Cyanobacterial macrophytes in an Early Silurian (Llandovery) continental biota: Passage Creek, lower Massanutten Sandstone, Virginia, USA

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LETHAIA

A compression macrofossil with structure consisting of mineral-replaced filaments embedded in an amorphous carbonaceous matrix is described as a macrophytic cyanobacterial colony from continental assemblages of the Early Silurian (Llandovery) Passage Creek biota, in the lower Massanutten Sandstone (Virginia, USA). Filaments are predominantly multiseriate and consist of spheroidal crystalline aggregates representing early pyrite (subsequently replaced by iron hydroxides) precipitated preferentially inside cells. Interpretation of the fossils as cyanobacteria is based on close similarities to modern organisms in terms of overall morphology and production of copious extracellular investments, filament and cell sizes, and continental epigeal (freshwater or terrestrial) habitat. This interpretation incorporates data on cyanobacterial taphonomy and mechanisms of diagenetic mineral precipitation. These fossils are part of the oldest macrofossil assemblages documenting well-developed and diverse communities on continents. They provide the earliest direct evidence for cyanobacteria in strictly continental habitats, corroborating the commonly held but poorly documented view that cyanobacteria were among the initial colonizers of continents. □ *Continental, cyanobacteria, fluvial, macrofossils, multi-trichomous, pyrite, Silurian.*

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The beginnings of life on emergent land masses marked a fundamental change in the biosphere (Knoll & Bambach 2000) that has long been the subject of speculation, and that remains shrouded in mystery today. Geochemical signatures (Watanabe *et al.* 2000) suggest that photosynthetic organisms were present on land as early as the beginning of the Proterozoic, 2,600 million years (Ma) ago, and cyanobacteria have been proposed as the primordial terrestrial photoautotrophs (Golubic & Campbell 1979; Watanabe *et al.* 2000; Dott 2003). Stromatolite-building cyanobacteria have been a major component of biotas in peritidal environments for at least 2,000 Ma (Golubic & Seong-Joo 1999), and sedimentary structures produced by potentially photosynthetic microbial mats are known from 3,200 Ma siliciclastic tidal deposits (Noffke *et al.* 2006). However, to date there is no compelling body fossil evidence for cyanobacteria in strictly continental settings prior to the origin of vascular plants.

Although cyanobacteria are the principal focus of paleobiological studies of early marine life on Earth (Schopf 1992; Golubic 1994; Knoll 2003), organically preserved cyanobacteria are much less commonly reported in Phanerozoic deposits (Golubic & Knoll 1993). Likewise, despite their present day ubiquity in all continental environments (Whitton & Potts 2000), cyanobacteria have a surprisingly scarce fossil record outside the marine realm. The oldest cyanobacteria previously documented in such a setting are Devonian, about 400 Ma old (Croft & George 1959; Edwards & Lyon 1983; Taylor *et al.* 1995, 1997). This gap and the general paucity of the continental fossil record of cyanobacteria can be attributed to the much more severe factors controlling the preservation of organic matter on continents, combined with a low preservation potential of microbial organisms. However, this situation is also at least partly due to a change in the focus of paleobiological studies toward larger, more complex and more appealing fossils produced by the animal and plant

kingdoms, both of which were profusely radiating, in Phanerozoic deposits.

The current paper describes the oldest macroscopic evidence for cyanobacteria on continents, consisting of a carbonaceous fossil with filamentous internal organization, preserved in fluvial deposits of the Early Silurian (Llandovery) lower Massanutten Sandstone in Virginia (USA). Several facets of this discovery are addressed. These include: (1) the mode of preservation of the fossils; (2) the nature of diagenetic minerals replacing the cells; and (3) the most probable taxonomic affinity of the organisms at the origin of these fossils. The systematic treatment of these fossils will be published elsewhere.

Materials and methods

Fossil assemblages consisting of carbonaceous compressions occur in the fluvial deposits of the lower

Massanutten Sandstone, on the eastern slope of Green Mountain, at Passage Creek, Shenandoah County, Virginia (38°56′N, 78°18′W; for location map see Pratt *et al*. 1978). Compressions are preserved in numerous siltstone and silty shale layers present at multiple levels throughout the rock unit. Fossiliferous layers represent discontinuous fine-grained partings between much thicker sandstone and fine conglomerate beds, and are interpreted as representing sedimentation in overbank settings of the river systems that deposited the lower Massanutten Sandstone (Tomescu & Rothwell 2006).

The fossils can exceed 10 cm in greatest dimension and are abundant on multiple bedding planes within each layer. The majority of compressions exhibit thalloid morphologies (e.g. Fig. 1A, at arrow) of different sizes, but more extensive crustose forms, as well as strap-shaped fossils, are also present (Tomescu & Rothwell 2006). Among the largest fossils, one strap-shaped carbonaceous compression (Fig. 1A, B) preserved in siltstone provided the material described in the present
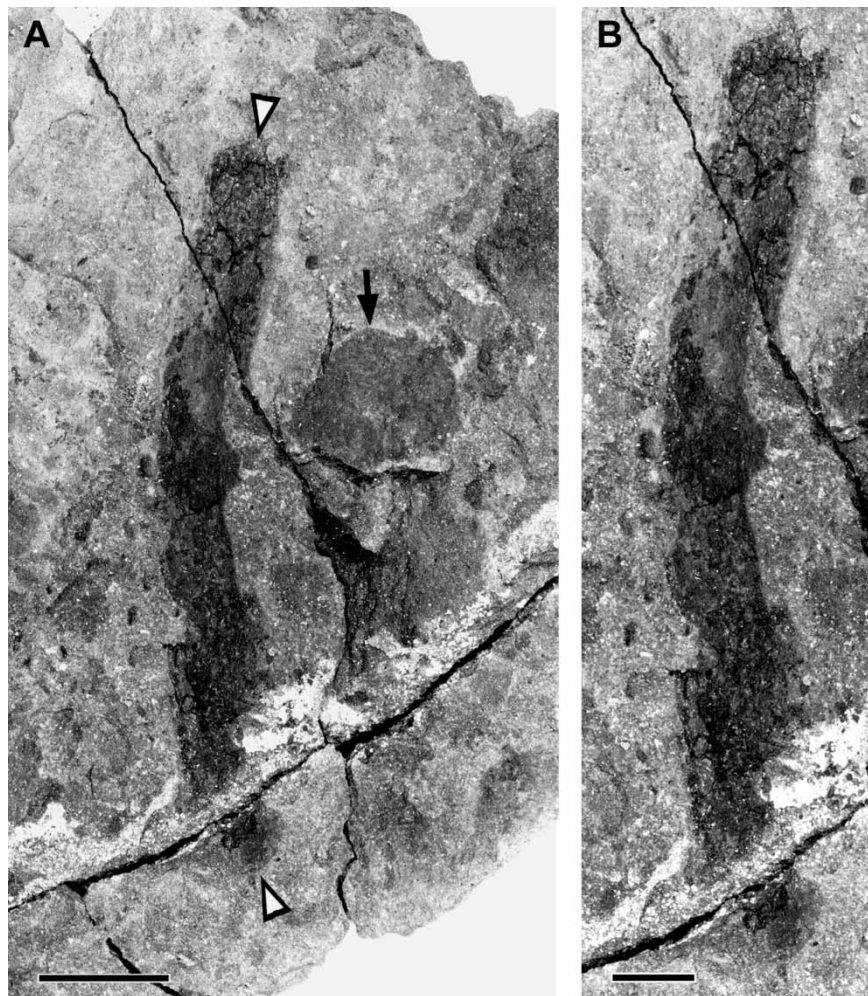

Fig. 1. Lower Massanutten Sandstone, Llandovery, Passage Creek, Virginia, USA. OUPH 15988. □A. General aspect of bedding plane with carbonaceous compression fossils: strap-shaped cyanobacterial colony (between arrowheads) and thalloid fossil (arrow); scale bar 10 mm. □B. Strap-shaped macroscopic aspect of macrophytic cyanobacterial colony; scale bar 5 mm.

paper. Filamentous organization of the type seen in this specimen has not been recorded in any of the other fossils examined to date in the Passage Creek assemblages, most of which are characterized by multilayered internal structures (Tomescu & Rothwell 2006). The compression documented here is 61 mm long, 7−9 mm wide and 250−300 μm thick. The internal structure of this compression has been documented in detail using light and electron microscopy of fragments removed from the fossil and subjected to different treatments.

For light microscopy, fragments were cleared in household-grade bleach (sodium hypochlorite) for 9 days, and rinsed in distilled water by progressive dilution of the sodium hypochlorite solution until complete removal in a 4-h interval. The material was then mounted on microscope slides after dehydration in graded ethanol and xylene series. For scanning electron microscopy (SEM) and energy-dispersive X-ray spectrometry (EDS) we used untreated fragments. Fossil fragments destined to transmission electron microscopy were cleaned of mineral matrix and inclusions in 30% hydrofluoric acid (1 h) and 40% hydrochloric acid (0.5 h). Fragments were then rinsed in distilled water, dehydrated in a graded ethanol series and embedded in Epon-type resin (Electron Microscopy Sciences, Fort Washington, PA) for thin and ultrathin sectioning, without fixation. Thin and ultra-thin sections were cut on Reichert Ultracut microtomes using glass and diamond knives. No staining was performed on the ultrathin sections. Imaging of specimens was realized with Leaf Lumina (Leaf Systems Inc., Southboro, MA) and PhotoPhase (Phase One A/S, Frederiksberg, Denmark) digital scanning cameras, using a macrolens mounted on a Leitz Aristophot bellows camera, or using the Aristophot in conjunction with a Zeiss WL compound microscope. SEM was performed on a Hitachi S4000 field emission microscope, and EDS spectra were measured on a FEI Quanta 400 FEG (field-emission gun) scanning electron microscope equipped with a Princeton Gamma-Tech thin-window energy-dispersive X-ray spectrometer. Ultra-thin sections were observed and imaged using a Hitachi H7000 transmission electron microscope. Specimens are deposited in the Ohio University Paleobotanical Herbarium as nos. 15988, 15996, 16001, and 16002.

Geological setting

As the effects of the Late Ordovician glacial pulse (Brenchley *et al*. 2003) were dissipating, vast alluvial plains developed 25−30°S of the paleo-Equator, along the coasts on the Early Silurian continent of Laurentia. Extending between the recently uplifted Taconic Mountains to the south and a shallow marine basin to the north, and covering areas located today in the Appalachians of eastern North America, these alluvial plains were supplied with sediment eroded from the mountains by extensive northwardly flowing river systems (Yeakel 1962; Smith 1970; Cotter 1983; Castle 1998, 2001). Such alluvial deposits formed the lower Massanutten Sandstone, as well as the laterally equivalent and much more extensive Tuscarora Formation. These units, comprising most of the Early Silurian clastic sedimentation on the coastal plains of Laurentia north of the Taconic Mountains (e.g. Castle 1998), represent environments that range from alluvial fan complexes and braided river alluvial plains proximal to the sedimentary source, through coastal plains, to beach, lagoon, estuary, and shelf facies distally (Smith 1970; Cotter 1983; Castle 1998). Within this clastic apron, the lower Massanutten Sandstone is the sector most proximal to the sedimentary source (Dennison & Wheeler, 1975; Pratt *et al*. 1978), and includes continental deposits of river braidplains (Tomescu & Rothwell 2006 and references therein). Marine fossils being absent, the age of the lower Massanutten Sandstone is constrained biostratigraphically by marine assemblages of the underlying and overlying strata, as well as by stratigraphic equivalence with the Tuscarora Formation (Rader & Biggs 1976; Pratt *et al*. 1978). These are consistent with an early- to mid-Llandovery age, ca. 440 Ma old (Pratt *et al*. 1978; Cotter 1983).

Results and discussion

Filamentous organization of the fossils and nature of spheroidal units forming the filaments

Oxidative clearing of the black carbonaceous material of the compression using sodium hypochlorite (bleach) rendered this material translucent and revealed a structure consisting of numerous long filaments embedded in an amorphous matrix. In cleared material the filaments are lighter in colour than the amorphous matrix when viewed under reflected light (Fig. 2A, C), and they are darker than the surrounding matrix in transmitted light (Fig. 2B, D). The filaments are usually straight or gently curved, and rarely more sinuous. They are unbranched and run more or less parallel to each other and to the long axis of the compression. Closer examination in light microscopy (Fig. 2F, G) and SEM (Fig. 3A−E), showed that the filaments consist of spheroidal units.

The amorphous matrix which includes the filaments represents the coaly material that forms the bulk of the compression, and is jet black when not cleared in bleach. Opaque in sections thicker than 0.9 μm, this material becomes translucent and appears colored in hues of brown when sectioned at thicknesses ranging between 0.5−0.7 μm. Sections cut perpendicular to the plane of
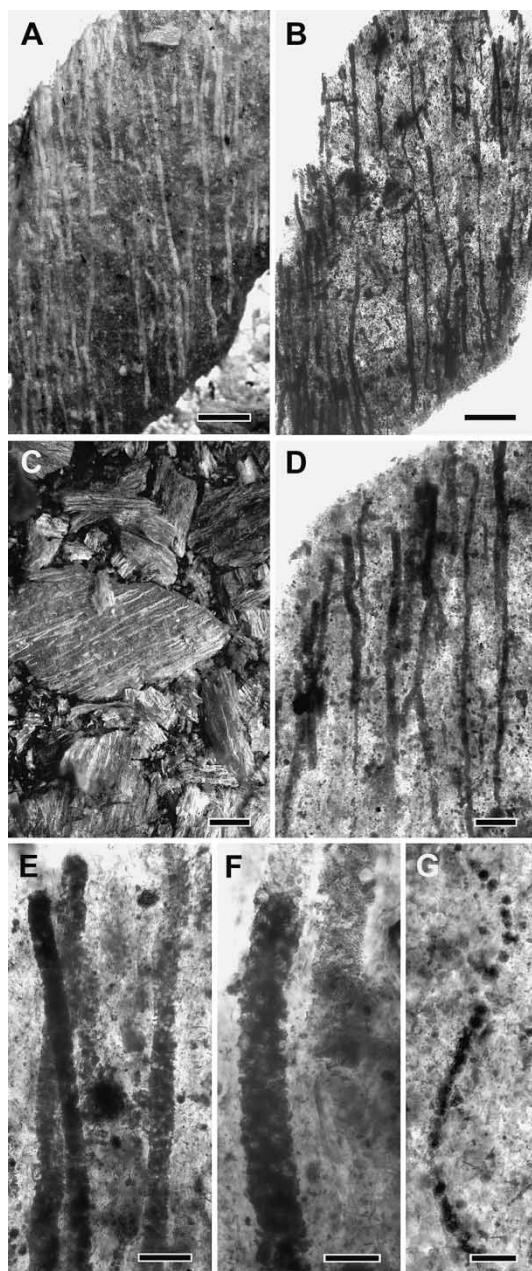
Fig. 2. Lower Massanutten Sandstone, Llandovery, Passage Creek, Virginia, USA. Light microscopy of fragments of the cyanobacterial colony cleared in sodium hypochlorite (A and C, reflected light; B, D and E–G, transmitted light). □A and B. Internal structure of the fossil consisting of numerous filaments embedded in amorphous carbonaceous matrix, seen with reflected (A) and transmitted (B) light in the same fragment. Filaments lighter than the matrix in reflected light and darker than the matrix in transmitted light; scale bar 250 μm. OUPH 16001. □C. Numerous fragments of different sizes during clearing; all display characteristic filamentous organization; scale bar 500 μm. □D. Detail of fragment with straight and undulating filaments of different diameters; scale bar 100 μm. OUPH 16001. □E and F. Multiseriate filaments in carbonaceous matrix; scale bar 50 μm in E and 25 μm in F. OUPH 16001. □G. Uniseriate filament in carbonaceous matrix; scale bar 25 μm. OUPH 16001.

the compression reveal a gross laminar structure of this coaly matrix (Fig. 3F) that is disrupted by tangential fissures and by the filaments running longitudinally,

parallel to the plane of stratification (material in the filaments has been dissolved by acid treatments in Fig. 3F, G).

The majority of filaments (94%) are multiseriate (Fig. 2E, F; 3A–D). They consist of 2–4 rows of closely spaced spheroids running more or less parallel to each other and forming cylindrical structures that may be >1 mm long and that have relatively constant diameters ranging 10.8–30.0 μm with a mode around 23 μm (Fig. 4A): mean $\pm$ SD (n) = 21.17 $\pm$ 4.44 μm (70). The spheroids in these filaments are 3.1–11.2 μm in diameter (Fig. 4A): 6.18 $\pm$ 1.35 μm (315). About 6% of observed filaments are thinner and uniseriate (Fig. 2G; 3A, B), can be >600 μm long and consist of contiguous or closely spaced spheroids 4.9–8.0 μm in diameter: 6.31 $\pm$ 0.85 μm (15).

Scanning- and transmission electron microscopy revealed that the amorphous carbonaceous matrix has a massive structure and that the spheroidal units in the filaments are microcrystalline aggregates of euhedral crystals (Fig. 3E, G). Crystals in the aggregates are around 1 μm in size and the spaces between crystalline aggregates in multiseriate filaments are filled with evenly distributed minute crystals ranging in size from <0.5–2 μm (Fig. 3C, D). More rarely, larger crystals the sizes of which fall within the same range as those of crystalline aggregates (4–12 μm) replace the latter in the filaments.

The spheroidal crystalline aggregates are highly reminiscent morphologically of pyrite (FeS_2) framboids or nodules (e.g. Love & Amstutz 1966; Canfield & Raiswell 1991; Schieber 2002a). However, energy-dispersive spectrometry (EDS) of the crystalline aggregates (Fig. 4B) indicates that they consist mainly of Fe with subordinate O, Si, Al, and C. If the aggregates were originally pyrite as their morphology seems to indicate, the latter must have been pseudomorphically replaced by iron oxides-hydroxides (e.g. goethite), a common process (A.H. Knoll, personal communication 2004; J. Schieber 2005: personal communication) that could explain the EDS spectra. The subordinate Si and Al probably represent clay minerals associated with the fossils, and the C reflects the organic matrix that surrounds the crystalline aggregates.

Biological significance of the filamentous structure and mode of fossil preservation

The biological origin of the filaments is clearly apparent from the relatively variable (in terms of filament geometry) and yet consistent (in terms of sizes of filaments and spheroidal units) morphology. The consistent arrangement and sizes of crystalline spheroids indicate that they replace biotic units – cells of filamentous organisms. As discussed below, the organisms are interpreted as representing cyanobacteria. The
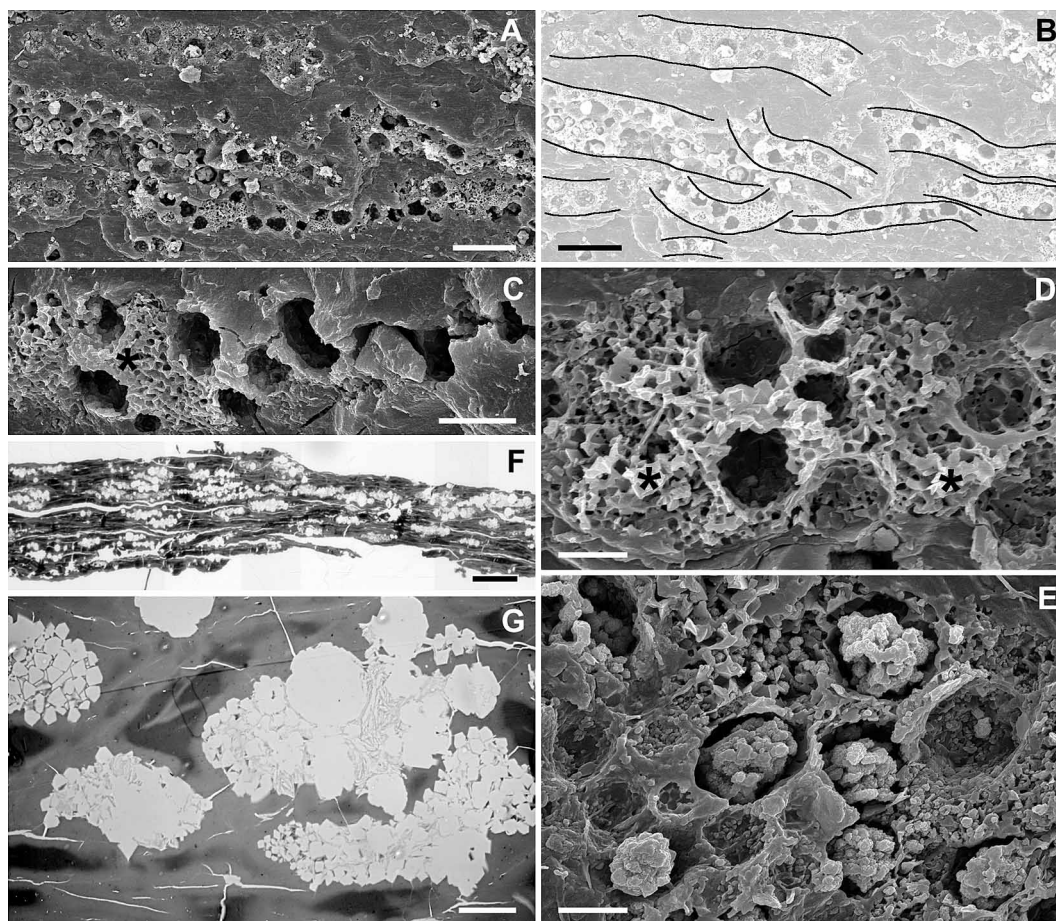
Fig. 3. Lower Massanutten Sandstone, Llandovery, Passage Creek, Virginia, USA. □A. SEM of several filaments embedded in amorphous carbonaceous matrix (most of the spheroids comprising the filaments have fallen from their sockets in the carbonaceous matrix); scale bar 30 µm. □B. Tracings of individual filament boundaries onto specimen in Fig. 3A. Note two uniseriate filaments at bottom; scale bar 30 µm. □C and D. SEM of multiseriate filaments with abundant evenly distributed crystals filling up the space between holes left by the crystalline aggregates (e.g. asterisks); scale bar 10 µm in C and 5 µm in D. □E. SEM showing morphology of spheroidal crystalline aggregates comprising the filaments; scale bar 5 µm. A through E OUPH 16002. □F. Transmitted light micrograph of transverse section of a fragment from the carbonaceous compression showing gross laminar organization of the amorphous matrix and voids left by minerals that make up the numerous filaments (removed with acids); scale bar 50 µm. OUPH 15996.G. TEM showing amorphous carbonaceous matrix (in dark and light grey) and voids left by crystals and crystalline aggregates removed with acids prior to sectioning. Note euhedral shapes of crystals in the aggregates; scale bar 5 µm.

amorphous coaly material incorporating the numerous filaments represents the common matrix of extracellular investments (slime), and the whole carbonaceous compression corresponds to a macrophytic colony or part of such a colony.

We propose that preservation of filaments within the common organic matrix entailed preferential precipitation of early diagenetic pyrite replacing the cell content or infilling spaces created by cell decomposition, and preservation of the common matrix. The euhedral shapes of crystals that form the crystalline spheroids (Fig. 3G) indicate that they grew within the fossil rather than being transported and deposited within biological material. Relationships between the crystals and the gross laminar structure of the fossil (Figs. 3F, G) reveal that crystal growth was mainly disruptive, suggesting that it occurred after the burial of the fossils, when it

could not be accommodated through expansion of the organic material, tightly embedded in the sedimentary matrix.

Pyrite is a common product of early diagenesis in sediments of varied environments (Love & Amstutz 1966; Berner 1984), including freshwater sediments such as the river floodplain deposits that formed the Early Silurian lower Massanutten Sandstone. Pyrite formation involves bacterial sulfate reduction and is enhanced by the anaerobic decomposition of organic material (Berner 1984; Canfield & Raiswell 1991; Wilkin & Barnes 1997). Replacement of cyanobacterial cells by diagenetic pyrite can be explained by a simple model for the conditions present in the floodplain deposits that preserve the Massanutten Sandstone fossils. Cyanobacterial colonies living or deposited on the floodplain would have been buried in sediments and would have
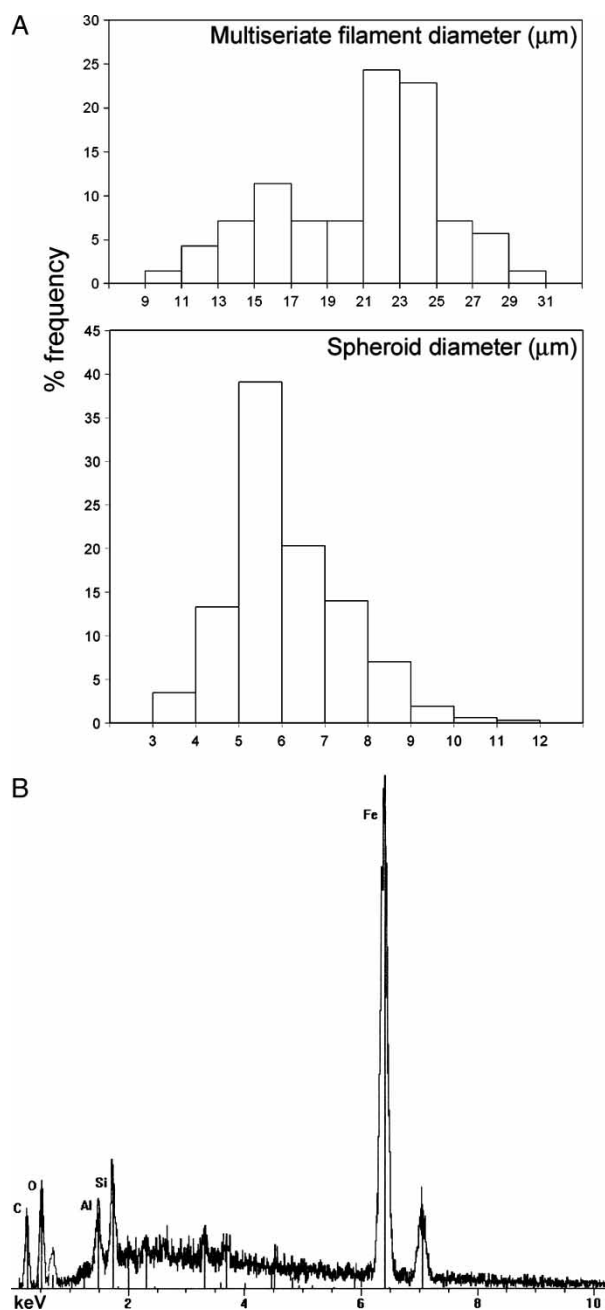
Fig. 4. Lower Massanutten Sandstone, Llandovery, Passage Creek, Virginia, USA. □A. Frequency distribution of multiseriate filament diameters (top) and spheroidal crystalline aggregate diameters (bottom). □B. Energy-dispersive X-ray spectra of crystalline aggregates comprising the filaments. The crystalline aggregates are characterized by high iron and subordinate O, Si, C and Al.

entered the redox zone below the sediment-water interface. Here pyrite precipitates as a result of bacterial sulfate reduction using sedimentary organic matter as a reducing agent, and of reaction of the H_2S thus produced with detrital iron minerals (Berner 1984). Polymers in the extracellular investment (amorphous carbonaceous matrix) of cyanobacterial colonies are highly resistant and durable (Helm *et al.* 2000; Kaz-

mierczak & Kremer 2002; Westall *et al.* 2003) when compared to the cyanobacterial cells. These cells, more susceptible to the activity of decomposers (e.g. bacteria), would have provided a more favorable 'culture medium' for the latter, including sulfate reducing bacteria (Schieber 2002b). Diagenetic pyrite would thus precipitate preferentially in voids created by decomposition of the cyanobacterial cells and in their immediate vicinity, creating pyrite replicas of the filaments in a process similar to the one described by Schieber & Baird (2001) for *Tasmanites* cyst fillings.

Previous work on lower Massanutten Sandstone fossil assemblages

The carbonaceous compressions of the Passage Creek biota were first mentioned by Pratt *et al.* (1978), who characterized the microfossils in the fine grained layers of the Massanutten Sandstone and attributed the fossil assemblages to the extinct and enigmatic nematophytes. However, affinities with this group are clearly excluded in the case of our fossils. Nematophytes are a group with unresolved affinities consisting exclusively of tubes of different types and sizes (Burgess & Edwards 1988; Strother 1993; Hueber 2001). The occurrence of dispersed tubular microfossils in palynological bulk macerates from the same source as the cyanobacterial fossils prompted Pratt *et al.* (1978) to propose nematophyte affinities for the carbonaceous compressions. Nematophyte tubes are highly resistant to degradation and are always preserved as discrete units, be they dispersed microfossils, or constituents of macrofossils. However, the amorphous matrix that includes the filaments described here shows no evidence of such primary tubular organization, irrespective of the method of observation, magnification, or chemical treatment used. Palynological acid maceration that commonly yields tubular dispersed microfossils and that was used by Pratt *et al.* (1978) to extract dispersed tubular microfossils from the Massanutten Sandstone, failed to produce tubes when applied to our fossils. These demonstrate that the filaments reflect the primary organization of the original organisms, rather than being a particular type of diagenetic infilling of nematophyte tubes.

Chemical analyses of carbonaceous material by Niklas & Pratt (1980) found constituents interpreted as potential degradative by-products of a lignin-like moiety. Later, Niklas & Smocovitis (1983) studied small (0.26–2.75 mm) compressions and described an interesting tissue fragment tentatively interpreted as conducting tissue, nevertheless emphasizing that systematic affinities of the fossils will remain conjectural until their anatomy and morphology are better characterized. Studying the Passage Creek assemblages, Tomescu &

Rothwell (2006) have demonstrated that a well-developed groundcover consisting of communities formed by a guild of thalloid organisms and/or associations of organisms comparable to extant biological soil crusts, was present in river floodplains by the Early Silurian. The predominantly thalloid morphologies of fossils suggest possible affinities with cyanobacteria, algae, fungi, lichens, or embryophytes.

Systematic affinities of the filamentous fossils

Sustained work on Precambrian biotas has led to the establishment of a comprehensive set of criteria for resolving the systematic affinities of microbial fossils (e.g. Golubic & Campbell 1979; Knoll & Golubic 1992). These are based on comparisons with living organisms in terms of morphology (including development), ecology and behavior, at the same time taking into account taphonomic effects. Applying this type of approach to the Massanutten Sandstone fossils described above, one has to look for extant organisms with multiseriate filamentous organization that produce copious amounts of extracellular investments and are characteristic of freshwater, fluvial environments.

Although developmental aspects have been masked by the leveling effects (in terms of relative cell sizes and positions) of diagenetic mineral replacement of cells, the multiseriate arrangement of the constitutive units of filaments suggests a multi-trichomous organization. The defining characteristic of such an arrangement is the presence of a sheath enclosing several filaments. However, as pointed out by Kazmierczak & Kremer (2002), the outer mucous envelopes that surround groups of cyanobacterial cells, subcolonies and colonies are transformed from more-or-less structured biological material into amorphous organic matter by diagenetic kerogenization. Although this is the case with our fossils, where diagenesis has blurred the differences between filament sheaths and the slime that incorporates the filaments, melting them together into the amorphous carbonaceous matrix of the fossil, the presence of sheaths is still indicated by the abundant, evenly distributed minute crystals that fill the spaces between 'cells' and form sharply defined domains around multiseriate filaments (Fig. 3C, D). Differentiation of such regions is best explained by the presence of physical boundaries (sheaths) originally separating two types of domains with dissimilar chemistries, one inside the sheath more favorable to mineral precipitation (in and around the cells) than the other (outside the sheath). Diagenetic homogenisation of the filament sheaths with the common amorphous slime matrix that incorporates the filaments is all the more plausible in view of the results of ultrastructural work by Bazzichelli *et al.* (1985). These authors have shown that cyanobacterial

slime and sheath behave as structural and morphological end-members of a reversible system composed of a fluid component and a reticular-fibrillar one.

Filamentous organization similar to the fossils described here is found in several prokaryotic and eukaryotic groups (Golubic & Seong-Joo 1999). Sizes of 'cells' fall within the size range characteristic of filamentous cyanobacterial prokaryotes (Schopf 1992, 2000). Moreover, multitrichomous filaments dimensionally comparable to the Massanutten Sandstone fossils are known only among the cyanobacteria (e.g. *Schizothrix*, *Microcoleus*) and in the morphologically similar chemotrophic sulfur-oxidizing bacterium *Thioploca* (Seong-Joo & Golubic 1998). Living multitrichomous cyanobacteria are known from the whole range of freshwater and terrestrial settings (Whitton & Potts 2000), and *Thioploca* also occurs in freshwater systems (Kojima *et al.* 2003). However, *Thioploca* does not form colonies with a common mass of extracellular slime: filaments of this genus occur interspersed within the sediment in various subvertical to subhorizontal positions (Schulz *et al.* 1996), and only free trichomes migrate outside the sheaths, above the sediment-water interface. This behavior is a result of the particular chemotrophic physiology of *Thioploca* whose trichomes shuttle along sheaths between nitrate-rich bottom water and sulfide-rich sediments (Huettel *et al.* 1996; Zopfi *et al.* 2001). Therefore only in cyanobacteria is the multitrichomous arrangement of filaments combined with production of an extracellular matrix that incorporates several to many filaments forming macrophytic colonies.

To summarize the information presented above, a combination of several types of evidence indicates the cyanobacterial origin of the Massanutten Sandstone fossils. Filaments occur in abundance within a macroscopic carbonaceous matrix which corresponds to the extracellular investment of the macrophytic colony. The constitutive units of filaments have a consistent size range that falls within that of cyanobacterial cells, and the shapes and sizes of filaments are consistent with those of living and fossil cyanobacteria. Additional evidence is provided by the mode of preservation represented by these fossils, which is consistent with the results of chemical, structural (Helm *et al.* 2000) and experimental taphonomic (Bartley 1996) studies, all of which have stressed the characteristic resistance to degradation of cyanobacterial extracellular polymeric substances (i.e. sheath and slime) in contrast to cell contents. Morphologically, the multiseriate filaments are comparable to multi-trichomous oscillatoriacean cyanobacteria in which several trichomes are bundled together inside a common sheath. The morphological variability is attributed to developmental stages of such organisms. Uniseriate filaments most likely represent

isolated trichomes of the taxon that forms the multi-trichomous filaments. Such free trichomes are the first stage in the development of multitrichomous organization in living cyanobacteria (Seong-Joo & Golubic 1998).

Palaeoecology

The fossils described here are preserved in fluvial deposits and therefore represent strictly continental organisms, adapted to life on land or in freshwater. Based on the occurrence of the Passage Creek fossils assemblages in overbank deposits, and on taphonomic observations, Tomescu & Rothwell (2006) were able to show that at least some of the organisms contributing to these assemblages lived in environments temporarily exposed to desiccation. The morphology characterized by macrophytic colonies consisting of important masses of extracellular slime enclosing the filaments is in accord with such a living environment (where the slime provides protection against desiccation; e.g. *Nostoc commune* or *N. flagelliforme*; Mollenhauer *et al.* 1999; Hill *et al.* 1994; Gao & Ye 2003), although copious production of extracellular investments is also known in some aquatic forms (e.g. *Nostoc pruniforme*; Dodds & Castenholz 1987). Be it on land or in the water, the macrophytic morphology characterizes organisms living on the surface of the sediment, rather than within the sediment. Moreover, the carbonaceous compression is oriented parallel to sediment stratification and filaments run parallel to the surface of the compression, with no evidence that their horizontal position could be merely the result of diagenetic compaction. The horizontal orientation of filaments is consistent with an epigeal life mode where it would maximize exposure to light and photosynthesis.

Palaeobiological context and significance of lower Massanutten Sandstone fossils

Fossils of the 440 Ma old Passage Creek biota hold a special place in the study of the early stages in the colonization of land, by providing the oldest macrofossil evidence for well-developed, diverse communities and for anatomically complex organisms on continents (Tomescu & Rothwell 2006). The cyanobacteria described here from the fluvial deposits of the lower Massanutten Sandstone are 40 Ma older than the oldest unequivocal cyanobacteria previously known from a continental setting (Rhynie Chert; Croft & George 1959; Edwards & Lyon 1983; Taylor *et al.* 1995, 1997) and represent the oldest cyanobacterial occurrence in a strictly continental setting. Pre-Silurian continental deposits have previously yielded dispersed microfossils in the form of spores, tubular fragments, and scraps of

tissue or cuticle-like material. Some of these are clearly attributable to embryophytes (Strother *et al.* 1996; Wellman *et al.* 2003). Others may represent algae (Strother & Beck 2000) or even cyanobacteria, but their affinities remain unresolved to date. The presence of organic matter and the chemistry of palaeosols in alluvial sequences also are interpreted as indications of primary producers on continents in the Precambrian (e.g. Watanabe *et al.* 2000; Kenny & Knauth 2001), but our ability to image these organisms directly has been very limited. Filamentous microorganisms 1,200 and 800 Ma old reported by Horodyski and Knauth (1994) were probably the only unequivocal Precambrian fossils previously known from continental settings. However their growth environments are uncertain (hypogeal *vs.* epigeal) and although they are compared to bacteria in general, their affinities have remained equivocal.

Conclusions

Filamentous structures in a macroscopic carbonaceous compression of the Passage Creek biota represent microorganisms replaced by early diagenetic minerals. The minerals form spheroidal crystalline aggregates that replace individual cells and represent iron oxide-hydroxide pseudomorphs after pyrite spheroids. The majority of filaments are multi-seriate and display well-defined zones of crystallization around each of them. These are interpreted as reflecting presence of an outer envelope (sheath), and therefore multi-trichomous organization. The whole carbonaceous compression represents a common matrix of extracellular investment (slime) incorporating the filaments. The sizes of cells and filaments, the shape and structure of filaments, and the macroscopic morphology of the fossil, together with its continental origin, indicate cyanobacterial affinities. The fossil represents a macrophytic cyanobacterial colony and the filaments are compared to multi-trichomous oscillatoriaceans, with the observed morphological diversity reflecting different developmental stages.

The Passage Creek cyanobacteria are remarkable because of the continental depositional environment and Llandovery age of the rocks that harbor them. Colonial organization within a common mass of extracellular slime indicates an epigeal living environment and corroborates taphonomic data on Passage Creek macrofossil assemblages that suggest exposure to desiccation of these Early Silurian floodplain communities. The Passage Creek biota comprises the oldest extensive assemblages of continental macrofossils known to date. These fossils record the transition from exclusively microbial continental communities of the Proterozoic,

to the essentially modern terrestrial ecosystems of the Late Paleozoic and, as such, hold a crucial place in understanding the early phases of the colonization of land. Documentation that cyanobacteria were one component of the Massanutten Sandstone assemblages marks a first step toward resolving the systematic affinities of this important biota. Forty million years older than the Rhynie Chert cyanobacteria, the Passage Creek fossils are the oldest cyanobacteria known from a strictly continental setting, providing the first direct evidence that cyanobacteria were important components of early continental ecosystems.

References

Bartley, J.K. 1996: Actualistic taphonomy of cyanobacteria: implications for the Precambrian fossil record. *Palaios 11*, 571–586.

Bazzichelli, G., Abdelahad, N. & Ventola, F. 1985: Structural modifications in the extracellular investment of *Nostoc commune* Vauch. During the life cycle. I. Motile and nonmotile hormogonium, biseriate stage. *Journal of Ultrastructure Research 91*, 174–181.

Berner, R.A. 1984: Sedimentary pyrite formation: an update. *Geochimica et Cosmochimica Acta 48*, 605–615.

Brenchley, P.J., Carden, G.A., Hints, L., Kaljo, D., Marshall, J.D., Martma, T., Meidla, T. & Nolvak, J. 2003: High-resolution stable isotope stratigraphy of Upper Ordovician sequences; constraints on the timing of bioevents and environmental changes associated with mass extinction and glaciation. *Geological Society of America Bulletin 115*, 89–104.

Burgess, N.D. & Edwards, D. 1988: A new Palaeozoic plant closely allied to *Prototaxites* Dawson. *Botanical Journal of the Linnean Society 97*, 189–203.

Canfield, D.E. & Raiswell, R. 1991: Pyrite formation and fossil preservation. *In* Allison, P.A. & Briggs, D.E.G. (eds): *Taphonomy: releasing the data locked in the fossil record*, 337–387. Plenum Press, New York, NY.

Castle, J.W. 1998: Regional sedimentology and stratal surfaces of a Lower Silurian clastic wedge in the Appalachian Foreland Basin. *Journal of Sedimentary Research 68*, 1201–1211.

Castle, J.W. 2001: Appalachian Basin stratigraphic response to convergent-margin structural evolution. *Basin Research 13*, 397–418.

Cotter, E. 1983. Shelf, paralic, and fluvial environments and eustatic sea-level fluctuations in the origin of the Tuscarora Formation (Lower Silurian) of central Pennsylvania. *Journal of Sedimentary Petrology 53*, 25–49.

Croft, W.N. & George, E.A. 1959: Blue-green algae from the Middle Devonian of Rhynie, Aberdeenshire. *Bulletin of the British Museum of Natural History (Geology) 3*, 341–353.

Dennison, J.M. & Wheeler, W.H. 1975: *Stratigraphy of Precambrian through Cretaceous strata of probable fluvial origin in southeastern United States and their potential as uranium host rocks*. 210 pp. Southeastern Geology Special Publication 5. Duke University, Durham, NC.

Dodds, W.K. & Castenholz, R.W. 1987: Effects of grazing and light on the growth of *Nostoc pruniforme* (Cyanobacteria). *British Phycology Journal 23*, 219–227.

Dott, R.H.J. 2003: The importance of eolian abrasion in supermature quartz sandstones and the paradox of weathering on vegetation-free landscapes. *Journal of Geology 111*, 387–405.

Edwards, D.S. & Lyon, A.G. 1983: Algae from the Rhynie chert. *Botanical Journal of the Linnean Society 86*, 37–55.

Gao, K. & Ye, C. 2003: Culture of the terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae), under aquatic conditions. *Journal of Phycology 39*, 617–623.

Golubic, S. 1994: The continuing importance of cyanobacteria. *In* Bengtson, S. (ed.): *Early Life on Earth. Nobel Symposium*, 334–340. Columbia University Press, New York, NY.

Golubic, S. & Campbell, S.E. 1979: Analogous microbial forms in recent subaerial habitats and in Precambrian cherts, *Gloeothece caerulea* Geitler and *Eosynechococcus moorei* Hofmann. *Precambrian Research 8*, 201–217.

Golubic, S. & Knoll, A.H. 1993: Prokaryotes. *In* Lipps, J.H. (ed.): *Fossil Prokaryotes and Protists*, 51–76. Blackwell Scientific Publications, Boston, MA.

Golubic, S. & Seong-Joo, L. 1999: Early cyanobacterial fossil record: preservation, palaeoenvironments and identification. *European Journal of Phycology 34*, 339–348.

Helm, R.F., Huang, Z., Edwards, D., Leeson, H., Peery, W. & Potts, M. 2000: Structural characterization of the released polysaccharide of desiccation-tolerant *Nostoc commune* DRH-1. *Journal of Bacteriology 182*, 974–982.

Hill, D.R., Peat, A. & Potts, M. 1994: Biochemistry and structure of the glycan secreted by desiccation-tolerant *Nostoc commune* (Cyanobacteria). *Protoplasma 182*, 126–148.

Horodyski, R.J. & Knauth, L.P. 1994: Life on land in the Precambrian. *Science 263*, 494–498.

Hueber, F.M. 2001: Rotted wood-alga-fungus: the history and life of *Prototaxites* Dawson 1859. *Review of Palaeobotany and Palynology 116*, 123–158.

Huettel, M., Forster, S., Kloser, S. & Fossing, H. 1996: Vertical migration in the sediment-dwelling sulfur bacteria *Thioploca* spp. in overcoming diffusion limitations. *Applied and Environmental Microbiology 62*, 1863–1872.

Kazmierczak, J. & Kremer, B. 2002: Thermal alteration of the Earth's oldest fossils. *Nature 420*, 477–478.

Kenny, R. & Knauth, L.P. 2001: Stable isotope variations in the Neoproterozoic Beck Spring dolomite and Mesoproterozoic Mescal limestone paleokarst: implications for life on land in the Precambrian. *Geological Society of America Bulletin 113*, 650–658.

Knoll, A.H. 2003: *Life on a Young Planet*. 277 pp. Princeton University Press, Princeton, NJ.

Knoll, A.H. & Bambach, R.K. 2000: Directionality in the history of life: diffusion fom the left wall or repeated scaling on the right? *In* Erwin, D.H. & Wing, S.L. (eds.): *Deep Time. Paleobiology's Perspective*, 1–14. The Paleontological Society.

Knoll, A.H. & Golubic, S. 1992: Proterozoic and living cyanobacteria. *In* Schidlowski, M., Golubic, S., Kimberley, M.M., McKirdy, D.M. & Trudinger, P.A. (eds.): *Early Organic Evolution: implications for mineral and energy resources*, 450–462. Springer-Verlag, Berlin.

Knoll, A.H., Swett, K. & Mark, J. 1991: Paleobiology of a Neoproterozoic tidal flat/lagoonal complex: the Draken Conglomerate Formation, Spitzbergen. *Journal of Paleontology 65*, 531–570.

Kojima, H., Teske, A. & Fukui, M. 2003: Morphological and phylogenetic characterizations of freshwater *Thioploca* species from Lake Biwa, Japan, and Lake Constance, Germany. *Applied and Environmental Microbiology 69*, 390–398.

Love, L.G. & Amstutz, G.C. 1966: Review of microscopic pyrite from the Devonian Chattanooga Shale and Rammelsberg Banderz. *Fortschritte der Mineralogie 43*, 273–309.

Mollenhauer, D., Bengtsson, R. & Lindström, E.-A. 1999: Macroscopic cyanobacteria of the genus *Nostoc*: a neglected and endangered constituent of European inland aquatic biodiverity. *European Journal of Phycology 34*, 349–360.

Niklas, K.J. & Pratt, L.M. 1980: Evidence for lignin-like constituents in Early Silurian (Llandoverian) plant fossils. *Science 209*, 396–397.

Niklas, K.J. & Smocovitis, V. 1983: Evidence for a conducting strand in early Silurian (Llandoverian) plants: implications for the evolution of the land plants. *Paleobiology 9*, 126–137.

Noffke, N., Eriksson, K.A., Hazen, R.M. & Simpson, E.L. 2006: A new window into Early Archean life: microbial mats in Earth's oldest siliciclastic tidal deposits (3.2 Ga Moodies Group, South Africa). *Geology 34*, 253–256.

Pratt, L.M., Phillips, T.L. & Dennison, J.M. 1978: Evidence of non-vascular land plants from the Early Silurian (Llandoverian) of Virginia, U.S.A. *Review of Palaeobotany and Palynology 25*, 121–149.

Rader, E.K. & Biggs, T.H. 1976: *Geology of the Strasburg and Toms Brook Quadrangles, Virginia. 91 pp. Virginia Division of Mineral Resources Report of Investigations 45*. Virginia Division of Mineral Resources, Charlottesville, VA.

Schieber, J. 2002a: Sedimentary pyrite: a window into the microbial past. *Geology 30*, 531–534.

Schieber, J. 2002b: The role of an organic slime matrix in the formation of pyritized burrow trails and pyrite concretions. *Palaios 17*, 104–109.

Schieber, J. & Baird, G. 2001: On the origin and significance of pyrite spheres in Devonian black shales of North America. *Journal of Sedimentary Research 71*, 155–166.

Schopf, J.W. 1992: Proterozoic prokaryotes: affinities, geologic distribution, and evolutionary trends. *In* Schopf, J.W. & Klein, C. (eds.): *The Proterozoic biosphere: a multidisciplinary study*, 195–218. Cambridge University Press, New York, NY.

Schopf, J.W. 2000: The fossil record: tracing the roots of the cyanobacterial lineage. *In* Whitton, B.A. & Potts, M. (eds.): *The Ecology of Cyanobacteria*, 13–35. Kluwer Academic Publishers, Dordrecht.

Schulz, H.N., Jorgensen, B.B., Fossing, H.A. & Ramsing, N.B. 1996: Community structure of filamentous sheath-building sulfur bacteria, *Thioploca* spp., off the coast of Chile. *Applied and Environmental Microbiology 62*, 1855–1862.

Seong-Joo, L. & Golubic, S. 1998: Multi-trichomous cyanobacterial microfossils from the Mesoproterozoic Gaoyuzhuang Formation, China: paleoecological and taxonomic implications. *Lethaia 31*, 169–184.

Smith, N.D. 1970: The braided stream depositional environment: comparison of the Platte River with some Silurian clastic rocks, north-central Appalachians. *Geological Society of America Bulletin 81*, 2993–3014.

Strother, P.K. 1993: Clarification of the genus *Nematothallus* Lang. *Journal of Paleontology 67*, 1090–1094.

Strother, P.K. & Beck, J.H. 2000: Spore-like micofossils from Middle Cambrian strata: expanding the meaning of the term cryptospore. *In* Harley, M.M., Morton, C.M. & Blackmore, S. (eds.): *Pollen and Spores: morphology and biology*, 413–424. Royal Botanical Gardens, Kew.

Strother, P.K., Al-Hajri, S. & Traverse, A. 1996: New evidence for land plants from the lower Middle Ordovician of Saudi Arabia. *Geology 24*, 55–58.

Taylor, T.N., Hass, H. & Kerp, H. 1997: A cyanolichen from the Lower Devonian Rhynie chert. *American Journal of Botany 87*, 992–1004.

Taylor, T.N., Hass, H., Remy, W. & Kerp, H. 1995: The oldest fossil lichen. *Nature 378*, 244.

Tomescu, A.M.F. & Rothwell, G.W. 2006: Wetlands before tracheophytes: thalloid terrestrial communities of the early Silurian Passage Creek biota (Virginia). *In* Greb, S.F. & DiMichele, W.A. (eds.): *Wetlands through Time*, 41–56. Geological Society of America Special Paper 399.

Watanabe, Y., Martini, J.E.J. & Ohmoto, H. 2000: Geochemical evidence for terrestrial ecosystems 2.6 billion years ago. *Nature 408*, 574–578.

Wellman, C.H., Osterloff, P.L. & Mohiuddin, U. 2003: Fragments of the earliest land plants. *Nature 425*, 282–285.

Westall, F., Walsh, M.M., Toporski, J. & Steele. A. 2003: Fossil biofilms and the search for life on Mars. *In* Krumbein, W.E., Paterson, D.M. & Zavarzin, G.A. (eds.): *Fossil and Recent Biofilms. A natural history of life on Earth*, 447–465. Kluwer Academic Publishers, Dordrecht.

Whitton, B.A. & Potts, M. (eds.) 2000: *The Ecology of Cyanobacteria*. 669 pp. Kluwer Academic Publishers, Dordrecht.

Wilkin R.T., & Barnes, H.L. 1997: Formation processes of framboidal pyrite. *Geochimica et Cosmochimica Acta 61*, 323–339.

Yeakel, L.S., Jr. 1962: Tuscarora, Juniata, and Bald Eagle paleocurrents and paleogeography in the Central Appalachians. *Geological Society of America Bulletin 73*, 1515–1540.

Zopfi, J., Kjaer, T., Nielsen, L.P. & Jørgensen, B.B. 2001: Ecology of *Thioploca* spp.: nitrate and sulfur storage in relation to chemical microgradients and influence of *Thioploca* spp. on the sedimentary nitrogen cycle. *Applied and Environmental Microbiology 67*, 5530–5537.