

# Phosphate biomineralization in mid-Neoproterozoic protists

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## ABSTRACT

**The origin and expansion of biomineralization in eukaryotes played a critical role in Earth history, linking biological and geochemical processes. However, the onset of this phenomenon is poorly constrained due to a limited early fossil record of biomineralization. Although macroscopic evidence for biomineralization is not known until the late Ediacaran, we here report biologically controlled phosphatic biomineralization of scale microfossils from mid-Neoproterozoic (pre-Sturtian) strata of northwest Canada. Primary biological control on mineralization is supported by the identification of apatite in both chert-hosted and limestone-hosted specimens, the conspicuously rigid original morphology of the scale microfossils relative to co-occurring organic-walled cyanobacteria and acritarchs, and the microstructure of the constituent phosphate. Cell-enveloping mineralized scales occur in a wide range of extant protists, but the apparent restriction of phosphate scales to one modern taxon of green algae suggests a possible affiliation for these fossils. Documentation of primary phosphate biomineralization in Fifteenmile Group (Yukon Territory, Canada) microfossils greatly extends the known record of biologically controlled mineralization and provides a unique window into the diversity of early eukaryotes.**

## INTRODUCTION

Biomineralization plays a critical role in understanding evolutionary history, as organisms that form mineralized components become fossilized much more commonly than those that do not; biomineralizing eukaryotes also play a major role in biogeochemical cycles. Although the first conspicuous records of biologically controlled mineralization are found in multicellular fossils from the late Ediacaran (Germs, 1972; Grotzinger et al., 2000), it is not until the Mesozoic and Cenozoic that unicellular organisms adopted a comparable level of biomineralization, potentially in response to escalated ecological pressures (Hamm et al., 2003). Even so, phylogenetic and molecular clock analyses (e.g., Sperling et al., 2010), combined with variably problematic paleontological reports (Horodyski and Mankiewicz, 1990; Porter et al., 2003), point to a significant pre-Ediacaran record of biologically controlled mineralization. Diverse scale-like microfossils from the Fifteenmile Group (Yukon Territory, Canada; formerly the Tindir Group; Macdonald et al., 2011) have often been noted as instances of early biomineralization, most likely utilizing silica (Allison and Hilgert, 1986; Knoll, 2003), though neither their age nor original composition was reliably constrained. Recent work has now confirmed

a mid-Neoproterozoic (717–812 Ma) depositional age for scale-bearing horizons of the Fifteenmile Group (Macdonald et al., 2010a, 2010b, 2011). Here we present new data from analyses by Raman and fluorescence spectroscopy and energy dispersive X-ray spectroscopy (EDS) that show the scales to be composed of calcium phosphate produced through biologically controlled mineralization.

## GEOLOGIC SETTING

Fossiliferous samples were collected from outcrops of the Fifteenmile Group from Mount Slipper (Yukon, Canada, N65°16', W140°57'), exposed on the west limb of a broad anticline that straddles the Yukon-Alaska border. Microfossils were recovered from organic-rich, chert-bearing limestone micrite that was deposited below fairweather wave base (Macdonald et al., 2010a) (Fig. DR1 in the GSA Data Repository<sup>1</sup>). Although these exposures were originally assigned to the upper Tindir Group and interpreted variously as late Cryogenian to early Cambrian in age (Allison and Awramik, 1989; Kaufman et al., 1992), recent mapping and chemostratigraphic analyses have reassigned the fossiliferous strata to the Fif-

teenmile Group (formerly lower Tindir Group) (Macdonald et al., 2010a, 2011). The age of the fossiliferous upper carbonate unit of the Fifteenmile Group is determined as between  $811.5 \pm 0.2$  Ma and  $717.4 \pm 0.1$  Ma by U-Pb isotope dilution–thermal ionization mass spectrometry ages on zircons from volcanic horizons in sections ~75 km to the east of Mount Slipper (Macdonald et al., 2010b).

## MATERIAL AND METHODS

Chert thin sections were prepared at thicknesses of 60 or 100  $\mu\text{m}$  for light microscopy, confocal laser scanning microscopy (CLSM), and Raman and fluorescence spectroscopy. In addition to the fossils present in black chert nodules (Macdonald et al., 2010a), we also discovered abundant specimens preserved in three-dimensional submicron-scale detail in acid-resistant residues of the associated limestone. Limestone hand samples were broken into ~2  $\text{cm}^3$  pieces and dissolved in 30% acetic acid; the resulting macerates were filtered through a 30  $\mu\text{m}$  mesh, dry mounted on copper tape, and coated with Pt/Pd or Au for study by scanning electron microscopy (SEM), focused ion beam (FIB)-SEM, and EDS (for additional information, see the Data Repository).

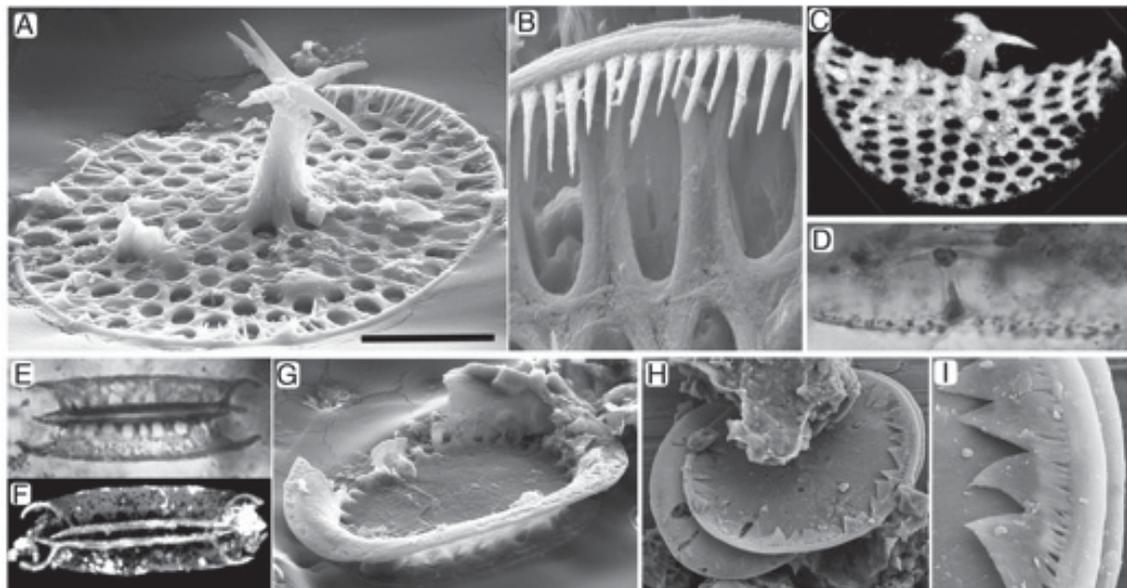
## MORPHOLOGY AND COMPOSITION

Diverse scale microfossils were identified in both chert and limestone lithologies. Three of the most common morphotypes are illustrated here: (1) imperforate circular scales 14–27  $\mu\text{m}$  in diameter (*Archeoxybaphon*; Figs. 1H and 1I); (2) ovoid scales 30–45  $\mu\text{m}$  in diameter having regularly perforated concave sidewalls (*Bicorniculum*; Figs. 1E–1G); and (3) ovoid perforated shield-like scales 26–52  $\mu\text{m}$  in diameter, composed of a hexagonal network of struts, from one surface of which arises a central shaft that terminates in a four- or six-pronged structure (*Characodictyon*; Figs. 1A–1D). CLSM and SEM of *Characodictyon* show these scales to be 1–2  $\mu\text{m}$  thick with the hexagonal lattice-work defining 0.5–1.5- $\mu\text{m}$ -diameter perforations (Fig. 1D). On the shaft-bearing surface, 1–2- $\mu\text{m}$ -long rigid, regularly oriented spines protrude from the intersecting nodes of the lattice-work (Fig. DR2A in the Data Repository),

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<sup>1</sup>GSA Data Repository item 2011174, Figures DR1–DR7 and extended Materials and Methods, is available online at [www.geosociety.org/pubs/ft2011.htm](http://www.geosociety.org/pubs/ft2011.htm), or on request from [editing@geosociety.org](mailto:editing@geosociety.org) or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

**Figure 1.** Optical, confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM) images of Tindir scale fossils. **A:** SEM image of *Characodictyon*. **B:** SEM image of shield-fringing spines and fibrous microstructure of *Characodictyon*. **C:** CLSM image of *Characodictyon*. **D:** Photomicrograph of *Characodictyon*. **E:** Photomicrograph of *Bicorniculum*. **F:** CLSM image of *Bicorniculum*. **G:** SEM image of *Bicorniculum*. **H:** SEM image of *Archeoxybaphon* sp. **I:** SEM image of *Archeoxybaphon* showing peripheral triangular teeth. Scale bar represents 5  $\mu\text{m}$  in A, 2  $\mu\text{m}$  in B, 15  $\mu\text{m}$  in C and D, 12  $\mu\text{m}$  in E and F, 8  $\mu\text{m}$  in G, 10  $\mu\text{m}$  in H, and 3  $\mu\text{m}$  in I.



whereas similar spines fringe the shield perimeter (Figs. 1A and 1B). A similarly fringing array of triangular tooth-like structures, lying flat against the scale surface, is present in *Archeoxybaphon* (Figs. 1H and 1I). The narrow spines of *Characodictyon* and the tooth-like structures of *Archeoxybaphon* have been observed only by SEM in acid-isolated specimens. In addition to these primary biological features, some specimens of *Characodictyon* exhibit a microfabric of oriented crystallites parallel to the latticework construction (Figs. 1B; Fig. DR2). Specimens are often found in clusters of monotypic scales, both in limestone and chert lithologies (Fig. 2A; Fig. DR7).

Raman and fluorescence spectroscopic analyses reveal the presence of morphology-defining apatite (shown in fluorescence spectra to contain minor concentrations of  $\text{Sm}^{+3}$ ) and kerogen, surrounded by the permineralizing quartz of the chert matrix (Fig. 2; Fig. DR3) in all three taxa studied. The presence of kerogen is also indicated by a pronounced fluorescence under CLSM (Figs. 1C and 1F). EDS results showing the presence of strong peaks corresponding to P, Ca, and C (Fig. 3; Fig. DR5) in acid-isolated specimens further identify the presence of calcium phosphate and kerogen in all scale fossils studied. Acid-macerated samples cut with a FIB-SEM and analyzed using EDS also show no internal compositional variation (Fig. DR6), indicating a homogeneous mix of kerogen and apatite. By contrast, apatite was not detected in any of numerous organic-walled coccoids, acritarchs, or particles of amorphous kerogen co-occurring with the scales in the Fifteenmile Group chert (Figs. DR3 and DR4). The quartz signal recorded in chert-permineralized specimens is not duplicated in any carbonate-hosted

counterparts, indicating that the fossils were not originally biomineralized silica. The  $\text{Sm}^{+3}$  signal in the fluorescence spectra of chert-hosted scales derives from Sm replacement of Ca-1 ions in the apatite, a characteristic of substitution under anoxic conditions (Gaft et al., 2001). These analyses show that the scale microfossils are consistently composed of apatite and organic carbon, in both host-rock lithologies.

#### ORIGINAL COMPOSITION

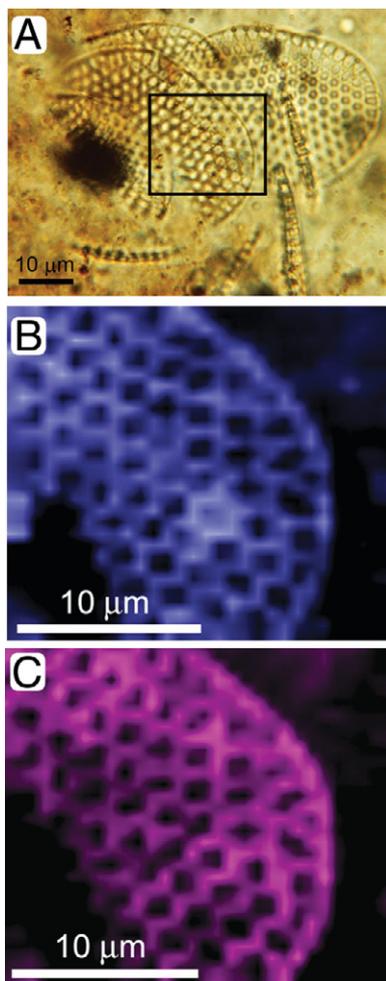
The consistently undistorted morphology of the scale microfossils in both chert and limestone combined with the intact, fully three-dimensional specimens recovered from compacted limestone point to an original rigidity that is typically associated with biomineralization. Even the delicate marginal spines of specimens isolated from the limestone show no deformation. By contrast, the walls of co-occurring organic-walled acritarchs, coccoids, and cellular filaments in silicified samples were originally pliable, as evidenced by their deformation during early, precompactional, diagenesis.

Despite such arguments, any definitive identification of biomineralization must rule out the possibility of secondary, early diagenetic mineralization. Phosphate replacement is common throughout the fossil record, including instances of precompactional soft tissue phosphatization. None of these, however, is comparable to the preservation of Fifteenmile Group scales. In the best known examples, i.e., apatite-permineralized Ediacaran embryos, middle Cambrian mid-gut glands, and Cretaceous muscle, there is a clear histological selectivity to mineralization, with early phosphate preferentially replacing only the most labile cellular tissues (Butterfield, 2003). Diagenetic phosphate also accounts for

the preservation of more recalcitrant features, including the cell walls of Ediacaran acritarchs and cuticles of Cambrian ecdysozoans, but these fossils all exhibit evidence of original plastic deformation and secondary encrustation. Paleozoic “mazuelloid” acritarchs display a somewhat similar style of deformation, and combined with the presence of discrete carbonaceous walls composed of zoned arrays of perpendicularly oriented crystallites, indicate a diagenetic origin of phosphate (Kremer, 2005). By contrast, none of the scales in the Fifteenmile Group are deformed or show any evidence of either phosphate replacement or secondary apatite overgrowths. The possibility that these fossils were originally composed of calcium carbonate can be discounted due to the absence of (1) carbonate pseudomorphs, (2) any carbonate signal in the Raman spectra, and (3) cast and mold structures characteristic of phosphate replacement (cf. Feng and Sun, 2003).

The presence of nanometer-scale crystallites in some specimens of *Characodictyon* (Fig. 1B; Figs. DR2B and DR2C) derives from localized diagenesis, but the orientation of these microstructures, i.e., elongate forms extending parallel to the perforation-defining primary struts, differs distinctly from the granules or microspheres observed in replacing or encrusting phosphate (Wilby and Briggs, 1997). Given the nanometer-scale crystallinity of this material and the higher order orientation of most biologically precipitated phosphate (Schmahl et al., 2008), the formation of this microcrystalline fabric can be attributed to a diagenetic merging of preexisting phosphate crystallites aligned along an organic template.

The fossiliferous lithologies of the Fifteenmile Group show no evidence of background

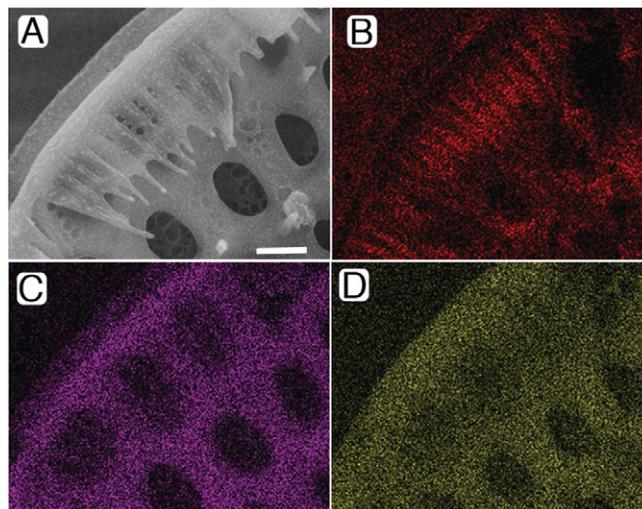


**Figure 2.** Optical and fluorescence spectroscopic images of *Characodictyon* sp. **A:** Photomicrograph of cluster of specimens; black rectangle shows area imaged in **B** and **C**. **B:** Fluorescence image showing spatial distribution of kerogen (acquired in spectral window centered on kerogen fluorescence band at ~570 nm shown in Fig. DR4 [see footnote 1]). **C:** Fluorescence image showing spatial distribution of apatite (acquired in spectral window centered on  $\text{Sm}^{+3}$  fluorescence band at ~599 nm shown in Fig. DR4).

phosphogenesis or phosphatic overgrowths, as would be expected in sediments hosting phosphate-replaced fossils. Taken together, the chemical, textural, and distributional expression of the scales indicate primary biologically controlled calcium phosphate biomineralization.

#### TAXONOMIC AFFINITY

Taxonomic assignment of the Fifteenmile Group scale microfossils has been frustrated by the disparate range of extant protists that develop comparable armatures of scale-like plates, including prasinophytes, chrysophytes, *Raphidiophrys* (members of the centrohelid heliozoans), haptophytes, ciliates, and testate amoebae (Nicholls and Durrschmidt, 1985;



**Figure 3.** Energy-dispersive X-ray spectroscopy maps of portion of specimen of *Characodictyon* sp. acquired at 5 keV. **A:** Scanning electron microscopy image of examined area. **B:** Carbon elemental map. **C:** Calcium elemental map. **D:** Phosphorous elemental map. Scale bar in **A** represents 1  $\mu\text{m}$  for all images.

Domozych et al., 1991; Anderson, 1994; Graham, 2000; Foissner, 2005). Our identification of phosphate biomineralization potentially offers an additional character for phylogenetic analysis.

The use of phosphate in cell-enveloping scales is much less common among extant unicellular eukaryotes than that of other biominerals such as silica and calcium carbonate (Knoll, 2003). Only a handful of such examples are known, including the adventitiously encrusted stalks of a chrysophyte alga (Lee and Kugrens, 1989), the tests of the lobose testate amoeba *Cryptodiffugia operculata* (Hedley et al., 1977), and the scales of the streptophyte green alga *Mesostigma viride* (Domozych et al., 1991). The brevity of this list is in part due to the rarity of studies on cell coverings of modern protists. Of those that have been studied, only the phosphatized scales of *M. viride* are structurally and functionally comparable to the Fifteenmile Group fossils; indeed, the intricate latticework of the basket scales of this taxon is strikingly similar to that of *Characodictyon*, despite their markedly smaller size. The absence of primary silica or carbonate in the scales distinguishes them from the strictly siliceous biomineralized scales of crown-group chrysophytes, diatoms, or *Raphidiophrys*, and from the strictly calcareous biomineralized scales of coccolithophores. Other affinities, such as within the greater haptophyte or centrohelid heliozoan clade, are plausible given that stem groups may have independently evolved the capacity for apatite biomineralization prior to the appearance of silica and calcium carbonate biomineralization in the crown group clades, combined with the fact that only a fraction of modern protist diversity has been described. However, in light of their mineralogical and morphological similarity to the scales of *M. viride*, there is a strong argument for identifying the scale microfossils as an extinct sister group of the Streptophyta, one of

the two principal clades that constitute the Viridiplantae (green plants) (Rodríguez-Ezpeleta et al., 2006).

#### DISCUSSION

The data presented here indicate that the Fifteenmile Group scale microfossils represent the earliest compelling evidence of biologically controlled eukaryotic biomineralization known in the fossil record. Although two other records of comparable age (Porter et al., 2003; Horodyski and Mankiewicz, 1990) are intriguing, they are less robust because neither definitively document primary, nondiagenetically sourced biominerals.

Like their modern analogs, the Fifteenmile Group scales were likely formed intracellularly in specialized endomembrane-derived vesicles and then extruded to the surface of the cell (Okuda, 2002). In modern protists, such biomineralized cell coverings are thought to be a response to selective pressure induced by eumetazoan predation (Smetacek, 2001; Hamm et al., 2003; Kuwata and Tsuda, 2005). The paucity of biomineralized fossils known from the Precambrian may thus reflect the lack of a selective need for mineralized structures due to the absence of eumetazoans in mid-Neoproterozoic seas. While predation plays an important role in inducing biomineralization, many other factors are also involved, including the regulation of buoyancy, protection from ultraviolet radiation, nutrient storage, and predation by other protists (Graham, 2000; Sikes and Wilbur, 1982). In the absence of eumetazoans, the biomineralization of the scale microfossils thus may be a response to protistan predation, or other factors, exhibited by only a small subset of protists.

The approach of isolating mineralic microfossils from limestone using weak acid maceration has rarely been applied to the Precambrian in the search for such small-sized objects as the Fifteenmile Group scale microfossils. The

success of this technique illustrated here and the new search image provided by the scale microfossils' phosphatic composition provides a promising new approach to studies of the Precambrian fossil record.

## CONCLUSION

Our evidence of biologically controlled mineralization in a putative green alga indicates that a capacity for biologically controlled biomineralization existed among eukaryotes relatively early in the Neoproterozoic, but suggests that the selective pressures necessary for its widespread development were not met until the Phanerozoic. In addition, the calcium phosphate composition of the scale microfossils indicates that some early biomineralizing protists did not utilize the mineralogies dominant in their modern analogs, a finding consistent with the wide variation in biominerals found across the eukaryotic tree. Continued work on the Precambrian fossil record, further research on the selective pressures leading to biomineralization in modern protists, and more refined reconstructions of Neoproterozoic ecosystems will be required to fully understand the biology of these intricate biomineralized fossils.

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