Tonian Macroalgae Fossils from the Dolores Creek Formation, Canada

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Abstract.— The ecological expansion of algae in the Neoproterozoic (1000-539Ma) led to algae becoming the dominant bioproducers in marine systems and to the reorganization of marine food webs (Tang et al. 2020, Maloney et al. 2021). Here, new Tonian macroalgal fossils from the Dolores Creek Formation (950-900Ma) of Northwestern Canada are interpreted as a new genus and species of benthic green algae: Fillinilla Bifrangis (Maloney et al. In Review). This interpretation is based on multicellularity, cell shape, cell size and degree of cell differentiation. Fossils exhibit an unbranching, multicellular thallus with uniform width and elliptical to globose holdfasts. As on of the oldest known examples of green macroalgae, these organisms have important implications for molecular clock studies due to their reliance on fossil occurrence data.

Introduction

During the Neoproterozoic era (1000 – 541Ma), algae began to dominate the base of the food chain replacing cyanobacteria, resulting in changes to primary production in marine environments (Brocks et al. 2018; Maloney et al. 2020). This ecological expansion led to algae becoming the dominant bioproducers in marine systems and to the reorganization of marine food webs (Tang et al. 2020, Maloney et al. 2021). The base energy of the food chain increased with this organization, allowing the persistence of larger grazers, the advent of predation and the development of defense strategies (Brocks et al. 2018).

To understand the environmental drivers for the change of dominancy from cyanobacteria to algae, it is critical to examine and document fossil algae (Loduca et al. 2017). The goal of this project is to establish the taxonomy of Dolores Creek macrofossils and report a new species of Tonian fossil algae: *Fillinilla Bifrangis*. Reported macrofossils are a new genus and species of early macroalgae with a likely green algal affinity based on a characteristic assemblage of features, such as large size and rib wall ornamentation.

These fossils along with the 1 Ga morphologically-differentiated chlorophyte from North China have important implications for molecular clock studies and potential drivers of divergence in algal lineages (Tang et al. 2020). Molecular clock estimates are largely impacted by ambiguous taxonomic interpretations from the Precambrian. The inclusion of this fossil occurrence in the calibration of molecular clock studies can possibly adjust the timing of main algal divergences and be used to evaluate hypotheses regarding causes of algae diversification.

Geologic Setting

The Dolores Creek Formation (950 – 900Ma) is the basal unit of the Mackenzie Mountains Supergroup (950-775Ma) located in northwestern Canada. At the fossil locality, the Dolores Creek Formation is a one-kilometer-thick succession of dark grey siltstones and shales, with minor stromatolitic floatstone (Maloney et al. In Review). Fossils were found at four stratigraphic levels within the stromatolitic floatstone of the middle of the section (~500m), which was likely formed by gravity flows beneath the storm wave base (Maloney et al. In Review).

Methods

A total of 4 double-sided slabs and 57 specimens were examined. Slabs contained 3-40 total specimens, with 1-22 per bedding surface. Some slabs were susceptible to fracturing, which exposed additional bedding surfaces that contained specimens. Such surfaces were included during data collection. In slabs with high proportions of specimen overlap, individual organisms were difficult to discern. These specimens were excluded from both the total specimen number and organism length data.

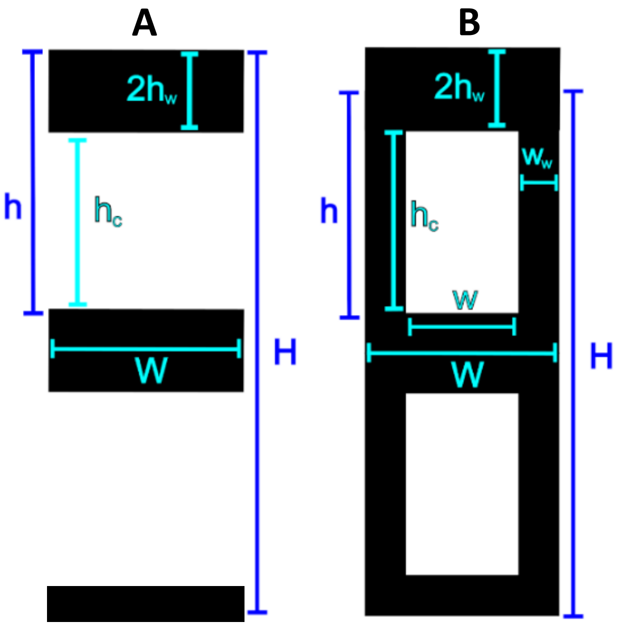

Figure 1: (A) The dimensions obtained from specimens when lengthwise cell walls not being preserved. (B) The measurements obtained from specimens with complete cell wall preservation. *W* depicts width. The height (total length) is represented by *H*. *h* depicts the height of a single cell, where h_w represents the height of each inner/widthwise cell wall and h_c the inner height of the cell. It should be noted that the individual width-wise cell walls were frequently indistinguishable, and measurements are 2hw. w_w, the width of the lengthwise cell wall was also obtained where possible.

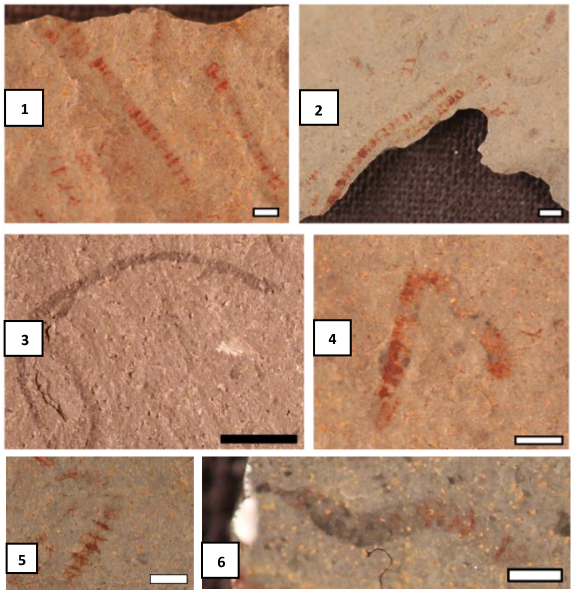

Figure 2: Images demonstrating the different positions specimens can occur in, a of which indicate that the specimens were not preserved *in situ*. (1) Straight. (2) Slightly Curved. (3) U-shaped.) (4) V-shaped. (5) J-Shaped. (6) S-Shaped. White scale bars are 1mm, black scale bars 5mm.

The open-source program ImageJ was used to obtain morphometric data including: total organism length, cell height and width, cell wall thickness, differentiated cells, specimen features (false branching, twisting/deformation, overlapping, tapering ends), and specimen position (straight, slightly curved, U-shaped, V-shaped, J-shaped, or S-shaped; see Figure 2).

Both general organism descriptions and unique features were documented to determine what type of organism the fossils represented.

Systematic palaeontology

Kingdom Archaeplastida, Adl et al. 2005
Phylum Viridiplantae, Cavalier-Smith 1981
Genus Fillinilla new genus

Type species.— Fillinilla bifrangis

Diagnosis.— As per species.

Occurrence.— Dolores Creek Formation, Mackenzie Mountains Supergroup, Yukon Territory, Canada; Tonian (Maloney et al. 2020)

Etymology.— From Latin, Fillinilla meaning ‘little thread’.

Remarks.— As per species.

Fillinilla Bifrangis new species

Holotype.— Specimen 59.28, seen in Figure 3 Image 2.

Diagnosis.— Multicellular, uniseriate thallus. Simple, unbranched filaments with consistent width (0.3-0.7mm). Cells are rectangular with double septa between adjacent cells. Ellipsoidal to globose holdfasts may be present.

Occurrence.— Dolores Creek Formation, Mackenzie Mountains Supergroup, Yukon Territory, Canada; Tonian (Maloney et al. 2020)

Description.— The thallus is a simple, unbranching, uniseriate filament with septa, indicating multicellularity. Well-preserved specimens may possess differentiated cells, or cells with a unique form and function, and/or false branching. Thallus length ranges from 0.8-32.7mm, depending on the number of segments within a specimen. The sharp terminations at one or both ends of the filaments are indicative of fragmentation, making the length of specimens a minimum value. Width is consistent along the thallus and is more diagnostic of the species than the length.

Cells within the thallus are rectangular, ranging 0.2-1.0mm in length and 0.3-0.7mm in width. Cells are arranged in single chains with differing degrees of cell wall preservation between internal and external cell walls (see Figure 2). External cell walls, or lengthwise cell walls, have lower rates of preservation compared to internal cell walls (width-wise cell walls). This is likely attributed to the double septa of internal cell walls (averaging 0.1mm in thickness), which are generally 1.7 times the thickness of external cell walls (0.06mm on average), creating a higher preservation potential. The presence of double septa is suggested by the occurrence of a clear division between two adjacent interior cell walls in an extremely well-preserved specimen (see Figure 3). This is presented as a clear line perpendicular to the length of the organism.

Differentiated cells included elliptical to globose holdfasts at the termini of the thallus (see Figure 3). Holdfasts range from 0.2-0.7mm in length and 0.2-0.5mm in width.

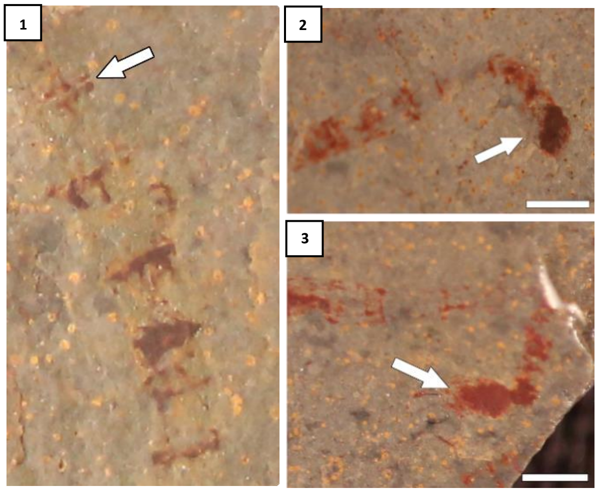

Figure 3: (1) Evidence of a separation between double septa in the preservation of the fossils. (2-3) Multiple specimens possessing ellipsoidal to globose holdfasts marked by white arrows. All scale bars are 1mm.

Fossils can be distinguished from the matrix through color, reflectivity, and, in rare cases, exceptionally preserved fossils with three-dimensional features. Colors include orange, red, black and of-white, which stand out against the dark-grey matrix. Fossils also have higher reflectivity than the surrounding rock, allowing them to be differentiated at various lighting angles.

Etymology.— Species name is derived from Latin terms meaning ‘two’ and ‘to break or bend’, describing the tendency to have double septa and be fragmentary in nature.

Materials.— 57 specimens from the Dolores Creek Formation in Northern Canada.

Remarks.— The new species was erected based on a unique suite of morphological features including an unbranched and segmented thallus, the presence of an elliptical to globose holdfast, and the size and shape of individual cells.

Of key importance in determining the genus and species was the consistent cell width and cell intervals, suggested a lack of specialized cells beyond the holdfast. The size, shape and preservation are consistent throughout specimens with clear segmentation, however, the quality of preservation differs between the external and internal cells walls of *F. bifrangis* n.sp.. There is a possibly that this a taphonomic artifact created by the higher preservation potential of internal double septa in comparison to length-wise cell walls.

There were occurrences of false branching in multiple specimens that exhibited minimal fragmentation (see Figures 4 and 5). This feature is created by the ends of a filament interacting with the thallus of another filament, although the two are not properly attached (Barsanti and Gualtieri 2014). False branches can be created through breakage of a filament, often resulting in a dead cell between the thallus and start of the branch (see Figure 5). In comparison, true branching is made through cell division in multiple directions (Barsanti and Gualtieri 2014). There are three features in *F. bifrangis* n.sp. that indicate the branching is false: First, the branches have tapering (thinning) ends where they interact wit the thallus of another filament, making them appear somewhat triangular. Second, there is minor curvature at the branch-thallus junction. Lastly, the preservation of double septa stops abruptly before the branch meets the thallus, but a slight

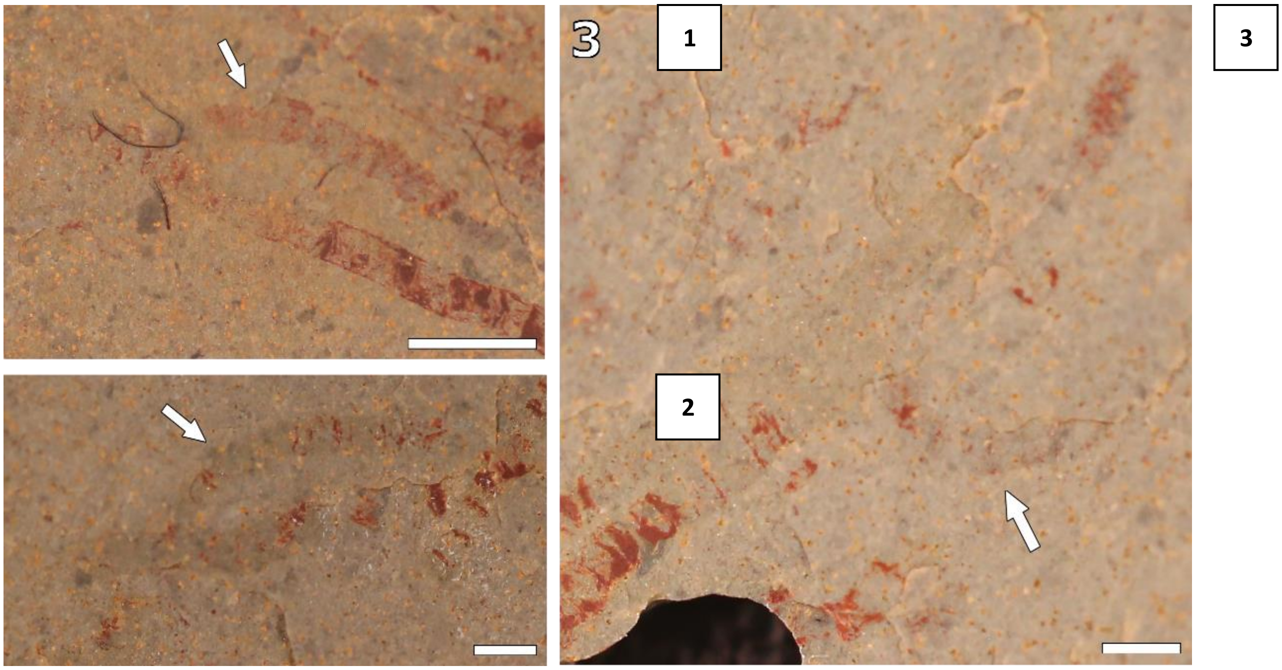

Figure 4: Examples of false branching seen within specimens. False branches are marked by white arrows. (1) The longest specimen showing two false branches. (2-3) Specimens of moderate length with a singular case of false branching. All scale Bars 1mm.

discoloration indicates the path of the branch (see Figure 4). All three of these features suggest that the branch attachment is not that of true branching, and that a dead cell may have been filling the gap at the branch-thallus junction before preservation (see Figure 4). The empty space where the branch interacts with the thallus is a possible indication that the cell was not able to be preserved and/or that the branch was not properly attached as it would have been through true branching.

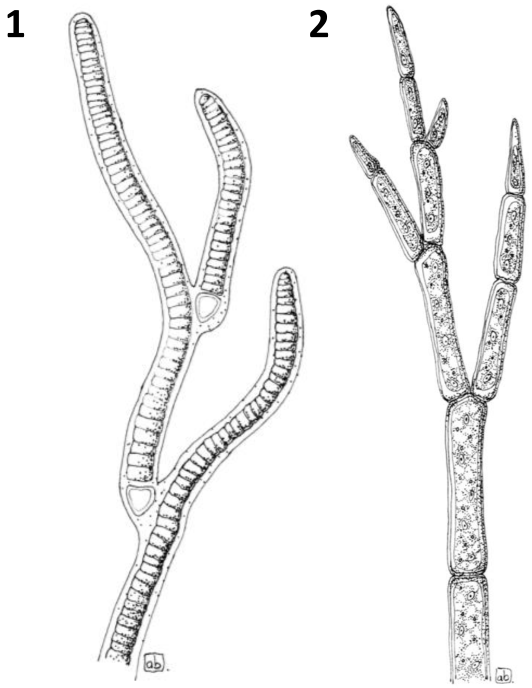

Figure 5: Diagrams comparing false branching (1) and true branching (2) seen in both algae and cyanobacteria (Barsanti 2014). Diagram 1 shows a dead cell where false branches interact with the main thallus.

DISCUSSION

Regarding the phylogenetic affinity of *F. bifrangis* n.sp., it is unlikely the fossils are forms of early animals based on the cell shape and filamentous construction. There are no known examples of animals that exhibit a filamentous construction with regular intervals of septa, and the rectangular shape of cells in *F. bifrangis* n.sp. are more suggestive of a fungal, cyanobacterial or algal affinity (Maloney et al. In Review).

The mycelium structure of fungi contain filamentous hyphae, but this affinity is unlikely based on the branching tendency of hyphae, the lack of evidence for spore-producing structures in *F. bifrangis* n.sp. filaments, and the typical size of hyphae. Specimens of *F. bifrangis* n.sp. do not exhibit any form of true branching, and false branching is not able to create the network of hyphae seen in mycelium. Hyphae, as well as spore-producing structures asci and basidia, are also on the micrometer scale (Maloney et al. In Review, Stephenson 2010). Based on the macroscopic size of cells in fossils presented in this paper, it is improbable that *F. bifrangis* n.sp. is a form of early fungi.

General characteristics of *F. bifrangis* n.sp. indicate the possibility of cyanobacterial affinity, however, the collective suite of morphological features in presented specimens excludes this possibility. Both false and true branching occurs in cyanobacterium, however, cyanobacterium do not possess holdfast structures (Barsanti and Gualtieri 2014, Maloney et al. In Review). There is also no indication that specimens of *F. bifrangis* n.sp. possess an extracellular sheath, a layer of extracellular polymeric substances that surround cyanobacterium, as well as a key feature indicating cyanobacterial affinity (Lee 2019, Maloney et al. In Review). Lastly, cyanobacterial cells range from the micrometer scale to a general maximum of 0.2mm in width, whereas *F. bifrangis* n.sp. cell width range from 0.3-0.7mm, providing cell widths are fully preserved and no deformation has occurred in the specimen. This culmination of evidence suggests that *F. bifrangis* n.sp. is not probable.

The majority of modern red and brown algae possess parenchymatous and pseudoparenchymatous forms rather than creating individual filaments. With parenchymatous thalli, cells grow in all directions and there is a complete loss of filamentous structure (Barsanti and Gualtieri 2014). In contrast, pseudoparenchymatous forms are accumulations of multiple filaments that are intertwined and/or branched, preserving the filamentous construct (Barsanti and Gualtieri 2014). Modern brown algae favour the usage of parenchymatous thalli, while red algae is mainly pseudoparenchymatous species (Barsanti and Gualtieri 2014). Both forms build sheets that experience cell division in multiple directions, whereas *F. bifrangis* n.sp. only exhibits growth in one direction. Due to the uniseriate, filamentous nature of *F. bifrangis* n.sp., it is unlikely that the specimens presented here are a form of red or brown algae.

Multiple lineages of green algae demonstrate macroscopic, filamentous seaweeds with holdfasts. Among forms of algae, benthic green algae is the best comparison for the features of *F. bifrangis* n.sp.. Oedogonialeans (Chlorophyta), Ulotrichaleans (Chlorophyta), Klebsormidiophyceans (Streptophyta) and Zygnematophyceans (Streptophyta) contain examples of filamentous algae alike *F. bifrangis* n.sp., with the chlorophytes forming holdfasts. With these morphological comparisons, it is evident the *F. bifrangis* is likely a form of green alga, making it the oldest known example in the fossil record.

CONCLUSIONS

The addition of new taxonomic information is essential in understanding the history of life and the origins of photosynthetic eukaryotes (Tang et al 2020; Xiao and Tang 2018). Molecular clock studies produce phylogenetic trees through calibration with fossil occurrences and interpretations, making the incorporation of these fossils imperative to interpreting the timing of Neoproterozoic algal divergences (Maloney et al. In Review, Tang et al. 2020). The introduction of these fossils into phylogenetic analyses has the potential to impact the current depiction of divergence in algae and land plants.

F. bifrangis n.sp. is indicative that algae were able to reach sizes on the centimeter scale early than previously predicted, which has important implications for both the ecological expansion of algae and hypotheses on the environmental influences on algal diversification. Hypotheses related to algae diversification and ecological expansion correlated with the snowball earth glaciations will need to be re-evaluated. Further research directions would benefit from the reconsideration of current algal divergence hypotheses and the examination of other Tonian successions for similar fossil material.

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