Tiny vampires in ancient seas: evidence for predation via perforation in fossils from the 780–740 million-year-old Chuar Group, Grand Canyon, USA

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One explanation for the Early Neoproterozoic expansion of eukaryotes is the appearance of eukaryovorous predators—i.e. protists that preyed on other protists. Evidence for eukaryovory at this time, however, is indirect, based on inferences from character state reconstructions and molecular clocks, and on the presence of possible defensive structures in some protistan fossils. Here I describe 0.1–3.4 μm circular holes in seven species of organic-walled microfossils from the 780–740 million-year-old Chuar Group, Grand Canyon, Arizona, USA, that are similar to those formed today by predatory protists that perforate the walls of their prey to consume the contents inside. Although best known in the vampyrellid amoebae, this ‘vampire-like’ behaviour is widespread among eukaryotes, making it difficult to infer confidently the identity of the predator. Nonetheless, the identity of the prey is clear: some—and perhaps all—of the fossils are eukaryotes. These holes thus provide the oldest direct evidence for predation on eukaryotes. Larger circular and half-moon-shaped holes in vase-shaped microfossils from the upper part of the unit may also be the work of ‘tiny vampires’, suggesting a diversity of eukaryovorous predators lived in the ancient Chuar sea.

1. Introduction

Predators exert a strong influence on the evolution of their prey, driving both innovation and diversification [1,2]. Although most attention has been paid to the role of metazoan predators in shaping the Phanerozoic history of life [3], predatory behaviour—killing for nutritional purposes—is much more ancient and widespread. Bacteria were probably preyed on by other bacteria in Earth’s earliest ecosystems [4], and were almost certainly preyed on by eukaryotes by Late Mesoproterozoic time, when we first see evidence for red algae and therefore primary plastids, acquired via phagocytosis of a cyanobacterium [5]. It is not clear, however, when eukaryotes themselves became prey. A recent study suggests that the earliest eukaryotes were bacterivorous, and that eukaryovory—the ability of protists to eat other protists—evolved much later, arising independently in several eukaryotic clades that diversified ca 800 million years ago (Ma) [6,7]. This is consistent with other indirect evidence for the rise of eukaryovory around this time [6,8,9], including the first appearance of mineralized skeletons and organic tests in protists, possibly used for defence [10,11]; biomarker evidence for the eukaryotic production of lytic toxins that functioned either in defence or as a weapon for killing eukaryotic prey [12]; and fossil evidence for arcellinid testate amoebae, protists whose modern relatives consume both bacteria and eukaryotes [10,13].

Here I report the presence of circular holes in the walls of protistan fossils from the 780–740 Ma Chuar Group, Grand Canyon, Arizona, USA, similar to holes made today by predatory protists that perforate the walls of their prey to consume the cell contents inside. These holes provide the earliest direct fossil evidence for predation on eukaryotes and support the view that by 740 Ma, predation was an important agent shaping eukaryote ecology and evolution [6,8,9].
2. Background and methods

The 1600 m-thick Chuar Group comprises mostly shales and siltstones exposed over a 15 km² area in the eastern Grand Canyon [14]. Chuar sediments were deposited in a shallow marine restricted seaway [14] located within 18° of the palaeoequator [15]. A U–Pb detrital zircon age of ca 782 Ma from the underlying Nankoweap Formation [16] and a U–Pb zircon age of 742 ± 6 Ma from an ash at the top of the Chuar Group [17] place these strata in the late Tonian Period of the Neoproterozoic Era, about 20 Myr prior to the ‘snowball Earth’ glaciations [18], during the first major diversification of eukaryotes [9].

The holes described here occur in organic-walled fossils found throughout the Chuar Group. Although originally spheroidal vesicles, the fossils now occur as 1–2 μm-thick discs, a few tens to hundreds of micrometres in diameter. Twenty-three species of organic-walled fossils have been described from the Chuar Group, including the 1–3 mm compression Chuaria circularis [19,20]. In addition, 17 species of vase-shaped microfossils (VSMs), interpreted as the remains of arcellinid and possibly euglyphid testate amoebae occur in the upper part of the unit [10,13].

Fossils were released from their host rocks via HCl and HF acid maceration and viewed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) systems housed at UCSB’s Earth Science Department and Materials Research Laboratory (see [20] for more details).

3. Description of holes

The holes occur in seven species and an unnamed form. Five of these species and the unnamed form are interpreted to be the remains of vegetative cells or resting cysts (figures la,c,f,g and 2b; [20]); a sixth species is interpreted to be a possible scale-bearing protist (figure 1e; [20]) and a seventh species, represented by 30–100 μm sized smooth-walled fragments, is assumed to be C. circularis (figure 2a).

The holes are circular in...
shape and occur in specimens that may otherwise show no signs of pitting or degradation (e.g. figure 1c). In some cases, they are bevelled (e.g. figures 1b and 2), with the diameter narrowing towards the interior of the fossil (figure 1b). The holes do not always perforate the wall completely; in some cases, they extend only through the outer wall of a double-walled fossil (figure 1b,f). They range in size from 0.1 to 3.4 µm in diameter, but exhibit much narrower size ranges within species and within specimens (figure 3). Within ‘Trachysphaeridium’ laufeldii, for example, hole diameters range from 0.1 to 0.6 µm (mean = 0.3 µm, n = 34); within individual specimens the range narrows to 0.2 µm (figure 3). Similarly-sized holes are found in Leiosphaeridia sp. and Valeria lophostriata, as well as in two new species and an indeterminate form (described in [20]; figure 3). By contrast, larger holes are found in Cerebrosphaera globosa (=C. buickii; see [20]) (figure 2b; 1.2–2.1 µm in diameter; mean = 1.3 µm; n = 8) and C. circularis (figure 2a; 0.6–3.4 µm in diameter; mean = 2.0 µm, n = 42). Holes of different sizes may occur in fossils from the same rock sample, but always in different species (figure 3). The difference in hole sizes among species broadly corresponds to size differences among the species: species with vesicles typically tens of micrometres in diameter have smaller holes (0.1–0.6 µm); those with vesicles hundreds to thousands of micrometres in diameter have larger holes (0.6–3.4 µm).

Holes typically number between 1 and 10 in a single specimen (median = 5 observed per specimen), although holes in C. circularis may be more densely distributed, in some cases, overlapping (figure 2a; overlapping holes have not been observed in other species). In specimens with multiple holes, their spatial distribution is scattered rather than regular (e.g. figure 1a,d,f). For most species, only a few (one to six) specimens were examined under SEM, making it difficult to estimate the frequency of those with holes. However, in ‘T.’ laufeldii, a species that was both abundant and a focus of close study [20], about a tenth of the specimens examined (9 of 108) exhibited holes, although this is probably an underestimate given that the holes can easily escape notice.

4. Origin of holes

Both the irregular spatial distribution of the holes within individual specimens and the fact that they occur in a variety of disparate species but are not widespread in any single one, suggest the holes are not an original character of the organism, such as pores in the cell wall. Similarly, while minerals can perforate fossils during sediment compaction and diagenesis, the pits and holes they leave are polygonal in shape and irregular in size, and may extend through both walls of a flattened fossil (S. M. Porter 2015, personal observation; [21])—characteristics never observed in the holes described here. Post-mortem degradation of the wall material itself (i.e. scavenging by
In summary, several observations support the view that the Chuar holes were made by predatory protists: their similarity in shape and size to holes made by modern predators (e.g. figure 4); the fact that a single microfossil may have multiple similarly-sized holes, consistent with the fact that a single predatory protist may make numerous holes in its prey ([26,36]); and apparent specificity in hole size and microfossil species (figure 3), consistent with species-specificity in hole size and prey preference observed in vampyrellids [30,34].

Given that the ability to perforate cell walls is apparently widely convergent (occurring even in some bacteria [38], it is not possible to identify with confidence the predators that made the holes in the Chuar fossils; they may be early members of one of the modern vampire-like clades, or they may represent an extinct clade that independently evolved the ability to perforate resistant organic walls. What can be identified with confidence, however, is their prey: at least four of the species are eukaryotes, as indicated by their size, wall ornamentation and excystment structures; the most commonly perforated of these, 'T.' laufeldii, is characterized by networks of thin filaments arising from the vesicle wall, rounded excystment structures and bud-like protuberances, all hallmarks of eukaryotic organization [20,39]. (A prokaryotic affinity cannot be ruled out for the other three prey species—C. circularis, Leiophasaeridia sp. and new species 2.) Thus, the Chuar holes provide direct evidence for predation on eukaryotes ca 780–740 Ma, corroborating the viewing that predators were an important influence on protistan ecology and evolution by mid-Neoproterozoic time [6,8,9].

### 5. Other vampires in the Chuar Group?

An earlier study of VSMs from the Chuar Group revealed the presence of half-moon-shaped holes in several specimens, interpreted to reflect predation (figure 5a–d; [13]). The half-moon holes are 15–35 μm in length and are consistently oriented with their flat edge parallel to the long axis of the VSM test, suggesting stereotypy in prey manipulation. In addition, a few VSM specimens exhibit circular holes, 13–30 μm in diameter, in one case occurring in a test that also exhibits a half-moon hole (figure 5e–g; not previously reported). Like the much smaller holes that are the focus of this paper, these holes seem to be best explained by the action of vampire-like predators, though their much larger size would indicate different species of predators at work. Together with the VSMs themselves, interpreted to be the remains of arcellinid testate amoebae, and the biomarker cryostane—thought to be derived from eukaryotic sterols that protected their producer from lytic toxins [12]—these holes suggest that a diversity of eukaryovorous predators lived in the Chuar sea.

### 6. Other ancient vampires?

The very small size of many of the Chuar holes (e.g. 0.1–0.6 μm) means that they are only visible via SEM, a technique not routinely used in the study of Proterozoic microfossils.

*Figure 4. Perforations in spores of the fungus Cochliobolus sativus made by vampyrellid amoebae [32,33]. (a) Overview of spores, several showing circular perforations. (b) Close-up view of spore, showing several circular perforations, each approximately 0.2 μm in diameter. Scale bar is 20 μm in (a), 2 μm in (b). Reprinted with permission from [32]. Copyright © Canadian Science Publishing or its licensors.*
7. Conclusion

Body fossils and biomarkers from approximately 1200 to 740 Ma rocks record taxonomic and ecological expansion of eukaryotes, evidenced by an increase in eukaryotic micro- and macrofossil diversity; the appearance of morphologically disparate forms, including scale- and test-bearing taxa; increases in the relative contribution of eukaryotic algae to primary productivity; and the appearance of multicellularity in several eukaryotic clades [6,9]. It has been suggested that eukaryovorous predators played an important role in this expansion by driving diversification, increased productivity and morphological innovation in their prey [6,8,9]. It has been difficult to test these hypotheses, however, because unlike the Phanerozoic fossil record, in which the widespread occurrence of drill holes and shell repair scars has allowed predation intensity to be quantified through time (e.g. [46]), the Precambrian record of predation is virtually non-existent. The recognition that Neoproterozoic microfossils preserve minute ‘drill’ holes made by perforating predators, and that these holes may be widespread in Neoproterozoic assemblages, offers the prospect that a record of predation intensity can be extended much further back in time, making it possible to test the extent to which predator–prey interactions shaped the early evolution of life.

Data accessibility. All data associated with this manuscript are available as the electronic supplementary material. Additional information on specimen locations and accession numbers can be found at Dryad digital repository: http://dx.doi.org/10.5061/dryad.6pr6c.

Competing interests. I declare I have no competing interests.

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