



This is a repository copy of *The Nonesuch Formation Lagerstätte: a rare window into freshwater life one billion years ago.*

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/171464/>

Version: Published Version

Article:

Strother, P.K. and Wellman, C.H. orcid.org/0000-0001-7511-0464 (2021) The Nonesuch Formation Lagerstätte: a rare window into freshwater life one billion years ago. *Journal of the Geological Society*, 178 (2). jgs2020-133. ISSN 0016-7649

<https://doi.org/10.1144/jgs2020-133>

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:
<https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>



The Nonesuch Formation Lagerstätte: a rare window into freshwater life one billion years ago

Paul K. Strother^{1*} and Charles H. Wellman²

¹ Weston Observatory, Boston College, Department of Earth & Environmental Sciences, 381 Concord Road, Weston, MA 02493-1340, USA

² Department of Animal & Plant Sciences, University of Sheffield, Alfred Denny Building, Western Bank, Sheffield S10 2TN, UK

PKS, 0000-0003-0550-1704; CHW, 0000-0001-7511-0464

* Correspondence: strother@bc.edu

Abstract: The Nonesuch Formation in the clastic sedimentary Oronto Group on the Keweenaw Peninsula of the Upper Peninsula, Michigan, USA most likely represents an ancient lake that formed between 1083 and 1070 Ma. Exceptional preservation, seen in palynological preparations, provides a snapshot of cell morphology, biological complexity and ecology at an early stage in the evolution of the eukaryotes. A wide range of unicellular organization is documented in both vegetative and encysted cell morphologies, but the extent to which multicellularity is developed seems very limited at this time. Overall, the Nonesuch microbiota, when viewed as a Lagerstätte, opens up a window onto the early evolution of unicellular eukaryotes, presenting an essential baseline of both eukaryotic diversity and cell structure well in advance of eukaryotic diversification documented in marine deposits from the later Neoproterozoic.

Supplementary material: Materials and methods with a detailed specimen list are available at: <https://doi.org/10.6084/m9.figshare.c.5183652>

Received 13 July 2020; revised 21 October 2020; accepted 22 October 2020

A Precambrian palynological Lagerstätte

The Nonesuch Formation provides a rare glimpse of life on Earth one billion years ago, with only the Torridonian deposits of Scotland in any way comparable. Both may be considered as Lagerstätten, the Torridonian because of exceptional preservation in phosphate nodules (Wacey *et al.* 2014, 2019) and the Nonesuch Lagerstätte because of the exceptional quality of the preserved palynomorphs. The latter is a function of the clay-rich nature of the host sediment that has experienced a maximum temperature of only 125–150°C (Gallagher *et al.* 2017; Hren and Sheldon 2019). The Nonesuch Lagerstätte allows us to address important evolutionary questions with respect to eukaryotes during an extended period of time when diversification within major clades appears to have been in stasis (Knoll *et al.* 2006; Knoll 2015). The apparent biodiversity in the Nonesuch microbiota (Wellman and Strother 2015) stands in contrast with the somewhat depauperate marine microfossil assemblages from the late Mesoproterozoic–early Neoproterozoic interval, as documented by Knoll *et al.* (2006). This has led to speculation that evolution in terrestrial settings may have outstripped that in the oceans during the so-called ‘boring billion’ interval in eukaryotic evolution (Strother *et al.* 2011; Wellman and Strother 2015; Sánchez-Baracaldo *et al.* 2017; Jackson *et al.* 2018; Slotznick *et al.* 2018).

Early palaeontological reporting, based on samples collected in the White Pine copper mine, gave the impression that the organic preservation of microfossils in the Nonesuch Formation was poor (Barghoorn *et al.* 1965; Moore *et al.* 1969). Beginning in 2009, the sampling of drill cores through the entirety of the Nonesuch Formation, including sites distant from the White Pine mine, has revealed remarkably well-preserved and diverse palynological assemblages (Strother and Wellman 2010, 2016; Wellman and Strother 2015). Palynomorphs, recovered through acid maceration, are the actual remains of buried cells and cysts. Even though the

majority of these microfossils are not yet classified within the eukaryotic phylogeny, a review of their basic cellular morphology can help paint a picture of what life was like at the very beginning of the Neoproterozoic Eon.

Location and geological setting

The Nonesuch Formation is the shale-dominated middle unit of the clastic sedimentary Oronto Group on the Keweenaw Peninsula of the Upper Peninsula, Michigan, USA. This conformable sequence begins with the Copper Harbor Conglomerate (including the interbedded Lake Shore Traps), followed by the Nonesuch Formation and the Freda Sandstone. The Oronto Group was deposited in a failed rift basin called the Midcontinent Rift System (MRS), represented by predominantly volcanic sequences deposited between 1109 and 1083 Ma (Swanson-Hysell *et al.* 2019). The MRS was an interior continental feature that overlapped in time with the early development of the Grenville Front at the Laurentian margin c. 1000 km distant (Fig. 1c) beginning at c. 1090 Ma (Fairchild *et al.* 2017).

The Oronto Group has long been considered to be terrestrial in nature (VanHise and Leith 1911). The basal 100–2000 m thick red conglomerates and pebbly sandstones of the Copper Harbor Conglomerate are interpreted as braided fluvial deposits, complete with alluvial fans (Elmore 1984). Stromatolitic horizons near the top of the formation mark the onset of lacustrine deposition (Elmore 1983; Fedorchuk *et al.* 2016). The conformably overlying Nonesuch Formation is 50–200 m thick and consists predominantly of dark shale–fine sandstone (Fig. 1h) (Elmore *et al.* 1989; Stewart and Mauk 2017). The lacustrine shales of the Nonesuch Formation grade into the overlying Freda Sandstone, marking a return to fluvial settings as up to 3660 m of coarse red sandstones progressively infilled the rift basin (Daniels 1982; Elmore *et al.* 1989).

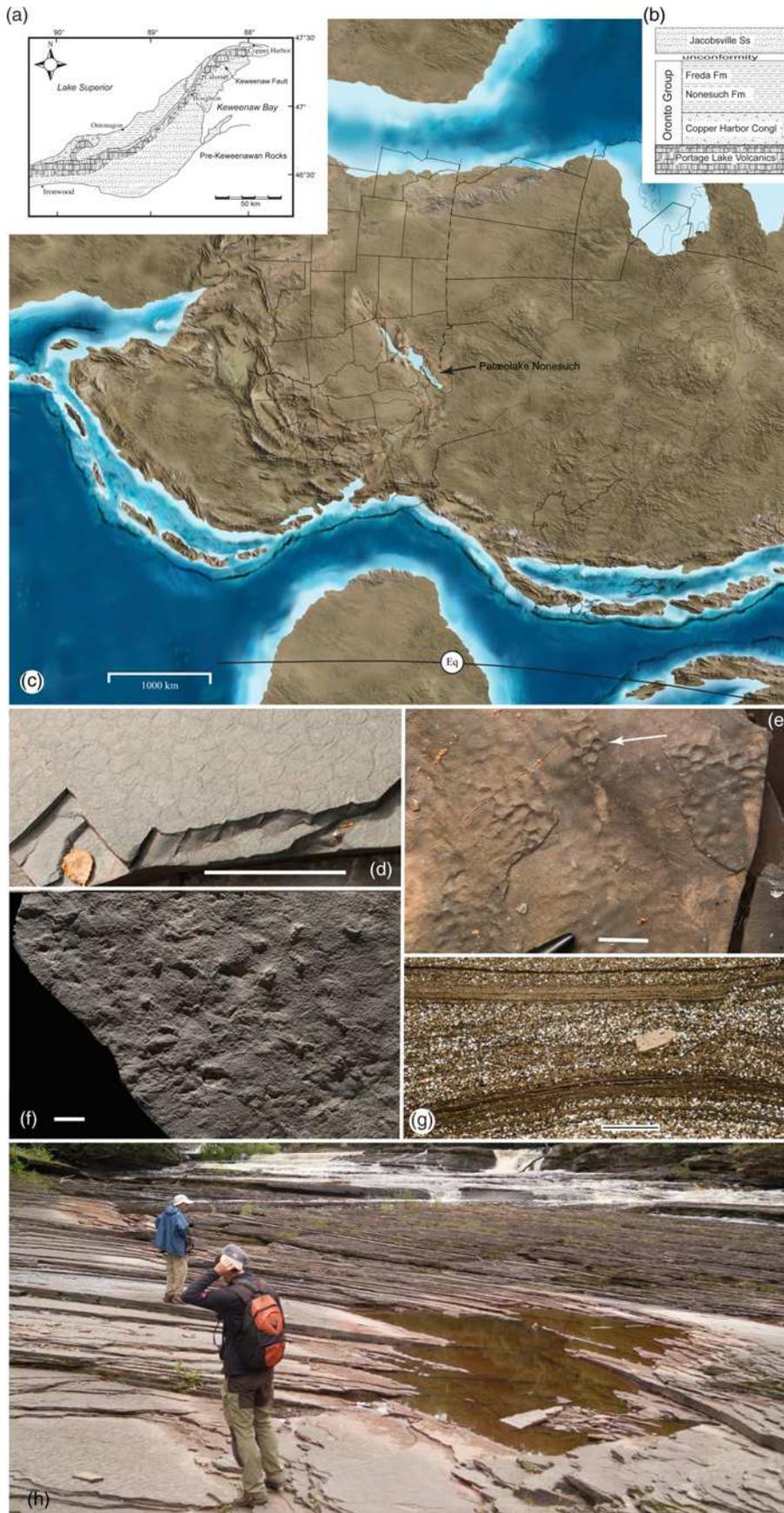


Fig. 1. Location, palaeogeography and depositional features of the Nonesuch Formation. (a) Outcrop map of the Nonesuch Formation in the Keweenaw Peninsula. (b) Stratigraphy of the Oronto Group; the fill patterns correspond to the map in part (a). (c) Palaeogeographical reconstruction prior to the deposition of the Nonesuch Formation showing the extent of the palaeo-lake Nonesuch. Eq corresponds to the estimated location of the palaeoequator. Image ©2013 Colorado Plateau Geosystems Inc. (d) Desiccation cracks from an outcrop along the Big Iron River near Silver City, Michigan. Scale bar 10 cm. (e) Raindrop impressions from Big Iron River Section. Note the circular form impinging on adjacent pits (arrow), indicating the dynamic nature of this sedimentary feature. Scale bar 1 cm. (f) Pustular microbially induced sedimentary structures similar to the proposed euglenid microbial mats found elsewhere in the Oronto Group. Scale bar 1 cm. (g) Thin section showing mat-like features seen in wispy organic laminae. Scale bar 1 mm. (h) Monotonous grey shales and siltstones characteristic of the Nonesuch Formation in outcrop from the Big Iron River Section between Silver City and Bonanza Falls, Michigan.

The Oronto Group contains a significant sediment-hosted copper system that has long been mined (Chamberlin 1883; White and Wright 1954; Bornhorst and Williams 2013). In close proximity to the White Pine mine, the host sediments have been altered by

post-depositional hydrothermal fluid circulation (Mauk and Hieshima 1992); however, away from the effects of this system, the rocks remain remarkably unaltered (Imbus *et al.* 1988). The low thermal maturity (Gallagher *et al.* 2017; Hren and Sheldon 2019)

and preservation of primary biogeochemical signals (Imbus *et al.* 1992) reflect the shallow burial of these deposits and the absence of significant tectonic effects.

The Nonesuch Formation contains numerous sedimentary indicators of shallow water deposition, including extensive microbially induced sedimentary structures (Sheldon 2011), desiccation polygons (Fig. 1d), mud cracks (Jones *et al.* 2020) and raindrop impressions (Fig. 1e). These and other sedimentary fabrics may occur in a range of shallow water habitats and are not necessarily diagnostic of lacustrine settings. This, along with purported geochemical indicators of saline conditions, have led to proposals that the Nonesuch formed in shelfal (Jones *et al.* 2020) or perhaps estuarine (Stüeken *et al.* 2020) settings. However, new mineralogical studies focused on the redox chemistry of the Nonesuch environment continue to support a lacustrine interpretation (Slotznick *et al.* 2018), whereas the geochemical evidence suggestive of marine conditions may actually be an artefact of the associated hydrothermal activity and copper mineralization.

Shallow water fabrics are documented from both sides of the MRS (Sheldon 2011) and palaeocurrent studies (Wolff and Huber 1973) indicate that sediments were shed locally into the basin from both sides of the rift, emphasizing the closed nature of this sedimentary system. In favouring a lacustrine depositional setting, Suszek (1997) points out that the Nonesuch Formation is conformably sandwiched between an alluvial fan complex and a fluvial flood plain – two clastic red bed sequences that are unambiguously terrestrial in character. Palaeogeographical reconstructions place the MRS near the centre of a continental crustal block (Fig. 1c) and, although it may not be possible to exclude a lateral marine incursion into the failed MRS, there is currently no tectonic model supporting a narrow marine incursion into the centre of the continent.

Because the Nonesuch Formation is an organic-rich shale unit, there has been a continuing interest in its organic geochemistry and biomarker chemistry. The initial recovery of phytane and pristane, thought to be evidence of chlorophyll breakdown products from eukaryotic algae (Barghoorn *et al.* 1965), was followed by early biomarker studies (Hieshima and Pratt 1991) that reported the presence of steranes, including low levels of 24-*n*-propylcholestane, originally interpreted to indicate marine deposition. However, the biomarker evidence here is not exclusively marine, as pointed out later by Cumming *et al.* (2013), who found additional support for a lacustrine provenance based on Os geochemistry.

Age of the Nonesuch Formation

The Lake Shore Traps, which interdigitate with the Copper Harbor Conglomerate, are precisely dated to 1085.57 Ma ± 0.25 myr (Fairchild *et al.* 2017). Volcanism continued in the rift basin until at least 1803.52 Ma ± 0.23 myr to the east of the Keweenaw Peninsula on Michipicoten Island. An undated ash bed was observed in the Copper Harbor Conglomerate c. 20 m below the base of the Nonesuch Formation in a core from well JBY-002 in the northeastern Keweenaw Peninsula (Jones *et al.* 2020). The end of volcanic activity therefore overlapped the start of a period of clastic sedimentation that includes the Nonesuch Formation, but sedimentation of the shale itself appears to have started shortly after the end of surficial igneous activity. A Re–Os isochron date of 1078 Ma ± 24 myr (Cumming *et al.* 2013) has been reported from the lower Nonesuch Formation. Subsequent radiometric dates are lacking, but palaeomagnetic data, which plot apparent polar-wandering paths, are consistent with an age of c. 1080–1070 Ma for the entire Oronto Group (Fairchild *et al.* 2017). Given the considerable thickness of the overlying Freda Sandstone (Daniels 1982), we consider 1083–1073 Ma to be a reasonable range estimate for the age of the Nonesuch Formation, aligning with the 1078 Ma date obtained by Cumming *et al.* (2013).

Overview of the microflora of the Nonesuch Formation

The vast majority of the organisms recovered in palynological macerations are acritarchs, an artificial group into which organic-walled microfossils are systematically parked until their biological affinities can be determined (Evvitt 1963; Servais *et al.* 1996). Butterfield (1997, 2015) has argued that Precambrian acritarchs should be considered as eukaryotic protists in the broadest possible sense. This includes their potential to represent those species capable of building cell walls composed of organic polymers. In addition, these microfossils may represent either vegetative or sexual (cyst) phases of heterotrophic or photoautotrophic species. Although the systematic affinities of the vast majority of these microfossils are unknown, some tentative phylogenetic assignments have been proposed within the broad morphological categories presented here.

Sphaeromorphs

Following the precedent of Jankauskas *et al.* (1989) and Butterfield *et al.* (1994), simple, unornamented Precambrian sphaeromorphs are assigned to one of four species of *Leiosphaeridia* based on a 70 µm diameter size boundary and wall thickness. Examples of the four species of *Leiosphaeridia* are shown in Figure 2a–d. The walls of *Leiosphaeridia* species are meant to be smooth, but, as can be seen in these well-preserved specimens, the actual surface texture varies considerably. Exceptional preservation in the Nonesuch Formation allows us to distinguish between the wall surface, the applied sculptural elements and the underlying wall structure itself. Instead of representing taphonomic artefacts, the cracks and folds in the vesicle walls preserved here reflect the physical properties of the underlying walls (e.g. the rigidity and tensile strength). Brittle wall failure during burial compression, for example, is the defining characteristic of *Leiosphaeridia ternata* (Fig. 2e). Sphaeromorphs rarely exhibit true sculpture. Acritarchs that look like the Paleozoic *Lophosphaeridium* (Fig. 2f) present protruding granae that are an integral part of a blotchy wall and not discrete sculptural ornamentation. The shape of sphaeromorphs can vary considerably from perfectly circular when compressed (Fig. 2g) to fairly irregular in outline (Fig. 2h, i). This important characteristic could potentially lead to a better understanding of the wall properties and cell functions. The pre-flattened sphericity can also be assessed: originally discoidal forms will lack medial crescentic folds (Fig. 2i), whereas the originally spherical forms will display numerous large folds (Fig. 2a–c).

Spent cysts

In the Nonesuch Lagerstätte, spent cysts are clearly indicated by excystment features in the vesicle wall. This may be in the form of a partial slit (Fig. 2j) or a medial suture that can split the cyst into two equal halves (Fig. 2k). This later form has been well documented in one of the oldest eukaryotes (Peng *et al.* 2009) from c. 1700 Ma. Evidence of encystment is also seen when a thick-walled sphaeromorph is found enclosed within a thinner walled sac that can be interpreted as a primary vegetative cell (Fig. 2l). Paleozoic palynological assemblages rarely contain organic-walled microfossils where both a vegetative cell wall and its enclosed cyst persist. The numerous such occurrences in the Nonesuch assemblage are a testament to the ability of these sediments to preserve a range of cell wall biopolymers, not just those necessarily related to sporopollenin.

Another general feature of the Nonesuch sphaeromorphs, but one that may or may not be related to encystment, is the occurrence of inner bodies or other distinct subcellular structures. When forming a single dense sphere, such structures are informally known from

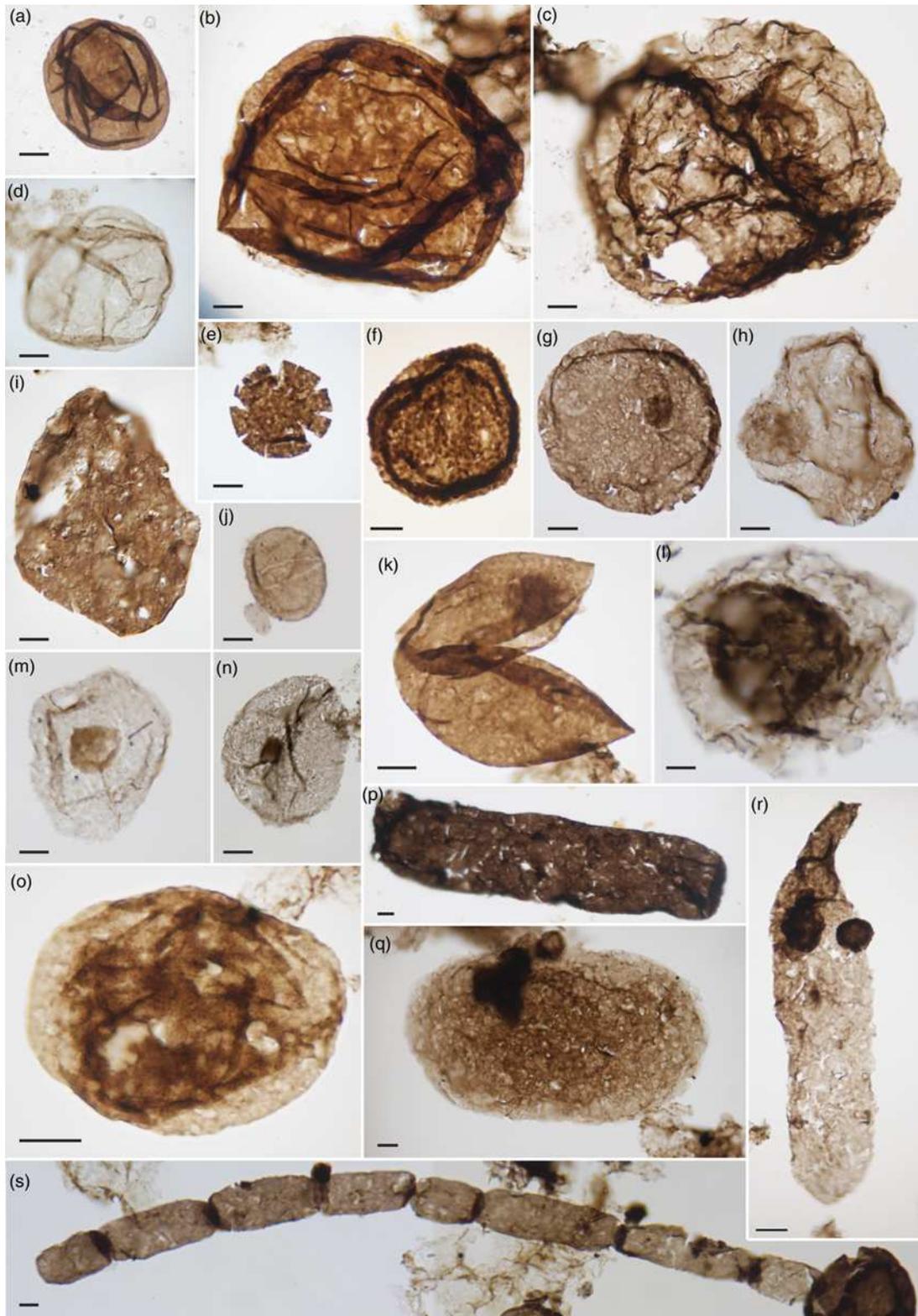


Fig. 2. Biological diversity in the Nonesuch assemblage. Sphaeromorphs and related forms. Scale bar in all images is 10 μm unless stated otherwise. (a) *Leiosphaeridia crassa*, the most common sphaeromorph acritarch, robust wall with crescentic folds <70 μm in diameter. (b) *Leiosphaeridia jacuta*, similar to *L. crassa* but with a diameter >70 μm . (c) *Leiosphaeridia tenuissima*, thin-walled sphaeromorph with diameter >70 μm . (d) *Leiosphaeridia minutissima*, thin-walled form <70 μm in diameter. (e) *Leiosphaeridia ternata* is recognized on the basis of its pie-shaped splitting on compression. (f) Form similar to the Paleozoic acritarch *Lophosphaeridium*, but, in this instance, the granae that appear to be a surface ornament are integral to the wall itself. (g) *Nucleosphaeridium* sp. with a small circular patch (spot). (h) *Nucleosphaeridium* sp. with a larger circular patch (spot). (i) Flat acritarch with subangular outline showing microbial perforations. (j) Disc-shaped organic-walled microfossil with distinctive medial (excystment?) suture. (k) *Schizofusa* sp. is an example of a spent cyst that has split into two valves, which remain attached distally. (l) *Zonosphaeridium* sp., in this case a darker, thick-walled cyst preserved within a thin, somewhat delicate envelope. (m) *Nucleosphaeridium* sp with a granular envelope enclosing a blockish inner body. (n) *Nucleosphaeridium* sp. with a granular envelope enclosing a small inner body (spot). (o) *Zonosphaeridium* sp. with large diffuse inner body. (p) Cylindrical cell, cf. *Archaeoellipsoides*. (q) Unnamed large, diffuse ovoid cell. (r) Cylindrical form similar to *Germinosphaera*. (s) Linear filament of *Archaeoellipsoides*-like cells.

studies of fossils preserved in both cherts (Schopf 1968) and phosphates (Wacey *et al.* 2019) as central bodies or ‘spot cells’. Here, in cells released from the rock matrix, such internal structures range considerably in both size and form (Fig. 2g, m–o). The central body in Figure 2o is interesting in that it consists of a rather diffuse specular central body made up of very tiny, submicron diameter dots. This same enigmatic feature is found in several distinct organic-walled microfossils, including the well-known striate sphaeromorph, *Valeria lophostriata* (Box 1).

Ellipsoids

Cell shapes that extend beyond simple spheres begin with extension along a single axis to produce elongate or ellipsoidal forms. These may be solitary (Fig. 2p–r) or attached end-to-end to form short chains (Fig. 2s). Ellipsoids may be cigar-shaped, including those with blunt ends (Fig. 2p), or they may be ellipsoidal in overall outline (Fig. 2q). They may possess a stalk (Fig. 2r), although this is not common. Ellipsoids include the largest individual cells yet recovered from the Nonesuch Lagerstätte. Here, as seen in Figure 4a, a pair of very large, cigar-shaped cells achieved lengths of >300 µm each. Extant protists rarely produce individual cells of such magnitude, but several orders of chlorophyte algae, including the Cladophorales and Dasycladales, achieve similarly grand cell sizes through endo-nuclearization to create coenocytic cells. So, regardless of the systematic affinities of these large ellipsoidal cells, it seems likely that they represent a coenocytic form of cellular organization. Prior studies of Precambrian cherts have described ellipsoidal taxa, such as *Archaeoellipsoides*, as possible akinetes of heterocyst-forming cyanobacteria (Tomitani *et al.* 2006). However, the morphological diversity and extreme size range of the ellipsoids seen in the Nonesuch Lagerstätte would seem to discount such an affinity.

Cell clusters: *Synsphaeridium*, *Symplassosphaeridium* and cellular sheets

It is common to find more or less random clusters of similar cells in the Nonesuch assemblage (Fig. 4b), implying that these are genetically related populations. Distinctly spheroidal cell clusters

range considerably in terms of the kinds of cells that make up such clusters. Although not terribly distinctive, clusters of very tiny, thin-walled cells can be accommodated by long-established genera such as *Symplassosphaeridium* (Fig. 4c). Here, the individual cells comprising the clusters are not usually well preserved and they appear to represent vegetative, rather than encysted forms. Another interesting cluster form includes a ‘morula’-type in which cells, some of which may possess internal contents, appear to be embedded in an amorphous organic groundmass (Fig. 4d, e).

Sheets of cells forming planar monolayers are distinctive (Fig. 4f, g) and similar forms have been noted elsewhere in the Precambrian and early Paleozoic. However, none of the planar cell sheets in the Nonesuch Lagerstätte show the tessellations that characterize some modern Hydrodictyacean coenobial taxa, such as *Pediastrum*. Nevertheless, such forms could be related to chlorophytic plankton. *Synsphaeridium*, a form genus that accommodates simple clusters of sphaeromorphs, is fairly common throughout the Nonesuch Lagerstätte, ranging from smaller thick-walled cells (Fig. 4b) to thinner walled forms (Fig. 4h), shown here with only three cells, but which vary considerably in the number of attached cells. The individual cells within the cluster are clearly related, but their attachment itself appears haphazard and not based on a well-defined attachment mechanism. Crudely formed linear clusters (e.g. *Arctacellularia ellipsoidea*; Fig. 4i), with their irregular alignment of a single file of cells, appear distinct from extant filamentous algae. Multiseriate irregular linear clusters, similar to *Gloeodiniopsis* (Fig. 4j), are highly variable in their overall morphology. These forms have yet to be compared with any extant algal counterparts, but could possibly be ascribed to the chlorophyte algae.

Filamentous and branching forms

Although filamentous microfossils occur throughout the Nonesuch Lagerstätte, they are never a dominant component of the assemblage. Simple filaments may be difficult to distinguish from cyanobacterial trichomes, especially when the cross-walls are indistinct. Some filaments can be fairly large, reaching almost 1 mm in length, and may still retain unbroken terminal cells.

Box 1. Key biological discoveries

***Valeria lophostriata*.** This distinctive acritarch is recognized by its wall patterning of concentric circular striae that emanate from two distinct points at opposite poles. The striae are typically less than a micron apart and are not always visible in published images (Fig. 3a, b) and only evident at higher magnifications (Fig. 3c). Hofmann (1999) reviewed the stratigraphic and geographical distribution of this taxon and found it largely associated with nearshore deposits, which is consistent with its occurrence here in freshwater settings. *V. lophostriata* is associated with some of the very earliest evidence of eukaryotes (Adam *et al.* 2017; Miao *et al.* 2019), but its stratigraphic longevity (as much as 700 myr) and wide environmental distribution (Hofmann 1999) indicate that the taxon may represent an entire class of organism, rather than a single biological species.

***Germinosphaera*-like forms.** This acritarch consists of a vesicle with a single tubular, tail-like extension. Such a simple description belies the lack of any phylogenetic significance to this form, although *Germinosphaera* has been discussed in terms of its potential fungal affinity (Butterfield 2015). Here, we see *Germinosphaera* as a taxon showing examples of recent germination and tip growth of a filamentous or tubular cell. A significant variety of forms show this characteristic in the microflora (Fig. 3d–i), many of which appear to have retained an actively growing tip (Fig. 3a, b). Other forms show what appears to be a more complex germination from an open cyst-like cell (Fig. 3g).

Possible euglenids. Several lines of evidence lead to the possibility that euglenids may have existed during Nonesuch time. The first is the presence of organic-walled microfossils that could belong to *Spurimoyeria* Wicander & Loeblich, a taxon thought to be close to *Moyeria*, which is now considered to be a Paleozoic euglenid (Strother *et al.* 2020). *Spurimoyeria* is characterized by a series of ridges that encircle the vesicle, forming a rectangular pattern when compressed (Fig. 3j). The second is the occurrence of a circular form, resembling the Precambrian acritarch *Simia* (Fig. 3k), but which resembles Recent freshwater and estuarine cysts formed by present day euglenids (Hindák *et al.* 2000). There is a third, more indirect line of evidence of potential euglenids in the Nonesuch ecosystem and that is reports of pustular microbially induced sedimentary structures in both the underlying Copper Harbor Conglomerate (Wilmeth *et al.* 2014) and the Nonesuch Formation (Fig. 1f). These ancient microbially induced sedimentary structures morphologies appear similar in form to microbial mats dominated by *Euglena mutabilis*, a species known to form pustular and laminated mats today (Brake *et al.* 2002; Brake and Hasiotis 2008).

Evidence of microbial decay. Elsik (1971) documented many examples of microbial degradation in various palynomorphs of different ages, including acritarchs, spores and pollen grains. Similar kinds of microbial perforations are illustrated here in Figure 3l and 3m. Today, pollen grains in waterlogged settings, such as soils and lake bottoms, are commonly infested by chytrids, which are capable of solubilizing sporopollenin and penetrating into the pollen protoplast. Chytrids are documented in the Devonian Rhynie Chert (Taylor *et al.* 1992). Although the systematic affinities of the microbes that produced these trace fossil perforations in Precambrian palynomorphs is not known with certainty, they are compatible with a fungal origin (Berbee *et al.* 2020) and could represent an early example of parasitism.

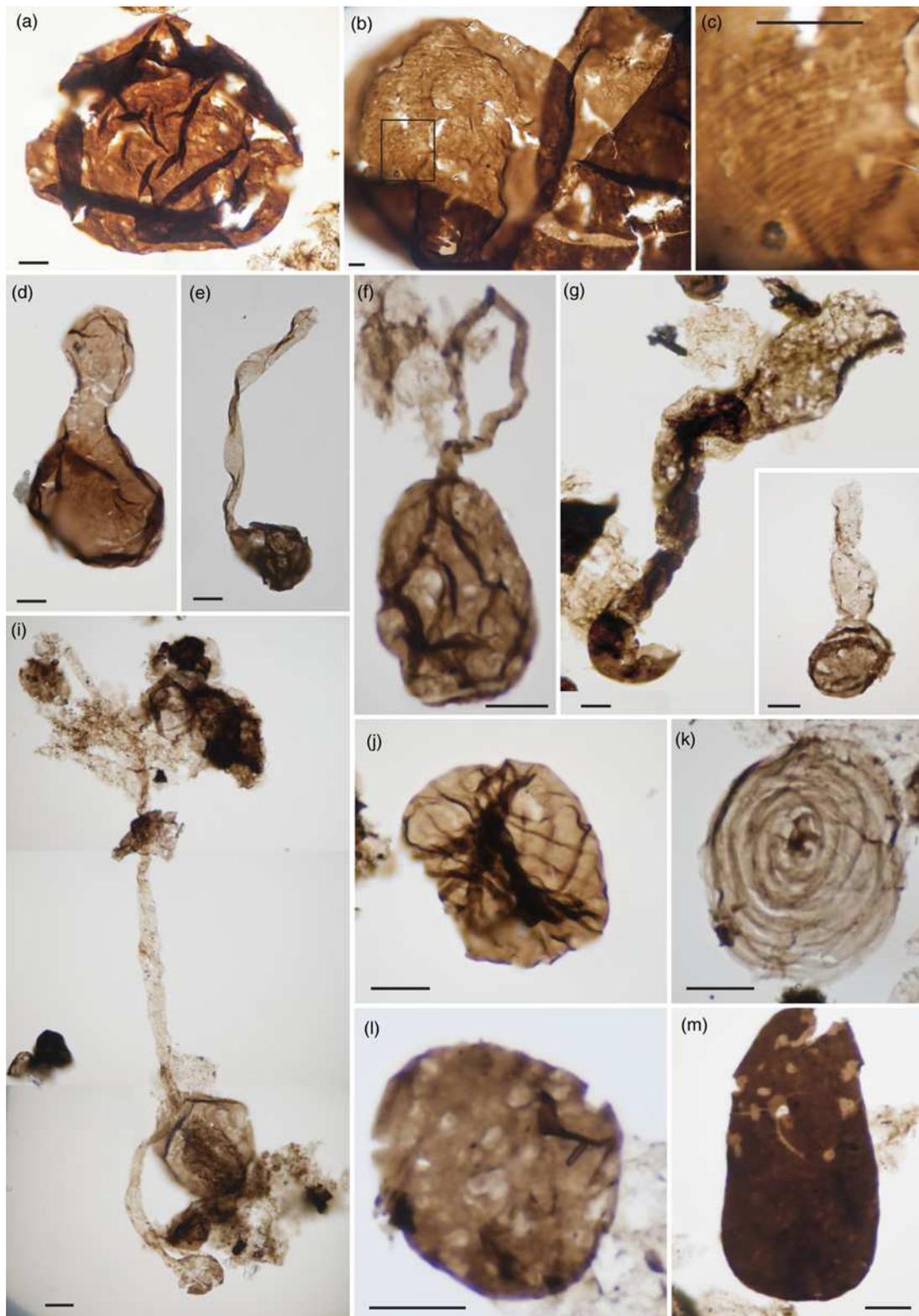


Fig. 3. Case studies. Some interesting taxa and their palaeoecological significance. (a) *Valeria lophostriata*, a long-ranging early eukaryote. (b) *V. lophostriata* split open and demonstrating its cyst-like character. (c) Enlargement of the wall seen in the box in part (b), showing the fine striations that characterize the inner wall surface in this taxon. (d) *Germinosphaera*-like specimen appearing to show recent extension of the cell wall. (e) Form similar to that in part (d), but with a more developed tubular extension. (f) More typical *Germinosphaera* form with a long thin tube, which is folded back on itself. (g) This specimen appears to show an open cyst with its germinating protoplast having elongated into a roughly linear, globular mass. Note that the cellular nature of the mass is not evident. (h) Another example of a thin-walled extension from a typical leiospherid. (i) A rather long extension in *Germinosphaera*. (j) A 'striate' form similar to *Spuromoyeria*, an Ordovician acritarch of probable euglenid affinity. (k) a *Simia*-like form that has similarities to Recent cysts of *Euglena* (see text). (l) A sphaeromorph with wall perforations similar to chytrid-based parasitized Phanerozoic palynomorphs. (m) Another example of microbial degradation in the upper portion of an unnamed protist.

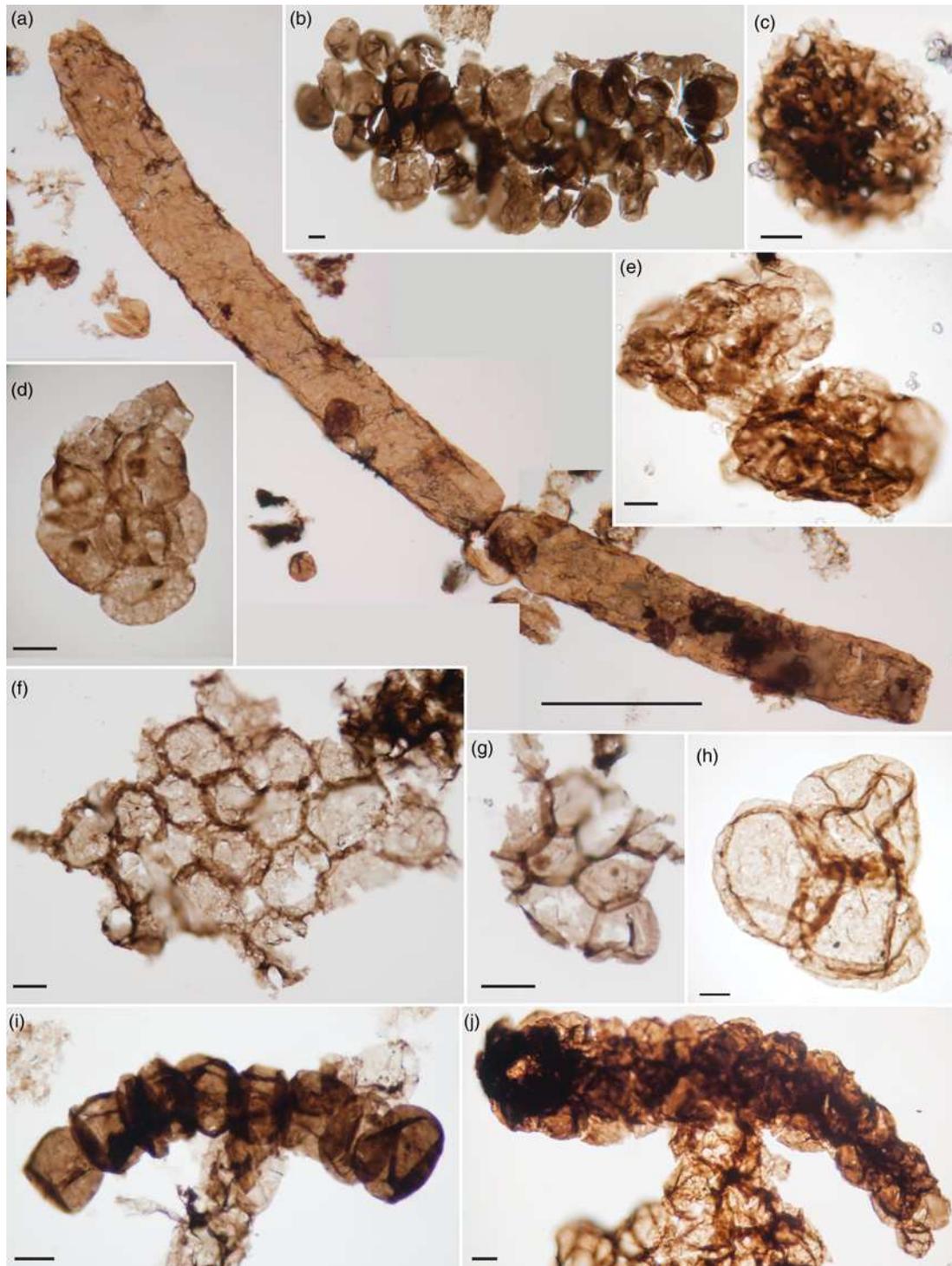


Fig. 4. Biological diversity in the Nonesuch assemblage. Cell clusters and simple multicellularity. Scale bar in all images is 10 μm unless stated otherwise. (a) An attached pair of a very large *Archaeoellipsoides*-like form, these cells are almost certainly coenocytic. (b) Cluster of small sphaeromorphs corresponding to *Synsphaeridium*. (c) *Symplastosphaeridium* sp.; note that the individual cells that make up the cluster are indistinct, possibly reflecting a vegetative habit. (d) This form of *Synsphaeridium* shows cells with a distinctive wall surface and interior spots. (e) Two clusters with cells that are embedded in a common EPS (extra-cellular polymeric substances), characteristic of the morula-like form. (f) Planar sheet of roughly isodiametric cells. (g) Fragment of a planar sheet of cells; note the possession of internal spots and the distinctive appearance of the cell contacts indicating cell-cell adhesion. (h) Triad of large, thin-walled cells. (i) Linear array of large rounded cells. (j) Linear multicellular cluster, comparable with *Gloeodiniopsis*, adjacent to a disorganized cell cluster.

These septate forms are possibly eukaryotic algae; their large size would seem to preclude classification as trichomes of filamentous cyanobacteria, however, for the most part, transverse walls (septa) are only preserved as darkened bands, not as distinct walls, so it is possible that even these larger forms could be the remains of cyanobacterial sheaths. Figure 5a shows one such an example, where a portion of a filament with diffuse cross-walls transitions to a

region without transverse walls, looking, in this instance, like an empty cyanobacterial sheath, such as that illustrated in Figure 4b.

Several interesting examples of pseudo-branching filaments are known from the assemblage. For example, a probable *Proterocladus* (Fig. 4c), with its diagnostic sub-septal branching pattern (Tang *et al.* 2020), may indicate that siphonaceous green algae were present at this time. Other non-septate forms show simple

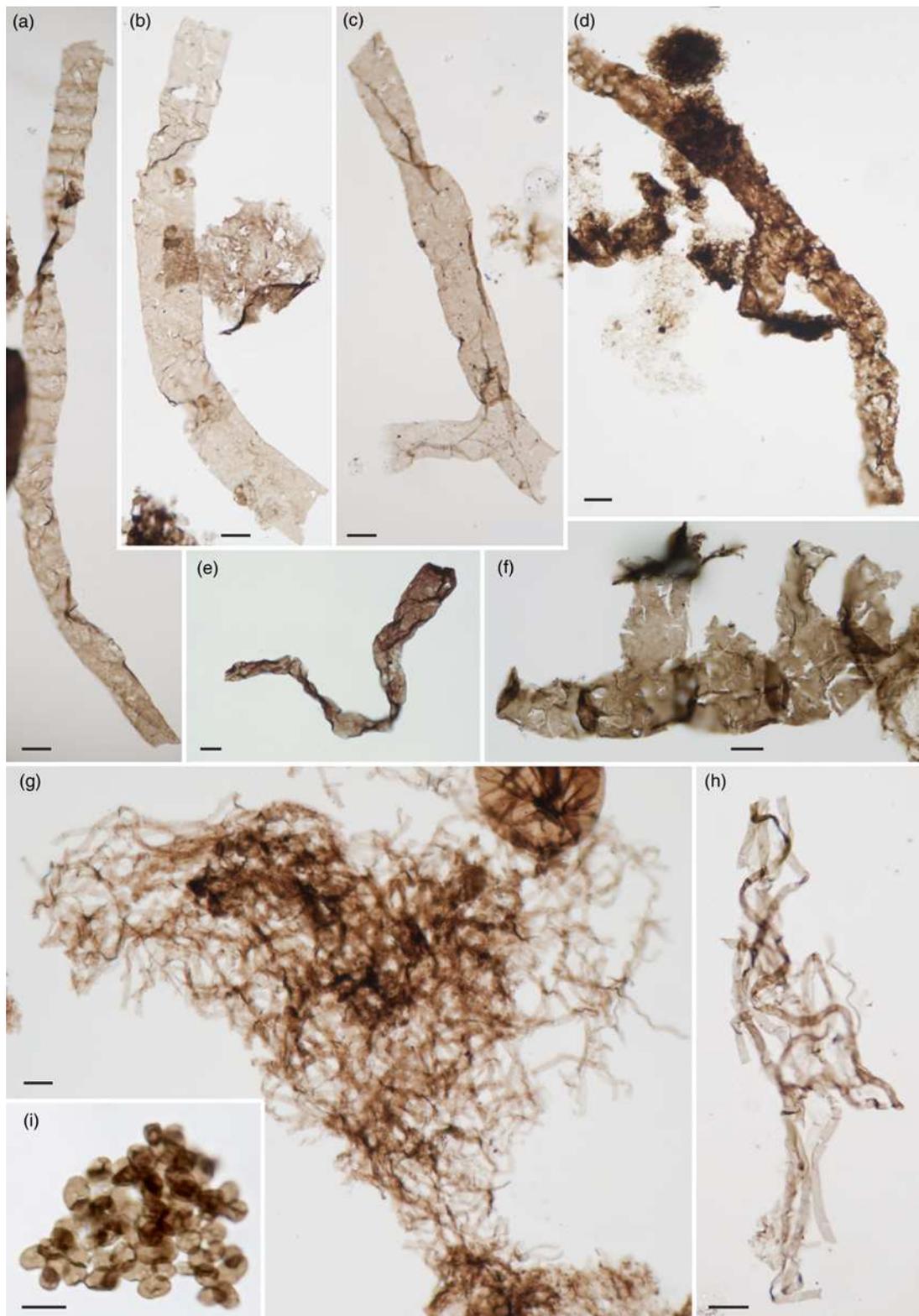


Fig. 5. Eukaryotic filaments and probable prokaryotes (cyanobacteria) in the Nonesuch assemblage. All scale bars 10 μm unless stated otherwise. (a) Large septate filament, but note the loss of the septae in the lower portion of the specimen, leading to the possibility that this form may be a large cyanobacterial sheath rather than a filamentous alga. (b) *Siphonophycus kestron*, considered to be an empty cyanobacterial sheath. (c) *Proterocladus* sp.; note the pre-septate branching pattern characteristic of this genus. (d) Single branched axis, but the lack of a clear underlying cellular structure makes this a problematic form. (e) Interesting sinuous, tapering axis, without obvious underlying cellularization. (f) Pseudo-branching in a linear filament of *Archaeoellipsoides*-like cells (compare this specimen with the 'unbranched' form in Fig. 2s). (g) Large tangled mat of *Siphonophycus* sp., probably representative of a microbial mat rip-up. (h) A smaller, more typical example of entangled sheaths that are probably the remains of cyanobacterial mats. (i) A cluster of *Eohalotheca lacustrinus*, which is typical of the planktic form of this presumed cyanobacterial species.

branches in tubes with a very rough wall structure that does not reveal an underlying cellular structure (Fig. 4d). False branching is apparent in linear sets of ellipsoidal cells that possess lateral wall

extensions (Fig. 4f). This form of false branching has yet to be reported from marine deposits of Precambrian age and the phylogenetic affinity of such forms is not known. They are an

interesting alternate form of *Archaeoellipsoides* that can form linear chains of cylindrical cells with rounded ends (e.g. Fig. 2s). It is unlikely that they represent cyanobacterial akinetes, as has been proposed for some isolated specimens of *Archaeoellipsoides* recovered from marine deposits (Golubic *et al.* 1995; Tomitani *et al.* 2006). This is another example of a taxon, *Archaeoellipsoides*, the simple morphology of which precludes a specific assignment to a single clade.

One interesting elongate organic-walled microfossil that has yet to be reported in the Precambrian has a tapered linear form, but without any preserved underlying cellular pattern. This unnamed organism always retains a sinuous shape and individual specimens may be fairly large, often in excess of 100 µm, as seen in Figure 4e.

Cyanobacteria in the Nonesuch assemblage

Direct evidence of prokaryotes in the Nonesuch assemblage is limited, but is primarily based on the assumption that non-septate, flattened tubes are the remains of allochthonous cyanobacterial sheaths. These may be isolated, as in the example of *Siphonophycus kestron* (Fig. 5b), but are most convincing when found as entangled masses (Fig. 5g, h). These allochthonous remains of microbial mat rip-ups are common throughout the formation. The Nonesuch Formation is the stratotype locus for *Eohalotheca lacustrinus* (Fig. 5i), a presumed cyanobacterium with an ecology similar to the modern day *Microcystis aeruginosa*. It is extremely abundant, occurring in most of the fossiliferous samples and ranging in number up to the order of 10⁴ specimens per slide. *Eohalotheca* may be preserved in cell clusters, including clathrate forms, which parallel the morphologies of colonial phytoplankton in lakes today (Strother and Wellman 2016). Individual isolated cells are also found, most typically associated with the amorphous organic groundmass characteristic of benthic settings. Within the greater terrestrial ecosystem of the Oronto Group, cyanobacteria have also been indirectly documented in the form of stromatolites from the underlying Copper Harbor Conglomerate (Elmore 1983; Nishioka *et al.* 1984; Sheldon 2011; Fedorchuk *et al.* 2016).

Palaeoecology

Recent questioning of the lacustrine nature of the Nonesuch Formation (Jones *et al.* 2020; Stüeken *et al.* 2020) is a reminder of the difficulty in proving a freshwater origin for rocks that lack fossils of an unambiguous terrestrial provenance. However, biological clues can help to distinguish between marine and non-marine depositional settings. For example, *Eohalotheca* is also found in the roughly contemporaneous Diabaig Formation (Torridon Group) in NW Scotland (Strother and Wellman 2016), a deposit long thought to represent a lake (Stewart 2002). Its distribution in both benthic settings (as scattered individual cells) and in large clusters representing planktic colonies parallels a seasonally partitioned distribution of *Microcystis* in lakes today (Preston *et al.* 1980; Verspagen *et al.* 2005; Misson *et al.* 2012). This behaviour has not been documented in marine settings.

Spines in acritarchs are often associated with a planktic habit in the marine water column, where they are hypothesized to increase the effective Stokes' number (Strother 1994; Butterfield 1997). Perhaps spines do not carry such a selective advantage in lakes, but the lack of this feature presents a striking difference between the Nonesuch Formation and the stratigraphically younger large acritarchs of the Ediacaran Period (Grey 2005; Liu *et al.* 2014) and acanthomorph acritarchs that characterize marine deposits of the early Paleozoic.

If low oxygen levels in marine settings during the Proterozoic (Anbar and Knoll 2002; Arnold *et al.* 2004) were responsible for moderating the rates of eukaryote evolution and diversification, then

determining the redox state of terrestrial settings becomes important to the idea that terrestrial ecosystems may have acted as refugia for continued eukaryotic evolution during the early Neoproterozoic. The excellent organic preservation that characterizes the organic-walled microfossils of the Nonesuch Formation is favoured under conditions of reduced oxygen availability during deposition and sediment accumulation. In spite of this tendency for organic preservation to generally act as an indicator of anoxia, recent work on magnetic susceptibility and iron mineralization indicates that the water column of the Nonesuch lake was well oxidized (Slotznick *et al.* 2018). Taken in the context of the kilometre-thick under- and overlying red beds, this would seem to release any potential evolutionary restraints due to large-scale anoxia in freshwater ecosystems at this time.

State of biological/evolutionary complexity of early eukaryotes

In spite of tentative taxonomic assignments for the vast bulk of fossils found in the Nonesuch Formation, it is still possible to gain some insight into the general state of eukaryotic evolution at 1.1 Ga. Basic descriptive cell morphology does indicate a measure of organismal complexity at this time. For example, it seems clear that protists of this era were more capable of growing large cells than they were of gaining size through building multicellular structures. This seems to be the case in spite of the fact that some basic cell adhesion molecules were already in place, as evidenced by the abundance of cell clusters in the assemblages (Fig. 4).

The presence of large, apparently empty, cells (e.g. Fig. 4a) speaks to the likelihood that the coenocytic habit, in which a single cell has multiple nuclei, was a common characteristic of cell biology at this time. Such large cells presage those of the Ediacaran 'large ornamented eukaryotic microfossils' (Grey 2005; Cohen *et al.* 2009). Likewise, there is little evidence here that macroscopic, tissue-level multicellularity, such as that seen in benthic macroalgae today, existed at this time because we have not recovered any cellular fragments that appear to derive from larger organisms. In fact, Figure 4 stands as a snapshot of biological complexity with respect to multicellularity in non-marine settings at 1.1 Ga. The levels of multicellularity that we do see are more likely to represent stages within more or less complex protistan life cycles, particularly those that might have included aggregated stages. The 'morula-like' clusters seen in Figure 4d and 4e may represent examples of this form of aggregated stages within a largely unicellular life cycle. The only exceptions to this condition are the planar cellular clusters of relative isodiametric cells, such as those illustrated in Figure 4f and 4g. Similar cellular sheets are known from Ordovician non-marine deposits (Navidi-Izad *et al.* 2019), so this may represent a persistent vegetative morphotype, probably belonging to freshwater, planktonic chlorophytes.

Discussion

Our understanding of the evolution of life during the Proterozoic is largely restricted to samples from marine settings (Knoll *et al.* 2006); prior to the discovery of the microbiotas of the Nonesuch (and Torridonian) lakes, there was no guarantee that any eukaryotic evolution was taking place in freshwater habitats by 1 Ga. Longstanding questions on the lethality of ultraviolet B (UV-B) radiation (Berkner and Marshall 1965) in terrestrial settings remain in play, but the very existence of these assemblages indicates that UV-B attenuation, either through atmospheric O₃ absorption or in combination with absorption in shallow water, was sufficient to quench the lethality of UV-B radiation. Evidence from character trait evolution (Blank 2013; Dagan *et al.* 2013; Sánchez-Baracaldo *et al.* 2017) and phylogenomics (Sánchez-Baracaldo *et al.* 2005;

Box 2. Outstanding questions stemming from the study of the Nonesuch microbiota

- Which organisms are providing the photoautotrophic source of carbon in this ancient lake ecosystem: cyanobacteria, eukaryotic chlorophyte algae, or some combination of both? This is one topic where biomarker studies may contribute significantly, so this represents a clear direction for new research.
- What can this microflora and its associated organic microfibrils tell us about life in subaerial settings at 1 Ga? How extensive were microbial crusts and soils at lake margins and can we find direct evidence of subaerial organisms washed into the lake sediments?
- Why do we not see adaptive morphology associated with the planktic habit in eukaryotes? Is it possible that specialized morphologies for maintaining position in the water column are only found in deeper waters that are more characteristic of marine settings? Or, as Butterfield (1997) has suggested, are many of the Proterozoic sphaeromorphs benthic organisms, including heterotrophs?
- What are the outstanding differences between marine and terrestrial biology at 1 Ga? Are we over-extending uniformitarianist assumptions by using modern community compositions as analogues of billion-year-old organisms and ecology?
- Which of the eukaryote supergroups are represented here, and how do we assess autotrophic v. heterotrophic components of the microflora as we attempt to reconstruct a picture of what life on Earth was like a billion years ago? Based on the exceptional preservation of the Nonesuch palynomorphs, they should be amenable to transmission electron microscopy studies of wall ultrastructure, which, combined with organic geochemical evidence, may provide clues to biological affinity.

Ponce-Toledo *et al.* 2017) indicate that the primary endosymbiotic origin of the green plastid took place in freshwater settings. Intriguingly, the same appears to be the case for the secondary origin of the chloroplast in the photosynthetic euglenids (Jackson *et al.* 2018).

General models of the evolution of eukaryotes during Precambrian time have been driven by the correlation of molecular time-trees (e.g. Parfrey *et al.* 2011) with environmental constraints on evolutionary diversification. Oxygen availability, for example, has long been seen as a promoter of eukaryotic evolution (Berkner and Marshall 1965) and the Proterozoic geochemical record of marine anoxia (Anbar and Knoll 2002) has been recommended as the cause of evolutionary stasis in eukaryotes prior to 800–850 Ma (Knoll *et al.* 2006). Under this model, the presence of diverse eukaryotes in freshwater settings at 1.1 Ga could be seen as evidence of enhanced evolution in oxygenated environments not subject to benthic marine anoxia. Porter *et al.* (2018) have challenged the very notion that the first-order patterns in early eukaryote evolution were driven by environmental oxygen availability. Their upgraded assessment of eukaryotic diversity, however, was based solely on assemblages recovered from marine settings. In either case, the Nonesuch Lagerstätte takes on a more important evolutionary role if fundamental aspects of eukaryotic evolution during the Precambrian were restricted to entirely non-marine settings.

It seems likely that a mix of photoautotrophs and osmotrophic, and probably phagotrophic, heterotrophs occupied the bottom muds of the Nonesuch lake ecosystem. This occurrence of such a wide range of cell wall morphology attests to the efficacy of natural selection in freshwater and subaerial settings by the end of the Mesoproterozoic. Even though the overall level of biological complexity seems rather unimpressive at this time, simple multicellularity (Bonner 1988), as seen here in the Nonesuch cell clusters, represents an important stage in the evolution of biological complexity, leading to the eventual evolution of both plants and animals (Niklas 2013; Newman 2016). The main outstanding questions arising from our study of the Nonesuch microbiota are listed in Box 2.

Acknowledgements We thank M. Humphries for access to the Michigan State core repository in Marquette and W.A. Taylor and B. van de Schootbrugge for field assistance. We thank the reviewers for their insightful comments and the editor for his contributions to the clarity and structure of this paper.

Author contributions PKS: conceptualization (equal), data curation (equal), formal analysis (equal), funding acquisition (equal), investigation (equal), methodology (equal), project administration (equal), resources (equal), visualization (lead), writing – original draft (lead), writing – review and editing (equal); CHW: conceptualization (equal), data curation (equal), formal analysis (equal), funding acquisition (equal), investigation (equal), methodology (equal), project administration (lead), resources (lead), writing – original draft (supporting), writing – review and editing (equal).

Funding This research was funded by NERC Standard Grant NE/R001324/1. Research on the palynology of the Nonesuch Formation was initiated under NASA 06-EXB06-0037.

Data availability Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Scientific editing by Philip Donoghue

References

- Adam, Z.R., Skidmore, M.L., Mogk, D.W. and Butterfield, N.J. 2017. A Laurentian record of the earliest fossil eukaryotes. *Geology*, **45**, 387–390, <https://doi.org/10.1130/G38749.1>
- Anbar, A.D. and Knoll, A.H. 2002. Proterozoic ocean chemistry and evolution: a bioinorganic bridge? *Science*, **297**, 1137–1142, <https://doi.org/10.1126/science.1069651>
- Arnold, G.L., Anbar, A.D., Barling, J. and Lyons, T.W. 2004. Molybdenum isotope evidence for widespread anoxia in mid-Proterozoic oceans. *Science*, **304**, 87–90, <https://doi.org/10.1126/science.1091785>
- Barghoorn, E.S., Meinschein, W.G. and Schopf, J.W. 1965. Paleobiology of a Precambrian shale: geology, organic geochemistry, and paleontology are applied to the problem of detection of ancient life. *Science*, **148**, 461–472, <https://doi.org/10.1126/science.148.3669.461>
- Berbee, M.L., Strullu-Derrien, C., Delaux, P.-M., Strother, P.K., Kenrick, P., Selosse, M.-A. and Taylor, J.W. 2020. Genomic and fossil windows into the secret lives of the most ancient fungi. *Nature Reviews Microbiology*, **18**, 717–730, <https://doi.org/10.1038/s41579-020-0426-8>
- Berkner, L.V. and Marshall, L.C. 1965. History of major atmospheric components. *Proceedings of the National Academy of Sciences of the USA*, **53**, 1215–1226, <https://doi.org/10.1073/pnas.53.6.1215>
- Blank, C.E. 2013. Origin and early evolution of photosynthetic eukaryotes in freshwater environments: reinterpreting Proterozoic paleobiology and biogeochemical processes in light of trait evolution. *Journal of Phycology*, **49**, 1040–1055, <https://doi.org/10.1111/jpy.12111>
- Bonner, J.T. 1988. *The Evolution of Complexity*. Princeton University Press.
- Bornhorst, T.J. and Williams, W.C. 2013. The Mesoproterozoic Copperwood sedimentary rock-hosted stratiform copper deposit, Upper Peninsula, Michigan. *Economic Geology*, **108**, 1325–1346, <https://doi.org/10.2113/econgeo.108.6.1325>
- Brake, S.S. and Hasiotis, S.T. 2008. Eukaryote-dominated biofilms in extreme environments: overlooked sources of information in the geologic record. *Palaios*, **23**, 121–123, <https://doi.org/10.2110/palo.2008.S02>
- Brake, S.S., Hasiotis, S.T., Dannelly, H.K. and Connors, K.A. 2002. Eukaryotic stromatolite builders in acid mine drainage: implications for Precambrian iron formations and oxygenation of the atmosphere? *Geology*, **30**, 599–602, [https://doi.org/10.1130/0091-7613\(2002\)030<0599:ESBIAM>2.0.CO;2](https://doi.org/10.1130/0091-7613(2002)030<0599:ESBIAM>2.0.CO;2)
- Butterfield, N.J. 1997. Plankton ecology and the Proterozoic–Phanerozoic transition. *Paleobiology*, **23**, 247–262, <https://doi.org/10.1017/S009483730001681X>
- Butterfield, N.J. 2015. Early evolution of the Eukaryota. *Palaeontology*, **58**, 5–17, <https://doi.org/10.1111/pala.12139>
- Butterfield, N.J., Knoll, A.H. and Swett, K. 1994. Paleobiology of the Neoproterozoic Svanbergfjellet Formation, Spitsbergen. *Fossils & Strata*, **27**, 1–76, <https://doi.org/10.1111/j.1502-3931.1994.tb01558.x>
- Chamberlin, T.C. 1883. The copper-bearing series of Lake Superior. *Science*, **1**, 453–455, <https://doi.org/10.1126/science.ns-1.16.453>
- Cohen, P.A., Knoll, A.H. and Kodner, R.B. 2009. Large spinose microfossils in Ediacaran rocks as resting stages of early animals. *Proceedings of the National Academy of Sciences of the USA*, **106**, 6519–6524, <https://doi.org/10.1073/pnas.0902322106>

- Cumming, V.M., Poulton, S.W., Rooney, A.D. and Selby, D. 2013. Anoxia in the terrestrial environment during the late Mesoproterozoic. *Geology*, **41**, 583–586, <https://doi.org/10.1130/G34299.1>
- Dagan, T., Roettger, M. *et al.* 2013. Genomes of Stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. *Genome Biology and Evolution*, **5**, 31–44, <https://doi.org/10.1093/gbe/evs117>
- Daniels, P.A. 1982. 7C: Upper Precambrian sedimentary rocks: Oronto Group, Michigan–Wisconsin. *GSA Memoirs*, **156**, 107–134, <https://doi.org/10.1130/mem156-p107>
- Elmore, R.D. 1983. Precambrian non-marine stromatolites in alluvial fan deposits, the Copper Harbor Conglomerate, upper Michigan. *Sedimentology*, **30**, 829–842, <https://doi.org/10.1111/j.1365-3091.1983.tb00713.x>
- Elmore, R.D. 1984. The Copper Harbor Conglomerate: a late Precambrian fining-upward alluvial fan sequence in northern Michigan. *GSA Bulletin*, **95**, 610–617, [https://doi.org/10.1130/0016-7606\(1984\)95<610:TCHCAL>2.0.CO;2](https://doi.org/10.1130/0016-7606(1984)95<610:TCHCAL>2.0.CO;2)
- Elmore, R.D., Milavec, G.J., Imbus, S.W. and Engel, M.H. 1989. The Precambrian Nonesuch Formation of the North American mid-continent rift, sedimentology and organic geochemical aspects of lacustrine deposition. *Precambrian Research*, **43**, 191–213, [https://doi.org/10.1016/0301-9268\(89\)90056-9](https://doi.org/10.1016/0301-9268(89)90056-9)
- Elsik, W.C. 1971. Microbial degradation of sporopollenin. In: Brooks, J., Grant, P., Muir, M., van Gijzel, P. and Shaw, G. (eds) *Sporopollenin. Proceedings of a Symposium Held at the Geology Department, Imperial College, London, 23–25 September, 1970*. Academic Press, 480–509.
- Evitt, W.R. 1963. A discussion and proposals concerning fossil dinoflagellates, hystrichospheres, and acritarchs. I. *Proceedings of the National Academy of Sciences of the USA*, **49**, 158–164, <https://doi.org/10.1073/pnas.49.2.158>
- Fairchild, L.M., Swanson-Hysell, N.L., Ramezani, J., Sprain, C.J. and Bowring, S.A. 2017. The end of Midcontinent Rift magmatism and the paleogeography of Laurentia. *Lithosphere*, **9**, 117–133, <https://doi.org/10.1130/L580.1>
- Fedorchuk, N.D., Dombos, S.Q., Corsetti, F.A., Isbell, J.L., Petryshyn, V.A., Bowles, J.A. and Wilmet, D.T. 2016. Early non-marine life: evaluating the biogenicity of Mesoproterozoic fluvial–lacustrine stromatolites. *Precambrian Research*, **275**, 105–118, <https://doi.org/10.1016/j.precamres.2016.01.015>
- Gallagher, T.M., Sheldon, N.D., Mauk, J.L., Petersen, S.V., Gueneli, N. and Brooks, J.J. 2017. Constraining the thermal history of the North American Midcontinent Rift System using carbonate clumped isotopes and organic thermal maturity indices. *Precambrian Research*, **294**, 53–66, <https://doi.org/10.1016/j.precamres.2017.03.022>
- Golubic, S., Sergeev, V.N. and Knoll, A.H. 1995. Mesoproterozoic *Archaeoellipsoides*: akinetes of heterocystous cyanobacteria. *Lethaia*, **28**, 285–298, <https://doi.org/10.1111/j.1502-3931.1995.tb01817.x>
- Grey, K. 2005. *Ediacaran Palynology of Australia*. Association of Australasian Paleontologists, Memoirs, **31**.
- Hieshima, G.B. and Pratt, L.M. 1991. Sulfur/carbon ratios and extractable organic matter of the Middle Proterozoic Nonesuch Formation, North American Midcontinent Rift. *Precambrian Research*, **54**, 65–79, [https://doi.org/10.1016/0301-9268\(91\)90069-M](https://doi.org/10.1016/0301-9268(91)90069-M)
- Hindák, F., Wolowski, K. and Hindáková, A. 2000. Cysts and their formation in some neustonic *Euglena* species. *Annales de Limnologie – International Journal of Limnology*, **36**, 83–93, <https://doi.org/10.1051/limn/2000010>
- Hofmann, H.J. 1999. Global distribution of the Proterozoic sphaeromorph acritarch *Valeria lophostriata* (Jankauskas). *Acta Micropalaeontologica Sinica*, **16**, 215–224.
- Hren, M.T. and Sheldon, N.D. 2019. Terrestrial microbialites provide constraints on the Mesoproterozoic atmosphere. *The Depositional Record*, **6**, 4–20, <https://doi.org/10.1002/dep2.79>
- Imbus, S.W., Engel, M.H., Elmore, R.D. and Zumberge, J.E. 1988. The origin, distribution and hydrocarbon generation potential of organic-rich facies in the Nonesuch Formation, Central North American Rift System: a regional study. In: Mattavelli, L. and Novelli, L. (eds) *Organic Geochemistry in Petroleum Exploration. Proceedings of the 13th International Meeting on Organic Geochemistry, Venice, Italy 21–25 September 1987*. Elsevier, 207–219, <https://doi.org/10.1016/b978-0-08-037236-5.50027-0>
- Imbus, S.W., Macko, S.A., Elmore, R.D. and Engel, M.H. 1992. Stable isotope (C, S, N) and molecular studies on the Precambrian Nonesuch Shale (Wisconsin–Michigan, U.S.A.): evidence for differential preservation rates, depositional environment and hydrothermal influence. *Chemical Geology*, **101**, 255–281, [https://doi.org/10.1016/0009-2541\(92\)90007-R](https://doi.org/10.1016/0009-2541(92)90007-R)
- Jackson, C., Knoll, A.H., Chan, C.X. and Verbruggen, H. 2018. Plastid phylogenomics with broad taxon sampling further elucidates the distinct evolutionary origins and timing of secondary green plastids. *Scientific Reports*, **8**, 1523, <https://doi.org/10.1038/s41598-017-18805-w>
- Jankauskas, T.V., Mikhailova, N.S. and Hermann, T.N. (eds) 1989. *Precambrian Microfossils of the USSR*. Leningrad, “Nauka”.
- Jones, S.M., Prave, A.R. *et al.* 2020. A marine origin for the late Mesoproterozoic Copper Harbor and Nonesuch Formations of the Midcontinent Rift of Laurentia. *Precambrian Research*, **336**, 105510, <https://doi.org/10.1016/j.precamres.2019.105510>
- Knoll, A.H. 2015. Paleobiological perspectives on early microbial evolution. *Cold Spring Harbor Perspectives in Biology*, **7**, <https://doi.org/10.1101/cshperspect.a018093>
- Knoll, A.H., Javaux, E.J., Hewitt, D. and Cohen, P. 2006. Eukaryotic organisms in Proterozoic oceans. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **361**, 1023–1038, <https://doi.org/10.1098/rstb.2006.1843>
- Liu, P., Xiao, S., Yin, C., Chen, S., Zhou, C. and Li, M. 2014. Ediacaran acanthomorphic acritarchs and other microfossils from chert nodules of the Upper Doushantuo Formation in the Yangtze Gorges area, South China. *Journal of Paleontology*, **88**, 1–139, <https://doi.org/10.1666/13-009>
- Mauk, J.L. and Hieshima, G.B. 1992. Organic matter and copper mineralization at White Pine, Michigan, U.S.A. *Chemical Geology*, **99**, 189–211, [https://doi.org/10.1016/0009-2541\(92\)90038-7](https://doi.org/10.1016/0009-2541(92)90038-7)
- Miao, L., Moczydlowska, M., Zhu, S. and Zhu, M. 2019. New record of organic-walled, morphologically distinct microfossils from the late Paleoproterozoic Changcheng Group in the Yanshan Range, North China. *Precambrian Research*, **321**, 172–198, <https://doi.org/10.1016/j.precamres.2018.11.019>
- Misson, B., Sabart, M., Amblard, C. and Latour, D. 2012. Benthic survival of *Microcystis*: long-term viability and ability to transcribe microcystin genes. *Harmful Algae*, **13**, 20–25, <https://doi.org/10.1016/j.hal.2011.09.010>
- Moore, L.R., Moore, J.R.M. and Spinner, E. 1969. A geomicrobiological study of the Pre-Cambrian Nonesuch Shale. *Proceedings of the Yorkshire Geological Society*, **37**, 351–394, <https://doi.org/10.1144/pygs.37.3.351>
- Navidi-Izad, N., Hashemi, H., Cascales-Miñana, B., Régnier, S., Wellman, C.H. and Servais, T. 2019. Colonial palynomorphs from the Upper Ordovician of north-eastern Iran: ‘Thalli’, coenobial Chlorophyceae (Hydrodictyaceae) or cyanobacteria? *Palynology*, **44**, 575–585, <https://doi.org/10.1080/01916122.2019.1657197>
- Newman, S.A. 2016. ‘Biogeneric’ developmental processes: drivers of major transitions in animal evolution. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, **371**, 20150443, <https://doi.org/10.1098/rstb.2015.0443>
- Niklas, K.J. 2013. The origins of multicellular organisms. *Evolution & Development*, **15**, 41–52, <https://doi.org/10.1111/ede.12013>
- Nishioka, G.K., Kelly, W.C. and Elmore, R.D. 1984. Copper occurrences in stromatolites of the Copper Harbor Conglomerate, Keweenaw Peninsula, northern Michigan. *Economic Geology*, **79**, 1393–1399, <https://doi.org/10.2113/gsecongeo.79.6.1393>
- Parfrey, L.W., Lahr, D.J.G., Knoll, A.H. and Katz, L.A. 2011. Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proceedings of the National Academy of Sciences of the USA*, **108**, 13624–13629, <https://doi.org/10.1073/pnas.1110633108>
- Peng, Y., Bao, H. and Yuan, X. 2009. New morphological observations for Paleoproterozoic acritarchs from the Chuanlinggou Formation, North China. *Precambrian Research*, **168**, 223–232, <https://doi.org/10.1016/j.precamres.2008.10.005>
- Ponce-Toledo, R.I., Deschamps, P., López-García, P., Zivanovic, Y., Benzerara, K. and Moreira, D. 2017. An early-branching freshwater cyanobacterium at the origin of plastids. *Current Biology*, **27**, 386–391, <https://doi.org/10.1016/j.cub.2016.11.056>
- Porter, S.M., Agić, H. and Riedman, L.A. 2018. Anoxic ecosystems and early eukaryotes. *Emerging Topics in Life Sciences*, **2**, 299–309, <https://doi.org/10.1042/ETLS20170162>
- Preston, T., Stewart, W.D.P. and Reynolds, C.S. 1980. Bloom-forming cyanobacterium *Microcystis aeruginosa* overwinters on sediment surface. *Nature*, **288**, 365–367, <https://doi.org/10.1038/288365a0>
- Sánchez-Baracaldo, P., Hayes, P.K. and Blank, C.E. 2005. Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. *Geobiology*, **3**, 145–165, <https://doi.org/10.1111/j.1472-4669.2005.00050.x>
- Sánchez-Baracaldo, P., Raven, J.A., Pisani, D. and Knoll, A.H. 2017. Early photosynthetic eukaryotes inhabited low-salinity habitats. *Proceedings of the National Academy of Sciences of the USA*, **114**, E7737–E7745, <https://doi.org/10.1073/pnas.1620089114>
- Schopf, J.W. 1968. Microflora of the Bitter Springs Formation, Late Precambrian, Central Australia. *Journal of Paleontology*, **42**, 651–688.
- Servais, T., Brocke, R., Fatka, O., Le Hérisse, A. and Molyneux, S.G. 1996. Value and meaning of the term acritarch. *Acta Universitatis Carolinae – Geologica*, **40**, 631–643.
- Sheldon, N.D. 2011. Microbially induced sedimentary structures in the ca. 1100Ma terrestrial Midcontinent Rift of North America. *SEPM, Special Publications*, **101**, 153–162, <https://doi.org/10.2110/sepmssp.101.153>
- Slotznick, S.P., Swanson-Hysell, N.L. and Sperling, E.A. 2018. Oxygenated Mesoproterozoic lake revealed through magnetic mineralogy. *Proceedings of the National Academy of Sciences of the USA*, **16**, 201813493, <https://doi.org/10.1073/pnas.1813493115>
- Stewart, A.D. 2002. *The Later Proterozoic Torridonian Rocks of Scotland*. Geological Society, London.
- Stewart, E.K. and Mauk, J.L. 2017. Sedimentology, sequence-stratigraphy, and geochemical variations in the Mesoproterozoic Nonesuch Formation, northern Wisconsin, USA. *Precambrian Research*, **294**, 111–132, <https://doi.org/10.1016/j.precamres.2017.03.023>
- Strother, P.K. 1994. Sedimentation of palynomorphs in rocks of pre-Devonian age. In: Traverse, A. (ed.) *Sedimentation of Organic Particles*. Cambridge University Press, 489–502.
- Strother, P.K. and Wellman, C.H. 2010. Paleobiology of a nonmarine Precambrian shale: well-preserved eukaryotes from the 1.1 Ga Nonesuch Formation. *The Palaeontological Association Newsletter*, **75**, Supplement, p. 34.

- Strother, P.K. and Wellman, C.H. 2016. Palaeoecology of a billion-year-old non-marine cyanobacterium from the Torridon Group and Nonesuch Formation. *Palaeontology*, **59**, 89–108, <https://doi.org/10.1111/pala.12212>
- Strother, P.K., Battison, L., Brasier, M.D. and Wellman, C.H. 2011. Earth's earliest non-marine eukaryotes. *Nature*, **473**, 505–509, <https://doi.org/10.1038/nature09943>
- Strother, P.K., Taylor, W.A., Schootbrugge, B.v.d., Leander, B.S. and Wellman, C.H. 2020. Pellicle ultrastructure demonstrates that *Moyeria* is a fossil euglenid. *Palynology*, **44**, 461–471, <https://doi.org/10.1080/01916122.2019.1625457>
- Stüeken, E.E., Jones, S., Raub, T.D., Prave, A.R., Rose, C.V., Linnekogel, S. and Cloutier, J. 2020. Geochemical fingerprints of seawater in the Late Mesoproterozoic Midcontinent Rift, North America: life at the marine–land divide. *Chemical Geology*, **553**, 119812, <https://doi.org/10.1016/j.chemgeo.2020.119812>
- Suszek, T. 1997. Petrography and sedimentation of the middle Proterozoic (Keweenaw) Nonesuch Formation, western Lake Superior region, Midcontinent Rift System. *GSA, Special Papers*, **312**, 195–210, <https://doi.org/10.1130/0-8137-2312-4.195>
- Swanson-Hysell, N.L., Ramezani, J., Fairchild, L.M. and Rose, I.R. 2019. Failed rifting and fast drifting: Midcontinent Rift development, Laurentia's rapid motion and the driver of Grenvillian orogenesis. *GSA Bulletin*, **131**, 913–940, <https://doi.org/10.1130/B31944.1>
- Tang, Q., Pang, K., Yuan, X. and Xiao, S. 2020. A one-billion-year-old multicellular chlorophyte. *Nature Ecology & Evolution*, **4**, 543–549, <https://doi.org/10.1038/s41559-020-1122-9>
- Taylor, T.N., Remy, W. and Hass, H. 1992. Fungi from the Lower Devonian Rhynie Chert: Chytridiomycetes. *American Journal of Botany*, **79**, 1233–1241, <https://doi.org/10.1002/j.1537-2197.1992.tb13726.x>
- Tomitani, A., Knoll, A.H., Cavanaugh, C.M. and Ohno, T. 2006. The evolutionary diversification of cyanobacteria: molecular–phylogenetic and paleontological perspectives. *Proceedings of the National Academy of Sciences of the USA*, **103**, 5442–5447, <https://doi.org/10.1073/pnas.0600999103>
- VanHise, C.R. and Leith, C.K. 1911. The geology of the Lake Superior Region. Chapter XV. The Keweenaw Series. *US Geological Survey, Monographs*, **52**, 366–424.
- Versapen, J.M.H., Snelder, E.O.F.M., Visser, P.M., Jöhnk, K.D., Ibelings, B.W., Mur, L.R. and Huisman, J. 2005. Benthic–pelagic coupling in the population dynamics of the harmful cyanobacterium *Microcystis*. *Freshwater Biology*, **50**, 854–867, <https://doi.org/10.1111/j.1365-2427.2005.01368.x>
- Wacey, D., Saunders, M. *et al.* 2014. Enhanced cellular preservation by clay minerals in 1 billion-year-old lakes. *Scientific Reports*, **4**, 5841, <https://doi.org/10.1038/srep05841>
- Wacey, D., Sirantoine, E., Saunders, M. and Strother, P. 2019. 1 billion-year-old cell contents preserved in monazite and xenotime. *Scientific Reports*, **9**, 9068, <https://doi.org/10.1038/s41598-019-45575-4>
- Wellman, C.H. and Strother, P.K. 2015. The terrestrial biota prior to the origin of land plants (embryophytes): a review of the evidence. *Palaeontology*, **58**, 601–627, <https://doi.org/10.1111/pala.12172>
- White, W.S. and Wright, J.C. 1954. The White Pine copper deposit, Ontonagon County, Michigan. *Economic Geology*, **49**, 675–716, <https://doi.org/10.2113/gsecongeo.49.7.675>
- Wilmeth, D.T., Dornbos, S.Q., Isbell, J.L. and Czaja, A.D. 2014. Putative domal microbial structures in fluvial siliciclastic facies of the Mesoproterozoic (1.09 Ga) Copper Harbor Conglomerate, Upper Peninsula of Michigan, USA. *Geobiology*, **12**, 99–108, <https://doi.org/10.1111/gbi.12071>
- Wolff, R.G. & Huber, N.K. 1973. The Copper Harbor Conglomerate (Middle Keweenaw) on Isle Royale, Michigan, and its regional implications. *Geological Survey, Professional Paper*, **754-B**, B1–B15.