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Biological and taphonomic implications of Ediacaran fossil embryos undergoing cytokinesis

Yin Zongjun [a], Liu Pengju [b], Li Gang [c], Paul Tafforeau [d], Zhu Maoyan [a,*]

[a] State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China
[b] Institute of Geology, Chinese Academy of Geological Sciences, Beijing 100037, China
[c] Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[d] European Synchrotron Radiation Facility, Grenoble 38000, France

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ABSTRACT

A collective of three-dimensionally (3D) phosphatized animal embryos with elongated olive shapes was discovered from the fossiliferous Ediacaran Doushantuo Formation (635–551 Myr) in Weng'an, Guizhou, southwest China. In order to reconstruct 3D architectures of the specimens without destroying them, a non-destructive imaging technique termed propagation phase contrast synchrotron X-ray microtomography (PPC-SR-μCT) was applied in concert with scanning electron microscopy (SEM). The results show that the embryos were enclosed in a thin capsule with an ornament of cell-like polygons on the surface. Moreover, each elongated specimen consists of two blastomeres inside the sculptured capsule, and the geometric relationship of the two blastomeres in each specimen suggests that these specimens probably represent cleaving embryos during different phases of cytokinesis. This is the first detailed description of Doushantuo fossil embryos undergoing cytokinesis, and this new discovery supports the view that not all of the ornamented Doushantuo embryos are at diapause stage. Additionally, the rare phenomenon that the dynamic cytokinesis process was captured and fixed in fossils yields valuable insights into the taphonomic process of animal embryos before early diagenesis.

1. Introduction

As one of the oldest known fossil record of metazoans on the planet, three-dimensionally phosphatized embryos with excellent preservation of cellular and sub-cellular structures from the Ediacaran Doushantuo Formation in Weng'an, southwest China, provide a unique Precambrian window for exploring the origin and early evolution of animals and potentially for testing the hypotheses of evolutionary development (Xiao et al., 1998, 2007a; Xiao and Knoll, 2000; Chen et al., 2006, 2009a, 2009b; Yin et al., 2013). However, these exquisite embryo fossils are accompanied by ontogenetic biases and taphonomic artifacts (Hagadorn et al., 2006; Xiao et al., 2007a). On one hand, they are dominated by uncleaved eggs and early cleavage-stage embryos sculptured by various ornaments (Xiao and Knoll, 2000). The simple construction of these fossils yields very few phylogenetic signals, which has led to alternative interpretations as giant sulfur bacteria (Bailey et al., 2007) and encysting protists (Huldtgren et al., 2011, 2012). However, many recent publications provide direct evidence of later stage Doushantuo embryos of a variety of forms, including some with bilaterally symmetric blastomere arrangements, micromere caps overlying macromere blastomeres, and clearly diverse cell types (Chen et al., 2006, 2009a, 2009b; Yin et al., 2013). Furthermore, abundant indirect evidence indicates that these Doushantuo microfossils could not parsimoniously be interpreted as sulfur bacteria or encysting protists (Xiao et al., 2007b, 2012; Yin et al., 2007; Cunningham et al., 2012a; Schiffbauer et al., 2012). Even so, all the ornamented eggs and embryos were interpreted as resting eggs (dormant embryos) because similar ornament patterns can be observed in the living lower invertebrate resting eggs (Xiao et al., 1998; Xiao and Knoll, 2000).

On the other hand, the taphonomic processes and mineralization mechanisms of animal embryos are fascinating topics (Xiao and Knoll, 1999; Martin et al., 2003, 2005; Dornbos et al., 2005; Hubert et al., 2005; Dornbos et al., 2006; Donoghue et al., 2006; Raff et al., 2006, 2008; Gostling et al., 2008, 2009; Xiao and Schiffbauer, 2009; Dong et al., 2010; Cunningham et al., 2012a, 2012b; Schiffbauer et al., 2012) as more and more fossil embryos have been found from Precambrian and Cambrian deposits (Zhang and Pratt, 1994; Bengtson and Yue, 1997; Xiao et al., 1998; Dong et al., 2004; Steiner et al., 2004; Zhang et al., 2011). Despite the fact that recent studies, including taphonomic experiments under laboratory conditions (e.g., Martin et al., 2003, 2005; Raff et al., 2006, 2008; Cunningham et al., 2012a) and diagenetic analysis of embryo fossils (e.g., Xiao and Knoll, 1999; Xiao and Schiffbauer, 2009; Cunningham et al., 2012b; Schiffbauer et al., 2012), have greatly expanded our knowledge about the decay and mineralization processes of animal embryos, the

* Corresponding author. Tel./fax: +86 25 83282159.
E-mail address: myzhu@nigpas.ac.cn (M. Zhu).

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entire fossilization process and the underlying mechanisms still remain unclear.

Herein, some particular elongated, olive-shaped embryo fossils enclosed by a thin capsule with two categories of cell-like polygonal ornaments are present. To reveal their internal structures and avoid destroying them, propagation phase contrast synchrotron X-ray microtomography (PPC-SR-μCT) was utilized to reconstruct their three-dimensional architectures. In combination with scanning electronic microscopy (SEM) imaging, the new data from this study forces us to reappraise the scope of application of the "resting egg hypothesis" (Xiao et al., 1998; Xiao and Knoll, 2000) as a biological interpretation for the ornamented, spherical Doushantuo microfossils. More importantly, the well-preserved elongated embryo fossils, which captured a snapshot of the cytokinesis process, have contributed to our understanding of the early taphonomic processes of the embryo fossils. Therefore, this study is not only helpful for us to figure out the affinities and preservation mechanism of these Doushantuo embryo fossils, but also a contribution to the investigation of the origin and early evolution of animals in the Gondwana continent.

2. Materials and methods

The animal embryos are preserved in the Upper Phosphate Member (or Weng'an Phosphate Member, ca. 580 Myr) of the Doushantuo Formation at Weng'an phosphate mining area in Guizhou Province, southwest China (Zhu et al., 2007; Chen et al., 2009a). This member is composed of an upper gray dolomitic phosphorite layer and a lower black phosphorite layer (Zhu et al., 2007; Chen et al., 2009a). Samples for this study were collected from the gray lithofacies, and then digested in diluted acetic acid (acid concentration is 7%). The liberated microfossils in the acid residue were picked by hand under a stereomicroscope.

Selected embryo fossils were examined by scanning electron microscopy, and well-preserved candidates with potential internal structures were screened at experiment station BL13W1 of the Shanghai Synchrotron Radiation Facility (SSRF, Shanghai, China) using PPC-SR-μCT. Valuable specimens with resolvable and well-preserved internal details were then scanned at the beamline BM5 and ID19 of the European Synchrotron Radiation Facility (ESRF, Grenoble, France) with higher spatial resolution. PPC-SR-μCT is a powerful technique for non-destructive 3D imaging. It not only allows observation of exposed surface configurations of embryo fossils, but also reveals their internal details which are invisible or hardly visible by classical absorption contrast-based microtomography and conventional imaging methods such as SEM (Donoghue et al., 2006; Chen et al., 2009a, 2009b).

Experiment parameters used at ESRF are as follows: 1800 projections over 180° were obtained for each scan, 17.78 keV (BM5) and 19.19 keV (ID19) were adopted as X-ray energies. Depending on the sizes of fossil specimens, two CCD-based high resolution detectors with isotropic voxel sizes of 0.56 (ID19) and 0.75 μm (BM5) were used. In order to get phase contrast effect, 10 mm (ID19) and 30 mm (BM5) were adopted as the propagation distances (sample–detector distance). In addition to the simple edge detection mode, we applied a single distance phase retrieval process (Paganin et al., 2002) for partial embryos. This process permits retrieval of high quality differential contrast of almost the same quality achieved by holotomography, but with far easier acquisition and reconstruction protocols. 3D volume data processing was performed using software VGstudio Max 2.1 (Volume Graphics, Heidelberg, Germany).

3. Description of fossil embryos undergoing cytokinesis

A large number of sculptured animal eggs with various ornament patterns have been reported from the Weng'an Doushantuo phosphorite (Xiao and Knoll, 2000; Xiao et al., 2007b), and the majority contain only one internal body (cell) within the thick, multilayered and ornamented envelopes. However, a distinct form characterized by regular polygonal ornaments and more than one blastomere inside a thin single-layered capsule can be distinguished from them (Chen et al., 2009a; Yin and Zhu, 2012). All ornamented embryos (eggs) from the Weng'an biota can be assigned to two "types", the former, with multilayered envelopes, was named type-A and the latter, with single-layered capsules, type-B (Yin and Zhu, 2012). Two categories of polygons have been discovered in type-B embryos so far (Xiao et al., 2007b; Chen et al., 2009a), the small one is 10–15 μm in average diameter (Fig. 1A1–A2, B1–B2, D1–D3, E1–E2) while the big one is about 80–100 μm in diameter (Fig. 1C1–C3).

Intriguingly, some elongated type-B embryos have been discovered, a selection of them is shown in Fig. 1, exhibiting significant variation in the shape of the cell-like polygons in the equatorial part of specimens (Fig. 1A1–E2). We propose that the elongated olive shape rather than spherical shape of the specimens is not due to mechanical deformation during post-mortem processes, but represents developmental deformation during embryo cleavage (cell division) from a one cell stage to a two-cell stage.

This interpretation is based on the following observations: 1) the elongated shape of the cell-like polygons only appears in the equatorial region (Fig. 1), and the elongation of the polygons shows gradual transition from the equator to the two poles (Fig. 1A1–A2, D1–E2). 2) All the elongated polygons are in the same smooth peripheral plane as the normal ones (Fig. 1A1, D1–E1). It is hard to imagine that the surface would retain its smoothness if it had been mechanically deformed. 3) The first cytokinesis of an embryo, viz cell division from zygote to the two-cell stage, is a dynamic process during which the embryo shape changes from spherical to olive-shape. The shape changes displayed in the fossil specimens are comparable to that which occurs in the living forms. 4) The PPC-SR-μCT data illustrates that all the elongated specimens contain two blastomeres inside their capsules (Figs. 2–3). This is the direct evidence supporting our interpretation. And 5) all the features are not occasional phenomena but repeated traits developed in a number of specimens (Fig. 1).

In addition, the compaction degree of the two blastomeres in different specimens are slightly modified. For example, the two blastomeres of specimen WB1-F02 (Fig. 3B1–B2) are less compacted, leading to a larger major to minor axis ratio than that of the others (Figs. 2–3). The minute variations displayed in these specimens probably represent embryos at different phases of the first cytokinesis, because similar features are commonly observed in living animal embryos at the same developmental stage.

Recently, Huldtgren et al. (2011) claimed that they discovered evidence for ongoing mitosis in a Doushantuo embryo-like fossil, however, they failed to prove their argument. Because, on one hand, the "nuclei" during division (the elongated nuclei-like structures) found in a specimen are unlikely to be preserved true nuclei but taphonomic artifacts (Schiffbauer et al., 2012; Xiao et al., 2012). On the other hand, the shapes of the cells containing elongated and dumbbell-shaped nuclei-like structures, as illustrated by Huldtgren et al. (2011), still remain polyhedral, and no shape variations can be observed. This fact is inconsistent with the cytokinesis characteristic of cells (without cell walls) during ongoing mitosis. Moreover, the "nuclei during division" were observed in only one specimen, which was selected from hundreds of Doushantuo embryo-like fossils (Huldtgren et al., 2011). We also have checked a large number of Doushantuo fossils with PPC-SR-μCT, and no similar structure has been discovered. The striking scarcity of the so-called "nuclei during division" in Doushantuo fossils obviously cast a cloud over the interpretation proposed by Huldtgren et al. (2011, 2012).

4. Biological nature of embryos with ornament of cell-like polygons

Using the resting egg hypothesis, both type-A and type-B embryo (egg) fossils from the Weng'an biota have been interpreted as