REVIEW

National Science Review
1: 498–520, 2014
doi: 10.1093/nsr/nwu061
Advance access publication 17 October 2014

GEOSCIENCES

Special Topic: Paleontology in China

The Weng'an biota and the Ediacaran radiation of multicellular eukaryotes

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Received 28 July 2014; **Revised** 17 September 2014; **Accepted** 18 September 2014

ABSTRACT

The rise of multicellularity represents a major evolutionary transition and it occurred independently in multiple eukaryote clades. Although simple multicellular organisms may have evolved in the Mesoproterozoic Era or even earlier, complex multicellular eukaryotes began to diversify only in the Ediacaran Period, just before the Cambrian explosion. Thus, the Ediacaran fossil record can provide key paleontological evidence about the early radiation of multicellular eukaryotes that ultimately culminated in the Cambrian explosion. The Ediacaran Weng'an biota in South China hosts exceptionally preserved eukaryote fossils, including various acanthomorphic acritarchs, pseudoparenchymatous thalli, tubular microfossils, and spheroidal fossils such as *Megasphaera*, *Helicoforamina*, *Spiralicellula*, and *Caveasphaera*. Many of these fossils have been interpreted as multicellular eukaryotes, although alternative interpretations have also been proposed. In this review, we critically examine these various interpretations, focusing particularly on *Megasphaera*, which has been variously interpreted as a sulfur-oxidizing bacterium, a unicellular protist, a mesomycetozoean-like holozoan, a volvocine green alga, a stem-group animal, or a crown-group animal. We conclude that *Megasphaera* is a multicellular eukaryote with evidence for cell-to-cell adhesion, a flexible membrane unconstrained by a rigid cell wall, spatial cellular differentiation, germ–soma separation, and programmed cell death. These features are inconsistent with the bacterium, unicellular protist, and mesomycetozoean-like holozoan interpretations. Thus, the surviving hypotheses, particularly the stem-group animal and algal interpretations, should be further tested with additional evidence. The Weng'an biota also hosts cellularly differentiated pseudoparenchymatous thalli with specialized reproductive structures indicative of an affinity with florideophyte red algae. The other Weng'an fossils reviewed here may also be multicellular eukaryotes, although direct cellular evidence is lacking in some and phylogenetic affinities are poorly constrained in others. The Weng'an biota offers many research opportunities to resolve the life histories and phylogenetic diversity of early multicellular eukaryotes and to illuminate the evolutionary prelude to the Cambrian explosion.

Keywords: Ediacaran Period, Doushantuo Formation, Weng'an biota, multicellularity, eukaryotes, animals, algae

INTRODUCTION

The evolution of multicellularity, best exemplified by the rise of animals, represents a major transition in the history of life [1] and had transformative geobiological and ecological impacts. Multicellularity evolved independently at least 25 times among eukaryotes [2–4], and complex multicellularity—characterized by intercellular communication and, commonly, tissue differentiation controlled by regulatory gene networks—occurs in a handful of eukaryotic groups [5,6]. Given the evolutionary importance of multicellularity, there is surprisingly little paleontological discussion on this topic, in part due to the scant and ambiguous nature of early

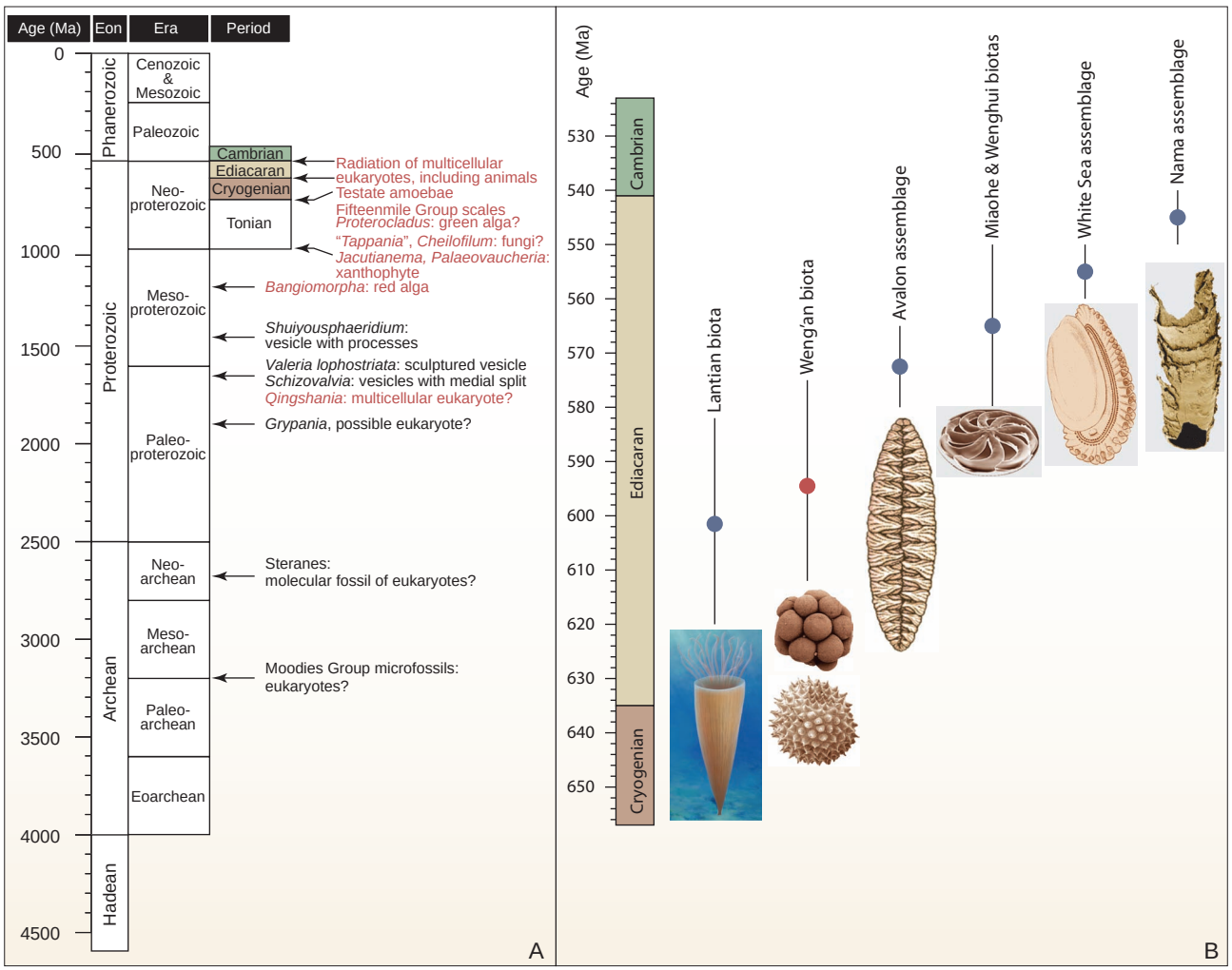

Figure 1. Proterozoic multicellular eukaryotic fossils. (A) Examples of Proterozoic eukaryote fossils, with multicellular eukaryotes highlighted in red color. (B) Exceptionally preserved biotas in the Ediacaran Period, with thumbnails showing representative taxa in each biota. The Weng'an biota is highlighted by a red dot. Vertical lines denote uncertainty in age estimates.

multicellular eukaryotic fossils. Eukaryotes evolved in the Paleoproterozoic (2500–1600 million years ago, Ma) if not earlier, and by the Cryogenian Period (~720–635 Ma), several eukaryote clades, including red algae, green algae, fungi, rhizarians, and amoebozoans, may have diverged [7–9]. Multicellular eukaryotes may have evolved in the late Paleoproterozoic and certainly by the Mesoproterozoic (1600–1000 Ma) [9–11], although their diversity remained relatively low until in the Ediacaran Period (Fig. 1).

The Ediacaran Period is a critical geological interval in the evolution of multicellular eukaryotes, not only because multicellular eukaryotes seem to have radiated in this time period, but also the fuse to the Cambrian explosion appears to be buried in Ediacaran rocks. To develop a better understanding of Ediacaran multicellular eukaryotes and their relationships to Cambrian animals, it is essential to have a fossil record of the Ediacaran Period on a par with that of the Cambrian Period. However, because virtually all life forms in the Proterozoic Eon—including those in the Ediacaran Period—are non-skeletal, our paleontological knowledge about the Ediacaran Period is generally poor and crucially dependent on exceptional taphonomy (the preservation of non-skeletal organisms through rapid authigenic mineralization processes) [12–14]. A handful of exceptionally preserved Ediacaran biotas stand above others because of their preservation of multicellular eukaryotes and hence their potential relevance to the Cambrian explosion (Fig. 1). These include the Lantian biota [15], Weng'an biota [16], Avalon assemblage [17], Miaohe biota and the equivalent Wenghui biota [18–21], White Sea assemblage [22], and Nama assemblage [23]. Among these biotas, the Weng'an biota hosted in phosphorite of the Doushantuo Formation in the Weng'an

area, central Guizhou Province, South China, is unique for its extraordinary cellular preservation. Because of the unparalleled preservation quality, the Weng'an biota provides direct evidence about the early evolution of cellularly differentiated eukaryotes and offers tantalizing but controversial insights into the early evolution of animals. In this review, we summarize the progress, problems, and prospect in the paleontological investigation of the Weng'an biota.

RESEARCH HISTORY

Geological investigation of the Doushantuo Formation in the Weng'an area started in the 1960s, but prior to the 1990s, paleontological reports on this geological unit were limited to the Chinese literature. Stromatolites have been known from Doushantuo phosphorite in the Weng'an area since the 1960s [24–26], and the first microfossils were discovered in 1984–1986 when Shixing Zhu (Fig. 2A) and others illustrated microfossils in thin sections and interpreted them as cellularly preserved eukaryotic algae related to red algae [27–29]. Also in 1984, microtunnels were described as *Microptychoites fuquanensis* [30] and interpreted as microscopic trace fossils [30–32], but these were later re-interpreted as pyrite trails [33,34]. Shortly afterwards, Meng'e Chen (Fig. 2B) described the first acanthomorphic acritarch (*Meghystrichosphaeridium wenganensis*) and spheroidal microfossil (*Megasphaera inornata*) from the Weng'an biota [35].

The Weng'an biota became internationally known in the late 1980s and early 1990s when Yun Zhang (Fig. 2C) published the first report of Weng'an paleontology in western literature [36] and Yaosong Xue (Fig. 2D) and colleagues extracted exquisitely preserved acanthomorphic acritarchs, spheroidal microfossils, and tubular microfossils from the Doushantuo Formation at Weng'an [37–39]. In 1998, two independent research groups reported the discovery of animal embryos from the Doushantuo Formation at Weng'an [16,40], opening a continuing debate on the phylogenetic affinities of various fossils in the Weng'an biota, including putative ciliates and other protists [41–43], possible lichenoids [44], red algae [45–48], blastula-like *Megasphaera* [49–55], putative sponge embryos [56,57], tubular cnidarian fossils [58–64], and putative bilaterian animals and their embryos [65–74]. Also in 1998, a systematic treatment of acanthomorphic acritarchs was published [75], followed by additional investigations using both thin section and maceration techniques [33,76–79]. In the past 10 years, three books on the Weng'an biota have been published [31,80,81]. The continuing interest in the Weng'an biota has been driven by the potential animal fossils and the biostratigraphic significance of acanthomorphs. Given the outstanding questions and controversies in early animal evolution and Ediacaran biostratigraphy, it is anticipated that the Weng'an biota will continue to be in the spotlight in decades to come.

STRATIGRAPHY, AGE CONSTRAINTS, AND DEPOSITIONAL ENVIRONMENT

The stratigraphy of the Doushantuo Formation at Weng'an has been described in several publications, most recently in Xiao *et al.* [79]. As in the Yangtze Gorges area where the Doushantuo Formation was first typified, the Doushantuo Formation at Weng'an overlies Cryogenian diamictite of the Nantuo Formation and underlies the upper Ediacaran Dengying Formation (Fig. 3A). It is about 40 m thick and can be divided into five units [79]. Unit 1 is a

Figure 2. Palaeontologists who pioneered the study of the Weng'an biota. (A) Shixing Zhu. (B) Meng'e Chen. (C) Yun Zhang. (D) Yaosong Xue.

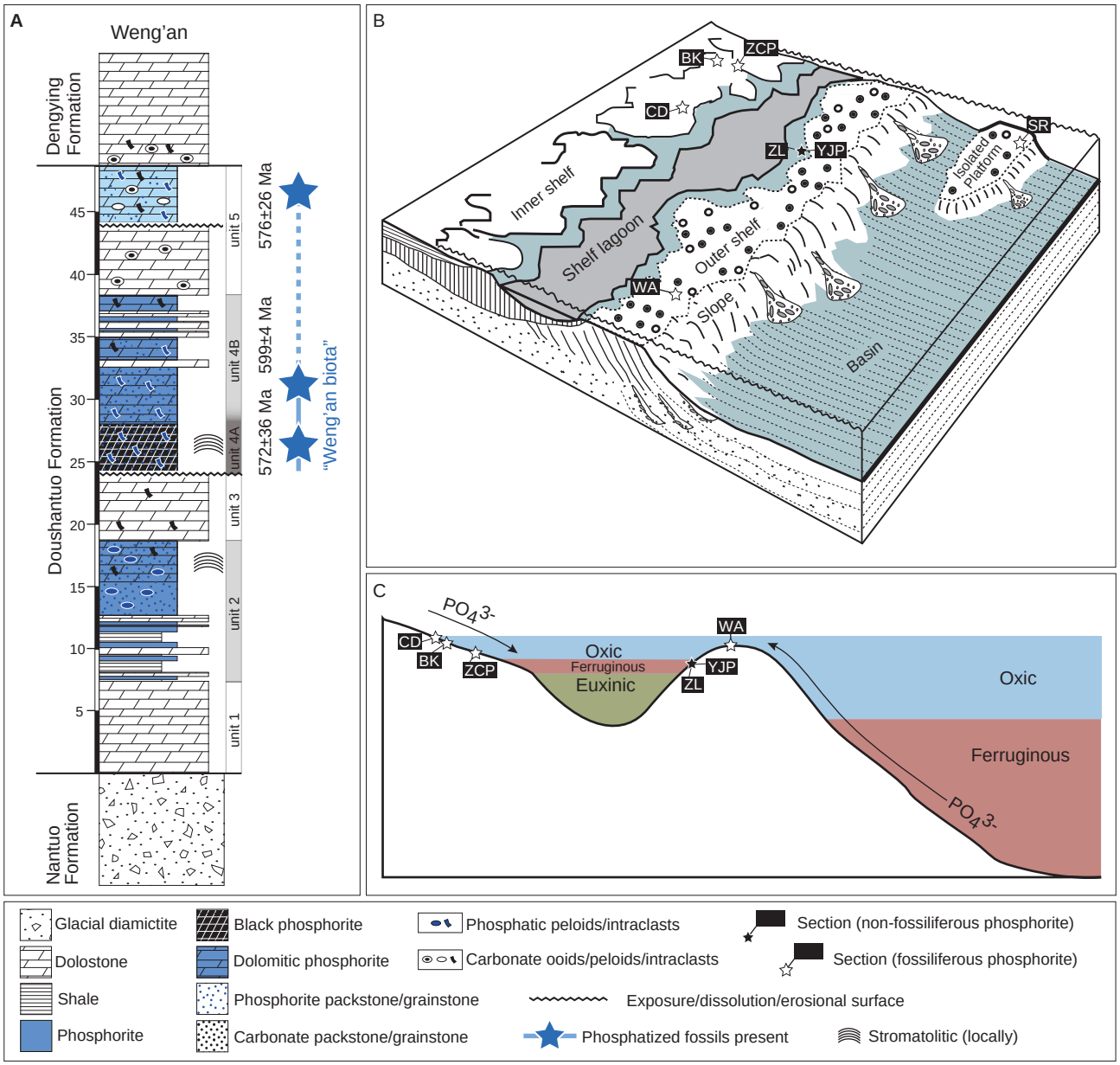

Figure 3. Simplified stratigraphic column (A) and depositional environment of the Doushantuo Formation at Weng'an (B: depositional model; C: transect across the Yangtze platform, with arrows showing possible source of phosphorus). Occurrences of Doushantuo phosphorite: Baokang (BK), Chadian (CD), Zhangcunping (ZCP), Zhongling (ZL), Yangjiaping (YJP), and Weng'an (WA). Pb–Pb isochron ages from [87–89]. Modified from [13].

5–10 m thick cap dolostone that is widely distributed in South China, and can be used as a marker bed for the correlation of the Doushantuo Formation deposited in different environments. The overlying unit 2 is 8–15 m thick, characterized by thin-bedded, peloidal phosphorite with interbedded dolostone and siltstone that contains rare pyritized microfossils [33]; this unit is also known as the lower phosphorite or phosphorite ore A [82]. Unit 3 is a 2–4 m thick massive dolostone, also known as the mid-Doushantuo dolostone. The upper surface of unit 3 is irregular and represents a prominent karstification surface. Unit 4 consists of 3–10 m thick intraclastic phosphorite, and is variously referred to as the upper phosphorite, phosphorite ore B [82], or Weng'an phosphorite [83]. The lower part of unit 4 (or unit 4A) is 0.5–5 m thick black phosphorite equivalent to the black facies of Dornbos *et al.* [83,84], whereas the upper part (or unit 4B) is 1–5 m thick gray dolomitic phosphorite equivalent to the gray facies of Dornbos *et al.* [83,84]. Unit 5 is ~10 m thick phosphatic dolostone, which may contain an additional exposure surface [85,86].

The Weng'an biota occurs in units 4 and 5 [79], which yield Pb–Pb isochron ages of 572 ± 36 Ma (unit 4A) [87], 599 ± 4 Ma (unit 4B) [88], and 576 ± 26 Ma (unit 5) [89]. These ages are consistent with zircon U–Pb ages from ash beds in

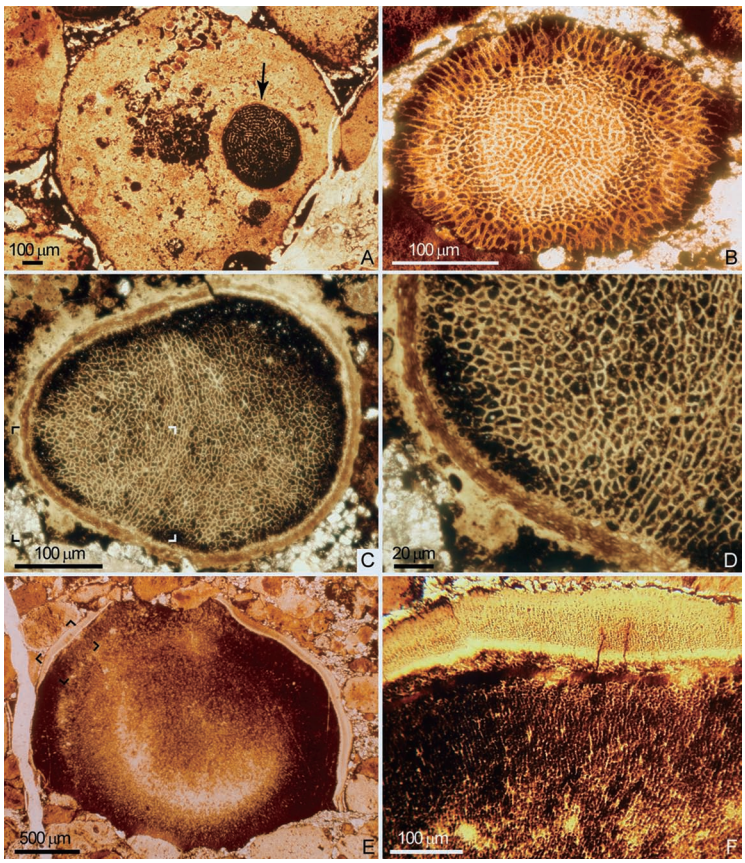

Figure 4. Transmitted light photomicrographs of multicellular algal thalli showing pseudoparenchyma and medulla–cortex differentiation. (A) *Wengania globosa* (arrow) in an intraclastic grain, indicating that the fossil was phosphatized elsewhere in the basin and then reworked and redeposited as intraclasts. (B) *W. globosa* specimen with a medulla and a cortex, which probably had filamentous construction with different cell sizes or filament orientations (e.g. medullar filaments are coaxially oriented but cortical filaments radiate outwardly). (C–F) Thalli with clearly differentiated cortex and medulla, with smaller cortical cells that are tangentially oriented (C–D) or radially oriented (E–F). (D, F) are magnifications of rectangle areas in (C, E), respectively. All fossils from unit 4A.

the Yangtze Gorges area, which constrain the Doushantuo Formation between 635.26 ± 1.07 and 551.09 ± 1.02 Ma [90,91]. Condon *et al.* [90] argue that the karstification surface atop unit 3 may be a glacioeustatic response to the ~582 Ma Gaskiers glaciation [92], implying that the Weng'an biota would be constrained between 582 and 551 Ma. However, the mid-Doushantuo dolostone (equivalent to unit 3) in the Zhangcunping area of Hubei Province contains a 'volcanic bed' dated at 614.0 ± 7.6 Ma [93], casting doubt on its temporal proximity to the 582 Ma Gaskiers glaciation. It is more likely that the exposure surface in unit 5 may represent a glacioeustatic response to the Gaskiers glaciation. This alternative interpretation is not contradicted by the 599 ± 4 Ma age from unit 4, and implies that the Weng'an biota is 600–582 Ma in age.

Biostratigraphic correlation of the Doushantuo Formation between the Weng'an and Yangtze Gorges areas has been attempted on the basis of newly described acanthomorphic acritarchs [79,94]. Briefly, two acritarch biozones can be recognized in the Yangtze Gorges area, with the lower zone dominated by *Tianzhushania spinosa* and the upper zone dominated by *Hocosphaeridium anozos* and *Tanarium conoideum* [95,96]. The Weng'an biota contains acanthomorph elements of both biozones and probably represents a transitional zone between the two biozones recognized in the Yangtze Gorges area [79,97]. This correlation is consistent with the radiometric age constraints currently available from both the Yangtze Gorges and Weng'an areas.

The Doushantuo Formation in South China was deposited on a southeast-facing passive continental margin on the Yangtze Craton [86]. Following the terminal Cryogenian glaciation, the Yangtze platform soon evolved from a ramp to a rimmed shelf, with mudstone-dominated shelf lagoon facies and grainstone-dominated outer shelf facies. Doushantuo phosphorites seem to be preferentially deposited in shallow water facies, including inner shelf and outer shelf environments [13]. The phosphatized Weng'an biota is no exception, occurring in the upper Doushantuo Formation deposited in the outer shelf facies (Fig. 3B and C). The host lithologies are characterized by stromatolites and intraclastic material [24–26,98], indicating deposition within the photic zone and above fair weather wave base [33,99]. Often, the intraclastic material consists of intrabasinally reworked phosphatic grains that are cemented by phosphate and silica (in black facies), or dolomite (in gray facies). Phosphatized microfossils are mostly found as or in reworked phosphatic grains (Fig. 4A), suggesting that fossil phosphatization occurred elsewhere in the basin and phosphatic grains were reworked, abraded, transported, winnowed, concentrated, and redeposited at Weng'an [33,50, 53]. The secondary reworking explains the high concentration of fossiliferous intraclasts that puzzled some geologists [49,100]. The shallow depositional environment implies that the Weng'an biota was likely preserved beneath oxic marine waters, despite the frequent occurrence of euxinic or ferruginous anoxia in deeper water or restricted environments, such as shelf lagoon, slope, and basinal facies [86,97,101–104]. Trace element geochemical data also indicate that the fossiliferous units 4 and 5 were likely deposited in oxic marine waters [105–108]. Thus, the Weng'an biota probably lived and was preserved in shallow and oxygenated environments, but this does not rule out the possibility that pore waters within sediments

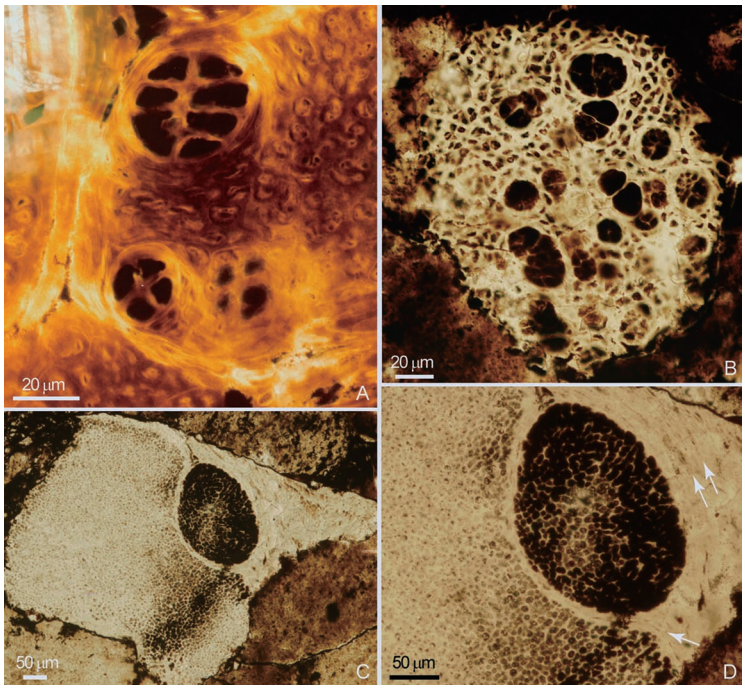

Figure 5. Transmitted light photomicrographs of possible reproductive structures of Weng'an algal thalli. (A–B) Monads, dyads, tetrads, octads, and cell islands. (C–D) Sorus-like cell island surrounded by light-colored cell layers with tangentially oriented cells (arrows). (D) is a magnification of (C). All fossils from unit 4A.

could have been anoxic during the phosphatization of Weng'an fossils. Indeed, it has been suggested that phosphogenesis and penecontemporaneous phosphatization probably did occur in anoxic sediments, with a strong and fluctuating redox gradient across the sediment–water interface facilitating the localized concentration of phosphate in sediments [12,13,109].

ACANTHOMORPH ACRITARCHS

An up-to-date review of acanthomorphic acritarchs in the Weng'an biota has been published recently [79]. Although acritarchs are typically regarded as the resting cysts of phytoplankton, for example the unicellular prasinophytes, it has been long recognized that they are polyphyletic and may include both unicellular and multicellular eukaryotes including animals [110]. The multicellular nature of some Doushantuo acanthomorphic taxa has been confirmed in cellularly preserved material in the Weng'an biota [79] and in the Yangtze Gorges area [94]. Indeed, the biozonal acanthomorph *T. spinosa* has been shown to be a multicellular eukaryote, perhaps representing animal diapause eggs [111], and vesicle wall ultrastructures of some Ediacaran acanthomorphs also suggest an affinity with diapause eggs of ancient animals [112,113]. Thus, acanthomorphic acritarchs in the Weng'an biota definitely include multicellular eukaryotes, although most of them have not been studied in detail to understand their life cycles and phylogenetic affinities.

MULTICELLULAR ALGAE

The first body fossils described from the Weng'an biota were multicellular algae, and it has since been recognized that these fossils are related to red algae [27–29,36,48,114]. For example, Xiao *et al.* [16] compared tetrad cell packets of the Weng'an fossil *Paratetraphycus giganteus*, later regarded as a junior synonym of *Archaeophycus yunnanensis* [115], with carposporangia of *Porphyra*, a modern bangialean genus [116,117]. However, given the morphological simplicity of tetrad cell packets, the possibility of convergent evolution among cyanobacteria, red algae, and green algae cannot be ruled out. Thus, a phylogenetic relationship between *Archaeophycus yunnanensis* and living bangialeans is an intriguing possibility, but at present is regarded as a tentative interpretation.

More convincing red algae in the Weng'an biota come from fossils with prominent pseudoparenchyma, cortex–medulla differentiation, and specialized reproductive structures (Figs 4–6) [47]. Although pseudoparenchyma—a multicellular construction with filaments that cut off cells along a single direction—occurs in red algae, green algae, and fungi [6], it is a conspicuous feature of florideophyte red algae, particularly corallinaleans [118] and their Paleozoic relatives [119]. Some Doushantuo fossils also show unambiguous evidence for thallus differentiation into a central medulla and a peripheral cortex (Fig. 4B–F). Often, medullar and cortical cells are different in size, shape, orientation, and preservation. Their differential preservation likely reflects underlying biological differences (e.g. in cell wall thickness) that affected their taphonomy. Also, some Weng'an algal thalli show evidence for apical meristematic growth and cell fusion [47], both features occurring in modern florideophyte red algae [117]. Perhaps the most diagnostic florideophyte features among Weng'an algal fossils are specialized structures (e.g. tetrads, octads, cell islands, and aggregate filaments embedded in and surrounded by vegetative cells) interpreted as reproductive structures such as tetraspores, octospores, cystocarps, and sori (Figs 5–6). Again, these reproductive structures are typically preserved differently from the surrounding vegetative thallus, probably reflecting biological differences (e.g. in cell size and cell wall thickness) between reproductive spores and vegetative cells. The life cycle of these Weng'an algal fossils is

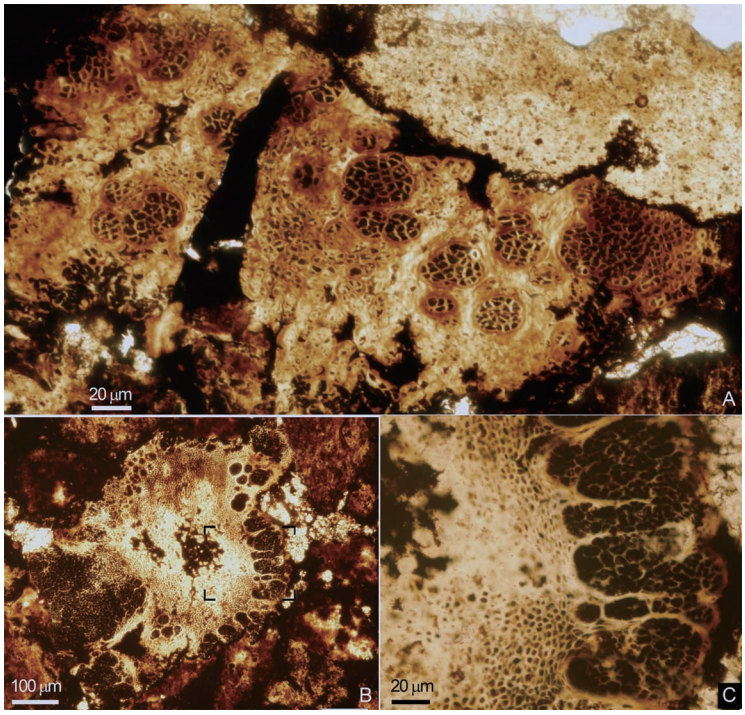

Figure 6. Transmitted light photomicrographs of possible reproductive structures of Weng'an algal thalli. (A) Cell islands. (B–C) *Paramecia incognata* thallus with peripheral chambers filled with dark-colored cells that are different in size and preservation from surrounding vegetative cells. (C) shows a magnification of rectangle area in (B). All fossils from unit 4A.

not yet completely understood; for example, it is uncertain whether the tetrads/octads developed into cell islands or were released to develop gametophytes as in modern florideophytes. Nonetheless, considering the geological age and their likely primitive nature, the Doushantuo multicellular algae with prominent pseudoparenchyma, cortex–medulla differentiation, and specialized reproductive structures can be plausibly interpreted as florideophytes, perhaps related to corallines [47].

Traditionally, the Rhodophyta is divided into the Bangiophyceae and Florideophyceae [120,121]. Recent molecular phylogenetic analyses, however, suggest that the traditionally recognized Bangiophyceae is a paraphyletic group [116]. The Florideophyceae, on the other hand, survives as a monophyletic clade consisting of the Hildenbrandiophycidae at the base, successively followed by the Nemaliophycidae, Corallinophycidae, Ahnfeltiophycidae, and Rhodymeniophycidae [122–125]. The Corallinophycidae includes three orders, Rhodogorgonales, Sporolithales, and Corallinales [125–127]. In light of this new phylogeny, it is possible that Weng'an multicellular algae with conspicuous pseudoparenchyma, spatial cell differentiation, and specialized reproductive structures could be stem-group corallinophycideans (Fig. 7). If so, much cladogenesis within the florideophyceans must have occurred in the Neoproterozoic.

TUBULAR MICROFOSSILS

Tubes with both complete and incomplete cross-walls are moderately abundant in the Weng'an biota. An up-to-date systematic treatment of these tubular microfossils has been published by Liu *et al.* [63]. Five species, *Ramitubus increscens*, *R. decrescens*, *Sinocyclocyclicus guizhouensis*, *Quadratitubus orbigoniatus*, and *Crassitubus costatus*, have been recognized on the basis of (1) whether and how the tube branches; (2) whether the tube is cylindrical or square; (3) whether the tube curves consistently; (4) whether the tube has a longitudinal ridge; (5) whether the tube has a multilaminate outer wall; and (6) whether the tube has both complete and incomplete cross-walls (Fig. 8).

The phylogenetic affinities of the Weng'an tubular microfossils are a matter of uncertainty. Some interpreted them as skeletal animals [61], comparing them with crinoid ossicles [37] or with Cambrian small shelly fossils [58]. Indeed, the tubular microfossils are sometimes preserved as internal molds consisting of stacked tablets that are similar to and were interpreted as secondarily phosphatized crinoid ossicles [37]. These tubular microfossils are often preserved three-dimensionally and sometimes show evidence of brittleness, giving the impression that they were skeletal organisms. However, when fossil mineralization occurs immediately after death (as is the case in the Weng'an biota), it can become difficult to determine whether mineralization occurred before death (biomineralization) or after death (taphonomic mineralization). Nonetheless, the rare occurrence of degraded cross-walls (Fig. 9A–C) and deformed tube walls (Fig. 9D–F) suggests that the Weng'an tubular microfossils were probably non-biomineralizing organisms that were phosphatized shortly after death but before significant decay [59,63]. Thus, apart from their lack of stereom and five-fold symmetry, their physical flexibility is also inconsistent with the crinoid ossicle interpretation.

Alternative phylogenetic interpretations of the Weng'an tubular microfossils relate them to filamentous cyanobacteria [64], algae [128], stem-group cnidarians somewhat similar to Paleozoic tabulate corals [59,63,64], or crown-group cnidarians [60]. If the described taxa of Weng'an tubular microfossils are closely related, then their collective morphological features make a cyanobacterial interpretation unlikely. These features include a square tubular morphology (in *Q. orbigoniatus*), dichotomous

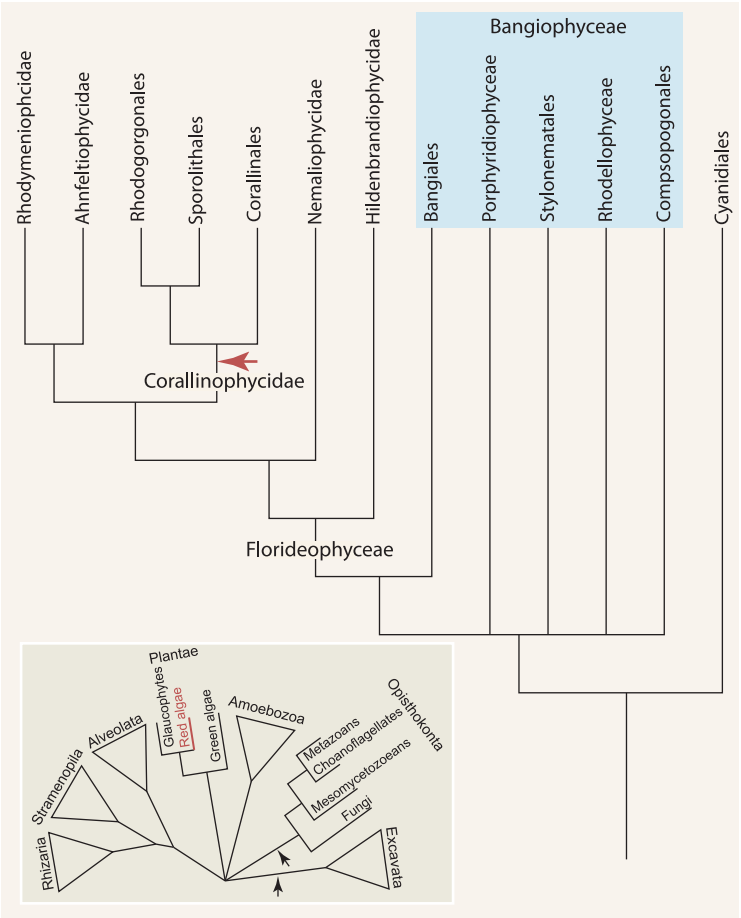

Figure 7. A phylogenetic tree of modern red algae [116, 122–127], with arrow denoting the possible phylogenetic position of Weng'an algal thalli with pseudoparenchyma, medulla–cortex differentiation, and specialized reproductive structures (Figs 4–6). Inset in lower left shows an unrooted phylogenetic tree of the eukaryotes to highlight the phylogenetic position of red algae, with arrows marking alternative rooting [170,171].

branching (in *R. decrescens* and *R. increscens*), a longitudinal ridge (in *C. costatus*), several ranks of incomplete cross-walls (in *C. costatus*, *Q. orbigoniatus*, and *S. guizhouensis*), and a pointed apical end (in *Q. orbigoniatus* and *S. guizhouensis*). An algal interpretation is intriguing, but the square tube and longitudinal ridge (always on the concave side of curved *C. costatus* tubes) are unusual features for algae; these features are indicative of tetraradial and bilateral symmetry, respectively. Moreover, some tubes seem to have nested side-walls (Fig. 9G and H) that do not find many comparisons among tubular or siphonous algae but are similar to the nested side-walls of *Cloudina riemkeae* (Fig. 9I), which is a weakly biomineralized Ediacaran tubular microfossil with some degree of physical flexibility [129]. Also, some Weng'an tubes have a wedge-shaped gap between successive sets of nested side-walls (Fig. 9J and K). This feature has been interpreted as a poorly preserved example of dichotomous branching [63]. Alternatively, it could be similar in origin to the wedge-shaped gap in *C. riemkeae* (Fig. 9L), which may have resulted from the release of one of the two daughter branches derived from asexual reproduction [129] or abrupt change in growth orientation after a period of arrested growth and subsequent regeneration [130–132]. We emphasize that *Cloudina* do not have internal cross-walls [133] and are thus fundamentally different from the Weng'an tubular fossils, but it is important to note their potential similarities in nested side-walls, dichotomous branching as an asexual reproduction strategy, or ecological response to arrested growth.

Whether the Weng'an tubes are related to cnidarians remains to be confirmed with more definitive evidence, but their tetraradiality/biradiality and the nested side-walls seem to be incompatible with the cyanobacterial and algal interpretations, and their non-biomineralizing nature and general morphologies refute the crinoid interpretation. Regardless, it is safe to conclude that they are likely multicellular eukaryotes with cellular and tissue differentiation because the construction of such morphologically complex tubes requires specialized tissues.

PUTATIVE ANIMALS AND ANIMAL EMBRYOS

Perhaps the most controversial fossils in the Weng'an biota are putative animals and animal embryos, which are potentially some of the most important fossils. Li *et al.* reported sponge embryos, larvae, and siliceous monaxonal spicules observed in thin sections [40], but some of these fossils appear to be multicellular algae or acanthomorphic acritarchs [56], and the putative spicules do not appear to be siliceous [134]. Cnidarian embryos, bilaterian embryos, and microbilaterian animals (e.g. *Vernanimalcula guizhouena*) have also been reported from thin sections [65,66,72], but the interpretation of these fossils is plagued by diagenesis, particularly multiple generations of cementation [59,71,135].

Ongoing debate, therefore, is centered upon the phylogenetic affinity of the spheroidal fossil *Megasphaera* (Fig. 10) [79,136]. The type species *M. inornata* was proposed to account for Weng'an spheroidal fossils with one large cell enclosed within a smooth envelope [35]. It was subsequently realized that some specimens are enclosed within an envelope ornamented with various sculptures (*M. ornata*), and others have multiple envelopes with the outermost being ornamented [136]. Thus, the distinction between *M. inornata* and *M. ornata* may be a taphonomic one, because the ornamented

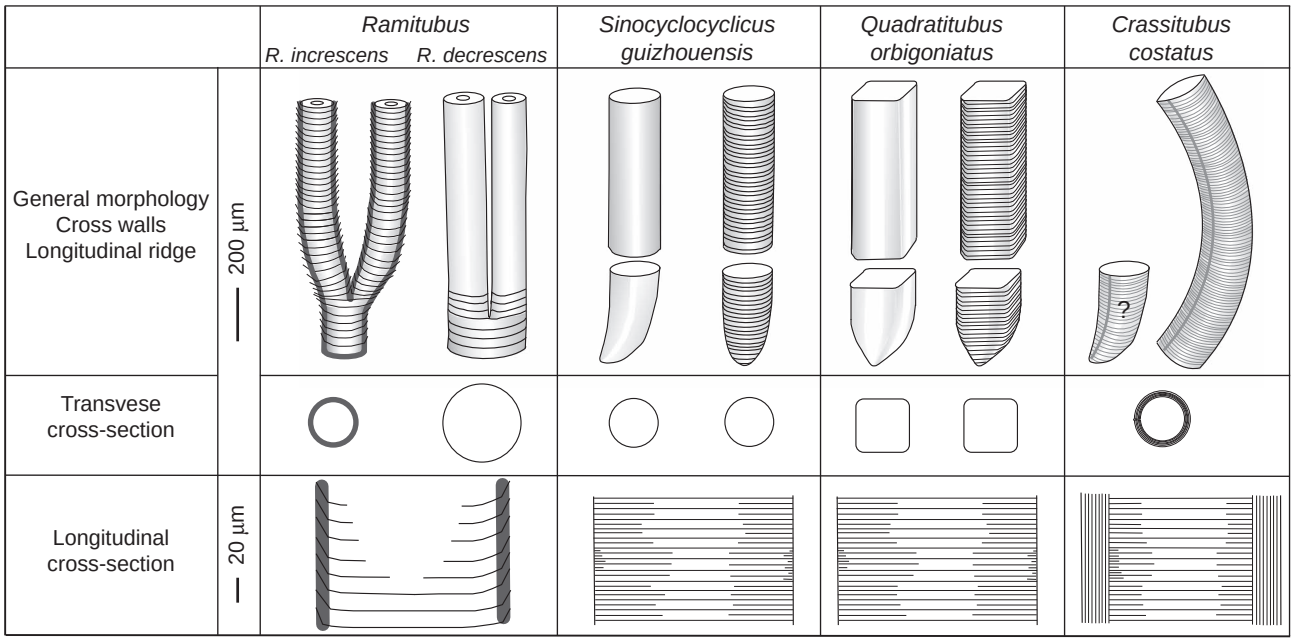

Figure 8. Schematic diagram showing diagnostic features of the five recognized species of tubular microfossils in the Weng'an biota. Modified from [63].

outer envelope may be lost taphonomically; indeed, sometimes only the internal cell is preserved, commonly with one or more craters of uncertain origin (Fig. 10C and D). It has also been shown that the one-celled *Megasphaera* probably underwent successive palintomic cell divisions, through which total cytoplasmic volume was conserved as cell number doubled and cell volume decreased exponentially, to generate forms previously described by Xue *et al.* [39] as *Parapandorina* (2–64 cells, typically polyhedral and tightly sutured; Fig. 10E and H) and *Megaclonophycus* (>64 cells, typically spherical and loosely packed probably due to degradation; Fig. 10I and J) [136]. The ontogenetic relationship between *Megasphaera*, *Parapandorina*, and *Megaclonophycus* is well accepted and they should be regarded as synonymous [79]. Thus, because *Megasphaera*, *Parapandorina*, and *Megaclonophycus* are synonymous, the first named genus *Megasphaera* takes nomenclature priority. The related genus *Tianzhushania* may have a cleavage sequence similar to that of *Megasphaera*, but its cleavage stages have not been completely documented [111]. It has been proposed that *Megasphaera* may be a junior synonym of *Tianzhushania* [137,138], but they are different in their envelope ornamentation and are regarded as two distinct genera because the diagnosis of *Tianzhushania* does not accommodate *Megasphaera* [79].

Megasphaera has been variously interpreted as large sulfur-oxidizing bacteria [52], volvocine green algae [39,139], mesomycetozoean-like holozoans [54], unicellular protists [71], stem-group animal embryos [51], and crown-group animal embryos [16,67–70,73,74,136]. The bacterial interpretation [52] is inconsistent with the morphologically complex envelope of *Megasphaera* [53] and the taphonomic volatility of large vacuole-bearing sulfur bacteria [140].

The current consensus is that *Megasphaera* is a eukaryotic organism, and most likely a multicellular eukaryote. Evidence for multicellularity comes from the way its cells are biologically deformed during successive cleavages [55,141]. In well-preserved *Megasphaera* specimens, particularly those at *Parapandorina* stages, polyhedral cells are joined at Y-shaped junctions (Fig. 10F and G). The Y-shaped junctions are not an artifact of the physical confinement of the cells within a rigid envelope, because they are preserved even when the *Megasphaera* cells are relaxed from the physical confinement during initial degradational shrinkage (Fig. 10G). The Y-shaped junctions of *Megasphaera* distinguish it from other eukaryotes in the Weng'an biota, such as the alga or cyanobacterium fossil *Archaeophycus*, which has cells joined at T-shaped junctions (Fig. 11). Thus, it is useful to compare cell configurations of *Megasphaera* and *Archaeophycus* in order to illustrate the processes during cytokinesis involved in the formation of Y-shaped cell junctions (Fig. 11). Y-shaped cell junctions form via deformation of T-shaped junctions when cell contraction—possibly caused by circumferential furrowing or invagination during cytokinesis [142–144]—results in the

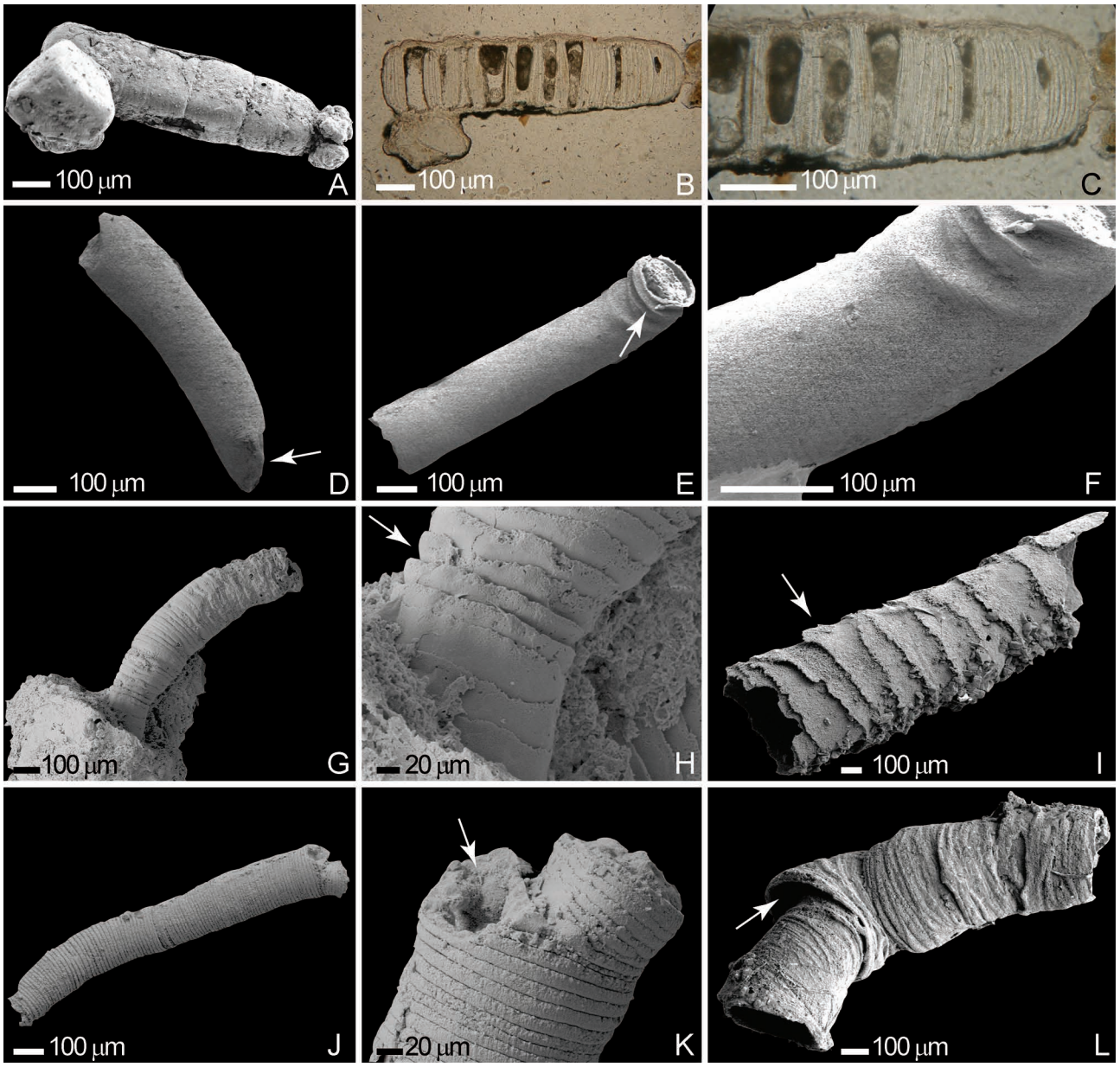

Figure 9. Tubular microfossils from the Doushantuo Formation at Weng'an (A–H, J–K) and Dengying Formation at Gaojiashan (I, L). (A, D–L) are scanning electron microscopic images and (B–C) are transmitted light microscopic images. (A–C) *Q. orbigoniatus*. (B–C) are longitudinal cross-sectional views of (A), showing degradation of non-biomineralizing cross-walls. Modified from [63]. (D–F) *S. guizhouensis*, showing flexible deformation (arrows) of non-biomineralizing tube walls. (F) shows a magnification of (E). (G–H and J–K) Internal molds of *R. increscens*, showing bent and nested side-walls (arrow in H) and a wedge-shaped gap or a poorly preserved dichotomy (arrow in K). (H, K) show magnifications of (G, J), respectively. (I and L) *C. riemkeae*, showing tube with nested side-walls (arrow in I) and a wedge-shaped gap (arrow in L), which could have resulted from the release of one of the two daughter tubes after dichotomous asexual branching [129] or abrupt change in growth orientation [131,132]. Modified from [133]. It is important to note that, unlike *R. increscens*, *C. riemkeae* tube consists of nested funnels and thus does not have internal cross-walls.

deformation of the cell membranes of the dividing cell and its adhered neighbors. This transformation requires that (1) the cell division planes of sister cells are offset or rotated relative to each other so that they meet the previous cell division plane at T-shaped junctions (Fig. 11A–C), (2) the cell membranes are flexible and not constrained by rigid cell walls, and (3) neighboring cells are adhered to each other by cell-to-cell adhesion proteins. This process of cell contraction transforms T-shaped junctions to Y-shaped junctions (Fig. 11C and D), resulting the well-known cross furrows at the four-cell stage (Fig. 11E and F) [145]. In contrast, the offset cytokinesis planes in *Archaeophycus* did not produce Y-shaped junctions, and the T-shaped junctions were preserved through successive cell

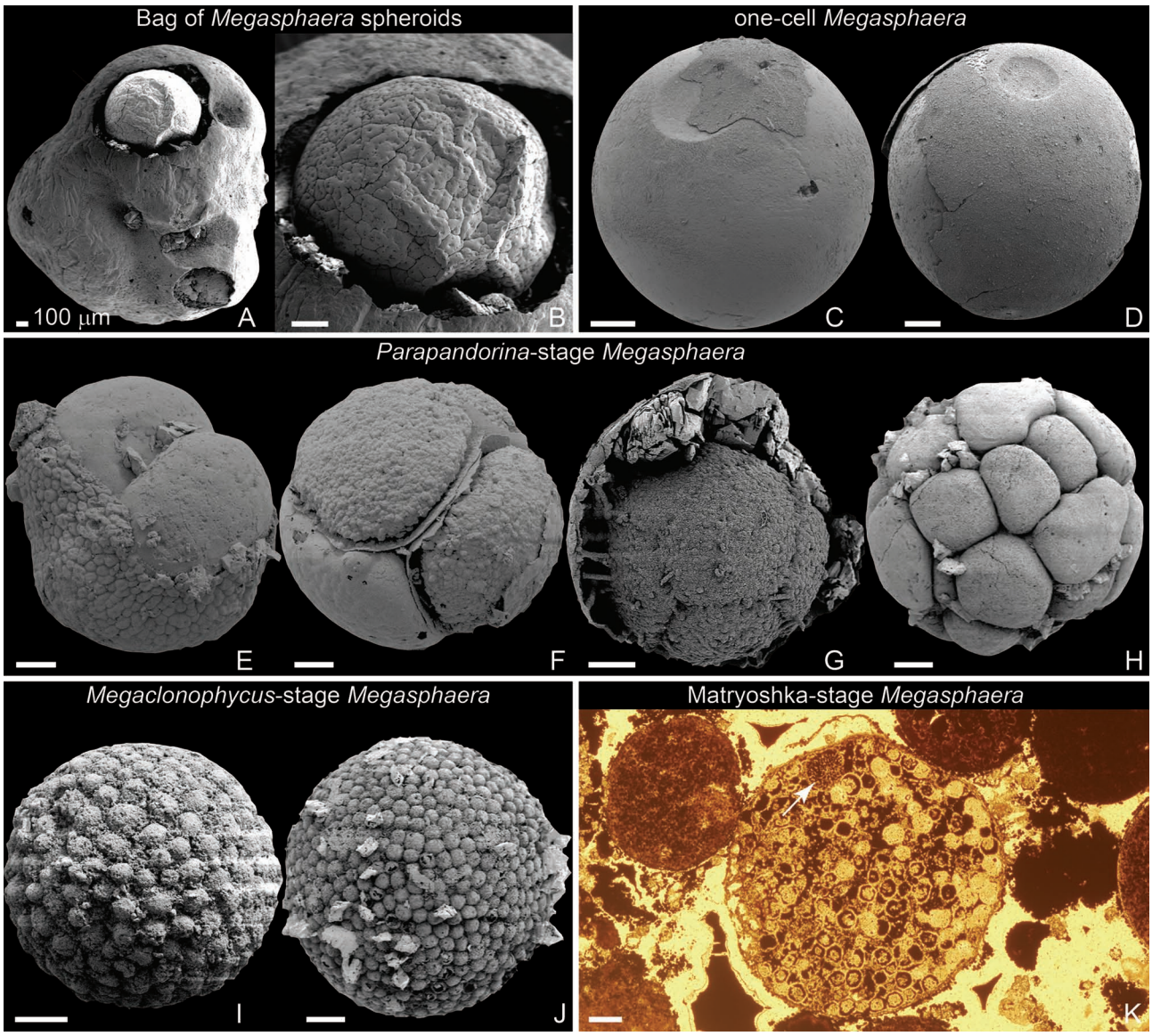

Figure 10. *Megasphaera* specimens, arranged according to a hypothesized developmental sequence. (A–B) A bag of at least three Megasphaera specimens, as confirmed by X-ray CT imaging, somewhat similar to oocytes and developing embryos within degenerating bodies of the placozoan animal *Trichoplax* (cf. fig. 1C of [152]). This is tentatively hypothesized as the parental stage of *Megasphaera*. (B) shows a magnification of (A) to show ornamented envelope. (C–D) One-cell stage specimens, each with a prominent crater (arrows). Ornamented envelope not preserved. (E–H) *Parapandorina*-stage specimens. An ornamented envelope is partially preserved in (E). Some cells became sub-rounded (H), probably due to degradation of cell-to-cell adhesion proteins [172]. (I–J) *Megaclonophycus*-stage specimens. (K) A matryoshka-stage specimen [148], with arrow pointing to a matryoshka structure. Scale bars = 100 μm.

divisions (Fig. 11G–K), presumably because *Archaeophycus* had a rigid cell wall that resisted the cytokinetic contraction force and/or its cytokinesis was accomplished through cell plate formation (as in some modern algae) so that the contraction force was weak. Thus, it is inferred that *Megasphaera* had cell-to-cell adhesion and a flexible membrane. Although the genetic toolkits and some protein families involved in cell adhesion may be present in unicellular and colonial protists [146,147], functional cell-to-cell adhesion is a diagnostic feature of multicellularity [3,5,6]. The flexible membrane suggests that a rigid cell wall was absent, at least in the *Parapandorina* stage and probably also in the *Megaclonophycus* stage [148]. However, because the life cycle of *Megasphaera* is not completely understood, it is uncertain whether a cell wall was absent in all or only certain developmental stages of the life cycle. If *Megasphaera* can be confirmed to have lacked a rigid cell wall through its entire life cycle, then it is likely

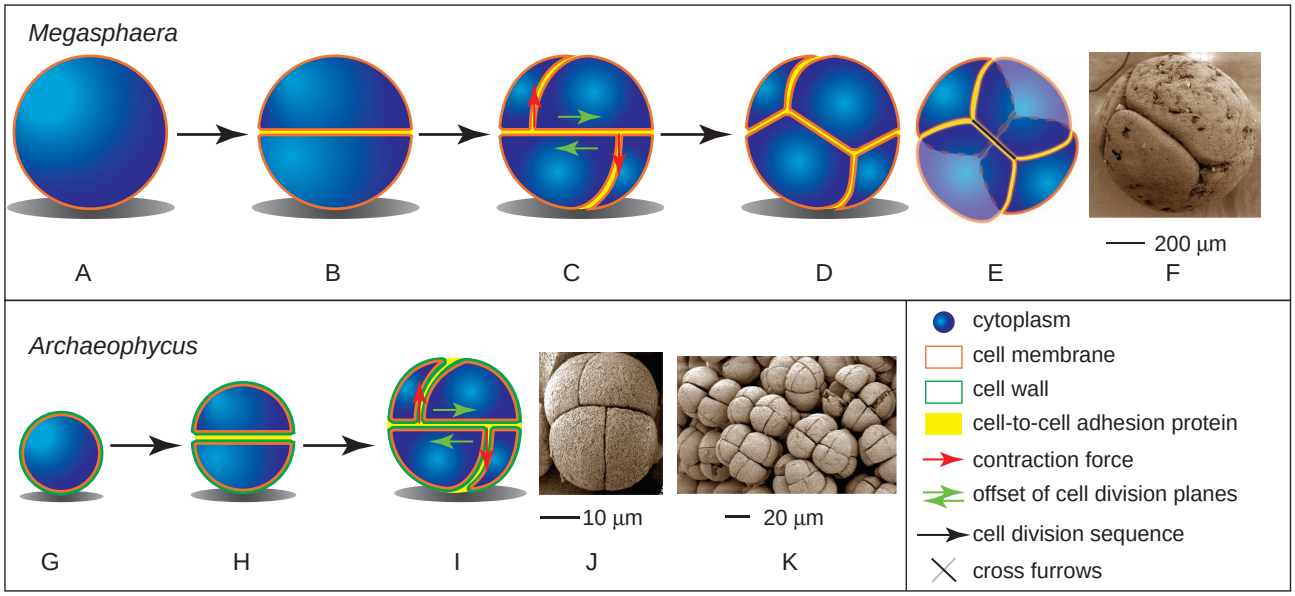

Figure 11. Schematic models showing the formation of T-shaped and Y-shaped junctions in successive cell divisions in *Megasphaera* (A–F) and *Archaeophycus* (G–K). (A) One-cell stage. (B) First cytokinesis. (C) Second cytokinesis, showing offset cell division planes to create T-shaped junctions. (D) Transformation of T-shaped junctions to Y-shaped junctions due to contraction force associated with cytokinesis. (E) Same as (D), but with upper-right and lower-left cells rendered semi-transparent to show three-dimensional configuration and cross-furrows (black lines). (F) A *Megasphaera* specimen in comparison with model shown in (E). (G) One-cell stage. (H) First cytokinesis after cell growth. (I) Second cytokinesis after further cell growth. T-shaped junctions are preserved despite offset of cell division planes, possibly due to the presence of rigid cell walls and/or a weak contraction force because cytokinesis is achieved through cell plate formation rather than simple furrowing.

a multicellular eukaryote with a possible animal affinity.

Megasphaera was compared with embryos of crown-group animals [16,136]. Recently, Knoll [5] has drawn comparison between *Megasphaera* and eggs/embryos of *Trichoplax*, a morphologically simple metazoan phylogenetically placed between sponges and cnidarians [149–152]. Indeed, multiple *Megasphaera* specimens can be housed in a 'bag' (Fig. 10A and B), somewhat similar to *Trichoplax* animals during oogenesis (cf. fig. 1C of [152]). If so, this 'bag' may represent the parental stage of *Megasphaera*, and this possibility needs to be investigated further in the future. The lack of gastrulation and epithelium formation in *Megaclonophycus*-stage fossils, however, led Hagadorn *et al.* to conclude that *Megasphaera* is likely a stem-group metazoan [51], although it is not entirely clear whether living sponges have (or the earliest animals had) true gastrulation and epithelia [153–155]. Huldtgren *et al.* [54], however, subsequently challenged the metazoan interpretation, instead comparing *Megasphaera* with modern mesomycetozoeans and interpreting it as a non-metazoan mesomycetozoean-like holozoan; it is important to point out that they did not claim that *Megasphaera* is a mesomycetozoean but they did explicitly rule out that *Megasphaera* could be a stem-group animal. Huldtgren *et al.*'s interpretation was based on nucleus-like intracellular structures (NISs) purportedly representing fossilized nuclei with evidence for closed mitosis, an inferred life cycle involving peanut-shaped endospore-bearing structures, and the assumption that modern mesomycetozoean cells divide palintomically. However, among modern mesomycetozoeans with known life cycles, cell cleavage is generally not palintomic, and cytoplasmic growth occurs between successive nuclear divisions [156,157]. Some modern mesomycetozoeans appear to have palintomic cell divisions during sporogenesis [158], but there is a large vacuole in the mother cell and cytoplasmic growth occurs between nuclear divisions at the expense of the vacuole volume [157–159]. Incidentally, these mesomycetozoeans seem to maintain large cell volume in the same way as giant vacuolated sulfur bacteria, and the taphonomic argument against giant sulfur bacteria as an interpretive analog to *Megasphaera* [140] also applies to these vacuolated mesomycetozoeans. In most mesomycetozoeans, karyokinesis is not immediately followed by cytokinesis, thus producing a multinucleate cell that simultaneously cleaves to form a large number of endospores [156,157], rather than producing a series of 2^n cells; although some mesomycetozoeans

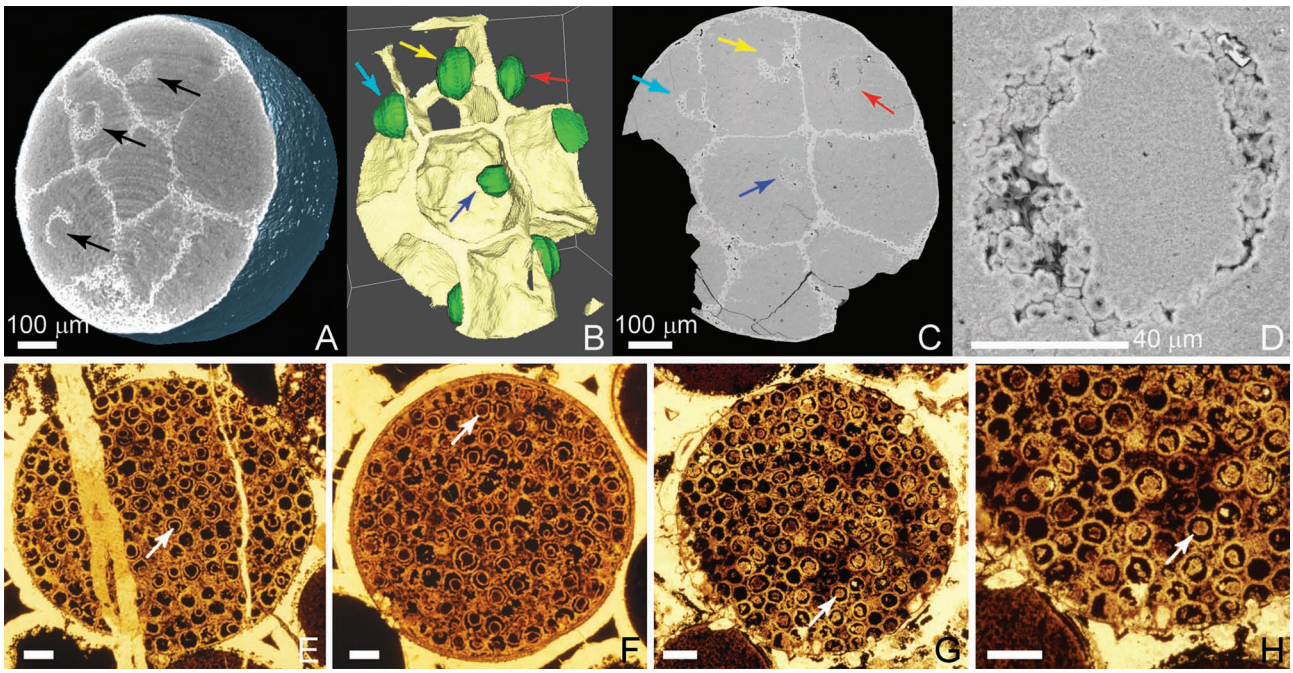

Figure 12. NISs. (A–D) A 16-cell *Parapandorina*-stage specimen analysed in [161]. (A) X-ray microCT volume rendition, with a cut-off view to show three NISs (arrows). (B) X-ray microCT volume rendition of a broken piece of the specimen, showing seven NISs (green color). (C) Backscattered electron (BSE) image of a polished surface of the broken piece, exposing four NISs (arrows). Colored arrows in (B, C) identify the same four NISs. (D) Enlarged BSE image of NIS identified by red arrows in (B, C), showing the lack of evidence for a nuclear envelope and the abundance of botryoidal cements. (E–H) *Megaclonophycus*-stage specimens with NISs denoted by arrows. Scale bars = 100 μm unless otherwise noted.

do produce a four-cell structure [160], their life cycle is not completely documented. These differences weaken modern mesomycetozoeans as an interpretive analog to *Megasphaera*.

The two lines of evidence—closed mitosis and a life cycle involving endospores—that Huldtgren *et al.* used to falsify the stem-group animal interpretation for *Megasphaera* can be critically evaluated by asking the following questions.

Are the NISs in *Megasphaera* indeed fossilized nuclei [51,54,69]? The NISs are 50–80 μm in diameter and spheroidal, ellipsoidal, crescentic, or dumbbell in shape, occurring centrally or peripherally as singleton, couplets, or triplets in *Megasphaera* cells [54] (Fig. 12). They are delineated by late diagenetic botryoidal cements [161, 162] (Fig. 12C and D), apparently contradictory to the taphonomic expectation that the easily degradable nuclei should be replicated by early diagenetic mineralization. This is true for NISs in both *Parapandorina*-stage (Fig. 12A–D) and *Megaclonophycus*-stage fossils (Fig. 12E–H). There is no evidence for a preserved nuclear envelope, either as a phosphatized membrane or as an organic substrate serving as a template for phosphate mineralization [161]. Thus, even if the NISs could represent the topographic position of nuclei, their shape must have been significantly modified by organic degradation, diagenetic cementation, and mineral overgrowth.

Assuming that the NISs indeed represent degraded nuclei, is there evidence for closed mitosis? Closed mitosis refers to the type of karyokinesis in which the nuclear envelope persists throughout the cell cycle [163,164], in contrast to open mitosis where the nuclear envelope is dissembled prior to anaphase and reassembled during late anaphase and telophase [163–165]. Thus, the confirmation of an intact nuclear envelope during karyokinesis is essential for the reliable identification of closed mitosis. However, because the NISs show no trace of a nuclear envelope, how could closed mitosis be recognized? Huldtgren *et al.*'s interpretation of closed mitosis was based on the observation of dumbbell-shaped NISs, assuming that the dumbbell structures represent dividing nuclei and are diagnostic of closed mitosis. However, as noted above, NIS morphology has been strongly modified by botryoidal cements and unlikely represents the original morphology of the nuclei. Moreover, even if we assume that the dumbbell-shaped NISs did represent the original morphology of dividing nuclei, they are not uniquely diagnostic of closed mitosis. In open mitosis, the nuclear envelope is reassembled during late anaphase and telophase, prior to the complete

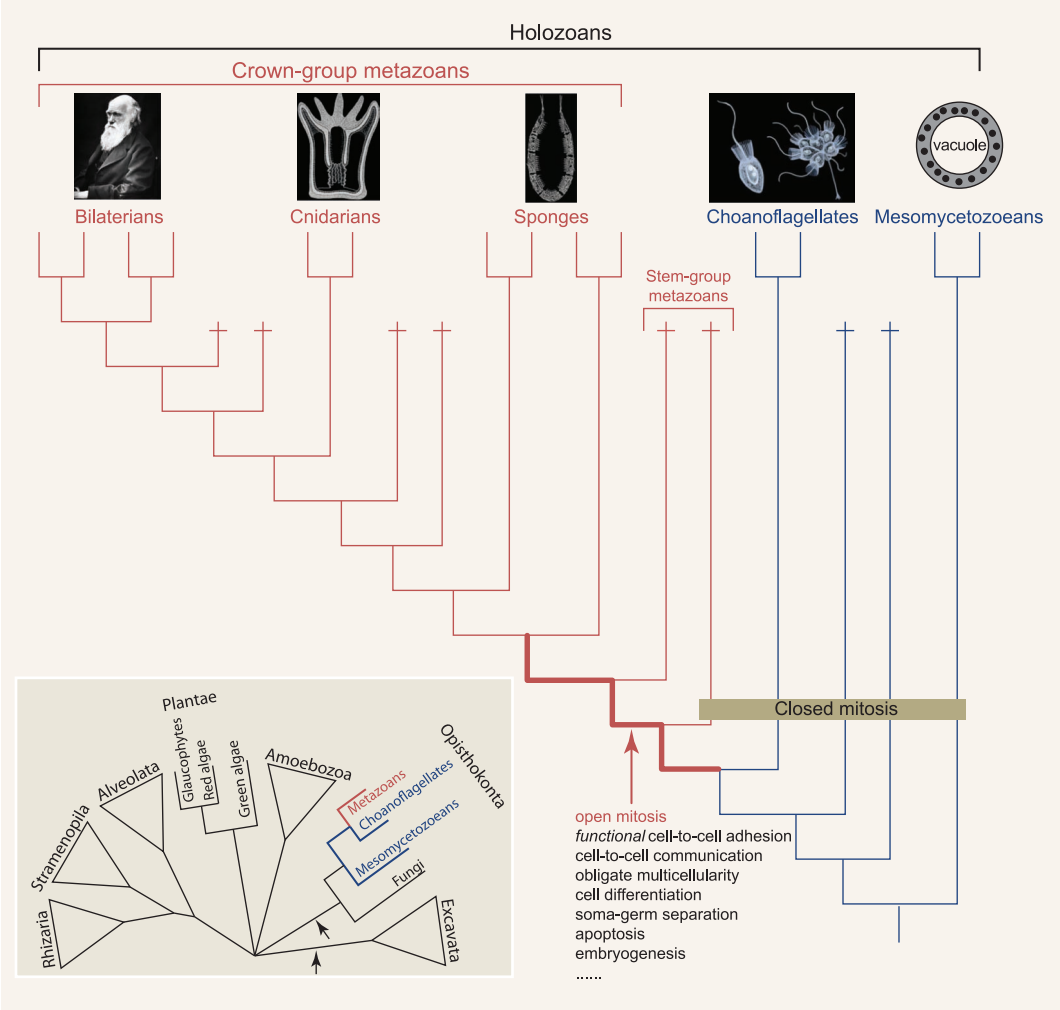

Figure 13. A phylogenetic tree of holozoans, showing the distribution of closed mitosis (blue horizontal bar) and open mitosis (red vertical arrow). Note the theoretical possibility that closed mitosis can occur in stem-group animals (red branches). Also note that the many features collectively defining crown-group metazoans must evolve in a step-by-step fashion along the stem (thick lines), i.e. in stem-group metazoans. Stem lineages are marked by crosses. Although some features (e.g. obligate multicellularity) may have evolved independently in other eukaryote groups, they apparently evolved only once within the holozoan clade. Inset in lower left shows an unrooted phylogenetic tree of the eukaryotes to highlight the phylogenetic position of holozoans, with arrows marking alternative rooting [170,171].

separation of the two daughter nuclei (e.g. fig. 5a of [163]). If these anaphase–telophase nuclei are preserved faithfully, they would also resemble a dumbbell. Thus, even if the NISs are fossilized nuclei, the dumbbell-shaped NISs are not uniquely diagnostic of closed mitosis.

Assuming that cell division in *Megasphaera* was indeed closed mitosis, does this feature falsify the stem-group animal interpretation? Both open and closed mitosis occur in opisthokonts (e.g. living animals, streptophytes, and some fungi), amoebozoans, SARs (= stramenopiles + alveolates + rhizarians), and archaeplastids (or Plantae), but cell division in excavates is exclusively closed mitosis [117,164,165] (see Fig. 13 inset for eukaryote phylogeny). Closed mitosis is regarded as the most ancient form of eukaryotic cell division, and open mitosis probably evolved multiple times in eukaryotes [117,164, 165]. Given that open mitosis occurs in crown-group metazoans and closed mitosis in other holozoans (e.g. mesomycetozoeans and choanoflagellates), it is likely that some stem-group animals had closed mitosis (Fig. 13). Thus, the presence of closed mitosis in *Megasphaera*, even if proved true, does not by itself falsify the stem-group metazoan interpretation.

Is the endospore-bearing peanut-shaped fossil part of the *Megasphaera* life cycle? There is a developmental gap between *Megaclonophycus*-stage *Megasphaera* fossils (each with $\sim 10^3$ cells) and

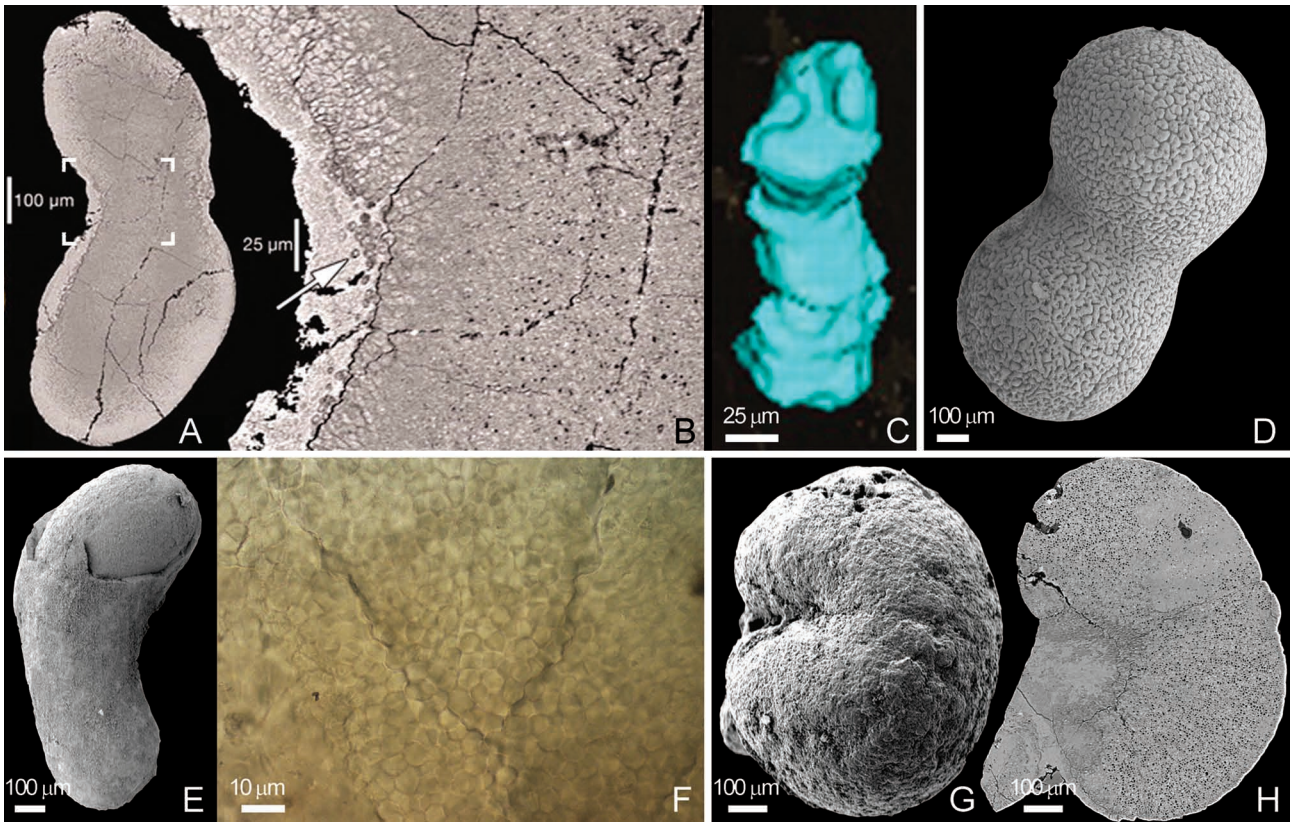

Figure 14. Peanut-, cashew-, and lima bean-shaped fossils. (A) Synchrotron X-ray CT slice image of a peanut-shaped fossil. (B) Magnification of rectangle area in (A), showing cells (arrow) interpreted as reproductive endospores of *Megasphaera* [54]. (C) NIS interpreted as a fossilized nucleus of *Megasphaera* [54], shown at the same scale as (B). Note that the NIS (C) is volumetrically $>10^3$ larger than the endospores (B) that are supposed to contain reproductive nuclei. (D, E, G) Scanning electron microscopic images of peanut-, cashew-, and lima bean-shaped fossils. (F) Transmitted light microscopic image of a thin section of (E), showing polygonal cells (possibly transverse cross-section of pseudoparenchyma). (H) Synchrotron X-ray CT slice image of (G), showing pseudoparenchyma (cf. B). (A–C) from [54], with permission from American Association for the Advancement of Science. (H) Courtesy of John Cunningham and Phil Donoghue.

peanut-shaped fossils (each with $\sim 10^6$ cells) stage. This gap needs to be filled with transitional forms in order to include the peanut-shaped fossils in the life cycle of *Megasphaera*. Another potential problem with the life cycle proposed by Huldtgren *et al.* [54] relates to the size difference between purported nuclei and endospores. As pointed out by Xiao *et al.* [55], the NISs ($\sim$50–80 μm in size) in *Parapandorina*-stage fossils are volumetrically $>10^3$ larger than the endospores (3–5 μm in size) in peanut-shaped fossils (Fig. 14A–C). Even the NISs in *Megaclonophycus*-stage fossils (Fig. 12E–H) are volumetrically orders of magnitude larger than the endospores. Endospores are supposed to be reproductive cells and each should contain a complete nucleus to be carried to the next generation. But their sizes do not fit. There are several possible solutions to this misfit. First, the NISs may not be nuclei. Second, the NISs may not be reproductive nuclei but may instead be non-reproductive macronuclei that are not passed down through generations. Third, they may represent reproductive nuclei but have been significantly modified and enlarged $>10^3$ times by botryoidal cements. Fourth, the NISs faithfully represent the size and shape of the nuclei, but the nuclei have been biologically reduced in size through successive cell divisions, leading to an isometric scaling relationship between NISs and their hosting cells. However, this possibility is inconsistent with measurements of NISs and their hosting cells, which show that NISs maintain a more or less constant size over successive cell divisions, at least for *Parapandorina*-stage fossils [54]. Comparison of NISs in *Parapandorina*-stage and *Megaclonophycus*-stage specimens (Fig. 12) also indicates that the NIS size is not isometrically scaled with cell size. Fifth, the endospore-bearing peanut-shaped fossils may not be part of the *Megasphaera* life cycle. These possibilities need to be considered thoroughly before the life cycle proposed by

Huldtgren *et al.* [54] can be used to constrain the phylogenetic affinity of *Megasphaera*.

Is the life cycle proposed by Huldtgren *et al.* [54] consistent with the unicellular protist and mesomycetozoean-like holozoan interpretations? The life cycle proposed by Huldtgren *et al.* [54] involves an endospore-bearing peanut-shaped fossil. A life cycle with an endospore stage occurs convergently in many eukaryote groups [117], and cannot be used to uniquely identify *Megasphaera* as mesomycetozoean-like holozoans. Peanut-shaped fossils in the Weng'an biota may be of diverse origins, with some of them being two-celled specimens [74] and other being one-celled specimens with a possible polar lobe-like extension [73]. However, the endospore-bearing peanut-shaped fossils are clearly multicellular in nature, showing excellent pseudoparenchymatous construction (e.g. fig. 3G of [54]). In this respect, the endospore-bearing peanut-shaped fossils are similar to other lobate pseudoparenchymatous thalli in the Weng'an biota (Fig. 14E–H), including cashew-shaped, lima bean-shaped, and ginger root-shaped fossils [47,79]. Some of these lobate fossils even have cellularly differentiated pseudoparenchyma [47]. If one accepts the peanut-shaped fossils as part of the *Megasphaera* life cycle (but see [148] and further discussion below), then these lobate fossils may also be ontogenetically or phylogenetically related to *Megasphaera*. If so, then *Megasphaera* would be a cellularly differentiated multicellular organism (possibly a multicellular alga), inconsistent with the unicellular protist or mesomycetozoean-like holozoan interpretations (or the stem-group metazoan interpretation, for that matter).

To summarize, the two lines of evidence—closed mitosis and a life cycle involving endospores—presented by Huldtgren *et al.* [54] are both problematic. Even if they are authenticated, they only have limited phylogenetic significance, given that closed mitosis is ancestral among eukaryotes and a life cycle involving endospores can be convergent. Thus, it is risky to use these problematic features to falsify *Megasphaera* as a stem-group animal or to confirm it as a mesomycetozoean-like holozoan.

The recent discovery of *Megaclonophycus*-like fossils with cell packets and matryoshkas brings further complications to the life history of *Megasphaera* [148]. These new fossils indicate that the peanut-shaped fossils are, at least, not the direct ontogenetic successors of *Megaclonophycus*-stage fossils, and they may not be ontogenetically related to *Megasphaera* at all. If the cell packets and matryoshkas in *Megaclonophycus*-like fossils are interpreted as asexual reproductive structures [148], the life cycle of *Megasphaera* is similar to that of volvocine green algae although there are good reasons to doubt a phylogenetic connection between them [148]. Nonetheless, it is safe to conclude that *Megasphaera* is a multicellular eukaryote with clear evidence for flexible cell membranes unconstrained by a rigid cell wall (at the *Parapandorina* and *Megaclonophycus* stages), cell-to-cell adhesion, palintomic cell division within a complexly ornamented envelope, cell differentiation, germ–soma separation, programmed cell death, and putative polar lobe structures [55,73,141,148]. These features help to constrain the evolutionary grade of *Megasphaera*, which is much more complex than mesomycetozoeans. This suite of features directs our search for the phylogenetic home of *Megasphaera* toward complex multicellular eukaryotes, including both animals and algae.

When assessing the phylogenetic affinities of ancient fossils such as *Megasphaera*, one has to be cognizant of the distinction between crown-group analogs and stem-group fossils. Crown-group animals, for example, are separated from their closest living sister group—the choanoflagellates—by significant morphological gaps. Compared to living choanoflagellates, living animals are characterized by open mitosis, cell-to-cell adhesion, cell-to-cell communication, obligate multicellularity, cell differentiation, soma–germ separation, apoptosis, embryogenesis, developmental regionalization, pattern formation, and many other features. Although similar characters may have evolved independently in other eukaryotic clades, within the holozoan clade these features evolve in the metazoan clade, and they have to evolve (or be functionalized by recruiting pre-existing genetic toolkits) in a step-by-step fashion along the stem leading toward crown-group animals. Thus, when interpreting Ediacaran fossils, one has to be reminded that stem-group animals are not expected to have all synapomorphies that collectively define the crown-group Metazoa, which would blur the morphological distinction between early animals and their unicellular relatives. Further, stem-group animals could evolve their own autapomorphies that are not present in crown-group animals, making them appear alien.

Because multicellularity and cell-to-cell adhesion are also present in multicellular algae [6] and it is uncertain whether a cell wall is completely absent through the entire life cycle of *Megasphaera*, it cannot be excluded that *Megasphaera* may be a multicellular alga. This possibility should be thoroughly investigated, given that multicellular thalli are known to be present in the Weng'an biota [47]. However, it is unlikely that *Megasphaera* could be a volvocine alga [50,136,148],

because (1) its tightly packed cells enclosed within a thick sculptured envelope would make flagellar locomotion (a universal feature of volvocine algae) impossible; (2) its matryoshkas are growing structures rather different from the gonidia of volvocine algae; (3) its stereoblastula-like cell organization is morphologically and functionally different from the coeloblastula-like cell organization in volvocines, and (4) multicellular volvocines are freshwater algae diverged in the Permian–Triassic periods according to molecular clock estimates [166].

HELICOFORAMINA, SPIRALICELLULA, AND CAVEASPHAERA

Helicoforamina (Fig. 15A–C) and *Spiralicellula* (Fig. 15D–F) are enigmatic spheroidal fossils in the Weng'an biota. Both genera are characterized by dextrally spiral internal bodies enclosed within a smooth or sculptured envelope. *Helicoforamina* has one internal body and its envelope has a dextrally helical structure (e.g. groove, tunnel, or ridge), whereas *Spiralicellula* has ≥4 internal bodies and its envelope lacks a helical ornamentation [39,79,136,167,168].

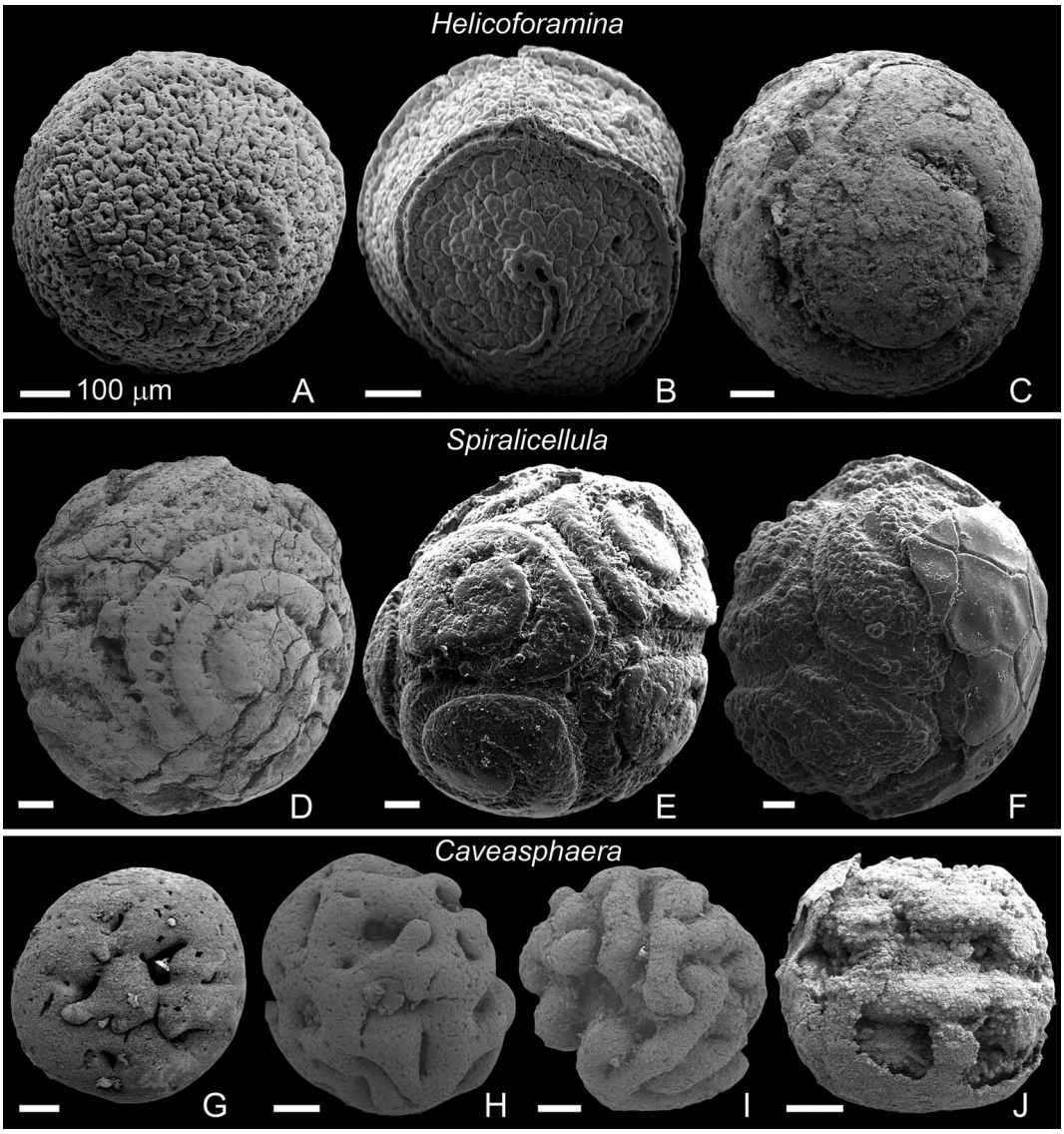

Figure 15. *Helicoforamina* (A–C), *Spiralicellula* (D–F), and *Caveasphaera* (G–J). (A) Ornamented envelope with a faintly visible helical structure. (B) Ornamented envelope with a clockwise helical groove. (C) A clockwise helical internal body. Presumably, ornamented envelope is not preserved. (D–E) *Spiralicellula* with four and eight internal bodies, respectively. (F) *Spiralicellula* within ornamented envelope. Note the lack of a helical structure on the envelope. (G–J) *Caveasphaera* with irregular ridges. Scale bars = 100 μm.

Spiralicellula was first interpreted as a volvocine alga [39], but this interpretation faces the same difficulties in the interpretation of *Megasphaera* as a volvocine [50,136,148]. *Helicoforamina* was interpreted as a late embryonic form ontogenetically related to *Megasphaera*, on the basis of transitional forms that bridge the sculptured envelopes of these two genera [167] (Fig. 15A). Subsequently, *Helicoforamina* was interpreted as a foraminifer [168]. These interpretations have been questioned [54,79,169]. Instead, it is proposed that *Helicoforamina* and *Spiralicellula* represent different developmental stages of the same organism—either a mesomycetozoean-like holozoan [54] or an alga [169], with *Helicoforamina* containing one spiral cell developing into *Spiralicellula* containing $\geq$4 spiral cells. This hypothesis predicts that the helical ornamentation on the sculptured envelope is ontogenetically lost during the developmental transition from *Helicoforamina* to *Spiralicellula* and that two-celled *Helicoforamina* or *Spiralicellula* should be present in the Weng'an biota. Thus far, these predicted transitional forms (e.g. one spiral internal body enclosed within an ornamented envelope without a helical structure, two spiral internal bodies enclosed within an envelope with or without a helical structure, or four spiral internal bodies enclosed within an envelope with a helical structure) have not been found. Zhang *et al.* [169] illustrated a specimen (their fig. 1.9–1.10) that was claimed to have eight internal bodies enclosed within a helically grooved envelope. Unfortunately, the specimen is too poorly preserved to determine whether there are eight internal bodies and whether the internal bodies are spiral. Further paleontological search for transitional forms is required to ascertain the hypothesized ontogenetic connection between *Helicoforamina* and *Spiralicellula*.

Caveasphaera (Fig. 15G–J) is a spheroidal fossil 300–500 μm in diameter, typically smaller than *Megasphaera*, *Helicoforamina*, and *Spiralicellula*. It is characterized by irregular ridges that form a hollow spherical cage [136] or a solid sphere [79] enclosed in an envelope (Fig. 15J). Its morphological variability makes it difficult to reconstruct its life cycle and phylogenetic affinity, although it is probably a multicellular eukaryote and has been compared with cnidarian embryos [136].

SUMMARY AND PROSPECT

The Weng'an biota is an important Ediacaran Lagerstätte, and it represents one of the few examples of phosphatized biotas in the Ediacaran Period. Fossils in the Weng'an biota are three-dimensionally phosphatized with cellular details. They were reworked, transported intrabasinally, winnowed, concentrated, and buried under oxygenated shallow seawaters above the fair weather wave base in outer shelf facies. The Weng'an biota contains a diverse assemblage of multicellular eukaryotes, including various acanthomorphic acritarchs, tubular microfossils, pseudoparenchymatous thalli, and spheroidal fossils such as *Megasphaera*, *Helicoforamina*, *Spiralicellula*, and *Caveasphaera*. Unambiguous evidence for complex multicellularity (with cellular differentiation and specialized reproductive structures) is apparent in pseudoparenchymatous thalli, which have been interpreted as florideophyte red algae. Although cellular preservation is rare or absent in tubular microfossils, the morphological complexity of these microfossils indicates that they are multicellular eukaryotes with tissue differentiation, possibly related to cnidarians. Acanthomorphic acritarchs in the Weng'an biota have diverse origins, but some of them are likely multicellular eukaryotes, although their life cycles and phylogenetic affinities have not been thoroughly investigated. The affinities of spheroidal fossils are most controversial. *Megasphaera*, for example, has been interpreted as a sulfur-oxidizing bacterium, a unicellular protist, a mesomycetozoean-like holozoan, a volvocine alga, a stem-group animal, or a crown-group animal. Among these interpretations, the bacterial interpretation can be falsified with confidence. The unicellular protist and mesomycetozoean-like holozoan interpretations are apparently inconsistent with the complex multicellularity, cell-to-cell adhesion, and recently discovered fossils relevant to the life cycle of *Megasphaera*. The proposed evidence in support of the mesomycetozoean-like holozoan interpretation—closed mitosis and a life cycle with an endospore-forming phase—is both problematic and non-diagnostic. *Megasphaera* is unlikely a volvocine alga because, among other things, it could not function as flagellated volvocine green algae, although this does not rule out the possibility of *Megasphaera* being a member of other multicellular algae (e.g. red algae which universally lack flagella). The crown-group animal interpretation is apparently inconsistent with the lack of gastrulation in *Megasphaera*, although the nature of 'gastrulation' in modern sponges is a matter of debate. Nonetheless, the stem-group animal interpretation is permissible given the presence of cell-to-cell adhesion and potential lack of a rigid cell wall. *Helicoforamina* and *Spiralicellula* have also been interpreted as algae, mesomycetozoean-like holozoans, or animals, but phylogenetic interpretations of these taxa are hampered by an incomplete understanding of their ontogeny. Finally, *Caveasphaera* is an enigmatic

form and its phylogenetic affinity is uncertain although a comparison with cnidarian embryos has been made.

The Weng'an biota holds great promise to illuminate the early evolutionary history of multiple multicellular eukaryote clades, including animals [16], algae [36], and fungi [44]. To fully realize its potential, the paleontological investigation of the Weng'an biota should explore both the grey and black facies, utilizing a combination of thin section, acid maceration, electron microscopy, X-ray microCT, and other micro-analytical tools. With more paleontological data and better characterization tools, it is possible to better understand the life cycle of pseudoparenchymatous thalli, to characterize their various reproductive structures, and to test whether they have biphasic or triphasic life cycles. Such data are important to refine or refute their phylogenetic affinities with the florideophytes. Similarly, new data are needed to discriminate the biological vs. taphonomic origin of NISs, to resolve the life history of *Megasphaera*, and to test whether *Megasphaera* could be ontogenetically related with pseudoparenchymatous algae in the Weng'an biota, thus further illuminating its evolutionary grade and constraining its phylogenetic affinity. The proposed ontogenetic connection between *Helicoforamina* and *Spiralicellula* should be tested with transitional forms to be discovered in the Weng'an biota. Convincing cellular structures have not yet been reported in tubular microfossils and *Caveasphaera*, despite the likelihood of cellular preservation in these taxa. There are many research opportunities in the Weng'an biota that have the potential to contribute to our understanding of the diversification of multicellular eukaryotes in the aftermath of the terminal Cryogenian snowball Earth glaciation.

ACKNOWLEDGEMENTS

This article is dedicated to Yun Zhang and Yaosong Xue, who pioneered the paleontological investigation of the Weng'an biota and also mentored three of the co-authors (SX, XY, and CZ). We would like to thank Stefan Bengtson, David Bottjer, Junyuan Chen, Meng'e Chen, John Cunningham, Phil Donoghue, Ganqing Jiang, Andy Knoll, Pengju Liu, Chongyu Yin, Leiming Yin, Zongjun Yin, Maoyan Zhu, and Shixing Zhu for sharing data and ideas, although we are solely responsible for the opinions and errors in this article. We would also like to thank three anonymous reviewers for their constructive comments.

FUNDING

This work was partially supported by the US National Science Foundation (EAR-1124062, EAR-1250800), Chinese Ministry of Science and Technology (2013CB835000), National Natural Science Foundation of China (41272011, 41130209), and Chinese Academy of Sciences (KZZD-EW-02).

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