

Putative Cryogenian ciliates from Mongolia

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ABSTRACT

Major lineages of modern eukaryotes, represented primarily by microscopic taxa, are thought to have originated during the Neoproterozoic, but microfossils older than 635 Ma rarely have unambiguous relationships to modern microscopic eukaryotes. Here we report exceptionally preserved 715–635 m.y. old eukaryotic tests in limestone strata of Mongolia. The ~100- μ m-long organic-rich three-dimensional tests have flask-like shapes, constricted necks, distinct and often thickened collars, and flexible walls composed of densely packed alveolar structures. The combined morphological and ultrastructural characters of these Cryogenian tests are remarkably similar to the tests of tintinnids, modern planktonic ciliates. Eukaryotes forming recalcitrant organic or mineral-rich tests before 635 Ma may have increased export and burial fraction of organic carbon, driving an increase in atmospheric oxygen and the subsequent radiation of metazoans.

INTRODUCTION

The diversification of modern eukaryotes may have had a major impact on geochemical cycling in the Proterozoic oceans (Logan et al., 1995; Rothman et al., 2003; Peterson and Butterfield, 2005; Holland, 2006). Previously, biological inventions including the construction of hard and recalcitrant body parts and the production of fecal pellets were suggested to account for the unusual geochemistry of the Ediacaran Period (635–542 Ma; e.g., references above), but molecular clocks suggest that at least some organisms with recalcitrant body parts, such as foraminifera, testate amoebae, ciliates (tintinnids), and dinoflagellates, were present in ecosystems >100 m.y. earlier (Douzery et al., 2004; Berner and Pawłowski, 2006).

Among microscopic taxa with rigid body parts, only testate amoebae from the supergroups Amoebozoa and Rhizaria (Porter et al., 2003) and scaly structures (Cohen et al., 2011) are known from strata older than 715 Ma (Knoll et al., 2006; Macdonald et al., 2010). The diversity of other testate or scaly eukaryotes in ecosystems between 715 and 635 Ma remains particularly poorly constrained, because few reported fossil assemblages can be unambiguously dated to 715–635 Ma (Bosak et al., 2011; Maloof et al., 2010). Here we report the discovery of exceptionally preserved fossil eukaryotic tests in the 715–635 Ma carbonate deposits from the Tsagaan Oloom Formation, southwestern Mongolia.

GEOLOGICAL SETTING

The Tsagaan Oloom Formation consists of <2 km of platformal carbonate rocks and glacial deposits (Fig. 1) that overlie the Dzabkhan Volcanics (dated as 803.4 ± 8.0 and 773.5 ± 6.6 Ma by U/Pb zircon laser ablation; Levashova et al., 2010). Cryogenian strata of the Tsagaan Oloom Formation consist of the Sturtian (ca. 715 Ma) Maikhan Ul diamictite, organic-rich limestone of the Tayshir member, and the Marinoan (ca. 635 Ma) Khongoryn diamictite (Fig. 1; Macdonald et al., 2009, 2010). The black cap carbonate at the base of the Tayshir member hosts a rise in $^{87}\text{Sr}/^{86}\text{Sr}$ from 0.7067 to 0.7073 (Shields et al., 2002), consistent with Sturtian cap carbonates in Namibia (Yoshioka et al., 2003) and northwest Canada (Kaufman et al., 1997). The carbon isotope profile through the Tayshir member begins with negative values in the Tayshir cap carbonate, increasing upsection to very positive values interrupted by an ~15% negative carbon isotope excursion (Fig. 1), referred to as the Tayshir anomaly (Macdonald et al., 2009). Fossil structures described here are most abundant in the carbonate strata at two different sections (F704 and F864) of the Tayshir member within the negative Tayshir carbon isotope anomaly, although they also occur in ribbonites and microbialaminites below this anomaly (Fig. 1).

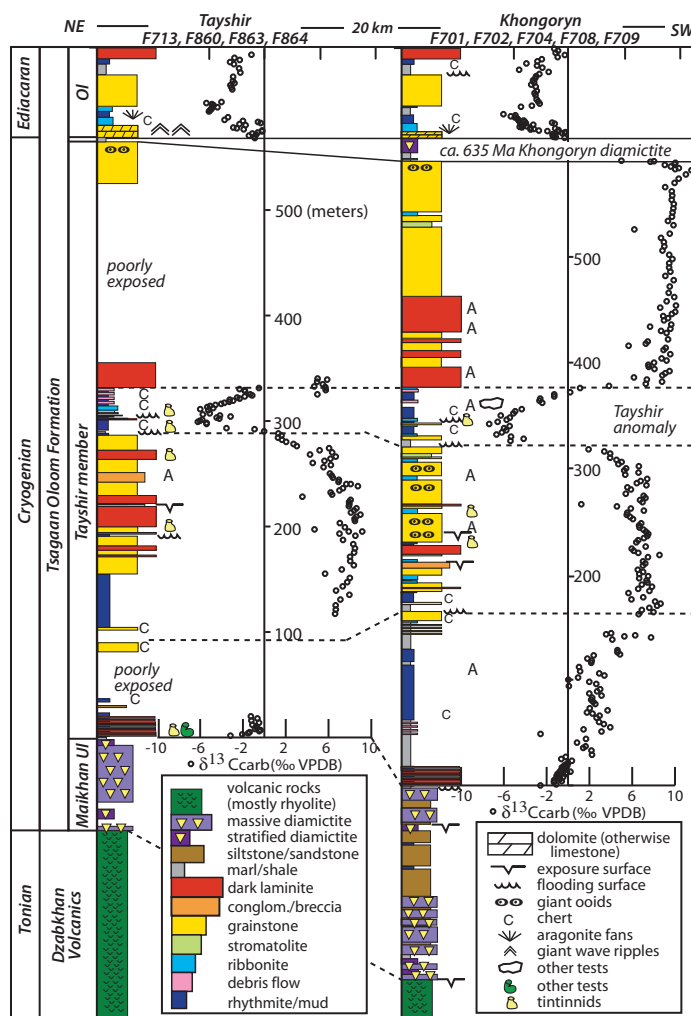


Figure 1. Lithostratigraphy, carbon isotope chemostratigraphy, and microfossil stratigraphy of Tayshir member of Tsagaan Oloom Formation (southwestern Mongolia). Conglom.—conglomerate; VPDB—Vienna Pee Dee belemnite. See Table DR1 in the Data Repository (see footnote 1) for coordinates, lithologies, stratigraphic positions of fossils, and isotopic data from Tayshir section.

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FOSSIL DESCRIPTION

Microsparitic mudstone from the Tayshir member encloses individual ~100- μ m-long dark orange-brown organic-rich structures (Figs.

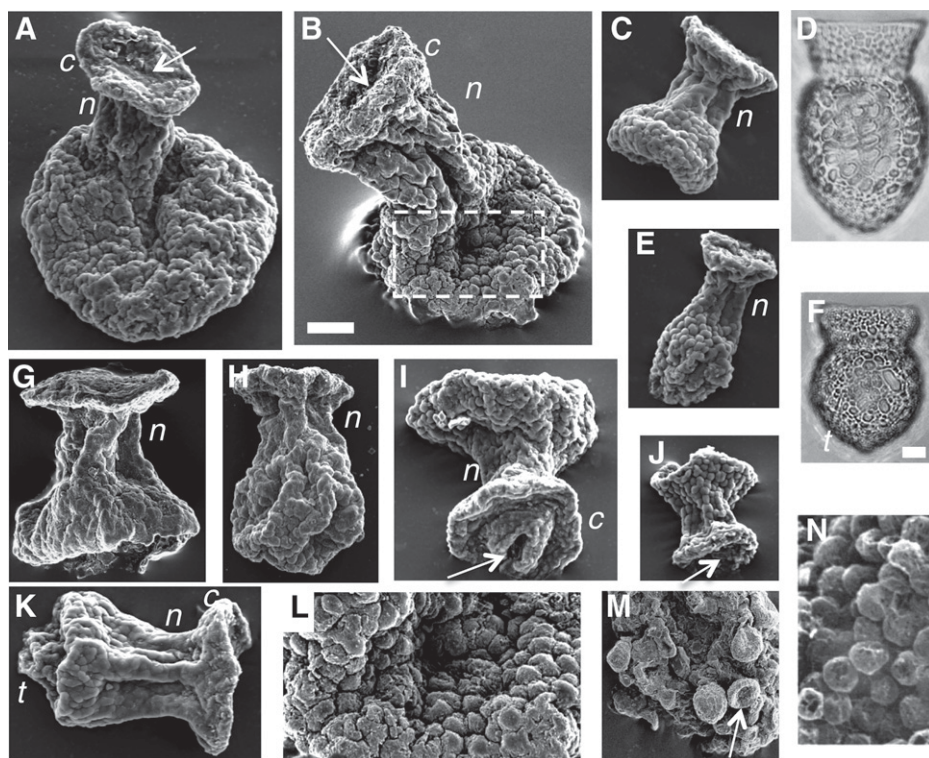


Figure 2. Scanning electron microscope micrographs of fossil tests (A–C, E, G–L are from F864 [height 30 and 60 m], M from F704 [height 242 m]), and comparisons with modern tintinnids (D, F, N). A, B: Side view of tests with deflated round bowls with constricted necks (n) and centrally invaginated collars (c, arrows). C, E–H: Side views of tests with flared collars and variably elongated bowls. D: Transmitted light micrograph (TLM) of polygonal alveolar structures on test of modern *Codonella elongata* (Fernandes, 2004). F: TLM of *C. apicata* (Fernandes, 2004) with tapering aboral end (t). I: Aperture in collar of fossil tests (arrow, front view). K: Small test with tapered bowl (t). L, M: Convex and collapsed, respectively (arrow in M), alveolar structures in walls of fossil tests in the areas outlined in B and Fig. DR3M [see footnote 1], respectively). N: Convex and collapsed (arrow) alveolar surface structures of modern *Tintinnopsis* sp. that are thought to facilitate flotation (Gold and Morales, 1976). Entire test is shown in Figure DR4. Scale bar in B: 20 μ m in A–E and G–K, 10 μ m in L–N. Scale bar in F: 20 μ m.

DR1 and DR2 in the GSA Data Repository¹), indicating that these structures were preserved during the deposition of the limestone and are not later contaminants. To date, the dissolution of calcite in acid yielded more than 100 three-dimensionally preserved dark orange-brown flask-shaped fossil tests (Fig. 2; for methods, see the Data Repository). The tests are 50–175 μ m long (median = 81 μ m, N = 105 specimens) and 46 ± 16 μ m wide (median = 43 μ m, N = 96), with one blunt end and one rounded, bowl-shaped end that is occasionally tapered (Fig. 2K; Fig. DR3). All tests have reticulated, ~5- μ m-thick walls formed by 2–5- μ m-diameter alveolar spheroids (Figs. 2 and 3; Fig. DR3). The blunt ends of all individuals thicken and/or widen into 16–55- μ m-wide, variably flaring collars (median = 36 μ m, N = 23) (Figs. 2A–2C, 2E, 2G–2K). Most individuals exhibit constrictions and less distinct reticulation immediately below the collar (Figs. 2C, 2E, 2G, 2H), but elongated, less constricted tests are also present (Fig. DR3). All examined collars preserve a central invagination consistent with a cemented or collapsed aperture (Figs. 2A, 2B, 2I, 2J; Fig. DR3) or remnants of such invagination. Carbonaceous material (Fig. 3B) is present both in and around the alveolate structures in the walls of all tests (Fig. 3D). The walls are flexible, and enclose a hollow interior, as shown by the expansion and collapse of tests upon hydration and desiccation (Fig. 2; Fig. DR3). The surfaces of tests from F704 (height 328 m) and F704 (height 242 m) were examined by the electron microprobe (N = 5) and found to contain Si, Al, Mg, K, Ti, and Ca (Fig. 3C; Fig. DR2), although the matrix contains primarily calcite (Figs. DR1 and DR2). Only quartz is detectable by X-ray diffraction analysis of ~15 extracted tests from F704 (height 242 m), indicating that other minerals constitute only a small proportion of the tests by weight and volume.

¹GSA Data Repository item 2011335, Figures DR1–DR4, is available online at www.geosociety.org/pubs/ft2011.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

INTERPRETATION

The combined characters of fossil tests from the Tayshir member closely resemble those used in taxonomic assignments of modern codonellid tintinnid ciliates (Alveolata: Ciliophora) (Figs. 2D, 2F, and 2N): the ~100- μ m-long tests have <50- μ m-wide apertures and distinctly constricted necks rimmed by ornamented or thickened collars (Fig. 2; Kofoid and Campbell, 1939). The pseudochitinous bowls of modern tintinnids can also taper into small or long horns (Gold and Morales, 1975). The similarity between modern tintinnid tests and the fossil tests extends to the ultrastructural level: the alveolar structures in the walls of exceptionally preserved fossil tests (Figs. 2L and 2M) match the closely packed ~2- μ m-diameter alveoli on the tests of various modern tintinnids (Gold and Morales, 1976; Fernandes, 2004). The rarity of tapering ends, the variable flaring of the collars, and the variable lengths and widths of bowls are consistent with the reported polymorphism within individual modern tintinnid species (e.g., Laval-Peuto and Brownlee, 1986). The oral diameter of the tests and the length of modern tintinnid tests vary independently, but the oral diameter strongly correlates both with the maximum and the preferred prey size of these predominantly herbivorous planktonic microbial eukaryotes (Dolan, 2010). The range of oral diameters of the fossil tests (Fig. 2) would suggest that their organismal precursors feasted on ~3–18- μ m-large particles or organisms.

Other groups of modern or fossil testate eukaryotes lack most of the characteristics preserved by the Tayshir tests. Some modern agglutinated arcellinids (lobose testate amoebae), *Diffugia biwae* (Yang and Shen, 2005), *D. urceolata*, and a number of psammobiotic (inhabiting sandy interstices) euglyphids (filose testate amoebae) like *Corythionella* sp., *Ogdeniella* sp., and *Alepiella* sp. (Meisterfeld, 2002), can have flask-shaped, ~100- μ m-long tests with flaring oral ends. However, these organisms lack thick collars, distinct constricted necks, and tapering bowls. Furthermore, all modern testate amoebae with flaring oral ends are heavily agglutinated and lack densely packed alveolar structures on the surface. Although the close association of aluminosilicates with the organic material in fossil tests is consistent with agglutination, a mechanism that

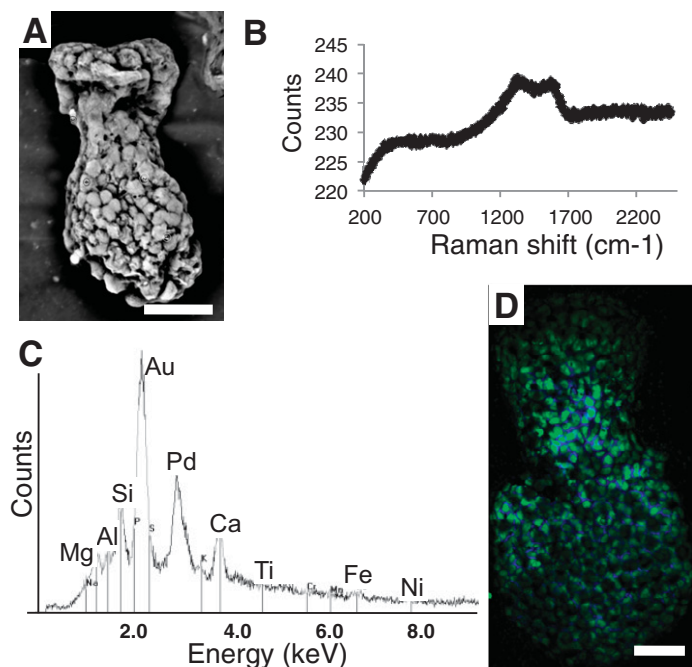


Figure 3. Compositional analyses of fossil tests. **A:** Backscattered scanning electron microscope micrograph of fossil test (F704 [height 242 m]). **B:** Raman spectrum of test showing two broad vibrational bands centered around 1340 and 1570 cm^{-1} ; characteristic of old carbonaceous material. **C:** Compositional spectrum of surface of sample shown in A (typical of other analyzed samples). Au and Pd are coating artifacts. **D:** Confocal epifluorescence micrograph of test (F704 [height 328 m]) stained by calcofluor white, fluorescent dye specific to glucans (Hageage and Harington, 1984). Alveolar structures (blue) and interstices (green) fluoresce differently, suggesting different organic compositions. Test became flattened during pre-staining NaOH treatment. Scale bars in A, D = 20 μm .

evolved during the Cryogenian (Bosak et al., 2011), the allochthonous origin of siliceous minerals in the Tayshir tests cannot be established unambiguously.

Flaring collars also characterize silicified or calcified vase-shaped tests from the late Ediacaran Dengying Formation (Hua et al., 2010), but the Dengying tests lack constricted necks, aboral processes, thickened and flaring collars, a flexible organic matrix composed of alveolar structures, and are 6–40 times longer than the Tayshir tests. Therefore, the Tayshir tests preserve a combination of morphological and ultrastructural characters that is diagnostic of modern tintinnids, and is absent from other fossil and modern eukaryotes.

IMPLICATIONS

Molecular clocks suggest that the Alveolata, a eukaryotic group that includes ciliates, dinoflagellates, and apicomplexans, diverged at 1360–950 Ma (Douzery et al., 2004; Berney and Pawlowski, 2006), and estimate the divergence of ciliates from Alveolata at 776 ± 92 Ma (Douzery et al., 2004), or at a much more recent time (after 500 Ma) (Berney and Pawlowski, 2006). The occurrence of possible ciliate tests in the Tayshir member supports the presence of Alveolata and ciliates much earlier than 635 Ma. This early date is also consistent with the organic biomarker record of gammacerane in the ca. 740 Ma Chuar Group (Summons et al., 1988), although the lipid precursor to gammacerane may be produced both by ciliates and by some bacteria (e.g., Kleemann et al., 1990). The oldest putative morphological fossils of ciliates, reported in the late Ediacaran phosphorites of the Doushantuo Formation in China (Li et al., 2007), are considerably younger and controversial (Dunthorn et al., 2010). There-

fore, putative tintinnids described here add to the currently sparse record of eukaryotes between the Sturtian and Marinoan glaciations (Knoll et al., 2006), extend the stratigraphic range of ciliate body fossils by >100 m.y., and testify to the increasing diversity of Neoproterozoic eukaryotes.

Signs of a transition from Mesoproterozoic oceans dominated by bacteria and unballasted organic vesicles to a modern carbon cycle that supported macroscopic heterotrophs are commonly sought in the latest Ediacaran Period (Logan et al., 1995; Butterfield, 2009). However, 715–635 Ma organic-rich tests of putative tintinnids illustrate new biological mechanisms that may have influenced the cycling of particulate and dissolved organic carbon much before the latest Ediacaran Period. Geochemical studies have identified the highly unusual and possibly quasi-static nature of the Neoproterozoic carbon cycle, marked both by large negative anomalies and prolonged times of very positive carbon isotope values (e.g., Kaufman et al., 1997; Shields et al., 2002; Rothman et al., 2003); the latter could have been the product of several conspiring factors, including enhanced carbonate weathering (Kump et al., 1999) and increased burial rates of organic carbon. The appearance of organisms with organic-rich recalcitrant tests (Holland, 2006) or those ballasted by mineral particles may have increased the export of organic carbon from the photic zone to deep oceans, and its subsequent burial: agglutinated and hyaline tintinnids constitute as much as 20% of the downward flux of organic matter in some areas of modern oceans and seas (Boltovskoy et al., 1993; Gonzalez et al., 2004). An increase in the fractional burial of isotopically light organic matter due to the Cryogenian radiation of planktonic organisms may have not only driven oceanic dissolved inorganic carbon to more enriched carbon isotope values, but may have also separated oxidants (O_2) from reductants (organic matter).

Modern tintinnids are primarily herbivorous planktonic phagotrophs and are preyed on by copepods (e.g., Dolan, 2010). Planktonic ciliates in the Neoproterozoic may have similarly fed the first animals. This increasing complexity of Cryogenian food webs may have driven the cycling of carbon to a quasi-static state (Rothman et al., 2003), without requiring a large reservoir of dissolved organic carbon. The addition of top-down control (predation by ciliates and other phagotrophic protists) to the bottom-up control (competition for nutrients) of bacterial and phytoplankton growth rates can stimulate the accumulation of organic carbon in a nutrient-rich water column (Thingstad et al., 1997), while the increasing complexity of food webs should enhance respiration in the upper ocean (Legendre and Rivkin, 2009). The emerging body fossil record and the organic biomarker record of the 716–635 Ma strata will help identify temporal relationships between the changes in trophic diversity, the emergence of various biological mechanisms and interactions (e.g., resistant polymers, hard body parts such as agglutinated or rigid tests, predation, herbivory), and changes in the cycling of carbon and oxidants during that time.

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