Microfossils of eukaryotic cysts through time – a study of Precambrian-Ordovician organic-walled microbiota

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Abstracts

The first manuscript


Light microscope and scanning electron microscope observations on the new material of unicellular microfossils Dictyosphaera macroreticulata and Shuiyousphaeridium macroreticulatum, from the Mesoproterozoic Ruyang Group in China, provide insights into the microorganisms’ biological affinity, life cycle and cellular complexity.

Gigantosphaeridium fibratum n. gen et sp., is described and is one of the largest Mesoproterozoic microfossils recorded. Phenotypic characters of vesicle ornamentation and excystment structures, properties of resistance and cell-wall structure in Dictyosphaera and Shuiyousphaeridium are all diagnostic of microalgal cysts. The wide size ranges of the various morphotypes indicate growth phases compatible with the development of reproductive cysts. Conspecific biologically, each morphotype represents an asexual (resting cyst) or sexual (zygotic cyst) stage in the life cycle, respectively. We reconstruct this hypothetical life cycle and infer that the organism demonstrates a reproductive strategy of alternation of heteromorphic generations. Similarly in Gigantosphaeridium, a metabolically expensive vesicle with processes suggests its protective role as a zygotic cyst. In combination with all these characters and from the resemblance to extant green algae, we propose the placement of these ancient microorganisms in the stem group of Chloroplastida (Viridiplantae). A cell wall composed of primary and secondary layers in Dictyosphaera and Shuiyousphaeridium required a high cellular complexity for their synthesis and the presence of an endomembrane system and the Golgi apparatus. The plastid was also present, accepting the organism was photosynthetic. The biota reveals a high degree of morphological and cell structural complexity, and provides an insight into ongoing eukaryotic evolution and the development of complex life cycles with sexual reproduction by 1200 Ma.
The second manuscript

Agić, H. & Moczydlowska, M. Reproductive cyst and operculum formation in the Cambrian-Ordovician algal microfossils and their seasonal blooms.

Unicellular organic-walled microfossils from the early Cambrian Lükati Formation and the Tremadocian Varangu Formation exposed in Estonia have been interpreted as reproductive cysts of the green algal phytoplankton. Both microfossil assemblages reflect the evolutionary history though the early Paleozoic: from the Cambrian radiation of morphologically innovative taxa to increasing in diversity and more disparate Ordovician forms. Combined light transmitted and scanning electron microscopy on the Tremadocian galeate plexus acritarchs *Caldariola, Priscogalea* and *Stelliferidium*, revealed exceptionally preserved morphological elements and rare structure among fossil and extant microbiota – an opening with an operculum (lid) in the reproductive cyst, in addition to lavish vesicle ornamentation and sculpture. Analogous morphology is observed in the extant alga *Dasycladales* (Chlorophyta), where it is determined by an intrinsic lid-forming apparatus during the organism’s reproductive stage. Unique morphology of operculum-bearing microbiota would have required a degree of intracellular complexity for its development, suggesting advanced intracellular machinery present already in the early Palaeozoic phytoplankton. A new Genus and Species A of minute, sphaeromorphic and aggregated microfossils, which possess a perforated by nano-scale pores vesicle wall and corrugated sculpture, is discovered. These early Cambrian microfossils have diagnostic characters of prasinophyte algae.
INTRODUCTION

Precambrian life is represented by minute organic-walled microfossils of prokaryotes and diversifying eukaryotes and their biosedimentary structures, i.e. microbial mats. As the eon encompassing most of Earth’s history, Proterozoic is a time of great biological innovations, major evolutionary divergences and environmental changes that have formed the atmosphere and hydrosphere. Records from this time are crucial to understanding of the early evolution of life and furthermore, the origin of eukaryotes and their radiation into known kingdoms. Precambrian microfossils are preserved mainly in cherts and shales, and represent a relatively abundant and globally distributed marine, hydrothermal and possibly lacustrine aquatic microbiota. While a significant progress has been achieved in understanding the earliest records of life (Schopf, 1993; Brasier et al., 2005), later, Mesoproterozoic organisms appeared to be vital in developing aerobic respiration. After the Great Oxygenation Event (Holland, 2006) and the endosymbiotic events leading to the origin of Eukarya (Margulis, 1970; McFadden, 2001; Keeling et al., 2005; Brasier, 2012), the two biggest steps in the evolution of biosphere were the origins of sexual reproduction and multicellularity (Butterfield, 2000; Knoll, 2011). Evidence for these innovations is preserved in records of protists from Siberia, Canada, China and Australia (Hermann, 1990; Arouri et al., 1999; Butterfield, 2000; Javaux et al., 2001; Knoll et al., 2006; Moczydłowska et al., 2011) and investigation of their features, allows inferences on their development and life cycle, intracellular complexity, as well as phylogeny.

Organic-walled microfossils are not only important for the study of Precambrian life, but are a key part of the early developing Paleozoic ecosystems. While significantly diversifying in morphology and size during Cambrian and Ordovician radiations, their diagnostic characters remained, reflecting their universal function as eukaryotic cysts.

Exact biological affinities of organic-walled microfossils have long been a matter of discussion. Evitt (1963) introduced and named a polyphyletic group Acritarcha, from ancient Greek words *achritos* and *arche* for “uncertain” and “origin”, as an umbrella term for early Paleozoic, marine fossil palynomorphs. The term has become counterfactual recently as most of the previous *incertae sedis* were allocated to specific groupings, mainly eukaryotic phytoplankton (Tappan, 1980; Colbath & Grenfell, 1995; Arouri et al., 2000; Tałyzina and Moczydłowska, 2000; Wicander, 2002; Moczydłowska and Willman, 2009; Moczydłowska et al., 2011; Zhang and Pratt, 2014). Additionally, individual case studies have identified
*Tianshushania* as a metazoan embryo (Xiao et al., 1998; Yin et al., 2007), some Ediacaran taxa as potential diapause egg cysts (Cohen et al., 2009) or holozoan protists (Huldtgren et al., 2011), and heterotrophic protists (Butterfield, 2005).

Further affinities of certain microfossils to modern classes have been revealed by the comparative morphology, wall ultrastructure and biochemical properties. Ultrastructural studies by Talyzina and Moczydlowska (2000) recognized shared characters between a sphaeromorph unicellular microfossil *Leiosphaeridia* and extant green algae in the order Chlo coccales. This key synapomorphy is the trilaminar sheath structure (TLS) in a distinct multi-layered cyst wall. Spheroidal organic-walled microfossils are phenotypically very similar, but SEM and TEM investigations of their ultrastructure or specific cyst wall features, may elucidate their phylogeny more accurately. While there is no TSL structure present in the Mesoproterozoic taxa *Dictyosphaera* and *Shuiyousphaeridium* studied here, their specific secondary wall might represent its precursor.

Diagnostic morphological elements in both Proterozoic and Paleozoic microfossils, such as excystment openings and opercula, also indicate their phylogenetic relationship to algae and are investigated here. Furthermore, the mechanisms behind their function and intercellular complexity governing the early unicellular organisms are of a major part of this thesis.

**Organic-walled microfossils as eukaryotic cysts**

The fossil record is strongly biased for shallow-marine organisms with skeletal elements (Allison and Briggs, 1993), which have long played a major role in palaeontological research. However, organically-preserved fossils and remnants of non-mineralising organisms are dominant in the Precambrian, such as microbial mats forming stromatolites, carbonaceous films and large Ediacaran impressions, alongside unicellular and colonial microfossils of prokaryotic and eukaryotic affinities (Mendelson and Schopf, 1992).

Organic-walled microfossils are mostly preserved in cherts and shales, and can be studied microscopically by preparation of thin sections or via extraction from the rock by acid maceration. Complex biopolymers comprising the organic matter allow its preservation in the fossil record and resistance to acetolysis (Horodyski et al. 1992). Modern analogues that utilise such biopolymers in cytogenesis are encysting eukaryotes, specifically green algae that produce protective cysts during reproduction. While bacteria may be preserved in secreted
sheaths or permineralised in cherts, and their traces fossilised as microbial mats, the only extractable individual organic fossils are eukaryotes.

Origin of Eukarya dates back to Palaeoproterozoic, with the exact first appearance datum (FAD) ages still uncertain between 2.5 and 2 Ga (Runnegar, 1994; Hedges et al., 2004, Yoon et al., 2004). Complex organic fossils of this time period possess recognisable eukaryotic features such as large cell size, wall ornamentation as processes or bulbous structures, and surface patterning (Porter, 2004; Knoll et al., 2006). Additionally, independent evidence of eukaryotic presence in Mesoproterozoic is provided by biomarkers–molecules produced by organism’s metabolism and refractory molecules resulting from their diagenesis, preserved in organic-rich sedimentary rocks (Summons et al., 2008; Halmann et al., 2011). Molecular signatures are derived from membrane components. Characteristic eukaryotic biomarkers are steranes, while bacteria are usually recognised by the presence of hopanes.

In addition to the broad classification among Eukaryota, Proterozoic organic-walled fossils exhibit diagnostic features that were used to narrow down their phylogenetic position further, and compare them to modern clades. There are several lines of evidence identifying Precambrian and Paleozoic acritarchs as green algae (Moczydłowska et al., 2011). Clues for biological affinity come from their biochemistry and functional morphology. Main diagnostic characters that are defining unicellular organic-walled microfossils as reproductive cysts are excystment openings, processes, wall structure and the acetylisis-resistant organic vesicle (Tappan, 1980; Colbath & Grenfell, 1995; Wicander, 2002; Moczydłowska, 2010).

Excystment opening structures are formed in the vesicle wall during the excystment process, presumably when daughter-cells are being released from the matured reproductive cyst as seen in extant algae. It is the most direct evidence of the fossil’s reproductive function. Variety of opening structures is found throughout Proterozoic and Phanerozoic organic-walled microfossils, in rare cases bearing additional features like a neck or an operculum. Partial or median-split openings and round openings (pylomes) occur in acritarchs of all ages, but the later are more common in Phanerozoic.

Processes in unicellular microfossils show comparable complexity and morphological patterns as those extant phytoplanktonic cysts. They are formed during the contraction of the cyst from the vegetative cell wall (e.g. Dale, 2001), and used in buoyancy control and sensory activity. Upon discarding the remnants of the vegetative wall / outer membrane, the mature, process-bearing (acanthomorphic) cyst will settle to the substrate and eventually release the offspring through the excystment opening. Ornamented microfossils in particular bear strong
similarities with encysted stages (zygotes) of extant unicellular algae in orders Chlorococcales, Volvocales and Zygnematales (Moczydłowska, 2010).

Organic-walled microfossils are extracted using a harsh method of maceration in hydrofluoric acid (HF) that dissolves the surrounding rock within a few days. Fossils themselves are unaffected by HF. This property of acetolysis-resistance is a result of complex, sporopollenin-like biopolymers building the vesicle wall (Horodyski et al. 1992; De Leeuw et al., 2006; Javaux and Marshall, 2006), synthesised primarily by autotrophic eukaryotes (Knoll et al., 2006; Moczydłowska et al., 2011). Presence of such sturdy walls during reproductive stages requires significant metabolic investment by the microorganism, which suggests wall’s function as protection of daughter-cells, that is, in propagation of genetic material. Tough walls are also considered to be ecological indicators due to their presence in the benthic sphaeromorph assemblages (Mendelson and Schopf, 1992), which probably represent sunken resting cysts.

Separated by almost billion years, *Dictyosphaera-Shuiyousphaeridium* plexus and the morphotypes of the *Caldariola-Priscogalea-Stelliferidium* galeate plexus studied here, exhibit these diagnostic, eukaryotic reproductive features and represent cysts of extinct microbiota.

**Dictyosphaera-Shuiyousphaeridium plexus**

In Paper 1, the focus of study is on the reproductive function and life cycle reconstruction as well as the biological affinity of *Dictyosphaera* and *Shuiyousphaeridium* from the Mesoproterozoic Ruyang Group in northern China. Upon assessing diagnostic characters of the *Dictyosphaera-Shuiyousphaeridium* plexus microfossils and studying their complex vesicle wall, a hypothetical life-cycle model was proposed. Both microfossils share a unique, complex cell wall composed of a protective, reticulated outer layer forming polygonal pits inside the vesicle. Pits are filled by polygonal platelets, comprising the inner vesicle wall for additional strengthening of the cyst. *Dictyosphaera* and *Shuiyousphaeridium* are differentiated only by absence or presence of processes, respectively. Such similarity suggests conspecificity of the two fossils, as distinct cyst stages (asexual and zygotic respectively) in the life cycle of a single species. Sexual heteromorphism and reproduction via the alternation of generations is common throughout Eukaryota and often reflects environmental conditions. The inferred life cycle reconstructed by comparison to living organisms (*Golenkinia radiata*, *Micrasterias thomasiiana* and *Staurastrum furgicerum*), consists of two stages (asexual and sexual), represented by each fossil morphotype (Fig. 1). Reproduction begins as the haploid
gametes fuse into a zygote that will form a protective cyst-wall, a process exhibited by the modern algae. The zygotic cyst will start to shrink and produce an acanthomorphic vesicle, preserved as the *Shuiyousphaeridium* fossil. Following meiosis, offspring cells will be released through the excystment opening. Upon reaching maturity, they can fuse into another zygote, or may reproduce asexually. In the latter case, as exemplified by some extant chlorophytes (*Chlamydomonas*), the mature vegetative cell will begin synthesising a protective wall. This stage is represented by the *Dictyosphaera* fossil. A sturdy aplanospore protects the organism while undergoing mitotic divisions and it eventually releases offspring cells through the excystment structure. A new genus and species *Gigantosphaeridium fibratum* uncovered from the same Ruyang Group assemblage, one of the largest unicellular organisms in Mesoproterozoic, also possesses a resilient vesicle with cyst-like, reproductive features.

Phylogenetic position of the *Dictyosphaera-Shuiyousphaeridium* organism is also discussed here. Both microfossils have been assigned to phytoplankton (Yin, 1997), undefined green algae (Kaufman and Xiao, 2003), photosynthetic eukaryotes (Yin et al., 2005), dinoflagellates (Meng et al., 2005), or broadly identified as protists without distinguishing between auto- or heterotrophic mode of life (Javaux et al., 2004; Knoll et al, 2006). Alternatively, *Shuiyousphaeridium* was suggested to be a possible fungus and a heterotrophic, benthic, multicellular organism (Butterfield, 2005). Recently, the form-genus *Shuiyousphaeridium* has been interpreted as a chlorophyte (Moczydłowska et al., 2011) based on phenotypic similarity of the vesicle morphology, ornamentation, wall ultrastructure and presence of excystment structures, to zygotic cysts known in various extant classes of microalgae. While broad similarities do exist between the *Dictyosphaera-Shuiyousphaeridium* organism and dinoflagellates and other protists, the sum of evidence and comparative morphology (Table 1, PAPER 1) identifies it as a primitive green alga with shared characters of Chloroplastida (Viridiplantae) and Chromalveolata (see Baldauf, 2008 for a model of eukaryote phylogeny). Considering the sequences of endosymbiotic events and a later evolution of chromalveolates (Yoon et al., 2004), *Dictyosphaera-Shuiyousphaeridium* was likely an autotrophic, planktonic protist in the early lineage of the Chloroplastida.
Figure 1. Life cycle model for *Dictyosphaera-Shuiyousphaeridium*. A, Zygote, start of the sexual reproduction. B, *Shuiyousphaeridium* morphotype, cyst shrinkage produces processes. Outer membrane is discarded, meiosis occurs. C, Haploid offspring is released through the excystment structure. It may fuse into another zygote (G) or form a resting cyst (D). E, Start of asexual reproduction and resting cyst formation, mitosis occurs in *Dictyosphaera* morphotype. F, Offspring is released through the excystment opening. G, Flagellated haploid offspring fuses into a zygote.

**Galeate plexus**

In Paper 2, the research on two microfossil assemblages from Estonia, from the early Cambrian Lükati Formation and the Ordovician (Tremadocian) Varangu Formation, is presented. The early Paleozoic evolution and diversification of phytoplankton is observed in both time intervals. Conventional taxonomy of the early Cambrian microbiota recognises variety of small sphaeromorphs, sphaeroidal taxa with pores, discoidal taxa with internal body, acanthomorphs with short thorn-like, long solid or branching hollow processes, as well as those bearing sculpture and distinct polygonal pattern on the cell wall (Volkova et al., 1983; Moczydlowska, 1991). These microfossils are thought to represent prasinophyte phycomata and chlorophyte cysts. Unlike large Proterozoic microbiota, the early Cambrian acritarchs tend to be small with less prominent ornamentation or sculpture, yet they exhibit a
great diversity during the Cambrian explosion, numbering c. 250 species (Moczydłowska, 2011). The trend of small size and relatively simple process changes around the Cambrian-Ordovician boundary and the Great Ordovician Diversification event when diversity and disparity of phytoplankton increases substantially. This time marks the first appearance of the vesicles with polar symmetry (Actinodissus), composite processes (Vulcanisphaera), polygonal flanges between the processes (Cymatiogalea) and remarkable excystment structure—opening with rim covered by opercula in galeates. The Ordovician radiation represents the second largest diversification of photosynthesising microbiota in Phanerozoic, after the Cambrian one, and almost reaching the present-day levels (Servais et al., 2008). While the modern phytoplankton (dinoflagellates, diatoms and coccolithophores) diversified in Mesozoic, quite late in the evolutionary history and parallel to the Modern evolutionary fauna (Sepkoski, 1992), the Ordovician radiation was important for the increasing diversification of the Paleozoic biota. The early Paleozoic phytoplankton is represented by acritarchs with innovative complex ornamentation, excystment structure with operculum, and polar symmetry of the vesicles.

Some of the most prominent members of the Cambro-Ordovician microbiota are the microfossils of the galeate plexus (Servais and Eiserhardt, 1995), comprising genera Caldariola, Priscogalea, Stelliferidium and Cymatiogalea. Galeates are characterised by a rare element among organic-walled microbiota—a reproductive opening (excystment) with a lid structure (operculum). Different stages of the operculum formation have been observed in all galeate morphotypes: from an encysted vesicle with a faint fault line developing on the wall, to vesicle with a fully formed operculum still attached to the excystment, and eventually to an opened vesicle with the operculum completely detached or collapsed inside the empty vesicle, presumably upon offspring release by the matured cyst (Fig. 2). Similar morphology is observed in the extant marine green alga Acetabularia mediterranea (Dasycladales, Chlorophyta). While Acetabularia is a derived unicellular alga with complex morphology and a very large cell, its reproductive cysts are very small, smooth, thick-walled and possess a lid-structure also seen in galeate microfossils. Morphologically, Acetabularia cysts are identical to the process-lacking morphotype of Caldariola. Culture studies on Acetabularia have shown that the development of the operculum during cyst formation is regulated by the lid-forming apparatus (LFA). LFA is an array of microtubules adjacent to the cell membrane, that will start forming circular band on the cyst wall that deepens into a fault line. This grove-like band will eventually separate the operculum from the cyst and allow a more controlled
reproductive opening and release of the offspring cells through the protective cyst wall (Neuhaus-Url and Schweiger, 1984).

Macroscopic dasycladalean algae have been documented in contemporaneous strata in Estonia and Canada (Nitecki et al., 2007; Young et al., 2007). Galeate plexus fossils consist only of lid-forming cysts and the morphology of the adult organism is not known. However, considering similarities in morphology and cyst formation to dasycladalean *Acetabularia*, and the Ordovician record of Dasycladales, galeate microfossils could belong to this order of chlorophytes.

Additionally in Paper 2, the potential conspecificity of the galeate plexus and algal diversifications during the Cambro-Ordovician transition is discussed. Genera *Caldariola*, *Priscogalea*, *Stelliferidium* and *Cymatiogalea* number a total of 84 species, appearing in the Upper Cambrian and diversifying through the Ordovician. While the main diagnostic character in galeates—the opening with an operculum, is identical in *Caldariola*, *Priscogalea*, and *Stelliferidium*, other features such as process length and shape are variable in a small degree. Morphometric analyses identified four morphological clusters, reflecting previously recognized four form-genera, with transitional forms between each, leading Stricanne and Servais (2002) to propose conspecificity for the plexus. Such variability of different cyst morphotypes of the same species is seen in Mesozoic and present-day dinoflagellates, whereby processes length, shape and distribution on the vesicle surface are governed by environmental factors such as temperature, salinity and nutrient availability, as well as the cyst development (Kokinos and Anderson, 1995; Servais et al., 2004; Reyes and Head, 2013). Rupture of the outer membrane before the full maturity of the cyst or low salinity conditions, may result in smaller processes.

Alternatively, the galeate-plexus microfossils may show the adaptive radiations and diversification of photosynthetic microorganisms, triggered by changing environmental conditions in the aftermath of the end-Cambrian glaciation, global warming, sea-level rise, and major tectonic rearrangement of palaeocontinents (Torsvik and Reinström, 2001; Barnes, 2004). The galeate microfossils are not the only novelty among innovative taxa of this time interval, when the taxonomic diversity reached more than 300 taxa and included complex morphotypes such as *Multiplicisphaeridium*, *Peteinosphaeridium*, *Vulcanisphaera* and *Saharidia*, and are a part of the Great Ordovician Diversification Event in general.

The Varangu assemblage represents a preservational window in the Tremadocian, while the galeate taxa persisted through the interval of about 30 million years. Therefore, the variability in fossil cysts can also represent a speciation event. While the number of galeate
species has been inflated and their taxonomy needs revision, a true speciation could have been under way, as a result of environmental adaptation.

Figure 2. Different stages of operculum formation in galeate plexus acritarchs. Scale bar represents 20 μm. A, *Priscogalea distincta* with formed operculum attached to the cyst. B, *Priscogalea simplex* with partially open operculum. C, *Priscogalea barbara* vesicle with excystment opening and dislocated operculum partially collapsed into the empty cyst.

**Vesicle morphology as a result of intracellular complexity**

Mechanism of cyst formation is a major theme in both papers. As the encysted stage of the microorganism’s life cycle, acritarchs often exhibit complex morphology. Apart from diagnostic processes or excystment structures, Proterozoic and Paleozoic microfossils possess a composite wall structure and additional cyst elements, such as necks, opercula or plugs. These features are present in modern phytoplankton and their formation has been studied in cultures. However, many of these cyst elements are considered derived and require a certain degree of developed intracellular machinery for their formation.

*Dictyosphaera-Shuiyousphaeridium* organism’s cyst is composed of the two walls: primary wall layer with pattern of polygonal reticulation, and the secondary wall layer formed by individual, ca. 2μm wide, polygonal platelets, additionally reinforcing the cyst. Similar wall construction during reproduction is known in the chlorophyte genera *Nephroselmis* and *Scherffelia*, where the additional wall elements are formed and secreted through the Golgi apparatus (GA) and the endoplasmatic reticulum (ER). By comparison, the organic platelets building the secondary wall in *Dictyosphaera-Shuiyousphaeridium* may be interpreted as the controlled secretion in cyst formation, governed by GA and ER. These organelles are also
crucial for the cell wall formation in Prasinophyceae and Chrysophyceae (Melkonian et al., 1991). This indirect evidence for GA and ER might have provided the time constraint on their origin in the eukaryotic cell, in the case of the studied Ruyang biota, by at least 1.2 Ga. Assuming the organism was photosynthetic (Kaufman and Xiao, 2003), a plastid may also be added on the list of present cell components.

Golgi apparatus is considered one of the least understood organelles and research into it has been marked by controversy (Hua and Graham, 2009). However its three main functions are clear: 1) serving as cell’s centre of the secretory pathway, protein sorting and trafficking station; 2) processing the waste material from the cytoplasm; and 3) cell wall construction. The last may be seen as a secondary function, that is, a means of dealing with and putting the excreted material to use. The timing of the GA invention in eukaryotic evolution is also unclear. Mowbrey and Dacks (2009) studied molecular and genomic data for the GA throughout Eukaryota, inferring that the stacked GA had already been present in the last eukaryotic common ancestor (LECA) and was subsequently lost or became unstacked eight times during cellular evolution. Dyer and Obar (1994) also argue for its presence in a “proto-eukaryote” before endosymbiotic events, while others propose its evolution along with the ER after the endosymbiosis of the archaeon and the bacterium (summarized by O’Malley, 2010). Nevertheless, considering this antiquity of the GA and its function of wall secretion in eukaryotic cells, this organelle may be expected as a cellular control for vesicle construction and ornamentation in the Precambrian microbiota. The oldest observable intracellular structures in microfossils have been reported in Ediacaran embryos (Hultgren et al., 2011; Schiffbauer et al., 2012). Although indirectly, the wall structure of the Mesoproterozoic unicellular biota studied indicates a complexity that requires an advanced level of cellular organization.

Another example of complex morphology depending upon certain cellular components, is found in the Cambrian-Ordovician galeate plexus microfossils, specifically Caldariola, Priscogalea and Stelliferidium. These taxa possess very rare character among unicellular organic-walled microfossils—an operculum (Fig. 2). Lid-like structure attached to the excystement structure is rare in the early Paleozoic and so far unknown from the Precambrian, with only a few other operculum-bearing taxa in Ordovician-Silurian, Cretaceous and Oligocene (Tappan, 1980). However, operculum is a defining feature of the galeate-plexus acritrachs (Servais et al., 2004), where it is preserved in most details and different stages of operculum formation. Such morphology is present in the extant chlorophyte genus Acetabularia, as described above. Operculum formation in Acetabularia is
governed by the “lid-forming apparatus” (LFA) (Neuhaus-Url and Schweiger, 1984). LFA is a structure on the cell periphery, close to the plasma membrane containing a layer of microtubules that will start forming a band in the membrane during the cyst maturation. The band will broaden into a fault-line and eventually separate the operculum (lid) from the rest of the vesicle. The pattern of cyst and lid-formation known in Acetabularia is inferred similarly in the galeate plexus microfossils. Some specimens contain a fault structure in the wall, some an attached operculum, while others have either completely lost their operculum or it has collapsed into the empty organic vesicle.

While intracellular structures of the galeate microfossils are impossible to observe like in modern Acetabularia culture studies, external features of the cyst may also indirectly reveal significant information about past intracellular processes and present organelles.

**FUTURE OBJECTIVES**

*Shuiyousphaeridium* and *Dictyosphaera* have revealed a lot of information about microorganism’s Palaeobiology and affinity, as well as the biological complexity in the Mesoproterozoic. Studies on much younger, Cambrian-Ordovician phytoplankton, have shown homologous key characters connected to organism’s reproductive function, and further demonstrated how external morphology of fossil cysts reflects internal mechanisms for its development. However, *Dictyosphaera* and *Shuiyousphaeridium* are not the only Precambrian organisms with complex morphology. Mesoproterozoic successions in Siberia, Canada, China and Australia have preserved organisms with unique vesicle sculpture and ornamentation. Among these taxa is *Valeria*, a protist of undefined affinities (Javaux et al., 2004) or a potential prasinophyte (Moczydłowska et al., 2011) which possesses a unique fingerprint-like vesicle sculpture consisting of concentric circles. This sculpture would have also required a certain degree of complexity for its synthesis, making *Valeria* a taxon of interest for studies on Mesoproterozoic palaeobiology and intracellular evolution. Future work on the Ruyang and Roper groups will focus on microfossils with complex ornamentation and sculpture to further elucidate their formation, and organisms’ biological affinity.

Additional work on the early Cambrian Lükati Formation is in progress. The new species “A” described in Paper 2 requires a more detailed taxonomical description and further SEM studies. Its wall structure of flexible vesicle filled with nano-scale pores seems to suggest relationship to prasinophytes. Several Proterozoic and early Paleozoic taxa are in need
of re-examination in terms of taxonomy and biological affinities, and are available in the current collections at the Palaeobiology Programme. New material from Proterozoic successions is under examination. Microfossil studies included in this Thesis and material planned to be examined, provide the evidence for clarifying phylogeny of the photosynthesising microbiota through different geological periods and for timing of major divergences leading to the rise extant clades.

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**LITERATURE**


AFFINITY, LIFE CYCLE AND INTRACELLULAR COMPLEXITY OF ORGANIC-WALLED MICROFOSSILS FROM THE MESOPROTEROZOIC OF SHANXI, CHINA

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ABSTRACT– Light microscope and scanning electron microscope observations on new material of unicellular microfossils Dictyosphaera macroreticulata and Shuiyousphaeridium macroreticulatum, from the Mesoproterozoic Ruyang Group in China, provide insights into the microorganisms’ biological affinity, life cycle and cellular complexity. Gigantosphaeridium fibratum n. gen et sp., is described and is one of the largest Mesoproterozoic microfossils recorded. Phenotypic characters of vesicle ornamentation and excystment structures, properties of resistance and cell-wall structure in Dictyosphaera and Shuiyousphaeridium are all diagnostic of microalgal cysts. The wide size ranges of the various morphotypes indicate growth phases compatible with the development of reproductive cysts. Conspecific biologically, each morphotype represents an asexual (resting cyst) or sexual (zygotic cyst) stage in the life cycle, respectively. We reconstruct this hypothetical life cycle and infer that the organism demonstrates a reproductive strategy of alternation of heteromorphic generations. Similarly in Gigantosphaeridium, a metabolically expensive vesicle with processes suggests its protective role as a zygotic cyst. In combination with all these characters and from the resemblance to extant green algae, we propose the placement of these ancient microorganisms in the stem group of Chloroplastida (Viridiplantae). A cell wall composed of primary and secondary layers in Dictyosphaera and Shuiyousphaeridium required a high cellular complexity for their synthesis and the presence of an endomembrane system and the Golgi apparatus. The plastid was also present, accepting the organism was photosynthetic. The biota reveals a high degree of morphological and cell structural complexity, and provides an insight into ongoing eukaryotic evolution and the development of complex life cycles with sexual reproduction by 1200 Ma.

INTRODUCTION

MOST OF the Earth’s geological past is represented by the record of minute organic fossils. Precambrian sediments preserved a relative abundance of globally distributed marine and some hydrothermal and lacustrine aquatic microorganisms that are fundamental for an understanding of the early evolution of life and especially the origin and diversification of eukaryotes (Mendelson et al., 1992; Javaux et al., 2004; Grey, 2005; Knoll et al., 2006; Porter, 2006; Moczydłowska, 2008; Butterfield, 2009; Lamb et al., 2009; Moczydłowska et al., 2011; Strother et al., 2011). An initial step in tracing this process is the identification of phenotypic characters, metabolic processes, and modes of life in order to recognize the affinity of microfossils and their phylogenetic relationships to extant biota. The appearance of new morphotypes and innovative features in the context of the geological time may unravel major divergences in lineages and their timing in the eukaryotic evolution. Traces of eukaryotes may extend as far back as at least 2 Ga (Knoll et al., 2006; Barton et al., 2007; Hackett et al., 2007; Bhattacharya et al., 2009), or even earlier at 2.5 Ga (Runnegar, 1994). Our study of the c. 1.2 Ga organic-walled microfossils Dictyosphaera and Shuiyousphaeridium from the Mesoproterozoic Ruyang Group of Shanxi Province in northern China, which are generally regarded as protists (Knoll et al., 2006), provides further evidence of their photosynthetic microalgal affinity. Gigantosphaeridium fibratum n.gen. et sp. is described and interpreted as belonging to the same group of microorganisms. New light
microscope (LM) and scanning electron microscope (SEM) observations on *Dictyosphaera* and *Shuiyousphaeridium* are conclusive for interpreting the organism’s conspecificity and biological affinities, and for reconstructing a potential reproductive life cycle. We have additionally inferred the presence of key eukaryotic organelles in these microbiota. Our increased understanding of the phylogenetic relationships of the fossils studied, allows us to delimit the time of origin of the primary “green” group of photosynthesizing eukaryotes (Archaeoplastida) prior to 1.2 Ga.

Previous studies of Mesoproterozoic biota established their relative diversity (Xing and Liu, 1973; Schopf, 1992; Yin, 1997; Xiao et al., 1997; Javaux et al., 2004; Huntley et al., 2006) and subsequently focused on their specific chemical properties (Kaufmann and Xiao, 2003; Marshal and Javaux, 2005; Meng et al., 2005; Javaux and Marshall, 2006). SEM and transmitted electron microscope (TEM) techniques (Javaux et al., 2004; Meng et al., 2005; Yin et al., 2005), in combination with ion and Raman spectroscopy analyses (Javaux and Marshall, 2006) illustrated the complexity of the vesicle wall and its biochemistry in *Dictyosphaera* and *Shuiyousphaeridium*. These are important features in distinguishing both a eukaryotic origin and their affinities. Several authors have interpreted these organisms as photosynthetic, unicellular plankton, at the base of green algae (Yin, 1997; Kaufmann and Xiao, 2003; Yin et al., 2005; Moczydlowska et al., 2011). We expand on this interpretation in the present paper through comparisons with extant eukaryotic and algal phenotypes.

Diversifications of green and red algal lineages, as well as heterotrophic protists occurred by a minimum age of 1.2 Ga, as indicated by the fossil record (Butterfield, 2000; 2004; Porter and Knoll, 2000; Porter, 2006). Single primary symbiosis and the origin of plastids that gave rise to the group alternatively called Plantae, Viridiplantae or Archaeplastida (Cavalier-Smith, 1981, 2003; Archibald and Keeling, 2002, 2004; Baldauf, 2008) is broadly estimated by molecular clocks to have evolved by 1.5 Ga (Yoon et al., 2004; Hackett et al., 2007). The timing of this event in the paleontological record could be prior to 1.8 Ga, if the green algal affinity of leiosphaerid microfossils described by Lamb et al. (2009) is accepted (Moczydlowska et al., 2011). However, the “cryptic” intracellular evolution through which a cell acquired a characteristic eukaryotic architecture might have occurred before c. 2.1 Ga (Runnegar, 1994). There are indications that the first eukaryotes and sexual reproduction might have evolved in the time interval c. 2.7–1.9 Ga (Barton et al., 2007).

The Mesoproterozoic Era (1.6–1.0 Ga) represents a crucial time for the rise of key innovations in cell complexity and genetic recombinations via endosymbiosis in single-celled organisms, as well as the origin of multicellularity. Often called the “boring billion” (Holland, 2006), this time interval may have been “dull” environmentally, but not cellularly. Brasier and Lindsay (1998) proposed a concept of eukaryotic evolution whereby a stable carbon cycle and low and stable nutrient levels allowed photosymbiotic relationships to stabilise over time, becoming an integral part of a eukaryotic cell. Furthermore, the increased complexity of cell walls observed in vesicles of organic fossils at this time (Javaux et al., 2004; herein) suggests intricate cellular machinery for cyst production and presence of the endomembrane system in addition to sexual reproduction.

Major characteristics of the studied Ruyang biota, such as the lavish processes, complex cell wall and excystment structures, share morphological similarities and biochemistry with the cyst stage of extant chlorophytes, charophytes, and dinoflagellates (Lacalli, 1981; Blackburn and Tyler, 1981; Raven et al., 2005; Moczydlowska and Willman, 2009; Moczydlowska et al., 2011; Guiry, 2013). As they represent elements of the reproductive cyst in modern taxa, sexual reproduction may also be assumed for Mesoproterozoic fossils. The argument in favour of sexual reproduction is further supported by the recognition of two morphotypes that form the *Dictyosphaera-Shuiyousphaeridium* plexus. These indicate a bimodal life cycle and reproduction via the heteromorphic alternation of generations from a sexual stage (represented by a zygotic cyst-like *Shuiyousphaeridium*) to an asexual stage (the resting cyst-like *Dictyosphaera*). The main function of a reproductive cyst as a protective vesicle for daughter cells explains the metabolically expensive, highly resistant, multi-layered cell walls that are present in Ruyang
biota. Present-day analogues secrete composite membranes and other wall elements via the Golgi apparatus and endoplasmatic reticulum. Such organelles presumably arose early in eukaryogenesis (Stanier, 1970; Margulis, 1981; Dacks and Field, 2004; Cavalier-Smith, 2006). The characteristic walls with reticulate sculpture and composite structure illustrated here suggest that key eukaryotic organelles were already present in the Mesoproterozoic.

GEOPOLICAL SETTING
Microfossils described here were extracted from the Mesoproterozoic sediments of the Ruyang Group exposed in the Shuiyou Section, Yongji district, Shanxi Province in northern China. Succession is well known both for its stromatolites and for the excellently preserved and readily extractable, diverse unicellular microfossils (Guan et al., 1988; Yin, 1997; Xiao et al., 1997; Kaufman and Xiao, 2003; Javaux et al., 2004; Yin et al., 2005; Meng et al., 2005; Yin and Yuan, 2007, Schiffbauer and Xiao, 2009). The Ruyang Group is un-metamorphosed and comprises a 380–429 m thick succession of siliciclastic and carbonate rocks, which (in ascending stratigraphic order) is divided into the Baicaoping and Beidajian formations. The Ruyang Group unconformably overlies the Archaean crystalline basement with the metamorphic age of c. 2350 Ma (Xiao et al., 1997) and the volcanoclastics of the Paleoproterozoic Xionger Group, dated to c. 1760 Ma (Zhao et al., 2002). The succession is overlain disconformably by several other Proterozoic formations and Neoproterozoic tillites and Cambrian strata (Guan et al., 1988; Yin, 1997; Yin and Yuan, 2005). Within the overlying succession, the Cuizhuang Formation has been dated at 1125±3 Ma, providing a minimum constraint for the age of the Ruyang Group fossils (Liu et al., 1999).

The Baicaoping Formation consists of purple quartzose sandstones intercalated with organic-rich shales, succeeded by stromatolitic dolomites. The Beidajian Formation contains alternating quartzose sandstones, shales, and mudstones in a minor lower portion, and then dolomites with stromatolites and cherts (Guan et al., 1988; Yin, 1997).

Sandstone bedding surfaces in the Baicaoping Formation display ripple marks and desiccation cracks, indicating deltaic and near-shore marine depositional environments. The Beidajian Formation accumulated on a tidally influenced, shallow carbonate shelf with stromatolitic flats (Yin, 1997; Xiao et al., 1997; Yin et al., 2005). Fossiliferous beds are found in the upper grey and black shales of the Baicaoping Formation and the lower grey shales of the Beidajian Formation. Microfossils described here were extracted from a thin shale bed in the lower Beidajian Formation (see Yin, 1997).

The age of the Ruyang Group is estimated to 1100–1300 Ma based on K-Ar dating of glauconite from the neighbouring Henan area (Guan et al., 1988; Yin, 1997; Yin and Yuan, 2007). Therefore the best estimate of the age of the microfossils is c. 1200 Ma (Xiao et al., 1997; Yin et al., 2005). The chemostratigraphic profile of the group and δ13C data are compatible with global values for Mesoproterozoic carbonates (Xiao et al., 1997)

MATERIALS AND METHODS
Microfossils were extracted from four samples (Shanxi 9, 11, 12, 13) of dark, thin-bedded shale from a stratigraphic level 60 m above the base of the Beidajian Formation in the Shuiyou Section of Yin (1997). Preparation followed standard palynological method (Vidal, 1988). Two samples were re-processed as a double-check of the record (Shanxi 9, Shanxi 12). Rock fragments weighing c. 100 g were split into large chips and immersed into hydrofluoric acid (40% HF) for several days. The resulting organic residue was separated into two batches, one of which was extracted after three and the other after six days, until the rock was completely dissolved. No difference in microfossil degradation was observed between the two batches. The residue was rinsed in water and decanted, then boiled in hydrochloric acid (30% HCl) for 10 min to remove fluorides, then filtered through 18 µm sieves. The residue was not oxidised. A Sartorius teflon membrane was used to filter the organic-rich residue. The residue was then rinsed in ethanol and acetone and centrifuged between rinsing for 3 min at 3000 rpm. Permanent strew mounts on microscope slides were produced using a synthetic resin EPOTEK 301. Slides were cured at 50°C for 1 hr. Rigorous laboratory
conditions and procedures prevented possible contamination.

Using high-concentration acids for extracting the microfossils revealed an important feature of their biochemical properties, namely that the complex biopolymers composing the vesicle wall make it remarkably resistant to HF and HCl treatment, which dissolves nearly all the enclosing minerals.

The microfossil collection and all illustrated specimens are stored in the collections of the Museum of Evolution at Uppsala University (under the prefix PMU). The position of the specimens in the microscopic slides is given using England Finder coordinates. The slides are orientated with their label to the left on the microscopic stage.

**SYSTEMATIC PALEONTOLOGY**

Group CHLOROPLASTIDA Adl et al., 2005

Phylum and class uncertain

Extinct Genera and Species

Genus **GIGANTOSPHAERIDIUM**

*Type species.*— *Gigantosphaeridium fibratum* n. gen. et sp., from China, Shanxi Province, Shuiyou Section, upper Mesoproterozoic Erathem, c. 1.2 Ga.

**Diagnosis.**— As for the type species.

**Etymology.**— From Latin *gigantes,-um* – giant, very large; *sphaera* – spheroidal in shape; referring to dimensions of the spheroid vesicle, which exceed the known size range of other organic-walled microfossils in the Mesoproterozoic.

**Remarks.**— The new, monospecific genus *Gigantosphaeridium* has three distinct features: 1) a robust, opaque vesicle of large diameter with a psilate wall surface; 2) abundant and tightly arranged, solid, thin fibrilar processes; 3) a thin spheroidal membrane enveloping both the vesicle and processes. The vesicle diameter is wide-ranging, but there is a continuous array of vesicle sizes and no distinct size classes are recognized. The membrane is not always completely preserved and fibrilar processes are free exposed in some parts of vesicles with a disrupted membrane, indicating that the processes were not welded into the membrane, and also that they were composed of more resilient material than the outer envelope.

There are no similar morphotypes known. The species distantly resembles the spheroidal vesicle covered densely by processes of the Ediacaran genus *Appendisphaera*. However *Appendisphaera* does not possess a membrane and its processes are hollow. The new genus and species has a smooth wall surface and in this it differs from *Shuiyousphaeridium pilatum* Li et al., 2012, which has a vesicle wall sculpture of a thin mesh diagnostic of the Dictyosphaera-Shuiyousphaeridium plexus.

Moreover, *S. pilatum* has branched processes intertwined at the tips, whereas *G. fibratum* n.gen et sp., only occasionally shows bifurcated process tips, which may form small loops. The diameters of the two species overlaps in size at their limits, but *G. fibratum* n.gen et sp. is considerably larger (ø 150-595 µm) by comparison with *S.pilatum* (ø 50-190 µm).

**GIGANTOSPHAERIDIUM FIBRATUM new species**

**Figure 1.1–1.5**

**Holotype.**— Specimen PMU-S13-1-12-(Z/38/3); illustrated in Fig. 1.4.

**Etymology.**— The species name derives from Latin *fibra,-ae* – fiber, filament; referring to the fibrilar or string-like shape of processes.

**Occurrence.**— Mudstone of the upper Baicaoping Formation, Ruyang Group, Northern China, Shanxi Province, Shuiyou Section.

**Material.**— Nine well-preserved and adequately preserved specimens, sampled level M 9206 (by Yin, 1997), upper Mesoproterozoic Erathem.

**Diagnosis.**— Organic-walled, acid-resistant microfossil consisting of a very large vesicle, circular to oval in outline (originally spheroidal). The vesicle is robust and has a firm wall with a psilate surface and bears abundant, solid, homomorphic fibrilar processes, evenly and densely distributed over the entire vesicle surface. The vesicle, together with the processes, is enveloped by a thin, translucent membrane. Processes are predominantly simple, straight or slightly bent, and occasionally bifurcated terminally and connected to other processes. Process terminations may form small loops through connected tips. Process tips and loops support the surrounding membrane.

**Dimensions.**— Vesicle diameter is 150-595 µm (holotype 595 µm); process
length 7-27 µm (holotype 14 µm; зов=10 µm). N=8.

**Preservation.—**

**Remarks.—** Considerable size variation among individuals (Fig. 1.) is indicative of growth phases. One specimen in the collection has much longer processes in a proportion to the vesicle diameter (Fig. 1.5) but the processes are of a similar morphology, being simple, string-like and resilient. They are free from any surrounding membrane. We include this specimen in the new species because only a single specimen with such remarkable process morphology is available. It is possible that it represents a separate species if the lack of outer membrane is a consistent morphologic feature and not a result of the state of preservation. Specimens are generally well preserved and the vesicle wall is very firm and almost opaque, varying in colour between dark-brown and black (TAI=4, value 2.5 on the Munsell pollen/spore colour standard). The colour reflects the thermal alteration to certain extent (taphonomy) but primarily results from the thickness of the cell wall and its robust structure. Some specimens display sharp fracture lines, which are taphonomically induced and confirm that the vesicle wall was rigid (Fig. 1.2 and 1.3). The degree of fracturing is generally lower than is observed in other Mesoproterozoic species, despite the large vesicle dimensions, a further indication of cell-wall strength and vesicle robustness. In instances when the membrane is broken, individual processes are seen to rise independently from the vesicle surface, and these also show a resilient structure (Figs. 1.4a, 1.4b, 1.5).

**Present record.—** Samples Shanxi.13-1, Z38-3, F44, G40, M40, M46-3, N42, O43-4, P40-3; Shanxi.13-3-O/49/2.

Genus DICTYOSPHAERA Xing and Liu, 1973

**Type species.—** Dictyosphaera macroreticulata Xing and Liu, 1973, from northern China, Yenliao region, Chih County of Hopei, Chuanlingkou Formation, lower Sinian system (Xing and Liu, 1973), now attributed to the upper Mesoproterozoic Erathem (Xia et al., 1997).

**Synonymy.—**


**Remarks.—** In the generic diagnosis (Xing and Liu, 1973, p. 22, 58), the vesicle diameter was given as “10 micrometres” but it should be “10 to tens of micrometres”, because recognized species range consistently in diameter from 10-45 µm. Both previously described species, D. macroreticulata and D. sinica, were described as having reticulate ornamentation on the vesicle surface and were distinguished by differences in the mesh diameter and the overall vesicle diameter. The given size differences are insignificant for identification and we consider the two morphotypes to be synonymous. D. macroreticulata is the senior synonym through priority in publishing. Several other subsequently described species of Dictyosphaera are treated as junior synonyms of D. macroreticulata in our taxonomic evaluation making the genus monospecific.

Similar patterns of surficial reticulation and overlap in vesicle size ranges of all previously described species of Dictyosphaera indicate that they are individual growth phases in ontogeny. We observe the same type of substantial size variability in Dictyosphaera (in the total record 10-274 µm, and 28-240 µm, herein), in a comparable way as in the other genus, i.e. Shuiyousphaeridium, which is considered to be conspecific with Dictyosphaera (Xiao et al 1997; Kaufman and Xiao, 2003; Butterfield, 2005).

In our opinion, the genera Dictyosphaera and Shuiyousphaeridium represent various developmental stages (generations) in the life cycle of a single biological species. Either the two form-taxa can be maintained as morphotypes of distinct life stages within a Dictyosphaera-Shuiyousphaeridium plexus or they can be attributed to a single species in which Dictyosphaera macroreticulata Xing and Liu, 1973 would have taxonomic priority. Following the first option, allows a better distinction of the morphology and clearer understanding of the various generations/life stages of extinct species. Such procedure is acceptable under the rules of
I.C.B.N., giving form-taxa names for parts of fossil plants, such as spores, pollen, sporophytes and gametophytes.

**DICTYOSPHAERA MACRORETICULATA**

Xing and Liu, 1973  
**Figure 2.1–2.9**  

**Holotype.–** Specimen inadequately illustrated for identification (Xing and Liu, 1973, pl. I:16) and not available for re-examination.

**Lectotype.–** The specimen originally assigned as the holotype of *Dictyosphaera sinica* Xing and Liu, 1973, and illustrated sufficiently well for identification by Xing and Liu (1973, pl. I:18) and which was derived from the same material as the holotype of *D. macroreticulata*, is here selected as the lectotype. The species *D. sinica* is considered here to be a junior synonym of *D. macroreticulata* Xing and Liu, 1973, and effectively, its holotype is chosen as the species lectotype because it has taxonomic priority.

**Synonymy.–**


1982 *Dictyosphaera delicata* Hu et Fu (sp. nov.) – Hu and Fu, p. 108, pl. II:3-6.  


1982 *Nucellosphaeridium areolosum* Fu and Hu (sp.nov.) – Hu and Fu, p. 109, pl. III:7-10.  

1982 Incertae Sedis Type 3 – Hu and Fu, p. 110, pl. IV, fig. 14-15.  

1982 Incertae Sedis Type 4 – Hu and Fu, p. 110, pl. IV, fig. 16-17.  

1982 *Tasmanites tenellus* – Hu and Fu, p. 113, pl. II:1.  

1982 *T. cf. volkovae* – Hu and Fu, p. 113, pl. II:2.  

1988 *Dictyosphaera delicata* Hu et Fu – Du in Guan et al., p. 133, pl. 11: 4, 12.  

1988 *Dictyosphaera gyrorugosa* (sic!) Hu et Fu – Du in Guan et al., p. 133, pl. 11:5.  

1988 *Dictyosphaera dyrorugosa* (sic!) Hu et Fu – Du in Guan et al., p. 200, pl. 11:5.  


1992 *Dictyosphaera incrassata* Yan sp. nov. – Yan and Zhu, p. 279, pl. II, figs 1, 2.  


1992 *Dictyosphaera gyrorugosa* Hu et Fu – Yan and Zhu, p. 280, pl. II, fig. 5.  


1997 *Dictyosphaera* sp. – Xiao et al., p. 205-206, figs. F, g.  

2001 *Dictyosphaera* sp. – Javaux et al., figure 1e.  

2005 *Dictyosphaera delicata* (Hu and Fu, 1982) – Yin et al., p. 52, 53, fig. 2:1, 2, 5, 7, 9, 10.  

2007 *Dictyosphaera delicata* Hu et Fu, 1982 – Yin and Yuan, fig. 1:1.  

2012 *Dictyosphaera delicata* Hu and Fu, 1982 – Li et al., pl. I, figs. 3-15.  

**Material.–** Around 100 specimens, including numerous entirely preserved specimens and, more commonly, fragmented specimens.

**Description.–** Spheroidal vesicle having a reticulate surface sculpture formed by pentagonal and hexagonal low ridges with positive relief delimiting concave pits (mesh). The vesicle wall is two-layered. The external layer is reticulate with ridges protruding both externally on the surface and inwards into the vesicle cavity. The internal layer is composite and consists of interlocked, polygonal, organic platelets, which are embedded within the network of ridges of the external layer. Individual platelets are bevelled along their sides and they interlock with each other with their shorter facets facing the cell cavity. On the internal vesicle-wall surface, the platelets also form a polygonal pattern but without relief. An excystment structure is present and consists of a partial rupture or simple median split, occasionally a circular opening (pylome) with a lid.

In our material, the vesicle diameter varies from 28 – 240 µm, and the average diameter of surface pits is 2 µm (N=83). The pits diameter is proportional to the diameter of the vesicle. Polygonal platelets from the internal layer range from 1-4 µm in width and are 0.7-1.0 µm thick, and are also dependent on the overall size of the specimen. Records compiled from previous reports show that *Dictyosphaera macroreticulata* and its junior synonyms have a wide-ranging vesicle diameter, of between10-274 µm, and the diameter of individual pits on the reticulate surface is 1-6 µm (Xing and Liu, 1973; Hu and Fu, 1982; Yan and Zhu, 1992).
Occurrence.—Northern China, Hopei Province, Chuanlingkou Formation, Precambrian (Sinian System) (Xing and Liu, 1973); Shaanxi Province, Luonan County, Gaoshanhe Group, Mesoproterozoic (Hu and Fu, 1982; Xiao et al., 1997); Shanxi Province, Shuiyou Section in the Yongji district, the Ruyang Group, Mesoproterozoic, c. 1.2 Ga (Guan et al., 1988; Yin and Zhu, 1992; Xiao et al., 1997; Yin and Yuan, 2003; Yin et al., 2005; Yin and Yuan, 2007); Henan Province, Ruyang Group (Li et al., 2012). Northern Australia, the Roper Group, Mesoproterozoic, c. 1.5–1.4 Ga (Javaux et al., 2004).

Remarks.—We retain Dictyosphaera macroreticulata as the type species of the genus by selecting the lectotype from the same material as the holotype derived (I.C.B.N., Article 7.5). We consider four species previously assigned to the genus, i.e. D. sinica, D. delicata, D. geyrorugosa, and D. incrassata, to be junior synonyms of the type species. There is no objective feature that can be used to distinguish them, and they overlap both in morphology and in size classes. Of the other species, D. “polycerata” and D. “rugosa” (Chen, 1980; Lei, 1982) are nomen nuda (Fensome et al., 1990), and D. yunanensis Xing, 1982, described from early Cambrian strata (Xing, 1982), should be attributed to the genus Retisphaeridium Staplin et al., 1965.

Some specimens, which were attributed to Tasmanites, are synonymised herein with D. macroreticulata, because they show small holes within a vesicle wall that have been left by the decay of pits in the reticulate surface layer and the disintegration of platelets. These holes are a taphonomic feature and not morphologic punctation of the vesicle wall, a feature which is diagnostic of Tasmanites.

Nucellosphaeridium areolosum is also similar to D. macroreticulata in morphology and dimensions, and which in addition possesses an internal body (Hu and Fu, 1982), is here regarded as a synonymous taxon. The internal body could be shrivelled cytoplasm, which coincidentally would appear as a lump of organic matter or an endocyst, if it is surrounded by its own wall. In the latter case, it would represent the reproductive stage of the same genus, i.e. Dictyosphaera (Fig. 2.1; see Life Cycle). Internal bodies preserved in D. delicata (Li et al., 2012, pl. I, figs. 3-12) are irregular lumps of organic matter, as are those in Shuiyousphaeridium pilatum (Li et al., 2012, pl. III, figs. 1, 2, 5, 7).

An internal body of very regular circular shape and delimited by a membrane, suggesting it is a true structural part of the vesicle, is preserved in one of the specimens previously reported (Yin et al., 2005, fig.2.5). We observe similar preservation in our material (Fig. 2.1). The internal body is indigenous to the vesicle because the vesicle is not broken and the body lies between the walls. We consider this structure as a putative endocyst developing inside a developmental stage of Dictyosphaera in which the meiotic division was occurring (Fig. 2.1; see Life Cycle). The endocyst is large in relation to the cell diameter, and this precludes interpreting it as a nucleus.

Opening of the vesicle by a rupture (Fig. 2.9) or median split (Fig. 2.5) is frequently observed. A large circular opening positioned in an apical part of the vesicle has also been recognized as an excystment structure in specimens attributed to Dictyosphaera sp. A (Yin et al., 2005, fig. 2: 3, 11, 12) and in D. delicata (ibidem, fig. 2:4). The presence of a small circular opening in a specimen of synonymous D. delicata by Yin et al., (2005, fig. 2:10) was suggested to be a pylome with a possible lid (Moczydłowska et al., 2011). The presence of a morphologically defined opening, i.e. pylome or apical opening, provides compelling evidence that the cell is a cyst. It has significant implications for the interpretation of life cycle of the Dictyosphaera-Shuiyousphaeridium plexus.

Genus SHUIYOUSPHAERIDIIUM Yan, 1992, emend. Yin, 1997, emended


*Diagnosis.* As for the type species emended diagnosis.

*Remarks.*– The generic diagnosis was considerably emended by Yin (1997). We here make minor modifications to include a multilayered wall and point out that the membrane may surround the entire vesicle, not just individual processes. The TEM studies of the vesicle-wall ultrastructure showed that the wall is multilayered (Javaux et al., 2004; Yin et al., 2005). The wall may alternate between uni- or multilayered, depending of the life stages (morphogenesis from vegetative to reproductive stage) in species with a complex life cycle and different ontogenetic developmental phases. Cell-wall ultrastructure may change as individuals grow and undergo metamorphosis between generations, especially where sexual reproduction and cyst formation is involved (Moczydłowska et al. 2010; 2011).

**SHUIYOUSPHAERIDIUM MACRORETICULATUM**

*Yan,* 1992, emend. Yin, 1997, emended

Figures 5.1-5.12, 6.1-6.7

*Synonymy.*–

1982 Incertae Sedis Type 3 and 4 – Hu and Fu, plate IV, figs. 14-17.
1992 *Shuiyousphaeridium macroreticulatum* (Du) Yan gen. et comb. nov. – Yan in Yan and Zhu, 1992, p. 272, 279, pl. 1, figs 1-6, 8.
1992 *Shuiyousphaeridium membraniferum* Yan gen. et sp. nov. – Yan in Yan and Zhu, 1992, p. 273, 279, pl. 1, figs 7, 9, 10.
1997 *Shuiyousphaeridium macroreticulatum* (Du) Yan, emend. – Yin, p. 19-20, pl. I, 1-3, 5, 8; pl. II, 2, 5, 7, 8; fig. 3.
1997 *Shuiyousphaeridium macroreticulatum* (Du) Yan – Xiao et al., fig. 3 a, b.

2004 *Shuiyousphaeridium macroreticulatum* (Yan, 1992) – Javaux et al., 2004, p. 127, fig. 5.
2009 *Shuiyousphaeridium macroreticulatum* – Schiffbauer and Xiao, figure 4A.
2011 *Shuiyousphaeridium macroreticulatum* – Schiffbauer and Xiao, fig. 13.6 c.

*Material.*– About fifty specimens, including numerous whole specimens as well as fragmented specimens.

*Diagnosis.*– Organic-walled microfossils consisting of a medium- to large sized, circular to oval, sharply defined vesicle (originally spheroidal), possessing a surface sculpture of polygonal reticulation with a positive relief and bearing complex cylindrical processes. The vesicle wall is thick and multilayered in structure. Processes are numerous, may be relatively short to long, and are evenly or irregularly distributed over the entire vesicle. Processes are heteromorphic, cylindrical, flaring towards bifurcating, branching or with funnel-shaped terminations, and either hollow and closed distally, or occasionally, simple and solid. Processes have conical bases or arise straight from the vesicle wall. Simple processes have truncate tips. Processes do not communicate with the vesicle cavity. A thin, psilate membrane may surround the whole vesicle or only certain processes or their branching terminations in a form of funnel or enclosing tube. The membrane closes the process but is concave towards the process basis, or it may stretch between neighbouring processes. Process branches are often connected laterally. Excystment is either by a median split or a partial rupture.

*Description.*– Vesicle diameter 80-240 µm; process length 7-18 µm; width of funnel-shaped process terminations 1-5 µm (N=43). The vesicle diameter (compiled from previous studies) is 50-357 µm; process length 2-45 µm; process branches 1-25 µm long; width of funnel-shaped process terminations 1-5 µm; and the width of process bases 0.5-3.0 µm. The vesicle wall is 0.5
μm thick (Yin, 1997; Xiao et al., 1997; Javaux et al., 2004; Yin et al., 2005; Yin and Yuan, 2007).

Occurrence.— Northern China, Shaanxi Province, Luonan County, Gaoshanhe Group (Hu and Fu, 1982); Shanxi Province, Shuiyou Section in the Yongji district, the Ruyang Group, Mesoproterozoic, c. 1.2 Ga (Guan et al., 1988; Yan and Zhu, 1992; Yin, 1997; Xiao et al., 1997; Yin and Yuan, 2003; Yin et al., 2005; Yin and Yuan, 2007). Northern Australia, the Roper Group, Mesoproterozoic, c. 1.5–1.4 Ga (Javaux et al., 2004).

Present record.— Samples Shanxi.S9-, S11-, S12-, S13-.

Remarks.— The type species of two genera, Dictyosphaera and Shuiyousphaeridium, were named “macroreticulata” and “macroreticulatum”, respectively, indicating that the same morphologic feature, a reticulate vesicle surface, is present in both. Xiao et al. (1997) suggested that the taxa are conspecific and that taphonomic loss accounted for the lack of processes in Dictyosphaera macroreticulata. If the taxa are conspecific, Dictyosphaera macroreticulata is the senior synonym and has priority. Taphonomic loss of processes is likely to be only partial, so would be insufficient to produce a process-less morphotype. We therefore suggest that Dictyosphaera and Shuiyousphaeridium are morphologically distinct cysts (Fig. 8) and consequently prefer to retain both form-genera, but recognise them as developmental stages in a life cycle of a single biological species (see Life Cycle).

THE CELL-WALL STRUCTURE

Our new LM and SEM observations on the cell-wall structure of the Dictyosphaera-Shuiyousphaeridium microfossil plexus extend previous studies based on various microscopic techniques (Kaufman and Xiao, 2003; Javaux et al., 2004; Yin et al., 2005; Schiffbauer and Xiao, 2009, 2011), and corroborate certain features of the wall layering and elements of its construction. Some features are reinterpreted, and morphological and structural differences between the two taxa infer a sequence of developmental changes in the life cycle of a single biological species. Overall morphology and the reticulate cell-wall sculpture suggest that the genera are conspecific. The lack of processes in Dictyosphaera was assumed to be due to diagenetic or sample processing loss, whereas their presence in Shuiyousphaeridium was viewed as a probable developmental feature (Xiao et al., 1997; Kaufman and Xiao, 2003; Butterfield, 2005). However, taphonomic processes or destructive laboratory procedures would not normally result in a total removal of processes in certain specimens (producing Dictyosphaera) or their complete preservation in others (Shuiyousphaeridium). Moreover, both morphotypes are present in the same samples and macerates. Loss of processes normally leaves attachment-point scars on the vesicle surface (Moczydłowska and Nagovitsin, 2012).

Xiao et al. (1997) assumed that processes were a developmental feature found only in larger individuals, as represented by Shuiyousphaeridium, after reporting that Dictyosphaera vesicles are smaller than those of Shuiyousphaeridium, thus implying that processes grew outwards from the cell surface in ontogenetically older individuals. This explanation cannot be sustained because both taxa largely overlap in their size ranges (see Systematic Paleontology). Additionally, both morphotypes are cyst-like in their morphology and acetylolation-resistance, and cyst processes grow contractionally from the cell surface. During morphogenesis from vegetative or zygotic cell into cyst, cytoplasm contracts from the cell wall, leaving strands that develop into processes, as observed in most extant algal microorganisms with known life cycle (Dale, 1983, 2001; van den Hoek et al., 1995; Belmonte et al., 1997; Raven et al., 2005). Multiple developmental mechanisms do involve processes growing outwards from the cytoplasm surface in a few dinoflagellate species (Kokinos and Anderson, 1995), but this reflects their highly derived phylogenetic position (Delwiche, 2007).

The wall structure of Dictyosphaera was previously examined in D. delicata by LM, TEM, a field-emission scanning microscope (FE-SEM), and a focused ion beam electron microscope (FIB-EM) by Yin (1997), Xiao et al. (1997), Kaufman and Xiao (2003), Yin et al. (2005), and Schiffbauer and Xiao (2009). We studied the synonymous D. macroreticulata using LM and SEM. Dictyosphaera was recognized as being multilayered, having a reticulate surface and consisting of interlocking polygonal plates (platelets herein) on the inner side of the wall...
(LM, TEM; Yin et al., 2005). In FIB-EM nanotomography, the wall was observed to be multilamellar and 200–500 nm in a total thickness (Schiffbauer and Xiao, 2009). The wall showed inner incisions corresponding to the boundaries between the polygonal plates, although the ridges on the wall surface were not depicted in cross-sections. From our material, it seems that the incisions are taphonomic rather than morphological and result from the plates breaking apart in a flattened specimen (see below). The outer edge of the vesicle wall in the FIB-EM section shows very regular chambers of unknown origin (Schiffbauer and Xiao, 2009, fig. 3K). Similar structures observed in *Shuiyousphaeridium macroreticulatum* were interpreted by the authors to be sections of the bulbous tips of processes beneath a membrane shrouding the processes. It seems more likely that these structures in both taxa are artefacts introduced during burial processes, or while preparing and coating the specimens. The “bulbous structures” are similar to artefacts of burial diagenetic processes affecting carbonaceous walls of other microfossils and producing apparent structures resembling “rounded chambers” or process-like “saw-tooth patterns” of carbonaceous material. Such structures were analyzed by FIB-SEM and FIB-TEM in cell walls of the spheroidal microfossil *Huroniospora* well preserved by permineralization from c. 1.9 Ga Gunflint chert (Wacey et al., 2012). The structures were recognized as the effects of taphonomy and fossilization, inducing an artificial wall texture (Wacey et al., 2012), although it was originally identified as a species-diagnostic morphologic wall surface reticulation (Barghoorn and Tyler, 1965; Hofmann, 1971; Schopf, 1992).

In FE-SEM images of *Dictyosphaera*, a pattern of high and low electron density areas seen on the outer and inner surfaces of the vesicle were explained as showing nanoscale pores (Kaufman and Xiao, 2003). The low electron density areas, i.e., the pores, are distributed on the ridges and in reticulate pits of the cell wall in various sizes and shapes, from dot-like to amygdaloidal and irregular strings. It is unclear why they are infilled with high electron density material on the interior surface of the wall only, in strong contrast to the wall’s carbonaceous material. The inferred porous structure of the wall is viewed here with reservation because of their irregular nature. Pores are normally consistent in size and shape when present as natural features in cell membranes and walls (Lodish et al., 1995). They are well known in other microfossils, such as those diagnostic of *Tasmanites* (Jux, 1968; Talyzina and Moczydłowska, 2000; Moczydłowska and Willman, 2009), and are characteristic of prasinophytes, especially order Pyramimonadales (Guy-Ohlson, 1996; Moczydłowska, 2011).

Our new reconstruction of cell-wall structure and of a life cycle for the *Dictyosphaera-Shuiyousphaeridium* plexus discriminates between taphonomic states and ontogenetic stages. Taphonomic processes are not always destructive and may enhance the visibility of certain features, such as the habit of individual platelets and their embedment in the cell-wall layer, a feature observed only in broken specimens. Alteration in progressively disintegrating specimens can be very informative about the robustness of discrete wall elements, and perhaps reflects the diverse biopolymers present at different layers in organic wall.

The cell-wall in *Dictyosphaera macroreticulata* is two-layered and consists of an outer layer with low polygonal ridges on both sides, and an inner layer formed by polygonal interlocked platelets (Figs. 3.7, 3.9, 4.1, 4.2). The outer layer has more prominent ridges on the wall surface (seen as reticulation with a positive relief) than those protruding inwards to form the pits which embedded platelets in the inner layer (Fig. 4.4).

The ridges are hexa- and pentagonal in planar view but in a three-dimensional (3D) reconstruction have a honeycomb structure. The inner layer is composite and comprised of laterally interlocking platelets that are tightly aligned in a polygonal pattern lacking relief. It is only when platelets begin to disintegrate that low ridges directed towards the cell cavity become visible, revealing that the platelet bases are embedded in pits formed by these ridges (Fig. 7). Platelet facets are hexagonal to occasionally pentagonal conforming to the polygonal ridges (Fig. 4.2). Platelets are trapezoidal in lateral cross-section.

The outer reticulate layer is composed of resistant organic matter so its ridges survive
prograding taphonomic alteration longer (Figs. 2.4–2.7). The ridge-delineated pit bottoms decompose more readily, turning into holes (Fig. 2.6). The reticulate outer layer acts as scaffolding for the cyst wall, and is the primary wall (PW), (Fig. 7). The inner composite layer is the secondary wall (SW). This is the first record of a composite organic secondary wall composed of individual platelets in microfossils. A secondary wall was previously recognized in Cryogenian Leiosphaeridia-like microfossils as a continuous and homogeneous sub-layer underlying the trilaminar sheath structure (TLS) in a multilayered cell wall (Moczydłowska et al., 2010). Similarly, a secondary wall lies beneath the TLS in the early Cambrian leiosphaerids with inferred chlorophycean affinities (Talyzina and Moczydłowska, 2000; Moczydłowska and Willman, 2009), and in Mesoproterozoic leiosphaerids interpreted as eukaryotic protists (Javaux et al., 2004, 2005; Schiffbauer and Xiao, 2009; Fig. 6.1, 6.3, 6.4). However, neither the process sections nor the surface reticulation relief are seen in TEM images. We consider the incisions in the second layer to be a taphonomic feature due to disintegration and separation of the interlocked platelets from their original spherical alignment. Seen in the TEM thin section of compressed wall of a once spheroidal cell, the platelets on the wall’s internal surface appear widely separated by incisions as a result of cell deformation and wall breakage. In the SEM images (Javaux et al., 2004), the platelets are tightly arranged, and this is also demonstrated in our material. Our interpretation is that the platelets belong to a composite (although separate) layer in the wall, i.e., the secondary wall, whereas the homogeneous layer forming the superficial and interior-facing ridges is the primary wall (Fig. 7). The third layer forming an inner lining of the cell wall (Javaux et al., 2004, fig. 5h) is difficult to see in the TEM section.

In Shuiyousphaeridium cell-wall by Javaux et al. (2004), it is not clear whether the first layer and the debris are remains of disintegrated surface layer that formed the processes, or the membrane that is seen in LM images and preserved in fragments on the cell-wall and surrounding the processes (Yin, 1997; Xiao et al., 1997; Yin et al., 2005; Schiffbauer and Xiao, 2009; Fig. 6.1, 6.3, 6.4). However, neither the process sections nor the surface reticulation relief are seen in TEM images. We consider the incisions in the second layer to be a taphonomic feature due to disintegration and separation of the interlocked platelets from their original spherical alignment. Seen in the TEM thin section of compressed wall of a once spheroidal cell, the platelets on the wall’s internal surface appear widely separated by incisions as a result of cell deformation and wall breakage. In the SEM images (Javaux et al., 2004), the platelets are tightly arranged, and this is also demonstrated in our material. Our interpretation is that the platelets belong to a composite (although separate) layer in the wall, i.e., the secondary wall, whereas the homogeneous layer forming the superficial and interior-facing ridges is the primary wall (Fig. 7). The third layer forming an inner lining of the cell wall (Javaux et al., 2004, fig. 5h) is difficult to see in the TEM section.

We consider the cell-wall structures of Dictyosphaera and Shuiyousphaeridium to be analogous because of the shared features. There are two layers: the outer reticulate layer as the primary wall, and the inner composite layer of platelets formed as the secondary wall for the additional strength and protection of the cyst (Fig. 7). The difference between the two taxa is the absence or presence of processes, respectively. We regard this difference as indication of distinct cyst stages (asexual and zygotic, respectively) in the life cycle of a single species (Fig.8). If correct, this is the earliest example of sexual heteromorphism in photosynthetic protists, although this represents generational variation (asexual vs sexual) rather than gender (female versus male morphs) differences.
LIFE CYCLE

The common morphological characteristics and biochemical properties (i.e., resistant organic vesicle, ornamentation and diverse processes, and excystment structures) in ancient organic-walled microfossils (acritarchs), have long been considered to be features of a reproductive cyst stage of phytoplanktic protists (Tappan, 1980; Moczydłowska, 1991, 2010; Grey, 2005; Wicander, 2007). Ultrastructural studies on organic walls in Proterozoic and Cambrian microfossils (Arouri et al., 1999, 2000; Talyzina and Moczydłowska, 2000; Kempe et al., 2002, 2005; Javaux et al., 2004; Willman and Moczydłowska, 2007; Moczydłowska and Willman, 2009; Schifflauer and Xiao, 2009; Willman, 2009; Moczydłowska et al., 2010) reinforced this interpretation by identifying significant complexity and multilayering comparable to that in modern photosynthesizing protists and certain microalgal species. Phylogenetically diverse protistan clades are known to produce preservable cysts as a part of their life and reproduction cycle, regardless of whether they are asexual (i.e., prasinophyte resting cysts) or sexual (zygotic cysts in chlorophytes, charophytes, and dinoflagellates) (Evitt, 1985; Margulis et al., 1989; Dale, 2001; Hagen et al., 2002; Raven et al., 2005; Damiani et al., 2006; Head et al., 2006). Cyst wall resistance (preservability without permineralization) demonstrates specific requirements for successful reproduction: the protection of the maturing zygote and meiotic subdivision or multiple mitotic divisions (Graham and Wilcox, 2000; Moczydłowska et al., 2011). The synthesis of sturdy walls in reproductive stages involves the secretion of additional biopolymers and layers to those present in vegetative cell walls. This requires a significant metabolic investment, but allows the release of multiple offspring-cells (swarmers). Additionally, sturdy walls provide an ecological advantage for coping with high deep-water pressure in cysts that rest periodically on bottom sediment (Mendelson, 1993; Reynolds, 2006).

The diverse processes in microfossils, including those in taxa studied here, show the same complexity and morphologic patterns as in extant phytoplanktic cysts, where their function is to maintain buoyancy and sensory activity. Ornamented-cyst processes act as flotation devices enabling passive migration or suspension in the water column (e.g. within the photic and oxygenated surface zone). Eventually they aid decent to the substrate, where cysts remain for releasing offspring or dormant under adverse environmental conditions (e.g., low nutrients supply, temperature change, acidity or anoxia) (Blackburn and Tyler, 1981; van den Hoek et al., 1995; Belmonte et al., 1997; Dale, 2001; Reynolds, 2006; Lee, 2008). Excystment structures (reproductive openings) develop once a cyst has matured to release offspring (Bold and Wynne, 1985; Raven et al., 2005; Wicander, 2007; Moczydłowska, 2010). The structure can be a simple split or a morphologically predetermined and physiologically controlled rounded or apical opening. All these kinds of excystment structures are present in Dictyosphaera and Shuiyousphaeridium. However, only low numbers of cysts are likely to be preserved after excysting because open cysts are more prone to biodegradation and disintegration. Consequently, fossil excystment structures are seldom observed (Moczydłowska and Willman, 2009).

Examination of key morphological and structural features and their function in the Dictyosphaera and Shuiyousphaeridium microfossils allows reconstruction of a life cycle as exemplified in the Cambrian organic-walled microorganism Skiagia (Moczydłowska, 2010). The structural evidence presented here supports the previous suggestion of Xiao et al. (1997) that the two morphotypes are conspecific and represent different life stages of a single biological species of eukaryotic affinity.

The cyst-like morphologies together with excystment structures in Dictyosphaera and Shuiyousphaeridium, further support the model of reproduction by alternation of generations (Fig. 8). The life cycle model is inferred from comparison with extant green microalgae (chlorophytes, charophytes, and dinoflagellates), (Margulis et al., 1989; Melkonian, 1989; van den Hoek, 1995). Alternation of sexual and asexual reproduction in individual species is genetically driven as part of a regular life cycle or induced ecologically as a means of overcoming adverse conditions (Lewontin, 1958; Pfiester and Anderson, 1987; Sarjeant and Taylor, 1999; Kaltz and Bell, 2002; Reynolds, 2006; Graham et al.,
Both generations usually differ in the morphologic appearance of their cysts, and are distinct from flagellate vegetative cells. This may be observed in a number of modern taxa, such as the chlorophyte *Chlamydomonas* (van den Hoek et al., 1995).

The reconstructed life cycle of the *Dictyosphaera* and *Shuiyouphaeridium* plexus is as follows. Sexual reproduction begins when the haploid, vegetative, motile cells (functioning as gametes) pair and fuse into a zygote, which loses its flagella and subsequently produces a diploid cyst with an ornamented wall (Fig. 8). The *Shuiyouphaeridium* macroreticulatum morphotype represents this stage and its morphology resembles cysts seen in several extant species. A common pattern of acanthomorphic zygotic cysts is known in microalgae, such as the chlorophycean *Golenkinia radiata* (Chodat, 1894; Guiry, 2013), the charophyte desmidiaceans *Micrasterias papillifera* (Kies, 1970) and *Micrasterias thomasiiana* (Blackburn and Tyler, 1981), and the zygnemophycean *Staurastrum fungicerum* (Lacalli, 1981), (Raven et al., 2005). During zygotic cyst (or zygospore) formation in *Micrasterias thomasiiana* (Blackburn and Tyler, 1981), the cytoplasm begins to shrink inwards and spiny processes are formed by contraction from the outer membrane enclosing the zygote (Belmonte et al. 1997; Lee, 2008). This ornamented cyst harbours the zygote and the ensuing meiotic subdivision, while the outer membrane is discarded. In *Shuiyouphaeridium*, remnants of the thin outer membrane, which may be analogous to the zygotic membrane, are observed as fragments surrounding processes (Fig. 6.3 and 6.4), either engulfling several processes (Fig. 5.4 and 6.1), or as conjoined, uneven, membranous flaps (e.g., in the synonymous *S. membraniferum*).

The thin outer membrane is discarded after the sturdy cyst represented by *Shuiyouphaeridium* has been formed (Fig. 8). This cyst is characterised by distinctive processes and the wall is reinforced by resistant-biopolymer(s) in the primary and secondary walls. It compares to the modern genus *Micrasterias* whose walls are reinforced by fusion of the Golgi septum vesicles with the plasma membrane (Lütz-Meindl and Broch-Salomon, 2000). Alternatively, the cyst may settle to the bottom as benthic plankton, a periodic mode of life common in extant microalgae (Dale, 1983). Meiosis occurs within the zygotic cyst, either floating or resting in the sediment. Haploid offspring is released through the excystment structure. Matured haploid vegetative cells may yield another zygotic cycle or may reproduce asexually.

During asexual reproduction, the cell loses its flagella, grows, and transforms into a resting cyst by secreting an extra protective wall within which mitosis occurs (Fig. 8). This stage is represented by the *Dictyosphaera macroreticulata* morphotype. The asexual cyst has no true ornamentation (processes) but only wall sculpture (reticulation) and it may settle immediately on to the bottom sediment and rest there during mitotic division. An extant example is *Chlamydomonas reinhardtii*, whose mother-cell wall swells after losing its flagella (e.g., Cavalier-Smith, 1974). In other chlorophyte species, asexual reproduction produces aplanospores, which differ in wall composition from their parent and do not possess flagella (Lee, 2008). Daughter cells, genetic copies of the mature cell, are released through a rounded or split excystment structure. Asexual reproduction can continue unless the gametes fuse into another zygotic cyst and begin the sexual cycle.

Apart from the *Dictyosphaera-Shuiyouphaeridium* microfossil plexus, the newly recognized taxon *Gigantosphaeridium* has a sufficiently robust, large, acanthomorphic vesicle to substantiate a eukaryotic affinity, and it too probably had a complex life-cycle with sexual reproduction and cyst formation.

Across the lineage Viridiplantae (including algae and plants), alternation between sexual and asexual reproductive strategies (heterogamy) is mostly dependant on environmental conditions (Lewontin, 1958; Kaltz and Bell, 2002). In a stable, nutrient-rich environment, cells can simply divide asexually. However, under unfavourable conditions, sexual reproduction and genetic exchange are more beneficial and cells that combine to form a zygocyst will produce more viable offspring, despite the metabolically costly process of cyst formation. Examples of such life-cycles with alternating generations, are known in a number of classes and individual species of extant microalgae (Chlorophytes, Charophytes). Similar patterns of cyst-like morphologies
associated with resistant walls with complex structures are recognizable throughout the Viridiplantae.

**BIOLOGICAL AFFINITY OF DICTYOSPHERA-SHUIYOUSPHAERIDIUM PLEXUS**

Review of previous hypotheses.– The biological affinities of organic-walled microfossils have long been a matter of discussion in pursuing the understanding of the evolutionary history of early microbiota. Term acritarchs, which was applied to such organisms in referring to their uncertain origins, is now somewhat misleading because many of these microfossils have been progressively allocated to specific groupings, mainly eukaryotic phytoplankton (Tappan, 1980; Moczydłowska, 1991, 2011; Colbath and Grenfell, 1995; Arouri et al., 1999, 2000; Talyzina and Moczydłowska, 2000; Wicander, 2002; Grey, 2005; Traverse, 2007; Molyneux, 2009; Kaźmierczak and Kremer, 2009; Moczydłowska et al., 2011).

Exceptions exist and individual case studies have identified the microfossil *Tianshushania* as a metazoan embryo (Xiao et al., 1998, 2012; Yin et al., 2004, 2007) or a holozoan protist (Huldtgren et al., 2011; Bengtson et al., 2012), and “*Tappania*” from the Wynniatt Formation as a fungus (Butterfield, 2005, 2009; Javaux 2007). Some unidentified Ediacaran taxa, together with *Alicesphaeridium* and *Gyalosphaeridium*, were claimed to be potential diapause egg cysts (Cohen et al., 2009), based only on morphology and despite any evidence of cell division (Moczydłowska et al., 2011).

Here, we focus our observations and inferences on the *Dictyosphera-Shuiyousphaeridium* plexus and comparisons with modern biota. Although their complete systematic position is difficult to establish, their affinity can be narrowed down. These microfossils have been assigned to phytoplankton (Yin, 1997), undetermined green algae (Kaufman and Xiao, 2003), photosynthetic eukaryotes (Yin et al., 2005) and more specifically dinoflagellates (Meng et al., 2005; Yin and Yuan, 2007), or broadly identified as protists without distinguishing between auto- or heterotrophic modes of life (Javaux et al., 2003, 2004; Knoll et al., 2006). By contrast, *Shuiyousphaeridium* was suggested as possible fungus and heterotrophic, benthic, multicellular organism (Butterfield, 2005). Recently, this form-genus has been interpreted as a chlorophyte (Moczydłowska et al., 2011). Palaeoecological conditions and the distribution of the microfossils studied in shallow marine facies point to a planktic life mode.

Kaufmann and Xiao (2003) inferred that *Dictyosphera* was a photosynthetic alga because the organic carbon isotopic fractionation values ($\delta^{13}C$) obtained from the microfossil wall are consistent with primary productivity via the Calvin cycle of carbon fixation. This interpretation was accepted with reservations by Butterfield (2005), through his interpretation of the microfossil as a fungal ascocarp, but without an alternative explanation of the light carbon isotopic ratio in *Dictyosphera*. A photosynthetic interpretation was also questioned by Javaux and Marshall (2006) on the grounds of “negative evidence” because of a lack of other diagnostic algal features, such as TLS in the wall or the presence of the biopolymer algaenan, rather than an evaluation of the isotopic signature significance. TLS and algaenan are not the only features diagnostic of algae, neither all algae possess them nor in all life stages. Inferences of a photosynthetic metabolism (Kaufman and Xiao, 2003; Meng et al., 2005) are supported by the striking phenotypic similarity of the microfossils to extant photosynthesizing protists, and we find the evidence compelling for such a metabolism for *Dictyosphera-Shuiyousphaeridium*.

The classification of *Shuiyousphaeridium* within dinoflagellates (Meng et al., 2005) remains dubious. Morphologically, the polygonal pattern of wall sculpture in the *Dictyosphera-Shuiyousphaeridium* plexus is not analogous to the paratabulation of dinocysts (Moczydłowska et al., 2011) and, therefore, does not support a dinoflagellate affinity. The double-walled vesicle similar to that of dinoflagellates (Meng et al., 2005), is recognized as a two-layered wall structure (Fig. 7). There is a long time gap between the age of these microfossils and the estimated chronology of secondary and tertiary endosymbioses leading to the origin of derived microalgal classes, such as Mesozoic dinoflagellates (Fensome et al., 1996; Delwiche, 2007). Some similar features of the cysts are common to both microfossils and dinoflagellates, but they are probably symplesiomorphic in dinoflagellates and inherited from symbiont(s) (Moczydłowska, 2010; Moczydłowska et al., 2011).
The main argument for dinoflagellate affinity came from the identification of the biopolymer dinosterane in the *Shuiyousphaeridium* cell wall (Meng et al., 2005). This molecule might have been derived from a dinoflagellate ancestor (Moczydłowska, 2010), or from another chromalveolate algae. The same compound family of dinosteranes was found in diatoms (stramenopiles) and prymnesiophytes (Volkman et al., 1993, 1998), which are derived and relatively recently evolved microalgae (Kooistra et al., 2007; Mustafa et al., 2009). Later evolved organisms may retain the ability to synthesize steranes from a common precursor (Moldowan et al., 2001). The name dino-sterane was coined because 4α-methyl-24-ethylcolestane compounds were originally described from dinoflagellates in Black Sea sediments (Boon et al., 1979). It is now ambiguous because it is not restricted just to dinoflagellates. This could explain the presence of “dinosteranes” in sediment bulk samples from Archaean (Brocks et al., 2003), Proterozoic, and Cambrian ages (Summons et al., 1992; Peng et al., 1998; Moldowan and Talyzina, 1998; Moldowan et al., 2001; Knoll et al., 2007), well before the appearance of dinoflagellates in the Permian-Mesozoic rock record (Fensome et al., 1996). If resistant “dinosteranes” were derived from dinocysts, then the fossils should be preserved, therefore the absence of dinocysts is less likely to be due to their poor preservation potential. While the biochemical pathway for dinosterane synthesis existed, the source may have been independent of dinoflagellates (Volkman, 2005).

*Shuiyousphaeridium*, and by default *Dictyosphaera* as its conspecific taxon, have also been compared to fungi (Butterfield, 2005). The author posited that the reticulate wall pattern corresponds to polygonal cells that form the multicellular ascocarp in primitive marine fungi (Pyneromycetae), while the processes in *Shuiyousphaeridium* are hyphae-like and the median-split represents a dehiscence structure that releases its spores. As has been shown in SEM images (Javaux et al., 2004; Meng et al., 2005), the reticulate sculpture is an integral part of the wall structure, here recognized as a primary wall (Fig. 7), and not a bundle or sheet of cells in a multicellular organism. *Shuiyousphaeridium* (and similarly the true *Tappania* morphotype) extracted repeatedly by palynological methods from the Ruyang Group (Yin and Yuan, 2007; Li et al., 2012; herein) have never demonstrated the presence of septae within the processes, a feature diagnostic of fungi. An additional reservation to postulating fungal affinity is that fungi do not produce a resistant wall of such structural complexity. Although the resistant wall can be synthesized in spores and fruiting bodies (de Leeuw and Largeau, 1993), it is of a very simple morphology (Elsik, 1996; Kalgutkar and Jansonius, 2000; Webster and Weber, 2007; Kalgutkar and Braman, 2008; Moczydłowska et al., 2011). Processes and excystment structures do not exist in fungi; fungal spores may have a pore or slit, but it has a different appearance.

Innovations in the form of more complex morphology and the synthesis of resistant tissue in fungi arose much later in their evolution and in terrestrial environments through adaptive opportunistic radiations following mass extinctions in the Mesozoic (Vajda and McLaughlin, 2004) and through their co-evolution with angiosperms (Elsik, 1996; Traverse, 2007; Kalgutkar and Braman, 2008). Fungal spores and fruiting bodies preserved in brackish-marine conditions and palynologically extracted from sediment are mostly terrestrial species. Spores of terrestrial ascomycetes were recovered from the Triassic and Jurassic (Elsik, 1996) and Cretaceous (Kalgutkar and Braman, 2008), with a few marine hyphomycetes known from the Cretaceous (Pirozynski and Weresub, 1979), and some marine fungi from the Permian (Elsik, 1996). A possible earliest record of ascomycete fungus extracted from the sedimentary rocks is of early Silurian age and is terrestrial (Edwards and Wellman, 1996). Fungal hyphae are exclusively found in the silicified material, such as the microfossils from the famous Lower Devonian Rhynie chert in Scotland that include spore-containing oogonia similar to extant Peronosporomycetes (Oomycota) (Krings et al., 2012). They were derived from freshwater pools and hot springs. Proterozoic fungal-like simple filaments and spheres were extracted from c. 950 Ma old marine sediments of the Lakhanda Formation in Siberia, together with algae (Hermann, 1990). None of the fungal microfossils from the geological record show morphologic complexity.
Some morphological similarities between ancient eukaryotes and extant fungi that do exist are exceptions. Wall ornamentation on the spore of the glomeromycotan fungus *Acaulospora denticulata* (Moore et al., 2011) is similar to the polygonal reticulation described here. The secondary fusion of processes in the zygospore of oomycetan *Phytophthora infestans* (Walker and West, 2007) may resemble the looped processes of the new genus *Gigantosphaeridium*. However, the possible resemblance is limited solely to morphology, whereas the acetolysis-resistant vesicle consists of a suite of characteristics that suggest affinities to cyst-producing taxa among extant photosynthetic protists.

**Comparison with other protists.**– The layered wall with platelets located on the internal side in *Dictyosphaera-Shuiyousphaeridium* plexus has no comparison, to our knowledge, to any heterotrophic protists, which also differ in having only external scale wall elements, polar symmetry, an oral opening, and generally do not produce a secondary wall (Table 1.). They may occasionally produce a wall with mineralized scales (test) that is potentially preservable, as observed in testate amoebzoans recognized in Neoproterozoic at c. 770–740 Ma (Porter, 2006), initially called the vase-shaped microfossils (Bloeser et al., 1977). They show polar symmetry, and well-defined oral opening for exuding the cytoplasm, often with a plug. The test is rigid, composed of acetolysis-resistant organic matter, and may show a reticulate surface pattern of once attached scales (*Melicerion poikilon*). These microfossils have been recognized as heterotrophic biota since their discovery (Bloeser, 1985; Marti-Mus and Moczydłowska, 2000; Porter and Knoll, 2000; Porter et al., 2003; Porter, 2006).

Superficial morphological resemblances to *Dictyosphaera-Shuiyousphaeridium* are further found in ventral polygonal bulges of the ciliate *Codonella cratera* (Duff et al., 2008), and honeycomb membrane of the thecamoeba *Gromia sphaerica* (Gooday et al., 2000) (Table 1.). Sporophytes of red algae also share this appearance, but are much larger in size.

Among autotrophic protists other than green algae, a cell wall with organic and biomineralized scales or plate-like elements in the wall structure is known in haptophytes and chrysophytes, and is exclusively an external covering of vegetative cells and cysts. Haptophytes possess organic scales on the surface of the flagellate cell and calcitic coccoliths in the nonmotile stage (coccolithophorids) and their record is restricted to the last 200 Ma (Read et al., 2013). Chrysophytes produce mineralized cysts, but their vegetative cells are covered with silicified scales and spines, solely on the cell surface. Only one chrysophyte group, marine silicoflagellates, produces a lattice-like siliceous internal skeleton, but these are no older than the Cretaceous (McCartney, 1993).

The earliest record of biomineralized scale microfossils (composed of apatite and organic carbon) is in cherts of the Neoproterozoic Tindir Group, Canada, (Allison and Hilgert, 1986; Cohen et al., 2011). They were identified as a putative green alga, although they resemble the cell-covering scales of various extant algal groups like prasinophytes, chrysophytes, haptophytes, and streptophytes, as well as heterotrophic ciliates and testate amoebans (Cohen et al., 2011).

We argue that the microfossils studied here do not belong in any of these groups because of a primary diagnostic feature— the acetolysis-resistant cell wall –, which in a combination with the morphologic and cell-wall structural features recorded here are only found in the phenotype of green microalgae. Even more significantly, the primary wall and the composite secondary wall consisting of internal platelets are features known only in extant prasinophytes, chlorophytes and charophytes (see below). All these green algal lineages derived from an ancestral monophyletic lineage evolved by primary symbiosis (O’Kelly, 2007), making the microfossils studied candidates for this group. Resistant cell walls are typical of so-called “plant cells” produced by algae and vascular plants (Raven et al., 2005) and are recognized among unicellular organic-walled microfossils of various ages since ca. 1.8 Ga through the Phanerozoic (Moczydłowska et al., 2011).

In other words, the *Dictyosphaera-Shuiyousphaeridium* plexus has little to suggest it belongs in the dinocysts or heterotrophic protists or fungi (Table 1.), and there is no convincing evidence to associate it with a benthic mode of life.
Present interpretation.— As discussed above, the available evidence points towards this group of microfossils being cysts of photosynthetic planktic protists belonging to the green microalgae Chloroplastida (= Viridiplantae), following the eukaryotic phylogeny model of Baldauf (2008). Alternatively, they represent an ancestral lineage of photosynthetically protists of the supergroup Bikonta before divergence into the Viridiplantae and Chromalveolata lineages, following the phylogenetic tree of Cavalier-Smith (2003, 2004, 2010). Microfossils studied here share general morphological characters with green algae and chromalveolates, such as zygotic cyst-like vesicles with processes and excystment structures, structurally complex walls, as well as being photosynthetic. The resistant biopolymers in their walls are consistent with photosynthesizing biota (Moczydłowska and Willman, 2009). Yet due to more recent evolution of Chromalveolata (Archibald and Keeling, 2004; Yoon et al., 2004), these characters could be ancestral (plesiomorphic) and deriving from primary endosymbiotic green eukaryote and their position in Viridiplantae is more likely.

Acanthomorphic vesicles with lavish processes are characteristic of reproductive cysts and found in many extant microalgal species. Examples are provided by chlorophytes Golenkinia radiata (Chlorococcales; Chodat, 1894; Guiry, 2013), and Atractomorpha echinata (Sphaeropleales; Hoffman, 1983), charophytes Microasterias papillifera (Kies, 1970), Microasterias thomaisiana (Blackburn and Tyler, 1981), and Stauarstrum furgicerum (Lacalli, 1981) (all belonging to Desmidiales). Distally divided processes in Shuiyousphaeridium resemble the processes in these algal zygocysts. Different fibrilar and occasionally looped processes of Gigantosphaeridium fibratum n. gen. et sp., bear similarities to extant marine microalgae Sphaeropsis (Pterospermataceae, Chlorophyta) and Piropsis (Crasedophyta) from the North Sea and Arctic Sea (AlgaeBASE). Secondary wall synthesis during the cyst stage was observed in several extant taxa: chlorophyte Haematococcus pluvialis (Hagen et al., 2002; Damiani et al., 2006) and charophyte M. thomaisiana (Blackburn and Tyler, 1981), Volvocales (Hagen et al., 2002), resembling features in the studied taxa.

Our reconstruction of the Dictyosphaera-Shuiyousphaeridium life cycle based on the alternation of sexual and asexual generations is a common model of reproductive strategy across extant lineages of Viridiplantae, Chromalveolata, Fungi, and Amoebozoa (Table 1.), indicating either an ancestral character derived from the last eukaryotic common ancestor (LECA) or independent evolution postdating the split of major eukaryotic groups. The latter alternative could be understood as a method of coping with adverse conditions in different clades (Lewontin, 1957; 1958).

Considering the sequence of phylogenetic divergences within the lineage Chloroplastida, which also reflects ecological adaptations from marine to fresh water environments and colonization of terrestrial realm, the Charophytes and land plants are derived and later evolved lineages (Paleozoic; Graham and Gray, 2001; Wellman et al., 2003; O’Kelly, 2007; Steemans et al., 2009; Rubinstein et al., 2010) than primary lineages of Prasinophytes and Chlorophytes (Turmel et al., 2002, 2008; Raven et al., 2005; Baldauf, 2008). Prasinophytes are known to reproduce asexually and, although producing resistant cysts, these are morphologically distinct and lack superficial processes. Chlorophytes reproduce asexually and sexually, forming resistant cysts of high morphologic disparity.

In sum, the Dictyosphaera-Shuiyousphaeridium organism is inferred to be microalga (chloroplast containing) in the lineage Chloroplastida of the group Archaeplastida, following the phylogeny of Baldauf (2003, 2008). The group Archaeoplastida was recognized as monophyletic and all members of its distinct lineages, Glauco phyta, Rhodophyta, and Chloroplastida, are photosynthetic (Adl et al., 2005; Rodriguez-Espeleta et al., 2005; Reyes-Prieto et al., 2007). The lineage Chloroplastida (green algae and land plants) has been recognized by Adl et al. (2005) and previously classified in a rank of the kingdom Viridiplantae (Cavalier-Smith, 1981).

We accept the recognition of the group Viridiplantae as equivalent to the lineage Chloroplastida (Baldauf, 2008) which consists of two monophyletic groups: Chlorophyta (prasinophytes and chlorophytes) and Streptophyta (charophytes and land plants) (O’Kelly, 2007; Turmel et al., 2008). The
phylogenetic position of *Shuiyousphaeridium* has been suggested within the Viridiplantae and the early branch of chlorophytes (Moczydłowska et al., 2011). We may confirm the position of the *Dictyosphaera-Shuiyousphaeridium* organism in the stem lineage of Chloroplastida by present study of the wall structure and further conclusions on the cell and functional biology.

**INTRACELLULAR COMPLEXITY AND INNOVATION OF THE SECONDARY WALL**

Characteristic eukaryotic organelles, including a nucleus and the Golgi apparatus (GA), together with a cytoskeleton, cell architecture, and complex membranes containing sterols, are considered to have been acquired at the time of “cryptic prehistory” prior to c. 2.1 Ga (Runnegar, 1994). Intracellular complexity defining eukaryotic origin and early cellular differentiation in Proterozoic fossils has been noted by Butterfield (2000) and Knoll et al., (2006) with respect to three cell types of gametophytic *Bangiomorpha* and potential requirement for a cytoskeleton and cell shape regulation in *Tappania, Shuiyousphaeridium* and *Germinosphaera*.

The high phylogenetic position of the *Dictyosphaera-Shuiyousphaeridium* organism within a major group of eukaryotes is supported by its remarkable wall structure and the innovation of a secondary wall, thus requiring a degree of intracellular complexity for its synthesis. The advanced level of organization of an eukaryotic cell with endomembrane system, ER and the GA, cytoskeleton, organelles including mitochondrion and plastid, and sexual reproductive machinery functioning for metabolic and physiologic processes is inferred for these microfossils. The organelles thought to be present in early eukaryotes, primarily nucleus and mitochondrion, and structures providing the minimum requirement for eukaryotic cell functioning, i.e., endomembrane system and cytoskeleton (Stanier, 1970; Lang et al., 1999; Cavalier-Smith, 2003, 2010; Dacks and Field, 2004;) have been inferred to exist in some Mesoproterozoic taxa, such as *Tappania, Valeria,* and *Shuiyousphaeridium* (Javaux et al., 2001, 2003, 2004).

Accepting the hypothesis of the photosynthetic metabolism of the *Dictyosphaera-Shuiyousphaeridium* organism (Kaufman and Xiao, 2003; Meng et al., 2005; Moczydłowska et al., 2011), we consequently suggest the presence of plastid and most likely the chloroplast.

In the *Dictyosphaera-Shuiyousphaeridium* organism we can interpret for the first time the controlled secretion and arrangement of polygonal organic platelets building a secondary cell wall and thus playing a role in cyst formation. Such advanced wall elements could have been formed and secreted through the GA and the endoplasmic reticulum (ER). The GA, a major biosynthesis organelle found in most eukaryotes and all plants, may provide an explanation in understanding the evolution of this organism.

The organic platelets reinforcing the cell wall conform to the reticulation of the primary wall (Fig. 7). Such a pattern of wall construction is known in extant eukaryotes, whereby the GA and the ER secrete organic granules that become subsequently attached to the cell membrane. Such process occurs in the extant alga *Nephroselmis olivacea* (Suda et al., 2004). The GA and the ER are also crucial for cell wall formation in Chrysophyceae (Brown, 1969), and wall scales formation in *Scherffelia dubia* (McFadden et al., 1986) and other Prasinophyceae (Melkonian et al., 1991). They produce complex sugars and polysaccharides (Dupree and Sherrier, 1998; Goubert et al., 2009) that may explain the resistant property of the vesicle wall. Cell-wall secretion is mediated by GA in chlorophyte *Microasterias* (Meindl, 1993; Lütz-Meindl and Broch-Salomon, 2000) resulting in an ornamented cell shape, and in the formation of retaining wall conjoining the cells of colonial *Botryococcus braunii* (Weiss et al., 2012).

As discussed above, the secondary wall is a significant feature of the *Dictyosphaera-Shuiyousphaeridium* cysts, and its complexity is typical of eukaryotes. Protistan cell wall fulfils three roles: 1) defence against bacterial biodegradation, phagocytosis and (micro)predation; 2) survival under unfavourable environmental conditions; 3) protection during reproduction. The last function may be combined with the other two, as the developing offspring would require protection against both heterotrophy and adverse conditions. All these factors might have driven the evolution of more complex eukaryotic cyst walls in the Mesoproterozoic. Environmentally induced light intensity, nutrient supply, temperature changes,
anoxia, and access to metabolically important metals (Anbar and Knoll, 2002; Klein et al., 1992; Lee, 2008; Parnell et al., 2013) could have initiated the development of reproductive and resting stages. While the first true signs of micropredation (Porter et al., 2003) and biomineralized defense structures (Cohen et al., 2011) do not appear in the fossil record until Neoproterozoic, it is reasonable to assume that the first step in protection of early photosynthetic eukaryotes would have been the composite reinforcement of the organic cell wall, i.e. the evolution of a secondary wall as observed in the Dictyosphaera-Shuiyousphaeridium organism.

CONCLUSIONS
Unicellular organic-walled microfossils were recovered from the shallow marine shales of c. 1.2 Ga Beidajian Formation, Ruyang Group in northern China. They include ornamented morphotypes Dictyosphaera macroreticulata Xing and Liu, 1973, Shuiyousphaeridium macroreticulatum Yan, 1992, emended, and a new genus and species Gigantosphaeridium fibratum, which is among the largest known microfossils in Mesoproterozoic. Identical wall reticulation patterns and wall structures occur in two taxa, Dictyosphaera and Shuiyousphaeridium and appear to represent a single biological species and its cysts in heteromorphic life-cycle generations. Dictyosphaera is an asexual (resting) cyst and Shuiyousphaeridium is a sexual (zygotic) one, as can be inferred by comparing their phenotypic features and functional morphology to those of extant photosynthesizing microbiota. The simplest explanation for this is that the fossils represent haploid and diploid stages, and have a reproductive strategy of alternation between the asexual mitotic division into clonal daughter-cells and sexual meiotic division from a zygote.

The large size, robust body and lavish ornamentation of Gigantosphaeridium gen. n., demonstrates not only eukaryotic affinity but also the reproductive function of a cyst.

Dictyosphaera and Shuiyousphaeridium illustrate the evolution of complexity in cyst-wall structure and provide the earliest record so far of a secondary wall composed of polygonal platelets. The metabolic expenditure required to construct complex, resistant walls paid off in terms of increase protection during cell division, especially for meiotic division and for ensuing offspring. The construction of a protective cyst suggests that a sexual reproductive stage was already established by the Mesoproterozoic Era.

Intricate walls would require well-developed intracellular machinery for their production and for maintenance of large cells, such as that found in extant algae where organic components are secreted by the Golgi apparatus, transported through the endoplasmatic reticulum and integrated into the cell membrane. Indirect evidence for Golgi apparatus activity and the presence of endoplasmatic reticulum is provided by infilling of the primary wall by crude platelets. Accepting a photosynthetic mode of life, the plastids were among organelles of this early eukaryote.

The combination of several lines of evidence based on morphology, cell wall ultrastructure, and biochemistry, and comparisons with extant microbiota provides compelling argument that Mesoproterozoic Dictyosphaera-Shuiyousphaeridium microorganism belongs among the photosynthetic eukaryotes of the stem group Chloroplastida, as part of the lineage of modern eukaryotic phytoplankton.

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FIGURE CAPTIONS

FIGURE 1– Morphology of organic-walled microfossils from the Mesoproterozoic Ruyang Group, Beidajian Formation, in Shanxi Province, northern China. 1-5, Light photomicrographs of *Giganthosphaeridium fibratum* gen. et sp. nov. 1, 1a, Specimen PMU-S13-1-11- (M/40), shown at different scale. Image (1a) is under the same magnification as the holotype in (4), to demonstrate the
wide size range of the new species. 2, PMU-S13-1-10- (P/40/3). 3, PMU-S13-1-6- (M/46/3). 4, Holotype, composite digital image showing the large vesicle with fibrilar processes surrounded by translucent membrane. PMU-S13-1-12- (Z/38/3). 4a-4b, Magnified fragments of the vesicle in (4), showing upper right and lower right parts, respectively, at same magnification. 5, Specimen with much longer processes and tentatively assigned to the species, PMU-S13-3-20- (O/49/2). Scale bars equal 15 µm for 1 and 5; 25 µm for 2-3; 50 µm for 4 and 1a; 5 µm for 4a-4b. All illustrated specimens are housed in the collections of the Museum of Evolution, Palaeontological Section, at Uppsala University, and are marked by prefix PMU-, followed by the Shanxi collection number S13-1-11, etc., and the England Finder coordinates (in brackets) in biological microscopic slides orientated to the right side by their labels.

FIGURE 2– Light photomicrographs of *Dictyosphaera macroreticulata* in various states of preservation (1-9). 1, Specimen with internal body, PMU-S9-2-1- (O/44/3). 2, Vesicle empty inside, PMU-S9-2-12- (X/40/1). 3, Fragment of the vesicle wall with polygonal meshwork, showing the highest resistance to degradation, PMU-S9-3-2- (C/21). 4, 6, Pattern of penta-and hexagonal fields with different diameters on the single vesicle wall exposed in broken specimens, (4) PMU-S5-1-1- (K/53/4), (6) PMU-S5-1-1- (W/51/1-2). 5, Broken vesicle showing the disintegrating polygonal fields on the right side, PMU-S5-1-1- (M/53/2). 7, PMU-S13-1-33- (B/30/1). 8, PMU-S13-1-31- (Z/32/2). 9, PMU-S11-1-1- (W/49/4).

Scale bar in (1) equals 20 µm for 1, 2, 4-9, and scale bar in (3) equals 20 µm for 3.

FIGURE 3– Scanning electron microscope (SEM) images of *Dictyosphaera macroreticulata* (1-10). 1-2, Complete specimens, PMU-S12-1-18; PMU-S12-1-24. 3, Fragment of the vesicle showing the wall thickness, PMU-S13-1-1. 4-6, 8, Internal side of the vesicle wall with polygonal platelets, which are beginning to disintegrate (4-5), and their sides are seen at the lower central part of image in (4) and the lower right part of image in (8). Specimens PMU-S12-1-30b; PMU-S12-17b; PMU-S13-1-14a; MPU-
S13-1-9a, respectively. 7, 9, Polygonal pattern with positive relief of ridges seen on the external side of the vesicle wall. Specimens PMU-S13-1-6a; PMU-S13-1-11b, respectively. 10, Fragment of the wall with disintegrating polygonal platelets. Specimen PMU-S12-1-30a. Scale bar in (1) equals 20 µm for 1-3, otherwise stated for each image.

FIGURE 4– Cell wall structure of the Dictyosphaera macroreticulata in SEM images (1-4). 1, Internal wall surface with pattern of polygonal ridges without preservation of platelets. PMU-S12-1-18b. 2, Internal wall showing hexagonal and pentagonal platelets of various diameter beginning to disintegrate from their original tight alignment, thus showing their sides and thickness. PMU-S12-1-10d. 3a, 3b, Internal wall surface with details of platelets morphology in three-dimensional appearance, seen underneath a broken vesicle wall (lower part of 3a). Possible bacterial growth on the platelet (3a). PMU-S12-1-10e. 4, Two-layered wall seen from the interior of the vesicle consists of the inner layer formed by interlocked platelets (secondary wall) and the outer layer with low polygonal ridges forming pits in which the platelets were located (primary wall). Both layers are exposed in the lower right corner of the image, where disintegrating platelets are detached from the pits but still lying on the outer layer. Gradual taphonomic alteration of the wall is seen in the upper right corner, where individual platelets are fading away and only polygonal ridges are still visible (scaffolding of the wall). Wall breakage develops along the angular edges of the polygonal platelets (2, 3b, 4).

FIGURE 5– Light photomicrographs of Shuiyousphaeridium macroreticulatum in various states of preservation but with well-preserved processes (1-12). 1, Specimen showing thin solid processes with surrounding membrane forming distally flared portion (lower right side). PMU-S13-2-4- (D/46/2). 2, PMU-S12-1-14- (D/47-1). 3, PMU-S12-2-6- (P/48/3). 4, 7, Some processes are interconnected. PMU-S11-3-7- (N/50/4); PMU-S13-1-15- (S/21/1). 5, PMU-S11-3-22- (F/37/3). 6, PMU-S12-2-7- (S/50). 8, PMU-S12-1-34- (O/30). 9, PMU-S12-2-8- (X/48/3). 10, PMU-S12-2-9- (F/40/1). 11, PMU-S12-2-1- (X/34/3). 12, PMU-S12-2-3- (M/33/1). Scale bars equal 25 µm for all images.
FIGURE 6– Process morphology in *Shuiyousphaeridium macroreticulatum* in light photomicrographs (1-4) and SEM images (5-7). 1, PMU-S11-3-7- (N/50/4). 2, PMU-S12-1-34- (O/30). 3, PMU-S12-2-4- (D/46/2). 4, PMU-S13-1-15- (S/21/1). 5, PMU-S12-1-19b. 6, PMU-S12-1-21a. 7a, 7b, PMU-S12-1-28. Enlarged fragment of the vesicle with processes (7b) from the lower part of specimen in (7a). Scale bar in (3) equals 10 µm for photomicrographs in (1-3). Scale bar in 4 equals 15 µm. Otherwise stated in each SEM image.

FIGURE 7– Wall structure of microfossils in the *Dictyosphaera-Shuiyousphaeridium* plexus, Fossil morphotypes differ by the absence/presence of processes respectively, but have identical wall structure (1, 3). Two layers in the microfossils’ wall are inferred to constitute the primary wall (PW) and the secondary wall (SW) of the cyst stage, by comparison to wall structure in extant microalgae. Taphonomic changes, notably the compression of spheroidal cells, deformation of the walls and disintegration of platelets causing their separation, are herein distinguished from true morphologic and structural features. Length and thickness of processes, vesicle dimension and the wall structure elements and thickness are not to scale.

FIGURE 8– Reproductive life cycle model reconstruction for *Dictyosphaera-Shuiyousphaeridium*, based on reproductive cycles of modern chlorophytes. 1, Zygote, start of the sexual reproduction. 2, *Shuiyousphaeridium* morphotype, cyst shrinkage produces processes. 3, Outer membrane is discarded, meiosis occurs. 4, Haploid offspring is released through the excystment structure. It may fuse into another zygote (9) or form a resting cyst (5). 6, Start of asexual reproduction and resting cyst formation, mitosis occurs in *Dictyosphaera* morphotype. 7, Offspring is released through the excystment opening. 8, Flagellated haploid offspring. 9, Fusion into a zygote.

TABLE 1– Distribution of *Dictyosphaera-Shuiyousphaeridium* characters among protists.
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| Gromia sphaerica            |              |                |             |       |          | +           |

| Melicerion poskilon         |              |                |             |       |          | "honeycomb membrane" |
| Theratromyx webberi         |              |                |             |       |          | +           |

| acetolyis resistance        | +            | +              | n/a         | n/a   | n/a      | -           |
| property                    |              |                |             |       |          |             |
| cyst/spore formation        | +            | +              | +           | +     | n/a      | +           |
| processes                   | +            | +              | +           |       | process-like extensions |
| excystment structures       | +            | +              | -           | -     | n/a      | oral opening |
| complex life cycle          | +            | +              | +           | +     | n/a      | +           |
| alternation of generations  | +            | +              | +           | +     | n/a      | n/a         |
| undisputed                  |              |                |             |       |          | +           |
| Precambrian fossil record   | +            | -              | -           |       | single occurrence |

| Gromia sphaerica            |              |                |             |       |          | +           |
I. SEXUAL

II. ASEXUAL

1. Shulysphaeridium / zygospore

2. Dictyosphaera / resting cyst

3. n

4. n

5. n

6. n

7. n

8. n

9. n

2n
REPRODUCTIVE CYST AND OPERCULUM FORMATION IN THE CAMBRIAN-ORDOVICIAN ALGAL MICROFOSSILS AND THEIR SEASONAL BLOOMS

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Abstract: Unicellular organic-walled microfossils from the early Cambrian Lükati Formation and the Tremadocian Varangu Formation exposed in Estonia have been interpreted as reproductive cysts of the green algal phytoplankton. Both microfossil assemblages reflect the evolutionary history though the early Palaeozoic: from the Cambrian radiation of morphologically innovative taxa to increasing in diversity and more disparate Ordovician forms. Combined light transmitted and scanning electron microscopy on the Tremadocian galeate plexus acritarchs Caldariola, Prisciogalea and Stelliferidium, revealed exceptionally preserved morphological elements and rare structure among fossil and extant microbiota – an opening with operculum (lid) in reproductive cyst, in addition to lavish vesicle ornamentation and sculpture. Analogous morphology is observed in the extant algae of Dasycladales (Chlorophyta), where it is determined by an intrinsic lid-forming apparatus during the organism’s reproductive stage. Unique morphology of operculum-bearing microbiota would have required a degree of intracellular complexity for its development, suggesting advanced intracellular machinery present already in the early Palaeozoic phytoplankton. A new Genus and Species A of minute, sphaeromorphic and aggregated microfossils, which possess a perforated by nano-scale pores vesicle wall and corrugated sculpture, is discovered. These early Cambrian microfossils have diagnostic characters of prasinophyte algae.

Key words: Cambrian, Ordovician, galeate microfossils, cyst-formation, operculum, phytoplankton

The Cambrian radiations’ and Ordovician diversifications’ events established biodiversity, both in microbial and metazoan clades, that gave rise to the Phanerozoic biosphere. The preceding Proterozoic, including famous Ediacaran, organisms were mostly swept away by the end-Ediacaran extinction and left open ecological niches for newcomers (Vidal and Moczydłowska -Vidal, 1997; Grey, 2005; Knoll et al., 2006; Moczydłowska, 2008a; Vickers-
Rich and Komarower, 2007; Fedonkin et al., 2007). The vacant and new ecologic niches were
extended by global marine transgressions and climate fluctuations, and enhanced by sea-water
chemistry change and nutrient influx (Wilde and Berry, 1986; Brasier, 1991; Barnes, 2004;
Nielsen, 2004; Canfield et al., 2007; Shields-Zhou and Och, 2011). They provided suitable
life conditions for evolutionary burst of photosynthetic primary producers (Moczydłowska,
1991, 1998; Martin, 1993; Molyneux et al., 1996; Butterfield, 2001; Servais et al., 2004) and
heterotrophic consumers (Cowie and Brasier, 1989; Zhuravlev and Riding, 2001; Erwin and
Valentine, 2013).

The early Cambrian radiation of phytoplankton occurred in the stepwise sequence of
evolutionary events and the second such event is well documented in the Lükati Formation
(Moczydłowska, 1991, 2011), whereas the Tremadocian radiation in the Varangu Formation
(Paalits, 1995), both successions exposed in Estonia. These two palynological records of
organic-walled microfossils (acritarchs), which are very well-preserved and demonstrate
innovative morphological and developmental ontogenetic features, are investigated here and
interpreted in terms of reproductive and life cycles analogous to extant green microalgae.

The studied microfossil assemblages reflect the trends of the early Palaeozoic
phytoplankton development. Early Cambrian microbiota is characterised by minute to
medium-sized sphaeromorphic vesicles or those with pores, short and solid processes or
marginal fringes, as well as acanthomorphic vesicles with hollow, simple, divided or
branching processes (Moczydłowska, 1991, 1998). They substantially differ from the large
Proterozoic microbiota with dissimilar and lavish processes and sculpture (Grey, 2005; Knoll
et al., 2006; Moczydłowska and Nagovitsin, 2012; Xiao et al., 2014). Characteristic species of
the early Cambrian radiation, such as species of Asteridium, Comasphaeridium,
Lophosphaeridium, Tasmanites, Spiagia, and Archaeodiscina, are abundant in the Lükati
Formation. These taxa are recognized as members of green microalgae, prasinophytes and
chlorophytes (Talyzina and Moczydłowska, 2000; Moczydłowska, 2010; Moczydłowska et al., 2011). We also informally describe a new taxon in this assemblage, which is a minute vesicle preserved in clusters, with a prominent sculpture and pores in the wall. Microfossil’s size classifies it as a member of the picoplankton, adding to a wider array of photosynthetic biota and making marine ecosystem more similar to modern, which is dominated quantitatively by bacteria and picoplankton (Bradbury, 1998; Trujillo and Thurman, 2005).

Although the early Cambrian is marked by recovery of phytoplankton diversity, invention of new body plans and ecological expansion offshore (Moczydłowska, 2002; 2011), prominent increase of disparity begun at the Cambrian-Ordovician transition and continued through the Ordovician and early Silurian (Servais et al., 2008). Microfossils from the Varangu Formation, exhibit complex, derived morphologies such as polar vesicle asymmetry, ramifying processes communicating with the vesicle cavity, and the morphologically predetermined excystment openings with opercula. Many of the taxa with body plans characteristic of the Ordovician biota are not restricted to this period, but appeared already in the late Cambrian in Baltica (Volkova, 1990), Armorica (Martin, 1983), Laurentia (Martin and Dean, 1988; Martin 1984, 1992; Parsons and Anderson, 2000), and Gondwana (Rubinstein et al., 2003).

Some of the most prominent members of the Ordovician microbiota are microfossils of the so-called galeate (helmet-like) plexus, which are characterized by hemispheroidal-spheroidal vesicle and a large opening (pylome) with an operculum (lid) (Servais and Eiserhardt, 1995; Stricanne and Servais, 2002). It includes genera *Caldariola*, *Priscogalea*, *Stelliferidium* and *Cymatiogalea*, which were first discovered over half a century ago. They were established to differentiate between palynomorphs with large polar openings with opercula, and processes (with the exception of *Caldariola*) distributed randomly (*Priscogalea*,...
*Stelliferidium*, or in a polygonal pattern (*Cymatiogalea*) on the vesicle surface. These organisms were recently compared to chlorophytes and dinoflagellates (Servais et al., 2004).

Our studies focus on unique morphological structures observed in the Estonian galeate microfossils, which are rare among organic-walled biota, and which reflect their function and aid in understanding of fossil’s palaeobiology and affinities.

**MATERIAL AND METHODS**

Present study is based on light transmitted microscope (LM) and scanning electron microscope (SEM) observations of abundant and taxonomically diverse organic-walled microfossils of the early Cambrian and early Ordovician, i.e. Tremadocian, ages from Estonia. The two distinct and age-diagnostic assemblages comprise species listed in Appendix 1, and certain guide-species characteristic respectively of the Cambrian undefined Stage 3, and the Varangu Regional Stage, which is correlated with the upper part of the Tremadocian Stage.

Microfossils have been recovered from two samples from the Lükati Formation exposed in the Kunda Quarry, Ida-Virumaa County (samples number 987 and 988), and one sample of the Varangu Formation from the excavation section by Museum of Art, Kadriorg, Tallinn (sample number 992) by standard palynological technique. Samples were macerated in the Global GeoLab, Ltd., Alberta, Canada, following the laboratory procedure of dissolving 50 g samples and preparing un-oxidized kerogen slides and palynological permanent strew-slides. Additionally, organic residue remaining from previous studies of the Lükati Formation in the Kopli Quarry, Tallinn, and extracted by chemical processing at Micropalaeontological Laboratory at Uppsala University (Moczydłowska, 2011), provided some microfossils described here, including a new taxon.
The early Cambrian Lükati Formation consists of thinly bedded and weakly-consolidated claystone, which is almost horizontally lying and accumulated in shallow marine environments below the wave base, and is thermally and diagenetically unaltered (Mens and Pirrus, 1977; Mens et al., 1990). The depositional and burial conditions rendered the preservation of microfossils exceptional, which is further shown by the preservation of a new taxon of picoplanktonic microfossils (minute in size and at c. 10 μm or less in diameter) and in clusters as they were probably positioned in life. The Tremadocian Varangu Formation consists of clays with inter-layers of silty sandstone with glauconite, which are poorly lithified and not affected by diagenesis to any recognizable degree. The sediments represent shallow water facies with low rate of subsidence in the epicontinental basin at the margin of the Baltica palaeocontinent (Paškevičius, 2007; Dronov et al., 2011). Preservation of microfossils in this formation is comparable to these in the Lükati Formation, i.e. without thermal alteration or deformation, and allows observations of delicate wall ornamentation (processes, microsculpture) and wall structures, which show various developmental phases in reproductive cysts of discrete species. Such exceptional taphonomic state and abundance of microfossils provide unique opportunity for reconstruction of life cycle in ancient species.

The relative age of the strata studied is well established by the successions’ superposition in a regional scale and by fossil content. The Lükati Formation belongs to the Schmidtiellus mickwitzi Zone of the Cambrian biostratigraphic subdivision in Baltica (Mens et al., 1990; Moczydłowska, 1991), which by correlation with the global chronostratigraphic stages may be within the time interval of c. 521-514 Ma (Moczydłowska, 2011; Gradstein et al., 2012). The Varangu Formation, which defines the Varangu Regional Stage and is time-equivalent to the upper part of the international Tremadocian Stage, belongs to the Bryograptus broeggeri–Kiaerograptus graptolite regional zones, and the upper part of the Cordylodus lindstromi–Iapetognathus fluctivagus conodont stratotypic zone (Paškevičius,
2007; Nestor et al., 2007). Alternatively, the Varangu Stage is correlated with the *Ceratopyge acicularis-Shumardia pusilla* trilobite zone (Pärnaste et al., 2013). The time interval of the Tremadocian Stage is bracketed between c. 485 and 477 Ma (Gradstein et al., 2012).

LM observations and optical photography were done on an Olympus BX50 microscope with Olympus UC30 camera. SEM images are obtained from a Philips XL30 microscope and specimens were coated with gold (22 nm). The microfossil collection (microscopic slides and SEM stubs) is stored in the Palaeontological Section of the Museum of Evolution at Uppsala University, and signed with the prefix ME. The position of the specimens in the microscopic slides is provided by England Finder coordinates with the slides oriented with their labelled edge to the left side on the microscope stage.

**MICROPALAEONTOLOGY**

*General characteristic of microfossils*

Elements of morphology in Proterozoic and early Palaeozoic organic microfossils are characteristic of eukaryotic reproductive cyst (Tappan, 1980; Lacalli, 1981; Moczydłowska, 1991, 2010; Servais et al., 2004; Moczydłowska and Willman, 2009; Moczydłowska et al., 2011). Vesicle wall resistant to diagenetic processes and acid treatment in laboratory suggests cyst’s protective role, while the processes have likely been formed by vesicle contraction from the outer membrane during the process of cyst formation, and opening in the vesicle functions as the excystment structure for the release of offspring cells, as in extant green algae (Evitt, 1985; Tappan, 1980; Moczydłowska, 2010).

The trend of body-size reduction characteristic of the Cambrian phytoplankton in comparison with large dimensions predominant in the terminal Ediacaran is a typical phenomenon observed after major extinctions through the Phanerozoic records among both micro- and macrobiota (Jablonski, 1986; Urbanek, 1993; Richards and Wright, 2002; Brayard
et al., 2010). After the end-Ediacaran phytoplankton crisis, diversity of microfossil species increased through the Cambrian exceeding the Proterozoic levels in disparity and taxonomic quantity (Vidal and Moczydłowska-Vidal, 1997; Mullins et al., 2005). Characteristic species of the early Cambrian radiation, such as species of *Asteridium*, *Comasphaeridium*, *Heliosphaeridium*, *Alliumella* and *Estiastra*, are small by a microfossil standard, but others are of regular. Morphologically disparate and of regular sizes are species of *Lophosphaeridium*, *Tasmanites*, *Skiagia*, and *Archaeodiscina*, and they are abundant in the Lükati Formation.

The Ordovician diversification of microbiota is evident by radical change in body plan and the appearance of numerous new morphotypes. Representative are the taxa of the galeate plexus possessing a unique feature, the morphologically predetermined, round opening with operculum (lid). Such morphologic element is known in extant chlorophyte algae and its formation, which is regulated in cell ontogeny, has been well studied in *Acetabularia mediterranea* (Neuhaus-Url and Schweiger, 1984; see below).

**Species concept in acritarchs**

Recognition of distinct but strictly morphological genera and species has been widely used in taxonomy of microfossils, including acritarchs, and those having well-constrained by other index fossils and short stratigraphic ranges in biostratigraphy. However, when considering the microfossils palaeobiology: developmental ontogenetic stages and environmental variants, taxonomy of certain microfossils may need to be revised and they could be lumped into less numerous taxa. Consequently, re-evaluation of their biostratigraphic ranges would follow. It is clear that insignificant differences between the morphotypes could have been induced by environmental conditions, such as temperature and salinity (Servais et al., 2004) and, as such, should be treated with caution in biostratigraphy.
This concerns the galeate plexus, which comprises a substantial number of superfluous taxa, which are difficult to recognize objectively. Some of the species are conspecific and inferred to represent developmental ontogenic stages and ecological variants (Servais and Eisenhardt, 1995; Servais et al., 2004).

Although similarity to modern plankton and general cyst-like body plan have been observed, the organic-walled microfossils have been classified under the informal group of acritarchs, meaning “of uncertain origin” and refraining from natural systematics (Mendelson, 1992; Colbath and Grenfell, 1995). Increasingly, the traditional “acritarch” taxa are being attributed to known clades (chlorophytes, prasinophytes, cyanobacteria, even metazoan embryos (Moczydłowska, 1991; Colbath and Grenfell, 1995; Servais et al., 1997; Kaufmann and Xiao, 2003; Wicander, 2002; Grey, 2005; Moczydłowska and Willman, 2009; Moczydłowska et al., 2011; Xiao at al., 2014). Certain form-genera and form-species of various ages are recognised as developmental stages of a single biological species (Servais and Eisenhardt, 1995; Servais et al., 2004; Moczydłowska, 2010; Agić and Moczydłowska, 2014). Thus the number of named species seems to be exaggerated, if accepting the possible biological conspecificity between fossil taxa.

Modern analogues to fossil phytoplankton among chlorophytes and dinoflagellates show high disparity of cyst morphology in a single biological species (Kokinos and Anderson, 1995; Reyes and Head, 2013). The same may be assumed for ancient species. The case study of the Cambrian genus *Skiagia* has shown that several form-species represent developmental cyst stages and that they belong to a single biological species or at least less numerous biological species (Moczydłowska, 2010).

The issue of taxonomic over-splitting arises in fossil record because of the uncertainties in recognizing the entire life cycle of ancient microbiota. Only the cysts of organic-walled plankton have preservation potential due to their acetolysis-resistant wall, permitting only one
life stage to be represented in the rock record, without clear evidence of the vegetative stage. The problem of recognising natural species is a significant issue in palaeontology (Allmon, 2013), even more when dealing with organisms that become fossilised only during the reproductive stage. Considering the great morphological similarity between the members of the galeate-plexus and the identical mechanism of lid formation, we assume their close affinities and identical reproduction mode, likely as the members of dasycladalean green algae. Recognizing the developmental stages possible belonging to a single biological species (Stricanne and Servais, 2002) would lead to reduction of the number of fossil species and suggest potential conspecificity for *Caldariola*, *Priscogalea* and *Stelliferidium*. However, the differences between some galeate species, could also reflect the radiation of phytoplankton underway in early Paleozoic.

*The early Cambrian assemblage*

The taxonomic diversity of the Lükati microfossil assemblage is a result of the early Cambrian radiation that is revealed by the first appearance of numerous ornamented species and morphologically innovative body plans. This diversification event is remarkable in comparison to the preceding association of microfossils from the basal Cambrian, which consisted of ornamented (*Lophosphaeridium*) and process-bearing taxa (*Asteridium* and *Comasphaeridium*), but only a few species and of much lower grade of disparity and biodiversity (Moczydlowska, 1991). The Lükati assemblage (Volkova, 1968, 1969; Moczydlowska, 2011) and the time-equivalent records from other geologic sites document true radiation of phytoplanktonic microbiota and their rapid global dispersal (Moczydlowska, 1998, 2002; Zang et al., 2007). Assemblages of this age are recorded across the palaeocontinents of Baltica, Laurentia (including Greenland, Svalbard and Scotland), Gondwana (Turkey, Armorica and Iberia), Siberia, China and Australia (Downie, 1982; Knoll
and Swett, 1987; Moczydłowska and Vidal, 1988; Baudet et al., 1989; Vidal and Nystuen, 1990; Moczydłowska, 1991, 1998, 2002; Palacios and Vidal, 1992; Vidal and Peel, 1993; Vidal et al., 1995; Vidal and Moczydłowska-Vidal, 1997; Moczydłowska and Zang, 2006; Zang et al., 2007, Jachowicz-Zdanowska, 2013). Present study adds to this variety of microfossils a new species of minute size and unusual wall sculpture, and well preserved and abundant specimens of *Comasphaeridium molliculum*, that have never been observed in such details. The new species shows diagnostic features of prasinophyte algae and extends the spectrum of previously recorded microalgal and cyanobacterial fossils (Kirjanov, 1974; Jankauskas, 1975; Moczydłowska, 2011). The phytoplankton these microfossils are inferred to represent, have thrived in the early Cambrian marine ecosystems, which were rapidly developing and becoming more structured with the evolving faunas and a complex food web (Moczydłowska, 2002; Butterfield, 2001; Erwin and Valentine, 2013).

The outbreak of phytoplankton diversity occurred in pair with the Cambrian explosion of metazoans and was likely tied to the metazoan radiation and increase in the predation pressure. It has been argued that the early Cambrian diversification of primary producers fuelled the explosion (Vidal and Moczydłowska-Vidal, 1997; Moczydłowska, 2002; Vecoli et al., 2005) and initiated the trophic chain revolution (Servais et al., 2008). The subsequent predatory innovations and metazoan ecological expansion into pelagic realm as mesoplankton (Butterfield, 2001) might have led to rise in phytoplankton disparity and evolution of complex Ordovician forms.

We briefly describe the species recorded here from the Lükati Formation to summarise their morphologic disparity and features, pinpointing the reproductive function of microfossil cysts and supporting their phylogenetic position among the green microalgae (Talyzina and Moczydłowska, 2000; Moczydłowska, 2010; Moczydłowska et al., 2011). The order of species description follows the morphological grouping and complexity, not natural
systematics, and their biological affinities are commented on below. These species are common and distributed worldwide, and their occurrence in the Lükati Formation conforms to stratigraphic ranges recognized elsewhere by co-occurring diagnostic faunas (Moczydłowska, 1991; 2002; Moczydłowska and Zang, 2006).

Generic grouping *Leiosphaeridia* spp. includes spherical specimens with translucent wall without any sculpture, roughly 20-150 μm in diameter. They show excystment opening by partial rupture or median split. Genus *Leiosphaeridia* Eisenack 1958 emend. Downie and Sarjeant 1963, is a morphologically broad taxon, where the species recognition is highly subjective. The taxon comprises polyphyletic biota and counts at least two classes of green microalgae as revealed by ultrastructural studies: prasinophytes and chlorophytes (Talyzina and Moczydłowska, 2000; Moczydłowska and Willman, 2009; Moczydłowska et al., 2011).

Among taxa without ornamentation or sculpture is *Leiovalia tenera* Kirjanov 1974, which is diagnosed by oval, elongated, thin-walled vesicle with round poles. The species is recognizable by a combination of consistent shape and dimensions, ranging 43-70 μm in short axis and 105-144 μm in long axis of vesicle (Kirjanov, 1974). Studied specimens are 38-126 μm (n=3) in diameter. *Leiovalia* ranges through the upper part of the traditional lower Cambrian to middle Cambrian (Volkova et al., 1983; Jachowicz-Zdanowska, 2013). The occurrence in the Lükati Formation indicates its even earlier range, recorded here for the first time. Following the tentative correlation of the Cambrian stages with biozones of the Baltica palaeocontinent, the species range is within undefined Stage 3 to Stage 5 of the Cambrian System (Moczydłowska, 2011).

*Granomarginata squamacea* Volkova 1968 is a species characterized by the Saturn-shaped vesicle consisting of spheroid central body surrounded by spongy margin in the equatorial zone. The observed overall diameter is 45 μm, internal body diameter is 22-25 μm, and width of marginal rim is 9-11 μm (n=2). The genus *Granomarginata* Naumova 1960
shows morphology typical of the prasinophyte resting cyst (phycoma), and this feature prompted the assumption on the microfossil affinity (Tappan, 1980; Strother, 1996; Rubinstein et al., 2003; Moczydłowska et al., 2011).

Genus *Lophosphaeridium* Timofeev 1959 ex Downie 1963, emend. Lister 1970 is recognised by a slightly ornamented vesicle surface. Two species are recorded here. Spheroidal, thin-walled vesicle of *L. tentativum* Volkova 1968 (Fig. 3J) is covered by small granular sculpture c. 1 µm in size, and vesicle diameter ranges 31-63 µm (n=7). The excystment opening is by partial rupture or median split. *L. truncatum* Volkova 1969 differs by having clavate coarse grains as elements of sculpture, and its observed diameter is 47-59 µm (n=2). Excystment structure was not observed. This species is recorded for the first time in the Lükati Formation, but its stratigraphic range is consistent with this new occurrence.

Among species of *Tasmanites* Newton 1875, a group of microfossils characterized by spheroidal vesicles with wall perforated by pores, which is a diagnostic feature of modern prasinophytes, we record *Tasmanites bobrowskae* Ważynska 1967 (Fig. 3G, 5J), *T. tenellus* Volkova 1968, and *T. volkovae* Kirjanov 1974 (Figs. 3I, 4D, 5I). The species are differentiated by size and distribution of pores, which are terminations of hollow radial canals in the wall, and have been previously recognized in LM, SEM and TEM studies (Ważynska, 1967; Kirjanov, 1974; Talyzina and Moczydłowska, 2000; Moczydłowska, 2011). *T. bobrowskae* has thick-walled, but translucent vesicle with large pores distributed randomly. Pores are separated by up to 3 µm, and wall thickness is consistent over the entire vesicle. Observed diameter is 60-127 µm (n=6). *T. tenellus* is thin-walled bearing small and evenly distributed pores. Observed diameter is 67-147 µm (n=2). *T. volkovae* shows a thick wall (2 µm) perforated by conspicuous pores (0.5 µm), distributed evenly on the vesicle and separated at ~2 µm distance. Pores are located within small depressions of the wall making the vesicle outline wavy. Observed diameter is ca. 120 µm (n=34). Dimensions of all
observed species are within the previously recorded ranges, and the excystment occurs by partial ruptures.

Acanthomorphic species are dominant in this assemblage. Regardless of their size and shape of process tips, which are diagnostic features of discrete species, the processes show random, although often regular, distribution on the vesicle wall without any geometrical pattern. Principle characteristic of the processes used in taxonomy is their nature, being solid or hollow, and in the last case being connected with the vesicle cavity or separated by a plug structure or the wall (Eisenack et al., 1973; Tappan, 1980; Moczydłowska, 1991, 1998; Mendelson, 1992; Strother, 1996). The earliest Cambrian acanthomorphic species are consistently of very small dimensions (many below 20 µm) and are attributed to the form-genera *Asteridium* Moczydłowska 1991 and *Comasphaeridium* Staplin, Jansonius and Pockock 1965, characterized by having solid processes. They mark the base of the Cambrian and their new species evolved throughout the Cambrian (Moczydłowska, 1991). Interestingly, species of *Asteridium* maintained their small sizes, whereas subsequently appearing new species of *Comasphaeridium* were larger and these are recorded in the Lükati assemblage.

*Asteridium tornatum* (Volkova 1968) Moczydłowska 1991 recorded here is a minute spheroidal vesicle evenly covered by short, solid spines c. 1-2 µm in length. The vesicle diameter is 10-20 µm. Fossils may occur as single cells or in clusters of planar sheets, and this was interpreted to represent natural colonies, not taphonomic accumulation (Moczydłowska, 2011). Such colonies of small cells with sculpture or ornamentation are known among modern green microalgae of the order Chlorococcales (Batten, 1996). Phenotypic resemblance of *Asteridium* fossils to chlorococcalean colonies suggests its chlorophycean affinity (Moczydłowska, 2011). The new Genus and Species A recorded here is comparable to *Asteridium* by dimensions, clustering and presence of wall sculpture, which in LM may
appear similar to *A. tornatum*. However, SEM observations reveal a very different
morphology.

New Genus and Species A is discovered in the Lükati Formation and characterised by
individual minute spheroidal vesicles, 6-12 µm in diameter, without ornamentation but with
conspicuous surface sculpture and pores in wall, which are assembled together into elongate
clusters (Figs. 3D, 4A-B, 5A). This arrangement is, however, much different from many
erlier (Precambrian) cluster-like forms of *Myxococcoides*-type, which are typically bacterial
cell clusters bound together by a secreted sheath. The Lükati clusters do not show any
evidence of a sheath structure, are generally larger than individual bacterial cells, exhibit
vesicle sculpture, and have complex wall ultrastructure. Individual spheres are thin-walled
and transparent with an uneven, corrugated surface pattern. SEM observation has revealed
elaborate wall sculpture on a nanometre scale. Outer surface of the thin vesicle appears
creased and is covered by distinct wrinkles (Fig. 4A), which suggests a very flexible wall.
Entire vesicle, including corrugations, is perforated by tiny, round pores.

Cell clustering suggests colonial mode of life. Similar algal clusters are known across
the green algae, specifically in the order Chlorococcales and Volvocales (Batten, 1996; Lee,
2008). Planar clusters of organic-walled microfossils with or without ornamentation have
been reported previously in Cambrian successions, and were formed by several taxa, such as
*Cymatiogalea* (Rasul, 1974), *Leiosphaeridia* cluster (Fatka and Vavrdová, 1998), *Asteridium
tornatum* (Moczydlowska, 2011), acanthomorphic clusters of *Heliosphaeridium* and
*Pterospermella* (Jachowicz-Zdanowska, 2013) and unidentified “acritarch colony growths”
(Harvey et al., 2011). Rasul (1974) has illustrated an unnamed specimen (*Priscogalea*
morphotype) from the Upper Cambrian of Belgium (fig. 3.6, *ibidem*), which strongly
resembles thin and corrugated surface of the Lükati clusters presented here and its processes
are not conspicuous. The author further argued that these clusters were likely formed prior to fossilization rather than due to the process itself.

The genus *Comasphaeridium* is clearly distinguished by abundant processes, which are thin, flexible and tightly arranged. *C. brachyspinosum* Kirjanov 1974 (Fig. 5F) has spheroidal vesicle ca. 30 µm in diameter (single specimen), bearing numerous relatively short, thin, homomorphic processes 3-6 µm in length. Processes are solid and filamentous with pointed tips. Excystment opening was not observed. *C. molliculum* Moczydłowska and Vidal, 1988 (Figs. 3A, 5E, 5G) differs by having long, filiform and flexible processes and its vesicle is larger, ranging 40-80 µm in diameter observed here (n=27). Process length is 10-18 µm. Excystment opening is by median split.

Another prominent acanthomorph in the assemblage is the genus *Globosphaeridium* Moczydłowska 1991, diagnosed by spheroidal vesicle with numerous short, solid processes. *G. cerinum* Volkova 1968 (Fig. 3B-C, 3F) processes are distantly distributed, thick and spine-shaped, slightly widened at the base and tapering to pointed tips. They are 3 µm in length and vesicle diameter is c. 30 µm (n=18). Vesicle may be smooth or sculptured by tiny globular granules 1-2 µm thick. Excystment occurs by median split.

The genus *Skiagia* Downie 1982 emend. Moczydłowska 1991 is the most distinct among forms possessing spheroidal vesicle with hollow processes terminated by funnel tips, which do not communicate with vesicle interior. Its species are a major component of the early Cambrian assemblages and stratigraphically diagnostic for specific biozones. The *Skiagia* plexus comprises numerous morphotypes, which may not only show distinct fossil species but also developmental stages in a life cycle of a single biological species (Moczydłowska, 2010). Three species are recorded herein: *Skiagia compressa* (Volkova 1968) Downie 1982, *S. orbicularis* (Volkova 1968) Downie 1982 (Fig. 3E), and *S. ornata* (Volkova 1968) Downie 1982. *S. compressa* shows long, slender and cylindrical processes
with broad conical bases, which form wavy outline of the vesicle, and wide funnel-like tips. Plug structures at the base of processes separate their interior from the vesicle cavity. Vesicle diameter is 26-43 µm, process length is 5-9 µm (n=2). S. orbicularis has medium-length tubular processes, tapering towards small funnel tips, and processes are separated from vesicle cavity by the vesicle wall. Vesicle diameter is 22-53 µm (n=10), and process length is 5-10 µm. S. ornata has numerous, long, slender processes, cylindrical in shape and slightly tapering towards the tips, which are conspicuously widened and funnel-shaped. Process interior is separated by vesicle wall from its cavity. Vesicle diameter is 25-40 µm (n=11), and process length 10-20 µm. All observed Skiagia species have excystment opening by partial rupture or median split.

Double-walled vesicle occurs in Archaeodiscina Naumova 1969 emend. Volkova 1968. A. umbonulata consists of inner, spheroidal body with a single star-like, solid and convex structure on its surface and thin, translucent outer membrane surrounding the inner body. Walls of both the internal body and the outer membrane are psilate. Mostly preserved are specimens consisting only the inner body. Overall vesicle diameter is 23-43 µm (n=6). The excystment opening was not observed in studied specimens but in others occurs via median split of the inner body (Moczydłowska, 2011).

Another double-walled taxon is Pterospermella Eisenack 1972, characterised by having spheroidal inner body surrounded by an outer translucent and thin membrane. The membrane may be stretched in the equatorial zone or may surround the entire inner body. Pterospermella sp. recorded here shows thick-walled inner body and thin outer membrane, which is equally distant from the inner body. Overall vesicle diameter is 42-65 µm, inner body diameter is 34-53 µm, and distance between the walls 5-7 µm (n=1). Body plan of microfossil Pterospermella is remarkably similar to modern phycomata of prasinophytes and its genus Pterosperma (Parke et al., 1978; Tappan, 1980; Mendelson, 1992).
Large carbonaceous fossil fragments are common in the assemblage, and resemble problematic metazoan elements described as possible disassembled molluscan radula and the jaw apparatus (Harvey et al., 2011). Horn-like carbonaceous fragments were described as Ceratophyton vernicosum Kirjanov 1979, and found in the Lower Cambrian strata from across the world (Fatka and Konzálova, 1995). Fragments are conical with acute tips, over 100 µm in length, hollow inside and terminally open with irregular edge, as if broken apart. While initially grouped with palynomorphs of uncertain affinities (Volkova et al., 1979), Fatka and Konzalová (1995) placed Ceratophyton into Crustacea, based on its great similarity to the copepod exopodite segments. Carbonaceous microfossils of the Ceratophyton type have been frequently recorded in the early Cambrian successions (Knoll and Swett, 1987; Moczydłowska, 1991, Fatka and Konzálova, 1995; Jachowicz-Zdanowska, 2013), but have also been found in the late Ediacaran (Moczydłowska, 2008b).

The Lükati Formation assemblage comprises taxa, which are among the green microalgae. Inferred by the phenotypic features, biochemical properties and the cell wall ultrastructure available in some cases, the genera Tasmanites, Granomarginata, Pterospermella, are members of the class Prasinophyceae, whereas Asteridium, Lophosphaeridium, Comasphaeridium, Globosphaeridium, Archaeodiscina, Skiagia and Polygonium are in the class Chlorophyceae (Talyzina and Moczydłowska, 2000; Moczydłowska, 2010; Moczydłowska et al., 2011). The least diagnostic and polyphyletic Leiosphaeridia comprises both classes, which are respectively represented by spheroidal phycoma cyst (Prasinophyceae) and vegetative cell transforming into cyst (Chlorophyceae) (Moczydłowska, 2011).
The Tremadocian assemblage

The Varangu Formation assemblage is dominated by species of the *Priscogalea, Stelliferidium* and *Caldariola* among the galeate plexus. The two former microfossils bear evenly distributed processes (Deunff, 1961, 1964; Deunff et al., 1974), and the latter is smooth or with minute processes (Servais and Eiserhardt, 1995). *Cymatiogalea*, which is distinct by having additional membrane between the processes and forming polygonal pattern of their distribution (Deunff, 1961), is missing in the assemblage.

Most galeate-plexus species appeared as the outcome of the Tremadocian radiation, with a few species ranging from the middle Cambrian (Górka, 1974; Rasul, 1974; Vecoli, 1999; Moczydłowska, 1998; Parsons and Anderson, 2000; Rubinstein et al., 2003).

Diacromorphic microfossils, another morphologic grouping with polar distribution of processes on the vesicle, are also common in the studied assemblage and are characteristic of the Ordovician strata. Their earliest record is known from the Cambro-Ordovician transitional interval. A few acanthomorphic taxa with prominent bases of processes, which are diagnostic taxonomically (*Polygonium* and *Vulcanisphaera*), are present in the Varangu assemblage. All recorded here species have wide palaeogeographic distribution (Moczydłowska and Stockfors, 2004; Servais et al., 2004), what is typical of modern marine phytoplankton (Reynolds, 2006; Lee, 2008), as well as observed in ancient phytoplankton records (Molyneux, 2009; Le Hérisse et al., 2009). The late Cambrian-Ordovician biotic turnover is shown by the extinction of many Cambrian taxa, while certain taxa extended into the early Ordovician. However, survivors also became shortly extinct, and the major radiation of morphologically more complex taxa occurred at the beginning of Tremadocian, notably the galeate plexus (Servais et al, 2004).

New record from Estonia shows a great variety of morphotypes and transitional forms between recognizable species as well as developmental phases of cyst maturation. Genus
Actinodissus is an example of the new diacromorphic taxa radiating during the transitional Cambrian-Ordovician time interval and constituting the distinctive Ordovician biota. It is characterised by a polar body plan in which processes are situated on the opposite ends of an elongated, rounded or sub-angular vesicle, and are in communication with the vesicle interior. Two species are recognised in the Varangu material.

Actinotodissus achrasii Martin 1973 (Fig. 5K) is defined by a process-bearing, translucent vesicle, circular to elliptical in outline. Sharp-tipped processes of 7-12 μm in length, are located in groups of 4-6 on the two poles. Excystment occurs by median split. Processes are hollow and open towards the vesicle cavity, which distinguishes the genus Actinotodissus from similar acritrachs with polar processes (diacromorphs) such as Acanthodiacrodium (Moczydłowska and Stockfors, 2004).

Actinotodissus scytotomillei Martin, 1973 (Fig.5L) originally identified as Acanthodiacrodium scytotomillei, is a thin-walled, rectangular vesicle bearing six long processes on each pole, ca. 10 μm in length. The processes are hollow, in communication with the vesicle cavity and flexible, thus giving the appearance of curved tips. Round excystment structure is observed in A. scytotomillei, suggesting its role as a cyst.

Dasydiacrodium obsonum Martin 1988 is another taxon with polar distribution of processes, but unlike Actinodissus, its vesicle is assymetrical, with one polar area larger than the other. Processes are shorter, ca. 5-10 μm in length, conical and elongate, and their numbers vary between the poles. Dasydiacrodium is mostly known from the Upper Cambrian strata (Moczydłowska and Stockfors, 2004) and may represent an ancestor to true diacromorphs.

Polygonium martinae Moczydłowska and Crimes 1995, is a common acanthomorphic species in the lowermost Palaeozoic successions. Its vesicle is polygonal in outline, bearing
several conical and elongate processes 15 µm in length, which form wavy outline of the vesicle.

Another representative taxon of the Ordovician microbiota is *Vulcanisphaera africana* Deunff, 1961 (Fig. 5M). It is diagnosed by distinct, composite processes with multiple tips. Each process extends from the vesicle as a wide conical projection, hollow and in connection with the vesicle cavity. The distal part of the process is radically different from the basal and described as a “secondary process” (Rasul, 1976). It ramifies from the conical base as two thin, flexible, filamentous processes, 7-12 µm in length. Only the bifurcating secondary processes are observed in this material, but their number may range up to five (Rasul, 1976; Martin and Dean, 1981). The vesicle is spheroidal and smooth on surface, ca. 40 µm in diameter, and occasionally possesses wall thickenings in a shape of polygonal fields. *V. africana* is distributed worldwide, and common in the Lower Ordovician, but also found in the upper Cambrian strata (Martin, 1982).

The simplest among the galeate-plexus microfossils is *Caldariola glabra* Martin, 1973 (Figs. 2I-L). It differs from other galeate taxa in the absence of processes, or having only minute ones. The vesicle is circular in outline and smooth, 21-38 µm in diameter, and thick-walled (1.5-2.0 µm). Processes do not exceed 2 µm. Specimens often possess excystment opening with an operculum (ca. 15 µm in diameter). *C. glabra* is a typical species of the Baltica palaeocontinent, characteristic of the Cambrian-Ordovician transitional interval and first occurring in the upper Cambrian strata (Volkova, 1990).

The genus *Priscogalea* Denuff, 1961, consists of 13 species (Stricanne and Servais, 2002), which are defined by different length and distribution of processes. *Priscogalea* vesicle is spherical in outline (hemispherical in cases of a ruptured cyst phase), bears divided processes and, in some cases, excystment opening with an operculum. In a few well-preserved specimens, a thickened rim around the opening is observed, resembling a collar (Figs. 1B-D).
Operculum itself is also process-bearing (Figs. 1A-C, 1I, 1K, 2A). Wall thickness is variable (0.7-1.1 µm), which may indicate a different phase in the cyst maturity. Processes disparity also varies, which was the main criterion for the establishment of numerous species. By analysing morphological parameters in galeate taxa, e.g. process length, vesicle diameter, absence/presence of membrane or striae, Stricanne and Servais (2002) have shown that all Priscogalea species belong to the common size and shape cluster.

There are five species in the Varangu assemblage. Priscogalea barbara Denuff, 1961 (Figs. 1A right, 1H-I, 2A-B) is the type species. Vesicle is spheroidal (or hemispheroidal when opened), approximately 35 µm in diameter and bearing randomly distributed, thin and filamentous processes divided at the distal end. Process length is 7 µm. Opening is circular in outline and the operculum is found either attached or collapsed into the vesicle. Specimens without an operculum show a thickened rim around the opening.

Priscogalea distincta Rasul, 1974 (Fig. 1.B.) has the most complex processes observed in Priscogalea. They are wide and long at ca. 6 µm, with multifurcating terminations. Rim around the rounded excystment opening is very thick (4 µm) and well-defined. Varangu specimens are slightly larger, ca. 40 µm in diameter, than P. barbara, P. fimbria and P. simplex from the same material.

Priscogalea fimbria Rasul, 1974 (Figs. 1K) is characterised by a spheroidal vesicle about 40 µm in diameter and wide filamentous processes with divided terminations, usually longer, at ca. 8 µm, wider and up to 2 µm, and more flexible than the type species. Rounded operculum is a common structure, persistent in shape and size, ca. 20 µm in diameter. Operculum often bears processes itself (Fig. 1I). In some specimens, the vesicle displays a faint granular sculpture (Fig. 1F-H).

Priscogalea simplex Denuff, 1964 emend. Rasul 1974 (Figs. 1A, 1C, 1F-G, 2C-F) vesicle diameter is 35 µm. It is distinguished from other species of the genus by a thinner wall
and shorter processes, 3-4 µm in length, with sharp acicular or divided terminations. *P. simplex* may form clusters comprised by up to 17 individual specimens (Rasul, 1974). Rimmed opening with an operculum is observed.

*Priscogalea cuvillieri* Deunff, 1961 (Figs. 1D, 1J, 2G-H) is diagnosed by very short, stout processes, ca. 1-3 µm in length, with conical bases. The vesicle of 35-40 µm in diameter, possesses a thin wall. Overall vesicle size and smooth surface, aside from tiny processes, is similar to the sphaeromorphic galeate *Caldariola* (also seen in Marin, 1973, plate IX.6) and this species could be a transitional form in the development of these galeate cysts. Due to a well-defined operculum, slightly polygonal in some specimens, this species was emended as *Cymatiogalea cuvillieri* by Rasul (1974).

Genus *Stelliferidium* Denuff et al., 1974 was erected to distinguish specimens with larger processes and striate bases from that of *Priscogalea* and it comprises 22 species (Stricanne and Servais, 2002), only one of which is recorded here. *Stelliferidium trifidum* Rasul, 1974 (Fig. 1E) has vesicle 35-40 µm in diameter and with densely distributed, long (6-11 µm), hollow processes trifurcating at the tips and a large opening with an operculum (15-20 µm in diameter). Bases of processes are wide and conical, with star-shaped striations extending into the vesicle wall. In places, striae are placed very close giving the appearance of processes joined by sutural ridges. For this reason, *S. trifidum* was identified as *Cymatiogalea trifida* (Górka, 1964). However, unlike *Cymatiogalea*, which bears regularly arranged processes in a polygonal pattern, processes in *Stelliferidium* are randomly distributed across the vesicle surface, more like in *Priscogalea*.

The Estonian assemblage of operculum-bearing microfossils reveals different cyst development stages and possible palaeoenvironmental-seasonal variations. Great number of microfossils in the material possessed an opening (Figs. 1A, 2A-L, 3A, 3G, 3I, 5C, 5K-L), or an opening with an operculum (Figs. 1B-K). Openings in the cyst are formed during
maturation for the release of gametes and/or offspring cells, but are rarely preserved (Tappan, 1980; Raven et al., 2005). Accumulation of very abundant organisms with excystment opening, i.e. mature cysts, could represent an event of high reproduction rate over a short time interval necessary for burial and preservation. Fast and mass reproduction cycle in extant phytoplankton is known as the algal blooms and occurs seasonally, during water mixing and/or due to high nutrient input into the environment, or periodically as the natural environmental fluctuations (Guillard and Heliebust, 1971; Paerl, 1997; Lee, 2008; Dobson and Frid, 2009). The Varangu assemblage of microfossils could be interpreted as the record of algal bloom in reproductive cycle and could reflect the existence of regular seasonal changes at the Cambrian-Ordovician time interval. The latter possibility is consistent with the palaeogeographic position of the Baltica palaeocontinent at c. 30-60° palaeolatitudes of southern hemisphere during the Cambrian-Ordovician times (Torsvik and Rehnström, 2001; Cocks and Torsvik, 2002). Ecological conditions in shallow epicontinental seas around the continental masses in temperate climatic zone with seasonal temperature fluctuation, would prompt phytoplankton blooms, as observed today.

GALEATE PLEXUS: CONSPECIFICITY VERSUS BIODIVERSITY
The galeate-plexus taxa described over the last 50 years, account 84 species attributed to four form-genera: Caldariola, Priscogalea, Stelliferidium and Cymatiogalea (Stricanne and Servais, 2002). Their stratigraphic ranges are largely concurrent in the intervals of the upper Cambrian to lower Ordovician strata, with a single record of Stelliferidium from the middle Cambrian (Palacios, 2008). The latter specimen lacks characteristic striation, but shows long and branching processes resembling Timofeevia taheddirtensis described by Vanguestine and van Looy (1983). It seems that all genera appeared in the upper Cambrian (Vanguestine, 1967
in Rasul, 1974, Dean and Martin, 1982), with more complex galeates (some *Cymatiogalea* species) diversifying through the Ordovician radiation (Rasul, 1974).

The main diagnostic feature differentiating particular galeate species is process morphology – shape of terminations and bases, presence or lack of striae extending into the vesicle wall, their length, width, and density of distribution. Other features, such as wall thickness, vesicle diameter and size of the opening and operculum, are less variable. Due to similarities, Deunff (1974) proposed two “units” for galeate acritarchs, one with polygonal ornamentation (*Cymatiogalea*) and the other with process striation (*Stelliferidium*), while attributing *Priscogalea* to those groupings (Servais and Eiserhardt, 1995). *Priscogalea* was retained by Rasul (1974) after critically reviewing differences between *Priscogalea* and *Cymatiogalea*.

Stricanne and Servais (2002) studied morphologic variability of the galeate plexus by taking a statistical approach. Their evaluation of morphometric parameters used to describe galeate acritarchs and subsequent multivariate and cluster analyses have shown the current oversplitting state of galeates’ taxonomy. Instead, the fossil species were shown to fall into four broad morphological clusters, with intermediate forms present between each of them. Process length was recognised as the most variable parameter. Furthermore, Servais et al. (2004) have shown increase in process length and diversity of galeate assemblages through the transitional Cambrian-Ordovician succession studied in the borehole from Algeria. The authors have interpreted these morphological variations in processes as an environmental signal, following studies on laboratory cultures of extant dinoflagellates showing significant variability of features in response to environmental fluctuations (Dale 1976, 1996; Kokinos and Anderson, 1995).

Transitional morphotypes between galeates advocate their potential conspecificity, as seen in members of *Priscogalea* and *Stelliferidium* form-genera. Vesicle with the shortest
processes (ca. 2μm) is attributed to *P. cuvillieri* (Figs. 1D, 2G-H) and represents a transitional form at the overlap of the *Caldariola* and *Priscogalea* morphological clusters. A continuous trend in process length increase is seen through *P. simplex* (Fig. 1A), *P. barbara* (Fig. 1H-I), *P. fimbria* (Fig. 1K) and the elaborate *P. distincta* (Fig. 1B). The clear contrasting character between the most similar form-genera, *Priscogalea* and *Stelliferidium*, is a star-shaped striation extending from the process bases into the vesicle wall in *Stelliferidium trifidum* (Fig. 1E). More developed processes (longer, branching) are indicative of the cyst maturity, potentially making *Stelliferidium* an end-member of the two fossil taxa development.

Monospecific genus *Caldariola* contains two morphotypes (smooth and with short processes identical to *P. cuvillieri*) described throughout the literature and occasionally assigned to other galeate taxa (Montenari and Servais, 2000).

Increasing variability in complexity of microfossils within the assemblage and transitional forms between the taxa may be explained as the record of different phases of development and maturation of the cyst in a single biological species (Servais et al., 2004). However, the studied Estonian assemblage only illustrates a short time interval and may also represent a larger evolutionary diversification of the galeate acritarchs.

Taxonomically, the galeate species have not been revised or synonymised since 1991 (Fensome et al., 1991), and although some have been transferred between the genera or claimed as conspecific, their diversity inflated (Servais and Eiserhardt, 1995; Stricanne and Servais, 2002; Servais et al, 2004). Thorough taxonomic revision is needed for a more reliable recognition of fossil taxa and their ontogenetic stages among the galeate plexus microfossils, which is beyond the scope of this paper. Based on available material from very short stratigraphic interval we may recognize some developmental stages belonging to less numerous biological species preserved in the assemblage. However, the appearance of such
variability in fossil cysts of the galeate plexus could be evidence of speciation of phytoplanktonic microorganisms alongside development of complex life during the Cambrian and Ordovician radiations. This is a significant evolutionary event and diversification of green microalgae to which the microfossils are affiliated (see below), and one of the earliest ulvophycean algae or their ancestral lineage recognised in the fossil record.

Changes in morphology of extant microalgal cysts are environmentally driven in short-time intervals (seasons, periodic anomaly). After environmental recovery the cyst morphotypes (ecophenotypes) return to normal morphology characteristic of species (Dale, 1996; 2001). These differences in morphology are variable in a short-time amplitude and in regional context. The long term or permanent global environmental changes induce adaptive morphological changes, which are observed in a linear pattern through time and lead to speciation. The increase in disparity of process shape among the galeate taxa over tens of million years (c. 27 Ma) in the late Cambrian-Tremadocian transition, shows a steady evolutionary trend – a speciation event. This trend might have been related to global environmental change, which induced adaptive radiation (see in different ecophenotypes) and gave rise to several species of the galeate-plexus.

While rapid changes in the cyst morphology are observed in Cenozoic and modern dinoflagellates (Kokinos and Anderson, 1995; Reyes and Head, 2013), exact behaviour of the ancient, early Paleozoic algae is uncertain.

Galeate genus *Cymatiogalea* has not been observed in the Estonian material. *Cymatiogalea* is the most diverse of the galeate morphotypes, accounting 47 species of high disparity and complex morphologies (Stricanne and Servais, 2002). It is characterised by polygonal patterning of processes distribution on the vesicle surface, polygonal opening, and in some cases, flanges stretched between the processes. Opening in *Cymatiogalea*, often polygonal instead of rounded, is unlike that of other galeates, and is determined by polygonal
pattern of wall thickenings, possibly indicating that a different mechanism of lid-formation might have operated. Moreover, complex cyst ornamentation of geometrical polygonal fields and flanges between the processes would have required a more regulated cyst formation than by contraction. This renders it dissimilar from other galeate acritarchs and a separate species, more distantly related than other galeate form-genera. While it could be argued that the polygonal patterning in *Cymatiogalea* is similar to paratabulation in dinoflagellates, there is no indisputable dinoflagellate fossil record until Mesozoic (Fensome et al., 1996).

**AFFINITY OF THE GALEATE PLEXUS MICROFOSSILS**

Galeate microfossils share morphological and biochemical characters with cysts of extant unicellular microalgae, such as resistant vesicle wall, processes, and excystment openings with operculum. Opercula preserved at different phase of cyst opening, unique in galeates, suggest a complex intracellular mechanism of their formation, similar to the extant chlorophyte alga *Acetabularia* from the Order Dasycladales of the Class Ulvophyceae (Menzel and Elsner-Menzel, 1990; Mandoli, 1998). Previously, ulvophycean algae have been identified in the late Tremadoc-Arenig (Nitecki et al., 2004; Young et al., 2007).

The lid is a part of the excystment structure in reproductive cyst and is a phenotypic feature of modern algae. We consider the operculum in microfossil cyst to be homologous character because of its remarkable similarity and consequently infer algal affinities of microfossils (see below). Complexity of the vesicle wall in microfossils and features such as processes and opercula, required intrinsic cellular machinery for cyst production and lid formation in a specific phase of maturity. Present day analogues among dasycladalean algae (Chlorophyta) develop the operculum in a young reproductive cyst via the intracellular lid-forming apparatus. Comparing morphological features between *Acetabularia* cysts and
seemingly homologous once in the galeate-plexus microfossils, it is possible that such intracellular apparatus existed in the Cambro-Ordovician eukaryotic phytoplankton and produced the characteristic Ordovician morphologies. Phenotypic features and metabolic processes, mode of life and reproductive cycle, that are comparable to modern biota, suggest fossils’ biological chlorophyte affinity.

Previously, Vavrdová (1992) has demonstrated remarkable similarity between the Ordovician organic-walled microfossil *Cheleutochroa gymnобрachiata* and cyst of the recent chlorophyceae alga *Tetraedron caudatum*. Both extinct and extant species have robust processes with wide bases forming striae as they are extending from the vesicle. Comparable processes with striate bases are observed in *Stelliferidium*, which is interpreted as the mature cyst. While processes in *Stelliferidium* are more numerous and densely distributed across the vesicle, their striate bases may reflect the similar stage of algal reproduction wherein the vegetative cell or zygotic union of cells functioning as gametes contracts to produce a protective cyst. *Stelliferidium* has been suggested to be the zygotic stage of chlorophyceans (Moczydłowska, et al., 2011).

Present-day analogues of galeate microfossils suggest their affinity to the phylum Chlorophyta, as exemplified by the chlorophycean alga (Vavrdová, 1992) and the derived ulvophycean alga (herein).

**OPERCULUM (LID) FORMATION**

Unicellular organic-walled microfossils, more extensively studied early Palaeozoic but also Proterozoic palynomorphs, are thought to represent the reproductive cyst stage in the life cycle of eukaryotes, more specifically green algae (Lacalli, 1981; Moczydłowska 1991, 2010; Servais et al., 2004; Moczydłowska et al., 2011). Major characters in ancient organic-walled
microfossils, e.g. acetylasis-resistant vesicle wall, processes and other elements of ornamentation, and excystment structure (openings in the vesicle) support the cyst stage interpretation. However, more significant for evolutionary history, the diagnostic phenotypic features can shed light on the biological affinity of the fossils. Thick walls with complex ultrastructure, openings and diverse wall ornamentation (processes) are structures formed in cysts during the reproduction of green algae (Tappan, 1980; Moczydlowska and Willman, 2009; Moczydlowska et al., 2011). Resistant cyst walls play a protective role in algal reproduction and processes are formed as the cyst contracts from its external membrane, preparing for the cell division (Lee, 2008). Openings in the vesicle allow the release of gametes or offspring cells from the protective cyst. Morphology of the openings is variable from simple cyst ruptures as a median-split or a slit (eptyche), to regular round or subangular openings (pylome or archeopyle) that occasionally possess neck-like structures or opercula (Tappan, 1980; Vavrdová, 1992; Volkova, 1993). In some cases (Fig. 1F, 1I, 2H), the opening is surrounded by a low collar.

Operculum (or a lid in algal terminology) is a significant morphological character in acritarchs, helpful for diagnosing taxa and conclusive of the vesicle’s reproductive function. It is seldom preserved and only found in the minority of organic-walled phytoplanktonic fossils (Tappan, 1980; Mendelson, 1992), being exceptionally rare in the earliest Phanerozoic. However, operculum is a persistent feature in galeate acritarchs and present in some Cambro-Ordovician microfossil species (Servais et al., 2004). We consider the operculum in microfossil cysts to be a homologous character to lid in modern algal cysts, and will further refer to them alternatively to make a distinction between the ancient and extant taxa.

Operculum-bearing microbiota, such as the Middle Ordovician acritarchs Asketopalla and Polyanistotrodorus from Oklahoma (Loeblich and Tappan, 1971), and Silurian Cymbosphaeridium from England (Lister, 1970) usually have simple round openings with
remnants of the lid-structure that is either completely lost or collapsed inside the vesicle. Operculum attached at one side of the pylome, or unopened and fully attached to the rest of the vesicle may also be preserved, as seen in the Cretaceous Operculites and Oligocene Cyclopsiella (Tappan, 1980). However, one of the best examples of the operculum structure in phytoplanktonic microfossils is the galeate plexus, with morphospecies that exhibit a full formation of cyst’s operculum: from the initial formation as a fault line on the vesicle’s surface, to a separate, fully or partially detached operculum. All galeate genera Caldariola, Priscogalea, Stelliferidium and Cymatiogalea are characterised by a well-defined, round (or semi-polygonal in Cymatiogalea) openings with an either attached or collapsed operculum (Stricanne and Servais, 2002; Servais et al., 2004; present study).

Very similar morphology may be observed in the lid-bearing cyst of the green alga Acetabularia mediterranea (Neuhaus-Url and Schweiger, 1984; Menzel and Elsner-Menzel, 1990; Mandoli, 1998). Genus Acetabularia presently inhabits subtropical marine regions and it belongs to the derived chlorophyte order Dasycladales (Olsen et al., 1994; Lee, 2008). Despite being a unicellular organism, Acetabularia can produce very large and complex body forms composed of three main features: 1) rhizoid-bearing “foot” where the nucleus is located; 2) a long and narrow stalk supporting the 3) flat and disk-like cap which releases the reproductive cysts (Koop, 1975; Tappan, 1980; Lacalli, 1981; Mandoli, 1998). Unlike the vegetative/adult organism, the cyst is very simple: small and spherical, without surface ornamentation, but with a thick double wall. A round lid is formed on the cyst’s surface and daughter-cells are released through the opening, upon the lid detachment initiated by increase in cell’s hydrostatic pressure (Koop, 1975).

Acetabularia has been used as a model organism for the study of lid formation in green algae (Neuhaus-Url and Schweiger, 1984). Electron microscopy in A. mediterranea revealed circular banding in the peripheral cytoplasm of the 1-3 day old cyst, where the lid is about to
be formed. In the most mature cyst stage, this banded layer consists of rod-like structures (microtubules) and marks a specialised part of the cyst wall that will form a fault line (“circular microtubule band” observed with LM) and eventually a lid. When the lid-structure opens, gametes are released through the cyst opening. The cytoplasmic structure close to the plasma membrane containing both the amorphous layer and the underlying microtubule layer has been named the “lid-forming apparatus” (LFA). This mechanism of excystment is relatively rare and controlled by the cell’s secondary nucleus; the number of lids on a single cyst will be consistent with the number of nuclei within the *Acetabularia* cell (Lüttke and Bonotto, 1982). The purpose of a lid structure in green algae is a more efficient gamete release from a protective cyst (Menzel and Elsner-Mezel, 1990).

Such a degree of intracellular organisation is quite derived, yet there are parallels to be made between the morphology of *Acetabularia* cysts at different maturity stages and the galeate microfossils preserved in different states of the operculum formation and detachment. Only a single galeate morphospecies *Caldariola glabra* lacks processes, being morphologically almost identical to the smooth and thick-walled *Acetabularia* cyst. Other members of the plexus are characterised by short or long processes with split ends and in case of *Stelliferidium*, striae at the base of the processes. There are transitional forms between the end-member morphotypes, represented by *Caldariola*-like specimens with bumps and very short processes to *Priscogalea* with shorter processes (Fig. 1J, 2B, 2G-H, 2L). Identical pattern of the operculum formation however, is consistently found throughout the plexus and resembles the lid-forming process observed in modern *A. mediterranea* and *A. wettsteinii* (Neuhaus-Url and Schweiger, 1984).

While reproduction, cyst development and cell biology of extant algae are increasingly studied in cultures, such observations are impossible in fossil microbiota. Despite excellent preservation in the Estonian strata, any internal organelles, cytoplasm and intracellular
structures are preserved. They almost never occur in the fossil record with a few exceptions in the Ediacaran (Huldtgren et al., 2011; Schiffbauer et al., 2012) and Cryogenian (Marti-Muss and Moczydłowska, 2000). However, external features of the cyst vesicle may reveal significant information about intracellular mechanisms (Agić and Moczydłowska 2014, in press). Using the comparative morphology approach, galeate acritarchs may be placed in the green algae and a model of ancient operculum formation may be inferred.

There is no evidence of the vegetative stage of the algae forming the galeate cysts, but considering the present-day analogues, it was likely unicellular. During the reproduction, a protective cyst was formed that would eventually release the gametes or offspring cells. Defining characters of unicellular organic-walled microbiota, such as acetolysis-resistant wall, openings and processes are features of eukaryotic reproduction and most researchers accept acritarchs (especially acanthomorphs) as resting or zygotic cysts of eukaryotic phytoplankton (Tappan, 1980; Vavrdová, 1992; Colbath and Grenfell, 1995; Wicander, 2002; Servais et al., 2004; Moczydłowska and Willman, 2009; Moczydłowska, 2010; Moczydłowska et al., 2011).

The proposed model of operculum (lid) formation in the galeate plexus based on the lid-formation in modern *Acetabularia mediterranea* is as follows:

1) Reproductive, thick-walled cyst was formed inside the membrane-surrounded zygotic cell by secreting the secondary wall. The cyst contracted inwards from the membrane and the processes were formed. The outer membrane was discarded, with remnants preserved between the processes in rare cases (e.g. illustrated by Górka, 1969). In *Priscogalea* and *Stelliferidium* morphotypes, there is evidence of the outer membrane.

2) In a young cyst, a ridge-and-fault structure was formed on the surface of the vesicle, delineating the future opening (Figs. 1C-D). The mechanism may have been similar to LFA in *Acetabularia* whereby the secondary nucleus is organising microtubules near the plasma membrane to form a circular band. This banding eventually formed both external and internal
protrusions in the cyst wall (Neuhaus-Url and Schweiger, 1984, fig. 7), facilitating a controlled opening in the thick protective wall through which offspring may be released. In the present day analogue, this stage of the lid formation occurs in the 1-2 day old cyst.

3) Matured cyst had a fully formed operculum which started to detach (Figs. 1B, 1E). *Acetabularia* cysts reach maturity in 3 days.

4) The operculum began to open (Figs. 1F-G, 1I) and eventually becomes completely detached from the cyst (Figs. 1K, 2A, 2D). Offspring was released through the controlled opening.

5) In some cases, detached operculum collapsed into the emptied vesicle (Figs. 2B-C, 2F-L), or became completely separated from the organism, recorded as isolated opercula that may or may not bear any processes (Rasul, 1974).

Extant lid-forming algae mature within several days (Menzel and Elsner-Menzel, 1990). Operculum formation in the fossil species could have been formed equally fast, which could explain the assortment of stages preserved together in taphocoenosis. While the size of the lid-structure in *Acetabularia* is usually larger than in fossil galeates (40-60 μm as opposed to 10-15 μm in *Priscogalea*), the modern cell is slightly larger than the fossils and the overall size of the lid/opening is approximately 20-30% of the vesicle in most cases. Galeate operculum is uniform in size throughout the morphospecies and formation stages, which suggests that the lid formation in the cyst is developmentally predetermined and regulated by an intracellular mechanism, suggesting the presence of LFA or its analogues.

Significance of these striking similarities between modern and fossil cyst is the fact that such complex morphology and presumably intracellular mechanisms (LFA, secondary nucleus) may have been already present in the Ordovician.

Despite the derived complex morphology for a unicellular organism and intricate cyst-forming mechanism, the lineage of dasycladalean algae dates back to the early Palaeozoic.
Macroscopic receptaculitid Dasycladales were found in the late Tremadocian strata of Estonia (Nitecki et al., 2004), Ordovician strata of Manitoba, Canada (Young et al., 2007), and Silurian of Ontario, Canada (LoDuca, 1997). While only the cysts were studied in this material and morphology of the adult alga and its position in the order Dasycladales (based on comparison to Acetabularia) remains uncertain, complex elements of the cyst are characteristic of the phylum Chlorophyta.

CONCLUSIONS

The Cambrian and the Ordovician periods were a time interval of significant biodiversification of the eukaryotic phytoplankton, reaching the peak of diversity in the terminal Ordovician and Silurian (Strother, 1996), and paving the way to modern biota. Following the end-Ediacaran extinction of large ornamented microbiota, the Cambrian palynomorphs have undergone recovery and steadily increased in diversity, which further escalated with the appearance of the innovative Ordovician forms.

Microfossil assemblages uncovered from the early Cambrian Lükati Formation and the Tremadocian Varangu Formation in northern Estonia, reflect this evolutionary trend. Cambrian microfossils are morphologically innovative, of high disparity and various size classes, including pico- and microplankton (Moczydłowska, 1991; Moczydłowska and Vidal, 1992; herein), and reaching unprecedented number of at least 250 new species (Mullins et al., 2005; Moczydłowska, 2011), in parallel to the evolution of complex fauna and trophic dynamics. In the Lükati assemblage, we record a new Genus and Species A, which has features diagnostic of prasinophyte algae. We extend the range of Leiovalia tenera to a lower stratigraphic level within the lower Cambrian, previously known from the transitional lower-middle Cambrian series.
The Cambrian radiation was followed by further rise in disparity during the Cambrian-Ordovician transition and subsequent Ordovician phytoplankton diversification (Servais et al., 2008), characterised by origin of microorganisms with polar vesicle symmetry, additional elements of ornamentation, and excystment openings with opercula.

Among the most abundant microfossils of the Tremadocian Varangu Formation are the sphaeroidal and process bearing acritarchs of the galeate plexus. Unique morphological characters, rare in other organic-walled microbiota, reflect their function as a reproductive cyst as observed in the extant microalgae, and provide significant information on organism’s palaeobiology. Furthermore, there are transitional forms between the four galeate genera showing a natural progression in the cyst maturation. Some morphologic variants may also result from changes in local environmental conditions (ecophenotypes) in a similar way as the modern dinoflagellates are affected by salinity changes (Mertens et al., 2009). Abundance of fossils preserved as reproductive cysts with openings could represent the seasonal events of algal blooms.

Galeate-plexus microfossils possess an additional diagnostic character of their cyst morphology, an operculum, which is rare among microfossils due to low preservation potential. Such character is present in the modern dasycladalean algae. Operculum (lid) allows a more regulated release of offspring through the opening in the resistant cyst wall. The observations on the cyst formation in modern unicellular chlorophyte *Acetabularia mediterranea*, revealing the intrinsic intracellular mechanism necessary for operculum formation, are used to infer similar process in studied microfossils. The lid-forming apparatus (LFA), consisting of actin microtubules as a regulatory mechanism that creates a future opening in the walls of a young cyst of *Acetabularia* (Neuhaus-Url and Schweiger, 1984), is suggested to operate in the ancient microorganisms.
Remarkably preserved Estonian assemblage consists of fossils at different stages of the operculum formation; from the delineated opening in a fully closed cyst, and the operculum becoming disconnected from the rest of the vesicle, to the fully detached operculum that collapsed into the vesicle interior, which mirrors the operculum formation stages in *Acetabularia*. Due to these shared morphological features reflecting the organism’s life cycle and ontogenetic alteration, it is likely that a similar mechanism of operculum formation existed in the eukaryotic phytoplankton at the transitional Cambrian-Ordovician time interval. Galeate microorganisms attributed to *Caldariola*, *Priscogalea*, *Stelliferidium* and *Cymatiogalea* would have required a significant degree of intracellular complexity to be able to produce cysts of such unique morphology.

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FIGURE CAPTIONS


FIG. 2. Excystment openings with dislocated opercula in the galeate plexus acritarchs *Priscogalea* (A-H) and *Caldariola* (I-L) in light microscopy. A-B, *Priscogalea barbara* with A, dislocated process-bearing operculum, PMU-L988 (T42/0) and B, operculum collapsed inside the empty vesicle, PMU-L988 (M36/0). C-F, *P. simplex*, excystment openings and operculum inside the vesicle. C, PMU-L988 (M34/0), view through the excystment structure.


Scale bar represents: A, B, C, D, F, H, 10 μm; E, 5 μm; G, I, J, 20 μm.

**FIG. 4.** Wall structure and wall sculpture details of the Lükati formation microfossils in SEM. A-B, New Genus and Species A, PMU-Kob2-55; A, view of the whole vesicle; B, magnification onto vesicle wall corrugations with pores. C, granulate surface sculpture of *Globosphaeridium cerinum*, PMU-Kob2-33. D, hollow radial canals in the wall of *Tasmanites volkovae*, PMU-Kob1-34. Scale bar represents: A, 2 μm; B, 3 μm D, 10 μm.
