RECOGNIZING AND INTERPRETING THE FOSSILS OF EARLY EUKARYOTES*

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(Received 27 July 2002; accepted in revised form 10 December 2002)

Abstract. Using molecular sequence data, biologists can generate hypotheses of protistan phylogeny and divergence times. Fossils, however, provide our only direct constraints on the timing and environmental context of early eukaryotic diversification. For this reason, recognition of eukaryotic fossils in Proterozoic rocks is key to the integration of geological and comparative biological perspectives on protistan evolution. Microfossils preserved in shales of the ca. 1500 Ma Roper Group, northern Australia, display characters that ally them to the Eucarya, but, at present, attribution to any particular protistan clade is uncertain. Continuing research on wall ultrastructure and microchemistry promises new insights into the nature and systematic relationships of early eukaryotic fossils.

Keywords: chemistry, early eukaryotes, evolution, molecular phylogeny, morphology, Proterozoic, ultrastructure

1. Introduction

Fossils formed early in the evolution of a major group commonly defy paleobiological interpretation. Ediacaran impressions, for example, are widely accepted as the remains of early animals, but what kinds of animals remains contentious and alternative interpretations range from seaweeds (Xiao *et al*., 2002) to giant coenocytic protists, or even bacterial colonies (see Runnegar, 1995, for review). Fossils interpreted with confidence as stem group members of extant phyla appear more than thirty million years after the first Ediacarans, and unambiguous crown group members of most phyla appear later yet.

Early eukaryotic fossils present similar interpretational challenges. Phanerozoic (<543 Ma) rocks commonly brim with the fossils of eukaryotic organisms, both macroscopic and microscopic, and most can be related to major clades present in the modern biota. In contrast, Neoproterozoic (1000–543 Ma) fossil assemblages mix fossils of known systematic origin with remains that are unambiguously eukaryotic, but impossible to relate to specific clades within the domain. And receding backward still further, late Paleo- and Mesoproterozoic (ca. 1800 to 1000 Ma) assemblages include fossils that are problematic even at the level of domain.

* Paper presented at the Astrobiology Science Conference, NASA Ames Research Center, 7–11 April 2002.

Origins of Life and Evolution of the Biosphere **33:** 75–94, 2003.

Since the birth of modern research on Precambrian life, paleontologists have endeavored to recognize and interpret fossils of early eukaryotes on the basis of preserved morphology. Early on, size was suggested as a criterion (Schopf, 1977); after all, many eukaryotic cells are larger than 10–20 μm in diameter, while most bacteria are no more than 1–2 μm in maximum dimension. Unfortunately, however, size fails us at the size classes most commonly found in Precambrian rock.

Eukaryotes can form 1–10 μm spheres, but so can cyanobacteria as well as members of other bacterial and archaeal clades. Larger vesicles (up to a few hundred μm) can be the preserved walls of algae or heterotrophic protists, but, in principle, they can also be large sulfur bacteria (Schulz *et al*., 1996) or the preferentially preserved envelopes of colonial cyanobacteria (Waterbury and Stanier, 1978). Among the largest fossils known from mid-Proterozoic cherts are cylindrical forms more than 100 μm long interpreted as akinetes of nostocalean blue greens (Golubic *et al*., 1995).

Another early hope was that small blebs of organic matter found within microfossils might be the preserved remnants of nuclei or other organelles (Schopf, 1968). Again, however, confidence in any such interpretation was dashed by the recognition that nuclei have an extremely low preservation potential, and both field observations (Golubic and Barghoorn, 1977) and laboratory experiments (Knoll and Barghoorn, 1975) showed that organic remnants within fossils are mostly collapsed and partially decayed cytoplasm. Perhaps the strongest case for organellar preservation in Proterozoic microfossils was advanced by Oehler (1977) on the basis of TEM images of small unicells from the Neoproterozoic Bitter Springs Formation. Within fossilized walls or envelopes, Oehler found tiny electron-dense structures that she interpreted as preserved pyrenoids. Pyrenoids are proteinaceous microstructures commonly associated with storage products in algal chloroplasts – green algal cells sometimes accumulate starch bodies about pyrenoid structures. Proteins have a low probability of preservation, but starch bodies are more likely to survive in fossils; thus, one possibility is that the structures imaged by Oehler are preserved starch grains within fossilized green algae (Knoll, 1981). At present, however, we lack taphonomic studies of modern protists that might illuminate the relative probabilities or distinguishing features of pyrenoid, starch, or collapsed cytoplasm preservation.

To date, the most compelling interpretations of Proterozoic cells as eukaryotic have been drawn on the basis of morphology – by the identification of structural features known to be fashioned by one or more eukaryotic groups but unknown among Bacteria or Archaea. Thus, vase-shaped microfossils 100 μm long, with shapes and wall composition known today only within specific genera of lobose and filose amoebae, provide unambiguous records not only of eukaryotic microorganisms, but more specifically of testate amoeban clades (Porter and Knoll, 2000; Porter *et al*., in press). Large (commonly >200 microns) spinose fossils found globally in terminal Proterozoic rocks are likewise considered to be unambiguously eukaryotic based on their pattern of ornamentation and microchemistry (e.g., Zang

and Walter, 1992; Zhang *et al*., 1998; Arouri *et al*., 1999, 2000, see discussion below), although more specific systematic attribution is currently impossible.

In this article, we explore the biological relationships of spheroidal acritarchs from ca. 1500 Ma shales of the Roper Group, northern Australia. Roper fossils epitomize the challenges inherent in the recognition and interpretation of early eukaryotes. They also illustrate how new approaches may improve our understanding of mid-Proterozoic biology, among other things enhancing our ability to calibrate molecular phylogenies and relate them to Earth's physical history.

2. Eukaryotic fossils in the early Mesoproterozoic Roper Group, Australia

Biologists easily differentiate between prokaryotic and eukaryotic organisms using myriad features of molecular and cell biology, but these characters rarely survive fossilization and so are not generally available to the paleontologist. Phylogenetically informative lipid molecules may be preserved in carbonaceous sediments, and these provide a geochemical record of Proterozoic eukaryotes (e.g., Summons and Walter, 1990). Morphologically preserved fossils can be identified as eukaryotic based on a number of features thought to be diagnostic of the domain (Javaux *et al*., 2002a). These include: (1) wall structure and surface ornamentation, (2) processes that extend from vesicle walls, (3) excystment structures (openings through which cysts liberate their cellular contents), (4) wall ultrastructure and (5) wall chemistry. Other early eukaryotes can be recognized on the basis of a regular and well-defined *macroscopic* morphology (e.g., Walter *et al*., 1976; Walter and Du Rulin, 1990; Grey and Williams, 1990).

2.1. Geological setting

The Roper Group comprises a thick ramp-like succession of siliciclastic rocks deposited in a rapidly subsiding intracratonic basin, located in the Northern Territory of Australia, West of the Gulf of Carpentaria (Abbott and Sweet, 2000). The Roper Group is well dated at its base by U-Pb SHRIMP analyses of zircons from an ash bed within the Mainoru Formation that fix an age of 1492±3 Ma (Jackson and Raiswell, 1991). A Rb-Sr age of 1429±31 Ma, obtained from illite in dolomitic siltstones near the top of the succession is consistent with the zircon age, albeit less reliable (Kralik, 1982).

Our study of 5 drill cores revealed abundant and exceptionally well preserved microfossils distributed among four biofacies stacked repeatedly throughout the group (Javaux *et al*., 2001). There is a clear onshore-offshore pattern of decreasing abundance, declining diversity, and changing dominance among Roper microfossils. Highly carbonaceous shales in basinal deposits of the Velkerri Formation contain low abundances of steranes sourced from eukaryotic organisms (Summons *et al*., 1988a). Recent geochemical research by Shen and Knoll (2002) shows

that a strong redoxcline existed within the basin, likely within the photic zone. Shen and Knoll (2002) also identified a strong correlation between facies and the isotopic abundances of sulfur in early diagenetic pyrites – likely only if sulfate levels and, by implication, oxygen concentrations were much lower than in present day oceans (Canfield, 1998; Kah *et al*., 2002; Shen *et al*., 2002). Thus, paleontology and geochemistry concur in their recognition of a strong onshore-offshore pattern in the Roper seaway. Similar microfossil assemblages occur in the slightly younger Ruyang Group of China (Yin, 1998; Xiao *et al*., 1997); the ca. 1.3 Ga Totta Formation, Siberia (Sergeev, 2002, pers. comm.), and the poorly dated but broadly correlative Sanda Formation, Ganga Basin, India (Prasad and Asher, 2001).

2.2. Morphological evidence of Roper eukaryotes

2.2.1. *Surface Ornamentation and Wall Structure*

Resting cells and reproductive cysts of many protists display micron-scale patterns of lineations, fields, spines or bosses not known among prokaryotic organisms. This being the case, Neoproterozoic and Paleozoic acritarchs with similar ornamentation are ascribed to eukaryotes with confidence. Roper microfossils do not display the strong ornamentation seen in some younger populations. Nonetheless, the ornamentation and wall structure of some Roper taxa identify them as protists. For example, *Valeria lophostriata* is a spherical acritarch easily distinguished by its distinctive ornament of concentric striations (Figure 1: 1). SEM observation shows that these striations consist of parallel ridges spaced 1 μm apart that traverse the inner surface of the vesicle (Figure 1: 2). *Dictyosphaera*, as well, displays surface ornamentation, in this case small interlocking polygons (Figure 1: 3).

Many Bacteria and almost all Archaea show a surface layer ornamentation of oblique, square or hexagonal crystalline arrays of glycoprotein subunits (called the S-layer; Sara and Sleytr, 2000). S-layers function as a molecular sieve, providing a protection from bacterial parasites and viruses, a template for mineralization, and adhesion sites for exoenzymes. The subunits are synthesized intracellularly, then translocated through the cell wall and incorporated into the existing S-layer lattice. S-layers subunits are easily isolated by chemical agents, and they retain the ability to recrystallize into regular arrays in suspension or on surfaces (Sara and Sleytr, 2000). This type of proteinic surface ornamentation occurs at a nanoscale and would probably not be preserved since it is easily removed by chemicals and easily lost in cultures.

Some prokaryotic cells are ornamented with concentric rings of juxtaposed filaments, or fibrils, pili and fimbriae, but again these features occur at the nanoscale (Boone and Castenholz, 2001), not the microscale seen in Roper acritarchs. Pili or fimbriae are hair-like appendages that extend out from the bacterial cell surface. Their composition and assembly are well studied because of their role in the colonization of host cells during bacterial infections (Soto and Hultgren, 1999). Pili consist of a large variety of adhesive proteinic structures assembled through at least

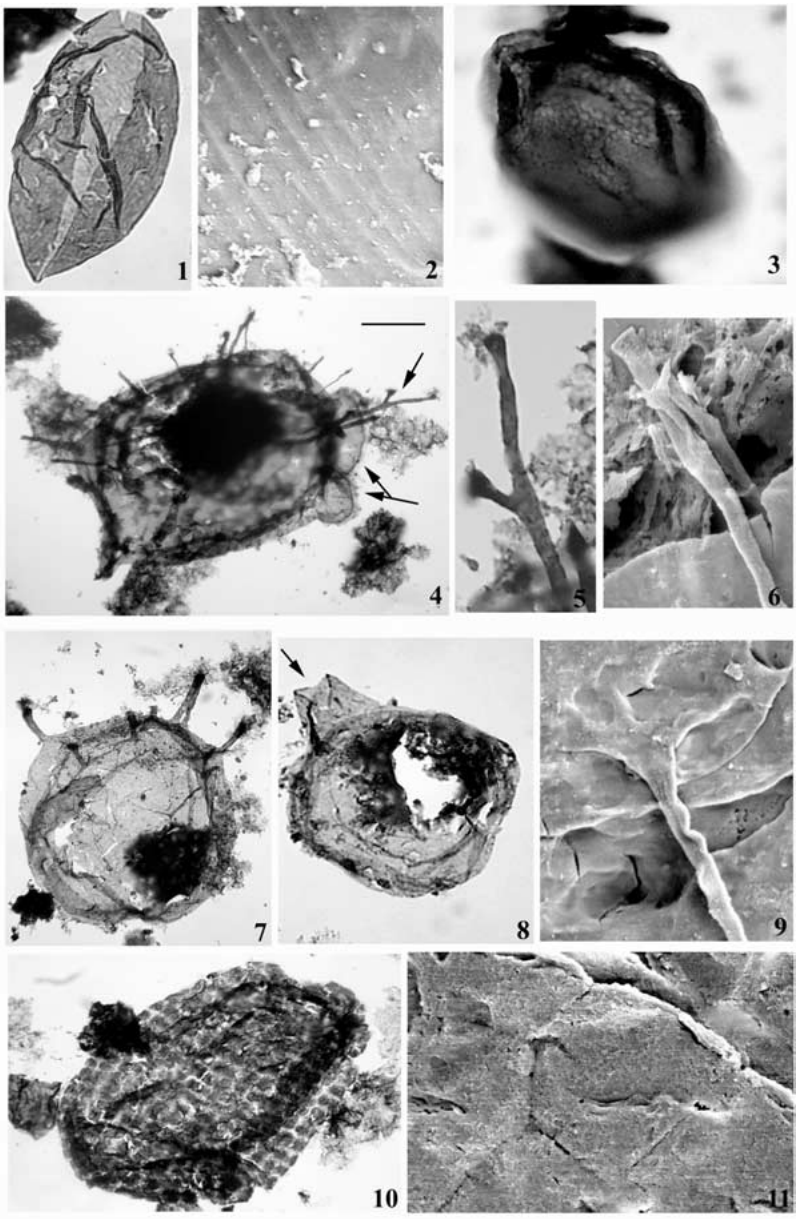

Figure 1. Eukaryotic microfossils from the Roper Group, Australia. 1–2: *Valeria lophostriata*, partially enrolled half vesicle, likely resulting from a medial split, 2: SEM showing striated ornamentation consisting of ridges spaced at 1 μm intervals on the internal surface of the vesicle; 3: *Dictyosphaera*, with polygonal ornamentation; 4–9: *Tappania plana*, 4: specimen with heteromorphic processes (including a branched process-see arrow) distributed asymmetrically about the vesicle and budding (double arrow), 5: detail of branched process in 4, 6: SEM of branched process, 7: specimen with heteromorphic processes, 8: specimen with excystment structure (arrow), 9: SEM showing structural continuity between vesicle wall and the base of a process; 10–11: *Satka favosa*, 10: specimen showing wall made of polygonal plates, 11: SEM showing detail of juxtaposed polygonal plates. The scale bar in 4 is 30 μm for 1, 2 μm for 2, 15 μm for 3, 35 μm for 4 and 7, 10 μm for 5, 5 μm for 6 and 11, 20 μm for 8, 3 μm for 9, and 40 μm for 10.

four distinct molecular pathways. These pathways are complex and range from secretion and precipitation on the cell surface where a specialized molecule serves for nucleation, to intracellular secretion and assembly followed by translocation of the assembled pili across the outer membrane to the surface, or two different chaperone mediated pathways where chaperone proteins form complexes with pilin components, forming fibers that are then transported across the membrane (Soto and Hultgren, 1999). Pili can reach sizes up to 4,000 nm long and width of 6–7 nm, and some types may branch. Thus, even if these nanoscale proteinic bacterial appendages could be preserved, they could not be confused with ornamentations of Roper acritarchs. Neither would myxobacterial cells that can produce an extracellular matrix of polysaccharide and proteinaceous fibrils with diameters of 10 nm and lengths of 60 to 100 nm (Kim *et al*., 1999). Myxobacteria live mostly in soils, dung, decaying plant material and bark, and their spores resistant to desiccation can sometimes be found in seashore sediments but few myxobacterium are known to be able to live in seawater (Reichenbach and Dworkin, 2001; Iizuka *et al*., 2002). Most myxobacteria enclose their myxospores in unornamented sporangioles up to 30 to 50 μm in diameter, with a wall up to 1 μm thick. Sporangioles may occur singly or in groups and may rest directly on or in the substratum or on simple or branched stalks. Nothing is known about the chemical composition of stalks, sporangioles and pigments composing myxobacterial fruiting bodies (Reichenbach and Dworkin, 2001), so their preservation potential is unknown although the spores are resistant to desiccation. Although vegetative cells of Actinobacteria can be relatively large (a few microns) and form complex branching colonies, their spores are 0.5 to 2 or 3 μm in diameter and form chains (Holt, 1984; Boone and Castenholz, 2001; Miyadoh, 1997). Some of these actinomycete spores are ornamented by rodlets, spines, warts, cristae, or hair-like tufts, but again, the scale differs from Roper and other Proterozoic fossils. In several species of *Streptomyces*, this ornamentation is formed by a fibrous protein sheath covering the peptidoglycan cortex of the spores (Miyadoh *et al*., 1997; Claessen *et al*., 2002). It is not known if this protein layer could be preserved in the fossil record, although the sheath can be dissociated into submicroscopic rods or tubules upon sonic oscillation (Dworkin, 1985). The soil actinobacteria *Actinoplanes*, can form sporangia of variable morphology and size (up to 5–15 μm) that may be covered by short hairs, but again at a nanoscale (Matsumoto *et al*., 2000).

Cyanobacterial cell walls are commonly covered by S-layers or by carbohydrate structures forming slime or sheaths. These sheaths are preserved in the fossil record, in preference to the peptidoglycan-rich cell walls, as shown by taphonomic experiments (Bartley, 1996). They are composed of polysaccharides such as cellulose-like homoglucan fibrils cross-linked by minor monosaccharides (*Phormidium, Nostoc*), pectin-like exopolysaccharides (*Microcystis*), or two different complex polysaccharides (*Anabaena*), and pigments like scytonemin (Hoiczyk and Hansel, 2000). These extracellular fibrillar carbohydrates provide a protective coat for the cells against UV radiation and desiccation as they maintain the cells in a highly

hydrated gel-like matrix. However, these tube-like or vesicle-like sheaths are not ornamented.

Thus it seems that while prokaryotic organisms can synthesize both cell wall ornament and preservable structures, wall ornamentation rarely occurs on the size scale observed in Proterozoic fossils, and ornamentation seldom is found on preservable structures.

For these reasons, we interpret Roper *Valeria* and *Dictyosphaera* as the preserved walls of mid-Proterozoic eukaryotes. More specific systematic interpretation will require TEM and microchemical analysis (see below).

Complex wall structure can also be a sign of eukaryotic affinity. *Satka favosa* wall is made of interlocking polygonal plates 10–15 μm in maximum dimension (Figure 1: 10–11). No prokaryotic cells build comparable walls. Note that the form genus *Satka* currently contains several species of potentially disparate biological origins. *Satka squamifera* consist of thin envelopes distended by ellipsoidal cells and could be prokaryotic. Only *S. favosa* is known to construct complex walls of interlocking plates.

2.2.2. *Processes*

Tappania plana comprises 20 to 160 μm vesicles that bear 0 to 20 or more elongated extensions or processes (Figure 1: 4–9). Those processes are heteromorphic, are distributed asymmetrically about the vesicle surface and have variable length (25 to 60 μm). Processes communicate freely with the vesicle interior, have dark slightly expanded closed ends and may branch (Figure 1:4–6). Specimens may also bear up to 3 bulbous protuberances and/or neck-like extensions. The irregular morphology and asymmetric distribution of processes in *Tappania* stand in marked contrast to the regular size and distribution of processes in Palaeozoic acritarchs and suggest that *Tappania* might have been an actively growing cell or germinating cyst rather than a metabolically inert spore (as acritarchs are generally assumed to represent). The bulbous protrusions in some specimens further suggest vegetative reproduction by budding (Figure 1: 4), although some neck-like extensions might be excystment structures (Figure 1: 8, see below).

As far as we are aware, the complex morphology of *Tappania* does not occur in prokaryotes. Some prosthecate bacteria have wart to tube-like appendages extending from the cell surface that are 0.5 to 2–3 μm in length and may bear bundles of fimbriae (Schlesner, 1987). *Caulobacter* produces a single stalk extending from its 1–2 μm cell, with a length usually 1 to 2 μm but ranging up to 20–30 μm in phosphate-depleted waters; the stalk terminates in a holdfast (composed of complex polysaccharide) that attaches to surfaces (Brun and Janakiraman, 2000). Because stalks increase the surface-to-volume ratio of the cells, they are thought to increase nutrient absorption in oligotrophic habitats. Stalks might also help maintain the cell at the air-water interface and unidirectional growth in biofilms. The cell surface layers are continuous with those of the cell body and include the cytoplasmic membrane, the peptidoglycan layer, and the outer membrane (Brun

and Janakiraman, 2000). The stalk is traversed by peptidoglycan rings or cross-bands synthesized at each cell cycle (Pondexter and Staley, 1996). Biogenesis of polar structures in Bacteria is linked to asymmetric protein localization near the cytoplasmic membrane (Lybarger and Maddock, 2001). In *Caulobacter*, localized signaling proteins are used to coordinate the cell-cycle so that dissimilar progeny is generated at each division: a swarmer cell with a polar flagellum and a stalked cell (Shapiro *et al*., 2002). Temporally controlled localization of structural and regulatory proteins to the cell pole and subsequent proteolysis and release of these polar components in preparation for the next cell cycle maintain asymmetry in *Caulobacter* (Shapiro *et al*., 2002). The biosynthesis of stalks is poorly understood but occurs only at one pole (or two poles in the case of *Asticcacaulis biprosthecum*) of the cell where the swarmer cell ejects its flagellum. One hypothesis is that FtsZ, the tubulin-like protein that polymerizes into a ring structure associated with the cytoplasmic membrane at the site of cell division, could also be involved in the topological reorientation of peptidoglycan synthesis required for stalk synthesis (Quardokus *et al*., 1996). It is unknown if the wall of these gram-negative bacteria would withstand fossilization and maceration in acids (technique used to extract organic-walled microfossils from shales). Moreover these morphologies differ from *Tappania* by their vesicle size; appendage diameter, number, distribution and morphology (not branching, not heteromorphic); and the absence of neck-like extensions (a morphology unknown in prokaryotes).

Some actinobacterial spores can produce up to three germ tubes, but these tubes originate within the spore interior and perforate the spore wall as they proliferate. Thus, their morphology bears little in common with *Tappania*; moreover, they are much smaller (Holt, 1984). *Streptomyces* can have 100 μm protoplasts from which hyphae emerge (Cavalier-Smith, 2002), but these consist of amorphous masses with open expanded tubes and do not closely resemble the *Tappania* vesicles illustrated clearly in a number of publications (Prasad and Asher, 2001; Xiao *et al*., 1997; Yin, 1998), or other Mesoproterozoic spiny acritarchs, despite cavalier claims to the contrary (Cavalier-Smith, 2002). Indeed, as Cavalier-Smith (2002, p. 37) pointed out, 'cysts with spines or reticulate surface sculpturing would probably have required both an endomembrane system and a cytoskeleton, the most fundamental features of the eukaryotic cell, for their construction'. We agree, and underscore the fact that such fossils appear in the fossil record only slightly later than 1500 Ma.

Could large prokaryotes form large appendages? The answer to this question is not known although no examples have been reported from nature, as far as we know. Heterotrophic and chemotrophic bacteria absorb nutrients over their external surface. Thus the surface-to-volume ratio of the cells is important and might explain why most bacteria are small rods, filaments, or vibrios (Koch, 1996). Another solution to optimize nutrient absorption is to branch and produce a colony with hyphae branching out in all directions as the actinobacteria and myxobacteria do. The largest bacteria live in special nutrient-rich environments such as fish gut

(heterotrophic gut bacteria *Epulopiscium*, 80×600 μm) or sulfide-rich seafloor (sulfur bacteria *Thiomargarita namibiense*, 750 μm; filamentous sulfur Bacteria *Beggiatoa*, bundles of filaments in sheath, 160×50 μm), where limitation of diffusion through a large cell would not be a problem (Schulz and Jorgensen, 2001). As mentioned above, some cyanobacteria can produce large colonial envelopes up to 30–50 μm in diameter (Waterbury and Stanier, 1978) or cylindrical akinetes more than 100 μm long (Golubic *et al*., 1995), and myxobacteria can produce sporangioles up to 50 μm in diameter. However none of these large bacterial structures are ornamented.

2.2.3. *Excystment Structures*

The cyst walls of Phanerozoic protists commonly contain well-defined openings through which motile cells escape. These excystment structures range from simple perforations that run the circumference of cyst walls ('medial splits') to the polygonal archaeopyles of dinoflagellates. Several preserved features of Roper microfossils may document excystment in mid-Proterozoic eukaryotes.

The simplest candidate excystment structures are, unsurprisingly, medial splits, as found in *Valeria lophostriata* (Figure 1: 1). One must be careful to distinguish between accidental tearing of the vesicle and biologically programmed splits. Observation of regular openings in a large population of vesicles would provide convincing evidence of excystment, but Roper populations display only limited numbers of splits. Thus, their interpretation remains conjectural. (Note that some pleurocapsalean cyanobacteria cells rupture to liberate their baeocytes (Waterbury and Stanier, 1978); these cells can reach sizes up to 30 μm, but ruptured walls do not separate clearly into two halves and do not roll-up.)

As noted in the preceding section, *Tappania plana* exhibits what appears to be a sophisticated excystment structure consisting of an opening at the end of a neck-like extension (Figure 1: 8). No prokaryotes, as far as we know, exhibit a comparable morphology.

2.2.4. *Wall Ultrastructure*

In conjunction with observations of living protists, TEM observations of wall ultrastructure may provide evidence for specific eukaryotic affiliation. For example, the walls around phycomata of some prasinophyte green alga have a diagnostic ultrastructure consisting of a homogeneous electron-dense wall punctuated by pore canals (Wall, 1962; Jux, 1968). Talyzina and Moczydlowska (2000) have identified such an ultrastructure in the Early Cambrian prasinophyte *Tasmanites tenellus*, demonstrating that this feature can be preserved in ancient microfossils. To date, few ultrastructural studies of Proterozoic acritarchs have been completed. Oehler (1977) reported unilayered walls of small unicells from the Neoproterozoic Bitter Springs Formation. Peat (1981) illustrated the homogeneous wall of three leiospherids from the McMinn Formation of the Roper Group. Arouri *et al*. (1999, 2000) studied the ultrastructure and chemistry of Neoproterozoic acritarchs from

Australia and suggested a dinoflagellate affinity for some species with a fibrillar multilayered wall and a chlorophycean affinity for other species with possibly preserved TLS (trilaminar sheath structure, characteristic of many green algae). Talyzina (2000) found a single-layered, electron-dense homogenous wall ultrastructure for the species *Chuaria circularis* from the Neoproterozoic Visingso Group, Sweden. The same species from the Neoproterozoic Pendjari Formation, West Africa, (Amard, 1992) showed a multilamellar ultrastructure with pore canals. We are currently conducting ultrastructural research on the walls of Roper and other Mesoproterozoic fossils. Preliminary results indicate that some Mesoproterozoic acritarchs display comlex, multi-layered wall ultrastructure similar in general to extant protists (Javaux *et al*., 2002b).

2.2.5. *Wall Chemistry*

Conventional extraction of lipid biomarker molecules can identify the presence of eukaryotic organisms in an ancient ecosystem (Summons and Walter, 1990), but correlation of biomarkers with specific microfossil taxa is fraught with difficulty. Moldowan and Talyzina (1998) reported the strong correlation of specific steranes such as dinosterane with particular acritarch populations in Lower Cambrian shales. It is certainly possible that a single species made both the biomarkers and the preserved cyst walls, but insofar as sterols (the parent molecules of geologically stable steranes) are not wall constituents (but, rather, part of the lipid bilayer of the cell membrane), it is hard to reject the alternative explanation that steranes and cyst walls reflect two different organisms that lived in ecological association. Paleobotanists, who commonly find isolated seeds and leaves on a single bedding plane, will immediately recognize the problem.

Another major difficulty resides in the fact that very little is known about the chemical composition of various potentially fossilizable structures (such as vegetative cells and cysts) of recent protists. The few biomarkers characteristic of eukaryotes include a variety of C_{28-30} steranes with side-chains alkylation patterns (Summons and Walter, 1990; Brocks, 2003) among which dinosteranes that are derived from dinosterol produced by dinoflagellates (and one diatom species; Volkman *et al*., 1993).

Recently developed geochemical techniques now permit microchemical analyses of individual microfossils (e.g., Arouri *et al*., 1999, 2000; Boyce *et al*., 2002; House *et al*., 2000; Kempe *et al*., 2002; Kudryavtsev *et al*., 2001; Schopf *et al*., 2002). In particular, laser micropyrolysis of single acritarchs may show biomarkers specific for eukaryotes (steranes) and even diagnostic of particular groups. However, it is possible that steranes would adhere to the surface of cell walls and not originate from the cell membrane that was once contained in that wall. Conclusive evidence (that the biomarker comes from the analyzed microfossil) would be obtained if various taxa from the same sample show different biomarker signatures that also differ from the host sediment. In conjunction with ultrastructural study, chemical analyses of single Neoproterozoic acritarchs (Arouri *et al*.,

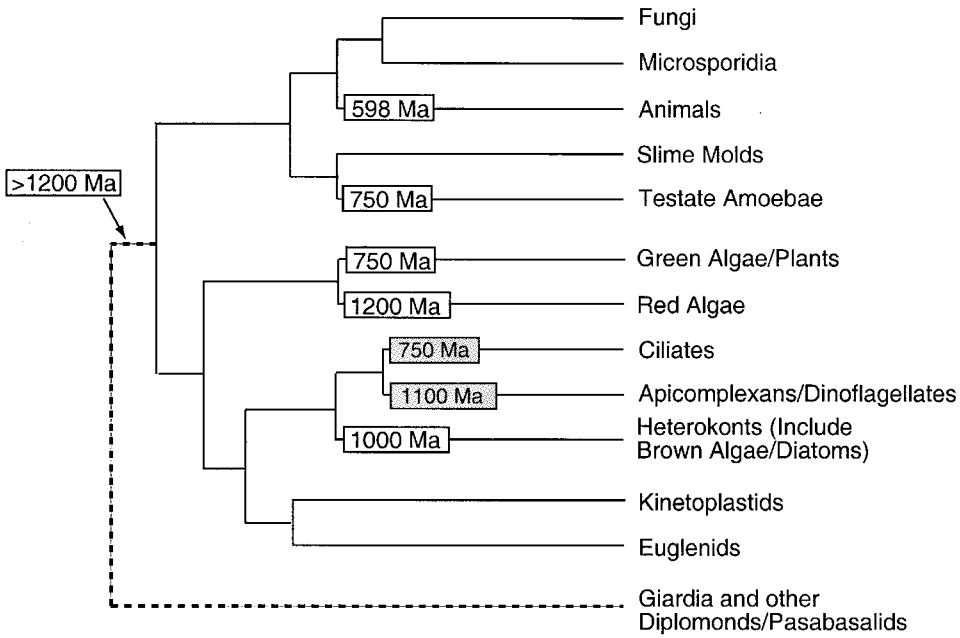

Figure 2. Phylogenetic tree for eukaryotes (modified from combined-protein tree of Baldauf *et al.*, 2000) calibrated using the paleobiological record (shaded boxes: biomarkers ; white boxes: body fossils – see text for details) and inferred minimum time of divergence.

1999, 2000) suggested possible affiliations with chlorophycean green algae (based on the finding of highly aliphatic biomacromolecules resembling the algaenan biomacromolecules of Chlorophyceae) and with dinoflagellates (among the two biomarkers of dinoflagellates, dinosterane and 4α-methyl-24-ethylsterane, only the latter rather not specific was found). Scanning transmission X-ray microscopy and spectroscopy provides another means of obtaining micron-scale chemical analysis of organically preserved acritarch walls (Boyce *et al.*, 2002). Microchemical studies of Roper fossils are planned, in conjunction with chemical analysis of acid-resistant structures of recent protists.

3. Discussion

The criteria for fossil interpretation outlined above will not eliminate all uncertainties in Proterozoic paleontology, but they will help us to fill in the gap between the origin of the eukaryotic kingdom and its Neoproterozoic morphological diversification. This, in turn, will permit more accurate and reliable geological calibration of eukaryotic phylogenies constructed using molecular sequence data (e.g., Baldauf *et al.*, 2000). At present, the fossil record provides minimum ages for a number of events in early eukaryotic evolution (Figure 2).

Biomarkers in 2.7 Ga kerogens of the Fortescue Group, Australia, indicate that contemporaneous cells were able to synthesize sterols (Brocks *et al*., 1999, 2003a,b). Sterol biosynthesis is a characteristic of eukaryotic cells – sterols have been found in bacteria, but known cases either result from the incorporation of molecules made by eukaryotic organisms (Bloch, 1994) or synthesis directed by genes transferred laterally from eukaryotic cells (Lamb *et al*., 1998; Gamieldien *et al*., 2002). As far as we know, only one bacterium (*Methylococcus capsulatus*) has been reported to produce methyl-sterols; these, however, differ structurally from eukaryotic sterols (Bouvier *et al*., 1976). Thus, accepting SSU rRNA phylogenies, Fortescue biomarkers would seem to set a minimum date for the divergence of Archaea and Eucarya. The presence in Fortescue rocks of carbon isotopic signatures thought to reflect methanogenic archaeans and methanotrophic bacteria independently suggests the same thing. By themselves, steranes in 2.7 Ga rocks do not necessarily imply the Archean evolution of eukaryotic cells with nuclei, cytoskeletons and endomembranes – although it is certainly not ruled out by the scanty evidence available in very old sediments.

Fossils thought to be eukaryotic occur in Paleo- and Mesoproterozoic rocks around the world, but at present, few of these can be related to specific protistan clades. Han and Runnegar (1992) interpreted millimetric coiled filaments found in the 1.87 Ga Negaunee Iron Formation, Michigan (Schneider *et al*., 2002), as eukaryotic, based on their broad resemblance to large helical fossils in 1.4 Ga rocks from China (Geoyuzhuan Formation; Walter and Du Rulin, 1990), the western United States (Walter *et al*., 1976) and India (Kumar, 1995) that are more clearly eukaryotic (based on morphological features such as coiling and transverse septae). Recently, however the interpretation of the 1.87 Ga coiled structures has been questioned, as there is no preserved microscopic detail (Samuelsson and Butterfield, 2001). Large (up to 200 μm) sphaeromorphic acritarchs possibly in the 2.4–2.5 Ga Hutuo Formation of China but very poorly illustrated (Sun and Zhu, 1998) and in the 1.8 Ga Chuanliggou Formation of China (Zhang, 1986) are also candidates for early eukaryotes, although none are known to exhibit the features of wall ornamentation, processes or excystment structures observed in Roper fossils.

As discussed above, more convincing microfossil evidence of eukaryotic microorganisms appears around 1.5–1.4 Ga in the Roper Group of Australia (Javaux *et al*., 2001) and other early Mesoproterozoic successions. Microfossils from the Ruyang Group, northern China, include not only the taxa found in Roper rocks, but also acritarchs with numerous regularly arranged cylindrical processes (Guan *et al*., 1988; Xiao *et al*., 1997; Yin, 1998). Such fossils strongly indicate that eukaryotic organisms of marked cytological and genetic complexity existed 1,500–1,300 million years ago. Relative to earlier assemblages, early Mesoproterozoic protists show higher diversity and more obvious ecological heterogeneity (Javaux *et al*., 2001).

At present, however, we do not know how to relate early Mesoproterozoic fossils to specific branches of the eukaryotic tree. More satisfying in this regard are

ca. 1.2 Ga fossils from the Hunting Formation in arctic Canada (Butterfield *et al*., 1990; Butterfield, 2000) that can be related with confidence to the bangiophyte red algae, based on observation of a nearly complete ontogenetic sequence for the preserved haploid generation, diagnostic cell division patterns, and morphologically distinct sexual dimorphs in a large and exceptionally well-preserved population. This provides a firm calibration point for molecular phylogenies, implying that by 1,200 million years ago, major branches of the eukaryotic tree were already in place.

At 1 Ga, *Palaeovaucheria*, a xanthophyte from the recently well-dated Lakhanda Formation, Siberia (German, 1990; Rainbird *et al*., 1998; Woods *et al*., 1998), indicates the appearance of stramenopiles (which include diatoms, xanthophytes, and brown algae) and of secondary symbiosis (involving a red alga-like endosymbiont). Populations of *Paleovaucheria* display morphological traits characteristic of vaucherian xanthophytes such as branching at right angles, 2 sizes of filaments on the same individual, and terminal pores and septae at filament ends (Woods *et al*., 1998). Vaucherians recently discovered in ca. 700–800 Ma shales in Spitsbergen display a more complete range of vaucherian morphologies (Butterfield, 2002).

Upper Mesoproterozoic/Lower Neoproterozoic rocks have also yielded biomarkers of alveolates (which include dinoflagellates and ciliates, among other groups). Dinosterane, derived from dinosterol produced by dinoflagellates, occurs in the 1.1 Ga Nonesuch Formation, United States (Pratt *et al*., 1991); the 830 Ma Bitter Springs Formation, Australia; and the 590–570 Ma Pertatataka Formation, also Australia (Summons and Walter, 1990; Summons *et al*., 1992; Moldowan *et al*., 1996). Gammacerane, derived from tetrahymenol produced by ciliates has been found in ca. 750 rocks of the Chuar Group, Arizona (Summons *et al*., 1988b) however it could also be derived from some bacteria (Kleeman *et al*., 1990). It is unknown whether Proterozoic dinoflagellates were photosynthetic.

Proterocladus, a *Cladophora*-like alga from the ca. 750 Ma Svanbergfjellet Formation of Spitsbergen (Butterfield *et al*., 1994), suggests that chlorophyte diversification was well advanced by the mid-Neoproterozoic. As noted above, filose and lobose testate amoebae from >742±6 Ma rocks of the Chuar Group, Arizona, provide a firm calibration point for the great clade that includes animals, fungi and the amoebozoans (Baldauf *et al*., 2000; Bapteste *et al*., 2002), not to mention direct evidence for heterotrophic eukaryotes and eukaryotic biomineralization (Porter and Knoll, 2000; Porter *et al*., in press).

Finally the Doushantuo Formation of China, recently dated at ca. 598±2 Ma (Barfod *et al*., 2002), hosts multicellular green, red and, possibly brown algae, as well as animal embryos, possible stem group cnidarians, and putative sponges (Xiao *et al*., 1998a, b, 2002; Li *et al*., 1998; Xiao and Knoll, 2000).

Molecular clocks estimate phylogenetic divergence times, whereas fossils record the evolution of recognizable body plans within clades that diverged earlier. Thus, Proterozoic fossils furnish only minimum dates for eukaryotic clade divergence. Nonetheless, the fossil record suggests that the Mesoproterozoic and early

Neoproterozoic eras were crucial times for eukaryotic diversification (Figure 2). By the time that large animals appear in the latest Proterozoic, much diversification among and within major eukaryotic clades had already taken place.

This view contrasts strongly with Cavalier-Smith's (2002) inexplicable view that eukaryotes evolved only 850 million years ago. (Actually, Cavalier-Smith's claim is inexplicable only from the standpoint of actual observations of Proterozoic fossils. It finds ready explanation in his argument that most principal clades of eukaryotes diverged rapidly from one another early in the history of the domain. Accepting Ediacaran fossils as faithful indicators of animal – and, hence, eukaryotic divergence – Cavalier-Smith is forced to doubt all significantly older records of nucleated organisms. There is, of course, a more reasonable reconciliation of fossils and molecular phylogeny – tissue-grade metazoans doubtfully reflect accurately (or even remotely) the timing of eukaryotic origins. Using Cavalier-Smith's own criteria for the recognition of eukaryotic fossils (2002, p. 37, quoted above), we must conclude that protists played a role in marine ecosystems 1,500 Ma and, conceivably, much earlier. Later Proterozoic diversification with eukaryotic clades reflects environmental opportunity (e.g., Anbar and Knoll, 2002) and the polyphyletic rise of complex multicellularity, likely in combination (e.g., Knoll and Carroll, 1999; Butterfield, 2000).

4. Conclusions

In combination with molecular biology and geology, detailed studies of organic-walled microfossils focusing on wall morphology, structure, ultrastructure, and chemistry will help us to understand better the early evolution of eukaryotic organisms. In particular, studies of Paleo- and Mesoproterozoic fossils, including our research on the Roper Group, Australia, will help to fill the gap between the earliest biogeochemical record of protists and their late Mesoproterozoic/Neoproterozoic diversification. Future developments of this work must include the refinement of TEM and microchemical techniques that hold out the promise of better systematic characterization of morphologically simple or enigmatic microfossils.

Acknowledgements

This article is a contribution to the Australian Geological Survey Organisation's NABRE project. We thank Jim Jackson, Peter Southgate and other members of the NABRE team for access to unpublished observations, helpful discussions, and advice on sampling. Core library staff in Canberra (AGSO) and Darwin (Northern Territory Geological Survey) greatly facilitated sample collection. We thank Nick Butterfield and an anonymous reviewer for helpful comments. We also thank Jochen Brocks for information on biomarker chemistry and Richard Losick for

discussion on bacterial morphology. Yin Leiming provided helpful information on Chinese stratigraphy, and Volodya Sergeev on Russian paleontology. Research supported in part by NASA Exobiology Grant NAG5-3645 and the NASA Astrobiology Institute, the Australian Research Council and Macquarie University.

References

Abbott, S. T. and Sweet, I. P.: 2000, Tectonic Control on Third-order Sequences in a Siliciclastic Ramp-style Basin: An Example from the Roper Superbasin (Mesoproterozoic), Northern Australia, *Austral. J. Earth Sci.* **47**, 637–657.

Amard, B.: 1992, Ultrastructure of *Chuaria* (Walcott) Vidal and Ford (Acritarcha) from the Late Proterozoic Pendjari Formation, Benin and Burkina-Faso, West Africa, *Prec. Res.* **57**, 121–133.

Anbar, A. D. and Knoll, A. H.: 2002, Proterozoic Ocean Chemistry and Evolution: A Bioinorganic Bridge? *Science* **297**(5584), 1137–1142.

Arouri, K., Greenwood, P. F. and Walter, M. R.: 1999, A Possible Chlorophycean Affinity of some Neoproterozoic Acritarchs, *Org. Geochem.* **30**, 1323–1337.

Arouri, K., Greenwood, P. F. and Walter, M. R.: 2000, Biological Affinities of Neoproterozoic Acritarchs from Australia: Microscopic and Chemical Characterisation, *Org. Geochem.* **31**, 75–89.

Baldauf, S. L., Roger, A. J., Wenk-Siefert, I. and Doolittle, F.: 2000, A Kingdom-level Phylogeny of Eukaryotes Based on Combined Protein Data, *Science* **290**, 972–976.

Barfod, G. H., Albarède, F., Knoll, A. H., Xiao, S., Télouk, P., Frei, R. and Baker, J.: 2002, New Lu-Hf and Pb-Pb Age Constraints on the Earliest Animal Fossils, *Earth Planet. Sci. Lett.* **201**(1), 203–212.

Bartley, J. K.: 1996, Actualistic Taphonomy of Cyanobacteria: Implications for the Precambrian Fossil Record, *Palaios* **11**, 571–586.

Bloch, K.: 1994, *Evolutionary Perfection of a Small Molecule, in Blondes in Venetian Paintings, the Nine-banded Armadillo, and other Essays in Biochemistry*, Yale University Press, Vol. 2, pp. 15–36.

Boone, D. R., Castenholz, R. W. (eds): 2001, *Bergey's Manual of Systematic Bacteriology. The Archea and the Deeply Branching and Phototrophic Bacteria*, Vol. 1, 2nd ed., Springer, N.Y.

Boyce, C. K., Cody, G. D., Feser, M., Jacobsen, Knoll, A. H. and Wirick, S: 2002, Organic Chemical Differentiation within Fossil Plant Cell Walls Detected with X-ray Spectromicroscopy, *Geology* **30**(11), 1039–1042.

Bouvier, P., Rohmer, M. and Benveniste, P.: 1976, $\Delta 8(14)$-Steroids in the Bacterium *Methylococcus capsulatus*, *Biochem. J.* **159**, 267–271.

Brocks, J. J., Logan, G. A., Buick, R. and Summons, R. E.: 1999, Archean Molecular Fossils and the Early Rise of Eukaryotes, *Science* **285**, 1033–1036.

Brocks, J. J., Buick, R., Summons, R. E. and Logan, G. A.: 2003a, A Reconstruction of Archean Biological Diversity based on Molecular Fossils from the 2.78–2.45 Billion Year Old Mount Bruce Supergroup, Hamersley Basin, Western Australia, *Geochim. Cosmochim*. Acta (in press).

Brocks, J. J., Buick, R., Logan, G. A. and Summons, R. E.: 2003b, Composition and Syngeneity of Molecular Fossils from the 2.78–2.45 Billion Year Old Mount Bruce Supergroup, Pilbara Craton, Western Australia, *Geochim. Cosmochim. Acta* (in press).

Brun, Y. V. and Janakiraman, R.: 2000, 'The Dimorphic Life Cycle of Caulobacter and Stalked Bacteria', in Y. V. Brun and L. J. Shimkets (eds), *Prokaryotic Development*, ASM Press Washington, DC, pp. 297–318.

Butterfield, N. J., Knoll, A. H. and Swett, K.: 1990, A Bangiophyte Red Alga from the Proterozoic of Artic Canada, *Science* **250**, 104–107.

Butterfield, N. J., Knoll, A. H. and Swett, K.: 1994, Paleobiology of the Neoproterozoic Svanbergfjellet Formation, Spitsbergen, *Fossils and Strata* **34**, 1–84.

Butterfield, N. J.: 2000, *Bangiomorpha pubescens* n.gen., n.sp.: Implications for the Evolution of Sex, Multicellularity and the Mesoproterozoic/Neoproterozoic Radiation of Eukaryotes, *Paleobiology* **26**, 386–404.

Butterfield, N. J.: 2002, 'A Vaucheria-like Fossil from the Neoproterozoic of Spitsbergen', *G. S. A. Annual Meeting, Denver, Co.*, 26–30 October 2002, Abstracts with Programs, 75–3, p. 169.

Canfield, D. E.: 1998, A New Model for Proterozoic Ocean Chemistry, *Nature* **396**, 450–453.

Cavalier-Smith, T.: 2002, The Neomuran Origin of Archaebacteria, the Negibacteria Root of the Universal Tree and Bacteria Megaclassification, *Int. J. Syst. Microb.* **52**, 7–76.

Claessen, D., Wosten, H. A. B., van Keulen, G., Faber, O. G., Alves, A. M. C. R., Meijer, W. G. and Dijkhuizen, L.: 2002, Two Novel Homologous Proteins of *Streptomyces coelicolor* and *Streptomyces lividans* are Involved in the Formation of the Rodlet Layer and Mediate Attachment to a Hydrophobic Surface, *Mol. Microb.* **44**(6), 1483–1492.

Derenne, S., Largeau, C., Casadevall, E., Berkaloff, C. and Rousseau, B.: 1991, Chemical Evidence of Kerogen Formation in Source Rocks and Oil Shales via Selective Preservation of Thin Resistant outer Walls of Microalgae: Origin of Ultralaminae, *Geochem. et Cosmochim. Acta* **55**, 1041–1050.

Dworkin, M.: 1985, *Developmental Biology of the Bacteria*, The Benjamin/Cummings Publ. Co. Inc., 255 p.

Gamieldien, J., Ptitsyn, A., and Hide, W.: 2002, Eukaryotic Genes in *Mycobacterium tuberculosis* could have a Role in Pathogenesis and Immunomodulation, *Trends Genet.* **18**(1), 5–8.

German, T. N.: 1990, *Organic World One Billion Years Ago*, Leningrad, Nauka.

Golubic, S. and Barghoorn, E. S.: 1977, 'Interpretation of Microbial Fossils with Special Reference to the Precambrian', in E. Flugel (ed.), *Fossil Algae*, Springer, Berlin, pp. 1–14.

Golubic, S., Sergeev, V. N. and Knoll, A. H.: 1995, Mesoproterozoic Archaeoellipsoides: Akinetes of Heterocystous Cyanobacteria, *Lethaia* **28**, 285–298.

Grey, K. and Williams, I. R.: 1990, Problematic Bedding-plane Markings from the Middle Proterozoic Manganese Subgroup, Bangemall Basin, Western Australia, *Prec. Res.* **46**(4), 307–328.

Guan, B., Geng, W., Rong, Z. and Du, H.: 1988, *The Middle and Upper Proterozoic in the Northern Slope of the Eastern Qinling Ranges, Henan, China*, Henan Science and Technology Press, Zhengzhou, 210 pp.

Han, T.-M. and Runnegar, B.: 1992, Megascopic Eukaryotic Algae from the 2.1-billion-year-old Negaunee Iron Formation, Michigan, *Science* **257**, 232–235.

Hoiczyk, E. and Hansel, A.: 2000, Cyanobacterial Cell Walls: News from an Unusual Prokaryotic Envelope, *J. Bacteriol.* **182**(5), 1191–1199.

Holt, J. G. (editor-in-chief): 1984, *Bergey's Manual of Systematic Bacteriology*, Vol. 4, Williams & Wilkins, Baltimore.

Iizuka, T., Jojima, Y., Fudou, R., Hiraishi, A., Ahn, J.-W. and Yamanaka, S.: 2002, *Plesiocystis pacifica* gen. nov., sp. nov., a Marine Myxobacterium Containing Dihydrogenated Menaquinone Isolated from the Pacific Coasts of Japan, IJSEM, http://dx.doi.org/10.1099/ijs.0.02418-0

House, C. H., Schopf, J. W., McKeegan, K. D., Coath, C. D., Harrison, T. M. and Stetter, K. O.: 2000, Carbon Isotopic Composition of Individual Precambrian Microfossils *Geology* **28**, 707–710.

Jackson, M. J. and Raiswell, R.: 1991, Sedimentology and Carbon-sulfur Geochemistry of the Velkerri Formation, a Mid-Proterozoic Potential Oil Source in Northern Australia, *Precambrian Res.* **54**, 81–108.

Javaux, E., Knoll, A. H. and Walter, M. R.: 2001, Morphological and Ecological Complexity in Early Eukaryotic Ecosystems, *Nature* **412**, 66–69.

Javaux, E., Knoll, A. H. and Walter, M. R.: 2002a, 'Early Eukaryotic Diversification', *Astrobiology Science Conference, NASA Ames Research Center, 7–11 April 2002*, p. 55.

Javaux, E., Knoll, A. H. and Walter, M. R.: 2002b, 'Recognizing and Interpreting the Fossils of Early Eukaryotes', G.S.A. Annual Meeting, Denver, Co., 26-30 October 2002, Abstracts with Programs, 31–1, p. 80.

Jux, U.: 1968, Uber den Feinbau der Wandung bei *Tasmanites* Newton, *Palaeontogr. Abt. B* **124**, 112−124.

Kah, L. C., Lyons, T. W. and Chesley, J. T.: 2002, Geochemistry of a 1.2 Ga Carbonate-evaporite Succession, Northern Baffin and Bylot Islands: Implications for Mesoproterozoic Marine Evolution, *Prec. Res.* **111**, 203–234.

Kempe, A., Schopf, J. W., Altermann, W., Kudryavtsev, A. B. and Heckl, W. M.: 2002, Atomic Force Microscopy of Precambrian Microscopic Fossils, *P.N.A.S.* **99**(14), 9117–9120.

Kim, S.-H., Rasmaswamy, S. and Downard, J.: 1999, Regulated Exopolysaccharide Production in *Myxococcus xanthus*, *J. Bacteriol.* **181**(5), 1496–1507.

Kleeman, G., Poralla, K., Englert, G., Kjosen, H., Liaaen-Jensen, S., Neunlist, S. and Rohmer, M.: 1990, Tetrahymenol from the Phototrophic Bacterium *Rhodopseudomonas palustris*: First Report of a Gammacerane Triterpene from a Prokaryote, *J. Gen. Microb.* **136**, 2551–2553.

Knoll, A. H.: 1981, 'Paleoecology of Late Precambrian Microbial Assemblages', in K. Niklas (ed.) *Paleobotany, Paleoecology, and Evolution*, Vol. I, Praeger, New York, pp. 17–54.

Knoll, A. H. and Barghoorn, E. S.: 1977, Archean Microfossils showing Cell Division from the Swaziland System of South Africa, *Science* **198**, 396–398.

Knoll, A. H. and Carroll, S. B.: 1999, Early Animals Evolution: Emerging Views from Comparative Biology and Geology, *Science* **284**(5423), 2129–2137.

Koch, A.: 1996, What Size should a Bacterium be? A Question of Scale, *Annu. Rev. Microb.* **50**, 317–48.

Kokinos, J. P., Eglinton, T. I., Goni, M. A., Boon, J. J., Martoglio, P. A. and Anderson, D. M.: 1998, Characterization of a Highly Resistant Biomacromolecular Material in the Cell Wall of a Marine Dinoflagellate Resting Cyst, *Org. Geochem.* **28**(5), 265–288.

Kralik, M.: 1982, Rb-Sr Age Determinations on Precambrian Carbonate Rocks of the Carpentarian McArthur Basin, Northern Territory, Australia, *Precambrian Res.* **18**, 157–170.

Kudryavtsev, A. B., Schopf, J. W., Agresti, D. G. and Wdowiak, T. J.: 2001, *In situ* Laser-Raman Imagery of Precambrian Microscopic Fossils, *Proc. Nat. Acad. Sci. U.S.A.* **98**, 823–826.

Kumar, S.: 1995, Megafossils from the Mesoproterozoic Rohtas Formation (the Vindhyan Supergroup), Katni Area, Central India, *Prec. Res.* **72**, 171–184.

Lamb, D. C., Kelly, D. E., Manning, N. J. and Kelly, S. L.: 1998, A Sterol Biosynthetic Pathway in *Mycobacterium*, *FEBS Letters* **437**, 142–144.

Li, C. W., Chen, J.-Y. and Hua, T.-E.: 1998, Precambrian Sponges with Cellular Structures, *Science* **279**, 879–882.

Lybarger, S. R. and Maddock, J. R.: 2001, Polarity in Action: Asymmetric Protein Localization in Bacteria, *J. Bacter.* **183**(11), 3261–3267.

Matsumoto, A., Takahashi, T., Seino, A., Iwai, Y. and Omura, S.: 2000, *Actinoplanes capillaceus* sp. nov., a New Species of the Genus Actinoplanes, *Antonie van Leeuwenhoek* **78**, 107–115.

Miyadoh, S.: 1997, *Atlas of Actinomycetes, The Society for Actinomycetes, Japan*, Asakura Publ. Co. Ltd., 223 p.

Moldowan, J. M. and Talyzina, N. M.: 1998, Biogeochemical Evidence for Dinoflagellate Ancestors in the Early Cambrian, *Science* **281**, 1168–1170.

Moldowan, J. M., Dahl, J., Jacobson, S. R., Huizinga, B. R., Fago, F. J., Shetty, R., Watt, D. S. and Peters, K. E.: 1996, Chemostratigraphic Reconstruction of Biofacies: Molecular Evidence Linking Cyst-forming Dinoflagellakes with Pre-Triassic Ancestors, *Geology* **24**(2), 159–162.

Oehler, D. Z.: 1977, Pyrenoid-like Structures in Late Precambrian Algae from the Bitter Springs Formation of Australia, *J. Paleont.* **51**(5), 885–901.

Peat, C. J.: 1981, Comparative Light Microscopy, Scanning Electron Microscopy and Transmission Electron Microscopy of Selected Organic Walled Microfossils, *J. Microscopy* **122**(3), 287–294.

Poindexter, J. S. and Staley, J. T.: 1996, Caulobacter and Asticcacaulis Stalk Bands as Indicators of Stalk Age, *J. Bacteriol.* **178**(3), 3939–3948.

Porter, S. M. and Knoll, A. H.: 2000, Testate Amoebae in the Neoproterozoic Era: Evidence from Vase-shaped Microfossils in the Chuar Group, Grand Canyon, *Paleobiology* **26**(3), 360–385.

Porter, S. M., Meisterfeld, R. and Knoll, A. H.: Vase-shaped Microfossils from the Neoproterozoic Chuar Group, Grand Canyon: A Classification Guided by Modern Testate Amoebae, *J. Paleontol.* (in press).

Prasad, B. and Asher, R.: 2001, Acritarch Biostratigraphy and Lithostratigraphic Classification of Proterozoic and Lower Paleozoic Sediments (Pre-Unconformity Sequence) of Ganga Basin, India, *Paleontograph. Indica* **5**, 151.

Pratt, L. M., Summons, R. E. and Hieshima, G. B.: 1991, Sterane and Triterpane Biomarkers in the Precambrian Nonesuch Formation, North American Midcontinent Rift, *Geochem. Cosmochim. Acta* **55**, 911–916.

Quardokus, E., Din, N. and Brun, Y. V.: 1996, Cell Cycle Regulation and Cell Type-specific Localization of the FtsZ Division Initiation Protein in Caulobacter, *P.N.A.S.* **93**, 6314–6319.

Rainbird, R. H., Stern, R. A., Khudoley, A. K., Kropachev, A. P., Heman, L. M. and Sukhorukov, V. I.: 1998, U-Pb Geochronology of Riphean Sandstone and Gabbro from Southeast Siberia and its bearing on the Laurentia-Siberia Connection, *Earth Pl. Sci. Lett.* **164**, 409–420.

Reichenbach, H. and Dworkin, M.: 2001, 'The Myxobacteria', in M. Dworkin *et al*. (eds), *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*, 3rd edn., Release 3.7, 2 November 2001, Springer-Verlag, New York, www.prokaryotes.com

Runnegar, B.: 1995, 'Vendobionta or Metazoa?: 1995, Developments in understanding the Ediacara 'Fauna' ', in W. E. Reif, F. Westphal and A. Seilacher (eds), *Festschrift for Professor Adolf Seilacher in Honor of Reaching his 70th birthday on 24 February 1995*, Neues Jahrb. fuer Geol. Palaeontol., Abhandl. Vol. 195, 1–3, pp. 303–318.

Samuelsson, J. and Butterfield, N.: 2001, Neoproterozoic Fossils from the Franklin Mountains, Northwestern Canada: Stratigraphic and Palaeobiological Implications. *Prec. Res.* **107**, 235–251.

Sara, M. and Sleytr, U. B.: 2000, S-Layer Proteins, *J. Bacteriol.* **182**(4), 859–868.

Schlessner, H.: 1987, *Verrucomicrobium spinosum* gen. nov., sp. nov., a Fimbriated Prosthecate Bacterium, *System. Appl. Microbiol.* **10**, 54–56.

Schneider, D. A., Bickford, M. E., Cannon, W. F., Schulz, K. J. and Hamilton, M. A.: 2002, Age of Volcanic Rocks and Syndepositional Iron Formations, Marquette Range Supergroup; Implications for the Tectonic Setting of Paleoproterozoic Iron Formations of the Lake Superior Region, *Can. J. Earth Sci.* **39**(6), 999–1012.

Schopf, J. W.: 1968, Precambrian Microorganisms from Central and South Australia, *Am. J. Botany* **55**(6P2), 722.

Schopf, J. W.: 1977, Biostratigraphic Usefulness of Stromatolitic Precambrian Microbiotas-preliminary Analysis, *Prec. Res.* **5**(2), 143–173.

Schopf, J. W., Kudryavtsev, A. B., Agresti, D. G., Wdowiak, T. J. and Czaja, A. D.: 2002, Laser-Raman Imagery of Earth's Earliest Fossils, *Nature* **416**(6876), 73–76.

Schulz, H. N., Brinkhoff, T., Ferdelman, T. G., Marine, M. H. and Teske, A.: 1999, Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments, *Science* **284**, 493–495.

Schulz, H. N. and Jorgensen, B. B.: 2001, Big Bacteria, *Annu. Rev. Microbiol.* **55**, 105–137.

Shapiro, L., McAdams, H. H. and Losick, R.: 2002, Generating and Exploiting Polarity in Bacteria, *Science* **298**, 1942–1946.

Shen, Y., Canfield, D. E., and Knoll, A. H.: 2002, Middle Proterozoic Ocean Chemistry: Evidence from the McArthur Basin, Northern Australia, *Am. J. Sci.* **302**, 81–109.

Shen, Y. and Knoll, A., 2002, 'Facies Dependence of S-isotopic Composition in Pyrites from the ~1.5 Ga Roper Group, McArthur Basin, Northern Australia', *Astrobiology Science Conference, NASA Ames Research Center, 7–11 April 2002*, p. 150.

Soto, G. E. and Hultgren, S. J.: 1999, Bacterial Adhesins: Common Themes and Variations in Architecture and Assembly, *J. bacteriol.* **181**(4), 1059–1071.

Summons, R. E., Brassell, S. C., Eglington, G., Evans, E., Horodyski, R. J., Robinson, N. and Ward, D. M.: 1988b, Distinctive Hydrocarbon Biomarkers from Fossiliferous Sediment of the Late Proterozoic Walcott Member, Chuar Group, Grand Canyon, Arizona, *Geochem. Cosmochim. Acta* **52**, 2625–2637.

Summons, R. E., Jahnke, L. L., Hope, J. M. and Logan, G. A.: 1999, 2-Methylhopanoids as Biomarkers for Cyanobacterial Oxygenic Photosynthesis, *Nature* **400**, 554–557.

Summons, R. E., Powell, T. G. and Boreham, C. J.: 1988a, Petroleum Geology and Geochemistry of the Middle Proterozoic McArthur Basin, Northern Australia. III. Composition of Extractable Hydrocarbons, *Geochim. Cosmochim. Acta* **51**, 3075–3082.

Summons, R. E., Thomas, J., Maxwell, J. R. and Boreham, C. J.: 1992, Secular and Environmental Constraints on the Occurrence of Dinosterane in Sediments, *Geochim. et Cosmochim. Acta* **56**, 2437–2444.

Summons, R. E. and Walter, M. R.: 1990, Molecular Fossils and Microfossils from Proterozoic Sediments, *Am. J. Science* **290-A**, 212–244.

Sun, S. and Zhu, S.: 1998, The Discovery of Micropaleophytes from the Doucun Subgroup, Hutuo Group in Wutai Mountain, Shanxi, *China Acta Micropalaeontologica Sinica* **15**(3), 286–293.

Talyzina, N. M.: 2000, Ultrastructure and Morphology of *Chuaria circularis* (Walcott, 1899) Vidal and Ford (1985) from the Neoproterozoic Visingso Group, Sweden, *Prec. Res.* **102**, 123–134.

Talyzina, N. M. and Moczydlowska, M.: 2000, Morphological and Ultrastructural Studies of Some Acritarchs from the Lower Cambrian Lukati Formation, *Estonia, Rev. Palaeobot. Palynol.* **112**, 1–21.

Tegelaar, E. W., De Leeuw, J. W., Derenne, S. and Largeau, C.: 1989, A Reappraisal of Kerogen Formation, *Geoch. et Cosmoch. Acta* **53**, 3103–3106.

Volkman, J. K., Kearney, P. and Jeffrey, S. W.: 1990, A New Source of 4-Methyl Sterols and 5α(H)-Stanols in Sediments: Prymnesiophyte Microalgae of the Genus Pavlova, *Org. Geochem.* **15**(5), 489–497.

Volkman, J. K., Barrett, S. M., Dunstan, G. A. and Jeffrey, S. W.: 1993, Geochemical Significance of the Occurrence of Dinosterol and Other 4-Methyl Sterols in a Marine Diatom, *Org. Geochem.* **20**(1), 7–15.

Wall, D.: 1962, Evidence from Recent Plankton Regarding the Biological Affinities of *Tasmanites* Newton 1875 and *Leiosphaeridia* Eisenack 1958, *Geol. Mag.* **4**, 353–362.

Walter, M. R. and Du Rulin: 1990, Coiled Carbonaceous Megafossils from the Middle Proterozoic of Jixian (Tianjin) and Montana, *Am. J. Sci.* **290-A**, 133–148.

Walter, M. R., Oehler, J. H. and Oehler, D. Z.: 1976, Megascopic Algae 1300 Million Years Old from the Belt Supergroup, Montana: A Reinterpretation of Walcott's *Helminthoidichnites*, *J. Paleontol.* **50**(5), 872–881.

Waterbury, J. B. and Stanier, R. Y.: 1978, Patterns of Growth and Development in Pleurocapsalean cyanobacteria, *Microbiol. Rev.* **42**(1), 2–44.

Woods, K. N., Knoll, A. H. and German, T. N.: 1998, Xanthophyte Algae from the Mesoproterozoic/Neoproterozoic Transition: Confirmation and Evolutionary Implications, G.S.A. Abstracts with Programs, 30, A 232.

Xiao, S., Knoll, A. H., Kaufman, A. J., Yin, L. and Zhang, Y.: 1997, Neoproterozoic Fossils in Mesoproterozoic Rocks? Chemostratigraphic Resolution of a Biostratigraphic Conundrum from the North China Platform, *Precambrian Res.* **84**, 197–220.

Xiao, S. and Knoll, A. H.: 2000, Phosphatized Embryos from the Neoproterozoic Doushantuo Formation at Weng'an, Guizhou, South China, *J. Paleont.* **74**, 767–788.

Xiao, S., Knoll, A. H. and Yuan, X.: 1998a, Morphological Reconstruction of Maiohephyton Bifurcatum. A Possible Brown Alga from the Doushantuo Formation (Neoproterozoic), South China, and its Implication for Stramenopile Evolution, *J. Paleont.* **72**, 1072–1086.

Xiao, S., Zhang, Y. and Knoll, A. H.: 1998b, Three-dimensional Preservation of Algae and Animal Embryos in a Neoproterozoic Phosphorite, *J. Paleont.* **72**, 1072–1086.

Xiao, S., Yuan, X., Steiner, M. and Knoll, A. H.: 2002, Macroscopic Carbonaceous Compressions in a Terminal Proterozoic Shales: A Systematic Reassessment of the Miaohe Biota, South China, *J. Paleont.* **76**(2), 347–376.

Yin, L.: 1998, Acanthomorphic Acritarchs from Meso-Neoproterozoic Shales of the Ruyang Group, Shanxi, China, *Rev. Palaeobot. Palynol.* **98**, 15–25.

Zang, W. and Walter, M. R.: 1992, Late Proterozoic and Early Cambrian Microfossils and Biostratigraphy, Amadeus Basin, Central Australia, *Memoirs Ass. Austr. Palaeont.* **12**, 1–132.

Zhang, Y., Yin, L. M., Xiao, S. H. and Knoll, A. H.: 1998, Permineralized Fossils from the Terminal Proterozoic Doushantuo Formation, South China, *J. Paleont.* **72**(4), Suppl. S, 1–52.

Zhang, Z.: 1986, Clastic Facies Microfossils from the Chaunlingguo Formation (1800 Ma) near Jixian, North China, *J. Micropalaeontol.* **5**(2), 9–16.