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ABSTRACT

Of various problems that have hindered progress in documenting the Precambrian history of life, the difficulty in distinguishing between bona fide microbial fossils and nonbiological microscopic pseudofossils has been among the most serious. Though errors in the interpretation of putative Precambrian fossil microbes have diminished greatly over recent years, mistakes continue to be made. We suggest that such errors can be avoided by the use of a multifaceted strategy based on a specified series of biologically definitive characteristics that document the presence of interrelated biological morphology and biologically derived chemistry. To illustrate this promising approach, we use optical microscopy, confocal laser scanning microscopy, and Raman spectroscopy, together, to distinguish between authentic microbial fossils and microscopic "look-alikes," both coccoidal and filamentous, rock-embedded in five Proterozoic and two Archean geological units: bona fide fossils of the ~

objects that we regard to be pseudofossils, analyzed in petrographic thin sections of seven Proterozoic and Archean geological units. As these studies show, the combined use of three analytical techniques (optical microscopy, confocal laser scanning microscopy, and Raman spectroscopy) seems to provide a firm basis by which to document the origin of such objects, a promising approach to the problem of biogencity that could also prove valuable in future studies of rocks acquired from Mars and other extraterrestrial bodies. To provide a context for these studies, we first summarize the characteristics of biological morphology and biologically derived chemistry to be expected of bona fide rock-embedded fossil microbes.

2. C[illegible] *bona fide* [illegible]

As has been suggested in earlier publications (Schopf and Walter, 1983; Schopf, 1992a, 1993, 1999a; Buick, 2001), authentic fossil microbes should be expected to satisfy five criteria: their host rock should be of known provenance and age; they should be indigenous to and syngenetic with the formation of the rock in which they occur; and they should be assuredly biogenic. This last criterion, almost always the most difficult to satisfy, is the focus of this article.

2.1. Biological morphology

Precambrian microbiotas are composed predominantly of morphologically simple, micron-sized, prokaryotic microorganisms, almost entirely either coccoidal (whether unicellular or colonial) or filamentous (whether cellular, like bacterial trichomes, or tubular, like the extracellular sheaths that enclose such trichomes). Table 1 summarizes the principal characteristics of such rock-embedded fossils that we suggest can be used to distinguish them from morphologically similar microscopic pseudofossils.

2.1.1. Coccoidal microfossils

Unlike minerals, the cells of prokaryotic microorganisms, composed of pliable organic matter, do not exhibit crystal-like sharp-cornered angularity. Nevertheless, because microscopic rounded mineral grains (and spheroidal fluid inclusions) can mimic the shape and size of fossilized coccoidal prokaryotes, we suggest that criteria in addition to simple spheroidal shape and minute size should be used to identify such fossils. Foremost among such criteria, listed Table 1, are those based on the genetics that determine the morphology and range of cell-sizes of such microbes (for modern species, a well-defined region of morphospace).

2.1.2. Filamentous microfossils

Suites of nonbiological mineral or carbonaceous structures that mimic convincingly the morphological characteristics of a diverse assemblage of threadlike prokaryotic fossils are unknown. Microbial filaments are cylindrical and commonly sinuous, not planar,

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Characteristics expected of bona fide fossil microbes.

Coccoidal prokaryotic fossils:
(1) should be spheroidal (or, if distorted, originally spheroidal), their shape defined by a distinct carbonaceous cell wall, such cells either being devoid of internal contents (i.e., "hollow," being mineral-infilled in permineralized specimens), their cell protoplasm having been leached away during diagenesis, or containing globular carbonaceous bodies, condensed protoplasmic remnants
(2) should have a unimodal size distribution and a species-specific limited range of cell diameters (viz., a plausibly biologic Divisional Dispersion Index; Schopf, 1976, 1992c), a pattern of size distribution and range of vegetative cell-size consistent with those of morphologically similar modern microbes and fossil taxa (for most species, having diameters <20 μm; Schopf, 1992b, Table 5.4.6)
(3) may include specimens that evidence stages of a life-cycle (ontogenetic) sequence including "lima bean-shaped" pairs in which the flat sides of adjacent cells evidence their derivation by the binary division of a parent cell
(4) if unicellular and planktonic, should occur as isolated specimens scattered across a substrate
(5) if colonial, should occur in close-packed groups of cells, commonly embedded in a carbonaceous (originally mucilaginous) envelope or diffuse matrix, an

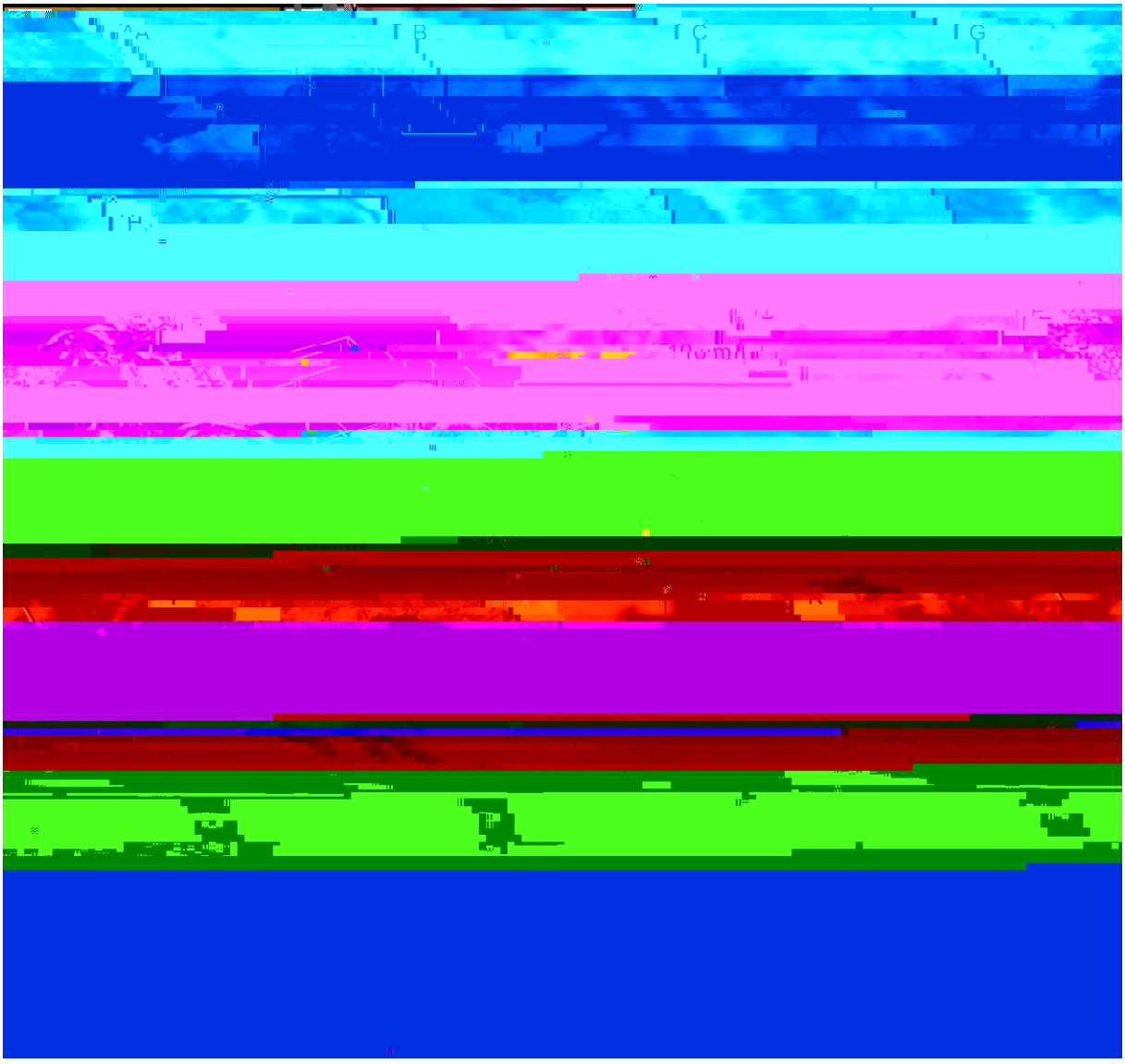

Fig. 1. Optical photomicrographs (A, D, G, I, J, L, M, N and O), Raman kerogen images superimposed on optical images (B, C, H, K and P), and confocal laser scanning micrographs (E and F) of bona fide microfossils in petrographic thin sections of cherts from the ~800 Ma Bitter Springs Formation of central Australia (A through H) and the ~3050 Ma Farrel Quartzite of northwestern Australia (I through P). Raman images, shown in blue, were acquired in a spectral window centered on the ~1605 cm^{-1} kerogen band (Fig. 5A). (A–F) Bitter Springs Colony A (composed of 63 cells); the red rectangles in (A) denote, from left to right, the Raman-imaged areas shown, respectively, in (B) and (C), and the

Fig. 4. Size distributions of bona fide microfossils measured in petrographic thin sections of cherts from the ~800 Ma Bitter Springs Formation of central Australia

prokaryotic microbes (259 species) and eukaryotic microalgae (234 species), provides a simple metric by which to relate the smallest to the largest cells of such populations (Schopf, 1976). Shown for populations both of living and fossil coccoidal microorganisms to span a characteristic range of values – clustering between 2 and 4 and having an average value of 3.3 (Schopf, 1976, 1992c) – such DDI values are one of many factors used here to assess the biological origin of putative coccoidal microfossils.

As with all measures of variability within populations, DDI values are subject to the limitations imposed by small sample size, for especially small groupings (e.g., those composed of fewer than 20 individuals, such as three of the colonies of bona fide fossils and one of the assemblages of coccoidal pseudofossils analyzed here; Fig. 4B–E), yielding values that are typically lower than those exhibited by larger populations. Such limitations are less applicable to the other four populations analyzed, each consisting of 50 or more individuals (Fig. 4A and F–H), and can be partially offset by comparing the DDIs of populations that contain comparable numbers of individuals – in the present study, microbial colonies composed of nine cells (DDI = 1; Fig. 4D), 15 cells (DDI = 2; Fig. 4C), and 17 cells (DDI = 2; Fig. 4B), compared with a "colony-like" group of 17 hematite pseudofossils (DDI = 4; Fig. 4E); and a microbial colony composed of 63 cells (DDI = 2; Fig. 4A) compared with three populations of 50 pseudofossils, having DDIs of 6, 8 and 8 (Fig. 4G, F and H, respectively), randomly selected for measurement from each of three large assemblages.

4.1.1. Bona fide coccoidal fossils

Shown in Fig. 1 are optical, CLSM, and kerogen Raman-overlay images of coccoidal microbe-like objects permineralized in cherts of the ~800 Ma Bitter Springs Formation of central Australia (Fig. 1A–H) and the 3050 Ma Farrel Quartzite of northwestern Australia (Fig. 1I–M). These objects are bona fide fossil microbes. Consistent with the criteria enumerated in Table 1:

- they are spheroidal or were originally spheroidal (Fig. 1D–G, L, O), their shape defined by a distinct carbonaceous cell wall (Fig. 1B, C, H, K P);
- they are three-dimensional and "hollow" (i.e., quartz-infilled) – shown especially well by the CLSM images in Fig. 1E and F – in some specimens containing bits of included organic matter (e.g., Fig. 1G, O, L, M);
- they have a unimodal limited size distribution – for the Bitter Springs cells ranging from ~7 to ~10 μm, a DDI of 2 ("Colony A": Figs. 1A–F and 4A) and ~10.5 to ~13.5 μm, a DDI of 2 ("Colony B:" Figs. 1G, H, and 4B); and for the Farrel Quartzite cells, from ~11.5 to ~13.5 μm, a DDI of 1 ("Colony C": Figs. 1I–M and 4C) and ~7 to ~10.5

4.1.2. Coccoidal pseudofossils

In Fig. 2 are shown optical and Raman-overlay images of coccoidal microscopic fossil-like objects in petrographic thin sections of a siltstone from the ~1020 Ma Lakhanda Formation of southeastern Siberia (Fig. 2

- due to its original flexibility, it is gently curved, its cylindrical shape being defined by distinct lateral walls;
- it has an essentially uniform diameter throughout its length and is partitioned by transverse septa into a uniseriate sequence of box-like cells of more or less uniform shape and size;
- some cells are subdivided by thin transverse partial septations (Fig. 3

unknown in nature, were they to be formed (for example, in silica gels in hypersaline environments or in silica-barite veins) and then preserved in the rock record, they would be distinguishable from microfossils by their lack of cellular structure.

To avoid mistakes in the interpretation of Precambrian microbe-like objects, we suggest the use of an interdisciplinary strategy that, based on a specified series of biologically definitive characteristics, can document the presence of interrelated biological morphology and biologically derived chemistry. As shown here, the combined use of optical microscopy, confocal laser scanning microscopy, and Raman spectroscopy represents one approach to address this problem, as would the use of comparable combinations of other analytical techniques. The effectiveness of this approach is illustrated by the results of comparative studies of coccoidal and filamentous bona fide microbial fossils and microscopic "look-alikes" rock-embedded in seven Precambrian units: the Proterozoic Chanda Limestone of southern India (~770 Ma), Myrtle Springs Formation of South Australia (~800 Ma), Bitter Springs Formation of central Australia (~800 Ma), Lakhanda Formation of southeastern Siberia (~1025 Ma), and Vempalle Formation of central India (~1700 Ma); and the Archean Marra Mamba Iron Formation of northwestern Australia (~2629 Ma) and Farrel Quartzite of northwestern Australia (~3050 Ma).

Colony-like groups of small (~10-μm-diameter) coccoids in cherts of the Bitter Springs Formation and Farrel Quartzite, and a narrow (~5-μm-wide) segmented filament of the Bitter Springs Formation exhibit all of the characteristics of biological morphology expected of bona fide permineralized microfossils. Thus, for example, they are composed of "hollow" (quartz-infilled) three-dimensional cells defined by distinct carbonaceous cell walls; their organismal morphology, cell-sizes, and cell-size distributions are

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