Precise age of *Bangiomorpha pubescens* dates the origin of eukaryotic photosynthesis

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**ABSTRACT**

Although the geological record indicates that eukaryotes evolved by 1.9–1.4 Ga, their early evolution is poorly resolved taxonomically and chronologically. The fossil red alga *Bangiomorpha pubescens* is the only recognized crown-group eukaryote older than ca. 0.8 Ga and marks the earliest known expression of extant forms of multicellularity and eukaryotic photosynthesis. Because it postdates the divergence between the red and green algae and the prior endosymbiotic event that gave rise to the chloroplast, *B. pubescens* is uniquely important for calibrating eukaryotic evolution. However, molecular clock estimates for the divergence between the red and green algae are highly variable, and some analyses estimate this split to be younger than the widely inferred but poorly constrained first appearance age of 1.2 Ga for *B. pubescens*. As a result, many molecular clock studies reject this fossil ex post facto. Here we present new Re-Os isotopic ages from sedimentary rocks that stratigraphically bracket the occurrence of *B. pubescens* in the Bylot Supergroup of Baffin Island and revise its first appearance to 1.047 ± 0.013/–0.017 Ga. This date is 150 m.y. younger than commonly held interpretations and permits more precise estimates of early eukaryotic evolution. Using cross-calibrated molecular clock analyses with the new fossil age, we calculate that photosynthesis within the Eukarya emerged ca. 1.25 Ga. This date for primary plastid endosymbiosis serves as a benchmark for interpreting the fossil record of early eukaryotes and evaluating their role in the Proterozoic biosphere.

**INTRODUCTION**

Photosynthetic eukaryotes (i.e., plants and algae) are responsible for most global primary production, and their evolution and diversification set the stage for today’s thriving biosphere. However, the timing and tempo of early eukaryotic evolution are unclear (Berney and Pawlowski, 2006; Parfrey et al., 2011; Shih and Matzke, 2013). While the origins of the Eukarya are debated, it is clear they became photosynthetic by engulfing a cyanobacterium in the primary endosymbiotic event that gave rise to the plastid, an organelle that houses photosynthetic machinery known as a chloroplast (Keeling, 2010). The fossil red alga *Bangiomorpha pubescens* provides the earliest unambiguous record of photosynthetic eukaryotic life and exhibits distinct morphological features of complex multicellularity and sexual reproduction (Butterfield et al., 1990; Butterfield, 2000). Therefore, this fossil is widely recognized as critical to calibrating the tempo of early eukaryotic diversification and constraining the acquisition of photosynthesis within the domain. Because concrete evidence for such evolutionary milestones is scarce, the appearance of *B. pubescens* in the fossil record presents a rare opportunity to anchor interpretations regarding early eukaryotic evolution.

The timing for the origin of the Eukarya is unresolved, with estimates spanning >1 b.y. (Roger and Hug, 2006). Isolated stem group eukaryotes may date as far back as ca. 3.2 Ga (Javau et al., 2010), whereas more widely occurring fossils with possible eukaryotic affinities, such as *Grypania*, occur in 1.9–1.8 Ga rocks (Han and Runnegar, 1992). Fossils from the Vindhyan Supergroup in India are suggested to represent ca. 1.6 Ga algae (Bengtson et al., 2017), but their age (Ray, 2006) and taxonomic assignment are uncertain. The first fossils with robust age control that conclusively display both the large cell size and complex ultrastructure or surface ornamentation characteristic of eukaryotes are 1.65–1.4 Ga sphaeromorphic acritarchs; i.e., *Tappania* and *Valeria* (Javaux et al., 2001; Lamb et al., 2009; Knoll, 2014; Adam et al., 2017).

Aside from *B. pubescens*, eukaryotic fossils remain taxonomically unresolved until ca. 0.8 Ga (Knoll, 2014; Cohen and Macdonald, 2015). Accepting that the last eukaryotic common ancestor had evolved by 1.65 Ga, the protracted early evolutionary history of stem group eukaryotes is poorly documented. While *B. pubescens* was long presumed to arise 1.2 Ga (see the GSA Data Repository!), its true age was only loosely constrained by conventional radiometric ages to a >500 m.y. interval. Here we present two new Re-Os isochron ages that precisely date the first appearance of *B. pubescens* in the Bylot Supergroup of Baffin Island. This revised fossil age helps resolve inconsistencies between molecular clocks and fossil evidence for early eukaryotic diversification (e.g., Berney and Pawlowski, 2006; Cavalier-Smith et al., 2006; Parfrey et al., 2011; Eme et al., 2014) and permits more precise calibration of primary plastid endosymbiosis and the split between the red and green algae.

**GEOLOGICAL BACKGROUND**

*B. pubescens* was first described in chert from peritidal carbonate facies of the Hunting Formation on Somerset Island (Butterfield et al., 1990) and later in similar facies in the correlative Angmaat Formation of the Bylot Supergroup on Baffin Island, both in northeastern Canada (Knoll et al., 2013; Fig. 1). The Hunting and Angmaat Formations occur within comparable stratigraphic sequences hypothesized to be remnants of previously interconnected basins in...
Figure 1. Location and geological context of the study area. A: Schematic lithostratigraphy of the Bylot Supergroup in the Borden Basin (age constraints from LeCheminant and Heaman, 1989; Heaman et al., 1992; Pehrsson and Buchan, 1999). Gp.—Group; sg.—subgroup; Fm.—Formation; Pt.—Point. B: Locations (red boxes) where Bangiomorpha pubescens occurs. C: Map of the Borden Basin (adapted from Turner, 2009) showing Re-Os sample localities (red stars) and B. pubescens fossil locality (yellow star; Knoll et al., 2013).

The Angmaat Formation records progradation and stabilization of a rimmed carbonate platform over black shale and siltstone of the underlying Arctic Bay Formation (Fig. 1). Regression of all Arctic Bay Formation samples yields a model 3 age of 1.046 ± 0.013/–0.017 Ga as the first appearance of B. pubescens. When combined with robust fossil data, molecular clocks offer an effective method to query the evolutionary history of eukaryotes (Hedges et al., 2004; Parfrey et al., 2011; Shih and Matzke, 2013). Although several potential sources of error exist in molecular clock analyses, one pivotal, geologically resolvable inconsistency is in the fossil ages required for calibration (Yoon et al., 2004; Berney and Pawlowski, 2006). As the first definitive fossil member of the red algae and photosynthetic eukaryote (Butterfield, 2000), B. pubescens is the oldest eukaryotic fossil included in these analyses and provides minimum limits on the age of both primary plastid endosymbiosis and the divergence between the red (rhodophytes) and green (viridiplantae) algae. To reexamine models for early eukaryotic evolution in light of the refined age of B. pubescens, we conducted cross-calibrated molecular clock analyses (BEAST2, https://www.beast2.org/ updated from Shih and Matzke, 2013), testing three different priors on the rhodophytes-viridiplantae divergence: (1) no constraint, (2) the previously reported age for B. pubescens of ca. 1.2 Ga (Butterfield, 2000), and (3) the age from this study of ca. 1.045 Ga (Table 1).

DISCUSSION

Much diversity within the Eukarya derives from various forms of plastid acquisition (Delwiche, 1999), but dating the original endosymbiosis of an ancient photosynthetic cyanobacterium has been elusive. Our molecular clock analysis using the refined B. pubescens age of...
ca. 1.045 Ga (run T09) suggests that primary plastid endosymbiosis occurred by ca. 1.25 Ga (Table 1). This analysis yields a decrease in the 95% confidence interval width for this event compared to the analysis using the previously accepted age of *B. pubescens* (run T08). The analysis with no age constraint for *B. pubescens* places the divergence between rhodophytes and viridiplanetae at 1.166 Ga, which is younger than the fossil’s previously inferred age, but consistent with this study and many previous molecular clock estimates (Berney and Pawlowski, 2006; Cavelier-Smith et al., 2006; Parfrey et al., 2011; Shih and Matzke, 2013; Eme et al., 2014). Together these confirm that the new age constraint better fits the existing molecular data set. It is important to note that these analyses utilize the most conservative (i.e., oldest) placement of the *B. pubescens* constraint. Various morphological features of *B. pubescens* suggest that it might belong to the Bangiales lineage within the Rhodophyta (Butterfield, 2000), and thus our analyses provide a conservative minimum age limit on primary plastid endosymbiosis.

Although the algorithms employed in molecular clock studies differ, the age constraints they implement exert by far the greatest control on their results. Accordingly, results from this study are calibrated with and improved by the revised first appearance age of *B. pubescens*. However, we note that these analyses differ from previous studies in other ways. Due to the paucity of available calibration points in deep time, Precambrian molecular clock studies often employ indirect or ambiguous constraints on the origins of key lineages (e.g., Archean lipid biomarkers and/or microfossils of uncertain taxonomic affinity) that affect their age estimates for evolutionary events. For example, Sánchez-Barracaldo et al. (2017) calculated, using controversial cyanobacterial microfossil calibrations (Butterfield, 2015), that both primary plastid endosymbiosis and the split between the red and green algae occurred ca. 1.9 Ga. Because divergence calculations from molecular data are only as valuable as their geological tie points, this study employs less controversial constraints from the fossil record of algae and plants; and by using a cross-calibration approach (Shih and Matzke, 2013), the value of these constraints is effectively doubled.

Various other Proterozoic fossils have been suggested to represent ca. 1.8–1.6 Ga primitive eukaryotic algae (Han and Runnegar, 1992; Moczydlowska et al., 2011; Bengtson et al., 2017). The Vindhyan Supergroup of central India was interpreted to host ca. 1.6 Ga crown-group rhodophyte fossils (Bengtson et al., 2017). Whereas Rafafajmazula chitrokkootensis and Ramathallus lobatus may have eukaryotic affinities, they are hundreds of millions of years older than most phylogenetic estimates (including those that exclude the age of *B. pubescens* as a constraint) for the rhodophytes-viridiplanetae divergence, more than 500 m.y. older than *B. pubescens*, and almost 1 b.y. older than the earliest green algae (Butterfield et al., 1988). Because early eukaryotic life was perhaps dominated by stem-group taxa (Javava and Knoll, 2017), if the age assignment for these fossils is correct (see the Data Repository), they may represent eukaryotes that diverged long before the origins of red and green algae. In this case, convergent evolution may account for their similarities to extant algae. Considering that plastids have been acquired at least twice within the Eukarya (i.e., algae and *Paulinella*; Marin et al., 2005), these fossils could represent an early failed experimentation in photosynthesis. In addition, it is difficult to distinguish the primary morphology of these fossils from secondary taphonomic features from phosphatization that obscure their taxonomic assignment. However, if the interpretations of the taxonomic identity and age of these fossils are correct, then they are discordant with the results of this study.

**CONCLUSIONS**

Given the remarkable preservation of *B. pubescens* and its morphological similarities to modern Bangia red algae (Butterfield et al., 1990; Butterfield, 2000), a precise first appearance age of 1.047 Ga cements its role as currently the most robust geological datum for reconstructing early eukaryotic evolution. This revised age narrows the window for when photosynthesis in crown eukaryotes first evolved and introduces a chronological framework for investigating the role this fundamental biological innovation played in shaping global biogeochemical cycles.

**ACKNOWLEDGMENTS**

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**REFERENCES CITED**


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**Figure 2. Re-Os isochron diagrams. A: The Arctic Bay Formation. MSWD-mean square of weighted deviates. B: The Victor Fm. Data-point error ellipses represent 2σ uncertainty. Elemental abundances and isotopic compositions are presented in Table DR1 (see footnote 1).**

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**Table 1. Effects of the New Age for Bangiomorpha pubescens on Molecular Clock Analyses**

<table>
<thead>
<tr>
<th>Run</th>
<th>Bangiomorpha age prior for red-green divergence (Ga)</th>
<th>Red-Green Divergence ± 2σ (Ga)</th>
<th>95% CI width (Ga)</th>
<th>Primary plastid endosymbiosis ± 2σ (Ga)</th>
<th>95% CI width (Ga)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T07</td>
<td>omitted</td>
<td>1.166 (1.450–0.883)</td>
<td>0.567</td>
<td>1.325 (1.164–1.029)</td>
<td>0.618</td>
</tr>
<tr>
<td>T08</td>
<td>1.198 ± 0.024 (previous)</td>
<td>1.194 (1.242–1.150)</td>
<td>0.092</td>
<td>1.422 (1.568–1.291)</td>
<td>0.277</td>
</tr>
<tr>
<td>T09</td>
<td>1.045 ± 0.015 (this study)</td>
<td>1.046 (1.046–1.106)</td>
<td>0.058</td>
<td>1.246 (1.368–1.142)</td>
<td>0.226</td>
</tr>
</tbody>
</table>

*Note: Multiple analyses using a lognormal relaxed clock in BEAST2 (https://www.beast2.org/) utilized either no constraint for the age of *B. pubescens* (run T07), its previously accepted age (run T08), or its new reported age from this study (run T09). The various analyses were compared on 95% confidence interval (CI) widths (in billions of years) of key nodes of interest, specifically the red-green divergence and plastid endosymbiosis. BEAST2 run outputs are presented in Figures DR1–DR3 (see text footnote 1).*