A biomechanical analysis of the early eukaryotic fossil *Valeria* and new occurrence of organic-walled microfossils from the Paleo-Mesoproterozoic Ruyang Group

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Abstract

The Paleo-Mesoproterozoic Ruyang Group of North China hosts early eukaryotic fossils such as *Dictyosphaera*, *Shuiyousphaeridium*, and *Valeria*, and thus offers valuable insights into the early evolution of single-celled eukaryotic life. In this paper, we report several additional forms of organic-walled microfossils from the Ruyang Group, including *Plicatidium latum*, *Spiromorpha* sp., and an unnamed form. *V. lophostriata* from the Ruyang Group is investigated using transmitted light microscopy, scanning electron microscopy, transmission electron microscopy, and biomechanical analysis. *V. lophostriata* is reconstructed as a spherical vesicle with two hemispherical halves bearing concentric striations resembling latitudinal circles. The formation of striations could be explained using the Belousov-Zhabotinsky reaction model or the Turing reaction-diffusion model. A biomechanical analysis

using the thin-walled spherical pressure vessel model suggests that the concentric striations of *V. lophostriata* may have functioned as a mechanism to guide biologically programmed excystment through medial split. Our analysis provides essential paleontological data to better understand the functional biology and life cycles of early eukaryotes such as *Valeria*.

1. Introduction

Considerable progress has been made in the past decade to resolve the phylogenetic relationships among eukaryotes (Parfrey et al., 2010; Katz, 2012; Katz et al., 2012; Burki, 2014; He et al., 2014), but the morphology, functional biology, and life cycles of early eukaryotes remain poorly constrained (Eme et al., 2014; Knoll, 2014). Molecular clock estimates suggest that the Eukarya clade may have split from their living sister group in the Archean (Hedges et al., 2001), but the divergence of major eukaryote crown groups from the last eukaryotic common ancestor (LECA) likely occurred by the Mesoproterozoic (Hedges et al., 2004; Yoon et al., 2004; Parfrey et al., 2011; Eme et al., 2014). Thus, the Paleo-Mesoproterozoic fossil record of eukaryotes is critical to resolve the morphological evolution of early eukaryotes. Acid-resistant organic-walled microfossils can offer important insights into the early evolution of eukaryotes. However, not all organic-walled microfossils are eukaryotes. For example, morphologically simple organic-walled vesicles (or acritarchs) from the 3.2 Ga Moodies Group, South Africa (Javaux et al., 2010) and ca. 3.0 Ga Farrel Quartzite of the Mount Grant area, Western Australia (Grey and Sugitani, 2009) may or may not be eukaryotes (Javaux, 2011; Xiao, 2013; Knoll, 2014). But morphologically complex acritarchs — those with vesicle sculptures and ornaments — are most certainly eukaryotic fossils (Javaux et al., 2001; Knoll et al., 2006), and they provide important information about the taxonomic diversity and morphological disparity of early eukaryotes (Huntley et al., 2006).

One of the oldest known morphologically complex sphaeromorphic acritarchs that have been widely accepted as a eukaryotic fossil is *Valeria lophostriata*, which has been reported from the 1600–1700 Ma Chuanlinggou Formation in North China

(Yan and Liu, 1993) and the 1650 Ma Mallapunyah Formation in Australia (Javaux et al., 2004), although it has a very long stratigraphic range extending from the late Paleoproterozoic to the Neoproterozoic (Hofmann, 1999; Javaux, 2011). First described as *Kildinella lophostriata* from the Riphean Zigalga Formation in southern Urals (Jankauskas, 1979), the species was later re-assigned to the genus *Valeria* by Jankauskas (1982). *Valeria lophostriata* is characterized by its diagnostic vesicle wall ornamentation — uniformly and closely spaced concentric striations (Jankauskas, 1979; Hofmann and Jackson, 1994). A second species of *Valeria*, *V. elongata*, has been reported from the Mesoproterozoic Kamo Group of the Central Angara Basin in southern Siberia (Nagovitsin, 2009). *Valeria elongata* is different from *V. lophostriata* in its spindle-shaped vesicles with meridional rather than concentric striations. *Valeria elongata* is somewhat similar to *Cucumiforma vanavaria* in their spindle-shaped vesicles and meridional ornaments, but the meridional ornaments in the latter species consist of curvy folds rather than striations (Konstantin Nagovitsin, personal communication, 2014).

The Paleo-Mesoproterozoic Ruyang Group in North China contains an assemblage of diverse organic-walled microfossils, including morphologically complex acritarchs and likely eukaryotic fossils such as *Dictyosphaera delicata*, *Shuiyousphaeridium macroreticulatum*, and *Valeria lophostriata* (Guan et al., 1988; Yan and Zhu, 1992; Xiao et al., 1997; Yin, 1997; Yin et al., 2005; Schiffbauer and Xiao, 2009; Pang et al., 2013). In our continuing effort to study the micropaleontology of the Ruyang Group, we discovered additional organic-walled microfossils that are illustrated here. We also carried out a biomechanical and functional biological investigation of *Valeria lophostriata* on the basis of detailed characterization using both transmitted light microscopy (TLM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Our data show that the striations of *Valeria* probably functioned as a mechanism to facilitate excystment through medial split, a feature further supporting the eukaryotic affinity of this morphologically complex acritarch.

2. Material and methods

Proterozoic strata are widely distributed along the southwestern margin of the North China Platform (Guan et al., 1988; Xiao et al., 1997). Based on lithostratigraphic characters, they can be divided into the Zhongtiaoshan-Leshan, Songshan-Jishan, Xiaoshan-Xiaoqinling, and Luanchuan stratigraphic provinces (Guan et al., 1988) (Fig. 1A). In the Zhongtiaoshan area, the Proterozoic sedimentary succession is underlain by Archean metamorphosed rocks or Paleoproterozoic igneous rocks, and overlain by Lower Cambrian conglomerates (Guan et al., 1988). At the most completely preserved sections, the Proterozoic sequence consists of the Ruyang and Luoyu groups, succeeded by the Huanglianduo, Dongjia, Luoquan, and Dongpo formations.

The siliciclastic Ruyang Group is subdivided into, in ascending order, the Yunmengshan, Baicaoping, and Beidajian formations (Guan et al., 1988). At the Shuiyongou section sampled in this study, the Yunmengshan Formation is missing, and the Baicaoping Formation sits directly on the Archean Sushui Group. In the northeastern Zhongtiaoshan area, however, the Ruyang Group rests on the 1.78–1.75 Ga Xiong'er Group (He et al., 2009). The youngest detrital zircons from the Ruyang Group in western Henan give a LA-ICP-MS U-Pb age of 1744 ± 22 Ma (Hu et al., 2014), providing a maximum depositional age for the Ruyang Group. A purported ash bed from the overlying Luoyu Group at the Yangpo section in Ruzhou of Henan Province (see Fig. 1A for location) yields a LA-ICP-MS U-Pb zircon age of 1611 ± 8 Ma, potentially providing a minimum age constraint for the Ruyang Group (Su et al., 2012). However, these dated zircons may be of detrital rather than igneous origin according to Lan et al. (2014), who reported both detrital zircon ages (youngest being 1937.8 ± 11.7 Ma) and diagenetic xenotime ages (oldest being 1411 ± 27 Ma) from the Sanjiaotang Formation of the Luoyu Group at the Laoleshan section in Zhumadian of Henan Province (see Fig. 1A for location). Together, these ages constrain the Ruyang Group between 1411 ± 27 Ma and 1744 ± 22 Ma, and possibly between 1611 ± 8 Ma and 1744 ± 22 Ma if the ash bed from the Luoyu Group is verified. Thus, the Ruyang Group is regarded as Paleo-Mesoproterozoic in age.

Organic-walled microfossils were obtained from shale and siltstone samples collected from the Beidajian (30 samples) and upper Baicaoping formations (6 samples) at the Shuiyongou section in Yongji, Shanxi Province, North China (Fig. 1B, C). The Ruyang acritarchs and other microfossils were extracted using a low-manipulation hydrofluoric acid maceration technique (Butterfield et al., 1994; Tang et al., 2013). Organic-walled microfossils were isolated from maceration residues and flat mounted on glass slides for TLM and SEM characterization. Selected specimens were embedded in epoxy and microtomed to make 75 nm thick ultrathin sections for TEM analysis on a Tecnai G² 20 (at 200 keV) at Peking University (Javaux et al., 2004; Pang et al., 2013). All illustrated specimens in this article are deposited at the Nanjing Institute of Geology and Palaeontology (NIGPAS).

We adapted the “thin-walled spherical pressure vessel” model to assess the functional morphology of the concentric striations in *Valeria*. This model estimates the membrane stress under internal pressure of a spherical shell, whose wall thickness t is less than 1/20 of the internal diameter d (Gere and Goodno, 2009). The same model has been applied in the analysis of the cell wall/membrane stress of the Proterozoic organic-walled fossils *Chuaria* and *Tawuia* (Sharma et al., 2009). Similar models have also been used to calculate the tension in the plant cell walls (Steudle et al., 1977), and to understand the energetics of volume regulation in extant freshwater algae (Raven, 1982). The thin-walled spherical pressure vessel model is employed here to estimate the stress on *Valeria* vesicles in order to understand the function of its striations.

3. Summary of microfossil assemblage from the Ruyang Group

Samples from the Baicaoping Formation yielded *Leiosphaeridia* spp., *Shuiyosphaeridium macroreticulatum*, and *Dictyosphaera delicata*. Organic-walled microfossils from the Beidajian Formation include the acritarchs *S. macroreticulatum*, *D. delicata*, *Tappania plana*, *T. tubata*, and *Valeria lophostriata*, which have been described previously (Yan and Zhu, 1992; Xiao et al., 1997; Yin, 1997; Yin et al., 2005) and no further description is needed here. The Beidajian samples also yielded

microfossils that have not been previously reported from the Ruyang Group: including *Plicatidium latum* (Fig. 2A, B), *Spiromorpha* sp. (Fig. 2C), and an unnamed form (Fig. 2E, F), which are briefly described below. We also distinguish two morphotypes of *V. lophostriata* that are characterized by different striation width.

Plicatidium latum is a thin, single-layered, unbranched tubular sheath, ornamented with closely spaced transverse plications (Fig. 2A, B). The specimens are 52–65 μm wide, 63–168 μm long, and constricted to form a truncated end about 28–35 μm wide. The plications or wrinkles are uneven in thickness (0.4–0.8 μm) and spacing (0.3–1 μm). Because both available specimens are incompletely preserved, it is uncertain whether the truncated end is a taphonomic artifact or a biological feature.

Spiromorpha sp. is characterized by its ovoidal or spindle-shaped vesicle with transverse stripes (Fig. 2C). The vesicle is ~ 47 μm long and ~ 34 μm wide, with possible granules but no processes. The stripes are slightly twisted and either circular or spiral in shape, 0.5–0.6 μm wide, and 0.8–1 μm apart. *Spiromorpha* sp. is similar to *Navifusa segmentatus* (= *Spiromorpha segmentata*) from the Sarda and Avadh formations in the Ganga Basin of northern India (Prasad and Asher, 2001), *S. segmentata* from the Ruyang Group (Yin et al., 2005), and two unnamed specimens (probably *S. segmentata*) from the Kamo Group of Siberia (Nagovitsin, 2009). But it differs from *S. segmentata* in its narrower and more closely spaced stripes and a smaller vesicle length to width ratio (Fig. 2D).

Our collection also includes an unnamed tubular fossil, which is compressed and randomly perforated by lacunae 3.5–4 μm in diameter (Fig. 2E, F). The tube is ~ 108 μm long and ~ 52 μm wide, ornamented with densely distributed granules 0.3–0.4 μm in size (Fig. 2F).

Most specimens of *V. lophostriata* are half vesicles or fragments. The vesicles are 85–436 μm in maximum dimension, ornamented with fine and closely spaced striations, resembling the tracks of gramophone records (Fig. 3A–H). It is clear in our material that the striations are concentric (Fig. 3B, D) rather than spiral as speculated by Hofmann (1999). A complete vesicle shows that there are two polar foci from which the striations emanate. The focus is about 0.3 μm in diameter and the striation

closest to the focus is about 0.7 μm in diameter (Fig. 3D). The striations can be observed on the vesicle surface using both TLM (Fig. 3G, H) and SEM (Fig. 3I), although they are really faint under SEM. If the fragmentary specimen examined under SEM (Fig. 3G–I) is folded inwardly, then the striations are represented by concentric ridges on the inner vesicle surface, consistent with previous interpretation based on SEM examination of an enrolled half vesicle of *V. lophostriata* (Javaux et al., 2004). However, observation of complete specimens is needed to confirm that the concentric ridges are indeed on the inner vesicle surface because half vesicles and fragmentary specimens can be enrolled inwardly or outwardly, although inward enrollment is arguably more likely than outward enrollment in half vesicles considering enrollment mechanisms.

Our collection of *V. lophostriata* contains two recognizable morphotypes that are distinct in striation width and spacing (Fig. 4A). Morphotype A has striations 0.3–0.5 μm in width (average = 0.4 μm , $n = 40$) and 0.1–0.3 μm in spacing (average = 0.2 μm , $n = 40$, Fig. 3A–D). Morphotype B has striations 0.7–1.2 μm in width (average = 0.9 μm , $n = 29$) and 0.3–0.8 μm in spacing (average = 0.5 μm , $n = 29$, Fig. 3E–I).

These two morphotypes may represent two different species of resting cysts or two different developmental stages of the same species. Seemingly consistent with the latter interpretation, *V. lophostriata* has been interpreted as prasinophycean phycomata (Vidal and Siedlecka, 1983; Moczydłowska et al., 2011), which do grow in size during their encystment (Tappan, 1980). If these two morphotypes were indeed developmental stages of the same taxon, then vesicle growth would have been achieved by insertion of new striations and/or the widening of existing striations. However, our measurements show that, among the 16 complete and half vesicles of morphotype A, the number of the striations is positively scaled with vesicle diameter (Fig. 4B; correlation coefficient $r = 0.91$; significant at $\alpha = 0.01$, two-tailed), whereas the striation width and spacing are not correlated with vesicle diameter (Fig. 4C, D). Thus, the two morphotypes cannot be linked ontogenetically through the widening of striations, because they have narrow and non-overlapping ranges of striation width and spacing (Fig. 4A). As discussed below, the positive relationship between vesicle

diameter and number of striations may result from reaction-diffusion processes, not from ontogenetic insertion of new striations during vesicle growth. Thus, it is possible that the two morphotypes may eventually prove to be resting cysts of two distinct *Valeria* species, although at the present they are regarded as two morphotypes of *V. lophostriata* because complete specimens of morphotype B are not sufficiently abundant to allow a biometric determination of the relationship of vesicle diameter vs. striation number, width, and spacing (cf. Fig. 4B–D for morphotype A).

TEM observations of ultrathin sections show that the vesicle wall of *V. lophostriata* is 70–182 nm in thickness and consists of a single homogeneous electron-dense layer (Fig. 5A, B), consistent with previous observations (Javaux et al., 2004). A novel observation herein is that the striations consist of raised ridges with somewhat more electron-tenuous material than the vesicle wall (Fig. 5B). For this particular specimen, it is uncertain whether the ridges are present on the inner or outer surface of the vesicle wall because the analyzed specimen was a fragment; however, observation of other more complete specimens suggests that the ridges are likely on the inner surface (Fig. 3G–I; Javaux et al., 2004).

4. The formation of *Valeria* striations

Like the processes and reticulate sculptures on Proterozoic acritarch vesicles, striations on *Valeria* vesicles are pattern formation that probably requires cytoskeletons and an endomembrane system (Cavalier-Smith, 2002; Xiao, 2013). Building material for vesicle wall and striations was synthesized by the endomembrane system and transported by vacuoles and/or cytoskeletons to the cell periphery. In addition, it is possible that physical processes related to diffusion and reaction may have also played a role in the pattern formation of *Valeria* striations.

Hofmann (1999) was the first to suggest that the Belousov-Zhabotinsky (B-Z) oscillatory reaction may have assisted in the formation of *Valeria* striations. The B-Z reaction model is an oscillating reaction-diffusion (R-D) system that has been used in various nonlinear biological systems and processes (Shanks, 2001), and it could be a potential physical mechanism that guides the formation of *Valeria* striations. Patterns

similar to the B-Z waves have been observed in the assembly of microtubules *in vitro* (Tabony and Job, 1990; Tabony, 1994), implying that the formation of a stationary chemical pattern of microtubules might be related to intracellular morphogenesis *in vivo* (Harrison, 1992). If true, then the B-Z reaction could have played a role in organizing the microtubules which then controlled intracellular transport and ultimately the pattern formation of *Valeria* striations.

Related to the Belousov-Zhabotinsky model is the Turing model, which is also a reaction-diffusion (R-D) model. Proposed by Turing (1952), the main idea is that two diffusible substances (morphogens) having catalytic and inhibitory interactions with each other could generate spontaneous spatial patterns with the trigger of random disturbance. Different from the B-Z reaction whose reactants have the similar diffusivities, the Turing model requires that the inhibitor diffuses much faster than the activator (Winfree, 1987; Harrison, 1992). The Turing model has been used to explain many biological examples of pattern formation, particularly in multicellular organisms where morphogens are a main control of pattern formation (Newman and Frisch, 1979; Shimizu et al., 1993; Kondo and Asai, 1995). In unicellular organisms, the Turing model can be applied to explain intracellular reaction-diffusion processes. For example, it has been used to understand the morphogenesis of the freshwater desmid green alga *Micrasterias* (Holloway and Harrison, 1999) and the marine dasyclad green alga *Acetabularia* (Harrison and Kolář, 1988; Harrison, 1992).

We suggest that a two-morphogen reaction-diffusion system may have also been involved in the pattern formation of *Valeria* striations. The morphogens (or equivalents) could be proteins or small molecules synthesized within the cell and transported to the plasma membranes (Harrison and Kolář, 1988; Holloway and Harrison, 1999). The R-D model can be modified to account for a spherical shell (Turing, 1952), which can be used to approximate a *Valeria* vesicle. Under certain chemical conditions, the homogeneity of morphogen concentrations within a *Valeria* vesicle can be broken and a new pattern with an axial symmetry (i.e., with two polar foci) would be formed. Basically, the two morphogens would form concentric and oscillatory cylinders around the polar axis. The spherical vesicle intersects with these

oscillatory cylinders that guide the formation of concentric striations on the inner surface of *Valeria* vesicles (Fig. 5C). Viewed in this light, the concentric striations in *Valeria lophostriata* could have been formed more or less simultaneously to prepare for a resting stage, rather than inserted sequentially during ontogenetic growth.

5. Biomechanical analysis of *Valeria*

After a period of resting stage and upon proper environmental cues, *Valeria lophostriata* apparently was able to excyst through biologically guided medial split, as indicated by the abundance of well-defined half vesicles (Fig. 6C–E; fig. 2f of Javaux et al., 2004). In this and following sections, we examine the biomechanics of *Valeria* vesicles and argue that the seeds of biologically programmed medial split were planted when the striations were formed in preparation for encystment.

On the basis of detailed morphological characterization of *Valeria* (Jankauskas, 1979; Hofmann, 1999; Javaux et al., 2004), we analyzed the stress on *Valeria* vesicle walls using the “thin-walled spherical pressure vessel theory”. Our analysis was based on the morphology of *V. lophostriata*, which is characterized by concentric striations. *V. elongata* Nagovitsin, 2009 is morphologically different because of its “meridional” striations, but the same principle can also be applied to that species as well. We assume that *Valeria* is a single-walled spherical vesicle, and its vesicle wall is internally ornamented with closely spaced concentric ridges surrounding two polar foci. As is shown below, the results would be the same if the concentric ridges are present on the exterior surface of the vesicle wall.

To illustrate our calculation, we first cut two sections of the vesicle, one along the equator between two ridges and the other along the meridian through the two polar foci. These two sections are shown in Fig. 5D, E where the internal diameter (d), net internal pressure (p , turgor pressure), vesicle wall thickness (t), ridge height (Δt), ridge width (w_1), and ridge spacing (w_2) are marked.

On the equatorial section (Fig. 5D), the pressure force (P) due to turgor pressure p is

$$P = \text{pressure} \times \text{area} = p \times \pi(d/2)^2 \quad (1)$$

The resisting area over which the tensile stress σ_1 (i.e., the circumferential stress of Sharma et al., 2009) acts on the vesicle wall is πdt . At equilibrium, the pressure force must be balanced by the tensile stress (Fig. 6B). Hence,

$$p \times \pi(d/2)^2 = \sigma_1 \times \pi dt \quad (2)$$

Thus, the tensile stress σ_1 on the vesicle wall along the equator where a medial split would be produced is

$$\sigma_1 = pd/(4t) \quad (3)$$

Similarly, consider a non-equatorial section between two concentric ridges; on a spherical vesicle, such a section generates a small circle with a diameter of d' (rather than a great circle with a diameter of d) and cuts the vesicle wall non-orthogonally so that the apparent wall thickness t' is greater than the true wall thickness t . The tensile stress σ_1' on the vesicle wall along this non-equatorial section is

$$\sigma_1' = pd'/(4t') \quad (3')$$

where d' is diameter of the small circle (i.e., $d' < d$) and t' is the non-orthogonal thickness of the vesicle wall (i.e., $t' > t$). Hence, $\sigma_1' < \sigma_1$. In other words, the stress acting along a non-equatorial plane is less than that along an equatorial plane.

Now consider an equatorial section cutting through a ridge, which essentially adds to the resisting area. The tensile stress σ_2 would be

$$\sigma_2 = pd/(4t + 4\Delta t) \quad (4)$$

Similarly, for a non-equatorial section cutting through a ridge, the tensile stress σ_2' would be

$$\sigma_2' \approx pd'/(4t' + 4\Delta t') \quad (4')$$

where d' is diameter of the small circle (i.e., $d' < d$), and t' and $\Delta t'$ are, respectively, non-orthogonal wall thickness and ridge height (i.e., $t' > t$; the relationship between $\Delta t'$ and Δt is not straightforward, but the difference is negligible relative to the significant change in d'). Hence, $\sigma_2' < \sigma_2$.

Next, consider the tensile stress σ_3 acting on the vesicle wall along the meridional section (Fig. 5E). To simplify calculation, we assume that the cross-sectional area of a raised ridge equals to the area of a triangle, i.e., $w_1\Delta t/2$. The number of ridges in a meridional cross section is $\pi d/(w_1 + w_2)$. Thus the area of

resistance is $\pi d[t + w_1 \Delta t / (2w_1 + 2w_2)]$. At equilibrium, the forces are balanced:

$$p \times \pi(d/2)^2 = \sigma_3 \times \pi d[t + w_1 \Delta t / (2w_1 + 2w_2)] \quad (5)$$

Thus, the tensile stress σ_3 is

$$\sigma_3 = pd / [4t + 2w_1 \Delta t / (w_1 + w_2)] \quad (6)$$

$$\sigma_1 : \sigma_2 : \sigma_3 = 1/t : 1/(t + \Delta t) : 1/[t + w_1 \Delta t / (2w_1 + 2w_2)] \quad (7)$$

Therefore, the tensile stresses σ_1 , σ_2 and σ_3 for *Valeria* can be arranged in descending order:

$$\sigma_1 > \sigma_3 > \sigma_2 \quad (8)$$

Applying the average values of t , Δt , w_1 and w_2 based on the observation of five ridges (*V. lophostriata* specimen in Fig. 5A), which are 81 nm, 80 nm, 1003 nm, and 628 nm, respectively, $\sigma_1 : \sigma_3 : \sigma_2$ is estimated to be 1 : 0.77 : 0.5 for this individual specimen.

Similarly, in a random section through the center of the spherical vesicle, the balance of forces leads to equation (9) that is similar to equations (3), (4), and (6).

$$\sigma = pd'' / (4t_{\text{avg}}) \quad (9)$$

where d'' is the internal diameter and t_{avg} is the average thickness of the vesicle wall along this section ($0 < d'' \leq d$; $t \leq t_{\text{avg}}$).

Thus, σ_1 is the maximal value of σ . In other words, the equatorial section between two ridges at the equator represents the biomechanically most vulnerable plane along which rupture could occur (Fig. 6A, B). Another prediction from the biomechanical analysis is that, everything else being equal, larger cells can withstand lower turgor pressures and, conversely, higher turgor pressures require thicker cell walls; observations consistent with this prediction have been documented in extant algae (Gutknecht et al., 1978; Bisson and Kirst, 1995). For taxa with similar turgor pressures and vesicle walls of similar mechanical properties, their vesicle wall thickness should be linearly scaled with vesicle diameter. In fact, the vesicle diameter is positively scaled with wall thickness in the microfossil *Dictyotidium coarctatum*, which is interpreted as prasinophycean phycomata (Colbath, 1983). However, a positive correlation between vesicle diameter and wall thickness is not a universal feature among acritarch fossils (Arouri et al., 1999; Talyzina and Moczyłowska,

2000; Javaux et al., 2004; Moczydłowska and Willman, 2009; Willman, 2009; Pang et al., 2013), possibly owing to variations in turgor pressures (Gutknecht et al., 1978), chemical compositions of biopolymers, and/or mechanical properties of vesicle walls.

6. The applicability of biomechanical analysis

An essential precondition for our biomechanical model is that the net internal pressure (p) should be greater than zero, i.e., a turgor pressure must exist to impose a tensile stress on the vesicle wall. Most marine algae are capable of maintaining hypertonicity inside their cells with respect to ambient seawater, and the osmotic potential inside the cells is usually 4–20 bar lower than the external environment (Raven et al., 1980; Yao, 1987; Lobban and Harrison, 1996). The net result is that water flows along an osmotic potential gradient into the cell, and the protoplast volume expands until it is restricted by the cell wall (Gutknecht et al., 1978; Lobban and Harrison, 1996). A turgor pressure then arises from these processes, and it is maintained almost constant over a wide range of external osmotic pressure because of the adjustment of the internal osmolarity (Hellebusi, 1976; Bisson and Kirst, 1995). It is likely that similar osmoregulation processes are applicable to *Valeria* vesicles and other ancient eukaryotic unicellular fossils. We note that protoplasts may biologically contract and consolidate in some algal cysts (Pang et al., 2013), but cytosol could fill the gap between protoplast and cell wall, and a turgor pressure may still exist to affect the biomechanics of *Valeria* vesicles, particularly during excystment.

Another prerequisite for the application of the “thin-walled spherical pressure vessel” model in the biomechanical analysis of *Valeria* is that its vesicle wall thickness must be $< 1/20$ of its vesicle diameter. The maximum wall thickness of *V. lophostriata* is less than 300 nm as determined in this and previous studies (Javaux et al., 2004), and the reported minimum vesicle dimension of *V. lophostriata* is 16 μm (Hofmann, 1999). Thus, the ratio of wall thickness t to vesicle diameter d is smaller than $1/20$. Finally, the application of the “thin-walled spherical pressure vessel model” requires that the vesicle wall is homogenous and continuous without any openings (Gere and Goodno, 2009; Sharma et al., 2009). TEM analyses of the *Valeria* vesicle

walls do show that they are homogenous (Fig. 5A, B; Javaux et al., 2004), and it is likely that *Valeria* vesicle walls do not have large openings to be permeable to proteins and other macromolecules.

The biomechanical analysis presented above is also applicable to spherical vesicles with external sculptures, because vesicle sculptures, whether on the internal or external surface, add to the effective thickness of the vesicle. Thus, if it turns out that the concentric striations were present on the external surface of *Valeria* vesicles, the analytical results would still be valid. Additionally, the biomechanical analysis can also be applied to *V. elongata*. Following the same calculations as outlined above, it is easy to arrive at the conclusion that the largest tensile stress in *V. elongata* is along the meridians between meridional ridges.

7. The function of *Valeria* striations

Processes in resting stages of phytoplankton could be functionally important in facilitating flotation, passive defense, information exchange, dispersal, or resistance to burial (Belmonte et al., 1997). However, these functions do not appear to be applicable to the striations in *Valeria*. Nor would such striations, particularly if they are present on the inner surface of *Valeria* vesicles, significantly change the exterior surface area of the vesicle.

The biomechanical analysis provides a hint of the function of *Valeria* striations. Our analysis shows that the striations increase the overall mechanical strength of the vesicle (equation 9). But perhaps more importantly, the most vulnerable areas to mechanical ruptures are along the equator between ridges for *V. lophostriata*, and along the meridians between ridges for *V. elongata*. Thus, we propose that the striations were formed through a reaction-diffusion process shortly before encystment, and they were used to guide biologically programmed excystment through medial split. Medial split has been reported as a putative excystment mechanism in *V. lophostriata* (Javaux et al., 2003, 2004). Enrolled half vesicles of *V. lophostriata*, with centrally located foci and co-marginal striations, are common in the Ruyang Group material (Figs. 3A, C, 6C–E), and these may also have resulted from medial split. No

excystment structures have been reported for *V. elongata* because of the limited number of available specimens (Nagovitsin, 2009). However, it is interesting to note that some longitudinally striated half vesicles (very similar to *V. elongata*) from the Paleoproterozoic Chuanlinggou Formation of North China appear to show meridional split (Peng et al., 2009), as predicted by the biomechanical analysis presented above. Thus, we conclude that the function of *Valeria* striations was to guide medial split during excystment upon favorable environmental cues, which may result in a change in the turgor pressure and a rupture of the vesicle along the most vulnerable plane.

It is important to note that Precambrian leiospheres without concentric or meridional striations can be split medially. The biological vs. taphonomic origins of medially split leiospheres have been much discussed in the literature (Tappan, 1980). In leiosphere populations with abundant and consistently medially split vesicles, a biological origin of such medial split has to be considered, because taphonomic rupture tends to produce irregular breakage with jagged margin or radially oriented incomplete splits (Tappan, 1980; Strother, 1996). For biologically controlled medial split to occur in leiospheres, there must be some biophysical or biochemical mechanisms (e.g., mechanically weak zones or biochemical dissolution of vesicle walls) other than concentric or meridional striations to guide such medial split. Thus, it is also important to point out that our analysis does not mean that concentric or meridional striations are the only way to guide medial split; instead, we argue that they are just one possible mechanism. And we hope that our study will stimulate more study to investigate other possible mechanisms for medial split or excystment in general.

8. Caveats and future research directions

As detailed in previous sections (particularly section 6 “The applicability of biomechanical analysis”), the biomechanical model is built upon certain assumptions and possible caveats must be stressed in order for the current analysis to be improved in the future. First and foremost, to the best of our knowledge, no modern analogs are known to have vesicle wall striations similar to those of *Valeria*. Thus, although we

and others (e.g., Hofmann, 1999) speculate that reaction-diffusion processes may have played a role in the formation of striations in *Valeria*, the exact biomolecular mechanism for striation morphogenesis remains elusive. Second, although the half vesicles of *Valeria* likely resulted from biologically programmed medial split during excystment, the life history of *Valeria* remains incompletely understood, and more evidence is needed to confirm that these half vesicles are indeed excystment products. As such, although our analysis raises the biophysical plausibility that *Valeria* striations may have facilitated medial split during excystment (regardless whether the striations are on the inner or outer vesicle surface), this interpretation must remain speculative at the present. Finally, the biochemistry of *Valeria* vesicle walls is unknown, and the possibility of an unusual vesicle wall with biophysical properties completely different from modern eukaryote cell walls could not be excluded, and such an unusual vesicle wall would compromise our biomechanical analysis.

In light of these caveats, we propose that, in order to improve our morphogenesis and biomechanical analysis, future work should more thoroughly explore possible modern analogs and should focus on biomechanical experiments. The discovery of modern analogs with striated vesicle walls will significantly help us to understand the morphogenesis and functional morphology of *Valeria* striations. Short of a modern analog, one can carry out laboratory experiments that subject modern resting cysts with different biochemistries and different ultrastructures to physical compression, in order to determine how physically produced vesicle breakage is different from or similar to biologically produced excystment structures. Additionally, it is possible to study the effect of environmental cues on excystment, for example by adjusting osmolarity (hence net internal pressure, p) in controlled experiments. Such experiments will give us a biophysical understanding of *Valeria* excystment even if no modern analogs are found. Finally, we hope our study will stimulate more ultrastructural investigation of other Proterozoic acritarchs (particularly leiospheres), which will eventually form a solid foundation for biomechanical analysis of ancient eukaryotes.

9. Conclusions

This study provides additional data about the diversity of organic-walled microfossils in the Paleo-Mesoproterozoic Ruyang Group, including *Plicatidium latum*, *Spiromorpha* sp., and an unnamed form. The new material clarifies the morphological details and allows a biomechanical analysis of *Valeria*, one of the earliest known eukaryotic fossils. It is shown that the vesicle of *V. lophostriata* is characterized by concentric (not spiral) striations surrounding two polar foci that are oppositely located on the spheroidal vesicle. It is proposed that, in addition to eukaryotic cytoskeletons and endomembranes, reaction-diffusion (R-D) processes may have played a key role in the formation of concentric striations in *V. lophostriata*. Our biomechanical analysis suggests that, while the striations in *Valeria* enhance the overall mechanical strength of the vesicle, they may have also functioned to guide excystment through medial split. With detailed biomechanical analyses built upon reliable ultrastructural characterizations of *Valeria* and other Paleo-Mesoproterozoic eukaryotes, we can begin to develop a deeper understanding of the morphogenesis and functional morphology of these earliest eukaryotic organisms, moving the field beyond simple tabulation of taxonomic and morphological diversity.

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Figure captions

Fig. 1. Geological map, fossil locality, and stratigraphic column. (A) Map showing Proterozoic stratigraphic provinces in the northern part of East Qinling Ranges, with stars marking the sampled Shuiyongou section, as well as the Yangpo and Laoleshan sections referred in the text; rectangle marks area enlarged in (B); I: Zhongtiaoshan-Leshan area; II: Songshan-Jishan area; III: Xiaoshan-Xiaoqinling area; IV: Luanchuan area. (B) Geological map of the Yongji area in southern Shanxi Province, with star marking the location of the sampled Shuiyongou section; gray scales indicate different stratigraphic units and match those in (C). (C) Stratigraphic column of the Shuiyongou section, with sample numbers (11-TJY-BDJ-01 to 11-TJY-BDJ-30; 11-TJY-BCP-01 to 11-TJY-BCP-06) and sampled horizons (black dots) marked against a detailed stratigraphic column of the Beidajian and upper Baicaoping formations. The 1611 ± 8 Ma age is from the Luoyukou Formation at the Yangpo section (Su et al., 2012), although the dated zircons may be detrital in origin (Lan et al., 2014). The Sanjiaotang Formation at the Laoleshan section is constrained between 1937.8 ± 11.7 Ma and 1411 ± 27 Ma, based on analysis of detrital zircons and diagenetic xenotime overgrowth (Lan et al., 2014). The detrital zircon age 1744 ± 22 Ma from the base of Ruyang Group provides the maximum depositional age for the Ruyang Group (Hu et al., 2014). LYK: Luoyukou Formation; SJT: Sanjiaotang Formation; CZ: Cuizhuang Formation. Modified from Guan et al. (1988) and Pang et al. (2013).

Fig. 2. TLM micrographs of organic-walled microfossils from the Beidajian Formation. (A, B) *Plicatidium latum*; (A) specimen 11-TJY-BDJ-23(4)-6, tubular sheath with closely spaced transverse plications; (B) specimen 11-TJY-BDJ-1(2)-1. (C) *Spiromorpha* sp., specimen 11-TJY-BDJ-9(1)-7, ovoidal or spindle-shaped vesicle with transverse stripes (arrow). (D) Schematic diagram comparing *S. segmentata* (left, redrawn from Yin et al., 2005) with *Spiromorpha* sp. (right). (E, F) Unnamed tubular form, specimen 11-TJY-BDJ-9(2)-21, showing compressed tube wall (E) with

randomly distributed lacunae (arrow in F, magnified view of rectangle in E) and submicron-sized granules (F).

Fig. 3. TLM (A–H) and SEM (I) micrographs of *Valeria lophostriata* from the Beidajian Formation. (A–D) Morphotype A, showing half vesicles with co-marginal striations and centrally located foci; (B) and (D) are magnified views of rectangles in (A) and (C), specimens 11-TJY-BDJ-5(2)-1 and 11-TJY-BDJ-6(1)-1, respectively; note that the striations are concentric and narrower than 0.5 μm in thickness. (E–I) Fragmentary specimens of morphotype B; (F) and (H, I) are magnified views of rectangles in (E) and (G), specimens 11-TJY-BDJ-13(1)-19 and 11-TJY-BDJ-6-SEM-7-3-12, respectively.

Fig. 4. Biometric analysis of *V. lophostriata*. (A) Cross-plot of striation width versus “wave-length” λ , showing two morphotypes with distinct and non-overlapping ranges of striation width and “wave length” (λ = striation width + spacing). (B) Cross-plot of the number of the striations versus vesicle diameter for morphotype A ($n = 16$ complete and half vesicles); correlation coefficient $r = 0.91$, $p \ll 0.01$ for $H_0: r = 0$; the number of the striations is estimated by vesicle diameter/ λ . (C) Cross-plot of striation width versus vesicle diameter of morphotype A; correlation coefficient $r = 0.067$, $p = 0.81$ for $H_0: r = 0$. (D) Cross-plot of striation spacing versus vesicle diameter of morphotype A; correlation coefficient $r = -0.079$, $p = 0.77$ for $H_0: r = 0$.

Fig. 5. TEM micrographs, reaction-diffusion (R-D) model, and biomechanical analysis of *V. lophostriata*. (A, B) TEM micrographs of *Valeria lophostriata*, showing vesicle wall with raised ridges (arrows in A) corresponding to cross sections of concentric striations, and the gradual transition from electron dense vesicle wall towards electron tenuous ridge (B, magnified view, with measurements marked). (C) Schematic diagram showing how an axial oscillatory reaction-diffusion (R-D) pattern can result in concentric striations on a spherical vesicle; oscillatory R-D reaction forms nested cylinders which intersect with the vesicle wall to form the concentric

striations surrounding two polar foci. (D, E) Biomechanical analysis (modified from Sharma et al., 2009), showing an equatorial cross-section (D) and a meridional cross-section (E); p = net internal pressure (turgor pressure), d = internal diameter, t = vesicle wall thickness, Δt = ridge or striation height, w_1 = striation width, w_2 = spacing between adjacent striations.

Fig. 6. Diagram showing distribution of tensile stress (A), medial split (B) and enrolled half vesicles of *Valeria lophostriata* (C–E) from the Beidajian Formation. (A) Schematic diagram showing the relative tensile stress acting on the vesicle along different latitudinal planes. This is a simplistic model based on equations (3, 3', 4, and 4') with the approximation of $t' \approx t$, $\Delta t' \approx \Delta t$, $t = \Delta t$, $w_1 = w_2$, and ridges at the latitude of 6° , 18° , 30° , ..., 78° , 90° on both hemispheres. Solid lines mark the position of ridges or striations, whereas dotted lines mark biomechanically weak zones between ridges. The relative stress is scaled so that the maximum stress at the equator between two ridges is set at 100%. (B) Vesicle is split into two hemispheres along the equator due to turgor pressure (modified from Sharma et al., 2009). σ_1 = tensile stress acting in the equatorial plane between ridges, p = net internal pressure (turgor pressure), t = vesicle wall thickness, d = internal diameter. (C–E) Enrolled half vesicles with foci located near the center, specimens 11-TJY-BDJ-5(3)-5, 11-TJY-BDJ-7(3)-7, and 11-TJY-BDJ-16(1)-18, respectively.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6