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Unraveling biological information from early single-celled eukaryotes in the Paleo-Mesoproterozoic Ruyang Group, North China

Pang, K.[1], Tang, Q.[2], Xiao, S.[2], Yuan, X.[1], Wan, B.[1] and Schiffbauer, J.D.[3]

[1]Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, No.39 East Beijing Road, Nanjing, 210008, China, kepang@nigpas.ac.cn
[2]Department of Geosciences, Virginia Tech, 4044 Derring Hall, Blacksburg, VA 24061, USA
[3]Geological Sciences, University of Missouri, 101 Geology Building, Columbia, MO 65211, USA

Molecular clock estimates suggest that the Eukarya clade may have split from their living sister group in the Archean [1], but the divergence of major eukaryote crown groups from the last eukaryotic common ancestor (LECA) likely occurred by the Mesoproterozoic [2–4]. Thus, the Paleo-Mesoproterozoic fossil record of eukaryotes is critical to resolve the morphological evolution of early eukaryotes. The Paleo-Mesoproterozoic Ruyang Group of North China Craton hosts some of the earliest known unambiguous eukaryotic fossils (acritarchs) such as, *Valeria*, *Dictyosphaera*, and *Shuiyousphaeridium*, and thus offers valuable insights into the origin and early evolution of single-celled eukaryotic life. In this study, we carried out a biomechanical and functional biological investigation of *Valeria lophostriata*, and critical and comprehensive analyses of intracellular inclusions (ICIs) in *Dictyosphaera delicata* and *Shuiyousphaeridium macroreticulatum*, to develop a thorough understanding of their biological information.

The Ruyang *V. lophostriata* is investigated using transmitted light microscopy (TLM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and biomechanical analysis. *V. lophostriata* is reconstructed as a spherical vesicle with two hemispherical halves bearing concentric striations resembling latitudinal circles. The formation of striations could be explained using the Belousov-Zhabotinsky reaction model or the Turing reaction-diffusion model. A biomechanical analysis using the thin-walled spherical pressure vessel model suggests that the concentric striations of *V. lophostriata* may have functioned as a mechanism to guide biologically programmed excystment through medial split. Our analysis provides essential paleontological data to better understand the functional biology and life cycles of early eukaryotes such as *Valeria*.

The debate on the nature and origin of ICIs in the early Nerproterozoic Bitter Springs microfossils and the Ediacaran Doushantuo animal embryo-like fossils [5–7] prompted a critical investigation of ICIs in the Ruyang *D. delicata* and *S. macroreticulatum* using a suite of characterization approaches: SEM, TEM, and focused ion beam scanning electron microscopy (FIB-SEM). Although the Ruyang acritarchs must have had nuclei when alive, our data suggest that their ICIs represent neither fossilized nuclei nor taphonomically condensed cytoplasm. We instead propose that these ICIs likely represent biologically contracted and consolidated eukaryotic protoplasts (the combination of the nucleus, surrounding cytoplasm, and plasma membrane). As opposed to degradational contraction of prokaryotic cells within a mucoidal sheath—a model proposed to explain the Bitter Springs ICIs, our model implies that protoplast condensation in the Ruyang acritarchs was an *in vivo* biologically programmed response to adverse conditions in preparation for encystment. While the discovery of *bona fide* nuclei in Paleo-Mesoproterozoic acritarchs would be a substantial landmark in our understanding of eukaryote evolution, the various processes (such as degradational and biological condensation of protoplasts) that

can produce nuclei-mimicking structures require that interpretation of ICIs as fossilized nuclei be based on comprehensive investigations.

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