

A merciful death for the “earliest bilaterian,” *Vernanimalcula*

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SUMMARY Fossils described as *Vernanimalcula guizhouena*, from the nearly 600 million-year-old Doushantuo Formation in South China, have been interpreted as the remains of bilaterian animals. As such they would represent the oldest putative record of bilaterian animals in Earth history, and they have been invoked in debate over this formative episode of early animal evolution. However, this interpretation is fallacious. We review the evidential basis of the biological interpretation of *Vernanimalcula*, concluding that the struc-

tures key to animal identity are effects of mineralization that do not represent biological tissues, and, furthermore, that it is not possible to derive its anatomical reconstruction on the basis of the available evidence. There is no evidential basis for interpreting *Vernanimalcula* as an animal, let alone a bilaterian. The conclusions of evolutionary studies that have relied upon the bilaterian interpretation of *Vernanimalcula* must be called into question.

INTRODUCTION

In 2004, a fantastic animal fossil crept into the scientific literature. *Vernanimalcula guizhouena* was reported from nearly 600 million-year-old Neoproterozoic (Ediacaran) phosphorites of the Doushantuo Formation in Guizhou, southern China (Chen et al. 2004a). Reconstructed as a flatulent “whoopie cushion” (Fig. 1), this broadly circular and flattened organism was interpreted to possess a protruding mouth region leading to a pharynx, a straight gut with an expanded mid region and glands, and an anus. It had a paired coelom. Sensory pits adorned its anterior margin. The animal was small, up to about 200 μm in diameter. It was hailed as the first bilaterian animal in the fossil record, the “small spring animal” following the winter of the Neoproterozoic glaciations.

This concept of *Vernanimalcula* has drawn criticism, at the most fundamental level. It has been contended that interpreted biological structures are layers of geological mineralization deposited on organic matter in decayed microfossils (Bengtson and Budd 2004; Donoghue and Purnell 2009; Xiao et al. 2009, 2010; Cunningham et al. 2012). In spite of the evidence that the reported tissues are artifacts, *Vernanimalcula* has attracted widespread interest for its conceived evolutionary significance. The original article by Chen et al. (2004a), has attracted more than 160 citations (Supporting Information Table S1), among which *Vernanimalcula* has been assigned a pivotal role in animal evolution (Bottjer 2005), in-

cluding the origin of swimming (Vargas and Aboitiz 2005), bilateral symmetry (Sarnat and Flores-Sarnat 2005; Corbalis 2009), the circulatory system (Sarnat 2008), eyes (Nilsson 2004), and even cancer (Saul and Schwartz 2007). In particular, its diminutive size has been cited as giving a decisive push to ideas featuring animal origins in meiofauna-sized creatures (Isaak 2007; Giere 2009).

Given this contrast between concerns over the veracity of the interpretation of *Vernanimalcula*, and the burden of evolutionary significance that has been placed upon this interpretation, we provide a review of its evidential basis, as given in the original descriptions (Chen et al. 2004a, 2004b) and in a recent study (Petryshyn et al. 2011) purporting to confirm the anatomy of *Vernanimalcula*.

LAYERED TISSUES OF INTERPRETATION

Vernanimalcula has been interpreted to preserve a mouth, pharynx, gut, glands, anus, paired coeloms, and an array of sensory pits on its external surface, much of which preserved to a cellular, even subcellular level of fidelity (Chen et al. 2004a, 2004b). There is no doubt that fossilization via mineral replication of biological structure occurs in this deposit: multicellular algae and the putative “fossil embryos,” which have recently been reinterpreted as reproductive stages of unicellular protists (Huldtgren et al. 2011), both preserve subcellular structures (Xiao et al. 1998; Hagadorn et al. 2006;



Fig. 1. Reconstruction of *Vernanimalcula guizhouena*. Artwork Amadeo Bachar (www.abachar.com). From Bottjer (2005).

Xiao and Schiffbauer 2008; Huldtgren et al. 2011; Cunningham et al. 2012; Schiffbauer et al. 2012). However, none of these fossils are precise lithographs of their original biological selves; all show evidence of decay prior to mineral replication of biological structure and the mineral infilling of spaces left after biological tissues have decayed. A significant effort has been expended in characterizing these different phases of mineralization in the Doushantuo fossils in order to constrain their biological and systematic interpretation (Xiao and Knoll 2000b; Xiao and Schiffbauer 2008; Cunningham et al. 2012; Schiffbauer et al. 2012).

The process of fossilizing soft tissues through the medium of apatite minerals (calcium phosphates), which characterizes the Doushantuo embryo-like fossils and the putative bilaterian, is among the best known of all. Paradoxically, decay is necessary for soft tissue fossilization, because although microbes (alongside autolytic processes) are the principal vectors of the decay, mineral replication of soft tissues is microbially mediated (Briggs 2003; Raff et al. 2008). However, there is no clear spatial dichotomy between the mineral impregnation of biological substrates, such as animal tissues, and the growth of later diagenetic minerals on the resulting organic or inorganic templates. These two phases of mineralization can, however, be distinguished by their microstructure (Fig. 2). Soft tissue replication is invariably associated with submicrometer scale crystals whose orientation reflects the fabric of the organic matrix. Later, diagenetic mineralization not associated with an organic matrix is characterized by coarser crystals growing perpendicularly from substrates, forming a characteristic fibro-normal to spherulitic/botryoidal texture (Cunningham et al. 2012; Schiffbauer et al. 2012).

Vernanimalcula was described to preserve layered ectothelial, mesothelial, and endothelial tissues and organs to a cellular or subcellular level (Chen et al. 2004a, 2004b). The primary reason for challenging this interpretation was that the layering is characteristic of generations of void-filling diagenetic mineralization that does not reflect original biological structure (Bengtson and Budd 2004). Putative cell boundaries were in that context reinterpreted as cracks propagated in the directions of the fibro-normal crystals (Fig. 3). Spherulitic structures interpreted originally as sensory organs, glands, pharynx, and a mouth (Chen et al. 2004a) are comparable to spherulitic fans of mineral crystals that nucleate on irregularities on the overgrown surfaces (Figs. 2–4).

Chen and colleagues ignored the indications of diagenetic origin (Chen et al. 2004b) and instead built onto their case with further unique anatomical, cellular, and subcellular features avowedly seen in the fossils. Their recent reconsideration in light of new specimens assigned to *Vernanimalcula* concludes, however, as did Bengtson and Budd (2004), that the structures identified as cellular layers were formed by diagenetic growth of apatite into voids. They concede (Petryshyn et al. 2011) that what may look like cell boundaries are likely optical features due to the growth habit of the crystals and write: “If these samples are indeed the remains of complex animals, the mode of preservation has obscured the original fine cellular detail.”

Evidently, the case for these fossils preserving the anatomy of a bilaterian animal is now more cryptic. Nevertheless, Petryshyn et al. (2011) speculate that the sections of the *Vernanimalcula* types are thinner, 15 μm (they are 50 μm according to Chen et al. 2004a), than ordinary 30- μm -thick petrographic sections and therefore may show primary cellular boundaries. Furthermore, they write: “The question is – what was originally phosphatized? Was it a decomposing complex metazoan, or a random collection of abiotic matter? We believe that the answer to this lies in the repetition of this form . . . it seems unlikely that the circumstances of rapid abiotic precipitation of phosphate would produce the same complex morphology at the same scales repeatedly. It is therefore more likely that these structures are the remnants of a Neoproterozoic bilaterally symmetrical organism.” This argument, however, sets up a classical false dichotomy by presenting a choice between two unlikely extreme alternatives that leaves out the proposed interpretation (Bengtson and Budd 2004; Xiao et al. 2010) that the objects are diagenetically mineralized, decayed microfossils.

At several intervals in the Doushantuo Formation, millimeter-scale spherical fossils are literally rock-forming. All of these fossils, from the most to the least remarkably preserved, show evidence of void-filling diagenetic mineralization after soft tissues have decayed (Cunningham et al. 2012). In many of these fossils, the generations of mineralization give the appearance of bilateral symmetry in the

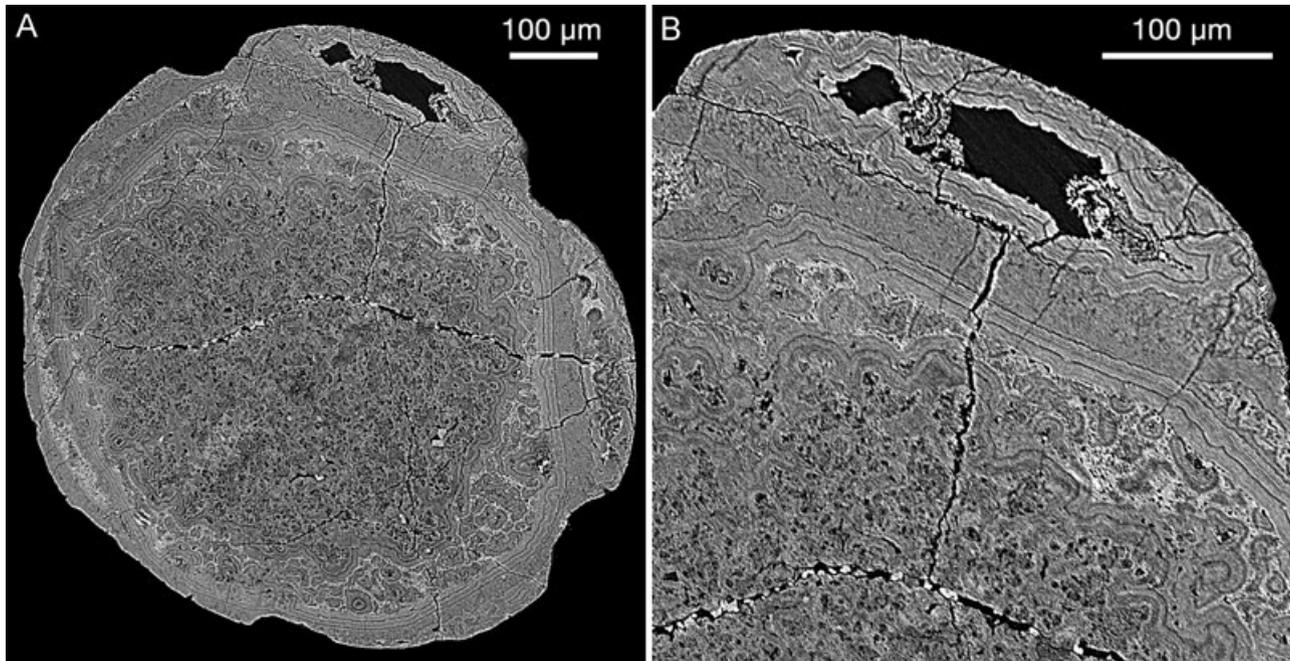


Fig. 2. Tomographic renderings (two-dimensional slices) of globular microfossil from the Doushantuo Formation, Weng'an, Guizhou, SMNH 4825. Note presence of early diagenetic cryptocrystalline apatite replacing organic tissue, as well as later diagenetic banded, spherulitic void-filling apatite (cf. the fabric in Fig. 3, A and C).

chance orientation of randomly oriented planes of section, as our examples from embryo-like and acritarch fossils demonstrate (Fig. 4). Chen et al. (2004a) reported the examination of 50,000 microfossils in thin sections, and so it is not unexpected that they identified such specimens; it is perhaps more surprising that they found so few. The recurrence of size and shape that so convinced Petryshyn et al. (2011) of the biogenic basis of the bilateral structure seen in *Vernanimalcula* fossils reflects the fact that almost all of the fossils in these horizons have the same approximate size and cross-sectional shape. This is a documented phenomenon, reflecting size sorting and grading at the time of sedimentation, after initial fossilization elsewhere (Xiao and Knoll 2000a).

ENTERING THE THIRD DIMENSION

The entire concept of *V. guizhouena* is based on 15 thin sections, one from each of 15 individual fossils, the anatomy of which is otherwise entirely unknown. Ten of these were rock sections described by Chen et al. (2004a; 2004b). The other five were made from isolated specimens without prior observation of their three-dimensional morphology (Petryshyn et al. 2011). In all, ten sections of *Vernanimalcula* have been figured, and these all show approximate bilateral symmetry in the occurrence of paired cavities within centripetally layered walls, surrounding a core of botryoidal structures.

It is important to note that there is no prior reason to interpret the fossil remains of *Vernanimalcula* as bilaterian. The fossils approach bilateral symmetry in the plane of section, but no insights are provided into the relationship of that plane of section to the fossil organism. It may seem glib, but it is important to remember that these are slivers of rock; they may preserve, through mineral replication, features of the biological structure of a once living organism, but that has to be demonstrated, not assumed a priori without justification. Similarly, the interpretative model, in light of which anatomical homologies are to be identified in the fossil remains, must be justified a priori, or else the conclusions are at risk of circular reasoning (Bengtson 1986; Donoghue and Purnell 2009).

When the general affinity of a fossil is unknown, the safest approach is to first determine the intrinsic geometric properties of the remains, taking taphonomy and diagenesis into account. Indeed, the best test of a hypothesis that the bilateral disposition of structures in *Vernanimalcula* reflects a bilateral organism is to reconstruct anatomy based on the geometric relations between structures preserved in the specimens. This is possible only if the available evidence includes three-dimensional information, in this case represented by a number of sections in different planes and at different angles.

Chen and colleagues (Chen et al. 2004a, 2004b; Petryshyn et al. 2011) assert that all sections of different individuals represent a coronal, or approximately coronal, plane. Thus,

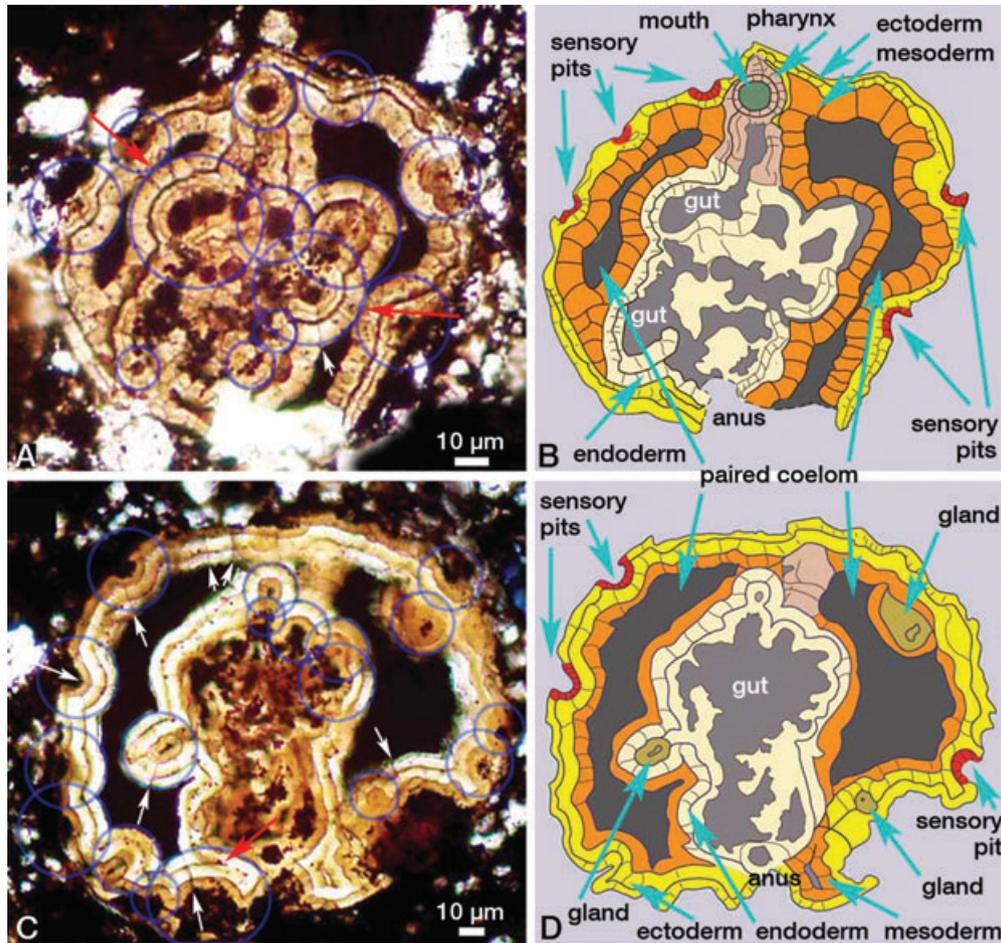


Fig. 3. *Vernanimalcula guizhouena* Chen et al. 2004a, holotype (A, B; X00305) and paratype (C, D; X08981). Microphotographs of thin sections (A, C) and interpretative drawings (B, D) from Chen et al. (2004a; reprinted with permission from AAAS); our additions are the rings and arrows in A, C to emphasize diagenetic features, as well as labels and arrows in B, D, representing Chen et al.'s (2004a) interpretations of bilaterian biological characters. The features indicating that the alleged cell layers represent successive generations of diagenetic overgrowth of apatite (Bengtson and Budd 2004) are: (1) regular banding of color and thickness that can be followed around the circumferences of the cavities but differs between specimens; (2) spherulitic fans radiating from irregularities on the overgrown surfaces (examples marked by bluish rings in A and C), (3) cavity-filling geometry, in particular the meeting of opposing spherulites (red arrows in A) and the absence of the last-formed layer in narrow spaces (red arrow in C); and (4) fibronormal texture of the bands, revealed as lineations and cracks that form “cell boundaries” that frequently persist across “cell layers” (examples marked by white arrows in A and C). Every “cell layer” and “organ” drawn in B and D corresponds to fibronormal apatite bands and concentric spherulites, respectively, in the actual specimens. A further alleged biological feature (Chen et al. 2004b), “inward bilateral ridges in the floor of the coelomic cavities,” corresponds to the meeting and intergrowth of opposing spherulites marked by red arrows in A.

although the sections were unoriented, no transverse, sagittal, etc., sectional planes through the allegedly bilateral body were discovered. However, without knowledge of the three-dimensional anatomy, derived from intersecting planes, it is impossible to determine the orientation of the planes of section, for example, as coronal or approximately coronal, let alone their relationship to one another. Conversely, without knowledge of the orientation and level of the plane of section even the presence of two separate “coelomic” cavities cannot be ascertained (cf. Fig. 4, D–G). In sum, the anatomical re-

construction of *Vernanimalcula* cannot be derived from the available data.

In contrast to the original specimens of *Vernanimalcula*, which were selected among 50,000 other specimens in rock sections, new specimens reported by Petryshyn et al. (2011) were isolated from the rock by chemical preparation prior to analysis. The specimens were found in phosphatic clasts, but unfortunately no attempt was made to use X-ray tomographic microscopy (e.g. Donoghue et al. 2006; Hagadorn et al. 2006; Chen et al. 2009) to analyze the full morphology

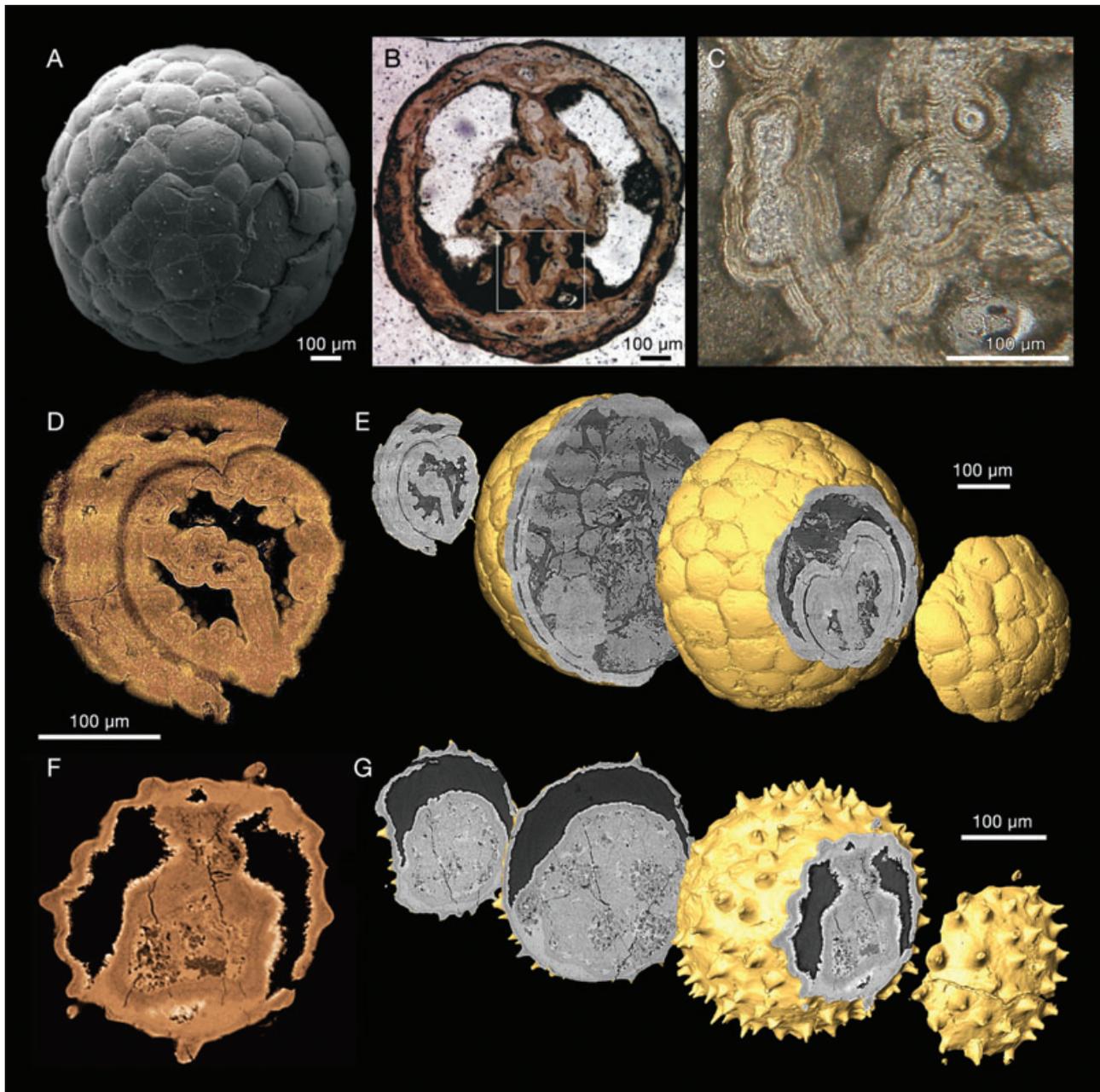


Fig. 4. *Vernanimalcula*-like sections through microfossils from the Doushantuo Formation, Weng'an, Guizhou. (A–C) Specimen of *Tianzhushania* (Museum of Earth Science, Institute of Geology, Chinese Academy of Geological Sciences MESIG 10025) before (A) and after (B, C) embedding and thin-sectioning (A and B from Liu et al. 2007). Frame in B shows position of C. The “bilateral symmetry” of this specimen appears to result from the plane of section passing through two partly degraded cells of an early cleavage stage. (D–G) Tomographic renderings of a *Tianzhushania* (D, E; Swedish Museum of Natural History SMNH X 4826) and a *Meghystrichosphaeridium* (F, G; SMNH X 4827). D and F are volumetric renderings of thin slices about 3 μm thick at levels of apparent bilateral symmetry. E and G show sections and two-dimensional slices at different levels through the fossils; “bilateral symmetry” is not a persistent feature in the specimens. Diagenetic void-filling apatite forms banding and spherulitic features in C and D (cf. the spherulites in Fig. 3, A and C). Lighter colors in tomographic slices correspond to higher X-ray attenuation.

of the “small bilaterian” before sectioning, to discover whether it was in fact bilaterally symmetrical, with a creeping sole, ventral mouth, peripherally placed sensory organs, etc. Thus, no evidence now remains for the three-dimensional morphology that would have provided a critical test of the *Vernanimalcula* hypothesis. In Fig. 4, we give examples of how *Vernanimalcula*-like morphologies may appear in sections of microfossils of known three-dimensional structure. Clearly, such morphologies can be derived from semidegraded specimens of *Tianzhushania* (cyst-forming protists; Fig. 4, A–E) and acritarch cysts (Fig. 4, F–G).

Evidently, the interpretation of *Vernanimalcula* as a bilaterian rests on an assertion based on little more than the approximately bilateral arrangement of cavities seen in the sectioned fossils. The anatomical reconstruction is without foundation.

EPISTEMOLOGY OF A SMALL BILATERIAN

“There is a bilaterian in that truck”—Chen Jun-Yuan quoted in Bottjer (2005).

The short history of research into *V. guizhouena* reminds us of the pitfalls, not merely of the interpretation of fossils, but of comparative anatomy more generally. The identification of anatomical homologies occurs within the context of a phylogenetic hypothesis—yet those interpreted homologies are then marshaled as evidence of phylogenetic affinity (Rieppel and Kearney 2007). The only means of escaping this circular logic is to establish an evidential basis for the phylogenetic milieu within which homologies are to be identified. Because anatomical homologies are ultimately based in the relations between parts, this is best achieved by establishing the geometric properties of intrinsic anatomical characters, seen through the filter of taphonomy.

The fossils referred to *V. guizhouena* can be rationalized on the body plan of a bilaterian animal, but only if it is assumed a priori that they represent the remains of a bilaterian animal. There is no intrinsic evidence to justify this interpretative model, however. The available evidence indicates little more than the fact that these objects are approximately circular in cross-section and filled with generations of void-filling mineral deposited after the internal biological structure intrinsic to the organism had decayed. There is no evidence for a bilaterian animal, or even an animal, interpretation.

It is likely that the fossils referred to *Vernanimalcula* were interpreted as bilaterians because this was, as our epigram betrays, the explicit quarry of its authors. If you know from the beginning not only what you are looking for, but what you are going to find, you will find it, whether or not it exists. As Richard Feynman (1974) famously remarked: “The first principle is that you must not fool yourself – and you are the

easiest person to fool. . . . After you’ve not fooled yourself, it’s easy not to fool other scientists.”

Conversely, once you have fooled yourself you will fool other scientists. And so *Vernanimalcula* has been marshaled in evidential support for the timing of bilaterian evolution and of multifarious bilaterian innovations. The “little spring animal” has taken on a life of its own, a life it never had in the Neoproterozoic. It is our hope that *Vernanimalcula* will now be laid to a merciful rest, freed from the heavy burden of undue evolutionary significance that has hitherto been heaped upon it.

Acknowledgments

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REFERENCES

- Bengtson, S. 1986. The problem of the Problematika. In A. Hoffman and M. H. Nitecki (eds.), *Problematic Fossil Taxa*. Oxford University Press, New York.
- Bengtson, S., and Budd, G. 2004. Comment on “Small bilaterian fossils from 40 to 55 million years before the Cambrian”. *Science* 306: 1291a.
- Bottjer, D. 2005. The early evolution of animals. *Sci. Am.* 293: 42–47.
- Briggs, D. E. G. 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annu. Rev. Earth Planet. Sci.* 31: 275–301.
- Chen, J.-Y., et al. 2004a. Small bilaterian fossils from 40 to 55 million years before the Cambrian. *Science* 305: 218–222.
- Chen, J.-Y., et al. 2009. Phase contrast synchrotron X-ray microtomography of Ediacaran (Doushantuo) metazoan microfossils: phylogenetic diversity and evolutionary implications. *Precambrian Res.* 173: 191–200.
- Chen, J.-Y., Oliveri, P., Davidson, E. H., and Bottjer, D. J. 2004b. Response to comment on “Small bilaterian fossils from 40 to 55 million years before the Cambrian”. *Science* 306: 1291b.
- Corballis, M. C. 2009. The evolution and genetics of cerebral asymmetry. *Philos. Trans. R. Soc. B Biol. Sci.* 364: 867–879.
- Cunningham, J. A., et al. 2012. Distinguishing geology from biology in the Ediacaran Doushantuo biota relaxes constraints on the timing of the origin of bilaterians. *Proc. R. Soc. B Biol. Sci.* 279: 2369–2376.
- Donoghue, P. C. J., et al. 2006. Synchrotron X-ray tomographic microscopy of fossil embryos. *Nature* 442: 680–683.
- Donoghue, P. C. J., and Purnell, M. A. 2009. Distinguishing heat from light in debate over controversial fossils. *BioEssays* 31: 178–189.
- Feynman, R. P. 1974. Cargo cult science. *Eng. Sci.* 37: 10–13.
- Giere, O. 2009. *Meiobenthology. The Microscopic Motile Fauna of Aquatic Sediments*. 2nd Ed. Springer, Berlin.
- Hagadorn, J. W., et al. 2006. Cellular and subcellular structure of Neoproterozoic animal embryos. *Science* 314: 291–294.
- Huldtgren, T., et al. 2011. Fossilized nuclei and germination structures identify Ediacaran ‘animal embryos’ as encysting protists. *Science* 334: 1696–1699.
- Isaak, M. 2007. *The Counter-Creationism Handbook*. University of California Press, Berkeley, CA.

- Liu, P., Yin, C., Tang, F., Gao, L., and Wang, Z. 2007. Progresses and questions on studying metazoan fossils of the Weng'an biota. *Geol. Rev.* 53: 728–735.
- Nilsson, D.-E. 2004. Eye evolution: a question of genetic promiscuity. *Curr. Opin. Neurobiol.* 14: 407–414.
- Petryshyn, V. A., Bottjer, D. J., Chen, J.-Y., and Gao, F. 2011. Petrographic analysis of new specimens of the putative microfossil *Vernanimalcula guizhouena* (Doushantuo Formation, South China). *Precambrian Res.*, online. DOI: 10.1016/j.precamres.2011.08.003.
- Raff, E. C., et al. 2008. Embryo fossilization is a biological process mediated by microbial biofilms. *Proc. Natl. Acad. Sci. USA* 105: 19360–19365.
- Rieppel, O., and Kearney, M. 2007. The poverty of taxonomic characters. *Biol. Philos.* 22: 95–113.
- Sarnat, H. B. 2008. Embryology and malformations of the forebrain commissures. In H. B. Sarnat and P. Curatolo (eds.). *Handbook of Clinical Neurology*. Elsevier, Amsterdam.
- Sarnat, H. B., and Flores-Sarnat, L. 2005. Normal development of the nervous system. In C. E. Coffey and R. A. Brumback (eds.). *Pediatric Neuropsychiatry*. Lippincott Williams & Wilkins, Philadelphia.
- Saul, J. M., and Schwartz, L. 2007. Cancer as a consequence of the rising level of oxygen in the Late Precambrian. *Lethaia* 40: 211–220.
- Schiffbauer, J. D., Xiao, S., Sharma, K. S., and Wang, G. 2012. The origin of intracellular structures in Ediacaran metazoan embryos. *Geology* 40: 223–226.
- Vargas, A. O., and Aboitiz, F. 2005. How ancient is the adult swimming capacity in the lineage leading to Echinodermata? *Evo. Dev.* 7: 171–174.
- Xiao, S., and Knoll, A. H. 2000a. Phosphatized animal embryos from the Neoproterozoic Doushantuo Formation at Weng'an, Guizhou, south China. *J. Paleontol.* 74: 767–788.
- Xiao, S., and Knoll, A. H. 2000b. Fossil preservation in the Neoproterozoic Doushantuo phosphorite Lagerstätte, South China. *Lethaia* 32 [year of imprint 1999]: 219–240.
- Xiao, S., Kowalewski, M., Shen, B., Dong, L., and Laflamme, M. 2009. The rise of bilaterians: a reply. *Hist. Biol.* 21: 239–246.
- Xiao, S., Kowalewski, M., Shen, B., Dong, L., and Laflamme, M. 2010. The rise of bilaterians: a few closing comments. *Hist. Biol.* 22: 433–436.
- Xiao, S., and Schiffbauer, J. D. 2008. Microfossil phosphatization and its astrobiological implications. In J. Seckbach and M. Walsh (eds.). *From Fossils to Astrobiology*. Springer Science.
- Xiao, S., Zhang, Y., and Knoll, A. 1998. Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite. *Nature* 391: 553–558.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. List of articles citing Chen, J.-Y., Bottjer, D. J., Oliveri, P., Dornbos, S. Q., Gao, F., Ruffins, S., Chi, H., Li, C.-W., and Davidson, E. H. 2004.

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