization, carbonaceous compression in fine-grained siliciclastic sediments and in amber, biomineral preservation, and trace fossil preservation. The study of prokaryotic microfossils faces many challenges. Given their microscopic sizes, simple morphologies, and possible confusion with biomorphs and eukaryotic microbes, it is a difficult task to demonstrate the syngenicity, biogenicity, and phylogenetic affinity of purported prokaryotic microfossils. Nonetheless, authentic prokaryotic microfossils are known in the geological record, and they extend as far back as 3200 Ma and perhaps 3500 Ma. Some of these microfossils can be assigned to phylogenetic or physiological groups, including cyanobacteria, iron-oxidizing bacteria, magnetotactic bacteria, sulfur-oxidizing bacteria, and methanogens. Of these, cyanobacteria have the richest record, one that goes back to 2000 Ma and perhaps 2700 Ma, and their identification is aided by ecological association with stromatolites and sometimes diagnostic morphological features.

Despite notable progress in the study of prokaryotic fossils since the 1950s, there

remain enormous opportunities for future research. Prokaryotic micropaleontology continues to be a frontier in scientific investigation. The vast majority of prokaryotic groups are poorly (or not at all) represented in the fossil record, including archaea and various nitrogen-metabolizing microbes, which are fundamental in the origin and function of the biosphere. The full spectrum of environmental distribution of prokaryotes is poorly documented in the geological record. This is particularly true for microbes in the terrestrial realm, cryptic spaces, deep-sea settings, deep lithosphere, and other extreme environments.

We know very little about how prokaryotes interacted with the environment and with other organisms in the geological record. It is likely that new advances will be made in the study of prokaryote micropaleontology at the interface with other sciences (e.g., geochemistry, sedimentology, microbiology, big data science) and advanced analytical techniques. Ultimately, the vast phylogenetic, physiological, and ecological diversity of bacteria and archaea evident today must surely have substantial geological and evolutionary roots, and much more awaits discovery.