ESRF	**Experiment title:** Sub-micron resolution non-destructive 3D imaging of oldest known fossil embryos from China by phase-contrast microtomography	**Experiment number**: EC138
Beamline: ID19	**Date of experiment**: **from: 28/02/2007 at 8:00 to: 04/03/2007 at 8:00**	**Date of report**: 2009-08-08
Shifts:12	Local contact(s)**:** Dr. Paul TAFFOREAU	*Received at ESRF:*

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Report:

During the beam time assigned to EC138, more than 130 3D preserved microfossils (300-700micron diameter) from the Precambrian Ediacaran Doushantuo Formation, Weng'an, Guizhou Province, China, were investigated non-destructive using x-ray synchrotron micro-tomography (SR-μCT) in phase contrast mode with sub-micron resolution (0.56 and 0.7 microns) at ID19. The Weng'an fauna have attracted great attention as the oldest fossil evidence yet found for multicellular animal life on Earth. The radiometric age dating indicates that the Doushantuo is older than 580my. SR-μCT permits non-destructive computational examination of the specimen from any vantage point, visualization of internal characters in virtual sections in any plane, as well as 3D virtual extractions of internal structures. In the case of the Weng'an fossils it can reveal complex internal features of structures that might have been ignored or misinterpreted because of their deceptive exterior forms. Our studies of Weng'an embryos based SR-μCT images made the following two discoveries.

First, we discovered two remarkable Doushantuo embryos that are 3-dimensionally preserved and displaying a complex organization similar to that found in modern bilaterian embryos. The analysis of SR-μCT data reveals that the organization of cells demonstrates several bilaterian features, including the formation of anterior-posterior, dorso-ventral and right-left polarities, and cell differentiation into several types. Unexpectedly, our observations show a noticeable difference in organization patterns between the embryos, suggesting that they represent two distinct taxa. These embryos provide further evidence for the presence of bilaterian animals in the Doushantuo biota. Furthermore these bilaterians had already diverged into distantly related groups at least 40 million years before the Cambrian radiation, indicating that the last common ancestor of the bilaterians lived much earlier than is usually thought. Please see Figure 1 and its caption for detail information. This research will be published in PNAS[1].

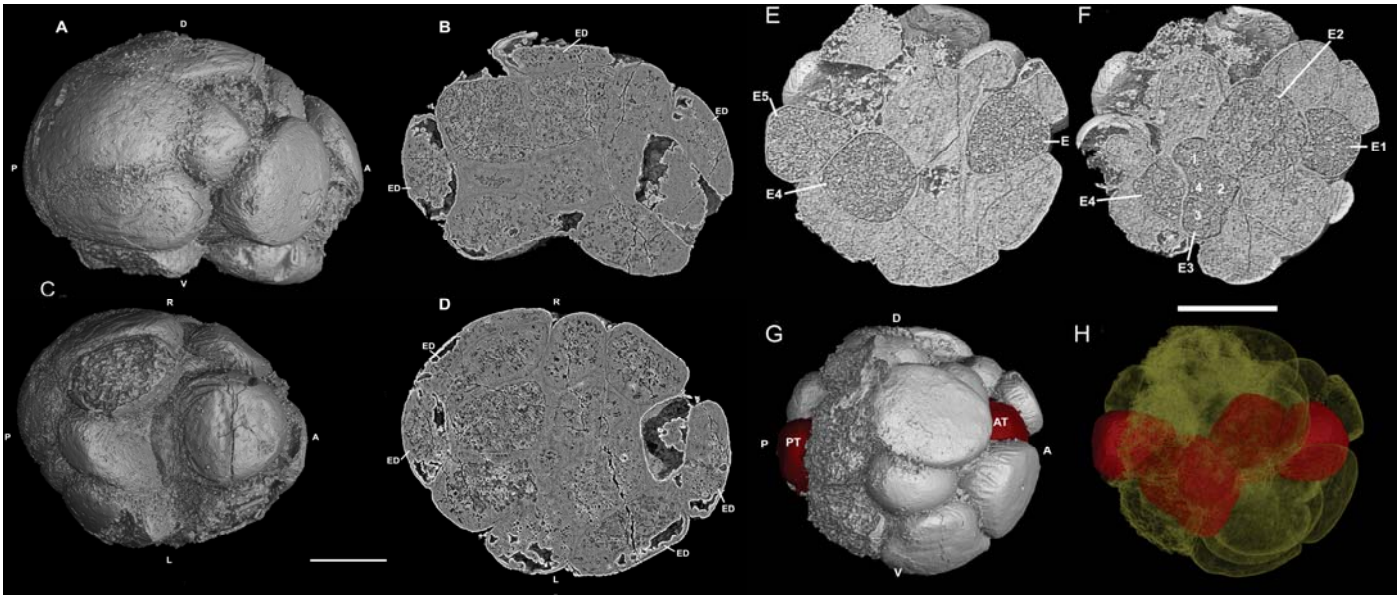

Fig.1A-D, Precambrian post-gastrular embryo (4F10) from the Weng'an Fauna. **(A)** External view from right side along the anterior (A) and posterior (P) axis. **(B)** Digital internal section cut half way into the embryo from the right surface (Fig.1A), showing maximal dorso-ventral asymmetry and ectodermal cells. **(C)** External digital view from the ventral side, revealing exposed, putative endodermal cells. **(D)** Digital internal section after 90^0 rotated from Fig.1B, internal section showing the plane of left-right bilateral symmetry. **Fig.1E-H**, Precambrian post-gastrular embryo (4F4) from the Weng'an Fauna. **(E)** Digital sections cut 35% from right surface, showing endodermal cells E5, E4 and E1. **(F)** 53% from right surface, showing most parts of the endodermal cord, close to the middle plane of the embryo, and cluster of four cells, apparently the daughter cells resulting from mitosis of E3. **(G)** Digital external view from right side along the A-P axis, showing: AT and PT terminations (in red) of the putative endodermal cord of cells, and presumed ectodermal cells on dorsal surface. **(H)** Transparent view from right side, showing the putative endodermal cord of cells (in red) that has a shallow S shape dorso-ventrally, and extends through the embryo from anterior to posterior ends. Scale bar in all figures, 250 μm. Key: A, anterior; P, posterior; D, dorsal; V, ventral; R, right side; L, left side; ED, ectodermal cell; BM, basal membrane; AT, anterior termination; PT, posterior termination.

Second, our studies of Doushantuo embryos based on SR-μCT images demonstrate the existence of a large suite of modern embryonic features, including macromeres and micromeres, cell lineage, polar lobes, compacted epithelia, equal and unequal cleavage, blastulation and gastrulation, and chorionic protection. Because embryos such as those here studied provide only a limited amount of phylogenetic information, and because adult metazoans of the types that produced these embryos have yet to be discovered in Doushantuo-age rocks, these fossilized embryonic forms can at present be assigned only to the various superclades represented amongst living Metazoa. The diversity of the embryos here studied suggests that the metazoan fauna of Weng'an may well have included animals of poriferan, cnidarian, and both protostomial (representatives possibly of basal protostome lineages) and deuterostomial affinity. If this interpretation is correct, it would then follow that the last common ancestor of the bilaterian metazoan lineage, as well as the last common ancestor of sponges, cnidarians and bilaterians, pre-dated deposition of the Doushantuo strata. SR-μCT is a powerful tool to study the diversity represented by the Weng'an microfossils. This research was published in Precambrian Research [2].

We also investigated some test type sample fossil, such as two different types of the developmental sequence comparable to the duet spiral cleavage and quartet spiral cleavage with a big polar macromere, known only among the phyla of Lophotrochozoa.

Traditional methods for the study of Weng'an embryos are by petrologic thin section, or alternatively, by examination of external form with scanning electron microscopy (SEM). But reconstruction of the whole form is difficult by the first of these methods, while examination of internal structure is impossible by the second. The work reported here shows SR-μCT is an effective way to address both these problems. It is not only non-destructive but also can reveal many structures that are invisible, or hardly visible, by the classical absorption contrast-based imaging technique. This report is the preliminary form for our new application of the beam time at ID19.

Reference:

1. Jun-Yuan Chen, David J. Bottjer, Gang Li, Michael G. Hadfield, Feng Gao, Andrew R Cameron, Chen-Yu Zhang, Ding-Chang Xian, Paul Tafforeau, Xing Liao, and Zhong-Jun Yin, Complex embryos displaying bilaterian characters from Precambrian Duoshantou phosphate deposits, Weng'an, Guizhou, China, will be published in PNAS.

2. Jun-Yuan Chen, David J. Bottjer, Eric H. Davidson, Gang Li, Feng Gao, R. Andrew Cameron, Michael G. Hadfield, Ding-Chang Xian, Paul Tafforeau, Quan-Jie Jia, H. Sugiyama, Rui Tang， Phase contrast synchrotron X-ray microtomography of Ediacaran (Doushantuo) metazoan microfossils: Phylogenetic diversity and evolutionary implications, Precambrian Research 173 (2009) 191–200