New observations of the ornamented Doushantuo embryo fossils from the Ediacaran Weng'an Biota, South China

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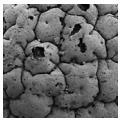

Phosphatized Weng'an embryo fossils from the Ediacaran Doushantuo Formation in Guizhou, South China are dominated by various ornamented spheroidal fossils, with one internal body or several blastomeres enclosed within a single or multiple layered envelope, which have been interpreted as the resting eggs or diapause embryos of metazoans. Based on the microstructure of the envelope, we separated these ornamented spheroidal fossils into two groups: 1) group one, represented by typical *Megasphaera ornata* and characterized by an envelope consisting of at least two thick capsules with various surface ornaments and a single internal body, which can be interpreted as resting eggs; 2) another group, exhibiting an envelope consisting of a thin capsule with cell-like surface structure and internal blastomeres (amount is equal to 2^n, n = 0, 1, 2, 3…), which are probably not diapause embryos but developing blastulae at various holoblastic cleavage stages. • Key words: Ediacaran, Doushantuo, fossil, embryos, resting eggs, Weng'an Biota.

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Since 1998, the phosphatized animal embryo microfossils with cellular and sub-cellular structures preserved in three-dimensional detail from the Ediacaran Doushantuo Formation at Weng'an phosphate mining area (Guizhou Province, southwest China), have been considered as one of the oldest fossil metazoan records on the planet (Li *et al*. 1998, Xiao *et al*. 1998, Condon *et al*. 2005). In spite of the fact that these Doushantuo embryo fossils are taphonomically biased (Chen 2004; Donbors *et al*. 2005, 2006), during the last decade, studies of animal fossils from this unique late Neoproterozoic taphonomic window have profoundly improved our outstanding of the evolution of multicellular animals in the Precambrian (Chen *et al*. 2000, 2002, 2004a, 2006, 2009a, b; Xiao & Knoll 2000; Hagadorn *et al*. 2006; Xiao *et al*. 2007a, b; Liu *et al*. 2008). The different embryo sizes and ornamentations (Xiao & Knoll 2000, Chen 2004), various embryo cleavage patterns (Chen 2004, Chen *et al*. 2006, 2009a) and potential fossil gastrulae (Chen *et al*. 2000, 2009a; Chen & Chi 2005; Xiao *et al*. 2007a), as well as problematic animal adults (Xiao *et al*. 2000; Chen *et al*. 2002, 2004; Liu *et al*. 2008) indicate that the diversity of multicellular animals before the Cambrian radiation was much higher than previously thought.

Except for the large ancanthomorphic acritarchs, some of which may represent the diapause embryos of metazoans (Yin L. *et al*. 2007, Cohen *et al*. 2009), the spheroidal embryo fossils from the Weng'an Biota were assigned to three morphological genera and four morphospecies (*Megasphaera ornata*, *M. inornata*, *Parapandorina raphospissa* and *Megaclonophycus onustus*) according to the envelope ornamentations and amount of internal cells (Xiao & Knoll 2000, Yuan *et al*. 2002). The morphological genus *Megasphaera* was established to describe the fossils without cell division. Based on the microstructure of the fossil envelope, this genus was further divided into two different morphospecies, *M. ornata* and *M. inornata*. The former is characterized by one cell enclosed within an ornamented envelope, while the latter refers to fossils with one cell encircled by a smooth envelope (Xiao & Knoll 2000, Xiao *et al*. 2007b). *Megasphaera* has been regarded as a synonym of the acritarch genus *Tianzhushania* by Yin C. *et al*. (2004), whereby *M. ornata* and *Tianzhushania tuberifera* may represent the same taxon preserved in different lithological facies. However, Yin C. *et al*.'s opinion was not widely accepted due to a lack of fossil evidence (Xiao *et al*. 2007a, b, Yin L. *et al*. 2007, 2008). Another group of spheroidal embryos with more than one internal cell within

a smooth or ornamented envelope was assigned to *Parapandorina raphospissa* and *Megaclonophycus onustus* (Xiao & Knoll 2000, Xiao *et al*. 2007b).

Recent investigations indicate that the dominant Weng'an spheroidal microfossils possess an ornamented envelope, but only a few contain more than one internal cell (Yuan *et al*. 2002, Chi *et al*. 2003, Chen 2004, Xiao *et al*. 2007b, Chen *et al*. 2009a). All these ornamented spheroidal microfossils were interpreted to be metazoan eggs or embryos at a diapause stage (Xiao *et al*. 1998, Xiao & Knoll 2000, Xiao *et al*. 2007b). However, this hypothesis was challenged by Chi *et al.* (2003) and Chen *et al*. (2009a), who interpreted the microfossils to be holoblastic blastomeres of embryos inside a sculptured envelope but not in the diapause stage. There two major reasons affect the interpretation of the Weng'an embryos. Firstly, these morphological taxa can be easily distinguished, however, the embryo fossils from different morphospecies may belong to different developmental stages of the same biological species, while different morphotypes of the same morphospecies may represent different biological species (Xiao & Knoll 2000). Hence, assignation of these ornamented spheroidal microfossils to these morphological taxa leads to confusion in biological interpretations of Doushantuo embryos. Secondly, it is difficult to distinguish taphonomic and diagenetic artifacts from the primary bio-structures, such as cell shrinkage, degradation, loss of blastomeres and embryo capsules, multiple episodes of coating and lining, *etc*.

In order to reassess the Weng'an embryos, the present paper focuses on investigation of the ornamentation patterns and envelope microstructures of *Megasphaera ornata* and new specimens of *Parapandorina raphospissa*. In the light of comparisons with modern embryos, we discuss the biological properties of the ornamented and smooth envelopes of the Weng'an embryos, as well as their possible relationship to cytomembrane, fertilization membrane and diapause cyst of metazoan embryos.

Materials and methods

The Weng'an Biota is found in the Upper Phosphate Member (= Weng'an Phosphate Member) of the Doushantuo Formation in the Weng'an phosphate mining area of Guizhou Province, South China (Chen 2004, Chen & Chi 2005, Dornbos *et al*. 2005, Zhu *et al*. 2007, Chen *et al*. 2009a). The Upper Phosphate Member consists of two different taphonomic facies, grey facies and black facies, represented by grey dolomitic phosphorite and black phosphorite, respectively (Chen & Chi 2005; Dornbos *et al*. 2005, 2006). All the rock samples for this study were collected from the grey facies, and then digested in dilute acetic acid (acid concentration is between 5% and 10%). The insoluble acid residue was then washed and dried. The liberated microfossils in the acid residue were checked under stereomicroscope and picked by hand with the help of a fine brush. Selected embryo fossils were examined with the scanning electron microscope. All the samples described in this paper are housed in Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences.

Taphonomic and morphological analysis of the ornamented embryos

Envelope ornamentation patterns of *Megasphaera ornata*

The phosphatized embryos from the Weng'an Biota are dominated by microfossils with an ornamented envelope. The envelope is typically sculptured with various ornaments which are grouped into five types, including well-seamed polygons (Fig. 1A), polygons with fractal branching (Fig. 1B), tubercles (Fig. 1C), tubercles with dimples on top (Fig. 1D), and anastomosing ridges (Fig. 1E) (Xiao & Knoll 2000, Yuan *et al*. 2002, Chen 2004). Recently, some large acanthomorphic acritarchs that coexist with the embryo fossils (*e.g*. *Tianzhushania*) have been considered to represent the diapause embryos of metazoans (Yin C. *et al*. 2004, Yin L. *et al*. 2007, Cohen *et al*. 2009). Thus, cylindrical processes on the envelope as exhibited by *Tianzhushania* should be considered to be another type of envelope ornamentation on the Doushantuo embryos. In fact, the morphological diversity of ornamentation pattern may be even higher as illustrated by Fig. 1H–J and Fig. 2A. In addition, some transitional forms between the ornamentation patterns mentioned above are very common (Fig. 1F, G, Xiao & Knoll 2000). It should be noted that the range of preserved ornamentation patterns may be overestimated because of taphonomic artifacts. For example, the pitted ornament as shown by Fig. 1L differs greatly from any others shown in Fig. 1 and it may be just an imprint of ornaments on the inner capsule as shown by Fig. 1K, L.

Eggshell or embryo chorion ornamentation is a useful characteristic for classification of extant embryos at high taxonomic or even the species level (Gilbert & Wurdak 1978, Belk 1989, Britz *et al*. 1995, Shen & Huang 2008). In this regard, remarkable morphological variations of the envelope ornamentation patterns imply a relatively high evolutionary level for the metazoans in the Weng'an Biota.

Ornamented spheroidal microfossils with cell-like structure

Among the Weng'an ornamented embryos, one distinct form is the one with a thin envelope sculptured with small

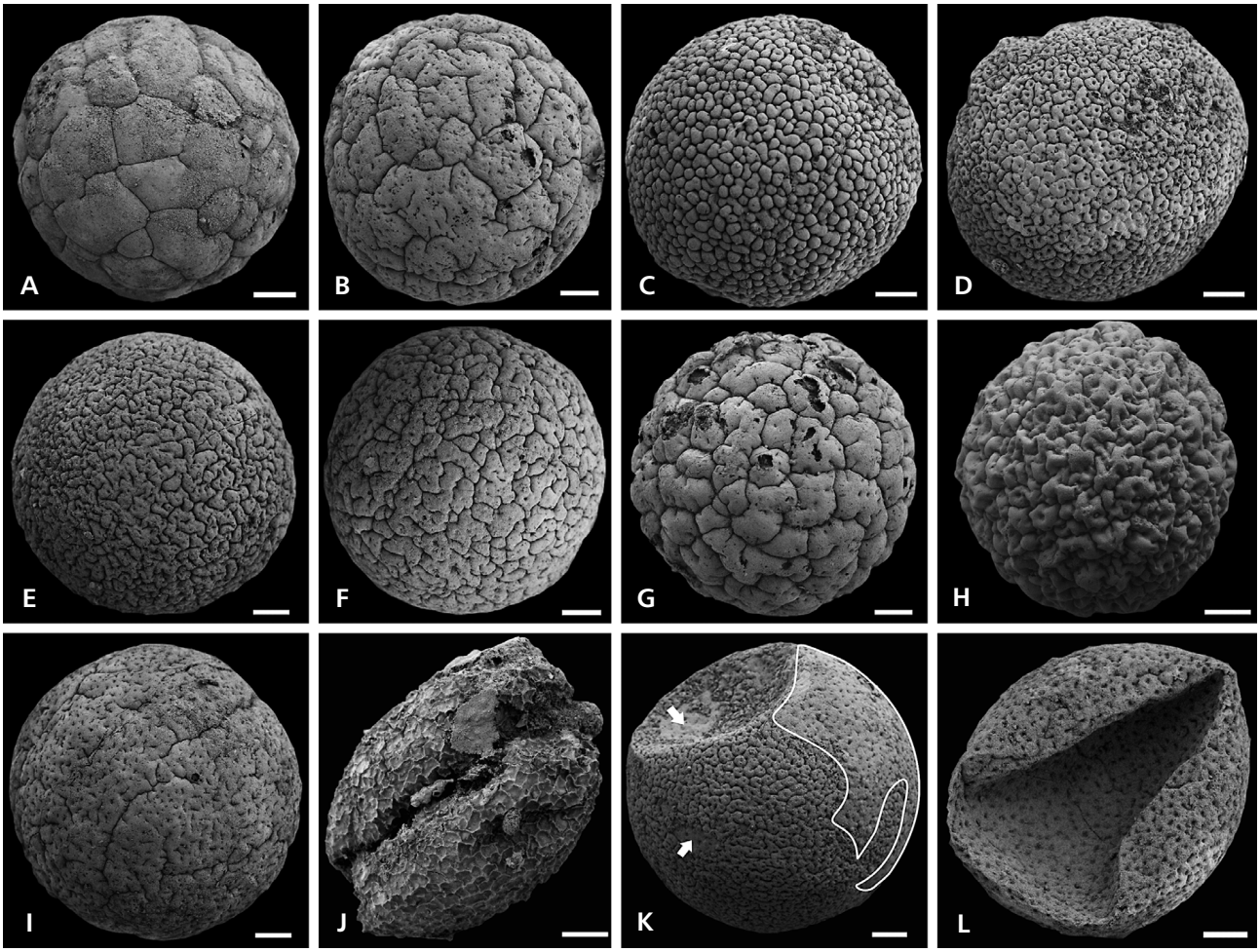

Figure 1. Ornamentation patterns of *Megasphaera ornata* from the Weng'an Biota. • A – polygons ornamentation. • B – polygons with fractal branching. • C – smooth tubercles ornamentation. • D, tubercles with dimples on top. • E – anastomosing ridges ornamentation. • F, G – transitional forms between B and E. • H–J – microfossils with new ornamentation patterns. H – cerebral cortex-like ornamentation without clear anastomosing ridges; I – discontinuous furrows, short rod-shaped lines and dots; J – network ornamentation. • K – a bad-preserved fossil showing transitional pattern between D and E, notice the imprinted ornamentation on the surface of exposed inner capsule (arrows and curve frame). • L – pitted ornamentation looks similar to that on inner capsule of K. All the scale bars represent 100 μm.

regular polygons (Fig. 2A–G). Similar specimens have been illustrated previously, but they were considered to be the same as *Megasphaera ornata* in morphology and affinity (Yuan *et al*. 2002, Xiao *et al*. 2007b). Since the polygons are preserved in three-dimensional detail (Fig. 2K) and their shape is comparable to cell shape, Chen *et al*. (2009a) interpreted the polygons as the follicle cells of embryos. Although the follicle cell hypothesis has to be tested further (see conclusion section of this paper), one point can be affirmed that the cell-like structure is absolutely different from all other ornaments, not only because of its unique morphology but also its extreme stability of shape. The statistical investigation (Figs 3, 4) indicates that the shape and size of these cell-like polygons are uniform in a single specimen and show only very slight variation among different specimens (Fig. 2A–I). In contrast, the shape and size of ornaments in other types are much more variable (Fig. 1). Take ornamented microfossils with "well-seamed polygons" for example, in which the so called "polygons" have no constant size (Figs 3, 4), and the pattern of boundaries between them is highly irregular (Fig. 1A). Additionally, the variation in ornamentation is further evidenced by the transitional ornamentation forms (Fig. 1F, G).

Several new elongated specimens, two of them shown in Fig. 2F, G, show significant variation in the shape of the cell-like polygons in the equatorial part (Fig. 2J). We propose that the elongated shape represents developing deformation during mitosis from a one cell stage to a two-cell stage, and are not due to mechanical deformation. This interpretation is based on the following observations: 1) the elongated shape of the cell-like polygons only occurs in the equatorial part; 2) the elongation of the cell-like polygons shows gradual transition from the equator to the two poles; and 3) more importantly, all the elongated polygons are

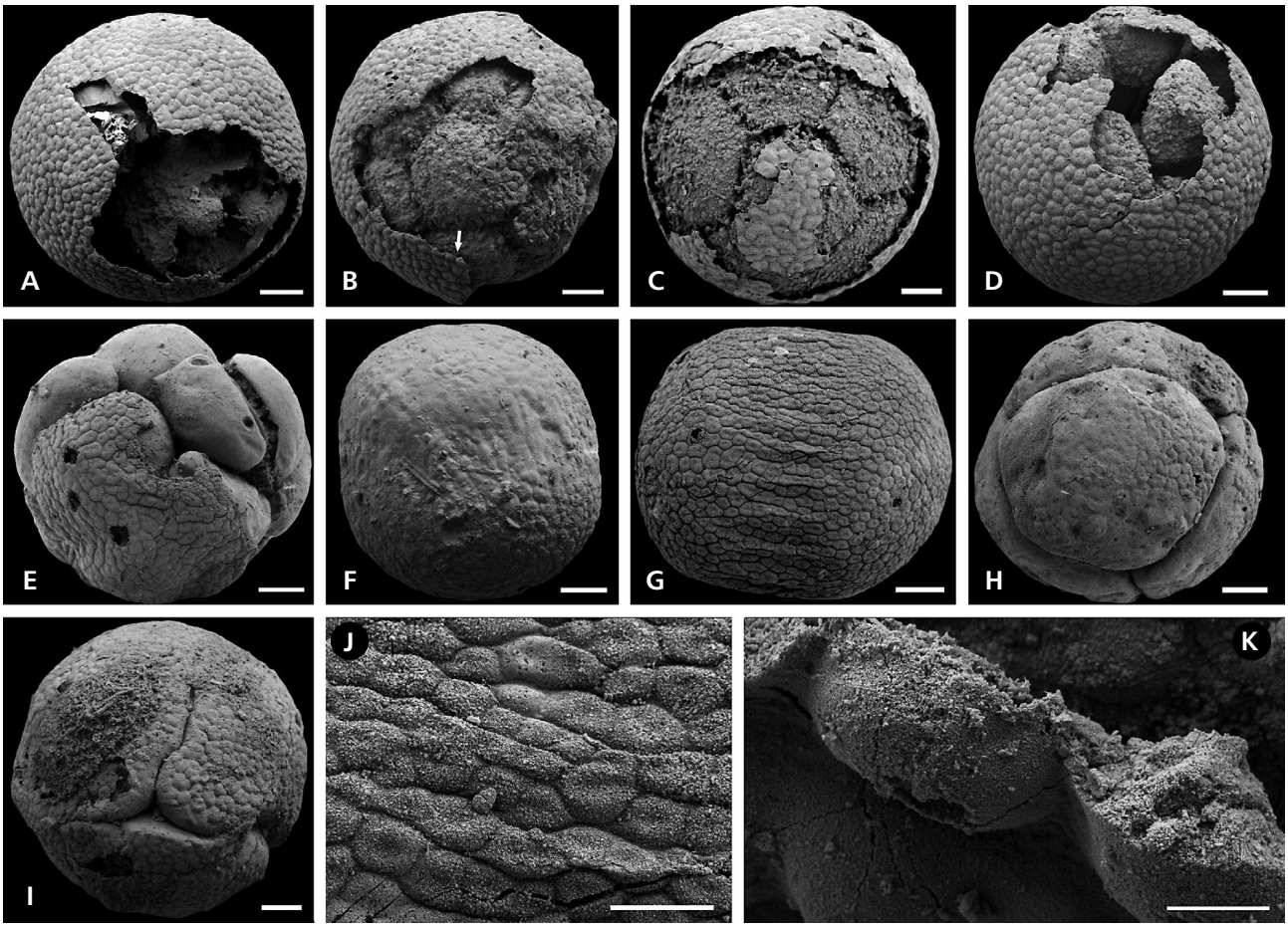

Figure 2. *Megasphaera ornata* (A) and *Parapandorina raphospissa* (B–I) with cell-like structure from the Weng'an Biota. • A – one internal body enclosed in an ornamented envelope with cell-like structure, notice the space between outer envelope and internal body. • B–E – samples show several internal blastomeres enclosed in a envelope with cell-like struture, notice no preservation of cytomembranes of blastomeres and phosphatic filiments and/or spherules on the surface of blastomeres in B, C and D. • F, G – embryo fossils probably undergoing mitosis, notice the shape change of polygons around the equator part. • H, I – 4-cell stage embryos without preservation of the outer capsule, showing imprint of the cell-like ornaments on the surface of blastomeres. • J – a close-up view of G. • K – magnified view of arrowed area in B, showing the details of envelope with cell-like structure. Scale bars represent 100 μm for A–I, 50 μm for J, and 10 μm for K.

in the same smooth peripheral plane as the normal ones (Fig. 2G, J). It is hard to imagine that the surface would retain its smoothness if it had been mechanically deformed.

Given the same size and envelope structure shared by the fossils shown in Fig. 2, they should be more closely related to each other than to the ornamented fossils shown in Fig. 1. From the perspective of developmental biology, these specimens with a different number of internal cells and the same cell-like polygons on the surface as shown in Fig. 2 may represent embryos of the same taxon at different cleavage stages. However, previous classification has treated these fossils as two different morphospecies. The fossil with only one internal cell (Fig. 2A) is assigned to *Megashaera ornata*, whereas the others with more than one internal cell (Fig. 2B–I) are named *Parapandorina raphospissa*. On the another hand, the fossils with various ornamentation patterns as shown in Fig. 1 may relate to different taxa (Xiao & Knoll 2000), but they have all been assigned to one morphospecies *Megashaera ornata*. It is therefore clear that the morphological taxonomy of the Weng'an embryos conceals the biological affinities between different morphospecies, and hampers the estimation of the original biodiversity of the Weng'an embryos.

Envelope microstructure of the ornamented spheroidal microfossils

Interpretations of the Weng'an phosphatized microfossils vary greatly because of the difficulty in distinguishing primary biological features from diagenetic artifacts (*e.g.* Bengstson & Budd 2004, Chen *et al*. 2004b). Unlike the phosphatized small shelly fossils, the Weng'an phosphatized microfossils show exquisite preservation of the cellular

and subcellular structures (*e.g*. Hagadorn *et al*. 2006, Chen *et al*. 2009b). Although it still remains unclear how these embryo fossils preserved cellular details following phosphatization (Martin *et al*. 2003, 2005), and why taphonomic bias exists favouring the preservation of embryos in earlier cleavage stages (Dornbos *et al*. 2005) or embryos with a fertilization envelope (Raff *et al*. 2006), preservation analysis indicates that impregnation and encrustation were two principal phosphatization processes affecting the cell tissues of the Weng'an embryos (Xiao & Knoll 1999, Xiao & Schiffbauer 2009). In general, impregnation provides the best preservation of the primary biological structures and is characterized by randomly oriented fine apatite crystals (Fig. 5H, L, N), whereas encrustation usually adds diagenetic artifacts by producing isopachous rims with acicular or prismatic apatite crystals which are always perpendicular to the surface of original substrate (Fig. 5I, J, M, N). Since the envelope microstructure of the ornamented spheroidal microfossils are critical to their interpretation, it is important to distinguish the primary envelope (*e.g*. embryo chorion, vitelline membrane, and even plasma membrane, *etc.*) from diagenetic coatings and linings.

Based on SEM analyses, two major envelope categories and fossil morphologies of the ornamented spheroidal microfossils can be distinguished as shown in Figs 5, 6: 1) fossils with a thick multilayered envelope with various surface ornamentation patterns and a single internal cell (Fig. 6a); and 2) fossils exhbiting a single-layered envelope with the cell-like surface structure and several internal cells (amount is equal to 2^n, n = 0, 1, 2, 3….) (Fig. 6b). In most cases, the thick multilayered envelope exhibits three layers, which are composed of randomly oriented fine apatite crystals, representing the primary structure (Fig. 5A–D, G, H). The inner layer tightly surrounding the internal body (cell) is interpreted to be phosphatized cytomembrane, which is usually thin and smooth (Fig. 5B, C). Sometimes, the inner capsule shows wrinkled and deformed structures which may be due to rapid syneresis or/and cytoplasm decay before mineralization, thus leading to an irregular space between the inner and middle layers (Fig. 5A, E). The phosphatic spherules and/or filaments within this space represent microbial activity during decay (Fig. 5A, E; Xiao & Knoll 1999, 2000; Xiao & Schiffbauer 2009). In such cases, an apatite coating could be deposited on the outer surface of the inner layer (Fig. 5E). Compared with the middle and outer layers, the inner cytoplasm membrane is poorly preserved (Fig. 5D). In some specimens, the inner layer is hardly distinguishable from the phosphatized cytoplasm (Fig. 5F), but an interface between the internal cell and the middle layer implies the presence of a flimsy membrane. One possible interpretation for the preservational bias is that the cytoplasm membrane is more fragile, allowing it to be more easily damaged during decay. In addition,

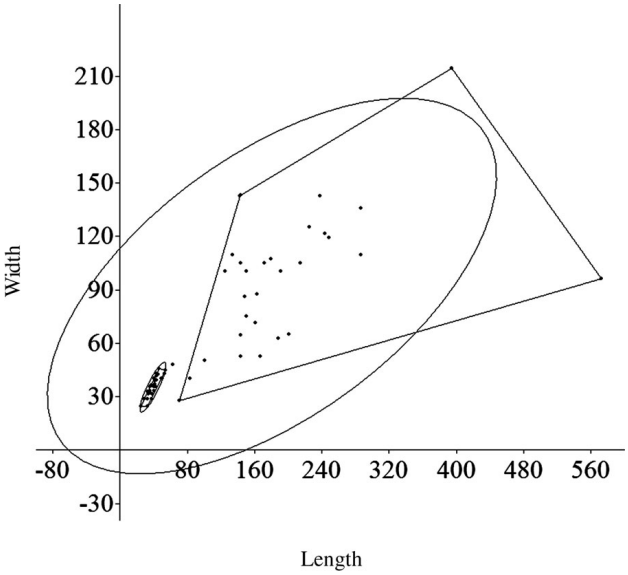

Figure 3. Scatter diagram showing the size stability of two kinds of polygons on the envelope surface of the ornamented spheroidal microfossils from the Weng'an Biota. The smaller 95% ellipse and convex hull represent data from cell-like polygons, while the bigger 95% ellipse and convex hull represent the data from well-seamed polygons. Original statistical data are given in the Table 1.

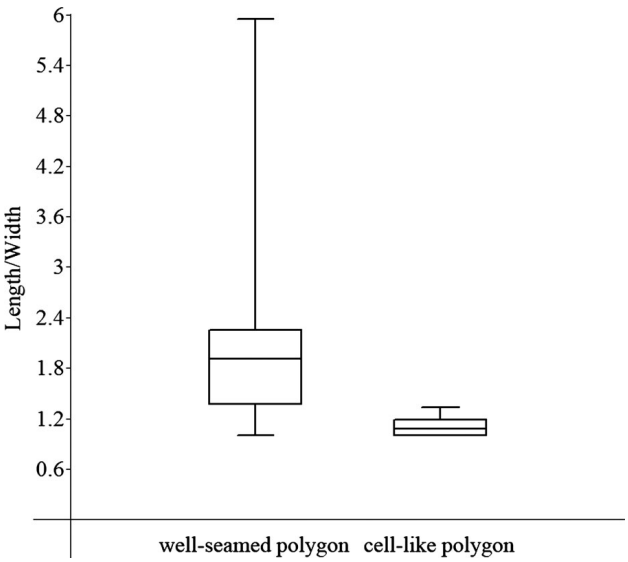

Figure 4. Box plot diagram showing the length/width ratio of two kinds of polygons on the envelope surface of the ornamented spheroidal microfossils from the Weng'an Biota. Original statistical data are given in the Table 1.

more microbial activity might be expected to affect the cytoplasm, since it is usually filled with nutrient-rich substances, such as yolk granules and lipid drops, rather than the nutrient-poor chorion or diapause cyst of embryos (Xiao & Knoll 1999).

The middle layer is much thicker than the inner cytomembrane (Fig. 5B, C, F). It is usually coated with an

Table 1. Statistical data for the well-seamed polygons and cell-like polygons* on the envelope surface of the ornamented spheroidal microfossils from the Weng'an Biota. Abbreviations: * "length" and "width" are used to describe the polygons which have obvious long axis and short axis, σ is standard deviation, and $\overline{A}$ is mean value.

Number	Well-seamed polygon			Cell-like polygon		
	Length (μm)	Width (μm)	L/W	Length (μm)	Width (μm)	L/W
1	150	100	1.5	28.6	28.6	1
2	142.5	142.5	1	32.6	24.5	1.330612
3	70	27.5	2.545455	40.8	36.7	1.111717
4	82.5	40	2.0625	32.6	28.6	1.13986
5	125	100	1.25	32.6	32.6	1
6	237.5	142.5	1.666667	36.7	28.6	1.283217
7	162.5	87.5	1.857143	28.6	28.6	1
8	187.5	62.5	3	24.5	24.5	1
9	150	75	2	37.1	36.4	1.019231
10	200	65	3.076923	41.4	39.2	1.056122
11	100	50	2	40.7	38.6	1.054404
12	225	125	1.8	36.4	35.7	1.019608
13	393.8	214.3	1.837611	40	35.7	1.120448
14	285.7	135.7	2.10538	42.9	39.2	1.094388
15	142.9	142.9	1	42.9	41.4	1.036232
16	160.7	71.4	2.2507	42.9	42.9	1
17	242.9	121.4	2.000824	37.1	35.7	1.039216
18	178.6	107.1	1.6676	36.4	31.8	1.144654
19	142.9	64.3	2.222395	40.9	36.4	1.123626
20	214.3	104.8	2.044847	45.5	45.5	1
21	142.8	104.8	1.362595	42.2	35.6	1.185393
22	133.3	109.5	1.217352	44.4	42.2	1.052133
23	247.6	119	2.080672	37.8	31.1	1.215434
24	147.6	85.7	1.722287	40	35.6	1.123596
25	285.7	109.5	2.609132	48.9	40	1.2225
26	190.5	100	1.905	40	33.3	1.201201
27	61.9	47.6	1.30042	35.6	35.6	1
28	171.4	104.8	1.635496	53.3	44.4	1.20045
29	142.8	52.4	2.725191	40	40	1
30	571.4	96.2	5.939709	35.6	33.3	1.069069
31	166.7	52.4	3.181298	33.3	31.1	1.07074
32	28.6	28.6	1	40	35.6	1.123596
33	52.4	42.8	1.224299	37.8	31.1	1.215434
σ			0.915794			0.091968
$\overline{A}$			2.023985			1.098572
$\sigma/\overline{A}$			0.452471			0.083716

isopachous rim (2–5 μm thick) consisting of prismatic apatite crystals perpendicular to inner, outer or both sides (Fig. 5I, M, Xiao & Knoll 1999, Xiao & Schiffbauer 2009). The outer layer (Fig. 6a) is more easily identifiable because of the various ornaments on its outer surface (Fig. 5A–F). The maximum thickness of the outer layer is up to 30–40 μm (Fig. 5C, M, N). A crescent cavity (view from cross-section) between the outer and middle layers is common and has a similar size and shape in different specimens (Fig. 5B, C, D, F). Phosphatic filaments and/or spherules representing microbial activities are also found within the cavity (Fig. 5C, D, F, I, J). The regular shape and uniform size of the cavity as well as the filaments inside not only indicate that the cavity existed before mineralization, but also suggest that it is probably a primary biological structure rather than taphonomic artifact. Beyond the area of the crescent cavity, a clear slit as the boundary between the middle and outer layers can still be identified at higher magnification (Fig. 5B, K, L). The randomly oriented apatite crystals on both sides of the slit suggest that they are impregnated primary capsules (Fig. 5L, Xiao & Schiffbauer 2009), but not secondary artifacts. The two layers usually merge into one thicker layer in most specimens (Fig. 5C, F, M). Only one internal body was found in the ornamented spheroidal microfossils with a multilayered envelope based on examination of more than ten thousand specimens of the ornamented microfossils.

The envelope of the spheroidal microfossils with cell-like surface structure is a thin capsule, about 2–10 μm in thickness (Fig. 2K). As shown by Fig. 6, these microfossils do not exhibit a middle layer except for the inner cytomembrane of internal cells or blastomeres (Fig. 2A–E), and the outer capsule with a cell-like structure (Fig. 2K). This inference is further supported by the impressions of cell-like structure on the surface of the internal blastomeres (Fig. 2H–I). All the observations above suggest that these morphological and structural differences between the two categories of ornamented embryos (eggs) are biogenetic rather than artifacts resulted from taphonomic process.

Conclusions and discussion

In summary, there are two gross categories (A-type and B-type in Fig. 6) of the sculptured spheroidal microfossils in the Weng'an Biota. With the exception of the phosphatized cytomembrane of internal cells or blastomeres (Fig. 6-aI, bI), the A-type fossils have a two-layered envelope (Fig. 6-aO, aM) enclosing only a single internal body, which has been previously assigned to *Megasphaera ornata.* The B-type fossils possess a single-layered envelope surrounding several internal bodies (2^n, n = 0, 1, 2, 3…), which have been previously assigned to *Megasphaera ornata* (when n = 0, Fig. 2A) or *Parapandorina raphospissa* (when n > 0). All these sculptured spheroidal microfossils have been previously regarded as metazoan eggs or embryos during the diapause stage. Based on comparisons

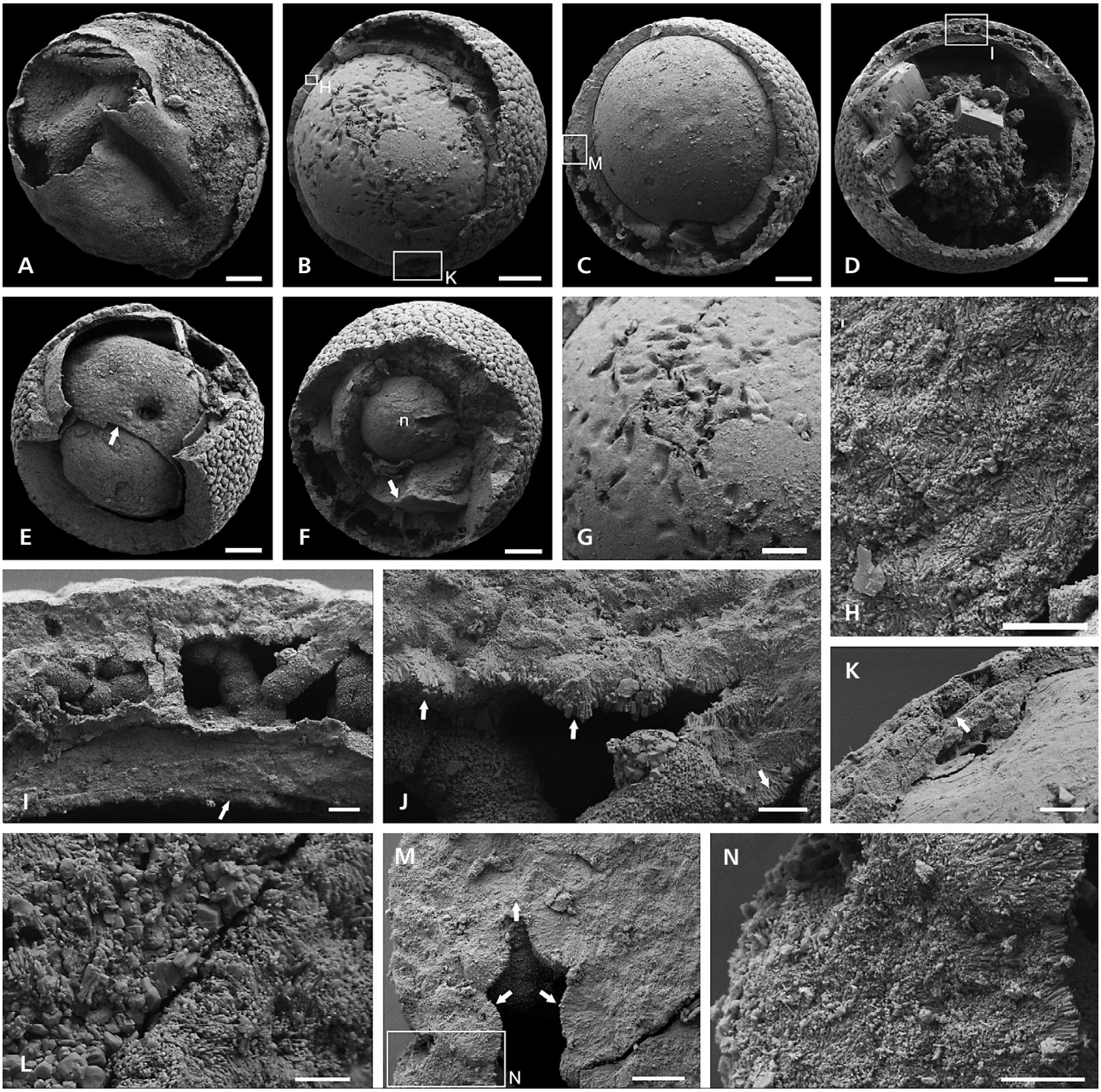

Figure 5. Envelope microstructures and internal body of *Megasphaera ornata* from the Weng'an Biota. • A–F – the ornamented fossils encircled by three original capsules. The inner and middle layers of A folded; notice a regular cresent cavity with phosphatized filiments and/or spherules between outer and middle capsules in B–D and F; the internal cell of D strongly degraded, notice the giant idiomorphic crystals of pyrite; the inner layer are covered by phosphatic spherules and coating in A and E; nucleolus is preserved in F (n). • G – magnified view of B, showing deformation on the surface of the inner layer. • H – magnification of the box area in B (left up), showing randomly oriented apatite crystals. • I – magnification of the box area in D, showing space between ornamented outer and middle layers, notice the phosphatic filiments inside the cavity and lining on inner surface of the middle layer (arrow). • J – close-up view of I, showing more details of the inner surface of the outer layer and filiments, notice the isopachous prismatic apatite crystals perpendicular to the inner surface of the outer layer and outer surface of filaments (arrows). • K – magnification of the boxed area of B (right down), showing slit between out and middle layers (arrow). • L – magnified view of K, showing randomly oriented crystals distributed on both sides of the slit. • M – magnification of the boxed area of C, showing no obvious boundary between the out and middle layers beyond the cavity (upper arrow). Notice coatings on the outer surface of the middle layer and the inner surface of the outer layer (lower arrows). • N – magnification of the boxed area of M, notice an isopachous rim with inward growing crystals of apatite perpendicular to the inner surface of the outer layer. Scale bars are equal to 100 μm for A to F, 50 μm for G, 10 μm for I and M, 25 μm for K, 5 μm for H, J and N, and 2.5 μm for L.

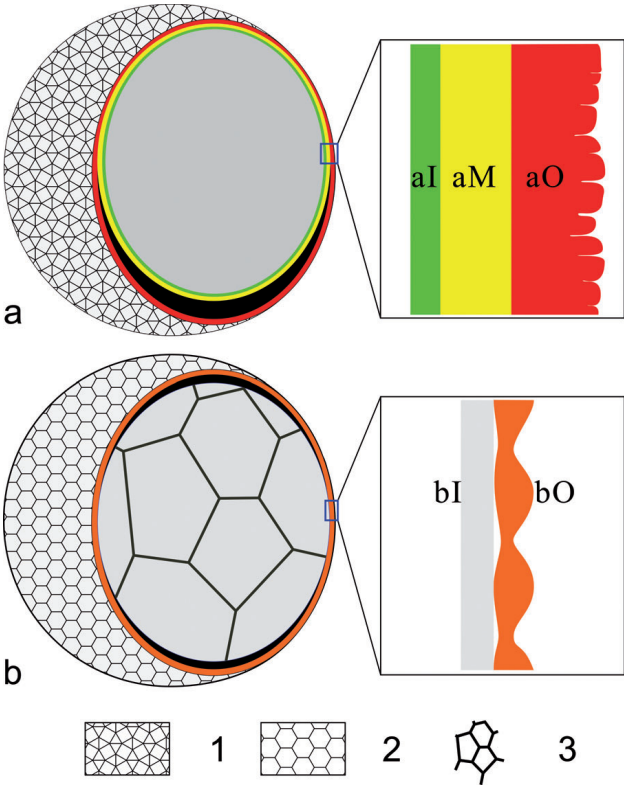

Figure 6. Sketch diagram showing two types of the Weng'an ornamented embryos. a – typical *Megasphaera ornata*; b – sculptured *Parapandorina raphospissa*; Legends: 1 – ornamentations on outer envelope of *Megasphaera ornata*; 2 – cell-like structure of envelope of *Parapandorina raphospissa*; 3 – internal blastomeres; I – inner layer; M – middle layer; O – outer layer.

with the diapause embryos of modern aquatic invertebrates, however, we propose that the diapause embryo hypothesis can be only adapted for the A-type fossils, while the B-type fossils probably represent normal developing embryos at an early holoblastic cleavage stage.

Diapause is a common physiological state for embryos in a number of extant invertebrates (Belmonte *et al*. 1997, Cáceres 1997), and even some vertebrates (Murphy & Collier 1997, Hrbek & Larson 1999). Diapause embryos are generally characterized by the following features: 1) thick and multilayered diapause cysts directly outside of the plasma membrane (Santella & Ianora 1990, Belmonte & Puce 1994, Marcus 1996, Castro-Longoria 2001, Couch *et al*. 2001); the cyst consists of at least the cortical and alveolar layers (Gilchrist 1978, Blades-Eckelbarger & Marcus 1992, Dharani & Altaff 2004, Liu *et al*. 2009); 2) taxon-specific ornamentation pattern on the outer surface of the diapause cyst (Gilbert & Wurdak 1978, Belk 1989, Shen & Huang 2008); and 3) diapause only occurs at a specific developmental stage for each species (Ianora & Santella 1991). The multilayered envelope and diverse ornamentation patterns of the A-type fossils are comparable to those known from extant diapause embryos. The outer and middle layers of the envelopes of the A-type fossils are comparable to the cortical and alveolar layers of the resting cysts, whilst the crescent-shaped cavity between the outer and middle layers in the fossils is a possible analog for the subcortical spaces of extant diapause embryos (Gilchrist 1978, Liu *et al*. 2009). The complex surface ornaments are similar to that of extant diapause eggs, such as those of branchiopod arthropods (Mura 2001).

Unlike for the A-type fossils, the envelope of the B-type fossils does not show multilayered microstructure, and it is only a thin capsule which is more alike the textured fertilization membrane (developed from vitelline membrane) of the non-diapause embryos of modern metazoans, *e.g*. annelids, molluscans, and arthropods (Santella & Ianora 1990, Conn 1991, Castro-Longoria 2001). The B-type fossils with a single capsule also resemble extant mature subitaneous eggs (Santella & Ianora 1990, Conn 1991, Ianora & Santella 1991, Couch *et al*. 2001). In addition, the outer capsule, which shows elongated cell structure in some specimens (Fig. 2F, G) and is reminiscent of mitosis, further rules out the interpretation of the thin capsule with cell-like structure as a diapause cyst. This is because once diapause initiates, the embryo enters a state of extremely low metabolism, during which embryonic development almost stops. By contrast, shape change of the fertilization membrane during cleavage in non-diapause embryos is a common phenomenon (Conn 1991). Another line of evidence to support the non-diapause interpretation of B-type fossil embryos is the discovery of specimens at various stages of holoblastic cleavage. As shown by Fig. 2A–E, some specimens have different numbers of blastomeres which are enclosed in an envelope with the same microstructure, suggesting they are non-diapause embryos of the same organism at various holoblastic cleavage stages. As mentioned above, diapause only occurs at a specific development stage for each species, for example, resting eggs of *Anomalocera patersoni* (copepod) arrest development at the 32-cell stage and remain at this stage until diapause breaks (Ianora & Santella 1991). Thus, the possibility of fossil embryos of one organism with preservation of the same number of blastomeres would be much higher if they were at a diapause stage which usually lasts for several months or even years, but this is not the case for the B-type fossils.

Chen *et al*. (2009a) interpreted the cell-like polygons on the surface of the B-type fossils as follicle cells. However, extant embryos which exhibit follicle cells usually possess capsules between the cell plasma membrane and the follicle cell layer (Villa & Patricolo 1987, Pennington *et al*. 1999, Buckland-Nicks & Hodgson 2000), but such capsules are not found in the B-type fossils. The document about ripe oocyte directly in the follicle cell layer without a vitelline membrane is unknown in Eumetazoa (Conn 1991, Gilbert & Raunio 1997, Schoenwolf 2009).

The A-type fossils are more abundant than the B-type fossils in the Weng'an embryos, providing additional support to our interpretation. Because diapause cysts are more resistant against decay than the fertilization membrane, and could survive in sediments for a very long time, even several years or more than a century (Hairston *et al*. 1995, Engel & Hirche 2004, Belmonte & Pati 2007), this would lead to taphonomic bias toward preservation of diapause cysts. In addition, the extreme high density of diapause eggs in modern sediments, for example, the density of copepod diapause eggs can reach 2×10^5 eggs/m^2 (Hairston *et al*. 1995), provide a reasonable interpretation for why the diapause embryo fossils are so abundant in the Weng'an Biota.

Interpretation of the Weng'an phosphatized embryos is like a "Rorschach Inkblot Test" to paleontologists; the answers are always different. Taphonomic bias and diagenetic artifacts provide reasons for this on the one hand, while the lack of knowledge about the complex embryonic development of the earliest animals provides a further challenge on the other. For example, the resting cyst of *Boeckella triarticulata* (copepod) can be seen to be composed of five layers under transmission electron microscopy (Couch *et al*. 2001). If such a kind of microstructure was present in the Weng'an embryos, it would have been lost during phosphatization. Likewise, the fertilization envelopes of the subitaneous eggs of some living animals are also multilayered, *e.g*. *Sinodiaptomus indicus* (copepod, Dharani & Altaff 2004). Nevertheless, we believe that assessment of the Weng'an ornamented embryos through taphonomic tests and comparison to extant embryos will provide further useful information to solve this dilemma.

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