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Chitin in microfossils from the ca. 1.0 Ga Lakhanda Group, Siberia and its implication for skeletal biomineralization

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The co-evolution of life and minerals had profoundly shaped Earth's biological and geological history. One striking example is the evolution of skeletal biomineralization in eukaryotes, which profoundly impacted ecological dynamics and Earth's geochemical cycles. Skeletal biomineralization emerged in eukaryotes >800 Ma years ago, likely driven by atmospheric oxygenation, changes in seawater chemistry, and advent of predation. Skeletal minerals are organo-mineral composites, with organic matrices guiding mineralization by binding ions and organic macromolecules to direct nucleation and crystal growth. Chitin, a long chain polymer of N-acetyl glucosamine, serves as a widely-used organic template in this process. While chitin is widespread as modern skeletal biomineral, when early eukaryotes acquired it remains uncertain. Until now, chitin in protist body fossils has only been identified in rocks as old as 505 Ma. To shed light on the evolutionary history of chitin, we investigated the presence of fossilized chitin in the exquisitely well-preserved eukaryotic fossils from the ca. 1 Ga Lakhanda Group (Siberia), using a combination of high-resolution spectroscopy and biochemical methods. To identify whether the Lakhanda Group microfossils contain chitin, shale thin sections were labelled *in situ* with Wheat Germ Agglutinin-FITC, a fluorescence dye that selectively binds to chitin. Several spherical fossils, measuring 100–200 µm in diameter, fluoresced under confocal microscopy, indicating the presence of vestigial chitin. To further confirm the presence of chitin, we used nano-IR technique, atomic force microscopy infrared spectroscopy (AFM-IR), to study *in situ* molecular composition of these fossils. The AFM-IR high-resolution infrared spectra showed peaks near 1650 cm^{-1} (corresponding to C=O stretching vibrations in chitin peptide bonds; amide I band) and 1550 cm^{-1} (amide II band, arising from N-H bending and C-N stretching vibrations), as well as polysaccharide-related bands at 1040–1080 cm^{-1}. These features diagnostic of chitin have been used to confirm the presence of chitin in both modern and fossilized material in previous studies [1,3]. Overall, these findings extend the timeline for chitin utilization in protists by 500 Ma, demonstrating its role in early protist evolution and in shaping biomineralization pathways.

[1] Ehrlich, H. https://doi.org/10.1080/00206811003679521
[2] Bonneville, S. *et al.*1126/sciadv.aax7599