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LIFE CYCLE OF EARLY CAMBRIAN MICROALGAE FROM THE *SKIAGIA*-PLEXUS ACRITARCHS

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ABSTRACT—Light microscopy studies on new materials and museum collections of early Cambrian organic-walled microfossils, informally called acritarchs, provide the observations on phenetic features that permit a comparison to certain Modern microalgae and the recognition of various developmental stages in their life cycle. The microfossils derive from various depositional settings in Estonia, Australia, Greenland, Sweden, and Poland. The exceptionally preserved microfossils reveal the internal body within the vesicle, the endocyst, and the process of releasing the endocyst from the cyst. Vegetative cells, cysts, and endocysts are distinguished, and the hypothetical reconstruction of a complex life cycle with the alternation of sexual and asexual generations is proposed. Acritarchs from the *Skiagia*-plexus are cysts, and likely zygotes in the sexual generation, which periodically rested as “benthic plankton.” Some microfossils of the *Leiosphaeridia*-plexus that are inferred to be vegetative cells were planktonic and probably haplobiontic. These form-taxa may belong to a single biological species, or a few closely related species, and represent the developmental stages and alternating generations in a complex life cycle that is expressed by polymorphic, sphaero- and acanthomorphic acritarchs. The morphological resemblance and diagnostic cell wall ultrastructure with the trilaminar sheath structure known from earlier studies suggest that the early Cambrian microfossils are the ancestral representatives and/or early lineages to the Modern class Chlorophyceae and the orders Volvocales and Chlorococcales.

INTRODUCTION

PHOTOSYNTHETIC ORGANISMS that are commonly termed algae are amazing in their phenotypical diversity and metabolic versatility; they live in salt, fresh, and brackish waters, soils, within ice and terrestrially. Their greatest biomass is in the oceans, but their species diversity dominates that of all plants in the freshwater environments. All algae derived, however, from marine ancestors, although they do not comprise a monophyletic group (Raven et al., 2005; Chapman and Waters, 2006; Fehling et al., 2007). They adapted to aquatic terrestrial environments in the Palaeozoic but have a long evolutionary history in the marine realm beginning in the Proterozoic (Knoll et al., 2006) or Archean (if cyanobacteria are included; Altermann and Schopf, 1995). The phylogeny of algae is ancient as revealed by the fossil record (Knoll et al., 2007) and by molecular clock analysis (Hackett et al., 2007; Turmel et al., 2008), but it remains poorly understood at its early branches.

The algae do not comprise a single high-level systematic unit, such as kingdom. Indeed, the common conception of algae includes all photosynthetic organisms from Bacteria (cyanobacteria) to many single and multicelled, water-dwelling Eukarya. Cyanobacteria are prokaryotic autotrophs with blue-green chlorophyll, which “invented” photosynthesis. The eukaryotic algae, single- or multicelled, are more complex and obviously larger, are all photosynthetic, and are sexual organisms (Falkowski and Raven, 2007). By transferring electron energy from sunlight to living systems, they, along with a number of bacterial groups, link the inorganic world with the biosphere. The evolution of unicellular algae has led to a complex life cycle with the alternation of sexual and asexual generations that may differ greatly from each other in morphology (polymorphic).

The red, brown, golden, and green algal lineages arose by endosymbiosis and acquired their plastids by primary, secondary, or tertiary symbiosis (Baldauf, 2003; Falkowski and Raven, 2007; Hackett et al., 2007). Those of “green lineage” include the classes Prasinophyceae, Chlorophyceae,

Ulvophyceae, and Charophyceae (which gave rise to terrestrial plants) (Mattox and Stewart, 1984; Melkonian, 1989; Guilloux et al., 2004; Lee, 2008). Green algae were the progenitors of the subsequent diversification of plants because of the efficiency of their biochemical pathway of photosynthesis (O’Kelly, 2007). In addition, their reproduction, which included a sexual cycle, resulted in an exponential increase in biodiversity (Margulis et al., 1989). Algae are essential to life on this planet and are the source of much of the oxygen we breathe (Chapman and Waters, 2006). They are beautiful in form and function—organisms that are fascinating for research.

The complete life cycle of living algae is understood in only a few taxa; in fossil taxa it is even more uncertain and difficult to uncover. However, new discoveries of microfossils with such diagnostic features as endocysts and cysts with excystment structures provide a new basis for reconstructing the complex life cycle of certain Cambrian microalgae.

The early Cambrian microfossils discussed here are determined to be unicellular, eukaryotic, marine, planktonic, and photosynthesizing organisms and thus are interpreted to be algae. Their cells are microscopic, in a range of a few tenths to over 200 micrometres in diameter, and many are elegantly ornamented (Moczydłowska, 1991; Zang, 2001). In terms of informal taxonomy, they have been incorporated with other organic-walled microfossils of polyphyletic origins in a group termed acritarchs (Tappan, 1980; Wicander, 2007). Optical microscopic studies of these microfossils from various depositional settings through geological time have been carried out in order to determine their functional morphology and phenotypic traits. These features are particularly important in elucidating the biological affiliations and life cycle of acritarchs. The ultrastructure of cell walls and biochemistry are combined with functional morphology as a proxy for recognizing their biological affinities.

Observations on unpublished materials from the Lükati Formation of Estonia and re-studied museum collections from South Australia, Greenland, Sweden, and Poland have proven to be complementary and conclusive. These materials

provided new evidence for reconstructing the developmental stages in a complex life cycle that included sexual reproduction in the Cambrian *Skiagia*-plexus microorganisms. The new specimens of microfossils actually show the release of the endocyst *in status nascendi*. The presence of endocysts within cysts is vital evidence that documents the reproduction cycle and supports the algal affinity of studied acritarchs.

MATERIALS AND METHODS OF STUDY

New light transmitted microscopy (LM) studies on acritarchs from the early Cambrian Lükati Formation have been performed as a continuation of the search for their palaeobiology and biological affinities. The exceptional preservation of organic-walled microfossils in this succession earlier prompted the examination of several acritarch form-species for their detailed morphology and cell wall ultrastructure by scanning electron microscope (SEM) and transmission electron microscope (TEM; Talyzina and Moczydłowska, 2000), and allowed the recovery of biomarkers (Moldowan and Talyzina, 1998).

The samples are from the thin-bedded claystone of the Lükati Formation in the Kopli Quarry, Tallinn, Estonia, which is assigned to the *Schmidtellus mickwitzii* Chron (Moczydłowska, 1991). These horizontally lying, shallow marine sediments are relatively soft, weakly compressed and insignificantly diagenetically altered despite a ca. 520–500 Ma estimated age (Gradstein et al., 2004). Chemical processing of the samples by hydrochloric (HCl) and hydrofluoric (HF) acids followed the standard palynological technique and the permanent microscopic mounts were made with the epoxy resin Epotek 301.

Museum collections allowed for complementary studies, and are notable for providing “the missing links” for the reconstruction of the complete acritarch life cycle. These collections are from the early Cambrian successions in South Australia, Sweden, Greenland, and Poland. Acritarchs in these collections are in various states of preservation because of different geological and thermal histories of the successions and their palaeogeographic settings. The successions are broadly of early Cambrian age (Moczydłowska, 1991; Vidal and Peel, 1993; Moczydłowska et al., 2001; Moczydłowska and Zang, 2006; Zang et al., 2007).

The illustrated specimens from the Lükati and Buen formations are stored in the collections of the Museum of Evolution at Uppsala University. The acritarchs from the Kaplonosy-Radzyn formations are in the collections of the University of Lund, Geology Department. The collection from the Arrowie Basin is housed in the Core Library, Department of Primary Industries and Resources, Geological Survey of South Australia at Adelaide. The position of the specimens in the microscopic slides is given by England Finder coordinates, with the slides oriented with their labelled edge to the left side of the microscopic stage.

GENERAL COMMENTS ON ORGANIC-WALLED MICROFOSSILS

This report is concerned with acritarchs, which are interpreted to be eukaryotic and autotrophic biota. Its scope is thus limited within the spectrum of organic-walled microfossils, which is highly diverse and heterogeneous, and includes prokaryotes, unicellular auto- and heterotrophic eukaryotes, and parts of multicelled plants and animals.

The morphological term “vesicle” describes a sac-like body with an internal cavity in organic-walled microfossils, including acritarchs, dinoflagellates, chitinozoans and some other unicellular biota, without reference as to whether it is a stage

of a vegetative cell or a resting/reproductive cyst in their life cycle (Tappan, 1980; Strother, 1996; Traverse, 2007). Despite the lack of knowledge of its function, the vesicle is understood to be a fossilized single cell. In living dinoflagellates, if recognized, it is then referred to as theca and cyst, respectively. The fossil record of the dinoflagellates represents one stage in the life cycle—the cyst (Dale, 1996). Acritarchs are assumed to comprise mostly resting stages, but not exclusively, and some are vegetative cells (Tappan, 1980; Moczydłowska, 1991). In chitinozoans, the vesicle is composed, despite their name, of a non-chitin biopolymer and may represent the egg-case of unknown metazoans (Paris and Növlak, 1999).

In acritarchs and dinoflagellates, the vesicle wall (cell wall) is composed of a recalcitrant biopolymer, the so-called sporopollenin-like material, which is now better defined as algaenan, which survives early diagenesis and chemical maceration in acids (Evitt, 1985; Kokinos et al., 1998; Allard and Templier, 2000; Versteegh and Blokker, 2004; Marshall et al., 2005). Although predominantly synthesized in protective resting cysts, the sporopollenin/algaenan biopolymer (termed algaenan below) occurs also in vegetative cells of marine and freshwater algae (van den Hoek, 1978; Derenne et al., 1996; Gelin et al., 1997, 1999; Allard and Templier, 2000). Algaenan gives mechanical strength, rigidity, and impermeability to the cell wall, which contributes to its potential to be fossilized.

PALEOBIOLOGICAL DESCRIPTION AND FUNCTIONAL MORPHOLOGY

Microfossils of the Skiagia plexus.—A number of organic-walled form-species is well defined under the generic grouping of *Skiagia* Downie, 1982 emend. Moczydłowska, 1991. This grouping reflects their high interspecific disparity but also accommodates certain intraspecific variations (Downie, 1982; Moczydłowska, 1991, 1998; Vanguetaine et al., 2002; Moczydłowska and Zang, 2006). The genus is morphologically one of the most conspicuous among the Cambrian taxa, and it ranges from the late early Cambrian unnamed Series 2, provisionally called “Stage 3,” to the middle Cambrian “Stage 5.” It is characterized by an acanthomorphic vesicle (i.e., process-bearing) and funnel-shaped, distally closed tips of the processes, which are numerous, long, evenly distributed and hollow inside, but not continuous with the vesicle cavity. The vesicle may possess an opening due to rupture or circular pylome. The morphological trait of having widened process terminations is diagnostic of the genus and novel in the context of the Cambrian radiation of organic-walled microbiota. During this event, new ornamented morphotypes appeared following the end-Ediacaran extinction, which almost entirely eliminated the pre-existing phenetic diversity (Moczydłowska, 1991, 2008; Moczydłowska and Zang, 2006). The origin of funnel-shaped processes is not only the expression of a different type of construction of the vesicle with more lavish surface elements, but it records a more complex functional morphology that reflects evolutionary changes and emergence of new genera or clades of eukaryotic microbiota with complex life cycle and reproduction.

In general, the function of the processes, which are present in vegetative cells but mostly in cysts of modern organic-walled microorganisms such as planktonic microalgae as green algae and dinoflagellates, is variable and depends on the ontogenetic stage. However, there are species, as the green algae *Cosmarium turpinii* (van den Hoek, 1978; Bold and Wynne, 1985) and *Staurostrum gladiusum* (Winter and Biebel, 1967), which have spiny vegetative cells and beautifully ornamented encysted zygotes with even more elaborate

processes of a very different pattern than in the vegetative generations.

Algae have strategies for maintaining their cells in suspension by producing the shapes that reduce the sinking rate. Small round or oval cells have low inherent sinking rates, while larger and heavier cells achieve this effect by forming star-like shapes, elaborate shapes with appendages (spines, processes), and different types of radial symmetry (Hansen et al., 1996; Beakes, 2006). They may also fill their cells with nitrogen gas vacuoles (Beakes, 2006).

The function of the processes in vegetative cells is to maintain buoyancy in the photic zone so the cell can harvest the sunlight energy (Tissot and Welte, 1978). The processes also allow vertical migration (diurnal) deeper into the water column for mineral nutrients uptake, which is depleted during the day at the ocean surface. Many algae exhibit positive phototaxis and move toward water masses with proper light-wave level (e.g., some in Volvocales; Bold and Wynne, 1985; Hoops et al., 1999; Lee, 2008). The cell regulates the passive vertical movement by secreting an extra-cellular organic matter (mucus) between the processes and thus increases the cell weight (for sinking) and then discharges it (for rising). Elaborately shaped cells improve the efficiency of nutrient uptake (Pahlow et al., 1997), and the processes increase the surface/cell volume ratio for osmotic exchange of nutrients and gases with the environment (Moczyłowska, 2002; Lee, 2008). Spines and appendages also help deter predators (Harris, 1986).

In cysts, the function of the processes is initially to suspend the cyst inside the vegetative cell during its formation and maturation but also to allow the released cysts to sink to the bottom sediment for resting. The sinking of the cyst is achieved by increasing the cell density by secreting mucilaginous material between the processes (Lee, 2008). The processes are formed by contracting from the cell membrane while the cyst is produced and the cytoplasm contracts inside the vegetative cell (Tappan, 1980; Evitt, 1985; Kokinos and Anderson, 1995; Dale, 2001; Grey, 2005; Head et al., 2006; Falkowski and Raven, 2007). The terminations of the processes are attached to the inner surface of the vegetative mother cell before it is shed (ecdosized).

The mechanism of process formation has been observed in dinoflagellate cultures, although only in a very few species. These observations show that the vesicles with processes of a particular shape are typical for dormant cysts (Kokinos and Anderson, 1995; Dale, 2001; Head et al., 2006), and similar processes are observed in certain acritarchs. In many green algae (e.g., the Zygnematales), the processes covering the cyst wall (at zygote stage) are attached either to the enveloping spheroidal wall of the gametangia in which they formed (Bold and Wynne, 1985) or to vegetative cells (Winter and Biebel, 1967; Kies, 1975; Tassigny, 1971; Coessel and Texeira, 1974).

The excystment structure, which is an opening in the cell wall, is another morphological indicator of the vesicle function. It is known in modern chlorophytes, dinoflagellates and chrysophytes, which reproduce both sexually and vegetatively (Bold and Wynne, 1985; Evitt, 1985; Margulis et al., 1989; Lipps and McCartney, 1993; Fensome et al., 1996b). The excystment structure is formed in a mature cyst in order to release endocysts, miospores, or gametes, or in a vegetative cell for liberating cysts, aplanospores, autospores, or zoospores. The opening structures are present in all cysts, but only in some vegetative cells. This is because the vegetative cells are mostly dissolved in order to release the offspring cell(s) (Bold

and Wynne, 1985; Raven et al. 2005), and are not preserved. They are poorly preserveable in any event, due to a different biochemistry of the cell wall in contrast to the cyst wall. The shape of the excystment structure may be morphologically pre-determined as a circular or geometrically patterned pylome, sometimes with extra elements such as collars, lids or microsculpture, or may be as a median split or partial rupture.

Because the excystment opening is formed in both resting cysts (i.e., the occasional or periodic asexual generation induced by environmental conditions) and reproductive cysts (the regular sexual generation in a life cycle), as well as in vegetative cells, this feature alone is not conclusive for recognizing the stage in a life cycle stage of a microorganism.

There are several morphologically relevant traits persistently observed among *Skiagia* species that may indicate the function of the vesicle. These are: 1), funnel-shaped process terminations and the regular arrangement of processes on the vesicle surface without any geometric pattern (which would resemble paratabulation); 2), equal length of processes that may reflect the mechanism of their formation by contracting from the vegetative mother cell during the process of encysting and their temporal attachment to the surrounding vegetative cell; 3), vesicles that open by a median split, partial rupture or circular apical opening that is consistent with the interpretation of being the excystment structure; and 4), double-walled vesicle (not to be confused with the two-layered ultrastructure of the cell wall) observed in some skiagias (*S. ciliosa*) or their developmental stages, which may show the beginning of the endocyst formation.

The primary evidence in support of the function of the vesicle is, however, provided by the presence of the internal body—the endocyst—within the cavity of the process-bearing vesicle [i.e., seen in *Skiagia ornata* and its junior synonym *Baltisphaeridium bimacerium*, Fig. 4.1, 4.2, 4.3, 4.4; and in *S. ciliosa* (not illustrated here)], which suggests that *Skiagia* is a cyst. Moreover, a newly discovered specimen of *S. ornata* actually records *in status nascendi* the release of the endocyst through the median split (Fig. 3.3, 3.4). The possibility that the internal body is a remnant of shrivelled cytoplasm is excluded because of its regular oval to globular shape. In addition, its sharp outline suggests that it is surrounded by a membrane and is not merely a lump of amorphous, disintegrating organic matter. The endocyst is centrally located within the cyst, as shown by an equal, albeit short, distance from the cyst wall (Fig. 4.1, 4.2, 4.3, 4.4), which is probably due to the infilling of this void by organic matter or fluid while alive, whereas shrivelled cytoplasm would have an irregular shape and be preserved in random locations in the cell cavity.

A set of features (presence of an endocyst, an excystment structure, the morphology of the processes and the overall vesicle habit, and the mechanism of excysting) are consistent with the interpretation that the *Skiagia* plexus of microfossils represent a resting/reproductive cyst in a complex life cycle of a number of microalgae. This stage is comparable to the cyst and its morphogenesis in some green microalgae, including dinoflagellates (Dale, 1983, 2001; van den Hoek, 1978; Bold and Wynne, 1985; Kokinos and Anderson, 1995; Head et al., 2006). However, this interpretation of *Skiagia* does not suggest a close biological affinity to dinoflagellates, but, rather, a similar functional morphology and ecological adaptation of diverse elements of a phytoplanktonic microbiota. Such adaptations are exemplified by certain Proterozoic

and early Palaeozoic acritarchs in the same way as Mesozoic dinoflagellates.

It is unclear how to treat the species of *Skiagia*. It is possible that they are cysts of different biological species (with each acritarch species corresponding to a biological species) or, alternatively, that they are different developmental stages and/or ecological variants of a single or merely a few biological species. The sequential chronological appearance of *Skiagia* species suggests that some of them are discrete biological species that evolved through time. The record of morphologically diverse natural populations of skiagias preserved in taphocoenosis (i.e., in the Lükati Formation) also indicates that various morphological types of cysts existed contemporaneously and might have been produced by different biological species instead of being ecological variants. Such morphological plasticity of cysts in a single species is less likely.

The species *Skiagia ornata* and *Baltisphaeridium bimacerium*, the latter considered herein to be a junior synonym, all show the mentioned diagnostic features, and *S. scottica* displays the most compelling morphology of processes that support the presence at some stage of a surrounding spheroidal envelope. These species are interpreted as developmental stages in a complex life cycle (Fig. 5), although not necessarily of the same biological species. At present, any specific *Leiosphaeridia*-type microfossil could be identified and combined with certain *Skiagia* species in the hypothetical reconstruction shown in Figure 5.

The type species of *Skiagia*, *S. scottica* Downie, 1982, mostly resembles the cysts of some modern and fossil microbiota by its vesicle habit and morphological appearance. The process terminations are wide and flat, face the exterior of the vesicle, and they may be connected with adjacent processes by lateral contact (Downie, 1982, fig. 9c-e; Moczydłowska, 1991, pl. 6E-F; Vidal and Peel, 1993, fig. 14 a-d; Fig. 2.1, 2.2), or by means of trabeculae (Downie, 1982, fig. 8k-l). In well-preserved specimens, the processes are distally attached to each other and form an almost continuous surrounding envelope (Fig. 2.1). Such a linkage between neighbouring processes is due to the equal length of the processes, their abundance, and dense distribution. It is assumed that at some stage in the life cycle, the process terminations of *S. scottica* were attached to the surrounding vegetative cell wall. There is a strong coincidence between the diameter of *S. scottica* and many leiosphaerids. In addition, an interpretation that these two forms represent alternating sexual and vegetative generations is further supported by the presence of vesicle openings in leiosphaerids (Fig. 1.3, 1.4), which are of appropriate size to release such a cyst. Indeed, leiosphaerids are ubiquitous in distribution and co-occur not only with *S. scottica* but with the *Skiagia*-type microfossil plexus in the same Cambrian units, where they overlap in size classes (see below).

Skiagia ornata, the most common and abundant species of *Skiagia*, is evidently a cyst, as shown by several records of an endocyst preserved inside its process-bearing vesicle, the cyst itself (Figs. 4.1, 4.2, 4.3, 4.4, 5C; see Zang, 2001; Moczydłowska, 2002; Zang et al., 2007). An example where the endocyst was arrested at the moment of being released through the median split (Fig. 3.3, 3.4) is particularly important for recognizing the mechanism of excysting and providing proof that such an opening is actually the excystment structure (Fig. 3.2). Furthermore, this example shows that the empty specimen without the opening (Fig. 3.1) is an immature cyst.

The endocyst is a dark or opaque, spheroidal to ellipsoidal internal body without any surface sculpture and has a diameter that is a few micrometres smaller than the process-bearing cyst (i.e., the microfossil *S. ornata*). The endocyst almost entirely fills the cavity of the cyst and is located centrally. Originally, the process-bearing vesicles with the internal body were assigned to *Baltisphaeridium bimacerium* Zang, 2001, and other specimens to *S. ornata* from the same sample in the Yalkalpo-2 drillcore, Arrowie Basin in South Australia (Zang, 2001; Fig. 4.3). *Baltisphaeridium bimacerium* has been diagnosed as a double-layered vesicle with robust and thick "interior layer" and membranous, translucent, and thin "outer layer" (Zang, 2001, pl. III, 1, 2). The shape, dimensions, and distribution of processes, as well as the median split in this species, match the characteristics of *S. ornata*. The "interior layer" is in fact a wall of the internal body—the endocyst. These microfossils are regarded herein as synonymous with *S. ornata*, a form-species which is also preserved with the internal body and is described as having a double-layered vesicle wall (Zang, 2001, pl. II, 1, 2). Illustrated specimens of *B. bimacerium* and *S. ornata* with internal bodies (Zang, 2001; Zang et al., 2004; Moczydłowska and Zang, 2006) are interpreted to be cysts with the endocysts contained inside and thus represent an immature stage before release of the endocyst.

Skiagia ornata with the endocyst preserved has been recovered from the lower Cambrian Grammajukku Formation, northern Sweden, and has been described as having condensed organic matter inside the vesicle (Moczydłowska, 2002). Numerous specimens without the endocyst or with the excystment structure only are preserved in the same stratigraphically thin interval (Moczydłowska et al., 2001). Remarkably, the Australian and Swedish assemblages contain microfossils with the endocyst together with numerous *Skiagia* specimens in different developmental stages (immature and closed to emptied cysts with the excystment structure). This type of association represents natural populations preserved as taphocoenoses.

The three-dimensionally preserved and unique specimen of *Skiagia ornata* shows the process of excysting *in status nascendi* (Fig. 3.3, 3.4). The vesicle is widely opened along the median split, and the cyst halves are attached at one edge, while the endocyst is essentially in the process of escaping from the cavity, although still occupying one half of the cyst. The assemblage from whom it derives consists of other species of *Skiagia* in various developmental stages, and leiosphaerids with and without the excystment structure and other taxa preserved as a death assemblage (Moczydłowska, personal data). Because the microfossils of the Lükati Formation are not thermally altered, both the endocyst and the *Skiagia*-cysts are translucent and have a light colouration of the organic matter. In this way, they differ from dark or opaque endocysts from other occurrences (Australia, Sweden), which are affected by thermal alteration.

The morphological variety of resting cysts produced by a single species of modern dinoflagellates depends on such environmental conditions as water temperature, salinity, insolation, and nutrient influx (Dale, 2001). Thus, it could be considered that several species of *Skiagia* may be ecophenotypes of a single biological species (Moczydłowska, 1991). However, no direct evidence exists to assess whether *Skiagia ornata* could be an ecological variant or a developmental stage of *S. scottica*. Although similar in gross morphology, the two morphotypes are distinguishable as discrete fossil species. It may be argued that *S. scottica* is an

FIGURE 1—4. Early Cambrian microfossils from the *Leiosphaeridia*-plexus interpreted to be vegetative cells in a complex life cycle of microalgae. Specimens are thin-walled sphaeromorphic vesicles with compaction wrinkles as taphonomic features and opening by median split (2 and 3), interpreted to be the excystment structure. 1 specimen Ko-96-5 (H/52/3); 2, specimen LO 6,237 t (K/44); 3, specimen LO 6,236 t (S/36/2); 4, specimen Ko-96-5 (X/40). Specimens derive from the Lükati Formation, Estonia (1 and 4), and the Kaplonosy-Radzyn formations, Kaplonosy IG-1 borehole, Poland (2 and 3). Scale bars equal 15 μ m for all micrographs. All are light microscope images.

earlier stage in the life cycle, which formed within the vegetative cell, matured and then discarded it, lost (degenerating) some of the processes, and “transformed” into the *S. ornata* morphotype. As mentioned above, because the two species are preserved in the same depositional layers, they may represent taxa that lived contemporaneously under the same ecological conditions. They are more likely to be different biological species, although closely related at the generic level, and sharing a common pattern of life cycle and cyst morphogenesis as inferred herein (Fig. 5), by comparison of their morphotypes and excystment structures.

The same may be inferred for other *Skiagia* species, such as *S. compressa*, *S. orbicularis*, *S. ciliosa*, *S. insignis*, and *S. pura*. These species are distinguished by various combinations of such morphological traits as process length and abundance,

complexity of process terminations and bases, and ratios of process length to vesicle diameter (Moczyłowska, 2001; Moczyłowska and Zang, 2006).

Some specimens of *Skiagia ciliosa* also possess a dark body inside the vesicle and have a double-walled vesicle (Volkova, 1969; Downie, 1982). These are features that are confined to the preserved endocyst and may include an excystment opening by a median split or partial rupture (Volkova et al., 1979; Moczyłowska and Vidal, 1992; Vidal and Peel, 1993). When present, these features are indicative of abandoned cysts. *Skiagia orbicularis* occasionally shows a less well-preserved opaque body inside the vesicle (Moczyłowska et al., 2001, fig. 4d) that could be the endocyst. *Skiagia pura* has prominent process tips and a circular apical opening (Moczyłowska, 1991, pl. 7G–H) that resembles the pylome

FIGURE 2—Early Cambrian *Skiagia scottica* Downie, 1982, interpreted to be a cyst in the complex life cycle of microalga. The specimens are without the vesicle opening and represent immature developmental stage before the excystment structure was formed. 1–2, specimens from the Buen Formation, North Greenland: 1, specimen MGUH 21.569 (Q/26-3) from GGU sample 184004-B; 2, specimen 891025 (C/29) from GGU sample 27479; 3, 4, specimens from the Lükati Formation, Estonia 3, specimen Ko-96-5 (D/35/2); 4, specimen Ko-96-5 (W/30/4). Scale bars equal 15 μm for 1–3, and 25 μm for 4. All are light microscope images.

in dinocysts and other microalgae (Tappan, 1980; Evitt, 1985; Le Hérissé et al., 2007).

In summary, microfossils of the *Skiagia* plexus have a functional morphology that allows a convincing interpretation as resting/reproductive cysts in a complex life cycle of different biological species, likely green microalgae (see under affinities). Having a cyst, they needed to have a vegetative cell, and the best candidate for it is the *Leiosphaeridia*-type microfossil (Fig. 5; see below).

Microfossils of the Leiosphaeridia plexus.—The genus *Leiosphaeridia* Eisenack, 1958, emend. Downie and Sarjeant, 1963 is an informal grouping of spheroidal unornamented microfossils with simple morphology. They lack diagnostic features and are probably of polyphyletic origins. The genotype *L. baltica* has been recognized by Eisenack (1958) from the Ordovician (Ashgillian). Subsequently, microfossils of various ages and un-determined systematics have been referred to *Leiosphaeridia*. Several Proterozoic and Cambrian leiosphaerid form-species are distinguished on the basis of size

classes and thickness of the vesicle wall (Butterfield et al., 1994). However, it is understood that a variety of phylogenetically un-related or distantly related microorganisms are assigned to *Leiosphaeridia*. These include prokaryotic cyanobacteria and eukaryotic protocists (Tappan, 1980; Martin, 1993), alete spores (Chaloner and Orbell, 1971), parts of a larger plant (Butterfield, 2004) and even metazoan eggs (van Waveren, 1993). Recent TEM studies on cell wall ultrastructure and biochemical analyses have demonstrated that some Proterozoic and Cambrian leiosphaerids are indeed green microalgae (Moczydłowska and Willman, 2009; Moczydłowska et al., 2009).

The vesicle diameter and its wall thickness are the only measurable parameters in leiosphaerids and they have been used statistically in an attempt to reveal their taxonomy. The size classes of leiosphaerid vesicles are, however, not reliable criteria for defining species (Moczydłowska, 1991), although they may, to some extent, coincide with natural taxa (Butterfield et al., 1994). Alternatively, as argued below, they

FIGURE 3—Early Cambrian *Skiagia ornata* (Volkova, 1968) Downie, 1982, interpreted to be a cyst in the complex life cycle of microalga. All specimens derive from the Lükati Formation, Estonia. 1, specimen Ko-96-5 (S/27/4), showing acanthomorphic vesicle without opening, in an immature developmental stage; 2, specimen Ko-96-5 (Z/46/4) with the excystment structure and representing mature and abandoned cyst; 3–4, specimen Ko-96-5 (Z/51/1), shown in two different focal images, *in status nascendi* of excysting the endocyst from the acanthomorphic cyst. The spheroid body (endocyst) is escaping from the *Skiagia*-cyst, which is opened by median split. Scale bars equal 20 μm for 1–2, and 25 μm for 3–4. All are light microscope images.

may reflect the developmental stages in the growth of vegetative cells and their morphogenesis into the resting/reproductive stage that harbours the cyst, aplanospores, or gametes. This interpretation does not contradict the assumption that leiosphaerids show a strong morphological convergence throughout geological time among various species of marine phytoplankton and other unrelated organisms. The convergence might be explained in terms of the functional morphology of the spheroid cell and its adaptation to certain modes of life. The spheroid shape has an advantage in keeping the cell suspended in the water and providing the most efficient cell volume/surface ratio for osmotic exchange of nutrients and gases across the cell wall during active metabolism. These features are meaningful in the biology of both vegetative and encysted stages (the latter at a much lower metabolic rate) in modern microalgae (Pahlow et al., 1997; Lee, 2008) but serve the same purpose equally well in cyanobacteria or metazoan egg capsules.

Observations on leiosphaerids' dimensions and excystment structure are not directly conclusive for distinguishing

vegetative cell or cyst, with these criteria equally inconclusive in modern phytoplankton. The spheroid morphology is typically known in vegetative cells produced by such common modern chlorophyceae microalgae as *Chlorella* and *Chlorococcum* (order Chlorococcales), but also occurs in the later, reproductive stages in a life cycle that includes the production of aplanospores, autospores, or zoospores inside the cell (van den Hoek, 1978; Bold and Wynne, 1985; Raven et al., 2005). As a result, there is no morphological distinction between the vegetative and hypnocyctic stages. In prasinophytes, the morphologically simple spheroid phycoma is a vegetative cell-reproductive cyst and thus is not morphologically differentiated in functionally different stages in their life cycle.

The excystment structure observed in many leiosphaerid specimens is by median split or partial rupture and, less frequently, a pylome in the vesicle wall [as is generally the case in acritarchs (Strother, 1996)]. Alternatively, its presence may suggest that these microfossils are resting cysts produced to survive the adverse environmental conditions, reproductive cysts in a regular life cycle, or vegetative cells.

FIGURE 4—Early Cambrian *Skiagia ornata* (Volkova, 1968) Downie, 1982, from the Yalkalpo 2 borehole, South Australia, containing the internal body and interpreted to be the maturing acanthomorphic cyst with the endocyst. 1, specimen from slide 7036RS137-3, D30; 2, specimen from slide 7036RS143-1, O43/1; 3, specimen from slide 7036RS137-3, D56/4; 4, specimen from slide 7036RS137-4, M34/1. Scale bars equal 10 μ m for all specimens. All are light microscope images.

Certain specimens and even easily distinguishable species of *Leiosphaeridia* (e.g., *L. bipyromifera* Palacios, 1998) possess morphologically pre-determined openings in the vesicle wall, which are true pylomes in terms of microalgal morphology. Such pylomes are seen in exceptionally well-preserved leiosphaerids from the Proterozoic Chuar Group in Utah, western United States (Vidal and Ford, 1985), early Cambrian Daroca Formation in Spain (Palacios and Moczydłowska, 1998), and some other occurrences. These microfossils can be confidentially interpreted as mature cysts after releasing the offspring cell(s) or gametes. However, most frequently, the leiosphaerids produce openings by median split (=equatorial splitting) or partial vesicle rupture (Fig. 1.3, 1.4, respectively). It is possible

to speculate that such vesicles represent vegetative cells after releasing either the cyst (Fig. 5, stage 1) or aplano-, auto- or zoospores.

DEVELOPMENTAL STAGES, LIFE CYCLE AND REPRODUCTION

Based on the observations of a great number of *Skiagia* and *Leiosphaeridia* specimens, consideration of their inferred biological affinities, and by analogue to modern microalgae, the life cycle of early Cambrian acritarchs has been reconstructed (Fig. 5). This complex life cycle comprises alternating generations and several developmental stages, which differ not only morphologically but also in their planktonic and benthic modes of life. The planktonic life is

FIGURE 5—The reconstruction of a complex life cycle of microalgae with alternating sexual and asexual generations and polymorphic developmental stages represented by microfossils from the *Leiosphaeridia*- and *Skiagia*-plexus. Numerical 1–6 indicate various stages in the morphogenesis of the cyst (2–4) from the vegetative cell (1) and released gametes or sack of offspring cells (5) or swimmers (6). The green outline of cell wall in stage 1 and 2 indicates the photosynthesizing vegetative cell (1) transforming into encysted stage (2). The dark blue spheroid internal body in stage 3 shows the developing endocyst inside the acanthomorphous cyst. The red coloured opening in the vegetative cell (stage 1) and matured abandoned cyst (stage 4) is the excystment structure.

a characteristic adaptation of these Cambrian forms (the forms are convincingly phytoplankton; see Moczyłowska, 2008). However, as part of their reproductive cycle and as a way to survive unfavourable environmental conditions, the cysts, being alternately the reproductive generation or dormant stage, are periodically/occasionally benthic. Developmental stages in the life cycle are represented by morphotypes, which are form-species of microfossils with features diagnostic for their function in particular generations (Stage 1–6 in Fig. 5). The two generations alternate as vegetative cells and resting/reproductive cysts that produced offspring cell(s) or gametes.

Morphologically, biochemically, and in conformance to the composite cell wall ultrastructure determined by TLS, the *Leiosphaeridia*-type vesicle may represent a vegetative cell of an actively photosynthesizing microalga (Fig. 5, stage 1). It is likely referable to the class Chlorophyceae, and possibly the orders Chlorococcales or Volvocales (Moczyłowska and Willman, 2009). This stage was planktonic and passively floated in the photic zone. Morphotypes of leiosphaerids with varying diameter and wall thickness, but particularly those with a multilayered cell wall, may represent growing and aging vegetative cells. The process of secreting additional sub-layers in cell walls, which differ in the texture of organic fabrics and thickness and also in biopolymer compounds between the sub-layers, is described in recent chlorophyceae microalgae of the order Volvocales during the growth of vegetative cells and their transformation into a resting stage (Hagen et al., 2002; Damiani et al., 2006). Morphogenesis into the resting stage (asexual aplanospore) in these algae is marked by the formation of TLS below the primary wall layer and then formation of the secondary wall beneath it. Comparable multilayered cell walls, some with preserved TLS, are recognized in leiosphaerids and are suggested to show developmental stages and various generations among ancient microbiota (Moczyłowska et al., 2009). The deposition of secondary wall layers and formation of three-layered cell walls in morphogenesis of the resting stages occur in a similar way in

other algal orders [i.e., the Zygnematales (Mix, 1969; Pickett-Heaps, 1972, 1975)], and thus does not preclude possible alternative affinities.

Resting and/or reproductive process-bearing cysts formed inside the vegetative cells in developmental stage 2, which was photosynthetic and planktonic up to the time of cyst maturation. Subsequently, the vegetative cell wall was discarded or the cyst was liberated through the excystment structure. The cyst of stage 2 is well illustrated by *S. scottica*. The mature cyst sank into the bottom sediment to rest, as modern microalgae do, while the endocyst developed and rested in a dormant stage (stage 3). Alternatively, the endocyst underwent reduction divisions to produce multiple offspring cells (swimmers) or gametes.

Stage 3 is represented by *Skiagia ornata* with the internal body. The abandoned, empty cyst of stage 4 is depicted by *S. ornata* with an opened excystment structure (Fig. 5), and it is commonly preserved among *Skiagia* plexus microfossils. Cysts of the developmental stages 3 and 4 spent their life in a benthic mode ("benthic plankton").

The endocyst from developmental stage 3 may represent a single offspring cell or a sac of cells (flagellated or naked swimmers or zoospores). After being released, the cell(s) began to swim or float in the water column for the short intervals of life stages 5 and 6. Eventually, the swimmers lost their flagella and rounded-up, and grew into adult vegetative cells, as did the single-celled endocyst, to close the life cycle and return to stage 1.

The two generations in the reconstructed life cycle (Fig. 5) may differ also by having sexual and asexual reproduction. By analogy to the reproduction cycles of modern microalgae, it is assumed that the vegetative cell was a haplobiontic generation, which in its asexual cycle produced haploid gametes that then united into a sexual zygote to form the cyst. The encysted zygote was a sexual generation that underwent meiotic subdivision and produced haploid juvenile organisms or zoospores that grew into a new adult vegetative cell. If the vegetative cell was diplobiontic, it would undergo meiotic

subdivision to produce haploid gametes, which fused and produced a zygote that then grew into an adult vegetative cell. The latter interpretation is less probable, as most modern microalgae are haplobiontic in their vegetative generation (Raven et al., 2005; Lee, 2008). In the asexual cycle, the haplobiontic (or diplobiontic) vegetative cell would produce haploid (diploid) juvenile cells or zoospores that matured as vegetative cells, all cycles being mitotic.

The alternation (perhaps seasonal) of sexual and asexual generations could be genetic and intrinsic to the species/clade of these microalgae. Morphologically complex cysts of the *Skiagia*-type are likely to be the sexual generation (zygote) in a regular life cycle. Alternatively, this type of cyst was produced to survive adverse environmental conditions and was produced irregularly in the life cycle. It could constitute the asexual generation that released a single endocyst with the restoration of suitable environmental conditions. Subsequently, it directly grew into the adult vegetative cell, perhaps even mitotically dividing into daughter cells. It may be speculated, however, that ecologically induced dormant cysts would be morphologically simple and reproduced asexually as aplanospores in modern algae (Bold and Wynne, 1985; Chapman and Waters, 2006; Lee, 2008).

BIOLOGICAL AFFINITIES

A strong resemblance of the microfossils *Skiagia* and *Leiosphaeridia* to modern microalgae in their morphology, cell wall ultrastructure and biochemistry, palaeoecology, and life cycle stages is compelling evidence that they are ancient members of the class Chlorophyceae.

Examples of morphologically analogous modern Chlorophyceae, Ulvophyceae and Charophyceae species are numerous and occur in various orders. Ornamented (process-bearing) morphologies occur predominantly, or almost as a rule, among mature zygotes or dormant zygotes (hypnospores) and “spores” but also among vegetative cells. Spheroid cell shape is very common in vegetative stages and cysts that represent zygotes, or are produced at the time of transformation to the cyst, even in taxa with such a distinctive morphology as *Acetabularia* (order Dasycladales). The classical examples of “coccoidal” organisms are nonmotile vegetative cells with a wide range of diameters that reflect growth stages from juvenile to mature cells. These organisms occur in the order Chlorococcales, and include such forms as *Chlorella*, *Chlorococcum*, *Pulchrasphaera*, and others (van den Hoek, 1978; Bold and Wynne, 1985; Lee, 2008). The spheroid cysts (=zygotes), which may be potentially preserved as fossils, are known in the Volvocales (*Chlamydomonas*, *Dunaliella*, *Volvox*), Chlorococcales and Dasycladales (*Acetabularia*) (Brackett, 1965; Bold and Wynne, 1985; Raven et al., 2005). Typically spheroid cells are stages in asexual reproduction as the aplanospores and autospores that occur even in species with the ontogenetic capacity for motility. Aplanospores that thicken their walls and rest (hypnospores) are preserveable and morphologically do not differ from the vegetative cells that comprise the somatic stage.

The ornamented cells, which bear spines and diversely shaped processes as a diagnostic morphologic trait, are known in encysted stages (zygotes, dormant zygotes, hypnozygotes) in modern microalgae among the Volvocales, Chlorococcales and Zygnematales (Winter and Biebel, 1967; Kies, 1975; Tassigny, 1971; Coessel and Texeira, 1974; van den Hoek, 1978; Bold and Wynne, 1985; Raven et al., 2005). In their superficial morphological appearance, some of these encysted stages are “acritarchs.” They are thick-walled and lavishly

ornamented with spines or processes, which may have divided or widened tips and conical bases. The cysts may be surrounded by spherical envelopes at an early developmental stage. Remarkably, certain species of *Chlamydomonas* (e.g., *C. moewusii*) in the Volvocales show in their zygote stage an opaque internal body within a thick ornamented wall (Bold and Wynne, 1985), which resembles the microfossils with endocysts (Fig. 5, stage 3). The mature zygote of *C. pseudogigantea* shows processes and, in general, a cell habit identical to that of the acritarch *Multiplicisphaeridium*. In *Chlamydomonas*, the ornamented zygote, which is the diploid sexual generation, undergoes meiosis and then germinates haploid zoospores. The zoospores grow to motile flagellate juvenile organisms that eventually fuse to form the zygote and close the life cycle (van den Hoek, 1978). A similar cycle is observed even in colonial *Volvox* (Volvocales), which also produces a spinose hypnozygote.

In the order Chlorococcales, the spinose wall of the dormant zygote stage is seen in *Chlorococcum* (van den Hoek, 1978; Raven et al., 2005). The genus may reproduce sexually, undergoing meiosis in the hypnozygote and releasing haploid, small, spheroid, juvenile cells that grow into haplobiontic vegetative cells. Such vegetative haploid cells may change into a sexual generation by a multiple bipartition that produces flagellate zoospores, which subsequently fuse into the zygote. The zygote loses its flagella, rounds up, and again secretes a thick wall with spines. Vegetative, spheroid nonmotile cells may reproduce asexually by forming inside aplanospores, which after their release grow in a short life cycle either into vegetative cells (all haploidal; van den Hoek, 1978), or into zoospores, which after losing flagella grow into vegetative cells (Deason and O’Kelly, 1979). This genus has asexual and sexual reproduction, and alternation of generations in a life cycle, in which the spheroid vegetative cells alternate with spinose cysts. A similar life cycle may be inferred for the Cambrian microfossils (Fig. 5).

Ornamented zygotes are formed also by *Golenkinia minutissima* (Chlorococcales), in which the spiny wall develops when the zygote enters a period of dormancy (Starr, 1963). Spiny and thick-walled dormant zygotes produced in sexual generation by *Atractomorpha echinata* of the order Sphaeropleales (Hoffman, 1983) are morphologically similar to acritarchs of the form-genus *Cymatiosphaera*. Interestingly, although not directly contributing to the reconstruction of life cycle, the encysted stage of *Atractomorpha* develops from a fusion of biflagellate gametes produced by long fusiform and multinucleate vegetative cells. This provides an example of a complex life cycle with very distinctive and diverse morphologies in alternating generations. If this alternation of generations was not observed in cultures, these generations would not be recognized as belonging to a single biological species.

The strongest cases for comparative morphology and inferences of the function of the ornamented cells in acritarchs are found in modern species of the order Zygnematales. The assumption that some acritarchs belonged to this clade has been earlier proposed (Tappan, 1980; Wicander, 2007; Moczydłowska, 2008), although without considering the life cycle. Zygnematales have different morphologies between vegetative and reproductive generations, and have ornamented cell walls that occur mostly in the mature and dormant zygotes. Although the Zygnematales are fresh- or brackish-water algae today, their ancestors have been marine (Bold and Wynne, 1985; Raven et al., 2005; Falkowski and Knoll, 2007). The zygotes have thick walls with surface ornaments in a

shape of symmetrical simple spines (*Spirotaenia condensata*, *Closterium calosporum*) or long processes with divided tips and conical bases (*Cosmarium*, *Micrasterias*, *Staurostrum*). They are surrounded by spheroidal, thin membranous envelopes supported by the process terminations. Morphologically, they all resemble various species of *Skiagia* microfossils—a comparison which is particularly appropriate—as the fragile membranous envelopes would not be fossilized. The ornamented zygotes in the Zygnematales originate from the sexual union of two haploid, non-flagellate (amoeboid) gametes. The diploid zygote undergoes meiosis and germinates into one to four juvenile cells that reinitiate the vegetative generation. The vegetative cells may reproduce asexually in a short life cycle by dividing equally into zoospores or juvenile cells, and then growing into adult vegetative cells. In sexual and asexual reproduction, the vegetative generations are haplobiontic and have haploid gametes, while meiosis occurs in the mature zygote (Bold and Wynne, 1985; Raven et al., 2005).

In sexual reproduction, the mature zygote of *Spirotaenia condensata* develops after the mating of a pair of cylindrical vegetative cells and the fusion of two amoeboid gametes. At the end of zygotic dormancy, meiosis occurs, and germination into four young, elongated vegetative cells takes place (Hoshaw and Hilton, 1966; Biebel, 1973). The zygote of *S. condensata* with its elegant, symmetrical, equally long processes surrounded by an external envelope is strikingly similar to *Skiagia scottica* (Fig. 2.1, 2.2).

Zygotes of *Cosmarium turpinii* (Starr, 1958; van den Hoek, 1978; Bold and Wynne, 1985) develop into acanthomorphic cells with processes like those in *Skiagia ornata* or *S. orbicularis*. In *Micrasterias papillifera*, the zygote has processes that resemble those in *S. ornata*, *S. orbicularis*, or *S. ciliosa*, and are enveloped in a translucent membranous sphere as it emerges from the gametangial walls after the conjugation of two vegetative cells (Kies, 1975; Tassigny, 1971; Coessel and Texeira, 1974; Bold and Wynne, 1985). In *Staurostrum gladiusum*, the isogamous conjugation forms a spiny-walled zygote, which at germination gives rise to one to four vegetative cells. The vegetative cells also possess superficial wall spines, but they are short and of a simple shape, and the vegetative cells are triangular in polar view, in contrast to the spheroidal zygote (Winter and Biebel, 1967). This is another example in which the zygote morphologically resembles the *Skiagia*-plexus—in particular the form-species *S. ciliosa*, *S. pura*, and *S. insignis*.

Developmental polymorphism (i.e., morphologically differentiated individuals that represent various stages in a life cycle that includes the alternation of vegetative and reproductive generations as a result of asexual and sexual reproduction cycles) is recognized in chlorophytes and streptophytes algae that include several orders and many species. The morphotypes, which resemble closely the *Skiagia*- and *Leiosphaeridia*-plexus microfossils, are taxa referable to the orders Volvocales, Chlorococcales, Sphaeropleales, and Zygnematales. The mechanism of excysting from the vegetative or gametangial cells in these algae is by rupture of the cell wall, as is commonly observed in Proterozoic and Palaeozoic acritarch microfossils. The external membranous spheres enveloping the maturing spiny zygotes in modern species would not be expected to be preserved in fossils but could be inferred to exist in *S. scottica*. The combination of morphological traits and developmental polymorphism among modern microalgae has an analogue in these microfossils.

TEM studies on the cell wall ultrastructure of some acritarchs, including *Skiagia* and *Leiosphaeridia*, have been

previously carried out on the material from the split sample from the Lükati Formation, as those reported here (Figs. 2.3, 2.4, 3.1, 3.2, 3.3, 3.4). *Leiosphaeridia* specimens with diagnostic trilaminar sheath structure (TLS), recognized as green algae of the class Chlorophyceae, and the order Chlorococcales (Talyzina and Moczyłowska, 2000), derive from the residuum used for microscope slides preparation. Therefore, the evidence that leiosphaerids are good candidates for being the vegetative stage of the *Skiagia ornata* cyst (and other skiagias), which co-occur in a natural taphocoenosis, allows a conclusion that they may represent the same biological entity. The interpretation based on shared morphological features, inferred live cycle, and co-occurrence is the microalgal origin of the *Skiagia* microfossils, and a likely systematic assignment to the order Chlorococcales.

This interpretation differs from the one suggested by Moldowan and Talyzina (1998), who inferred the affinity of *Skiagia*, among other acritarchs from the Lükati Formation, to the dinoflagellates because of the presence of some dinosteranes in the bulk sample with *Skiagia* microfossils. The occurrence of steranes, regarded diagnostic of dinoflagellates, has also been detected in Proterozoic sediments (Summons and Walter, 1990) in which neither skiagias nor dinoflagellates have been recovered and from a geological time interval long preceding the appearance of dinoflagellates in the Mesozoic (Fensome et al., 1996a). The steranes (and dinosteranes) might have been synthesized by an early common precursor to these later appearing organisms, which retained this secretory ability in distantly related clades (Moldowan et al., 2001). Similarly, the biopolymer chitin is synthesised by phylogenetically distant prototists, fungi and metazoans (Traverse, 2007). By comparison, algaenan is produced by algae and vascular plants, including a prominent group of land seed plants (Falkowski and Raven, 2007). Thus, the presence of “dinosteranes” in Proterozoic sediments alone is neither a proof of such an early evolution of dinoflagellates, nor does it prove that Cambrian acritarchs are dinoflagellates.

The biopolymers that compose the leiosphaerid, as well as other acritarch, vesicles are well known to be resistant to water, solution during diagenetic mineral formation, to humic acids that migrate through the sediment during burial, and to the inorganic acids (HCl, HF) and organic solvents (ethanol, acetone) used in laboratory maceration. This property is identical to that in sporopollenin/algaenan, and thus the acritarch biopolymers are inferred to be its fossil derivative. Indeed, acritarch composition was considered by earlier researchers, even prior to biochemical analysis, to indicate acritarchs' affiliation to marine phytoplankton (Evitt, 1963; Downie et al., 1963; Tappan, 1980).

Algaenan is synthesized by all plants in their reproductive generations (e.g., spores and pollen) and is produced in cysts (which are also reproductive generations) and, less frequently, in vegetative cells by various classes of microalgae (Chlorophyceae, Dinophyceae, Eustigmatophyceae; Atkinson et al., 1972; van den Hoek, 1978; Derenne et al., 1992a, 1992b, 1996; Allard and Templier, 2000; De Leeuw et al., 2006). For example, in the modern chlorococcalean green alga *Chlorella*, the vegetative cell is only partially composed of algaenan (=sporopollenin; van den Hoek, 1978), or may only be synthesized by a strain of the species (Versteegh and Blokker, 2004). It is inferred from genetic studies and phylogenetic trees that algaenan evolved in green algae and has been inhabited subsequently in evolutionary history by tracheophytes (vascular plants), and the ability to produce this biopolymer was readapted by land plants, to which green algae are ancestral,

to produce spores and pollen (Kenrich and Crane, 1997; Wellman, 2003; Keeling et al., 2005; Hackett et al., 2007; Taylor and Strother, 2008). In the process of evolution, the deposition of sporopollenin (=algaenan) from an ancestral diploid hypnozygote has been transferred to haploid spores (Hemsley, 1994). All these clades of organisms, regardless of their systematic position and time of radiation, are evidently photosynthetic, and thus the presence of algaenan in a fossil is a proof of its photoautotrophy.

However, the lack of algaenan does not exclude photosynthesis because some microalgae do not synthesise it. Thus, algaenan compounds, as those detected in certain Proterozoic *Leiosphaeridia* cell walls (Arouri et al., 1999, 2000; Javaux et al., 2004; Marshall et al., 2005, 2006), indicate that these were photosynthesizing organisms, and being single-celled, they were microalgae. The presence of algaenan present in a microfossil, however, does not permit a differentiation between vegetative cells or cysts. They may be both indeed. Algaenan has a specific nanoscale ultrastructure (Derenne et al., 1992a, 1992b, 1996). It is laminated with TLS, and provides mechanical strength and chemical resistance to the algal cyst that harbours the resting/reproductive cell(s) or the spores and pollen of higher plants. Therefore, they are all readily fossilizable.

Biosynthesis of algaenan by algae to produce predominantly resting/reproductive cysts but also vegetative cells and by land seed plants to produce reproductive spores and pollen promoted reproductive success and survival to the next generation. This biosynthesis ability was and is directed by the same genes and has the same evolutionary origin (Wellman, 2005, personal communication).

The genus *Leiosphaeridia* has been understood as embracing marine planktonic algal cysts and vegetative cells (Eisenack, 1958; Tappan, 1980), including those of prasinophycean and chlorophycean origins (Kjellström, 1968; Jux, 1969; Le Hérisse, 1989; Colbath and Grenfell, 1995). The ultrastructure and biogeochemical composition of the vesicle walls of some Proterozoic and early Palaeozoic leiosphaerids provided the evidence that these microfossils are algal (Arouri et al., 1999, 2000; Talyzina and Moczydłowska, 2000; Javaux et al., 2004; Marshall et al., 2005, 2006; Willman and Moczydłowska, 2007; Willman, 2009; Moczydłowska and Willman, 2009; Moczydłowska et al., 2009). Certain specimens (species are not recognized precisely) indeed represent microalgae of the class Chlorophyceae, because they show the diagnostic trilaminar sheath structure (TLS) of the cell wall that is known in vegetative cells and dormant cysts in modern genera of the orders Chlorococcales and Volvocales (Moczydłowska and Willman, 2009; Moczydłowska et al., 2009). Although TLS is a diagnostic feature of the cell wall ultrastructure in many chlorophycean microalgae, it is not present in all species (Brunner and Honneger, 1985; Derenne et al., 1992a, 1992b, 1996; Gelin et al., 1999; Allard and Templier, 2000). Thus, it should be remembered that the lack of such structure does not exclude a modern species or a microfossil from the class. Similarly, the lack of algaenan, which is a biopolymer diagnostic for algae although not all produce it, does not preclude algal affinities (Allard and Templier, 2000; Marshall et al., 2005, 2006, 2007).

The interpretation of certain Cambrian leiosphaerid specimens as metazoan eggs (Cohen et al., 2007) merely emphasizes a highly convergent and superficial morphological resemblance. A resemblance of spheroidal microfossils to the pelagic eggs of modern copepods and fish has been noted repeatedly (Tappan, 1980). This interpretation has been

elegantly demonstrated in studies of Holocene “leiosphaerids” that are identical morphologically and appearing to be the modern egg-envelopes of extant copepods (Van Waveren and Marcus, 1993).

It is apparent that there is no single and direct method, neither by ultrastructure nor biogeochemically, of recognizing the natural systematics of microfossils that lack a conspicuously diagnostic morphology. In the case of the *Leiosphaeridia* grab-bag, a group that essentially ranges through a great amount of geological time and occurs in most marine palaeoenvironments, some specimens have been proven to be microalgal (both prasino- and chlorophyceae), but the majority may only be circumstantially inferred to be phytoplanktonic, as discussed above. This interpretation is supported by their palaeoecological distribution in almost all marine facies, which is consistent with a planktonic habitat, their small size and great abundance, and it is even more convincing for early records that pre-date the appearance of metazoans. The available evidence and conclusions, as being chlorophyceans, as well as the likelihood that other systematic affinities are hidden by their simple morphology, make the microfossils of the *Leiosphaeridia*-plexus an intriguing subject for more sophisticated studies and new methodologies in order to determine their actual systematic relationships.

CONCLUSIONS

The studied early Cambrian microfossils are excellently preserved, and they show morphological details never observed before, such as endocysts and a cyst “giving birth” to an offspring cell. These specimens finally resolve aspects of the microfossil puzzle and allow reconstruction of the complex life cycle of Cambrian microalgae. A strong resemblance of the microfossils *Skiagia* and *Leiosphaeridia* to modern microalgae in their morphology, cell wall ultrastructure and biochemistry, palaeoecology, and developmental stages in a life cycle is compelling evidence for regarding them as ancient members of the class Chlorophyceae.

The inferred life cycle comprises several developmental stages and alternating generations, which differ not only morphologically but also in their planktonic and benthic modes of life. The two generations in the reconstructed life cycle may also differ by having sexual and asexual reproduction that formed haplobiontic vegetative cells and then the zygote. The zygote then closed the life cycle by undergoing meiosis, which again produced haploid cells that grew into vegetative cells.

Morphologically, and in agreement with a composite cell wall ultrastructure with TLS and its biochemistry, the *Leiosphaeridia*-type vesicle may represent a vegetative cell of actively photosynthesizing microalgae of the class Chlorophyceae, possible of the orders Chlorococcales or Volvocales (Moczydłowska and Willman, 2009). This stage was planktonic and passively floated in the photic zone. Morphotypes of leiosphaerids with varying diameter, wall thickness, and, most significantly, multilayered cell walls with variable number of layers may represent growing and aging vegetative cells that were undergoing a transformation to the resting stage. A resting and/or reproductive, process-bearing cyst (zygote) formed inside the vegetative cell, and it was initially actively photosynthetic and planktonic but settled onto the bottom and became benthic up to the moment of cyst maturation (“benthic plankton”). The *Skiagia*-type vesicles represent the cyst at different morphogenetic stages. The endocyst inside the *Skiagia* cyst is an offspring cell or a sac of swimmers.

Skiagia microfossils resemble the elaborate, process-bearing encysted stage (sexual zygote) of such modern algae as the Volvocales, Chlorococcales, Sphaeropleales, and Zygnematales. If combined with *Leiosphaeridia* as a vegetative cell, the *Skiagia-Leiosphaeridia* organism likely belonged to the orders Chlorococcales or Volvocales.

The biopolymer algaenan (previously called sporopollenin) is synthesized by various classes of microalgae and all seed plants in their reproductive generations. Algaenan is used in cysts and in vegetative cells at the beginning of their morphogenesis into cysts, and in spores and pollen. All these clades of organisms, regardless of their systematic position and time of evolution, are evidently photosynthetic, and thus the presence of algaenan in a fossil is a direct indication of its photoautotrophy. However, the lack of algaenan does not exclude the ability to photosynthesize because some microalgae do not synthesize it.

Algaenan compounds, as those detected in certain *Leiosphaeridia* cell walls, indicate that these single-celled organisms were photosynthetic and were microalgae. The presence of TLS indicates more precisely their affinities to green microalgae of the class Chlorophyceae, and more specifically to the order Chlorococcales and/or Volvocales (Moczyłowska and Willman, 2009; Moczyłowska et al., 2009).

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