

A new genus and species of filamentous microfossil of cyanobacterial affinity from Early Silurian fluvial environments (lower Massanutten Sandstone, Virginia, USA)

ALEXANDRU M. F. TOMESCU^{1*}, GAR W. ROTHWELL² and ROSMARIE HONEGGER³

¹Department of Biological Sciences, Humboldt State University, Arcata, CA 95521-8299, USA

²Department of Environmental and Plant Biology, Ohio University, Athens, OH 45701-2979, USA

³Institut für Pflanzenbiologie, Universität Zürich, Zollikerstr. 107, CH 8008 Zürich, Switzerland

Received 18 March 2009; accepted for publication 1 May 2009

Fossils reported previously from the Early Silurian (Llandovery) lower Massanutten Sandstone (Virginia, USA) are formally described here as *Prattella massanuttense* gen. & sp. nov. Organization into cellular filaments embedded in extracellular matrix, the sizes of cells and filaments and the fluvial origin of deposits that host the fossils are all consistent with cyanobacterial affinity. *Prattella massanuttense* combines preservation as carbonaceous compression at a macroscopic scale with cellular preservation by mineral replacement of cell contents at a microscopic scale. These fossils provide the earliest direct evidence for the occurrence of cyanobacteria in fluvial habitats and add to the knowledge of terrestrial ecosystems that hosted early stages of land plant evolution. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, 160, 284–289.

ADDITIONAL KEYWORDS: continental – diagenesis – extracellular polysaccharides – fossil preservation – Llandovery – mineral replacement – pyrite.

INTRODUCTION

The purpose of this paper is to formally name and describe fossils of cyanobacterial affinity reported by us elsewhere (Tomescu, Rothwell & Honegger, 2006). The distinctive combination of morphology (cellular filaments embedded in significant amounts of extracellular slime), living environment (fluvial habitats) and mode of preservation (combining carbonaceous compression and cellular replacement by precipitated minerals) of these fossils sets them apart from other fossil cyanobacteria and warrants formal description as a new taxon.

MATERIAL AND METHODS

LOCALITY AND AGE

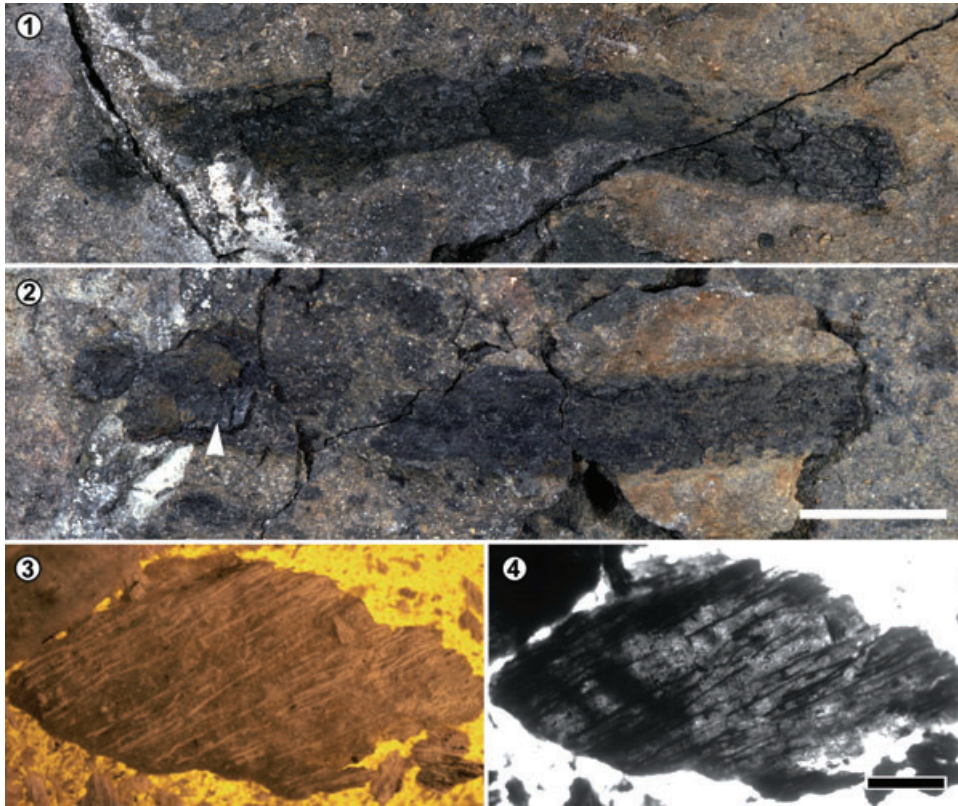
The specimens are preserved in siltstone layers of the lower Massanutten Sandstone at Passage Creek,

in northern Virginia (Shenandoah County, 38°56'N, 78°18'W). The general geological setting of the Massanutten Sandstone was discussed by Pratt, Phillips & Dennison (1978), who also provided a map of the locality. The lower Massanutten Sandstone represents deposits of a braided fluvial system of Early Silurian (early to mid Llandovery) age (Pratt *et al.*, 1978; Cotter, 1983). Within the fluvial sequence, the fossiliferous siltstone layers represent overbank deposits. Depositional environments and taphonomy of the fossils in the Passage Creek biota were addressed in detail by Tomescu & Rothwell (2006).

MATERIAL

Fossils are preserved at Passage Creek as macroscopic carbonaceous compressions which form rich assemblages in most of the 2- to 25-cm thick siltstone partings interspersed throughout the rock unit. Most of these compressions display a variety of thalloid morphologies, but others are strap-shaped and

*Corresponding author. E-mail: mihai@humboldt.edu



Figures 1–4. *Prattella massanuttense* gen. & sp. nov., Early Silurian (Llandovery), lower Massanutten Sandstone, Virginia, USA. Figs 1 and 2. Macroscopic aspect of cyanobacterial colony; part and counterpart; arrowhead in Figure 2 indicates area where the colony was sampled for microscopic investigations. Ohio University Paleobotanical Herbarium (OUPH) 15988. Scale bar, 10 mm. Figs 3 and 4. Structure of the colony consisting of numerous filaments embedded in an amorphous carbonaceous matrix, as seen in a specimen cleared in sodium hypochlorite. OUPH 16001. Scale bar, 0.5 mm. Fig. 3. Reflected light micrograph. Fig. 4. Transmitted light micrograph.

crustose films (Tomescu & Rothwell, 2006). One of these strap-shaped compressions is the fossil specimen discussed here (Figs 1, 2).

TECHNIQUES

The internal structure of the macroscopic carbonaceous compression has been characterized using light and electron microscopy of fragments removed from the fossil. For light microscopy, fragments of carbonaceous material sampled from the compression (Fig. 2) were oxidized (cleared) in household-grade sodium hypochlorite solution for 9 days; the treatment rendered the coaly material of the compression translucent and amenable to transmitted light microscopy. Other fragments were cleaned of mineral matrix and inclusions in 30% hydrofluoric acid (1 h) and 40% hydrochloric acid (0.5 h), embedded in Epon-type resin (Electron Microscopy Sciences, Fort Washington, PA, USA) and sectioned on Reichert Ultracut microtomes using glass knives. For scanning electron microscopy (SEM) we used untreated fragments of the carbon-

aceous compression. Imaging of specimens was realized with Leaf Lumina (Leaf Systems Inc., Southboro, MA, USA) and PhotoPhase (Phase One A/S, Frederiksberg, Denmark) digital scanning cameras, using a macro-lens 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.

REPOSITORY

Specimens described here are housed in the Ohio University Paleobotanical Herbarium (OUPH) as nos 15988, 15996, 16001 and 16002.

SYSTEMATICS

BACTERIA

CYANOBACTERIA

PRATTELLA TOMESCU, ROTHWELL & HONEGGER GEN. NOV.

Etymology: The generic name *Prattella* is proposed in honour of Dr Lisa M. Pratt, in recognition of her

contribution to our knowledge of early continental biotas.

Diagnosis: Filaments of single trichomes or composite, consisting of several unbranched, parallel or twisted trichomes, embedded in copious amounts of extracellular secretions forming an amorphous matrix.

Type species: *Prattella massanuttense* Tomescu, Rothwell & Honegger gen. & sp. nov.

Etymology: The specific epithet *massanuttense* refers to the Massanutten Sandstone (Virginia, USA) from which the fossils were collected.

Diagnosis: Filaments 10- to 30- μm thick, composed of 1–4 or more trichomes. Cell size and trichome diameter 3–11 μm . Filaments usually straight, sometimes slightly sinuous, generally parallel to each other and to the long axis of colony, widely spaced within a matrix of extracellular investment; filament length can exceed 0.5 mm. Colonies elongated, over 60 mm long and 8 mm wide, consisting of hundreds to thousands of filaments. Freshwater or terrestrial, epigeal. Preservation combining carbonaceous compression and mineral replacement of cells.

Holotype: Strap-shaped carbonaceous compression, illustrated in Figures 1 and 2 and reposit as the coalified compression OUPH 15988, plus various preparations from this fossil. These include cleared carbonaceous material from the compression mounted on microscope slides (OUPH 16001; Figs 3–7), microscope slides of thin sections through carbonaceous material of the colony (OUPH 15996; Fig. 8) and SEM stubs of carbonaceous fragments (OUPH 16002; Figs 9–11).

Locality: Outcrop on Green Mountain, left bank of Passage Creek, Shenandoah County, Virginia, USA, 38°56'N, 78°18'W.

Stratigraphy: Mid-section of lower Massanutten Sandstone; the lower Massanutten Sandstone is stratigraphically equivalent to the more extensive Tuscarora Formation in the Appalachian Basin.

Age: Early Silurian, Llandovery, c. 440 Ma.

DESCRIPTION

Prattella massanuttense is characterized by filaments of one to several trichomes (Figs 5–7) forming macroscopic colonies agglutinated by significant amounts of amorphous extracellular polysaccharides (slime)

(Figs 1–4, 8, 9). The shape of the colony is elongated, reaching 60 mm in length and 9 mm in width (Figs 1, 2). Trichomes consist of spherical crystalline aggregates of diagenetic minerals that replaced the cyanobacterial cells (Figs 9, 11). As shown by Tomescu *et al.* (2006), these crystalline aggregates consist of iron oxy-hydroxides that have replaced early diagenetic pyrite. They are interpreted as preserving the morphology of trichomes and reflecting approximately the sizes of cells that they have replaced (as discussed, e.g. by Kremer & Kazmierczak, 2005). Trichome diameters and cell sizes are 3.1–11.2 μm (mean \pm standard deviation: 6.19 \pm 1.33 μm). Diameters of filaments consisting of two or more trichomes are 10.8–30.0 μm (21.17 \pm 4.44 μm).

The presence of evenly distributed minute crystals around and between the cells, forming well-defined zones around many of the filaments (Fig. 10), was discussed by Tomescu *et al.* (2006) as potentially reflecting the presence of a common envelope (sheath) and, thus, a multitrichomous condition of *P. massanuttense* filaments. This mineral precipitation pattern could be as a result of the presence of two chemically distinct domains, that inside the sheath being more favourable to mineral nucleation than that outside the sheath. However, the ultrastructural features of the colony observed in transmission electron microscopy (data not shown) do not corroborate such an interpretation.

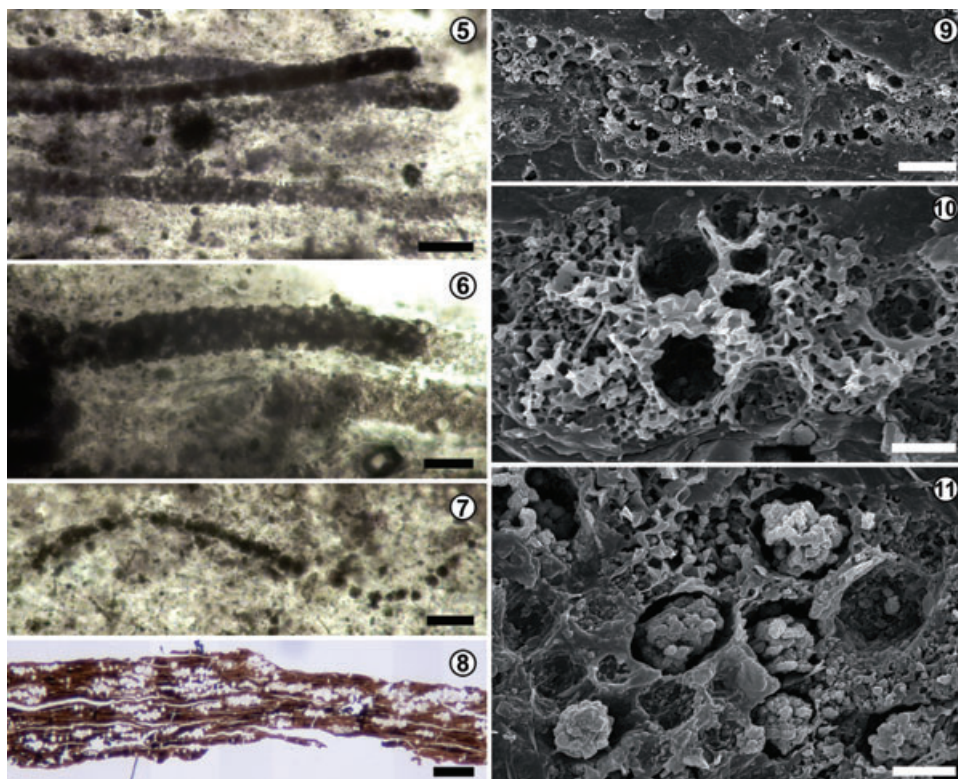
DISCUSSION AND CONCLUSIONS

TAXONOMY AND SYSTEMATICS

The systematic affinities of *Prattella* were discussed in detail by Tomescu *et al.* (2006), who showed that the cyanobacterial nature is supported by several types of evidence: (1) a filamentous structure consisting of trichomes and embedded in a common amorphous matrix of extracellular slime; (2) the sizes of the cellular units (spheroids) and sizes and shapes of filaments, consistent with those of extant cyanobacteria; (3) the type of fossil preservation combining cell replacement by minerals and carbonaceous preservation of the more resistant extracellular slime, consistent with cyanobacterial biochemistry and taphonomy; and (4) the fluvial living/depositional environment. All of these features are entirely consistent with those of extant cyanobacteria. The presence of filaments consisting of multiple trichomes and lack of true branching of the filaments together suggest that the affinities of *Prattella* most likely lie with oscillatoriacean cyanobacteria (e.g. *Microcoleus*).

COMPARISONS

Fossil cyanobacteria are overwhelmingly preserved either as carbonaceous remains (particularly in the



Figures 5–11. *Prattella massanuttese* gen. & sp. nov., Early Silurian (Llandovery), lower Massanutten Sandstone, Virginia, USA. Fig. 5. Multiseriate filaments in carbonaceous matrix (specimen cleared in sodium hypochlorite). Ohio University Paleobotanical Herbarium (OUPH) 16001. Scale bar, 50 μ m. Fig. 6. Multiseriate filament (specimen cleared in sodium hypochlorite). OUPH 16001. Scale bar, 25 μ m. Fig. 7. Uniseriate filament in a carbonaceous matrix (specimen cleared in sodium hypochlorite). OUPH 16001. Scale bar, 25 μ m. Fig. 8. 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 constitute the numerous filaments (removed with acids). OUPH 15996. Scale bar, 50 μ m. Fig. 9. Scanning electron microscopy (SEM) of several filaments embedded in amorphous carbonaceous matrix (most of the spheroids comprising the filaments have fallen from their moulds in the carbonaceous matrix). OUPH 16002. Scale bar, 30 μ m. Fig. 10. SEM of multiseriate filament with abundant evenly distributed crystals (e.g. arrow) filling the space between cells (or moulds of crystalline aggregates). OUPH 16002. Scale bar, 5 μ m. Fig. 11. SEM showing morphology of spheroidal crystalline aggregates comprising the filaments. OUPH 16002. Scale bar, 5 μ m.

Precambrian) or as mineral (micritic) rinds that coated the organisms (Golubic & Knoll, 1993). These two types of fossils correspond to the modes of preservation defined by Schopf (1975) as permineralization and, respectively, authigenic or duripartic preservation. In the particular case of filamentous cyanobacteria, permineralized specimens (e.g. *Eoschizothrix*; Seong-Joo & Golubic, 1998) preserve mainly the external cellular envelopes (sheaths), whereas cells and their contents are altered to various degrees or not preserved at all. As a consequence, diagnoses of taxa typified by such permineralized specimens are based on sheath characters and are idealized to different extents with respect to those characters that are not preserved. Such taxa are different from forms typified by authigenic/duripartic preservation fossils (e.g. *Girvanella*; Golubic & Knoll,

1993), in which sheaths are not organically preserved. Hence, taxonomic treatments of authigenic/duripartic fossils rely on different types of characters. Thus, the mode of preservation is an integral part of the diagnoses of fossil cyanobacterial taxa.

Prattella does not fall into either of those two categories of cyanobacterial fossil preservation. At a macroscopic scale, it is preserved as a carbonaceous compression (*sensu* Schopf, 1975) because of the important amounts of extracellular slime secreted by the cyanobacteria. At a microscopic scale, *Prattella* shows preservation by diagenetic mineral replacement of cell contents. Carbonaceous compressions are rare among cyanobacteria. In this respect, *Prattella* would be similar to the Neoproterozoic marine compression fossils *Chuarua* and *Tawuia* if the interpretation of these two taxa as cyanobac-

terial colonies (Sun, 1987) is confirmed. Cellular replacement of cyanobacterial cells by diagenetic minerals, including pyrite, is more widespread (e.g. Munneke, Servais & Vachard, 2001; Kremer & Kazmierczak, 2005), but has not been reported previously in conjunction with macroscopic preservation as carbonaceous compressions.

Prattella is distinctive among cyanobacteria by combining two modes of fossil preservation. It is also distinctive from the point of view of its ecology, being preserved in fluvial deposits. The combination of characteristic (1) morphology (macroscopic colonies consisting of cellular filaments embedded in a common matrix of extracellular slime), (2) mode of preservation (compression and mineral replacement of cell contents) and (3) environment (continental – freshwater/terrestrial; Tomescu & Rothwell, 2006) clearly distinguishes *P. massanuttense* from previously described cyanobacteria, warranting formal description of a new genus and species. *Prattella massanuttense* represents the oldest direct fossil evidence (c. 440 Ma) of cyanobacterial presence in strictly continental (i.e. fluvial) deposits.

CYANOBACTERIA ON CONTINENTS

Cyanobacteria are widely counted among the earliest photosynthetic residents of continental habitats and are believed to have played an important role in the colonization of land (Golubic & Campbell, 1979; Watanabe, Martini & Ohmoto, 2000; Dott, 2003). However, direct fossil evidence in support of those hypotheses has been frustratingly meagre (Golubic & Knoll, 1993). Until the recent discovery of the fossil material upon which the current work is based, the earliest unequivocal evidence for cyanobacteria on continents had been reported from Devonian sediments (Croft & George, 1959; Edwards & Lyon, 1983; Taylor *et al.*, 1995; Taylor, Hass & Kerp, 1997) that were deposited at least 15–20 Ma after the initial radiation of vascular plants (Bateman *et al.*, 1998; Edwards & Wellman, 2001; Tomescu & Rothwell, 2006). Older fossils of prokaryotic affinities were reported by Horodyski & Knauth (1994) from Proterozoic karst-infilling deposits interpreted as continental, but their cyanobacterial nature was not thoroughly assessed.

The history of the initial colonization of land can be regarded as an evolutionary parallel to the succession seen in the establishment of extant biological soil crusts. There, cyanobacteria and algae are the first to establish and are only later followed by lichens and bryophytes (Metting, 1981; St. Clair & Johansen, 1993); although rarely mentioned, heterotrophic prokaryotes and fungi likely play major roles in those communities. It has been proposed by numerous

authors that the initial terrestrial groundcover consisted of biological soil crust-like communities (Golubic & Campbell, 1979; Wright, 1985; Retallack, 1992), but direct fossil evidence has been lacking previously. Indirect evidence indicates that life was already present on land already in the Proterozoic, and possibly even as early as the Archean (Campbell, 1979; Gay & Grandstaff, 1980; Rye & Holland, 2000; Watanabe *et al.*, 2000); biogenic sedimentary structures reported from Proterozoic terrestrial settings (Prave, 2002) are consistent with microbial crust communities. The thalloid fossil assemblages of the lower Massanutten Sandstone (Tomescu & Rothwell, 2006) are now closing this gap, albeit at its shallower reaches, by providing direct evidence for the presence of soil crust-like communities on land prior to the advent of vascular plants. Identification of cyanobacteria among these early terrestrial communities corroborates the commonly held but poorly documented view that cyanobacteria were among the initial colonizers of the terrestrial realm.

ACKNOWLEDGEMENTS

Richard M. Bateman and an anonymous reviewer are thanked for helpful suggestions and outstandingly fast reviewing (if only reviewers everywhere were so diligent!). This work was supported by the National Science Foundation under Grants EAR-0308931 (GWR) and DEB-0308806 (GWR and AMFT). AMFT gratefully acknowledges support from the Geological Society of America, Sigma Xi, Ohio University Graduate Student Senate, the Systematics Association, the Paleontological Society and Botanical Society of America. RH gratefully acknowledges free access to the Electron Microscopy Laboratory of the Institute of Plant Biology, University of Zürich, financed by the Canton of Zürich, Switzerland.

REFERENCES

- Bateman RM, Crane PR, DiMichele WA, Kenrick PR, Rowe PR, Speck T, Stein WE. 1998. Early evolution of land plants: phylogeny, physiology, and ecology of the primary terrestrial radiation. *Annual Reviews of Ecology and Systematics* **29**: 263–292.
- Campbell SE. 1979. Soil stabilization by a prokaryotic desert crust: implications for a Precambrian land biota. *Origins of Life* **9**: 335–348.
- 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 WN, George EA. 1959. Blue-green algae from the Middle Devonian of Rhynie, Aberdeenshire. *Bulletin of the British Museum of Natural History (Geology)* **3**: 341–353.

- Dott RHJ. 2003.** The importance of eolian abrasion in super-mature quartz sandstones and the paradox of weathering on vegetation-free landscapes. *Journal of Geology* **111**: 387–405.
- Edwards D, Wellman C. 2001.** Embryophytes on land: the Ordovician to Lochkovian (Lower Devonian) record. In: Gensel PG, Edwards D, eds. *Plants invade the land. Evolutionary and environmental perspectives*. New York: Columbia University Press, 3–28.
- Edwards DS, Lyon AG. 1983.** Algae from the Rhynie chert. *Botanical Journal of the Linnean Society* **86**: 37–55.
- Gay AL, Grandstaff DE. 1980.** Chemistry and mineralogy of Precambrian paleosols at Elliot Lake, Ontario, Canada. *Precambrian Research* **12**: 349–373.
- Golubic S, Campbell SE. 1979.** Analogous microbial forms in recent subaerial habitats and in Precambrian cherts: *Gloeotheca coerulea* Geitler and *Eosynechococcus moorei* Hofmann. *Precambrian Research* **10**: 201–217.
- Golubic S, Knoll AH. 1993.** Prokaryotes. In: Lipps JH, ed. *Fossil prokaryotes and protists*. Boston: Blackwell, 51–76.
- Horodyski RJ, Knauth LP. 1994.** Life on land in the Precambrian. *Science* **263**: 494–498.
- Kremer B, Kazmierczak J. 2005.** Cyanobacterial mats from Silurian black radiolarian cherts: phototrophic life at the edge of darkness? *Journal of Sedimentary Research* **75**: 897–906.
- Metting B. 1981.** The systematics and ecology of soil algae. *Botanical Review* **47**: 195–312.
- Munnecke A, Servais T, Vachard D. 2001.** *Halysia* Hoeg, 1932 – a problematic Cyanophyceae: new evidence from the Silurian of Gotland (Sweden). *Neue Jahrbücher für Geologie und Paläontologie Monatshefte* **1**: 21–42.
- Pratt LM, Phillips TL, Dennison JM. 1978.** Evidence of non-vascular land plants from the Early Silurian (Llandoveryan) of Virginia, USA. *Review of Palaeobotany and Palynology* **25**: 121–149.
- Prave AR. 2002.** Life on land in the Proterozoic: evidence from the Torridonian rocks of north-west Scotland. *Geology* **30**: 811–814.
- Retallack GJ. 1992.** What to call early plant formations on land. *Palaios* **7**: 508–520.
- Rye R, Holland HD. 2000.** Life associated with a 276 Ga ephemeral pond?: evidence from Mount Roe 2 paleosol. *Geology* **28**: 483–486.
- Schopf JM. 1975.** Modes of fossil preservation. *Review of Palaeobotany and Palynology* **20**: 27–53.
- Seong-Joo L, Golubic S. 1998.** Multi-trichomous cyanobacterial microfossils from the Mesoproterozoic Gaoyuzhuang Formation, China: paleoecological and taxonomic implications. *Lethaia* **31**: 169–184.
- St. Clair LL, Johansen JR. 1993.** Introduction to the Symposium on soil crust communities. *Great Basin Naturalist* **53**: 1–4.
- Sun W. 1987.** Palaeontology and biostratigraphy of Late Precambrian macroscopic colonial algae: *Chuarua* Walcott and *Tauwua* Hofmann. *Palaeontographica Abt. B* **203**: 109–134.
- Taylor TN, Hass H, Kerp H. 1997.** A cyanolichen from the Lower Devonian Rhynie chert. *American Journal of Botany* **87**: 992–1004.
- Taylor TN, Hass H, Remy W, Kerp H. 1995.** The oldest fossil lichen. *Nature* **378**: 244.
- Tomescu AMF, Rothwell GW. 2006.** Wetlands before tracheophytes: thalloid terrestrial communities of the Early Silurian Passage Creek biota (Virginia). In: Greb SF, DiMichele, WA, eds., *Wetlands through time*. Geological Society of America Special Paper **399**: 41–56.
- Tomescu AMF, Rothwell GW, Honegger R. 2006.** Cyanobacterial macrophytes in an Early Silurian (Llandovery) continental biota: passage Creek, lower Massanutten Sandstone, Virginia, USA. *Lethaia* **39**: 329–338.
- Watanabe Y, Martini JEJ, Ohmoto H. 2000.** Geochemical evidence for terrestrial ecosystems 2.6 billion years ago. *Nature* **408**: 574–578.
- Wright PV. 1985.** The precursor environment for vascular plant colonization. *Philosophical Transactions of the Royal Society of London B* **309**: 143–145.