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Article

A 'Giant Microfossil' from the Gunflint Chert and its Implications for Fungal and Eukaryote Origins

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Abstract We report here a 'giant microfossil' resembling the beaked, muriform conidium (dictyospore) of the modern ascomycete fungus *Alternaria*. The specimen is preserved in stromatolitic black chert of the Gunflint Iron Formation (Paleoproterozoic Eon, Orosirian Period, 1.88 Ga) of southern Ontario, Canada. The rock slab that provided the thin section was apparently collected by Elso Barghoorn as part of the original discovery of the Gunflint microbiota. The large size of the fossil sets it apart from other (tiny by comparison) Gunflint microfossils. The fossil is 200 microns in length and has both transverse and vertical septa as seen in both transmitted light (with camera fusion optics) and spinning disc confocal microscopy. Individual cells are 30-46 microns in greatest dimension. The conidial beak is cap-shaped, and has partly separated from the rest of the structure. *Cloulicaria gunflintensis* gen. nov. sp. nov. may provide very early evidence for eukaryotes (fungi) in the fossil record.

Keywords Gunflint Chert, stromatolite, black chert, origin of eukaryotes, evolution of fungi, Proterozoic microfossils, muriform cells, conidium, dictyospore

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1. Introduction

The earliest known eukaryotes are sphaeromorph acritarchs assigned to *Valeria lophostriata* from the Changzhougou Formation, Changcheng Group, China. These fossils are dated to 1673±10-1638±14 Ma [1]. The oldest reported fungi are assigned to *Ourasphaira giraldae* from the Shaler Supergroup (Grassy Bay Formation) of Arctic Canada, and are dated to 1013±25-892±13 Ma [2]. We describe here a 'giant microfossil' from the 1900-2000 Ma Gunflint Iron Formation of Ontario, Canada that is considerably older than either *Valeria* or *Ourasphaira*. The great size of this microfossil indicates that it should be evaluated as a candidate for the earliest known eukaryote. The similarity of the fossil to beaked, muriform conidia (dictyospores) of modern *Alternaria* indicate that it constitutes a promising candidate for the earliest known fossil fungus.

2. Geological Setting

The Gunflint Iron Formation is known for its microfossiliferous black cherts. These cherts have produced compelling evidence for very ancient (1.88 Ga [3]) microbial life [4-12]. Tyler and Barghoorn [4] noted that as "far as we are aware, these [microbes] are the oldest structurally preserved

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organisms . . . and, as such, are of great interest in the evolutionary scheme of primitive life." This paper [4] is considered one of the great classics of American earth science ("a benchmark, a monumental 'first'" [6]). Indeed, this was one of the first definitive descriptions of very ancient Precambrian life.

Gunflint microfossils continue to serve as prime examples of early life on earth. Their authenticity is uncontested, in part due to evidence obtained using secondary ion mass spectrometry (SIMS) showing that Gunflint microfossils have a consistent ~19 per mil total fractionation ($\delta^{13}C$ of dissolved inorganic carbon minus $\delta^{13}C$ of biomass) "similar to that observed in living cyanobacteria, over a wide range of $\delta^{13}C_{carb}$ values" (-2.9 per mil to 3.4 per mil) [13]. Research is ongoing to distinguish authentic ancient microfossils from, say, todorokite filament pseudofossils. Reliable criteria for recognizing ancient microbial life forms will be essential in the search for extraterrestrial life [14].

The Animikie Group, associated with the Cuyuna, Gunflint, Mesabi, and Vermilion Ranges in the Lake Superior region of Minnesota, USA and the Thunder Bay district of Ontario, Canada, was deposited in the Animikie Basin during the Paleoproterozoic between 2.5-1.8 Ga. The stratigraphic sequence in the Mesabi Range has the Pokegama Quartzite at its base, overlain in turn by the Biwabik Iron Formation and the Virginia Formation. The Gunflint Range's similar stratigraphic sequence consists of a basal conglomerate, overlain in succession by the Gunflint Iron Formation and the Rove Formation.

The Gunflint Iron and Biwabik Iron Formations are correlative rock units [5]. The Gunflint "basal clastics consist of a half meter or so of poorly sorted and poorly lithified massive shaly material consisting of angular-to-subangular greenstone fragments . . . associated with well-rounded-to-angular grains of quartz, jasper, chert, and greenstone with occasional grains of fresh pink feldspar. The clastics are cemented by a minor amount of carbonate and chert" [5].

Animikie strata deposited at approximately 2.0 Ga are remarkable for their banded-iron formations (BIFs), marine deposits that reflect the oxidation of dissolved marine iron due to large scale oxygenation of the ocean and atmosphere during the Great Oxygenation Event. An ejecta layer associated with the Sudbury impact event occurs between the Gunflint and Rove formations and the Biwabik and Virginia Formations, respectively. Global catastrophe due to the 1.85 Ga Sudbury impact is implicated in the cessation of global-scale BIF deposition [15].

Gunflint Iron Formation microfossils are preserved in black chert and are associated with stromatolites. Some of these stromatolites attain 3 m diameter. A typical Gunflint stratigraphic sequence, for example in the vicinity of Flint Island and Winston Point on the Lake Superior shoreline, develops a tripartite division beginning with Neoarchean greenstone (2.7 Ga;, intruded by 2.5 Ga Algoman Granite), Gunflint breccia, and Gunflint stromatolites and black chert. A ca. 1.9 Ga paleosol is developed on the Neoarchean pillow basalts [16]. Iron-mineralized microfossils [17] and carbonates [18] occur in the sequence. Many of the stromatolites initiated growth on a cobble of Archean greenstone, and retain the greenstone clast in the core of the stromatolite. More distal facies, as for example in the vicinity of Trans-Canada Highway 11-17 one mile north of Nelson Road, develop interbedded light and dark chert that in selected horizons shows evidence for lateral sedimentation, developed as intraformational breccias in the form of edgewise conglomerates.

Specimens from the Gunflint sequences of Biwabik, Minnesota, USA show development of centimeter scale stromatolites in banded red jasper with either low domal or columnar forms. The fossil described here is from a stromatolitic black chert (Gunflint Range, Gunflint Iron Formation; Fig. 1), with stromatolite morphology strongly resembling that of Biwabik jasper stromatolites. Barghoorn and Tyler [4, 5] reported transitions from black to red cherts, and inferred that these reflected "a change from reducing to oxidizing conditions at the time of deposition."

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Figure 1. Thin section, Gunflint Iron Formation. Stromatolitic laminations are clearly visible in this black chert from the Gunflint Range. Oolitic grains have accumulated between the stromatolitic columns. Slide 1 of 1/30/92; IGM 5009. Specimen courtesy E. Barghoorn. Photograph by Destiny Treloar.

3. Provenance of Sample

The provenance of the sample is certain. It is derived from the Gunflint Iron Formation of southern Ontario, Canada. In fact, the rock sample was probably collected by Elso Barghoorn himself as part of the original Gunflint microbiota research programme, was later donated to Mount Holyoke College, and thus bears historical as well as scientific interest. The fossil described here is unquestionably embedded in chert as it is the same color as other organic matter in thin section. It is thus not a modern contaminant.

After arriving at Mount Holyoke College in 1984, one of us (MASM) found in the paleobotany collections an uncurated small slab (4.5 cm by 3.5 cm; Mount Holyoke Paleontology Collection No. 5052; sample 1 of 1/30/92) of black stromatolitic chert in a white envelope with "The Biological Laboratories/Harvard University" printed as a return address (Fig. 2) and marked in pencil "from preCambrian/from Barghoorn". The specimen was evidently presented as a gift to Mount Holyoke College by Elso S. Barghoorn. Recognizing its importance (in spite of the fact that it had been poorly curated prior to my arrival at Mount Holyoke), I had this slab thin sectioned to create slide 1 of 1/30/92.

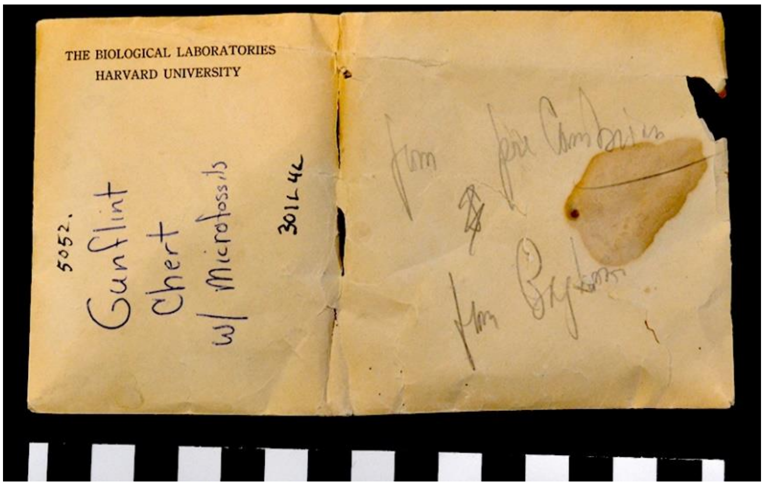

Figure 2. Envelope that contained the black chert specimen that was presented to Mount Holyoke by Elso S. Barghoorn of Harvard University. The return address is "The Biological Laboratories/Harvard University". The paper is old, yellowed, and cracked (evidently not acid-free paper) with a stain on the right side. Pencil label reads "from preCambrian/from Barghoorn." Additional writing refers to the specimen's former location in the Mount Holyoke College Paleontology Collection. Scale in centimeters. Photograph by M. A. S. McMenamin.

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Slide 1 of 1/30/92 is already known to the paleontological community due to its service providing the first specimen used to demonstrate stereophotomicrograph images of microfossils, namely, specimens of the filamentous microfossil *Gunflintia minuta* [19]. The fossils in slide 1 of 1/30/92 are clearly derived from the Gunflint Iron Formation.

Comparison with Barghoorn's own descriptions of the Gunflint Formation [4, 5] strongly suggest that this specimen belongs to the lower algal member of the black cherts of Schrieber Ontario. This chert crops out at several sites at the same stratigraphic horizon "over a distance of approximately 190 kilometers along the strike of the formation" [5]. Of these sites, the Schrieber locality has the best fossil preservation making it the focus of Barghoorn's research and the likeliest source of the slab.

The thin section (Fig. 1) shows three long columnar stromatolites and, in its stratigraphically lower part, several low domal stromatolites. The columnar stromatolite on the far right (Fig. 1) appears to have toppled over to the left, as its growth axis is no longer parallel to the undisturbed columns to the left. Allochem grains, including ooids, occur in the spaces between the columns. The ooids are very dark in color, and some show spalling of their outer laminations. Larger, rounded allochems appear to be faintly laminated fragments of microbial mat material. The large microfossil of interest occurs near the base of the columnar stromatolite (doubled to two conjoined columns in its middle part) in the left half of the slide.

4. Giant Gunflint Microfossil

Figures 3-7 show *Cloulicaria gunflintensis* gen. nov. sp. nov. The fossil somewhat resembles the conidium of modern *Alternaria alternata*. The fossil is clearly not a modern contaminant as it is completely embedded in the chert matrix. As noted above, its color matches that of the other organic material in the chert, and under cross-polarization cryptocrystalline quartz crystals of the chert are visible inside the fossil.

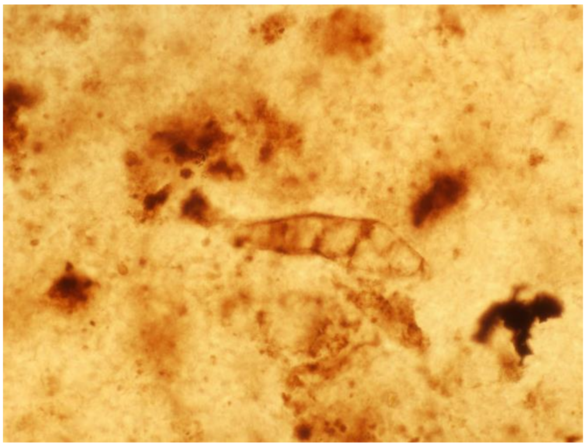

Figure 3. *Cloulicaria gunflintensis* gen. nov. sp. nov. Conidium-like structure preserved in Gunflint chert. Length of fossil 200 microns; maximum width 46 microns. IGM 5009. Photograph by M. A. S. McMenamin.

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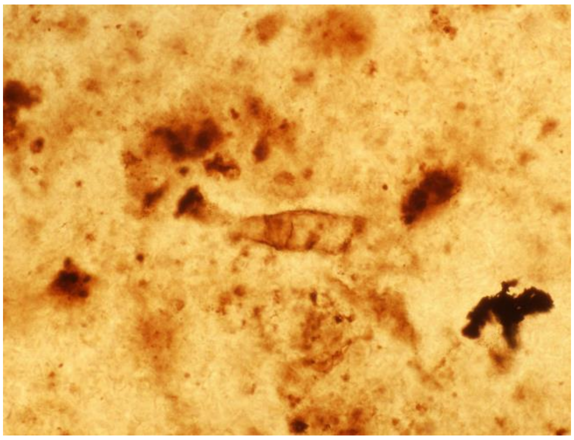

Figure 4. *Cloulicaria gunflintensis* gen. nov. sp. nov. Conidium-like structure. Length of fossil 200 microns. Photograph by M. A. S. McMenamin.

Figure 5 shows a modern conidium of *Alternaria* for comparison. *Alternaria* is an extremely widespread modern fungus [20], occurring almost ubiquitously in terrestrial [21] and marine [22] habitats. Barnett and Hunter [20] describe *Alternaria* as developing elongate conidia "typically with both cross and longitudinal septa." *Cloulicaria gunflintensis* gen. nov. sp. nov. shows both transverse and inclined septa, as seen in the modern species *Alternaria alternata* and *A. solani*.

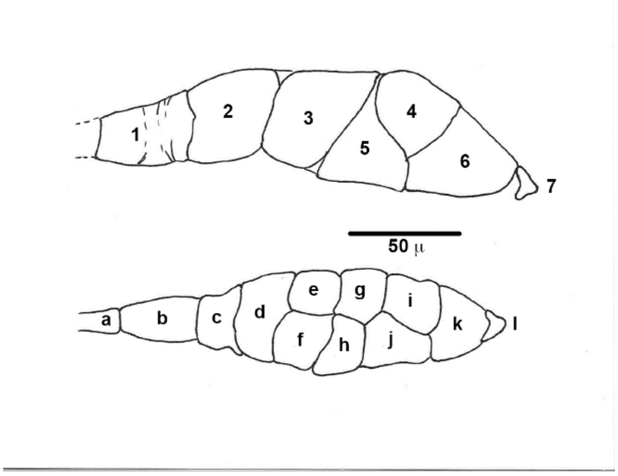

Figure 5. *Top*, sketch of *Cloulicaria gunflintensis* gen. nov. sp. nov., spore-like bodies labelled 1-7; *Bottom*, sketch of modern *Alternaria* sp. conidium, spores labelled a-l. Bottom image sketched from http://www.sporometrics.com/resources/fungal-descriptions/alternaria_spore/. Scale bar = 50 microns. Image credit: Mark McMenamin.

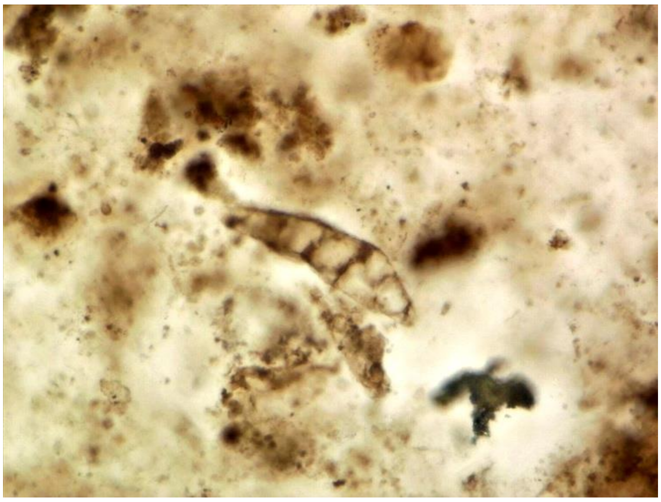

Figure 6. *Cloulicaria gunflintensis* gen. nov. sp. nov. Conidium-like structure. Length of fossil 200 microns. Photomicrograph taken on a Leica DVM6 using integrated camera fusion optics. Note: Not a multifocus (i.e., Z-stack all-in-focus image). Photograph by M. A. S. McMenamin.

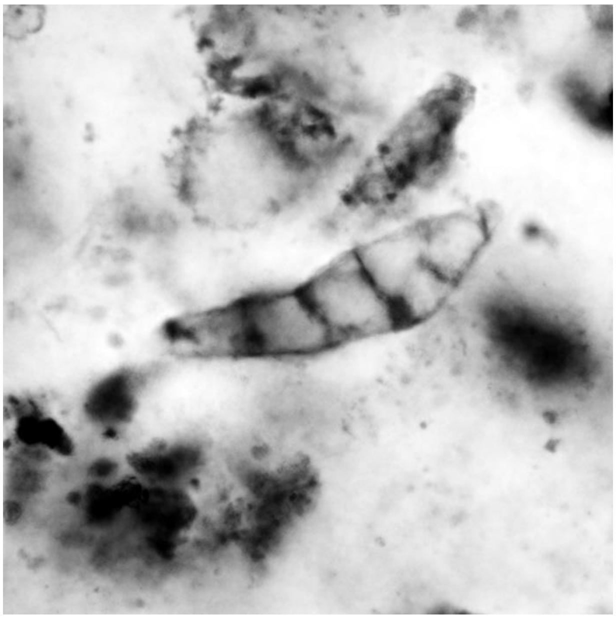

Figure 7. *Cloulicaria gunflintensis* gen. nov. sp. nov. Conidium-like structure. Length of fossil 200 microns. Photomicrograph taken on a Nikon Eclipse T/2 Spinning Disc Confocal Microscope (SDCM). Image taken with the thin section inverted with respect to the view in previous images, as the slide coverslip needs to be oriented downward for SDCM. Photograph by M. A. S. McMenamin.

Cloulicaria gunflintensis gen. nov. sp. nov. is huge (200 microns total length and 30-46 micron spore diameter) in comparison to other Gunflint microbes such as *Gunflintia minuta* (a typical filament is 1 micron thick) and *Huroniospora* (both prokaryotes are present in slide 1 of 1/30/92). *Cloulicaria's* large size could support a eukaryotic and even fungal interpretation of the structure.

From Fig. 5, it is possible to make the following cell/spore comparisions between *Cloulicaria gunflintensis* gen. nov. sp. nov. and *Alternaria* as shown in Table 1. Note the particularly close morphological comparison regarding cells/spores at the distal ends of each structure. Pairs 4/i and 7/l show particular similarity, with their respective length/width ratios of 0.95/0.93 and 0.63/0.73, respectively. The fact that *Cloulicaria* cell/spore 7 has split off from the main body is evidence that the tapering tubular structure from which it was derived served as a propagule that, on maturity, disaggregated to allow spore dispersal.

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Table 1. *Cloulicaria* and *Alternaria* Compared

spore/cell number (*Cloulicaria*)	L/W ratio	spore/cell letter (*Alternaria*)	L/W ratio	degree of form similarity
		a		n/a
1	1.41	b	1.71	moderate
2	1	c	0.66	moderate
3	1.16	d	0.66	moderate
		e		n/a
		f		n/a
		g		n/a
		h		n/a
4	0.95	i	0.93	good
5	0.81	j	1.23	good
6	1.25	k	0.8	good
7	0.63	l	0.73	high

Alternative interpretations of *Cloulicaria gunflintensis* gen. nov. sp. nov. must also be considered. Although cross walls can occur in certain types of bacteria, particularly in actinobacteria such as *Streptomyces* (as a stage in sporulation septation [23]), its aerial hyphae approaching sporulation are miniscule compared to the size of *Cloulicaria*. The possibility that *Cloulicaria gunflintensis* gen. nov. sp. nov. represents a partially degraded colony of giant prokaryotes cannot be completely eliminated at this point, but seems unlikely because what we interpret as septa show morphology that is much more characteristic for eukaryotes. The megascopic spiral, tubular form taxon *Grypania* is likely to have been prokaryotic early in its very long (1.2 Ga) temporal range, and eukaryotic in the later part of its range [24], with the two types linked by homoplasic shared body form that may have changed its position in the water column by rotating in a corkscrew fashion to ascend or descend. It seems unlikely that *Grypania* represents a single taxon that survived for over a billion years. In any case, its spiral tube morphology is very unlike that of *Cloulicaria*.

Four lines of evidence suggest that *Cloulicaria* is not bacterial. First, large filamentous structures with cross walls are unknown among ancient Archaea or the Bacteria. Second, the cap-shaped structure or beak at the distal end of the filament is often seen among fungi (*Alternaria* in particular) and protists such as chytrids (Chytridiomycota) but absent from sporulating bacteria. The tapered shape of the ‘conidium’ of *Cloulicaria* suggests determinate growth which is also uncharacteristic of bacteria [9]. Fourth, equally ancient organisms with both large size and cross wall morphology are unknown as fossils. Biogeochemical analysis might allow further characterization of *Cloulicaria gunflintensis* gen. nov. sp. nov., but we are loath to risk damage to a unique holotype. A search is underway in Gunflint chert for additional specimens of this species for geochemical analysis.

A second alternative interpretation, namely, that the structure is a pseudofossil, may be dismissed because the of the fossil’s evident biological morphology, along with its typical state of preservation in chert as seen for other fossils in the same slide, plus the absence of any plausible abiotic mechanism that could make such a complex pseudofossil.

A third alternative interpretation, that represents a non-fungal eukaryote such as a red alga (cf. *Bangiomorpha pubescens* from the 1.047 Ga Hunting Formation of Somerset Island, Canada [25]) is untenable on morphological grounds, as *Cloulicaria* is morphologically quite distinct from *B. pubescens*. An unnamed, enigmatic complex Precambrian (*ca.* 1.2 Ga) microfossil with an elongate shape showing internal structure [26] is unlike *Cloulicaria* due to both its small size and to the strange tubular nature of its internal structures.

5. Conclusions

The oldest “fungal-like” fossils are anastomosing filamentous microfossils from 2.4 Ga vesicular basalts [27], but classification of these fossils as fungi or even as eukaryotes is controversial. *Cloulicaria gunflintensis* gen. nov. sp. nov. appears to be an anomalously large and complex microbial structure for 1.88 Ga. It is a candidate for the earliest known eukaryote.

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We speculate further that the fossil may represent an conidium-like structure that drifted into the stromatolitic microbiotic assemblage, an environment that was not its usual habitat. Considering its rarity (a unique specimen) in the well-studied Gunflint assemblage, *Cloulicaria gunflintensis* gen. nov. sp. nov. may have settled into the stromatolite habitat after originating in a terrestrial habitat. If so, *Cloulicaria gunflintensis* gen. nov. sp. nov. represents the oldest direct evidence for eukaryotic (and possibly fungal) life on land.

Alternaria spores are wind-dispersed and widespread in many modern environments. Spores of *A. alternata* remain viable 'under storage conditions' for a decade or more [28]. Spores of *A. tenuis* were found to be highly variable in shape, with septation, 'wartiness', size, shape and color depending on the culture medium [29]. The ecological versatility of *Alternaria* is impressive, with two phytotypes of the genus providing (as fungal endophytes) competitive advantages to their invasive forb host *Centaurea stoebe* [30].

The assumption that the earliest fungi were aquatic/marine [31-32] may prove to be unfounded. The absence of ribbons, filaments and spinous ornamentation on the spores, characteristic of marine fungi such as *Corollospora* and *Carbosphaerella* [33], would be consistent with a terrestrial habitat for *Cloulicaria gunflintensis* gen. nov. sp. nov. An alternate interpretation is that *Cloulicaria gunflintensis* gen. nov. sp. nov. lived in an aquatic habitat that differed from the Gunflint stromatolite habitat, in which case the conidium-like structure described here drifted in to the stromatolite region and was trapped and bound onto a biomat lamina.

6. Systematic Paleontology

Repository data: IGM, Institute of Geology Museum, Departmento de Paleontología, Cuidad Universitaria, Delegacíon de Coyoacán, 04510, México..

Domain ?Eukaryota

Kingdom ?Fungi

Genus *Cloulicaria* nov. gen.

Type species: monotypic, *Cloulicaria gunflintensis* gen. nov. sp. nov.

Diagnosis: Elongate microfossil with cross walls, 200 microns in length, consisting of a chain (tapered at both ends) of presumed spores (30-46 microns), with a small cap structure ('beak') at the presumed apical end. Septa occur at transverse and longitudinal orientations along the length of the fossil to form a muriform conidium-like structure.

Description: A buckled, septate tube with multiple irregular partitions interpreted here as transverse and longitudinal septa. In the single known specimen, the conidium-like structure bends and buckles along its length. No sheath is visible surrounding the structure. *Cloulicaria gunflintensis* gen. nov. sp. nov. is 200 microns in length. Individual 'spores' are 30-46 microns in greatest dimension.

Discussion: The 'beak' is cap-shaped, as in many beaked, muriform conidia of the ascomycete fungus *Alternaria*. Figure 5 compares the muriform spore arrangement in both *Alternaria* and *Cloulicaria* gen. nov. The number and arrangement of septa in *Alternaria* varies. *Alternaria* has exclusively asexual reproduction by means of spores produced in conidia, and presumably *Cloulicaria* gen. nov. would share this characteristic if the comparison between the two proposed here is valid. The possibility thus exists that sex evolved in fungi independently from other eukrayotes. *Alternaria* also lacks conidiophores (specialized hyphae), as the conidia form at the ends of apparently normal hyphae.

Etymology: Named for Preston Cloud and Gary Licari for their contributions to Pre-Cambrian paleontology.

***Cloulicaria gunflintensis* nov. gen. nov. sp.**

Figs. 3-7, *GunflintEukaryote.mov*, *GunflintEukaryoteSDCM.mov*

Holotype: Sample 1 of 1/30/92. Position from reference X : 27.7 mm x 15.0 mm; IGM 5009.

Description: Same as for genus, by monotypy.

Etymology: Named for the Gunflint region of southern Ontario, Canada.

Age and Locality Information: Gunflint Iron Formation, lower algal member of the black cherts of Schrieber, southern Ontario (Paleoproterozoic Eon, Orosirian Period, 1.88 Ga). The rock slab from which the thin section was cut was probably collected by Elso Barghoorn himself.

Supplemental File

Video file: *GunflintEukaryote.mov*, *GunflintEukaryoteSDCM.mov*.

Competing Interests

Not applicable.

Authors' Contributions

Investigation, Mark McMenamin, Aurora Curtis-Hill, Sophie Rabinow, Kalyndi Martin and Destiny Treloar; Methodology, Mark McMenamin, Aurora Curtis-Hill and Kalyndi Martin; Supervision, Mark McMenamin; Visualization, Mark McMenamin, Aurora Curtis-Hill, Sophie Rabinow, and Destiny Treloar; Writing—original draft, Mark McMenamin; Writing—review & editing, Aurora Curtis-Hill.

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