Cyanobacterial fossils from 252 Ma old microbialites and their environmental significance

Ya Sheng Wu[1], Gong Liang Yu[2], Ren Hui Li[2], Li Rong Song[2], Hong Xia Jiang[1], Robert Riding[4], Li Jing Liu[1], Dong Yan Liu[3] & Rui Zhao[1]

[1]Institute of Geology and Geophysics, Chinese Academy of Sciences, Key Laboratory of Petroleum Resource Research, CAS, Beijing 100029, People's Republic of China, [2]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430071, People's Republic of China, [3]Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, People's Republic of China, [4]Department of Earth and Planetary Sciences, University of Tennessee, Knoxville, TN 37996, USA.

The end-Permian mass extinction was followed by the formation of an enigmatic rock layer with a distinctive macroscopic spotted or dendroid fabric. This deposit has been interpreted as microbial reef rock, digitate dendrolite, digital thrombolite, dendritic thrombolite, or bacterial deposits. Agreement has been reached in considering them as microbialites, but not in their formation. This study has revealed that the spotted and dendroid microbialites were composed of numerous fossil casts formed by the planktic cyanobacterium, *Microcystis*, a coccoid genus that at the present-day commonly forms blooms in modern lakes, rivers, and reservoirs. The abundance of the fossils and the diagenesis they experienced has determined the macroscopic fabric: where they abundant, the rock appears as dendroid, otherwise, it appears as spotted. The ancient *Microcystis* bloom might produce toxin to kill other metazoans, and be responsible for the oceanic anoxia that has puzzled so many researchers for so many years.

The most severe crisis in Earth history was the sudden disappearance of 90% of marine and most land species[1], just before 252.3 Ma ago[2], probably due to extreme oceanic and climate changes[3,4] caused by massive release of thermogenic carbon dioxide and methane[2]. The main extinction episode[5] was followed by the formation of a distinctive rock layer with a macroscopic spotted[6,7] or dendroid[8] fabric. This deposit has been interpreted as microbial reef rock built by *Renalcis* or *Renalcis*-like microbes[9–11], digitate dendrolite composed of lobate objects[8,12], or digital thrombolite and dendrolite[13] built by *Renalcis*-group calcimicrobes[14,15]. Other researchers have described them as spotted and dendritic thrombolite consisting of mesoclots with spheroids[16–18], or as deposits rich in bacterial colonies and altered by diagenesis[19,20]. Here we show that all these microbialites were formed by the planktic cyanobacterium, *Microcystis*, a coccoid genus that at the present-day commonly forms blooms in modern lakes, rivers, and reservoirs. *Microcystis* fossilization together with diagenetic alteration has determined the distinctive macroscopic appearance of these microbialites. *Microcystis* blooms could have contributed to the marine dysoxia or anoxia following this extinction event. Freshwater algal bloom in Permian-Triassic transition probably caused by green alga *Spirogyra* has been reported[21]. Here is the first report of a massive marine algal bloom preserved in fossil record.

Results

The microbialite interval in southern China is generally 2 to 10 m thick and consists of two parts[7,17–20]. The lower part, dominantly gray in color with brown spots (Fig. 1, A), has been described as spotted microbialite. The upper part is brown in color, with vertically-elongate gray patches (Fig. 1, B), has commonly been described as dendroid microbialite. Microscopic examination of the thin sections showed that both the brown "spots" of the spotted rock and the brown patches of the dendroid rock consist of abundant fossils in a micritic (fine-grained carbonate) matrix. The fossils are casts, composed of minerals filling the spaces left by decay of the original organisms. These preserves only the shape of the precursor organisms, but not their soft tissues. The cast fossils are all composed of calcite crystals (occasionally dolomite) that is coarser than the micritic matrix, with crystals generally 0.01 ~ 0.6 mm wide. The micritic matrix between the fossils consist of tiny calcite crystals (<0.005 mm).
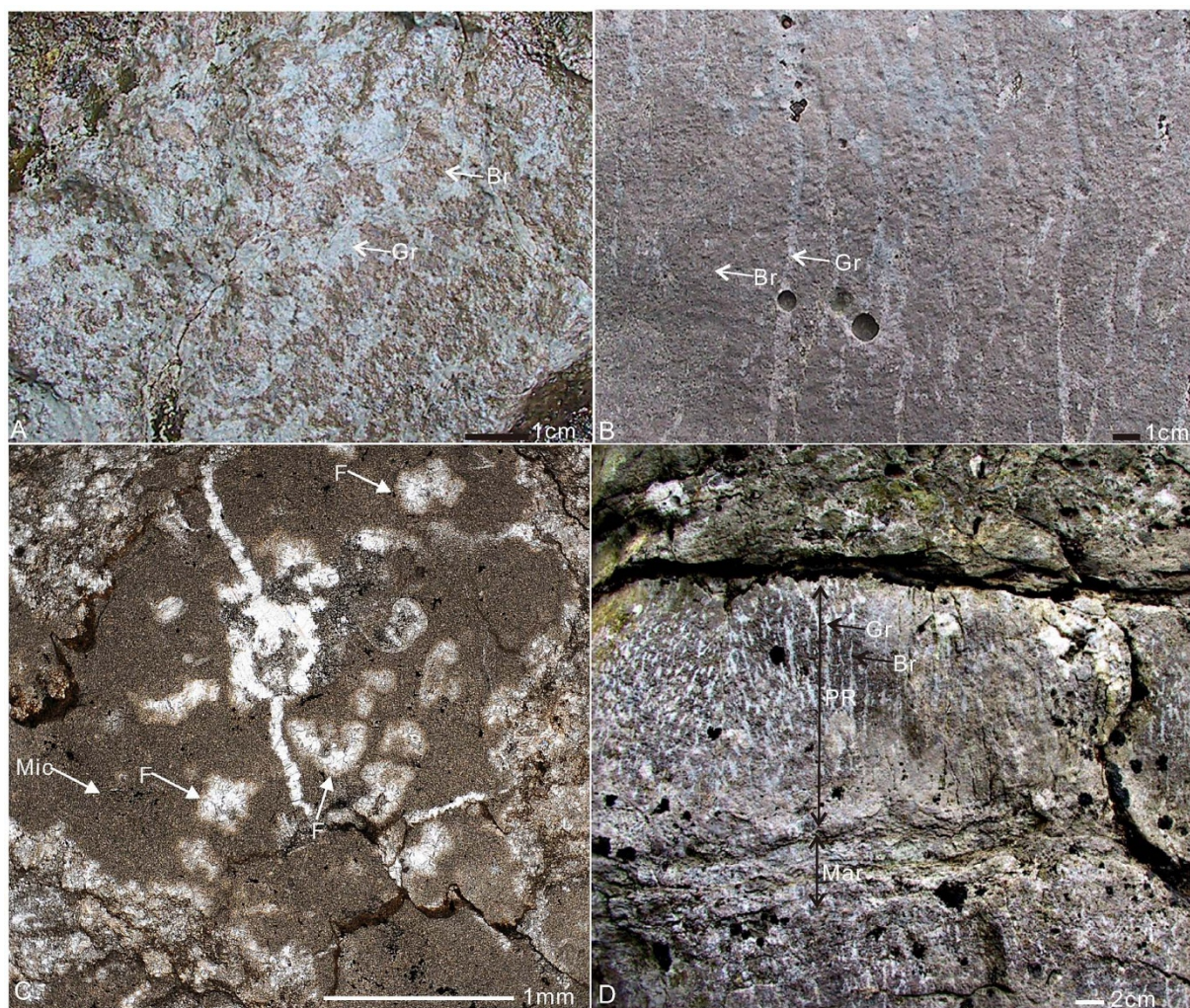
Figure 1 | Features of the spotted rock (A) and dendroid rock (B). (C): Fossils without orientation. (D): Thick dendroid rock bed and intercalated thin marl layer. Br-brown spot or patch; Gr-gray areas or patched; F-the problematic fossils, here interpreted as Microcystis colonies; Mic-rock of micrites; Mar-marl; PR-the dendroid rock.

The fossil casts are of different sizes, ranging up to more than 3.5 mm, and various shapes (Fig. 2). The smaller fossils (<0.5 mm) are generally subspherical to subelliptic. The medium sizes are 0.5 ~ 1.0 mm wide, in peanut-shape, reniform, trilobate, embryo-like and other irregular shapes. The large fossils (>1.0 mm) consist of partially connected small and medium fossils, in irregular or dendritic arrangements.

The internal composition and organization of the fossil casts includes at least three types: (1) needle-like calcites in which all the needles are perpendicular to the edges of the fossils and about 0.05 mm long and 0.01 mm wide; (2) needlelike calcite at the edge and large (e.g., 0.24 mm wide) blocky calcite in the interior (e.g., Fig. 2: D, F); (3) yellowish granular calcite (0.04 ~ 0.06 mm wide) or dolomite at the edge and colorless granular calcites in the interior (Fig. 2: H, K). We interpret the needle-like calcite as syndepositional cement that formed during organic matter decay.

The preservation of the shapes of the precursor organisms requires that the sediments were lithified prior to decay of the organisms. In present-day shallow marine environments, sediments can lithify within several months[22]. Thus, in order for their shapes to be preserved, the precursor organisms, or at least their external coverings, would needed to survive within the sediments for this period. Colonies of the cyanobacterium, *Microcystis*, can retain their shape in sediments for up to 15 years, due to their protective mucilaginous envelopes, which are difficult to decompose. Since there is no

evidence of a hard skeleton in the fossils, we infer that the precursor organisms might have possessed such a mucilaginous envelope.

The gray areas between the brown spots of the spotted rock and the brown patches of the dendroid rock are composed of micrite (i.e., calcites crystals < 0.005 mm; Fig. 1: C). Micrites is generally considered to have formed in calm or protected water[23]. The fossils in the spotted rock are randomly distributed, showing no preferred orientation (Fig. 1: C). If the organisms adhered to the sea floor during life, the fossils should remain in their upward growth orientation. However, this is not the case. Thus, we infer that the organisms were planktic.

The precursor organisms of the fossils are very similar in shape to the extant cyanobacterium *Microcystis*. As plankton, mainly in freshwater, *Microcystis* generally occur as greenish colonies (Fig. 3) consisting of masses of cells in a transparent shared mucilaginous envelope[24–27]. The colonies range 0.1 to more than 3.3 mm in size, and small ones are generally subspherical or subelliptic in form. The medium-sized colonies consist of two to several small colonies, in peanut-shape, reniform, trilobate, embryo-like, treelike, and other irregular shapes. The large colonies consist of numerous small and medium sized colonies, and can be treelike, netlike, or irregular in shape.

Three other cyanobacteria, *Woronichinia*, *Gloeocapsa*, and *Aphanocapsa* and one chlorophyte, *Botryococcus*, have some of the colonial shapes of modern *Microcystis*. However, they also show
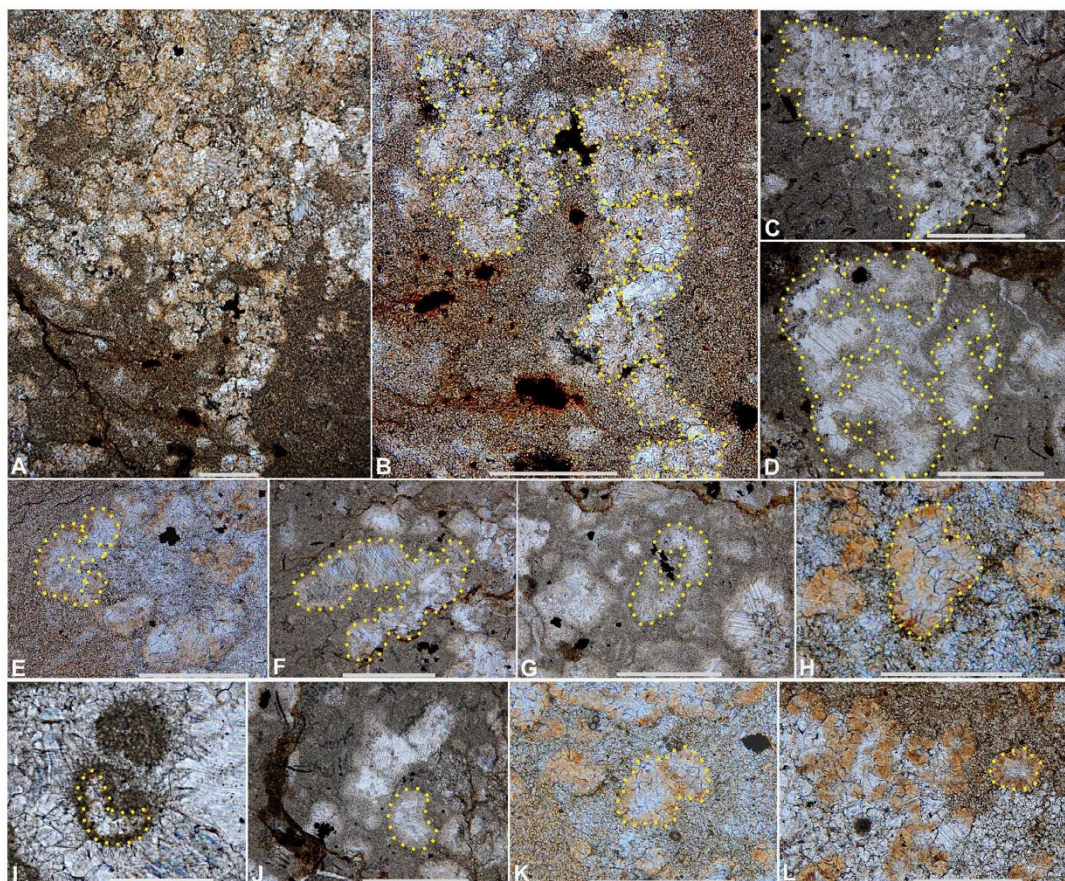
Figure 2 | Shapes and sizes of the fossils in the spotted and dendroid rocks. (A, B): large treelike; (C): medium treelike; (D): medium irregular; (E): medium embryo-like; (F): medium treelike; (G): medium circular; (H): medium trilobate; (I, J): medium kidney-like; (K): medium 8-like; (L): small subellipitical. The boundaries of some fossils are marked by yellow dotted lines. All bars 0.5 mm long.

differences. *Woronichinia* colonies are generally subspherical or embryo-like, but are small (<0.1 mm), and do not exhibit other shapes. The colonies of *Gloeocapsa* are generally subspherical or multiple-spherical, but also are small (<0.17 mm) and not varied in shape. The colonies of *Aphanocapsa* are generally subspherical, and <0.06 mm in size, even though their sheet-like colonies can reach 1.5 cm. The colonies of *Botryococcus* are generally multiple-subspherical, and do not show other forms, and are all small.

Both the precursor organisms of the fossils and modern *Microcystis* have mucilaginous envelopes, and are inferred to be able to stay alive in sediments for more than 14 years[28], sufficiently long for lithification of the sediments to occur. Modern *Microcystis*[29] is planktic, and so were the precursor organisms as inferred.

The precursor organisms are very similar in appearance to colonies of modern *Microcystis*, suggesting that they might be *Microcystis*, and that the cast fossils originated from them. However, it is also possible that some of the fossils also originated from ancient *Woronichinia*, *Gloeocapsa*, *Aphanocapsa*, and *Botryococcus*.

The formation of these fossils from *Microcystis* colonies requires four steps: (1) burial of *Microcystis* colonies by micritic sediments, (2) lithification that retained the shapes of the original colonies, (3) decay of the *Microcystis* colonies, leaving cavities, (4) growth of needle-like cements on the inner wall of the cavities with their basal ends perpendicular to the cavity walls, (5) further diagenesis. Lithification of the micritic sediments before the decay of the mucilaginous envelope was responsible for the preservation of the shape of the cyanobacterium. The decay left cavities, which were filled in by early needle-like aragonite and later blocky calcite cements. The spotted and dendroid structures have the same carbon and oxygen isotope

composition as the micritic matrix[6], which implies that the diagenesis has homogenized them.

Recognition of these *Microcystis* fossils clarifies several problems that have puzzled researchers for a long time. The first is the origin of these microbialites. Some researchers[14,15] thought the microbialites (or microbial framestones) were built by *Renalcis*-like microbes. Others[17,18] considered that they were constructed by unidentified spherical or globular objects, or by irregular bacterial colonies[19]. Our study identifies these Renalcis-like spheroidal objects and irregular colonial fossils as casts of *Microcystis* colonies. The appearance of the microbialites depends on the abundance of the cast fossils. Where the fossils are sparse, the microbialites appear look spotted. If they are very abundant, ∼>70% of the rock volume, then the microbialites have a dendroid or reticulate macrofabric.

Optimal temperatures for *Microcystis* growth are 15 ∼ 29°C[29]. Growth ceases below 14°C. *Microcystis* is tolerant of high temperatures, unless they exceed 45°C. These microbialites formed in low latitudes[15], with seawater surface temperatures in the range 27 ∼ 35°C[4], which partially overlaps with the optimal temperature of present-day *Microcystis*.

Fossil casts account for 50 ∼ 75% by volume of the dendroid microbialites in our samples, and for 30 ∼ 50% of the spotted microbialites. The dendroid microbialites always overlie the spotted microbialites, suggesting progression from low abundance to blooming.

Blooms of modern *Microcystis* are harmful to other organisms for two reasons. Firstly, some species of modern *Microcystis* produce toxins lethal to most metazoans including humans[30]. Secondly, *Microcystis* blooms can create anoxia[29] which will kill most other organisms. This effect is consistent with the scarcity of other

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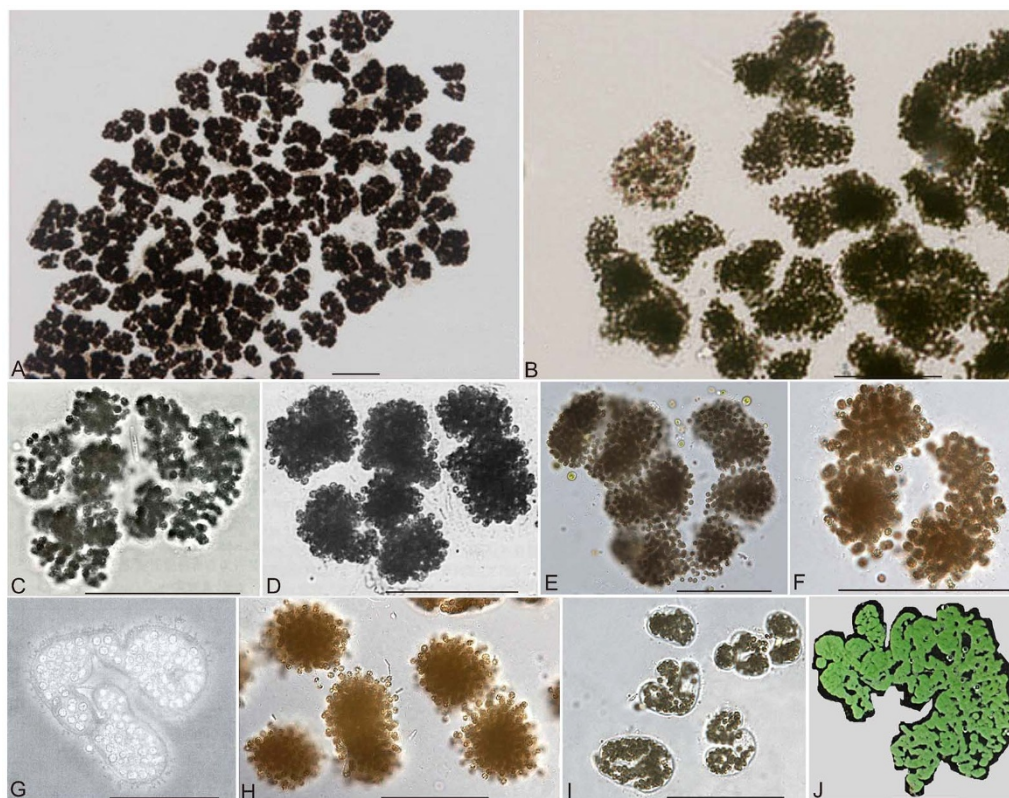
Figure 3 | Shapes and sizes of colonies of present-day *Microcystis*. (A, B): large tree-like; (C, D): medium irregular; (E): medium treelike; (F): medium circular; (G), medium embryo-like; (H): small subspherical, medium 8-shaped; (I): medium trilobate, embryo-like; (J): large irregular. Bars 0.1 mm in (A–I), and 1 mm in (J). (A) and (B) are cited from Fig. 7.2 of reference[29]; (C, D, E, F, H) cited from reference[26]; (I) cited from reference[27]; (G) from reference[25]; (J): taken in this study.

organisms associated with the microbialites. Our study shows 417 species of marine invertebrates in the crinoid limestone underlying the microbialites compared with 25 species in the microbialites, including ostracods, small gastropods, small worms, and tiny foraminifers.

Conflicting estimates have been made of the oxygen levels during formation of the microbialites in the Laolongdong section[15]. Some researchers found that the ostracods occurring in the microbialites were those tolerant of low oxygen[31]. We found pyrite grains (0.5 ~ 2 mm wide) in the microbialites, but not in the immediately underlying strata. We infer that the pyrite was transformed from framboid, which are considered to form in dysoxic water. The mean size of the framboid in the microbialites and the overlying strata is about 8.4 microns, and is considered to reflect dysoxic conditions[32], and upwelling of low-oxygen water has been proposed as the cause of the oceanic anoxia[15]. Since modern *Microcystis* bloom can cause dysoxia or anoxia of the water column[29], we suggest that dysoxia or anoxia of the sea water in which the microbialites formed was caused by *Microcystis* blooms.

Methods
Five outcropped profiles of the spotted and dendroid microbialites in southern Chin, three at Laolongdong, about 30 km north of Chongqing, one in Xiushui, Jiangxi Province, and the last in Xuanhan, Sichuan Province, were examined inch by inch. Details of the spotted and dendroid fabric were observed and pictured. More than 150 oriented samples were collected, slabbed and thin-sectioned (0.035 mm) using standard techniques. All thin sections were observed under 2×, 5×, and 10× objective lens of Olympus BX41P Microscope. All fossils were pictured, and compared with similar modern colonial cyanobacteria. Their similarities were evaluated carefully.

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Acknowledgments

Author contributions

Y.S.W. wrote the manuscript. G.L.Y. and R.H.L. helped in identifying the fossils. L.R.S. provided modern cyanobacterial samples. D.Y.L. gave helpful advice. R.R. revised the manuscript. H.X.J., L.J.L. and R.Z. joined the field work.

Additional information

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