

Reassessment of the Putative Ciliate Fossils *Eotintinnopsis*, *Wujiangella*, and *Yonyangella* from the Neoproterozoic Doushantuo Formation in China

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Summary. Three putative ciliate fossils were described from the Neoproterozoic Doushantuo Formation in China: *Eotintinnopsis*, *Wujiangella*, and *Yonyangella*. The identity of these fossils is important for our understanding of the origins and early morphological evolution within ciliate clades. Here we compare the homology of the fossil characteristics with those in their proposed ciliate relatives. *Eotintinnopsis* resembles a tintinnid, but its feathery tentacle-like apical structure is probably not homologous within any known ciliate. *Wujiangella* presents homology issues with the size and distribution of its putative somatic cilia. *Yonyangella* appears to be a suctorian with its tentacle-like structures, but the presence and size of its putative somatic cilia pose homology issues. We suggest that these three fossils are likely to be taphonomically and diagenetically distorted and altered acritarchs. These alterations include secondary mineral encrustations on the interiors of vesicles, the crushing, folding and other distortions of the vesicles, the bending and crushing of the acritarch spines, and the preservation of organic material in and outside of the cysts. The earliest known ciliate fossil remains a tintinnid that occurs in the Ordovician of Kazakhstan.

Key words: Ciliophora, Doushantuo Formation, fossils, homology, taphonomy, diagenesis.

INTRODUCTION

Ciliate (Ciliophora Doflein, 1903) fossils are rare and difficult to interpret. Only under exceptional conditions are these primarily unicellular and soft-bodied creatures preserved. Until recently, ciliate fossils have been limited to the Phanerozoic eon. Most are remnants of hard loricas from the Tintinnida Kofoid & Campbell, 1929, dating back to the Ordovician (Tappan and

Loeblich 1968). Remains of actual soft ciliate cells are known from Cretaceous amber (Schmidt *et al.* 2001, Schönborn *et al.* 1999, Martín-González *et al.* 2008, Ascaso *et al.* 2005, Poinar *et al.* 1993). Thus only a limited understanding of early ciliate evolution is available in the fossil record.

Recently, Li *et al.* (2007) studied the 580 MYA Wengan Phosphate Member of the Doushantuo Formation in China for microbial fossils. Three new microbial fossils were interpreted to be ciliates and assigned to extant clades (Li *et al.* 2007; Fig. 1). These three specimens were selected from tens of thousands of microstructures observed in thousands of thin-sections of the Wengan Phosphate Member. Three-dimensional models were

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constructed from images taken at many different focal planes of the thin-sectioned specimens. No other material was described, hence the variability within the species is undocumented as is the abundance of these particular fossils in the Wengan.

The validity of these fossils is important for the resolution of a number of questions with ciliates. For example, they could significantly affect any new attempts at dating the origins and expansion of various ciliate clades if used as calibration points in molecular clock estimates. They also could affect our understanding of early morphological evolution within the two clades to which the fossils are purported to belong. First we discuss the problems in interpreting fossils such as those in the Wengan Phosphate Member. Then we reevaluate the three fossils by comparing the potential homology between their characters with those in their proposed extant relatives, and propose a reinterpretation that these purported “ciliates” are diagenetically and taphonomically distorted and altered acritarchs. Our observations and conclusions are based only on the descriptions and specimen photos found in Li *et al.* (2007).

THE VAGARIES OF TAPHONOMY AND DIAGENESIS

The Wengan Phosphate Member of the Doushantou formation contains abundant organic and inorganic microstructures. Algae, acritarchs, putative metazoan embryos, putative bilaterians, and the putative ciliates discussed here have been described from among many tens of thousands of these microstructures (Chen *et al.* 2004, Li *et al.* 2007, Zhang *et al.* 1998; among many others). Not all of these fossils are what they may seem to be. Most Wengan microfossils have been subjected to both taphonomic (changes to the organisms themselves) and diagenetic (changes to the sediment or rock) alterations prior to, during, and after deposition. The processes of phosphatization are complex (Briggs 2003, Briggs and Kear 1993, Dornbos 2010, Kremer 2005, Martill 1988), but include particular depositional conditions, sufficient phosphate ions in the sediment and the mobility of the phosphate during sedimentation and soon after deposition.

Phosphate deposits such as those in the Doushantou formation form in areas of slow sedimentation and high rates of organic production. These conditions com-

monly coincide where upwelling waters, bringing nutrients into the photic zone where they enhance primary production, occur over shallow areas such as shelves, nearshore banks, offshore seamounts, and deeper parts of dysoxic basin slopes and floors. In such areas, the organic matter grown in the overlying waters undergoes bacterial decay on the sea floor, releasing phosphate in abundance. The phosphate dissolved in the pore and overlying waters preserves by permineralization and encrustation of hard- and soft-parts (complete or degraded) if it occurs quickly (Briggs 2003, Briggs and Kear 1993, Kremer 2005, Martill 1988). These processes occur in fossils of all sizes, including encrusting the internal and external surfaces of acritarchs (Kremer 2005).

Microfossils in the Wengan represent whole, broken or partially decayed specimens, some of which began fossilization on the sea floor or before the sediment was dewatered (Li *et al.* 2007). The most common fossils in the Wengan are acritarchs (Zhang *et al.* 1998, Xiao and Knoll 1999), which for the most part are generally spherical cysts, with various processes extending from them, of chiefly planktonic or benthonic algae. Usually these fossils consist of an outer spherical or deformed layer with a shriveled internal mass that may have connections to the outer layer, commonly with internal and external encrustations (Bengtson and Budd 2004, Xiao and Knoll 1999). Fossil embryos from the Wengan show a more or less continuous taphonomic decay ranging from well preserved to partially or completely collapsed or degraded specimens (Xiao and Knoll 1999, Dornbos *et al.* 2005). The interior voids of the microfossils commonly are encrusted with one or more layers of apatite (Bengtson and Budd 2004) creating the impression of a firm skeleton or of cell layers. Acritarchs also may be deformed (folded, twisted, bent), decayed, crushed, and encrusted on the interior, as occurs in even younger phosphates (Kremer 2005). Other diagenetic features include dewatering structures, pressure-induced extrusions and degraded spines. These taphonomic and diagenetic processes, mediated by bacteria, began as deposition was occurring and continued during resuspension and redeposition of the fossils into the pelletal dolostone of the Wengan member. These processes variously changed some of the fossils into a variety of microstructures that have been interpreted as the remarkable preservation of intricate and complex soft-bodied organisms; e.g., bilaterians (Chen *et al.* 2004, but see Bengtson and Budd, 2004).

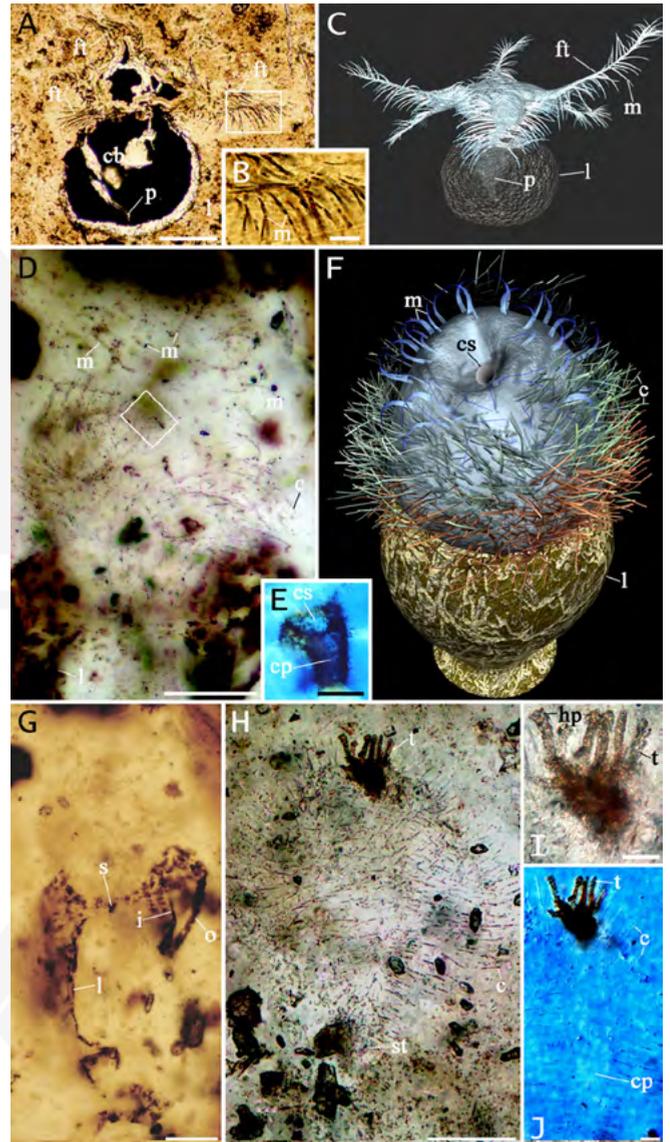
Putative bilaterian fossils from the Wengan Phosphate Member are therefore open to serious ques-

tion because of taphonomic and diagenetic effects. For example, putative bilaterian remains have been reinterpreted to be spherical structures with layers of secondary apatite lining the internal surfaces thus resembling cell layers (Bengtson and Budd 2004). The three purported ciliate fossils by Li *et al.* (2007) can be reinterpreted similarly. Most likely all of these fossils originally were spiny acritarchs that were decayed, deformed, permineralized, and encrusted (see Zhang *et al.* 1998 for descriptions and illustrations of Doushantou acritarchs, and Kremer 2005 for the diagenetic and taphonomic processes that affect acritarchs during phosphatization). The acritarch, putative bilaterian and ciliate fossils are also all within the same general size ranges (100–200 μm).

EOTINTINNOPSIS

Eotintinnopsis pinniforma Li *et al.*, 2007 is described as a tintinnid based on a single specimen. This fossil contains a putative organic cyst and a posterior peduncle (Fig. 1A–C), which would imply a tintinnid relationship (Li *et al.* 2007), if correct.

Just below the apex of the *Eotintinnopsis* specimen are six “strongly developed arms with feathery tentacle-like membranelles, 20 μm long and 1.5 μm wide” (Fig. 1A–C) that are interpreted to be similar to membranelles of extant tintinnids (Li *et al.* 2007). Membranelles are densely and serially arranged cilia that are fused or partially coalesced (Lynn 2008), and they may be up to 1.5 μm wide. In contrast to the fossil, oral ciliature of extant tintinnids consists mostly of a zone of membranelles that surround the perimeter of the oral region at the cell apex and are borne directly on the cell surface – not on arm-like projections (Agatha and Riedel-Lorjé 2006). We therefore do not view the feather-like arms of *Eotintinnopsis* to be homologous with the oral structures of tintinnids. Some extant ciliates have arm-like projections (e.g., *Dileptus* Dujardin, 1841, and *Teuthophrys* Chatton & de Beauchamp, 1923), but in these taxa both membranelles and loricas are absent, suggesting that they are likewise not homologous with those in *Eotintinnopsis*. At the apex of *Eotintinnopsis*, above the feathery arms, is a multi-tiered pointed structure that appears to be the same color and density as the cell body (Fig. 1A). Li *et al.*'s (2007) three-dimensional interpretation of the fossil does not include this pointed



Figs 1A–J. Putative ciliate fossils from the Neoproterozoic Doushantou Formation, China illustrated by Li *et al.* 2007 in their Fig. 1 (A–F here) and Fig. 2 (G–J here). **A** – *Eotintinnopsis pinniforma*, longitudinal section; scale bar: 50 μm . **B** – Close up of feather-like membranelles on *E. pinniforma*; scale bar: 10 μm . **C** – Reconstruction of *E. pinniforma*. **D** – *Wujiangella beidoushanese*, longitudinal section; scale bar: 50 μm . **E** – Close up of cytostome and cytopharynx of *W. beidoushanese*; scale bar: 10 μm . **F** – Reconstruction of *W. beidoushanese*. **G** – Longitudinal section of *W. beidoushanese*; scale bar: 50 μm . **H** – *Yonyangella ovalis*, longitudinal section; scale bar: 50 μm . **I** – Close up of tentacles of *Y. ovalis*; scale bar: 10 μm . **J** – Longitudinal section of *Y. ovalis*; scale bar: 50 μm . c= somatic cilia, cb= cell body, cp= cytopharynx, cs= cytostome, ft= feather-like membranelles, hp=haptocysts, l= lorica, m= membranelles, o= outer layer, p= peduncle, st= stalk, t= tentacles. © Geological Society.

structure (Fig. 1C). If this structure belongs to the fossilized organism, then that would further point to it not belonging with the tintinnids, as tintinnid cells do not extend beyond their apical membranelles.

Beyond the locria-like structure, we see no evidence that the fossil *Eotintinnopsis* is a ciliate. The subapical feather-like structure and the apical multi-tiered pointed structure are currently unknown in any ciliate. We therefore suggest that *Eotintinnopsis* be removed from the Ciliophora. We suggest that this fossil represents taphonomically and diagenetically altered remains of some other fossil. The internal masses and extensions (the putative peduncle) to the vesicle are common in acritarchs from the Wengan (see Zhang *et al.* 1998, Figs 5–5, 6, 10, Figs 7–3 to 11). At the top of the sphere of *Eotintinnopsis* is an irregular tripartite structure, called a “protruding partial cell body”, lined by a double layer of diagenetic mineralization (Fig. 1A). This kind of encrustation occurs on the interiors of acritarchs and not on cytoplasmic materials (Kremer 2005). The lower sphere and upper structures appear to be two separate spheres that have undergone two different taphonomic and diagenetic histories as evidenced by the encrustations and shapes of the spheres. The upper structure may have been crushed, folded, or otherwise degraded, and moved adjacent to the other sphere, perhaps as part of the degradation process or of compaction in the sediment. This upper structure is covered with short thin processes with purported tentacles and attached membranelles extending away from it. Some of these membranelles appear to be bifurcated and most have dark granular material within them. The tentacles themselves are flattened and bilamellar. On the whole, the upper part of *Eotintinnopsis* is best interpreted as a crushed and folded spiny acritarch, with the membranelles representing the thin spines of an acritarch and the tentacles representing part of the crushed and flattened cyst of an acritarch. Acritarchs known from the Wengan member that possess many thin and sometimes bifurcating spines are *Ericiasphaera magna* (Zhang Z.) Zhang *et al.* 1998 and *Ericiasphaera rigida* Zhang *et al.*, 1998, and both have spherical vesicles. *Ericiasphaera rigida* is common in the Wengan.

WUJIANGELLA

Wujiangella beidoushanese Li *et al.*, 2007 is also described as a tintinnid based on a single specimen. This fossil contains a putative organic cyst, oral ciliature,

cytostome, and cytopharynx (Fig. 1D–G), which seems consistent with a tintinnid relationship (Li *et al.* 2007).

Putative somatic cilia about 0.5 μm wide are found over the entire cell surface that extends beyond the lorica of *Wujiangella* (Fig. 1D, F). In contrast, cilia in extant ciliates are 0.25 μm wide. This discrepancy in width could be explained by the peculiarities of the fossilization process. The putative cilia in *Wujiangella* also appear to be randomly placed on the cell surface. In contrast, somatic ciliature in extant tintinnids is usually highly ordered into kineties (Agatha and Riedel-Lorjé 2006). A kinety is an integrated row of somatic kinetostomes (cilia basal bodies) and associated fibers (Lynn 2008). This discrepancy in the arrangement of cilia could also be explained by the fossilization process, and one can make the case that they are homologous with those found in extant tintinnids.

Given the putative lorica, cytostome, and cytopharynx-like structures, the fossil of *Wujiangella* as interpreted by Li *et al.* (2007) is probably the best candidate of the fossils discussed here to be related to extant ciliates. However, at this time the discrepancies of the ciliature between the fossil and extant ciliates suggests that fossil *Wujiangella* should be removed from the Ciliophora. We suggest that this fossil represents a poorly preserved and degraded spiny acritarch in which the spines radiate in different directions over the “cell body”. *Wujiangella* could also be a taphonomically and diagenetically degraded acritarch, perhaps a *Ericiasphaera* or *Meghystichosphaeridium* Zhang *et al.*, 1998, but its features are not preserved well enough to determine which acritarch it may be.

YONYANGELLA

Yonyangella ovalis Li *et al.*, 2007 was also described from a single specimen as a Suctorina Claparède & Lachmann, 1858. This fossil contains a putative apical cytostome and “at least 10 tentacle-like structures, each about 15 μm long and 3 μm wide” that have apical swellings (Fig. 1H–J), which is consistent with a suctorian relationship (Li *et al.* 2007). The tentacles and apical swellings in modern suctorians in no way resemble the shorter and somewhat stubby “tentacles” of the fossil, as they are longer, thinner and the swellings are at their apex.

The cell surface of *Yonyangella* is covered with putative cilia that are 0.5 μm wide (Fig. 1H–J) (but see above

about the problem of the width of these structures). In contrast, extant suctorians generally do not have cilia during the feeding stage that includes the tentacle-like projections (Lynn 2008, Matthes 1988, Dovgal 2002). The only suctorian group currently known to have cilia in the feeding stage is *Cyathodinium* de Cunha, 1914; these however are endocommensals of guinea pigs and retention of these cilia may be due to derived neoteny (Dovgal 2002). The putative cilia in *Yonyangella* might questionably be homologous with those in extant suctorians, and the retention of cilia in the feeding stage of *Yonyangella* merely represents an early period in suctorian evolution in which cilia are found in all life stages. Li *et al.* (2007) interpret a clear area in the fossil of *Yonyangella* to be a cytopharynx extending from a cytostome located at the center of the tentacle-like projections (Fig. 1J). In contrast, no oral structures (e.g., cytostomes, cytopharynxes) occur in the feeding stage of extant suctorians. Rather than using their tentacles to capture prey and direct it to a central mouth like cnidarian metazoans, suctorians suck out their prey's cytoplasm through the tentacles themselves (Dovgal 2002, Hull 1961).

Yonyangella is by far the strangest of the fossils with its tentacle-like structures in combination with putative cilia. The tentacles could possibly be homologous with suctorians, but cilia are not seen in the feeding stage of extant suctorians (except in derived taxa). Furthermore, the cytopharynx in *Yonyangella* is not homologous with any structure in the suctorians. We therefore suggest that the fossil *Yonyangella* be removed from the Ciliophora. We suggest that the tentacle-like structures in this fossil is a taphonomic anomaly caused by the extrusion of material from within an acritarch vesicle as part of the disintegration process or by external pressure squeezing it out through the slit-like aperture of the acritarch early in diagenesis. The purported "cytostome" in this fossil is lighter colored material inside the specimen running from below the "tentacles" into the center of the "cell". Other similarly colored areas also exist in the cell body in other places where they do not extend up to the tentacular area (Fig. 1J). Lighter colored regions in phosphatized fossils are common as the phosphate is usually pale (Kremer 2005). Nothing in this fossil unequivocally supports the interpretation that it is a ciliate. Instead, *Yonyangella* most closely resembles the acritarch *E. rigida* in its overall shape and nature of the thin, straight processes but slightly deformed early in diagenesis. Other features, such as the colors inside the

fossil, can be attributed to taphonomic and diagenetic processes as well.

CONCLUSIONS

Ciliates have a meager fossil record. Loricas from tintinnids have been found in rocks throughout the Phanerozoic, while soft-bodied cells have been limited to Cretaceous or younger deposits (Lynn 2008, Tappan and Loeblich 1968). Li *et al.*'s (2007) findings have the potential to push the ciliate fossil record back into the Proterozoic, which can affect future molecular clock estimates and our understanding of early morphological evolution. However, the morphological, taphonomic and diagenetic evidence that the fossils *Eotintinnopsis*, *Wujiangella*, and *Yonyangella* are closely related to extant ciliate groups is unsupported. Because of the numerous homology problems and alternative interpretations of their identity based on morphology and on taphonomic and diagenetic evidence, the three fossils should be removed from the Ciliophora altogether. Instead, the fossils *Eotintinnopsis*, *Wujiangella*, and *Yonyangella* are interpreted here as degraded and deformed acritarchs with numerous thin and straight spines. Indeed, the purported "ciliates" most closely resemble acritarch species in *Eri-ciasphaera* or perhaps *Meghystichosphaeridium*, both with known specimens from the Wengan Phosphate Member of the Doushantuo Formation.

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