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

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Abstract: The Nonesuch Formation in the clastic sedimentary Oronto Group on the Keweenaw 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.

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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; Slotnick 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 conformingly 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).
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. 2018) 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 (Hishima and Pratt 1991) that reported the presence of steranes, including low levels of 24-n-arylpropylcholstane, 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 Michigecoten 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 shelf 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 (Evitt 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 Lophospheariaidum (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 encystment 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 tenissima*, thin-walled sphaeromorph with diameter >70 µm. (d) *Leiosphaeridia minitissima*, 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) *Nucellospira* sp. with a small circular patch (spot). (h) *Nucellospira* 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) *Nucellospira* sp with a granular envelope enclosing a blockish inner body. (n) *Nucellospira* 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 lophostratiata (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 Archaeocellulipsidea, 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: Synphaeridium, Synplasmosphaeridium 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 Synplasmosphaeridium (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 Hydrodictyaceaen coenobial taxa, such as Pediasstrum. Nevertheless, such forms could be related to chlorophytic plankton. Synphaeridium, 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. Arcticaellulatrix ellipsoidae; 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. 4i), 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 lophostratiata. This distinctive acritarch is recognized by its well-patterned concentric circular spine 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 taxa and found it largely associated with nearshore deposits, which is consistent with its occurrence here in freshwater settings. V. lophostratiata 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 Spurimysoria Wicander & Loeblich, a taxon thought to be close to Moyeria, which is now considered to be a Paleozoic euglenid (Strother et al. 2020). Spurimysoria is characterized by a series of ridges that encircle the vesicle, forming a rectangular pattern when compressed (Fig. 3l). 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 putstular microbially induced sedimentary structures in both the underlying Copper Harbor Conglomerate (Wilmeth et al. 2014) and the Nonesuch Formation (Fig. 3f). These ancient microbially induced sedimentary structures morphologies appear similar in form to microbial mats dominated by Euglena mutabilis, a species known to form putstular and laminated mats today (Brake et al. 2002; Brake and Hasiotis 2008).

Evidence of microbial decay. Elak (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 (Beebee 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.
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 (septae) 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...
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 keström* (Fig. 2b), but are most convincing when found as entangled chains of cylindrical cells with rounded ends (Fig. 2s). It is extremely abundant, occurring in most of the fossiliferous samples and ranging in number up to the order of 10^4 specimens per slide. *Eohalothece* 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, *Eohalothece* 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 (Slotnick 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 vastbulk 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 microbats 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 microfabrics 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 sphaeromorph benthic organisms, including heterotrophs?
- What are the outstanding differences between marine and terrestrial biology at 1 Ga? Are we over-extending uniformitarian 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 vs. 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 promotor 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.

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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).

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Data availability

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

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