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Early embryogenesis of potential bilaterian animals with polar lobe formation from the Ediacaran Weng'an Biota, South China

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

[a] State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China
[b] European Synchrotron Radiation Facility, 6 rue Jules Horowitz, Grenoble 38000, France
[c] Institute of Geology, Chinese Academy of Geological Sciences, Beijing 100037, China
[d] Institute of High Energy Physics, Beijing 100049, China

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ABSTRACT

Exquisite phosphatized eggs and embryos from the Ediacaran Doushantuo Formation (Weng'an biota, ca.580Ma) of Guizhou, southwestern China have greatly expanded our knowledge of the diversity and palaeobiology of early multicellular animals, and contributed to our understanding of the origins of sponge, cnidarian and other potential eumetazoans. However, the key question of whether triploblastic bilaterian animals are present in the Weng'an biota remains controversial. In the present study, scanning electronic microscopy (SEM) and propagation phase contrast synchrotron X-ray microtomography (PPC-SR-μCT) are used to prove the presence of bilaterian embryos in the Doushantuo phosphorite. Based on the well preserved connection neck between the polar lobe (PL) and the egg or blastomere as shown by the high-resolution internal sections conducted by PPC-SR-μCT, three types of PL-forming embryos (calabash-shaped (1-cell stage), trefoil and L-shaped (2-cell stage)) have been confirmed in the Doushantuo phosphorite. These PL-forming embryos are distinct from the PL-forming-like embryos, multicellular algal fossils, and even diagenetic artifacts in morphology and anatomy. In addition, an early developmental sequence for these PL-forming embryos can be inferred for the first time. The results not only provide more convincing evidence for animal affinities among the Doushantuo embryos, but also indicate that polar lobes, which break the symmetrical distribution of morphogenetic determinants in cytoplasm during the early developmental stages of living bilaterian embryos, are a conserved device that occurred already during animal embryogenesis even in the Precambrian.

1. Introduction

As one of the oldest fossil records of metazoans, phosphatized animal embryos with three dimensionally preserved cellular and sub-cellular details from the Ediacaran Weng'an biota (Doushantuo Formation) in Guizhou, southwest China have opened a unique window for exploring the origin and early evolution of metazoans and even triploblastic bilaterians (Li et al., 1998; Xiao et al., 1998, 2007; Chen et al., 2006, 2009a,b; Hagadorn et al., 2006). These Doushantuo animal fossils are dominated by uncleaved eggs and cleavage-stage embryos; specimens at later developmental stages such as gastrulae, however, are extremely rare (Chen et al., 2009b). Up to now, no compelling evidence for fossil larvae and adults has been discovered. Presumed animal adults, including several morphospecies of tubular microfossils (Xiao et al., 2000; Chen et al., 2002; Liu et al., 2008) and microscopic bilaterian *Vernanimalcula* (Chen et al., 2004), are not yet widely accepted (Bengtson and Budd, 2004; Donoghue et al., 2006; Liu et al., 2010). Despite the fact that the different sizes, various ornament types and cleavage patterns may imply a relatively high biodiversity for these cleavage-stage embryos (Xiao and Knoll, 2000; Chen, 2004; Chen et al., 2009a), they provide very limited phylogenetic information because of their simply constructed morphologies. Therefore, whether bilaterian animals exist in the Weng'an biota remains a mystery.

To resolve the mystery, comparison of cleavage patterns and developmental sequences of these embryo fossils with those of extant bilaterians might provide an effective resolution. In extant bilaterians, a variety of lophotrochozoan animals, including many mollusks and annelids, utilize polar lobe (PL) formation as an important mechanism to break symmetric localization of cytoplasmic egg substance during early embryogenesis (for review, see Dohmen, 1983). PL-forming embryos have characteristic morphologies including calabash-shaped, trilobed, L-shaped and even five-lobed structures (Fig. 1, Tompa et al., 1984). It is no wonder that the first report of fossilized PL-forming embryos from the Doushantuo phosphorite in Weng'an attracted widespread

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

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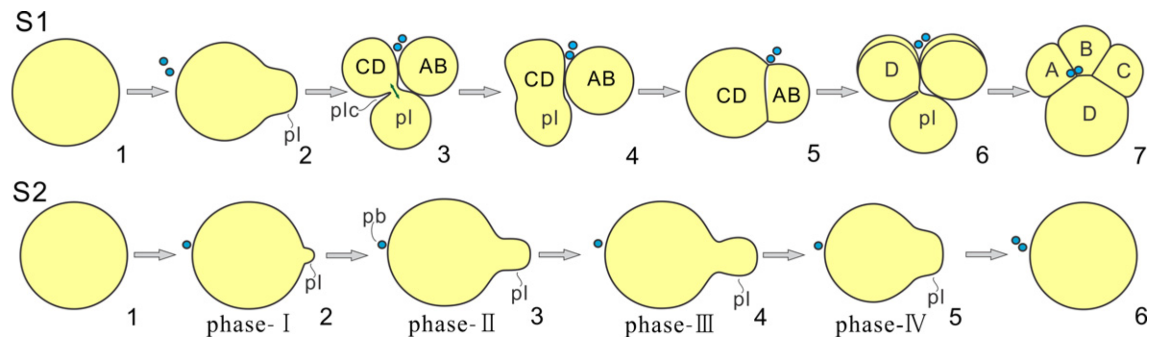

Fig. 1. Diagram showing early cleavage stages of polar lobe-forming embryos of living bilaterian animals (Conrad, 1973; Gilbert and Raunio, 1997). S1 is a development sequence from zygote to 4-cell stage embryo. S1-1, zygote; S1-2, calabash-shaped embryo; S1-3, embryo at trefoil stage, note the polar lobe connects with CD blastomere via a polar lobe neck (polar lobe constriction, plc); S1-4, L-shaped embryo with a large diameter of plc; S1-5, 2-cell stage embryo, CD blastomere is bigger than the AB; S1-6, five-lobed embryo; S1-7, 4-cell stage embryo with a big D blastomere. S2 is a development sequence displaying dynamic shape changes of the polar lobe before the first cell division. The whole process could be divided into four phases according to length of egg and diameter of plc (Conrad, 1973). The embryos have the biggest length and the smallest diameter of plc at Phase-III. Abbreviations: pl, polar lobe; pb, polar body; plc, polar lobe constriction.

attention (Chen et al., 2006). Nevertheless, it is difficult to differentiate the PL-forming fossils from other fossils with similar morphology in the Doushantuo phosphorite. Meanwhile, due to the insufficient contrast level of synchrotron X-ray microtomographic images presented by Chen et al. (2006), the key internal structures of these PL-forming fossils remain unclear, which has further cast doubt on PL-forming embryos in the Doushantuo phosphorite.

In order to test the reliability of the PL-forming embryo fossils from the Doushantuo Formation, the present study proposes identification criteria to distinguish the PL-forming embryos from other embryos, multicellular algae and diagenetic artifacts based on investigations of morphological characteristics and internal microstructures of abundant Weng'an fossil specimens, using improved propagation phase contrast synchrotron X-ray microtomography (PPC-SR-μCT) with phase retrieval process, and scanning electron microscopy (SEM). Our results confirm the presence of PL-forming embryo fossils in the Doushantuo phosphorite. In addition, a putative developmental sequence of the PL-forming embryos is reported here for the first time.

2. Materials and methods

The Weng'an biota is preserved in the Upper Phosphate Member (also called Weng'an Phosphate Member by some authors, e.g., Dornbos et al., 2005, 2006; Chen et al., 2009a) of the Doushantuo Formation at Weng'an phosphate mining area in Guizhou Province, southwestern China (Zhu et al., 2007). The Upper Phosphate Member consists of two different taphonomic facies: a grey facies and a black one (Dornbos et al., 2005, 2006). They are represented by grey dolomitic phosphorite and black phosphorite, respectively. All samples for this study were collected from the grey lithology, and then digested in dilute acetic acid (acid concentration is between 5% and 10%). The insoluble acid residue was then washed and dried. The liberated microfossils in the acid residue were checked under a stereomicroscope and picked by hand with the help of a fine brush. Selected embryo fossils were examined under SEM and by means of PPC-SR-μCT.

PPC-SR-μCT provides a powerful nondestructive technique which allows analysis of both exposed surface configurations and internal microstructures of 3D specimens. Thanks to the monochromaticity, high beam intensity and partial coherence of hard X-ray beams produced by third generation synchrotron radiation facilities, such as the European Synchrotron Radiation Facility (ESRF, Grenoble, France), PPC-SR-μCT can bring far better data quality than conventional microtomographs by means of the reducing beam hardening effect, submicron resolution and applying propagation phase contrast imaging to reveal microstructures which are invisible or poorly visible using the traditional absorption contrast X-ray imaging technique (Tafforeau et al., 2006).

All the microtomographic volume data for this study were obtained at the beamline ID19 of ESRF. We used a recently installed new undulator source delivering a single harmonic at 17.68 keV. The relative monochromaticity of that source is so good that it is not necessary to use any monochromator, leading to a perfectly stable high quality beam. Depending on the sizes of fossil specimens, two CCD-based high resolution detectors with isotropic voxel sizes of 0.56 and 0.7 μm were used. In order to get a phase contrast effect, 10 mm and 12 mm 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 derived from the Paganin algorithm (Paganin et al., 2002; Friis et al., 2007). When applied to some fossils, it permits retrieval of high quality differential contrast very similar to those achieved by holotomography, but requiring far more simple acquisition and reconstruction protocols. 3D digital volume data processing was performed using software VGstudio Max 2.1 (Volume Graphics, Heidelberg, Germany). All the digital data containing original reconstructions of the scans, fossil plates and supporting movies are available online in the free access paleontological database at http://paleo.esrf.eu. All the samples described in this paper are housed at the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences.

3. Phosphatized Doushantuo polar lobe-forming embryos

3.1. Previous reports of polar lobe-forming embryo fossils

Chen et al. (2006) reported three dimensionally phosphatized microfossils similar to modern bilaterian embryos with PL formation, and figured putative trefoil stage samples which consist of two blastomeres (AB and CD) and a PL. They employed synchrotron X-ray microtomography to study these early embryos for the first time, and tried to reveal the relationships between the three lobes of trilobed samples. However, due to the limited number of scanned specimens (Fig. 2 in Chen et al., 2006) and insufficient contrast level of virtual slice images (e.g., Fig. 2a-a[1] in Chen et al., 2006), they were unable to detect the expected connection neck between the CD blastomere and PL. Hence, these presumed polar lobe-forming embryo fossils were identified mainly based on fossil shape and volume ratio of AB blastomere to CD blastomere + PL [$V_{AB}/(V_{CD} + V_{pl})$]. Since the internal structures of these fossils are unclear, there are three possible arguments against the hypothesis that these are PL-forming embryos. First, calculation of the volume ratio might not provide a reliable diagnostic indicator for PL-forming embryos