Review Focus
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The Weng’an Biota (Doushantuo Formation): an Ediacaran window on soft-bodied and multicellular microorganisms

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Abstract: The Weng’an Biota is a fossil Konservat-Lagerstätte in South China that is c. 570 – 609 myr old and provides an unparalleled snapshot of marine life during the interval in which molecular clocks estimate that animal clades were diversifying. It yields fossils that are three-dimensionally preserved in calcium phosphate with cellular and sometimes subcellular fidelity. The biota includes candidates for the oldest animals in the fossil record, including embryonic, larval and adult forms. We argue that, although the Weng’an Biota includes forms that could be animals, none can currently be assigned to this group with confidence. Nonetheless, the biota offers a rare and valuable window on the evolution of multicellular and soft-bodied organisms in the prelude to the Cambrian radiation.

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The origin and evolutionary assembly of animal body plans comprises one of the most formative episodes in the history of life. Animals are ecosystem engineers and their appearance fundamentally changed our planet’s ecology (Butterfield 2011a). Despite the importance of this evolutionary episode, many aspects of the timing and nature of the event remain poorly constrained. Molecular-clock analyses estimate that animals originated by the Cryogenian and diversified through the Ediacaran (Petterson & Butterfield 2005; Erwin et al. 2011; dos Reis et al. 2015), but fossil evidence of animals from before the Cambrian is controversial (Erwin et al. 2011; dos Reis et al. 2015; Cunningham et al. 2017). The Weng’an Biota is one of the few Lagerstätten from the critical interval in which early animals are expected according to molecular-clock studies. In this Ediacaran fossil assemblage, organisms are phosphatized in cellular and even subcellular detail, providing a rare glimpse of soft-bodied and multicellular life at this time. Early research appeared to fulfill expectations of the presence of metazoans with reports of embryonic (Xiao et al. 1998), larval (Chen et al. 2000, 2002) and adult (Xiao et al. 2000; Chen et al. 2002, 2004; Yin et al. 2015) animals from the Weng’an deposit. However, subsequent analyses have cast doubt on this view, and there is currently much disagreement over these interpretations (Bailey et al. 2007a; Huldhgren et al. 2011; Bengtson et al. 2012; L. Chen et al. 2014, Xiao et al. 2014a). Here, we review the stratigraphic position, geological age and environmental setting of the deposit, and present an overview of the biota and an assessment of the phylogenetic affinities of the various taxa.

Stratigraphy and age

The Weng’an Biota occurs within the Ediacaran Doushantuo Formation (551 – 635 Ma, Condon et al. 2005) of south China (Fig. 1). In addition to the phosphatized microfossils from Weng’an, this formation has yielded silicified microfossils (Yin et al. 2004) and macrofossils that are preserved as 2D carbonaceous compressions including macro-algae (Xiao et al. 2002) and the putative cnemophore Eoandromeda (Tang et al. 2008, 2011). The Weng’an Biota itself is known from localities in Weng’an County, Guizhou Province. The Doushantuo Formation overlies the Marinoan glacial tylites of the Cryogenian Nantu Formation that can be dated to 635 Ma (Condon et al. 2005). It is overlain by the Ediacaran Dengying Formation, which contains fossils of the classical Ediacara macrobiota (Sun 1986; Xiao et al. 2005; Z. Chen et al. 2014). The base of the Dengying Formation can be dated to 551 Ma (Condon et al. 2005). In Weng’an, the Doushantuo Formation is composed of five units that have been described in detail by Xiao et al. (2014b) and Yin et al. (2015). The Weng’an Biota occurs mainly in Unit 4, the Upper Phosphorite Member, but also in Unit 5. Unit 4 is divided into 4A, a black phosphorite, and 4B, a grey dolomitico phosphorite (Oombos et al. 2006; Xiao et al. 2014a,b; Yin et al. 2015).

The age of the biota has been debated (Budd 2008; Erwin & Valentine 2013; Xiao et al. 2014a; Yin et al. 2015), with arguments focusing on the correlation of two karstic surfaces, one at the top of Unit 3 and the other within Unit 5 (for detailed discussion of Doushantuo correlation see Zhu et al. (2007), Zhu et al. (2013) and Yang et al. (2015)). If the lower surface is correlated to the c. 582 Ma Gaskiers glaciation (Condon et al. 2005) then the biota would be younger than 582 Ma. However, the lower surface may be older (Yin et al. 2015) and, if the upper karstic surface correlates to the Gaskiers glaciation (Xiao et al. 2014a), then the biota would be older than 582 Ma. Direct radiometric dates for Unit 4 at Weng’an have been inconclusive, giving Pb-Pb isochron ages of 572 ± 36 Ma for Unit 4A (Y. Chen et al. 2009) and 599 ± 4 Ma for Unit 4B (Barfod et al. 2002). However, a recent U–Pb date of 609 ± 5 Ma from a tuff immediately above Unit 4 at Zhancumping, in Hubei Province (Zhou et al. 2017), suggests that the Weng’an biota is probably older than 609 ± 5 Ma and probably older than the...
Depositional environment, preservation and reworking

The Weng’an Biota is interpreted as having been deposited in an outer-shelf environment on a SE-facing passive margin (Jiang et al. 2011). Abundant wave ripples and cross-bedding features indicate deposition above fair-weather wave base (Xiao & Knoll 1999). The fossils were probably deposited in oxic conditions (Shields et al. 2004), although phosphatization may have occurred in anoxic sediments (Muscente et al. 2014; Schiffbauer et al. 2014). The soft-bodied organisms of the biota are three-dimensionally preserved in calcium phosphate and can be preserved at a subcellular level (Hagadorn et al. 2006; Hultgren et al. 2011). However, even the best-preserved specimens are a complex amalgam of cements, making it challenging to determine which aspects represent preserved biology (Xiao et al. 2000; Bengtson 2003; Bengtson & Budd 2004; Cunningham et al. 2012a; Schiffbauer et al. 2012). The preservation of Weng’an fossils is discussed in Box 1. The fossils occur either as, or within, phosphatic grains that have been abraded and rounded, indicating transport from other parts of the basin after initial preservation (Xiao et al. 2007b). In unit 4A, a c. 5 m thick black phosphorite, the fossils occur in reworked phosphatic clasts. As a result, they cannot be released by acetic acid maceration and have generally been studied in petrographic thin sections (e.g. Chen

**Box 1: Preservation of Weng’an fossils**

The Weng’an organisms, like all exceptionally preserved soft-bodied remains, were subjected to both post-mortem decay and later diagentic and geological processes (Donoghue & Purnell 2009). These processes alter the morphology of the fossils in ways that can be unpredictable. Palaeontologists must take these factors into account rather than simply comparing the fossils with extant or freshly dead modern organisms. This is particularly true given the simple nature of the biological structures relevant to the interpretation of the Weng’an Biota. In the case of decay, carefully designed experiments can help constrain which features can feasibly be preserved, elucidate likely preservation pathways, and identify biases introduced by the decay process (Sansom 2014). Experiments have shown that animal embryos have a relatively high preservation potential, particularly when enclosed in a fertilization envelope, whereas primary larvae have an extremely low likelihood of being preserved (Raff et al. 2006; Gostling et al. 2008, 2009). In addition, these experiments have identified the likely mechanisms for Doushantuio-type preservation: 3D replication of cells by robust bacterial pseudomorphs followed by phosphate mineralization (Raff et al. 2008). The diagentic and subsequent geological processes that the fossil is subjected to can introduce artefacts (e.g. through crystal nucleation and growth) that have no connection to the original anatomy. These processes are difficult to simulate experimentally because of the timescales involved. An alternative approach is to characterize the mineral phases that preserve biological anatomy. This has been carried out in fossils where there is agreement regarding affinity and anatomy, and has allowed textual and chemical criteria to be established and then applied to more contentious Weng’an material (Cunningham et al. 2012a, 2014). In particular, geological artefacts tend to have larger, euhedral crystals with a preferred orientation, higher X-ray attenuation and characteristic void-filling textures. Geological artefacts typically also have high relative abundances of P, Ca and F, and low abundances of C and S. Failure to consider these processes and/or the 3D nature of the specimens can lead to misinterpretations of the fossils as discussed in Box 2.
et al. (2004); L. Chen et al. (2014); see Box 2 for a comparison of 2D and 3D analytical techniques). In unit 4B, a c. 10 m thick grey dolomitic phosphorite, the microfossils are abundant and in places are so concentrated that the layers resemble oolites. This is a grainstone composed largely of microfossils that have been phosphatized before being reworked, transported and winnowed (Xiao & Knoll 1999; Xiao et al. 2007b). The fossils can be extracted by dissolution of the carbonate constituents of rock samples in weak acetic acid and manual sorting of the resulting residues. Specimens can then be studied using scanning electron microscopy (e.g. Xiao et al. 1998) or tomographic techniques (e.g. Hagadorn et al. 2006) and can be re-embedded in resin and sectioned for further petrographic and geochemical analyses (reviewed by Cunningham et al. 2014).

### Overview of the Weng’an Biota

#### Algae

A variety of algal taxa have been reported from Weng’an (Zhao 1986; Zhang 1989; Zhang & Yuan 1992; Xiao et al. 1998, 2004; Xiao 2004). Some simpler forms, such as Archaeophycus (Fig. 2i), show interesting similarities to extant bangialean red algae (Xiao et al. 1998), although other affinities cannot be ruled out (Xiao et al. 2014a). More complex forms such as Thallophyca and Paramecia share characters with floridophyte red algae including pseudoparenchymous construction, differentiated thalli and possible reproductive structures (Xiao et al. 2004). However, the putative algae have received relatively little attention because of the focus on the search for animals. Many specimens lack the overall form of a photosynthetic organism. For example, the blades typical of various seaweeds have not been recovered and it is hard to envisage how photosynthetic organism. For example, the blades typical of various seaweeds have not been recovered and it is hard to envisage how photosynthetic organism. For example, the blades typical of various seaweeds have not been recovered and it is hard to envisage how they may have undergone unequal division. It is currently difficult to test between these possible interpretations.

#### Acritarchs

The acritarchs (Fig. 2k) are acritarchs from Weng’an have been reviewed comprehensively by Xiao et al. (2014b) and form part of the Doushantuo–Pertatakata microbiota (Zhou et al. 2001, 2007; Liu et al. 2014). The phylogenetic affinities of acritarchs are unknown and they probably represent a polyphyletic assemblage of euakaryotes (Huntley et al. 2006). Some Doushantuo acritarchs contain embryole-like fossils (Yin et al. 2007), leading to the suggestion that other Ediacaran acritarchs might be resting cysts of these organisms (Cohen et al. 2009). The interpretation of the Doushantuo–Pertatakata acritarchs is therefore at least partially linked to that of the embryo-like fossils, although they may well represent a polyphyletic assemblage. The affinities of taxa should be considered on a case-by-case basis.

### Embryo-like fossils

Weng’an fossils that have been interpreted as the embryos of early animals (Xiao et al. 1998) have been the focus of most attention and debate (Fig. 2a–f). They have been interpreted as metazoans (Xiao et al. 1998) including bilaterians (Chen & Chi 2005; J. Chen et al. 2006, 2009a,b; Yin et al. 2013), as stem-group metazoans (Hagadorn et al. 2006; Schiöblé et al. 2012; L. Chen et al. 2014) or as members of non-metazoan clades (Bailey et al. 2007a,b; Butterfield 2011b; Huldtgren et al. 2011, 2012; L. Chen et al. 2014; Zhang & Pratt 2014). We consider the various claims below.

#### Giant sulphur bacteria?

Bailey et al. (2007a,b) proposed an interesting hypothesis that the embryo-like fossils might be giant sulphur bacteria similar to the living Thiomargarita. These bacteria can be similar in size and shape to the embryo-like fossils from Weng’an and are capable of undergoing at least a few rounds of palintomic division. However, subsequent analyses have shown that these bacteria cannot account for key morphological aspects of the fossils such as the presence of ornamented envelopes, outer acritarch vesicles, and probable lipid vesicles and nuclei (Donoghue 2007; Xiao et al. 2007b; Huldtgren et al. 2011; Cunningham et al. 2012b). Moreover, evidence from experimental taphonomy showed that Thiomargarita cells are not replicated by biofilm-forming bacteria, meaning that they do not form the stable bacterial pseudomorphs that are thought to be the precursor to exceptional phosphatization (Cunningham et al. 2012b; see Box 1).

#### Bilaterians or eumetazoans?

Reports of embryonic bilaterians do not withstand scrutiny. Some are based on identifications of cell geometries argued to be unique to bilaterians. These include specimens purported to preserve endodermal cords (J. Chen et al. 2009b), polar lobes (J. Chen et al. 2006, 2009a; Yin et al. 2013), embryonic polarity (J. Chen et al. 2009a,b) and duct cleavage (J. Chen et al. 2009a). In each case, the specimens can be alternatively interpreted as examples of embryo-like fossils that have undergone taphonomic and diagenetic processes (Huldtgren et al. 2011; Cunningham et al. 2012a). Specimens interpreted as possible bilaterian or cnidarian gastrulae and larvae (J. Chen et al. 2000, 2002; Chen & Chi 2005) are more probable deformed cysts filled with phosphatic cements (Xiao et al. 2000). The presence of meroblastic embryos (Yin et al. 2016) would represent the post-sedimentary confirmation of the presence of animal embryos. A possible alternative interpretation of these specimens, which are associated with a large population known to undergo asynchronous division (Hagadorn et al. 2006), is that they have undergone unequal division. It is currently difficult to test between these possible interpretations.

#### Total group animals?

The embryo-like fossils probably represent one taxon dividing from a single cell to thousands of cells (Fig. 2a–f). This taxon has been
Fig. 2. Scanning electron microscope images of fossils from the Weng’an biota. (a–f) Tianzhushania specimens at various stages of division from a single cell (a) to many hundreds of cells (f) Swedish Museum of Natural History (SMNH) X 6449–SMNH X 6454. (g) Helicoforamina SMNH X 6455. (h) Spiraliscellula (from Tang et al. 2008). (i) Caveasphaera SMNH X 6456. (j) Archaeophycus, a putative red alga SMNH X 6457. (k) Mengeosphaera, an acritarch SMNH X 6458. (l) Eocyathispongia, a putative sponge, Nanjing Institute of Palaeontology and Geology (NIGPAS) 161760 (from Yin et al. 2015). Scale bar: (a) 320 µm, (b) 265 µm, (c) 265 µm, (d) 200 µm, (e) 245 µm, (f) 280 µm, (g) 395 µm, (h) 380 µm, (i) 250 µm, (j) 255 µm, (k) 130 µm, (l) 415 µm.
Fig. 3. Synchrotron radiation X-ray tomographic microscopy (SRXTM; a–h) and light microscopy (i–k) images of Weng’an fossils. (a, b) a possible alga SMNH X 6459, comparable with *Paramecia*. (c, d) a peanut-shaped fossil SMNH X 6460. (e, f) *Sinocyclocyclicus* SMNH X 5322. (g, h) *Ramitubus* SMNH X 5326. (i–k) Light microscopy images of putative algae from Weng’an. Scale bar: (a, b) 270 µm, (c, d) 280 µm, (e, f) 180 µm, (g, h) 175 µm, (i, j) 140 µm, (k) 115 µm.
**Box 3: Taxonomy of the embryo-like fossils**

The embryo-like fossils have been described under various genus and species names that are now considered to be different developmental stages or taphonomic variants of a single taxon (Huldtgren et al. 2011; Cunningham et al. 2012a; L. Chen et al. 2014; Xiao et al. 2014a,b). The names Megasphaera (single-celled specimens), Parapandorina (multiple polyhedral cells) and Megaclonophycus (large numbers of usually spheroidal cells) are now widely considered to be synonyms (e.g. Huldtgren et al. 2011; Cunningham et al. 2012a; Xiao et al. 2014a,b). However, because of different taxonomic interpretations, different researchers have referred to this taxon as either Megasphaera (e.g. L. Chen et al. 2014; Xiao et al. 2014a,b) or Tianzhushania (e.g. Yin et al. 2004; Huldtgren et al. 2011; Cunningham et al. 2012a). The genus Tianzhushania and its type species T. spinosa were described in 1978 for acanthomorphic acritarchs with cylindrical processes that were known from thin sections (Yin & Li 1978). Yin et al. (2001) subsequently described T. tuberifera based on specimens with both cylindrical processes and sculptured ornament. Megasphaera, with the type species *M. inornata*, was described in 1986 for smooth envelopes (Chen & Liu 1986) and later expanded by Xiao & Knoll (2000) to accommodate specimens with sculptured envelopes (*M. ornata*). Yin et al. (2004) showed that *M. ornata* specimens, when viewed in thin sections, could be surrounded by an outer wall identical to that of *Tianzhushania*. They therefore argued that *T. tuberifera*, which had been studied in thin sections, was the same species as *M. ornata*, which had mainly been studied in specimens isolated from acid residues. Yin et al. (2004) proposed *Tianzhushania ornata* as the valid name for this taxon. As the various embryo-like stages can be found inside these specimens, Huldtgren et al. (2011) argued that *Tianzhushania* is the senior synonym of *Megasphaera*, Parapandorina and Megaclonophycus. Xiao et al. (2014a,b) noted that the diagnosis of *Tianzhushania* had never been formally emended to include specimens with sculptured envelopes. They therefore proposed to retain *Megasphaera* for smooth or sculptured specimens that lack processes and *Tianzhushania* for specimens that have smooth envelopes and processes. The new genus *Yimitianzhushania* (basionym *T. tuberifera*) was erected to accommodate those specimens that have sculptured envelopes and cylindrical processes. However, this classification differentiates *Megasphaera* from the other genera based on the absence of tubular processes, which probably result from taphonomic loss rather than a biological difference. We also find it unsatisfactory to place specimens from acid residues into *Yimitianzhushania* if they have a sculptured envelope and *Tianzhushania* if they do not. This would result in a specimen with a sculptured envelope and tubular processes being placed in one genus (*Yimitianzhushania*) if it had lost only its processes and another (*Tianzhushania*) if it had also lost its sculptured envelope. It is therefore preferable to place all of these taxa in a single genus, *Tianzhushania*, which we consider to be the senior synonym of *Yimitianzhushania*, as well as of *Megasphaera*, Parapandorina and Megaclonophycus, despite the fact that the diagnosis of *Tianzhushania* has not yet been formally revised to include specimens with sculptured envelopes.

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**Fig. 4.** Schematic representation of eukaryote phylogeny, modified after Rensing (2016), showing the distribution of characters relevant to the interpretation of the embryo-like fossil *Tianzhushania*. Here multicellularity includes both aggregative multicellularity (e.g. slime moulds) and clonal multicellularity (animals, plants, fungi, various algae), as well as both facultative (e.g. choanoflagellates) and obligate multicellularity.
tetraids, which show evidence for palintomy, and larger ‘matryoshka’ clusters, which do not (Tang 2015; Cunningham et al. 2016). Although there must be a switch from palintomy at some point (Chen et al. 2017), the lack of intermediates weakens the evidence for an endogenous origin (Tang 2015; Cunningham et al. 2016).

The argument for apoptosis is that, because the enclosing envelope imposes a constant volume throughout ontogeny, the only way to create the space required for the putative reproductive structures to grow is for other cells to die off. However, the volume of *Tianzhushania* may well not be constrained in this way, given that early stages often do not occupy the full envelope, and putative later stages provide evidence for distension and then rupture of the envelope (Liu et al. 2009; Huldtgren et al. 2011). The evidence for differentiation, germ–soma separation and programmed cell death is therefore unconvincing. Moreover, there is also uncertainty regarding the other proposed animal characters. There is no evidence that the walls of *Tianzhushania* are any less rigid than those of non-animal groups such as non-metazoan holozoa (Marshall & Berbee 2011), amoebozoans (Olive 1975), ciliates (Park et al. 2005) or volvocalesc (Hallmann 2006), which also have Y-shaped cell junctions. Nor is there evidence that cell adhesion is different from that seen in groups such as non-metazoan holozoa (vovlcoalesce algae achieve adhesion through cytoplasmic bridges, which are absent in the fossils).

To summarize, *Tianzhushania* does not exhibit characters that are sufficient to identify it as an animal. Evidence for the presence of characters gained in the animal stem lineage is equivocal. There is therefore no justification for an interpretation of *Tianzhushania* as an animal, although a stem-animal affinity cannot be definitively rejected on the basis of current data (Huldtgren et al. 2011, 2012). Above all, the available evidence does not yet allow these fossils to be used to substantiate hypotheses on the timing of animal diversification.

**Other possibilities**

A number of alternative interpretations have been proposed, yet the affinities of these organisms remain uncertain. Comparisons with non-metazoan holozoa (Huldtgren et al. 2011), algae (Butterfield 2011b; L. Chen et al. 2014; Zhang & Pratt 2014) or ciliates are plausible but as yet unsubstantiated and require further investigation. The primary factor that has hindered interpretation of these fossils is poor understanding of the later stages of the organisms’ ontogeny. A number of candidates have been proposed, but none are widely accepted. Xiao et al. (2007a) suggested that helically coiled specimens (Fig. 2g), now named Helicoforamina, might be later stages, perhaps representing a coiled vermiform animal. However, this taxon is enigmatic and has also been argued to be an embryo of the cnidarian-like eumetazoans (Xiao et al. 2007; Chen et al. 2002; Liu et al. 2008). Other possibilities regarding the other proposed animal characters. There is no evidence to support a placement of these tubular fossils within eumetazoans or animals.

**Vernanimalcula**

*Vernanimalcula* has been described from thin sections as a miniature, adult bilaterian from the Weng’an Biota (Chen et al. 2004; Petrushev et al. 2013). It is purported to preserve bilaterian characters such as a mouth, gut, anus and paired coelomic cavities. However, all of the putative bilaterian characters can be alternatively interpreted as artefacts resulting from abiological diagentic apatite cements, which are ubiquitous in the deposit (Bengtson 2003; Bengtson & Budd 2004; Bengtson et al. 2012; Cunningham et al. 2012a). Moreover, 3D analyses show that Weng’an fossils such as acritarchs and fertilization envelopes that clearly lack bilateral symmetry can exhibit *Vernanimalcula*-like morphology when sectioned in particular orientations (Bengtson et al. 2012). There is no evidential basis for interpreting *Vernanimalcula* either as a bilaterian or as an animal of any kind.

**Summary and prospects**

Research into the Weng’an Biota is currently in a transitional phase. Early research involved many attempts to demonstrate the long-expected presence of animals, including bilaterians, in the Ediacaran. This has been followed by a spell of critical analysis of these claims that has shown that none of these fossils can so far be confidently identified as stem- or crown-group metazoans. The research is now entering a phase where more targeted analysis of the palaeobiology of each taxon is helping to constrain wide-ranging hypotheses of affinity more rigorously. Many fossils are known only from a few specimens and have received limited attention. One such example is the enigmatic fossil *Caveasphaera* (Fig. 2i), which has been tentatively compared with cnidarian embryos (Xiao & Knoll 2000) but requires further investigation. Such taxa betray a cryptic diversity that has been overlooked because of the focus on *Tianzhushania*.

The Ediacaran was a critical interval in the history of life (Butterfield 2007) and the Weng’an Biota offers a unique glimpse of microscopic, multicellular and soft-bodied organisms at this time.


