*REPRODUCTIVE STRUCTURES AND TAXONOMIC AFFINITIES OF SOME NANNOFOSSILS FROM THE GUNFLINT IRON FORMATION**

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It is difficult, and crucial, in studying possible records of life from very ancient rocks, or from extraterrestrial sources, to establish both that elements observed could not have resulted from nonvital processes, and that they are surely endemic to the substrates in which found. In the case of the diverse and well-preserved microbiota found in cherts of the approximately 1.9 billion-year-old Gunflint Iron Formation of southern Ontario, convincing evidence for a vital origin and compelling evidence for the endemic nature of vital elements observed has already been advanced.[1-4] We present here what we take as both a final link in the chain of evidence for a biological origin and the basis for more satisfactory conclusions as to the taxonomic affinities of some of the conspicuous biotal elements (Figs. 1–20).

This new evidence consists mainly of features that closely resemble the reproductive structures of certain living algae, but displayed by specimens of the Gunflint microbiota.

All of the fossil material here described comes from chert in the pre-Paleozoic Gunflint Iron Formation at Schreiber Beach, on the north shore of Lake Superior, about 6.5 km west-southwest of Schreiber, Ontario (Cloud's locality 1 of 8/25/1963), as previously discussed.[2] It was studied optically, in thin sections of the rock, using oil-immersion techniques and phase-contrast lighting. Electron microscopy studies are yet to be made of wall structure and the zones of junction of the seemingly dividing globular bodies here described. Pending availability of necessary facilities for such studies, however, results already obtained seem sufficiently significant to warrant the notice here given them.

Structures indicative of blue-green algal affinities

The most abundant entities observed are nonbranching, generally septate, tubular filaments that range from 0.5 to about 6.0 μ or as large as 10 μ in diameter. Such filaments were interpreted by Barghoorn and Tyler[1] as most probably blue-green or myxophycean algae, and were assigned to three genera and four species described by Barghoorn in the same paper. The two most numerous forms were both referred to the new genus *Gunflintia,* but to different species because of differences in diameter of the filaments and the shapes of individual cells. The smaller filaments, clustering around 1–1.5 μ in diameter and with regular elongate cells, were called *Gunflintia minuta,* in contrast to *Gunflintia grandis,* with equidimensional to elongate cells and a larger diameter of 2.5–5 μ. Cloud[2] and Cloud and Hagen,[3] however, preferred to designate these organisms in a collective sense simply as procaryotes (comprised of nonmitosing cells). Cloud considered it possible that the smaller-diameter threads of *G. minuta* might be either myxophycean algae or thread bacteria, the latter primarily on the basis of their minute size. Certain of the larger filaments in the range of 5–6 μ in diameter, and with short discoidal cells reminiscent of the living nostocalean alga *Oscillatoria*, were referred by Cloud to the myxophycean algae. These are also referrable to Barghoorn's genus *Animikia*, having a range in filament diameter from 5 to 10 μ.

Continuing study of these various filaments by the present authors reveals a marked variation in size and shape of cells spaced along some of them. From analogy with living blue-green algae, this confirms an assignment to the Myxophyceae for all *Gunflintia* threads. At the same time, it raises questions about the validity of thread diameter or varying cell length as criteria for assignment to two different species.

Heterocysts.—Numerous specimens referable to *G. minuta* Barghoorn show enlarged, clear, subspherical cells 2.5–3.0 μ in diameter spaced along the filaments (Figs. 7–9). Similar enlarged, transparent, thick-walled cells termed heterocysts are found among modern, filamentous blue-green algae (Fig. 6). There they apparently serve a reproductive function, either as points where threads (or trichomes) may be broken, or as actual germinating bodies that produce new threads.[5, 6] The near transparency in probable fossil heterocysts suggests differences in mineralization between nonpigmented and adjacent pigmented cells. This is consistent not only with their identification as heterocysts, but also with an interpretation that the now darker cells were once pigmented, photosynthesizing units of life.

Akinetes.—Another differentiated type of cell found along the length of modern myxophycean filaments is the enlarged, thick-walled resting cell, or akinete (Fig. 5), which also germinates to produce new filaments. Unlike heterocysts, akinetes possess photosynthetic pigmentation. They occur adjacent to heterocysts as single cells, or in a series. In many living genera of the family Nostocaceae such as *Wollea*, *Aphanizomenon*, *Anabaena*, and *Cylindrospermum*, the akinetes are not only larger in diameter than heterocysts but considerably longer.[6] That the greatly elongated cells of some *Gunflintia* filaments are akinetes that may alternate with heterocysts is implied by specimens such as those illustrated in Figure 4. Numerous filaments also demonstrate a change in diameter from about 1.4 μ to about 2.5–3.5 μ and a change in gross morphology of cells consistent with an interpretation of the larger-diameter portions as being long series

FIGS. 1–9.—Bar scale of 20 μ applies to all illustrations except Figs. 5 and 6. Thin section and finding coordinates: Fig. 1, C71(6), 48.6/109.3; Fig. 2, C71(6), 38.4/96.5; Fig. 3, C71(6), 38.0/97.8; Fig. 4, C71(6), 48.4/109.0; Fig. 7, C71(4), 54.5/109.1; Fig. 8, C71(7), 39.7/109.1; Fig. 9, C71(6), 32.4/104.1.

FIG. 1.—Photomicrograph of Gunflint chert showing profusion of "*Huroniospora*" ellipsoids and *Gunflintia* filaments.

FIGS. 2, 3.—Filaments exhibiting change in diameter from that characteristic of *Gunflintia grandis* Barghoorn to that of *Gunflintia minuta* Barghoorn.

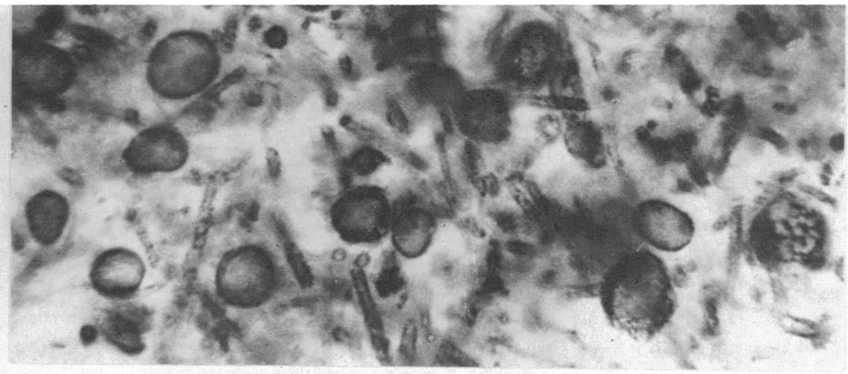
FIG. 4.—Differentiated cells of *Gunflintia* filaments interpreted as heterocysts (*h*) and akinetes (*a*). Compare with Fig. 5.

FIG. 5.—Living *Anabaena oscillarioides* Bory showing differentiated cells: akinetes (*a*) and heterocysts (*h*). Compare with Fig. 4. From Fritsch, ref. 6, p. 834.

FIG. 6.—Heterocyst (*h*) along a trichome of living *Aulosira implexa* Bornet and Flahault. After Smith, ref. 12, p. 583. (×435)

FIGS. 7–9.—*Gunflintia minuta* Barghoorn showing probable heterocysts (*h*). Compare with Fig. 6.

FIGS. 1–20.—All illustrated structures in this article have been located by coordinates of the Leitz Orthoplan microscope used in this investigation. Each thin section is also marked with a diamond-scratched reference *X*. Thus the thin section may be transferred to another microscope and the structure relocated when oriented with the label at the right and the reference *X* in the front left corner. Coordinates of reference marks for thin sections referred to are: C71(4), 68.1/111.3; C71(5), 69.8/109.8; C71(6), 69.8/109.7; C71(7), 69.3/111.39.



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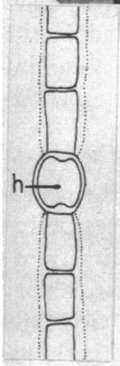

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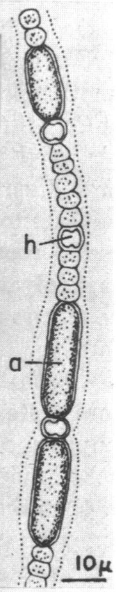

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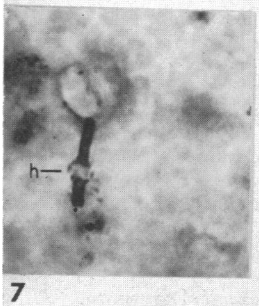

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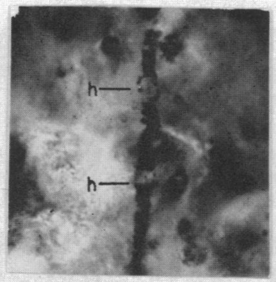

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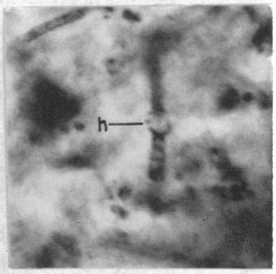

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of heterocysts, akinetes, or alternating heterocysts and akinetes (Figs. 2, 13). Such a change in the size and morphology of cells approximately parallels the taxonomic change of *G. minuta* to *G. grandis* as defined by Barghoorn.[1] The presence in many filaments of long series of larger-than-normal cells suggesting akinetes and heterocysts obviously complicates taxonomic description, particularly if one is dealing with a short filament. We must ask, therefore, whether some or all of the filamentous arrays of larger cells (2.5–4 μ in diameter) of irregular length associated with abundant smaller-diameter *G. minuta* may represent not a separate species (*G. grandis*), but only sequences of reproductive cells of *G. minuta*. Because a high percentage of all the filaments are broken, it would be expected that series of heterocysts and akinetes would commonly become separated from the normal cells, especially since such breaking of filaments at heterocyst boundaries is an important means of vegetative reproduction among living Myxophyceae.[7] At the same time the closely and regularly septate filaments of *Animikia* can hardly be explained as a chain of akinetes or heterocysts and clearly do represent a distinct taxonomic entity morphologically similar to living *Oscillatoria*. The bulbous filaments of Barghoorn's *Archaeorestis* are another problem, not considered here.

Radial Colony.—A group of radiating *Gunflintia*-like filaments (Fig. 10) was observed in one slide. The multiple threads appear to originate in a limited area, then branch out to distances of tens of microns. This group of filaments appears to possess a flat side which may have been a site of attachment. Such radial groupings of filaments, usually attached on one side, are found in the myxophycean family Rivulariaceae, although the filaments of the Gunflint colony do not show the false branching or "whiplike tails" which characterize this family.

Conclusions.—The morphological characteristics presented above, if correctly interpreted, require assignment of the septate *Gunflintia* filaments to the myxophycean order Nostocales. In addition, the presence of probable reproductive cells having the morphology and arrangement of heterocysts and akinetes, as well as the general size and shape of cells and the apparent lack of branching, supports assignment to the living family Nostocaceae. One grouping of filaments observed resembles filamentous colonies of the myxophycean family Rivulariaceae. However, evidence is as yet insufficient to indicate whether this represents an early colonial habit among the Nostocaceae or a taxonomically distinct forerunner of the Rivulariaceae. The difference between clear heterocysts and dark adjacent cells can be interpreted to imply the presence of original pigmentation in the latter—presumably a photosynthetic pigment.

FIG. 10.—Photomicrograph of *Gunflintia*-like filaments interpreted as a radiating colony.

FIG. 11.—*Synechocystis sallensis* Skuja, a living, nonmotile unicelled blue-green alga showing: (*a*) and (*c*) vegetative cell division and (*b*) single cell. From Fritsch, ref. 6, p. 789 (greatly magnified).

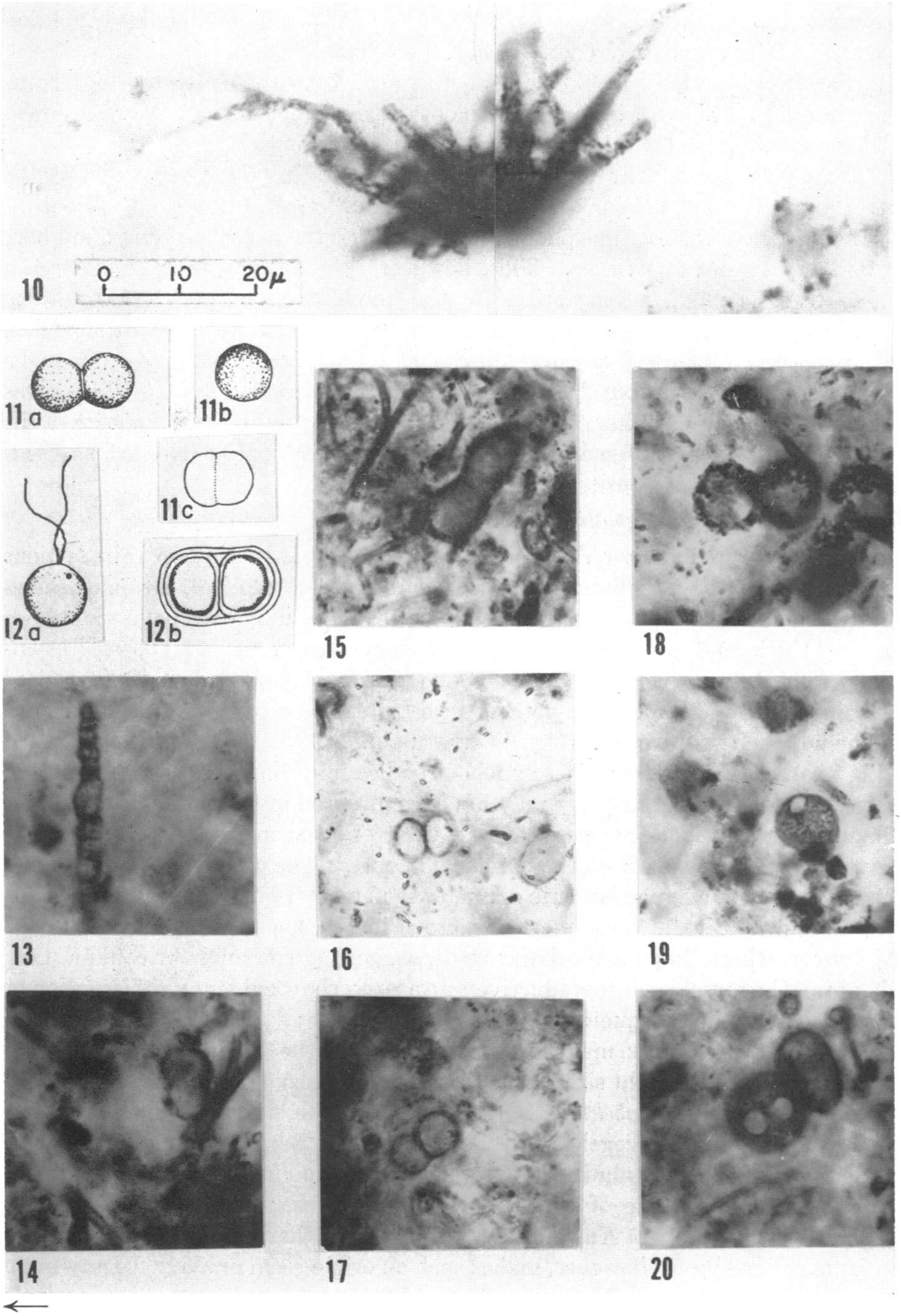
FIG. 12.—*Desmocapsa gelatinosa* Pascher, a simple living dinoflagellate showing: (*a*) swarmer and (*b*) cell division. From Fritsch, ref. 7, p. 671. (×780)

FIG. 13.—Differentiated cells terminating a filament of *Gunflintia grandis* Barghoorn.

FIGS. 14–18.—Photomicrographs of "*Huroniospora*" interpreted as representing stages of vegetative cell division.

FIGS. 19, 20.—Porelike openings of "*Huroniospora.*"

FIGS. 10–20.—Bar scale of 20 μ applies to all illustrations except Figs. 11 and 12. Thin section and finding coordinates: Fig. 10, C71(5), 42.1/105.6; Fig. 13, C71(6), 34.4/95.0; Fig. 14, C71(4), 43.3/112.0; Fig. 15, C71(4), 35.3/107.3; Fig. 16, C71(4), 55.6/103.9; Fig. 17, C71(4), 43.0/112.2; Fig. 18, C71(6), 32.6/104.6; Fig. 19, C71(7), 46.2/103.9; Fig. 20, C71(4) 35.0/107.9.



This new evidence lends additional force to the already expressed conclusion[1-4] that the Gunflint microbiota is dominated by procaryotic green-plant photosynthesizers that are related to living blue-green algae and which presumably produced free oxygen as a byproduct of photosynthesis. Geological evidence, however, implies that such oxygen did not *accumulate* before about 1.8–2 billion years ago, near or slightly younger than the probable age of the Gunflint strata. Rather, it was probably removed as fast as formed by the conversion of ferrous iron in solution to a precipitate of ferric iron oxide responsible for the widespread deposition of banded iron formation at this and older times.[2, 8, 9]

The nonseptate tubular filaments of similar size to *Gunflintia* may be either the result of partial degradation of septate filaments or procaryotic "fungi," or both.

Living genera that are morphologically similar to the organisms described here are mainly fresh-water forms. This, however, does not discriminate between a fresh-water versus a marine origin for the Gunflint microbiota. Many living genera of blue-green algae thrive in either fresh or salt water—morphological conservatism, evolutionary euryhalinity, and ecological plasticity seem to be characteristic of the group.[10] In view of this, the fossils do not of themselves require modification either of current theory about the origin and nature of the early hydrosphere, or of geochemical conclusions that imply their enclosing sediments to be marine deposits.

Globular bodies of problematical affinity

Numerous circular and elliptical microstructures are visible in thin sections of chert from the Gunflint Iron Formation (Fig. 1). Optical sectioning shows that such bodies are ellipsoidal to spheroidal, 1–15 μ in diameter, have walls 0.5 μ or less thick and apparently cellular in some instances, and possess essentially smooth-to-reticulate surfaces. Some also display one or a pair of terminal openings (Figs. 19, 20). The generic term *Huroniospora* was used by Barghoorn to include such bodies of probably diverse origin.

It has been suggested that some such bodies may be fungal spores. It has also been considered that they may have been reproductive bodies of *Gunflintia* filaments, and it is indeed possible that some of them may be heterocysts that have become detached from the parent filaments. The ellipsoidal-to-spheroidal bodies, however, average larger in diameter than most *Gunflintia* (including heterocysts and akinetes), being most numerous in the 4 μ to 8 μ diameter range. Moreover, where they have cellular walls, suggesting sporangia or conidia, they should, if they were such reproductive aggregates, show evidence of disruption or give rise to a greater frequency of colonial growths.

Some of these bodies may well be "spores" in the broad sense. Globular opaque bodies of the right size range (2–8 μ in diameter) are structurally a part of the unusual fossil *Kakabekia*. By analogy with the similar living soil microorganisms isolated by Siegel *et al.*,[11] it appears that Barghoorn and Tyler[1] were probably correct in postulating a reproductive function for the terminal bodies of *Kakabekia*. Thus some of the detached bodies observed may be *Kakabekia* "spores." The rarity of *Kakabekia* in samples from the Schreiber Beach locality so far examined by us, however, makes such an origin seem unlikely for any but a small fraction of the globular bodies observed (unless "spore" generation is erratic or seasonal and some of the numerous minute radiating structures observed are *Kakabekia* without terminal "spores"). The genus *Eosphaera*, with peripheral sporelike bodies,[1] is another possible source of the *Huroniospora* spheroids and ellipsoids. In addition, Cloud and Hagen[3] have observed that some of the smaller reticulate and seemingly encapsulated spheroids strikingly resemble the spores of certain living bacteria. This too presents difficulties. Spores of living bacteria are generally much less than 1 μ in diameter, and the bacteria to which such spores would have given rise remain unidentified or small in number.

Affinity with the planktonic blue-green algae has been suggested for the larger globular bodies,[1, 2] and Cloud[2] suggested that some of them might be colonial, like the living chroococcalean genus *Coelosphaerium*. Barghoorn and Tyler[1] postulated that they might be dinoflagellates.

Many of the noncellular globular bodies possess equatorial constrictions which suggest an intermediate stage in cell division (Figs. 14 and 15). Other bodies show other possible stages of cell disjunction (Figs. 16–18). Although fortuitous juncture of pairs of such globular bodies can be visualized, it seems unlikely that such bodies would produce configurations of the regularity and frequency observed. On the other hand, nothing suggesting nuclear material is observed among either the numerous single bodies or the dividing sequences, which thus presumably represent vegetative division. In size, shape, surface reticulation, and discrete nature, such bodies resemble the myxophycean family Chroococcaceae and the range of form and solitary to colonial habit found in the genera *Synechocystis* (Fig. 11) and *Coelosphaerium.*

The presence of a single, round, terminal aperture or pair of such apertures in some of the globular bodies (also reported by Cloud and Hagen[3]) adds another variable (Figs. 19, 20). Heterocysts may have pores at one or both ends, and it is conceivable that the aperturate bodies are detached heterocysts of larger algal filaments. If they were *Kakabekia* spores, the apertures might also mark the sites from which the stalk of the *Kakabekia* "umbrella" became detached. Otherwise, such aperturate bodies are inconsistent with analogy to the modern blue-green algae or bacteria. Such openings, in more advanced forms, might provide exits for flagella, or apertures through which plant protoplast swarmers might have escaped. They are also found in some of the less specialized dinoflagellates, such as the family Desmomonadaceae (Fig. 12), in keeping with the above-mentioned postulation of Barghoorn and Tyler.[1] Genera of this family may also possess not only flagellate motile cells, but also spherical cysts and flagellated swarmers, all of about the same size.

Considering the above, it can only be said that the globular bodies are almost certainly a heterogeneous mix, and that their affinities are as yet uncertain. Nevertheless, there are reasons for considering all of them to represent procaryotic forms and, as yet, no compelling evidence to the contrary.

Conclusions

Examination of new material from chert in the Gunflint Iron Formation reveals a variety of subordinate microstructures significant for the nature and taxonomy of the larger structures with which associated.

Filaments of the genus *Gunflintia* bear differentiated cells analogous to the heterocysts and akinetes that function in vegetative reproduction among living nostocalean blue-green algae of the family Nostocaceae. This striking morphological convergence implies affinity between *Gunflintia* and the Nostocaceae. Although such affinity does not require identity of physiological or biochemical functioning, it is sufficient to imply, in addition, that the origin of oxygen-releasing green-plant photosynthesis had already happened 2 billion years ago.[2, 8]

Abrupt change in diameter along the filaments of *Gunflintia,* implying transition from sequences of normal cells to chains of heterocysts and akinetes, also presents problems of classification, suggesting possible synonymy between *G. minuta* and *G. grandis.*

The existence of probable stages of vegetative cell division among globular bodies assignable to the form-genus "*Huroniospora*" indicates that this taxon, as presently conceived, includes more than "spores" of other microorganisms. Clearly the group is composite and needs intensive analysis of fine structures. Evidence as to whether the presumably algal forms belong exclusively to the procaryotic Myxophyceae or include representatives of more advanced groups such as the dinoflagellates is as yet inconclusive. If some of these bodies should turn out to represent dinoflagellates they would be the oldest yet recorded eucaryotes (comprised of mitosing cells). However, although they occur near the point in geologic time where Cloud[8] has postulated conditions marginally suitable for the emergence of the eucaryotic cell, this is too important a development in evolution to be dated on such evidence. More definitive structures and other explanations must be sought.

* The material on which this article is based was obtained while Cloud was working with the support of National Science Foundation grant GP-1804. Laboratory studies and preparation of the report were supported by the University of California, Los Angeles.

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