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21 May 1975; revised 23 July 1975

Precambrian Eukaryotic Organisms: A Reassessment of the Evidence

Abstract. *Comparison of partially degraded unialgal cultures of* Chroococcus turgidus *with coccoid microfossils from the Late Precambrian Bitter Springs formation, Australia, suggests that the Precambrian fossil record has been seriously misinterpreted. Use of degradational features as taxonomic characters has resulted in unrealistically high estimates of Precambrian algal diversity. There is at present no compelling evidence for the presence of eukaryotic microfossils in rocks from the Bitter Springs formation or any older sedimentary sequences.*

A decade ago one of us (*1*) suggested that certain coccoid microfossils occurring in profusion in the then recently discovered cherts of the Late Precambrian Bitter Springs formation, central Australia, represented the earliest evidence of eukaryotic organisms in the evolutionary record of life. These fossil algae, which were found either as isolated cells or as loose aggregates of few to many individuals, were sometimes devoid of cellular contents, but often possessed an internal discrete structure excentrically or peripherally located with respect to the delimiting "wall." Cytoplasmic remnants (interpreted as inner cell walls) surrounded the internal body in certain cells. The well-defined internal structures were interpreted as nuclear or organellar bodies (*2–4*), and a series of supposedly discrete taxa of both prokaryotic and eukaryotic algae was erected on the assumption that the presence or absence of these blebs and coagulated protoplasmic remnants constituted valid taxonomic characters (*2, 3*). This interpretation was seriously questioned by Awramik *et al.* (*5*), who emphasized that this taxonomy failed to take into account the variability of internal structure caused by partial degradation of blue-green algae.

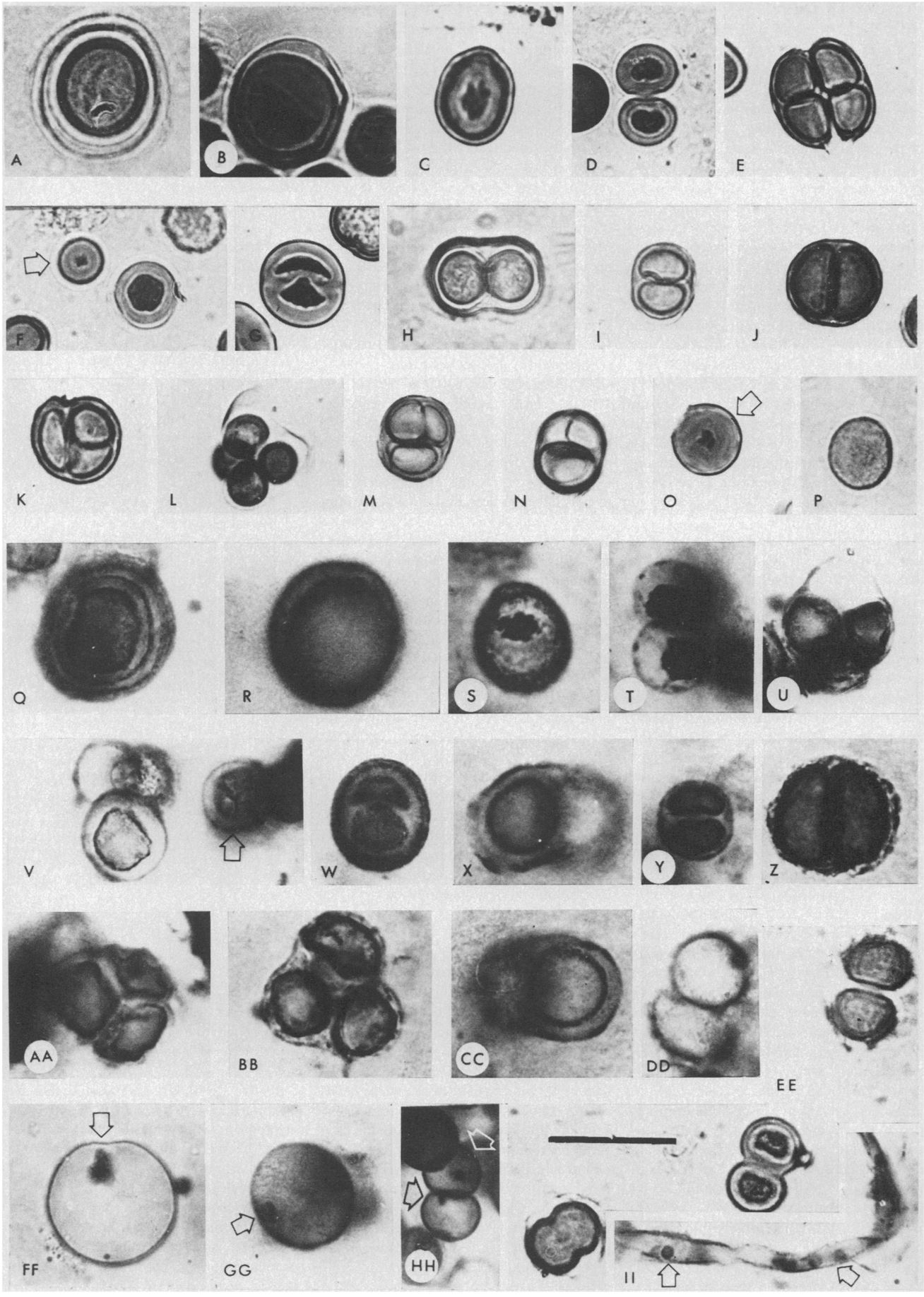
Because of the importance of proper interpretation of Precambrian fossils, we undertook a new series of degradation experiments and found that the entire range of morphologic variation exhibited by a dozen taxa of coccoid algae (supposedly both prokaryotic and eukaryotic) from the Ellery Creek locality of the Bitter Springs formation could be duplicated in detail by partially degrading a unialgal culture of the chroococcalean species *Chroococcus turgidus* (Kütz.) Nägeli. Our results suggest that the Precambrian fossil record has been seriously misread. The three-dimensional morphology of microfossils is generally not preserved by the cell wall as has been previously assumed, but rather by one or more mucoidal sheaths (*6*). The putative organellar remnants are in reality degraded protoplasm representing the entire contents of the cell. This fact, coupled with an appreciation of the morphologic variability within living chroococcalean species, indicates that there is no compelling evidence for the presence of eukaryotes in the Bitter Springs formation or any older rocks. It also demonstrates that published estimates of the taxonomic diversity of the Bitter Springs and other Precambrian floras are excessive.

The experimental procedure is simple. Unialgal cultures of cyanophytes were grown at room temperature in reduced natural light on mineral agar slants prepared with Bold's basal medium (*7*). As the cultures became stale, smears were prepared and stained with a dilute aqueous solution of safranin to enhance observation and photomicrography. A *Chroococcus turgidus* culture was selected for detailed examination because of its almost uncanny resemblance to described Bitter Springs fossils; however, other chroococcalean cultures exhibited a similar pattern of variation and degradation. This pattern has also been observed in algal mats growing in Massachusetts, Australia, and the Persian Gulf by Golubic and his colleagues (*5, 6*).

Figure 1, A to P, illustrates the range of morphologies which result from the partial degradation of a naturally morphologically varied population of blue-green algal cells. Diameters of individual cells of *Chroococcus turgidus* range from 6 to 20 μm or more; the average diameter is approximately 10 to 12 μm. The number of cells per packet varies from one to four, although unicells and dyads are by far the most common. The shape of the cells forming dyads is variable, ranging from spherical, gibbous, or hemispherical to lunate (Fig. 1, A to H). The number of sheaths per cell also varies, as does the degree of protoplast degradation (*6*). Particularly important is the observation first made by Awramik *et al.* (*5*) that decomposition leaves the sheath intact, but condenses the protoplast into a globular remnant identical in every respect to the "nuclei" and "organelles" observed in Precambrian fossils (Fig. 1O). The final product of this process is an empty sheath (Fig. 1P). In spite of the wide range of variation of the organisms pictured here, all represent a single species from a pure culture.

Comparison of the *C. turgidus* culture with coccoid fossils from the Ellery Creek locality of the Bitter Springs formation (Fig. 1, Q, DD, and GG) demonstrates a remarkable similarity between the two populations, which are separated in time by almost a billion years. It is no exaggeration to state that the Bitter Springs assemblage could easily represent the remains of a single species of *Chroococcus*, a species having the same range of variability as *C. turgidus*. The Bitter Springs organisms in question have been assigned to the following taxa: *Globophycus rugosum, Bigeminococcus lamellosus, B. mucidus, Eozygion grande, E. minutum, Myxoccoides reticulata, Glenobotrydion aenigmatis, G. majorinum, Gloeodinopsis lamellosa, Caryosphaeroides pristina, C. tetras*, and *Eotetrahedron princeps*. The last six taxa have been described as eukaryotes. All of the Bitter Springs taxa listed above can be found in three petrographic thin sections

Fig. 1. All photomicrographs × 1100; the bar in EE equals 20 μm. (A–P, EE). *Chroococcus turgidus* (Kütz.) Nägeli, demonstrating the variation in morphology and degree of degradation observable in a single unialgal culture. Arrows in (F) and (O) refer to pseudonuclear protoplasmic remnants within undegraded sheaths. The three groups of blue-green algae in (EE) duplicate in detail the putative mitotic sequence suggested for coccoid microfossils from the Bitter Springs formation. (M and N) Two views of a single tetrad. (Q–DD, GG) Microfossils found in slides TBS-22-1A, 1B, and 1C from the Ellery Creek locality of the Bitter Springs formation, the taxonomy according to Schopf and Blacic (*3*); (Q) *Gloeodinopsis lamellosa* Schopf; (R) *Globophycus rugosum* Schopf; (S and T) unnamed morphologic entities; (U) *Bigeminococcus mucidus* Schopf and Blacic (?); (V) *Eotetrahedron princeps* Schopf and Blacic. Arrow points to fold in degraded protoplasm which in this plane simulates a trilete scar; (W and Y) *Eozygion minutum* Schopf and Blacic; (X, Z, and CC) *E. grande* Schopf and Blacic. (X) and (CC) show two views of the same organism; (AA) unnamed triad; (BB) *Eotetrahedron princeps* Schopf and Blacic; (DD) *Myxococcoides reticulata* Schopf; (GG) *Glenobotrydion aenigmatis* Schopf; note pseudonucleus. (FF) Coccoid alga from the Gunflint formation, Ontario, exhibiting a pseudonucleus. (HH) *G. aenigmatis* Schopf from the Ross River locality of the Bitter Springs formation, showing prominent pseudonuclei. (II) Pseudonuclei within a blue-green algal sheath in chert from the Ross River locality of the Bitter Springs formation.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cut serially from a single piece of rock. Thus, it is not unlikely that they constitute a single population of a single species of blue-green algae.

Modern algal mat communities usually contain from one or two to more than a dozen species of blue-green algae, of which only a few may be coccoid. Forty-five algal species have been described from Bitter Springs mats, including 18 spheroidal taxa. One can infer from Fig. 1 that the apparent discrepancy in diversity between the ancient and modern mats is due primarily to taxonomic treatment rather than to major differences in cyanophytic community structure between the Late Precambrian and the present. Precambrian fossils are similar to those of the Phanerozoic in that they must be treated as populations rather than as individuals. Similarly, they resemble younger fossils in that they are not preserved in perfect cytological detail. All Precambrian microfossils were probably subject to degradation prior to silicification, and it is essential to discuss them in this light.

Of greater significance to students of evolution is the bearing of this study on the origin of the nucleated cell. Because the rise of the eukaryotic cell from its prokaryotic ancestors was the single greatest quantum step in evolutionary history, it is important to attempt to ascertain when this transition occurred. Although the Bitter Springs fossils were unknown only a decade ago, it has become the conventional wisdom to assume that they include the earliest assured eukaryotic remains. This view has become so entrenched that it may seem heterodox to challenge it, but the fact is that there is simply no compelling evidence for the presence of nucleated cells in the Bitter Springs formation or in any other rocks which significantly antedate sequences containing Ediacaran assemblages. Pseudonuclei can be found in fossils from many Precambrian localities (*4*), some of which are nearly 2 billion years old (*8*) (see Fig. 1FF). The oldest such structures to be expressly considered as evidence of eukaryotic life are found in the 1300-million-year-old Beck Springs Dolomite of southern California (*9*); however, degradation studies of modern cyanophytes are applicable to these older microbiotas, as well as to the Bitter Springs flora. All are most reasonably interpreted as blebs of degraded protoplasm within undecomposed sheaths. The presence of pseudonuclei within filamentous cyanophytic sheaths of some Bitter Springs fossils (Fig. 1II) corroborates this view. If one infers that these blebs are in fact genuine nuclear remnants, the problem arises as to why the cytoplasm has disappeared completely, while molecularly similar nuclear material persists. Such a sequence of protoplasmic degradation is illogical. Figure 1EE shows a single field of *C. turgidus* cells which fortuitously presents all stages in classical cyanophytic cell division. This sequence is identical to that which has been hypothesized to be a mitotic sequence (*2*). Again, the assumption that the internal bodies present in the fossils chosen to illustrate petrified stages of mitosis are nuclear remnants leads to problems, for if these pseudonuclei are indeed organellar, the question arises why cytokinesis has preceded mitosis (*10*). If the pseudonuclei are interpreted as protoplasmic remnants, the fossil sequence can be considered to illustrate simple cyanophytic fission.

The tetrahedral structure shown in Fig. 1BB has been hypothesized to represent the product of meiosis, but in fact it is a member of a large cell population which conforms in every way to a population of *C. turgidus*. Although true cyanophytic tetrads have not been described in the literature, "pseudotetrads" caused by two successive cell divisions in different planes or cell slippage within a single sheath are common among blue-green algae (*11*). The "trilete mark" pictured in Fig. 1V is the product of a fortuitous relationship of folded protoplasm internal to the sheath. It does not occur on the outer cell surface where one would necessarily find a true trilete scar.

In short, there is no good evidence for the presence of eukaryotes in Bitter Springs cherts. Similarly, all reports of older eukaryotes do not withstand critical examination. It would be hazardous for us to state that eukaryotes did not exist 900 million years ago, but if they did, their remains have yet to be found. Alternatively, multicellularity as evidenced by the several known Ediacaran faunas may have evolved quite rapidly following the origin of the nucleated cell. That is, eukaryotic cells may not have existed until very near the end of the Precambrian. Only further paleontological investigation of Precambrian rocks can elucidate this problem.

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21 May 1975

Freeze-Etching Nomenclature

Freeze etching (*1*) is now widely used as a preparatory technique for electron microscopy of biological materials. Observation of many freeze-etched prokaryotic and eukaryotic specimens have provided extensive views of their membranes. Because membranes are split during the fracture process used in freeze etching (*2*), two new fracture faces from the hydrophobic interior of the membrane are seen in addition to the true surfaces that can be exposed by etching alone. We wish to propose a simple, uniform nomenclature to describe and label these fracture faces and surfaces.

The recent publication of a symposium on freeze etching (*3*) shows the bewildering assortment of nomenclatures in current use:

1) The A, B, C, and D designations, which are hard to remember and are used in differing ways by different workers.

2) "Convex" and "concave" designations, which are useless for all but the simplest vesicle systems.

3) Descriptive designations (inner fracture face, outer fracture face, outer surface, inner surface), which are confusing, particularly when applied to complex tissues or membrane infoldings.