Der **Biologie-Preis 2016** wurde Dr. Zongjun Yin, Berlin, in Anerkennung seiner bahnbrechenden Forschungen über den Ursprung der Metazoa (Tiere) und deren frühen fossilen Bericht im Ediacarium von China verliehen.

Zongjun Yin

The Early Evolution of Animals: Insights from the 600 million-year-old Ediacaran Weng'an biota

Introduction

Dr. Zongjun Yin, Institute of Geology and Paleontology, Nanjing, China, Träger des Biologie-Preises 2016

The past three decades have witnessed profound progress in our understanding of the origin and early evolution of metazoans. New insights are being derived from molecular phylogeny and comparative development biology (Davidson and Erwin, 2009; Erwin et al., 2011, Cannon et al., 2016). In addition, discoveries of early animal fossils, especially the animal embryos from the Ediacaran and Cambrian deposits in China, provide evidential tests of evolutionary hypotheses (Xiao et al., 1998; Li et al., 1998; Dong et al., 2004; Chen et al., 2006, 2009a, 2009b; Yin et al., 2007).

Since 1998, the phosphatized animal embryo microfossils with cellular and sub-cellular structures preserved in three-dimensional detail from the Ediacaran Doushantuo Formation at Weng'an phosphate mining area (Guizhou Province, southwest China), have been considered as one of the oldest fossil metazoan records on the planet (Li et al. 1998, Xiao et al. 1998). In spite of the fact that these Doushantuo embryo fossils are taphonomically biased, during the last decade, studies of animal fossils from this unique late Neoproterozoic taphonomic window have profoundly improved our outstanding of the evolution of multicellular animals in the Precambrian (Chen et al. 2000, 2002, 2004a, 2006, 2009a, b, Xiao & Knoll 2000, Hagadorn et al. 2006, Xiao et al. 2007). The different embryo sizes and ornamentations (Xiao & Knoll 2000), various embryo cleavage patterns (Chen et al. 2006, 2009a) and potential fossil gastrulae (Chen et al. 2000, 2009a, Xiao et al. 2007), as well as problematic animal adults (Xiao et al. 2000, Chen et al. 2002, 2004) indicate that the diversity of multicellular animals before the Cambrian radiation was much higher than previously thought.

However, these exquisite Precambrian animal fossils remain to some extent controversial because of alleged taxonomic and ontogenetic biases. The majo-

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rity of the Ediacaran Doushantuo animal fossils initially discovered were early cleavage-stage embryos undergoing equal holoblastic cell division which led to interpretation of giant sulfur bacteria (Bailey et al., 2007) and encysting protists (Huldtgren et al., 2011). Though some 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 quartets or duets, and clearly diverse cell types (Chen et al., 2009a, 2009b), moreover, some cleavage stage forms with very particular structures still to be found in modern forms such as polar lobe-bearing embryos and duet cleavage patterns have been identified (Chen et al., 2006, 2009a), the actual phylogenetic affinities of all bilaterian-like Precambrian embryo fossils remain enigmatic, except for the general point that they represent a considerable variety of forms.

Recently, we discovered some new animal fossils from the Ediacaran Weng'an biota, including embryonic and adult forms (Yin et al., 2015, 2016). These discoveries are not only helpful to resolve the controversial problems mentioned above, but also cast new lights on the early evolution of animals.

Fossil materials and method

The microfossils from the Weng'an biota are preserved in the Upper Phosphate Member of the Doushantuo Formation at Weng'an phosphate mining area in Guizhou Province, southwest China. This member is composed by upper grey dolomitic phosphorite layer and lower black phosphorite layer. Fossil samples for this study were collected from the grey lithology, and then digested in dilute 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 ones with potential internal structures were scanned at the beamline ID19 of the European Synchrotron Radiation Facility (ESRF, Grenoble, France) using propogation phase contrast based synchrotron X-ray microtomography (PPC-SR-μCT).

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. We used an undulator source which can deliver a single harmonic x-ray with the energy of 17.68 keV at beamline ID19. The relative monochromaticity of the beam is so good that it is not necessary to use a monochromator. Depending on the sizes of fossil specimens, a CCD-based high resolution detector with isotropic voxel sizes of 0.56μm was used. In order to get phase contrast effect, 10mm were adopted as the propagation distances. In addition to the simple edge detection mode, we applied a single distance phase retrieval process for partial embryos. This process permits retrieval of high quality differential contrast nearly the same as that achieved by holotomography, but requiring far more simple acquisition and reconstruction protocols.

3D volume data processing was performed using software VGstudio Max 2.1 (Volume Graphics, Heidelberg, Germany). All the samples described in this paper are housed at the Nanjing Institute of Geology and Paleontology, Chinese Academy of Sciences.

Results

Meroblastic embryos

Ediacaran Doushantuo embryo-like fossils (EDEFs, ca. 600 Ma) from the Weng'an biota display cellular and sub-cellular structures and provide a unique window on the early evolution of multicellular eukaryotes.

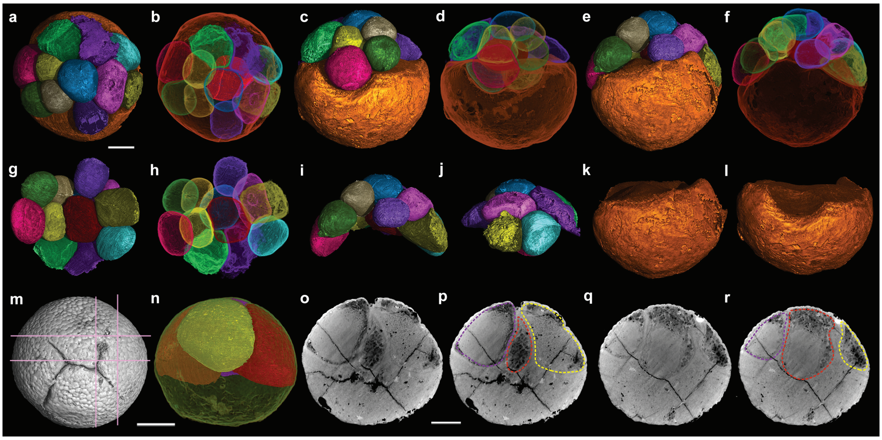

Fig. 1: Meroblastic embryos from the Weng'an biota (synchrotron X-ray tomographic data): a-f, different views of the same specimen. b, d and f are transparent patterns of a, c, and e, respectively. g-j, different views of the cellular disc. k and l, big yolk cell. M, surface rendering. N, transparent pattern. o and q, digital slices, the cell boundaries were marked in p and r. The scale bar represents 100 microns

But there have been widely disparate interpretations of these fossils. Recently, the debate on the EDEFs has begun to crystallize into two competing interpretations: (1) that the EDEFs represent crown metazoans, or (2) that they represent stem metazoans or nonmetazoan holozoans. These two competing interpretations have very different implications for the timing and tempo of animal diversification. If the EDEFs are crown metazoans, a deep Precambrian history of animals is implied, whereas if they are stem or non-metazoans, the fossils do not reduce the gap between molecular clockestimates and the fossil evidence for the early divergence of metazoans. It is difficult to reconcile these competing interpretations because these morphologically simple EDEFs yield very little phylogenetic information.

In 2016, we reported fossil embryo-like forms from the Weng'an biota that exhibit a meroblastic cleavage pattern (Yin et al., 2016). The results from high-resolution propagation phase contrast synchrotron radiation X-ray microtomography demonstrate that these fossils preserve features directly comparable to those of modern meroblastic animal embryos that utilize discoidal cleavage. The cell cleavage occurred on the animal pole, and the other pole of the embryos is a big uncleaved cell full filled with yolk mass.

As informative biological clues, the cleavage patterns are useful for testing the diverse interpretations of the EDEFs, but until now they have been overlooked. The initially described EDEFs display simple holoblastic cleavage during early stages of ontogeny. Such a palintomic cleavage pattern is found in animal embryos but also in some non-metazoan holozoan clades (Huldtgren et al., 2011). However, within total-group Holozoa, the discoidal-type meroblastic cleavage patterns like those presented here are only reported in bilaterians (Gilbert and Raunio, 1997; Rensing, 2016).

Given that discoidal-type meroblastic cleavage occurs only in metazoans, the phylogenetic positions of these fossils probably fall into the animal branch of the holozoan tree. Meroblastic as well as holoblastic cleavage forms were thus present by ca. 600 million years ago, substantiating the conclusion derived from molecular clock estimates that a variety of metazoan lineages had evolved by the mid-Ediacaran after the termination of the Marinoan glaciation (635 million year ago), if not earlier.

Our findings also support the conclusion that at least some EDEFs possibly represent crown-animals, although their phylogenetic affinity cannot be established because discoidal-type meroblastic cleavage has evolved independently in a variety of animal groups, e.g., scorpions and cephalopods as well as many vertebrates, including some fishes and amniotes (Gilbert and Raunio, 1997). Meroblastic cleavage is invariably seen in direct-developing forms where it is related to an unusually large egg yolk mass. The increased yolk load enables large highly cellularized embryos, which have sufficient nutrients and cells to support development into feeding juvenile stages. The data provided here suggest that direct development with massive yolk was present during the mid-Ediacaran. Previous studies indicated direct development in some Cambrian eggs (Dong et al., 2004; Chen et al., 2007), which suggests at least a late Ediacaran origin of this developmental mode. It is clear that a variety of animal forms, increasingly likely to have included advanced stem eumetazoans such as the meroblastic embryos reported here, were already present as conditions improved in the early Ediacaran following the last snowball Earth event. This substantiates the conclusion derived from molecular clock estimates that the evolutionary diversification of the metazoans was under way even earlier (Erwin et al., 2011).

The first Sponge

Adult forms of animals from the Weng'an biota are well expected. Several animal taxa have been proposed including small bilaterians (Chen et al., 2004), tabulate corals (Xiao et al., 2000), and some unnamed cnidarians (Chen et al., 2002), however, most of them are not yet widely accepted. Against this background, an extraordinarily well preserved, 600-million-year-old, three-dimensionally phosphatized fossil displaying multiple independent characters of modern adult sponges was discovered recently (Yin et al., 2015). The specimens has been analyzed by SEM and synchrotron X-ray tomography. The fossilized animal (named as *Eocyathispongia qiania*), slightly more than 1.2 mm wide and 1.1 mm tall, is composed of hundreds of thousands of cells, and has a gross structure consisting of three adjacent hollow tubes sharing a common base. The main tube is crowned with a large open funnel, and the others end in osculum-like openings to the exterior. The external surface is densely covered with flat tile-like cells closely resembling sponge pinacocytes, and this layer is punctuated with smaller pores. A dense patch of external structures that display the form of a lawn of sponge papillae has also survived. Within the main funnel, an area where features of the inner surface are preserved displays a regular pattern of uniform pits. Many of them are surrounded individually by distinct collars, mounted in a supporting reticulum. The possibility cannot be excluded that these pits are the remains of a field of choanocytes. The character set evinced by this specimen, ranging from general anatomy to cell type, uniquely indicates that this specimen is a fossil of probable poriferan affinity.

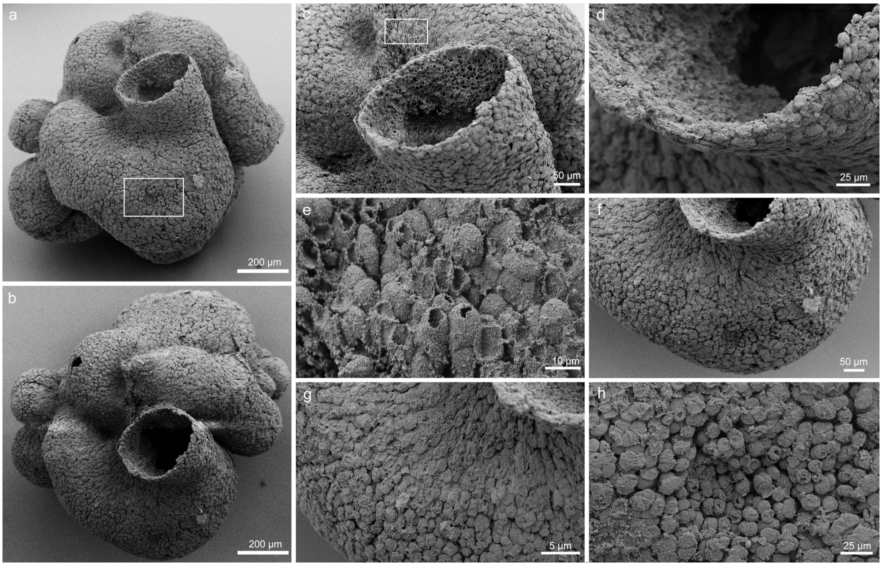

Fig. 2: Sponge grade animal from the Weng'an biota (SEM images): a, later view. b, top view, c-h, close-up views of the specimens, showing detail of the cells and the opening

Phylogenomic extrapolations indicate the last common ancestor of sponges and eumetazoans existed deep in the Cryogenian, perhaps 200 million years before the Cambrian (541 million year ago). This inference implies a long Precambrian history of animals phylogenetically allied with sponges. However, there is yet little unequivocal paleontological evidence of Precambrian sponges. Here, we present a newly discovered 600-Myrold fossil preserved at cellular resolution, displaying multiple poriferan features. The animal was covered with a dense layer of flattened cells resembling sponge pinacocytes, displaying a hollow tubular structure with apparent water inflow and outflow orifices. Although requiring additional specimens of similar form for confirmation, this finding is consistent with phylogenomic inference, and implies the presence of eumetazoan ancestors by 60 million years before the Cambrian. So far, we have only this single specimen, and although its organized and complex cellular structure precludes any reasonable interpretation that its origin is abiogenic, confirmation that it is indeed a fossilized sponge will clearly require discovery of additional specimens.

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