

Swiss light shed on the nature of fossilised embryos from the dawn of animal evolution

Philip Donoghue, Neil Gostling, *Dept. of Earth Sciences, University of Bristol, UK*; Stefan Bengtson, Therese Huldtgren, *Dept. of Paleozoology, Swedish Museum of Natural History, Sweden*; Xiping Dong, Fan Peng, *School Of Earth & Space Sciences, Peking University, Beijing, P. R. China*; Marco Stampanoni, *Research Department Synchrotron Radiation and Nanotechnology, PSI*

Although only recently discovered, the fossil record of embryonic development has already begun to challenge cherished hypotheses on the origin of major animal groups. Synchrotron-based X-ray Tomographic Microscopy has provided unparalleled insight into the anatomy and preservation of these fossil remains and this has allowed us to test competing hypotheses on their nature. With knowledge of both adults and embryos from the time of diversification of the major animals groups, it is now possible to test models of developmental evolution based on modern model organisms using information from their long-extinct ancestors.

Fossils represent the only direct record of evolutionary history, but that record has long been denigrated for its failure to provide any insight into embryology. Changes to embryology represent the most potent mechanism of evolutionary change, but until recently fossilisation processes had not been known to preserve structures as delicate as embryos. Remarkably, however, fossilised embryos have now been discovered in rocks deposited at the dawn of animal evolution [1-2]. These remains offer great opportunities for palaeontology to understand developmental evolution, but also great challenges to develop suitable methods of analysis of these microscopic remains. The scanning electron microscope is the routine tool of choice in palaeontology, but it only provides insight into surface morphology [1-2], while embryology mainly concerns events within. Methods such as sectioning have been employed [3] but interpretations of these data have proven highly equivocal [4], and desktop computed tomography provides insufficient resolution and contrast.

SLS provides fundamental insights

To overcome these limitations we have employed synchrotron-based X-ray Tomographic Microscopy (SRXTM) which provides an unparalleled high-resolution, non-destructive approach. It has provided fundamental new insights into the anatomy, development, and fossilisation of early animal embryos, generating data to challenge cherished hypotheses on the role of embryological evolution during the emergence of major animal groups.

Our research has so far focused on attempting to resolve the manner in which embryos are fossilised to determine the veracity of controversial claims of fossilised embryos and larvae from before the Precambrian–Cambrian transition 542 million years ago [3,5]. Embryos underwent a two-stage process of fossilisation: the first in which biogenic structure was replicated by mineral-precipitating bacteria, and a second later stage in which the preserved structure was encrusted by minerals. The distinction between the two phases is picked out by differing levels of X-ray attenuation. This advance has allowed us to reject many claims of embryos and larvae in which the alleged biogenic structures can be shown to be features of post-mortem mineralization.

The second focus of our research has been to unravel the internal structure and anatomy of bona fide fossil embryos recovered from rocks of Cambrian age. In particular, we have elucidated the anatomy of *Markuelia*, the oldest embryo of a complex animal known from the geological record. *Markuelia* has been controversial because of its worm-like form and apparent direct mode of embryonic development; annelids undergo indirect development, passing through metamorphosis from larva to the adult.

SRXTM analysis has allowed us to resolve the anatomy of *Markuelia*, demonstrating that its similarity to annelid worms is superficial and its possession of a terminal mouth, armed with circumferential rows of retractable teeth, together with a host of other features, demonstrate its close evolutionary relationship to the arthropods and a delightfully obscure ensemble of animals including the round worms, hair worms and priapulid worms.

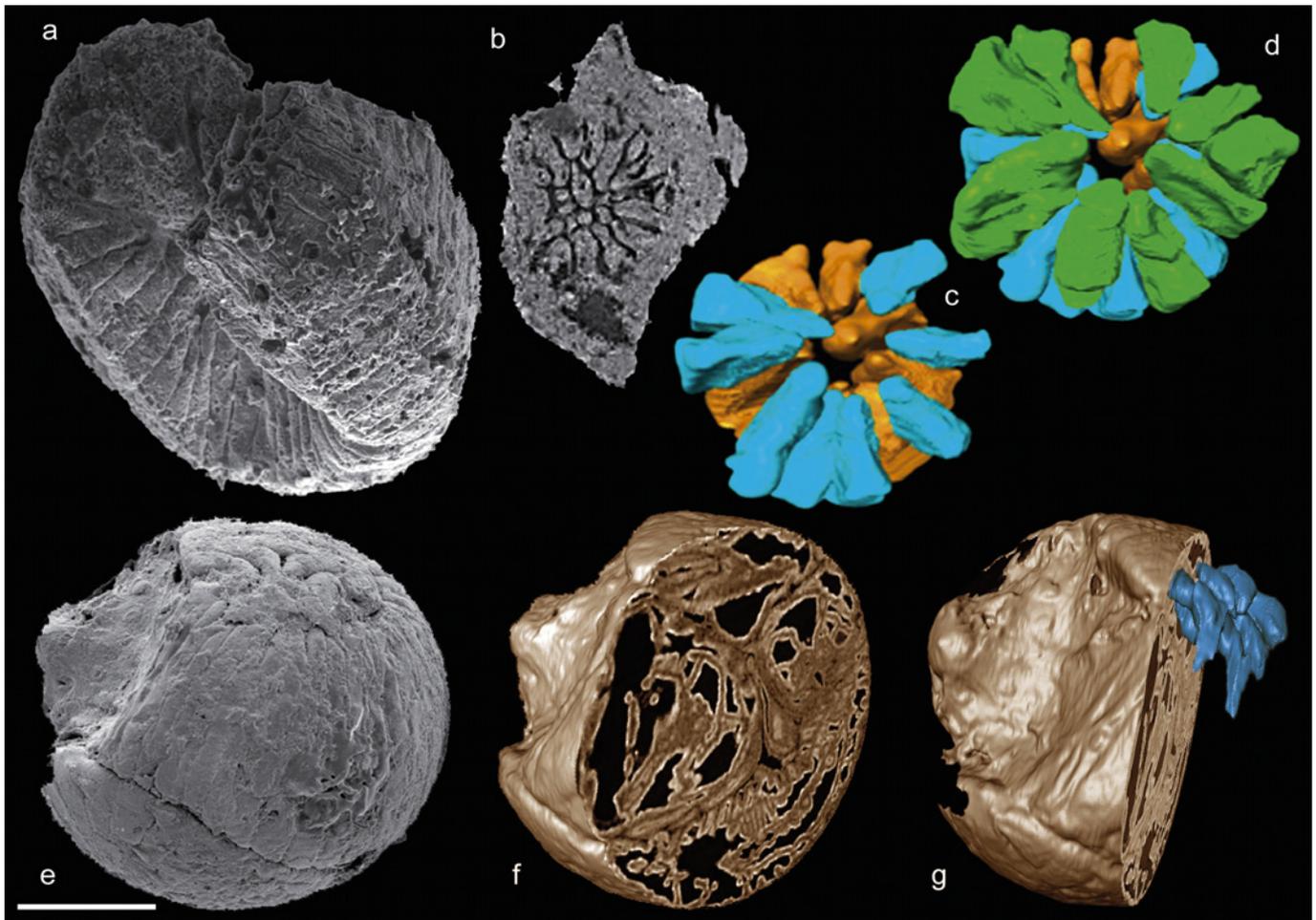


Figure 1: The more than half a billion year old embryos of *Markuelia hunanensis* (a–d) and *Markuelia secunda* (e–g). (a) SEM image of the head region, (b) an XTM image showing the internal structure of the mouth, (c–d) renderings of the teeth in the mouth. (e) SEM image of enrolled embryo (head end at upper left; tail end at top), (f) rendered and virtually sectioned to show internal structure, (g) segmented to show the structure of the tail. Relative scale bar: (a–b) 110 μm , (c–d) 45 μm and (e–f) 150 μm .

Clearly the direct mode of development exhibited by *Markuelia* conflicts with the long held view that metamorphosis is a primitive feature among marine animals [6], and this contradiction is increasingly supported by further discoveries of fossilised embryos [7].

The real significance of *Markuelia* arises from its genealogical proximity to the ancestor of the two main model animals in molecular developmental genetics, *Caenorhabditis elegans* (nematode) and *Drosophila melanogaster* (fruit fly). *Markuelia* provides insight into the characteristics of this ancestor, including issues such as whether or not it was segmented. It thus provides constraint on models of developmental evolution within a major branch of the animal kingdom.

None of these insights would have been possible without SRXTM, a technology that explores the limits of fossil preservation. SRXTM is set to provide a revolution in the palaeontological study on par with the introduction of the electron microscope.

References

- [1] Bengtson, S. & Yue, Z. , Science 277, 1645-1648 (1997)
- [2] Dong, X., Donoghue, P.C.J., Cheng, H. & Liu, J., Nature, 427, 237 (2004)
- [3] Chen, J. et al., Proceedings of the National Academy of Sciences, USA 97, 4457 (2000)
- [4] Xiao, S., Yuan, X. & Knoll, A. H. , Proceedings of the National Academy of Sciences, USA 97, 13684 (2000)
- [5] Chen, J.-Y. et al., Science, 305, 218 (2004)
- [6] Haeckel, E., Quarterly Journal of Microscopical Science, 14, 142-165, 223 (1974)
- [7] Donoghue, P. C. J. & Dong, X. in Evolving form and function: fossils and development (ed. Briggs, D. E. G.) 83 (Yale Peabody Museum, New Haven, 2005)