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Precambrian microbe-like pseudofossils: A promising solution to the problem

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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 ~800 Ma Bitter Springs Formation of central Australia and ~3050 Ma Farrel Quartzite of northwestern Australia; and objects we regard to be pseudofossils from the ~770 Ma Chanda Limestone of southern India, ~800 Ma Myrtle Springs Formation of South Australia, ~1020 Ma Lakhanda Formation of southeastern Siberia, ~1700 Ma Vempalle Formation of central India, and ~2629 Ma Marra Mamba Iron Formation of northwestern Australia. The results demonstrate that no single criterion, by itself, is sufficient to establish the biological origin of such objects. Instead, as shown here, this problem appears solvable by the use of an interdisciplinary approach that combines the data and techniques of geology, biology, and chemistry.

1. Precambrian microbe-like pseudofossils

1.1. Introduction

The Precambrian fossil record, encompassing some 85% of the documented history of life, is dominated by diverse types of coccoidal and filamentous microscopic prokaryotes. Because such microbes are minute, incompletely preserved in geological materials, and exhibit simple morphologies that can be mimicked both by microscopic contaminants and nonbiologic mineral microstructures, discrimination between *bona fide* microbial fossils and microscopic pseudofossils can be difficult. As a result, non-fossil microscopic objects have been misinterpreted over the years as "Precambrian microfossils" (for extensive tabulations, see Hofmann and Schopf, 1983; Schopf and Walter, 1983; Mendelson and Schopf, 1992). As illustrated in this study that compares authentic Precambrian fossil microbes with nonbiologic "look-alikes," the combined use of optical microscopy, confocal laser scanning microscopy, and Raman spectroscopy appears to provide a solution to this problem. Although we focus here only on prokaryotic microorganisms, the most abundant and morphologically simplest fossils of the Precambrian and the most likely to be confused with nonbiologic artifacts, these techniques are also applicable to analyses of morphologically more complicated Precambrian organic-walled fossils such as acritarchs.

1.2. Nature and history of the problem

In the 1960s and 1970s, when active studies of the Precambrian fossil record were in their beginning stages (Cloud, 1983; Schopf, 2009a), most mistakes in the interpretation of putative fossil microbes derived from studies of contamination-prone acid-macerated residues of ancient rocks, palynological preparations from which were reported such "fossils" as ball-

Abbreviations: CLSM, confocal laser scanning microscopy; DDI, divisional dispersion index; Ma, million years; PAH, polycyclic aromatic hydrocarbon; RIP, Raman index of preservation; SIMS, secondary ion mass spectrometry.

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shaped dust particles, lint fibers and strands of hair, shreds of resin abraded off rods used to stir the acid-rock sludge, airborne pollen and spores, and water-borne fungi and microalgae. In subsequent years, spurred by the first reports of authentic organic-walled microfossils permineralized in Proterozoic cherts (Barghoorn and Schopf, 1965; Barghoorn and Tyler, 1965; Cloud, 1965; Schopf, 1968), work refocused on studies of petrographic thin sections, preparations in which the detected fossil-like objects can be demonstrated to be rock-embedded. Yet this work, too, has been subject to error, primarily by misinterpretation of "life-like" microscopic mineral grains and other nonbiologic artifacts.

As studies of the Precambrian microbial fossil record have matured over the past half-century, such errors have become much less common. This is particularly so for the Proterozoic (2500 to ~542 Ma) record, known from hundreds of fossiliferous stromatolitic and fine-grained clastic units in which fossilized microbes are abundant, often well-preserved, and commonly so similar in morphology to modern prokaryotic taxa that both their biogenicity and biological affinities can be readily established (e.g., Mendelson and Schopf, 1992; Schopf, 1994, 2009b). However, the record of older, Archean (>2500 Ma), microbes is not nearly as well-documented. Because of geological recycling, the rock record that has survived to the present rapidly diminishes with increasing geological age, a petering-out that markedly limits the Archean terrains available for study. And not only is the Archean rock record sparse and patchy – estimated to represent only ~5% of strata that have survived to the present (Garrels and Mackenzie, 1971) – but a large proportion of this surviving rock mass has been subject to fossil-obliterating metamorphism. As a result, the Archean fossil record pales in comparison with that of the Proterozoic, being composed of microbial stromatolites recorded in about 50 geological formations, and *bona fide* microbial fossils, comprising six broad bacterium-like morphotypes, known from some 40 units (Hofmann, 2000; Schopf, 2006a,b).

Driven largely by the evident differences, both in quantity and quality, between the Proterozoic and Archean fossil records as now known, the interpretation of putative evidence of Archean life has been subject to considerable controversy (e.g., Buick et al., 1981; Awramik et al., 1983, 1988; Buick, 1984, 1988, 1990, 2001; Mozjsis et al., 1996; Altermann, 2001; Brasier et al., 2002; van Zuilen et al., 2002; Altermann and Kazmierczak, 2003; Lepland et al., 2005; Schopf, 2004, 2006a; Summons et al., 2006; Waldbauer et al., 2009). Indeed, doubt has been raised about the biologic origin of all claimed evidence of life older than even the mid-Paleoproterozoic (Moorbath, 2005).

Such skepticism is unwarranted. Not only are scores of stromatolitic and microfossiliferous deposits known from the Archean but, notably, uncertainties about the two most significant questioned claims of early life have been laid to rest: (1) the biological origin of putative fossil microbes from the Paleoarchean (~3465 Ma) Apex chert of northwestern Australia (Schopf, 1992a, 1993) – queried by Brasier et al. (2002) – has been confirmed by Raman spectroscopy and 2-D and 3-D Raman imagery (Schopf et al., 2002, 2007), techniques that establish the biologic cellularity and kerogenous composition of the Apex filaments, and by secondary ion mass spectrometry ("SIMS"), synchrotron-based scanning-transmission X-ray microscopy, and X-ray absorption near-edge spectroscopy, analyses that document the biologically derived elemental composition and functional-group chemistry of the Apex kerogen (De Gregorio et al., 2009); and (2) ^{13}C-enriched graphite particles reported to be included in quartz-hosted apatite grains of Eoarchean (~3830 Ma) Akilia supracrustal rocks of southwest Greenland and regarded by some to be of biological origin (Mozjsis et al., 1996) – the existence of which had been challenged by Lepland et al. (2005) – have been shown to be present by Raman spectroscopy, 3-D Raman imagery, and SIMS (McKeegan et al., 2007).

These recent results, backed by the stromatolites, microfossils, and evidence of biological carbon isotopic fractionation known from a large number of other Archean deposits (Strauss and Moore, 1992; Hofmann, 2000; Schopf, 2004, 2006a,b; Allwood et al., 2006, 2007; Sugitani et al., 2007, 2009; Van Kranendonk et al., 2008; Oehler et al., 2009; Sugahara et al., 2009; Waldbauer et al., 2009), show that there is a continuous record of flourishing microbial life that extends to ~3500 Ma.

1.3. Source of the problem

As demonstrated by the studies of the Apex filaments outlined above, current techniques are fully capable of establishing the biogenicity of ancient fossil microbes. Nevertheless, and though errors in the interpretation of claimed "Precambrian microfossils" are now much less common than in the 1960s and 1970s, mistakes continue to be made. What is the source of this problem and why has it persisted?

This problem does not exist for Phanerozoic plants and animals: fossil logs, leaves and seeds, and trilobites, clams and brachiopods and the like are sufficiently morphologically complicated that they are not likely to be confused with similarly shaped pieces of rock (although in the early 1900s, errors in the interpretation of Precambrian megascopic "fossils," regarded usually as remnants of invertebrate animals, were not uncommon; Raymond, 1935). Like modern studies of Phanerozoic fossils, those of Precambrian organic-walled acritarchs have been largely immune from this problem, chiefly because such fossil eukaryotes are large, relative to microbes, and commonly are thick-walled and have rather complex morphologies (e.g., exhibiting spiny processes and other suficial ornamentation). Yet for a vast segment of the Precambrian, where the fossil record is composed solely of morphologically simple microorganisms – for example, tiny coccoidal fossil microbes that can be mimicked by minute ball-shaped mineral grains – misinterpretation of fossil-like objects has long hindered progress.

Because of the microscopic size, simple organismal form, and, especially, the diverse physiological characteristics of Precambrian fossil microbes – microorganisms that not only dominated Earth's biota for much of Precambrian time but that originated all of basic metabolic biochemistry on which the modern ecosystem is founded – their study has come to involve a rather broader spectrum of evidence than that typical of morphology- and paleoecology-based Phanerozoic paleontology. In particular, the use of carbon isotopes to track the records of photosynthesis (Park and Epstein, 1963; Hoering, 1967) and methanogenesis (e.g., Hayes, 1983) over geological time, and of sulfur isotopes to document the presence of ancient sulfate reducing bacteria (e.g., Thode et al., 1962; Thode and Monster, 1965; Schidlowski et al., 1983), were pioneered by analyses of Precambrian rocks.

Thus, studies of the Precambrian microbial fossil record have evolved to require a merging not just of geology (e.g., mineralogy, petrology, sedimentology, paleontology) and biology (e.g., microbiology, phycology, ecology, evolutionary biology), but, in addition, of biological and biologically derived chemistry (e.g., organic chemistry, biochemistry, and organic and isotopic geochemistry). These requirements, coupled with the often less than optimal interaction between practitioners of the Physical and the Life Sciences at most universities and research institutions, suggest that the misinterpretation of putative Precambrian microfossils, and the explanation for the persistence of this problem, may be rooted in the differing educational backgrounds of geologists and biologists, the two groups of scientists who pursue such studies.

Paleontologically trained geologists, those most likely to search for Precambrian microbial fossils, commonly have had limited

expertise in the biology of prokaryotic microorganisms and/or organic geochemistry. As a result, prime evidence needed to establish the biogenicity of fossil microbe-like objects – their biological morphology (the presence of cells and cell lumina, the distribution of cell-sizes in species-populations, evidence of cell division, etc.) and their biologically derived carbonaceous chemistry (the organic, kerogenous, composition expected of microbial fossils) – has not always been adequately assessed.

Although the deficiencies are different for microbiologists and phycologists, the biologically educated scientists most commonly called-on to evaluate such finds, they are no less detrimental. Having expertise in the study of living microbes and microalgae, most such workers have had no training in petrology, mineralogy, or the study of petrographic thin sections, and have had little or no experience in the interpretation of the degraded fossilized cellular remains of microorganisms. Unacquainted with what can plausibly be expected of the fossil record, such biologists have on occasion been misled by "fossil-like" mineral artifacts.

Finally, workers in the field, whether trained as geologists or biologists, have not always recognized that the use of plane-polarized optics (those of a petrographic microscope) can help to identify common modern contaminants (e.g., lint fibers, pollen, and microalgae), the cellulosic components of which are birefringent (in contrast to the non-birefringent, molecularly disordered, organic geopolymers of authentic fossils). And still others may not have appreciated the usefulness of (or may not have had an opportunity to document their finds by) publishing full-color photomicrographs of putative organic-walled fossil microbes, a form of presentation important not only because the color of such fossils evidences their fidelity of preservation (viz., the geochemical maturity of the kerogen of which they are composed; Peters et al., 1977; Schopf et al., 2005), but, also, because the color of such objects can provide a basis by which to distinguish *bona fide* fossil microbes from morphologically similar mineralic "look-alikes."

The effects of such difficulties are illustrated by the following example. In 1923 and 1924, geologist John Gruner, of the University of Minnesota, published papers reporting filamentous Precambrian microfossils from Paleoproterozic iron-ores of northern Minnesota (Gruner, 1923, 1924). Gruner, however, reported to have been an expert mineralogist–petrologist–microscopist (pers. comm. from P.E. Cloud to J.W.S., 1972), was not schooled in biology, so prior to publication he turned for help to his colleague at the university, biologist Josephine Tilden, among the foremost phycologists of the day and author of the highly regarded textbook *The Algae and Their Life Relations* (Tilden, 1935). Though Gruner's filaments were almost assuredly the first "Precambrian fossils" Tilden had ever examined, she confirmed Gruner's biological interpretation and identified them as "blue-green algae" (cyanobacteria) similar to modern *Microcoleus* (Tilden, 1935, p. 5). Given this affirmation, Gruner formally established the genus *Inactis* to which he referred the threadlike specimens (Gruner, 1923). Four decades later – to highlight the inferred cyanobacterial affinities of Gruner's filaments (and, perhaps, to emphasize the priority of Gruner's work, now being overshadowed by recently announced discoveries of diverse Precambrian fossil microbes; Barghoorn and Schopf, 1965; Barghoorn and Tyler, 1965; Cloud, 1965) – two noted Soviet geologists – paleontologists, A. G. Vologdin and K. B. Korde, renamed Gruner's filaments *Paleomicrocoleus gruneri* (Vologdin and Korde, 1965). However, this re-naming of Gruner's *Inactis* was based solely on the earlier published photomicrographs, rather than a restudy of Gruner's specimens, and neither of these workers, authorities on calcified megascopic Phanerozoic algae, had training in microbiology or previous experience in the interpretation of fossil microbe-like objects.

All of these experts, both the geologists (Gruner, Vologdin, Korde) and the biologist (Tilden), were mistaken. Rather than being microscopic cyanobacteria, Gruner's fossil-like filaments are now known to be of inorganic origin, threadlike trails produced by pyrite grains as they migrated sinuously through their encompassing chert matrix (Tyler and Barghoorn, 1963; Knoll and Barghoorn, 1974; Hofmann and Schopf, 1983; Mendelson and Schopf, 1992). Although such misinterpretations always look clearer in hindsight – and this mistake might have been avoided had these workers detected the trail-producing pyrite grains that terminate the threadlike microstructures (grains not depicted in Gruner's 1923 and 1924 papers) – it seems most likely that Gruner and the others erred not so much because of a lack of expertise in their own disciplines but, instead, because of a lack of knowledge of ancillary relevant fields.

1.4. An interdisciplinary solution to the problem

How can the biogenicity of ancient putative fossil microbes be established? As discussed above, it seems evident that expertise in neither geology nor biology, by itself, is likely to provide the answer. Rather, we suggest that a more promising approach is to be found in an interdisciplinary merging (rather than a multidisciplinary separation) of geology, biology and chemistry.

Such interdisciplinarity has a long history in the natural sciences (e.g., Vernadsky, 1926; Mawson, 1929; Black, 1933; Baas Becking, 1934), and the combined use of geology and biology to establish the ecological setting and biogenicity of Precambrian microbes, including their morphological comparison with modern analogues, dates from some of the earliest studies in the field (e.g., Barghoorn and Schopf, 1965; Barghoorn and Tyler, 1965; Cloud, 1965; Schopf, 1968). Nevertheless, it is only in recent years that direct evidence of the chemistry of such fossil microbes, the third component of this interdisciplinary strategy, has become available.

As discussed below, we suggest that there are three prime factors to be assessed in the evaluation of the biogenicity of putative microbial fossils, namely, their occurrence in a "plausible biological setting," their "biological morphology," and their "biologically derived chemistry." Of the three components of such studies – geology, biology and chemistry – geology typically plays a less prominent role, not because the geological context of such claims is not critical to their interpretation but, rather, because thriving microbial communities are known from virtually all environments on Earth where water is available, whether oceanic or terrestrial and ranging from deep sea fumaroles to glacial ice fields, making the requirement of a "plausible biologic setting" relatively easy to satisfy. Similarly, though post-depositional alteration of the host rocks of such claims must be thoroughly assessed, such changes, in the Precambrian due primarily to metamorphism, typically produce crystalline angular textures not readily confused with *bona fide* fossils. For this and related reasons, putative microfossils reported from highly metamorphosed Precambrian terrains (e.g., Pflug, 1978) have been discounted (e.g., Bridgwater et al., 1981; Schopf and Walter, 1983).

Notably, however, the other two factors – biology and chemistry – merge together, the biologically definitive morphology of *bona fide* fossil microbes (e.g., their genetically determined organismal form, variability, and cell-size distribution) being a product of their original biochemistry and fossilized organic composition. And the two must be assessed together, as each is subject to error: reports of "life-like" morphology, alone, have often been mistaken; and, as shown below, organic (carbonaceous) composition, by itself, is not sufficient to demonstrate the biogenicity of fossil-like microscopic objects.

We therefore use here the interrelated "biological morphology" and "biologically derived chemistry" of such specimens to establish their biogenicity, a strategy illustrated by comparative studies of authentic fossil microbes and morphologically similar microscopic

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. Characteristics of *bona fide* fossil prokaryotes

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, so only rarely have they been confused with mineral veins (perhaps the most common "filament-like" morphology present in petrographic thin sections). Other rock fabrics, such as splays of acicular diagenetic pseudomorphs of gypsum and aragonite (e.g., Cao and Zhao, 1978), have also been mistaken for biogenic structures (cf. Cloud, 1983, p. 26), as have spheroidal aggregations of radiating fibrous chalcedonic quartz, interpreted to be colonies of filamentous (rivulariacean) microbes (Barghoorn, 1971). Such reports, however, are not common.

Filamentous microbes are represented in the fossil record by two differing morphologies: trichomes, threadlike strands composed of uniseriate box-like or spheroidal cells; and sheaths, the tubular extracellular investments secreted by some trichomes. Ensheathed trichomes are formally referred to as "filaments." Like the size and shape of coccoidal microbes, those of the trichomic cells of filamentous prokaryotes are genetically determined, for members of each species occupying a discrete morphospace. Table 1 lists the principal characteristics that we expect to be exhibited by fossil filamentous prokaryotes.

2.2. Biologically derived chemistry

2.2.1. Modes of preservation

Microbial fossils can be preserved in the rock record by two principal processes: compression, their carbonaceous remnants flattened along bedding planes during the compaction and lithification of fine-grained silty or shaley sediments; and permineralization, their cells infused during diagenesis by, and then three-dimensionally preserved within, quartz, calcite, or apatite matrices. Of all modes of fossil preservation, cellular permineralization provides the most faithful life-like representation of biologic form and cellular anatomy. Though the present study focuses on rock-embedded permineralized microorganisms, many of the characteristics of *bona fide* fossils listed in Table 1 apply also to compression-preserved fossil microbes.

Permineralization (known commonly, but incorrectly, as "petrifaction") was first described more than a century ago (White, 1893). The misnomer "petrifaction" (from petrify, "to convert into stone") dates from this early study when the process was misinterpreted as reflecting "molecule-by-molecule . . . replacement" of cellular organic matter by minerals (White, 1893). In contrast with this stoichiometrically implausible account of the process, permineralization is now known to result from the pervasion of mineral-charged solutions into biologic tissues during early stages of diagenesis, prior to their decay and cellular disintegration (Schopf, 1975) – a process analogous to the epoxy-embedding of the tissues of modern organisms in prepartion for their study by transmission electron microscopy. The organic structures of such fossils, in many specimens preserved in microscopic detail, are not mineral-replaced. Rather, the permeating fluids infill micellar, intercellular, and intracellular spaces – replacing the watery milieu of the biomolecular components, not the molecules of the organic structures themselves – to produce a mineral-infused inorganic–organic mix that in microbes serves to preserve their physically robust and organic-rich cell walls and encompassing envelopes and sheaths.

For Phanerozoic fossils, preservation by "mineral replacement" – for example, of calcite by pyrite in brachiopods, crinoids, or sea stars – is not uncommon. However, unlike such biomineralized eukaryotic metazoans, prokaryotic microbes are composed solely of organic matter. Fossilization by mineral replacement of the organic matter of such prokaryotes is virtually unknown. For microbial fossils, probably the closest analogy to such replacement is the infilling or particulate coating of cells by precipitated iron minerals (e.g., LaBerge, 1967; Cloud and Licari, 1968; Gutstadt and Schopf, 1969; Walter et al., 1976). Such microbial fossils are rare, known almost entirely from iron formations, and in no instance have their organic constituents been shown to be converted to mineral.

2.2.2. Carbonaceous (kerogenous) chemistry

Given the foregoing, it seems evident that the biologically derived chemistry of microbial fossils – their organic (carbonaceous) composition – is a key indicator of their biogenicity. The presence in Precambrian rocks of biologically derived carbon, including that in molecular biomarkers (e.g., Summons and Walter, 1990), has been long established (e.g., Hoering, 1967; Schidlowski et al., 1983; Strauss and Moore, 1992). Until recently, however, no techniques were available by which to characterize the chemistry of individual rock-embedded microscopic fossils, reports of their carbonaceous composition being based solely on optical microscopy

Table 1
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 organization evidencing their derivation from a common parent cell; if benthic, the lower surface of such colonies should be defined by the flat to undulating substrate across which the colony propagated, with the upper surface, where cell growth was less constrained, being commonly more irregular (e.g., bulbose, pustulose, etc.)
(6) should be represented by many specimens that exhibit the same limited range of morphological characters (if one example of a given species can be preserved, others should be too), typically in varying states of preservation (ranging from degraded to partially altered to their more or less original morphology), that are part of a multicomponent community (monospecific microbial communities being unknown in natural environments) that can occur over an extensive area (occupying contiguous paleoecologically similar settings)
Filamentous prokaryotic fossils:
(1) should be cylindrical (or, if distorted during preservation, initially cylindrical), their shape defined by distinct carbonaceous lateral walls
(2) should have a species-specific unimodal limited range of widths, a pattern and range of size distribution consistent with those of morphologically comparable fossil taxa and modern microbes (for most species, having widths <20 μm; Schopf, 1992b, Tables 5.4.8 and 5.4.9); due to the break-up of such microscopic threads into shorter fragments (a mode of propagation dependent on the energetics of the inhabited environment), specimen-lengths can be variable, ranging from tens to a few hundreds of microns
(3) should have an essentially uniform diameter throughout their lengths (in some trichomic species tapering toward their apices); if permineralized tubular sheaths, should be "hollow" (mineral-infilled); if cellular trichomes, should terminate in species-specific morphologically distinctive end cells (e.g., flat, rounded, hemispheroidal, conical, pillow-shaped, etc.)
(4) if trichomic, should be partitioned by transverse septa into discrete cells of more or less uniform size and shape that are "hollow" (mineral-infilled) in permineralized specimens and that may occur in pairs or be partially divided and/or exhibit partial septations (evidence of cell division; Schopf and Kudryavtsev, 2009), with elongate cells being typical of relatively narrow trichomes and short disc-shaped cells of broader trichomes (Schopf, 1992b)
(5) due to their original flexibility, should exhibit a sinuosity correlated with both their length and breadth, longer specimens tending to be more sinuous than shorter ones (just as a longer string can be wound into a greater number of coils than a shorter one), and narrower specimens tending to be more sinuous than broader ones (just as a length of rope can be wound into fewer coils than a similar length of string)
(6) should be represented by many specimens that exhibit the same limited range of morphological characters (if one example of a given species can be preserved, others should be too), typically in varying states of preservation (ranging from degraded to partially altered to more or less original morphology), that are part of a multicomponent community (monospecific microbial communities being unknown in natural environments) that can occur over an extensive area (occupying contiguous paleoecologically similar settings)

(primarily, their typically brown to black "coal-like" appearance). The first study to document the presence of elemental organic carbon (and its isotopic composition) in such fossils is that of House et al. (2000) in which SIMS was used to analyze microbial fossils of the Proterozoic Bitter Springs and Gunflint Formations. Although used since in several studies (Oehler et al., 2006, 2008a,b,c, 2009; Robert et al., 2008), SIMS has a major drawback of being capable of providing data only about the elemental and isotopic compositions of specimens exposed at the upper surface of polished petrograhic thin sections.

Two other methods by which to demonstrate the carbonaceous composition of rock-embedded fossil microbes at submicron spatial resolution – both rock-penetrating and, thus, of broader usefulness than surface-limited techniques – have also been recently introduced to such studies: confocal laser scanning microscopy, "CLSM" (Schopf et al., 2006), and Raman imagery (Schopf et al., 2002; Schopf and Kudryavtsev, 2005). These analytically complementary, non-destructive and non-intrusive techniques can be used to analyze thin section-embedded specimens to depths of ~150 μm.

Organic-walled fossils are composed of carbonaceous matter derived from the geochemical alteration of their original organic components, coaly material properly referred to as "kerogen." Such kerogen, for fossil microbes derived from a mix of lipids, mucopeptides and other cellular biochemicals, is a geochemically stable, three-dimensional macromolecular complex of interlinked polyaromatic hydrocarbons, "PAHs" (Vandenbroucke, 2003; Schopf et al., 2005; Vandenbroucke and Largeau, 2007). CLSM detects laser-induced fluorescence emitted from the PAHs of kerogen, whereas Raman imagery analyzes its molecular structure, provides spectral data by which to assess its organic geochemical maturity, and identifies associated minerals. The strengths and limitations of the two techniques have recently been discussed (Schopf and Kudryavtsev, 2009). Suffice it here to note that CLSM is not capable of detecting non-fluorescing minerals (such as those that comprise putative pseudofossils studied here) or of imaging specimens that are composed of geochemically mature, partially graphitized kerogens (such as those of the Marra Mamba Iron Formation and Farrel Quartzite discussed below).

3. Materials and methods

3.1. Materials

All illustrated specimens are in petrographic thin sections of Precambrian fine-grained sediments, either siltstone (those of the Lakhanda Formation) or chert (all other specimens), from the following seven geological units: the ~770 Ma Chanda Limestone of southern India (Bandopadhyay, 2007); ~800 Ma Myrtle Springs Formation of South Australia (cf., but stratigraphically 100 m above, PPRG sample 1283; Moore and Schopf, 1992, p. 630); ~800 Ma Bitter Springs Formation of central Australia (PPRG sample 1253; Moore and Schopf, 1992, p. 631); ~1020 Ma Lakhanda Formation of southeastern Siberia (Podkovyrov, 2009; German and Podkovyrov, 2009); ~1700 Ma Vempalle Formation of central India (Schopf and Prasad, 1978); ~2629 Ma Marra Mamba Iron Formation of northwestern Australia (PPRG sample 520; Walter et al., 1983, p. 402); and the ~3050 Ma Farrel Quartzite of northwestern Australia (Sugitani et al., 2007, 2009).

3.2. Optical microscopy

Transmitted-light optical photomicrographs were obtained by use of UCLA #0026535 Leitz Orthoplan 2 microscope (Leitz, Wet-

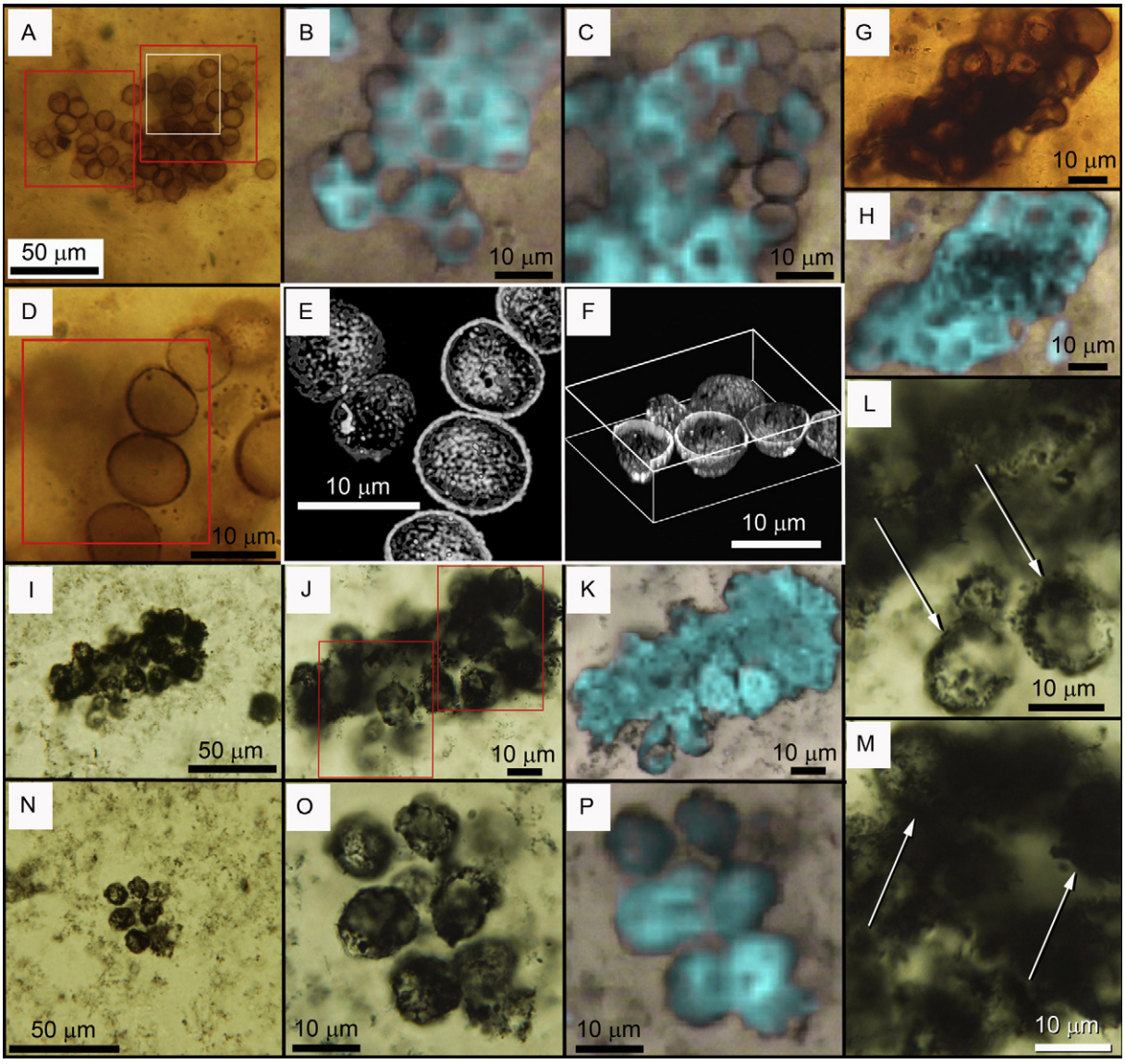

Fig. 1. Optical photomicrographs (A, D, G, I, J, L, M, N and O), Raman kerogen images superimposed on optical images (B, C, H, K and P), and confocal laser scanning micrographs (E and F) of *bona fide* microfossils in petrographic thin sections of cherts from the ~800 Ma Bitter Springs Formation of central 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 white rectangle denotes the area shown at higher magnification in (D); in (D), the red rectangle denotes the area shown in the CLSM images in (E) and (F) that encompasses a linear series of four cells that transect the upper surface of the thin section, in (E) shown from the same perspective as in (D), and in (F), tilted and rotated clockwise to illustrate the cells from an oblique side-view. (G, H) Bitter Springs Colony B (composed of 17 cells); the Raman image in (H) establishes the carbonaceous (kerogenous) composition of the close-packed unicells. (I–M) Farrel Quartzite Colony C (composed of 15 cells); the red rectangles in (J) denote, from left to right, the areas shown, respectively, in (L) and (M), parts of the colony situated at a lower focal depth than the cells shown in (J). (N–P) Farrel Quartzite Colony D (composed of 9 cells); the Raman image in (P) establishes the carbonaceous (kerogenous) composition of the colonial unicells.

zlar, Germany) equipped with an Olympus DP12 Microscope Digital Camera (Olympus, Melville, NY). For oil-immersion microscopy (whether by optical microscopy, CSLM, or Raman analyses), the thin sections were covered by a ~1-μm-thick veneer of Type FF, fluorescence-free, microscopy immersion oil (R.P. Cargille Laboratories, Inc., Cedar Grove, NJ).

3.3. Confocal laser scanning microscopy (CLSM)

CLSM images were obtained by use of an Olympus Fluoview 300 confocal laser scanning biological microscope system equipped with two Melles Griot lasers, a 488 nm 20 mW-output argon ion laser and a 633 nm 10 mW-output helium–neon laser (Melles Griot, Carlsbad CA). The techniques and instrumental settings used for image acquisition were the same as those specified by Schopf and Kudryavtsev (2009). Following their acquisition, all CLSM images were processed by use of the VolView v2.0 3-D-rendering computer program (Kitware Inc., Clifton Park, NY) that permits their manipulation in three dimensions.

3.4. Raman spectroscopy

Raman spectra were obtained by use of the T64000 (JY Horiba, Edison NJ) triple-stage laser-Raman system described by Schopf and Kudryavtsev (2009). Use of a Coherent Innova (Santa Clara, CA) argon ion laser to provide excitation at 457.9 nm permitted Raman data to be obtained simultaneously over a range from ~300 to >3100 cm^{-1} by use of a single spectral window cen-

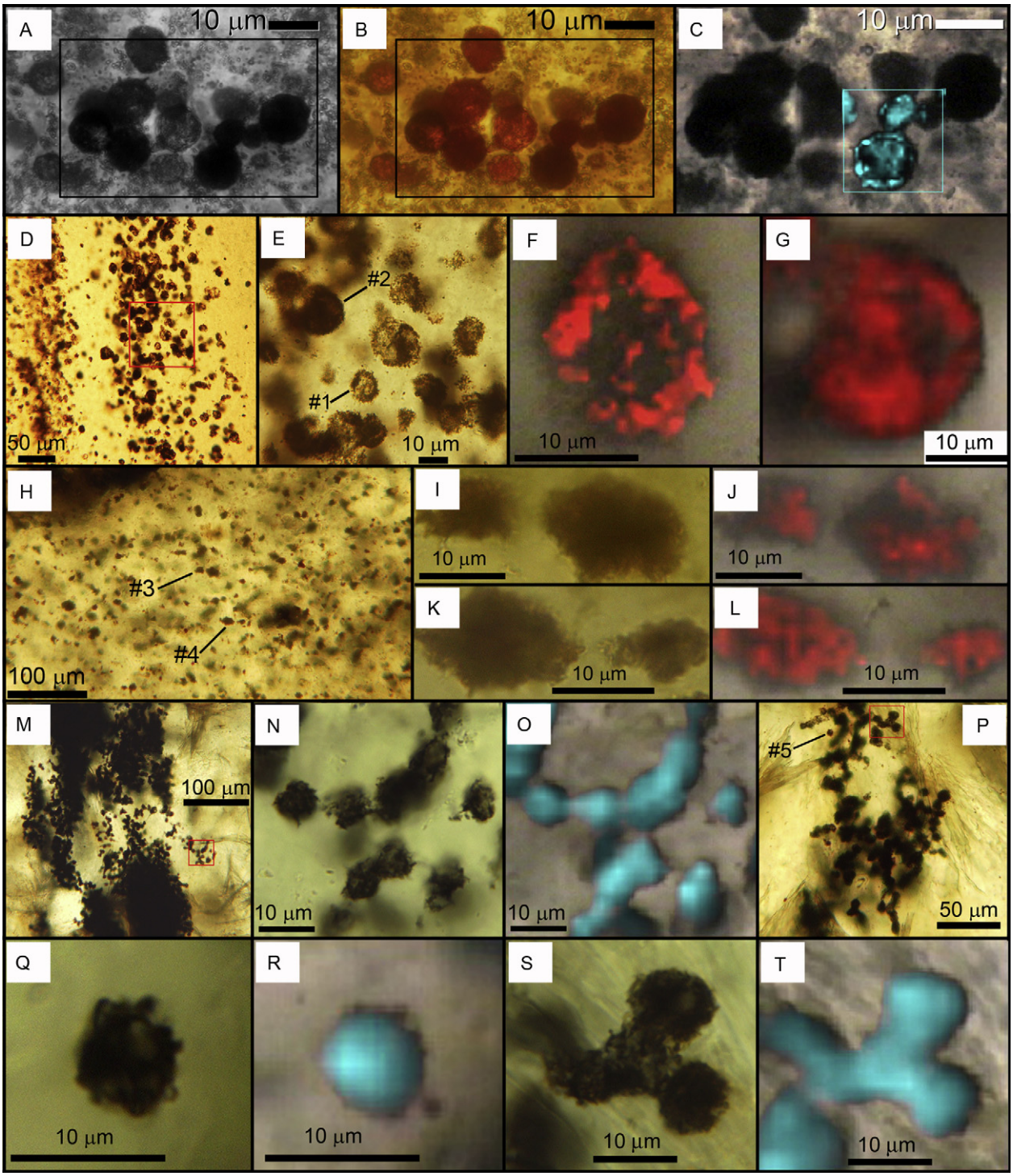

Fig. 2. Optical photomicrographs (A, B, D, E, H, I, K, M, N, P, Q and S) and Raman images superimposed on optical images (C, F, G, J, L, O, R and T) of spheroidal and ellipsoidal pseudofossils in petrographic thin sections of siltstone (A through C) and cherts (D through T) from the ~1020 Ma Lakhanda Formation of southeastern Siberia (A through C), the ~770 Ma Chanda Limestone of southern India (D through L), and the ~2629 Ma Marra Mamba Iron Formation of northwestern Australia (M through T). Raman images, shown in blue or red, were acquired in spectral windows centered on the Raman bands shown in Fig. 5B, at ~409 cm^{-1} for hematite (C), at ~394 cm^{-1} for goethite (F and G) and at ~678 cm^{-1} for magnetite (J and L), or in Fig. 5A, at ~1605 cm^{-1} for kerogen (O, R and T). (A–C) Spheroidal hematite pseudofossils, in (A) shown in a black and white image for comparison with the color image in (B); the black rectangles in (A) and (B) denote the area shown in (C) in which the blue rectangle encloses the Raman-analyzed area. (D–G) Spheroidal and ellipsoidal goethite pseudofossils; the red rectangle in (D) denotes the area shown in (E) that encloses Raman-analyzed specimens #1, shown in (F), and #2, shown in (G). (H–L) Spheroidal and ellipsoidal magnetite pseudofossils, in (H) indicating the locations of the Raman-analyzed specimens #3, shown in (I) and (J), and #4, shown in (K) and (L). (M–T) Solitary (Q and R) and interlinked (M through P and S and T) kerogen pseudofossils; in (M), the red rectangle denotes the Raman-analyzed area shown in (N) and (O); in (P) is shown the location of specimen #5, the Raman-analyzed spheroid illustrated in (Q) and (R), and enclosed in the red rectangle, the Raman-analyzed area shown in (S) and (T).

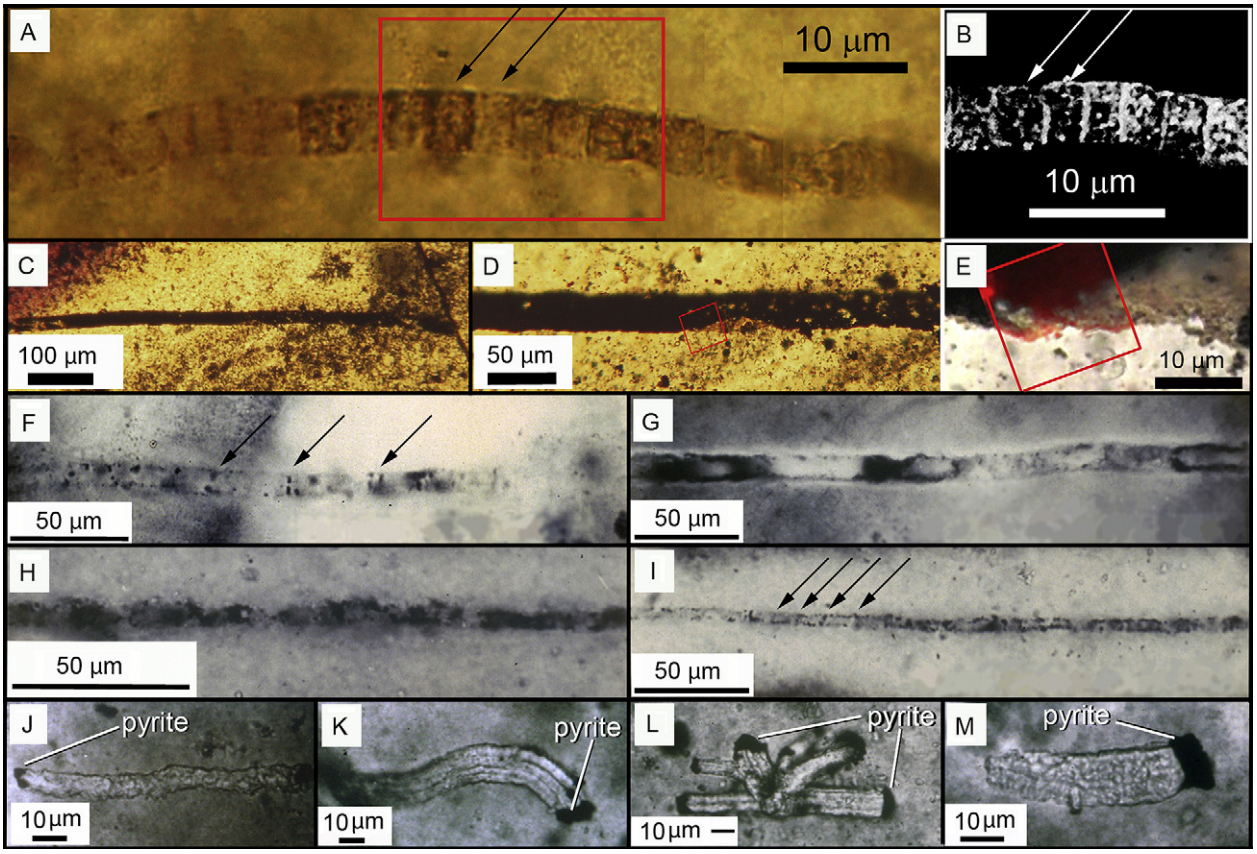

Fig. 3. Optical photomicrographs, in color (A, C and D) and in black and white (F through M), a confocal laser scanning micrograph (B), and a Raman image superimposed on an optical photomicrograph (E), showing a *bona fide* filamentous microfossil (A and B) and filamentous pseudofossils (C though M) in petrographic thin sections of cherts from the ~800 Ma Bitter Springs Formation of central Australia (A and B), the ~770 Ma Chanda Limestone of southern India (C through E), the ~1700 Vempalle Formation of central India (F), and the ~800 Ma Myrtle Springs Formation of South Australia (G through M). The Raman image (E) was acquired in a spectral window centered on the ~557 cm^{-1} manganite band indicated in Fig 5B. (A, B) A cylindrical oscillatoriacean cyanobacterial trichome composed of regularly spaced box-like cells; the brownish amber carbonaceous cell walls shown in the optical image (A) are distinctly defined in the CLSM image (B) by the laser-induced fluorescence emanating from the interlinked polycyclic aromatic hydrocarbons of their kerogenous components; arrows in (A) and (B) point to partial septations, thin incipient cell walls. (C–E) A dark-brown filamentous pseudofossil, a planar vein-filling oriented perpendicular to the upper surface of the thin section; the red rectangle in (D) denotes the Raman-imaged area shown in (E) to be composed of manganite. (F–I) Filamentous pseudofossils, planar quartz-filled veinlets oriented perpendicular to the upper surface of thin sections; arrows in (F) and (I) point to pseudosepta, veinlet-included opaque minerals that produce a "cell-like" appearance. (J–M) Filamentous pseudofossils produced by ambient pyrite, striated quartz-filled trails formed by grains of pyrite as they moved through the encompassing chert matrix.

tered at 1800 cm^{-1} and, thus, acquisition of spectra of matrix and vein-filling quartz (SiO_2, having a major band at ~465 cm^{-1}) as well as the materials comprising the specimens analyzed: kerogen (having major bands at ~1365 and ~1605 cm^{-1} and second-order bands at ~2800 cm^{-1}), goethite ($HFeO_2$, having a major band at ~394 cm^{-1}), hematite (Fe_2O_3, at ~409 cm^{-1}), manganite (MnO[OH], at ~557 cm^{-1}), and magnetite (Fe_3O_4, at ~678 cm^{-1}).

Two-dimensional Raman images were acquired by use of techniques and instrumental settings previously described (Schopf and Kudryavtsev, 2009). To prepare "Raman-overlay images," presented here for the first time, that show the spatial relations of a specimen to the kerogen and/or mineral of which it is composed, 2-D Raman images acquired in narrow spectral windows centered on the major bands of the material detected were superimposed on optical photomicrographs of the analyzed specimen.

4. Results

The optical, CLSM, and Raman images in Figs. 1 through 3 show *bona fide* coccoidal and filamentous microbial fossils and microscopic objects interpreted here as pseudofossils from seven Precambrian geological units. Figs. 4 and 5 present, respectively, a summary of the size distributions of the objects studied and the Raman spectra of the kerogenous or mineral materials of which they are composed. The data presented in these figures, taken together, provide the evidence of biological morphology and biologically derived chemistry that we suggest can be used to distinguish authentic microbial fossils from morphologically similar "look-alikes."

4.1. Coccoidal microscopic fossil-like objects

Of the morphological characters exhibited by coccoidal fossil microbes, cell-size ranges are among the most easily determined and commonly reported. However, because populations of microscopic spheroidal mineral grains can exhibit limited size distributions that are similar to those of authentic coccoidal microfossils, a "narrow size range" is not necessarily indicative of biogenicity. Rather, the size range of coccoidal microorganisms, specified in all taxonomic species descriptions, is a genetically determined trait produced by sequential cell divisions in a species-population that is correlated with the mean cell-size of the species. Thus, for example, the vegetative cells of a population of a microbial species that has a mean cell-size of 5 μm typically range from ~3 to ~7 μm, a "narrow" range of ~4 μm, whereas such cells of a population centered at 20 μm typically range from ~11 to 29 μm, a much broader range of ~18 μm. The Divisional Dispersion Index ("DDI"), devised from studies of ~500 species of modern coccoidal

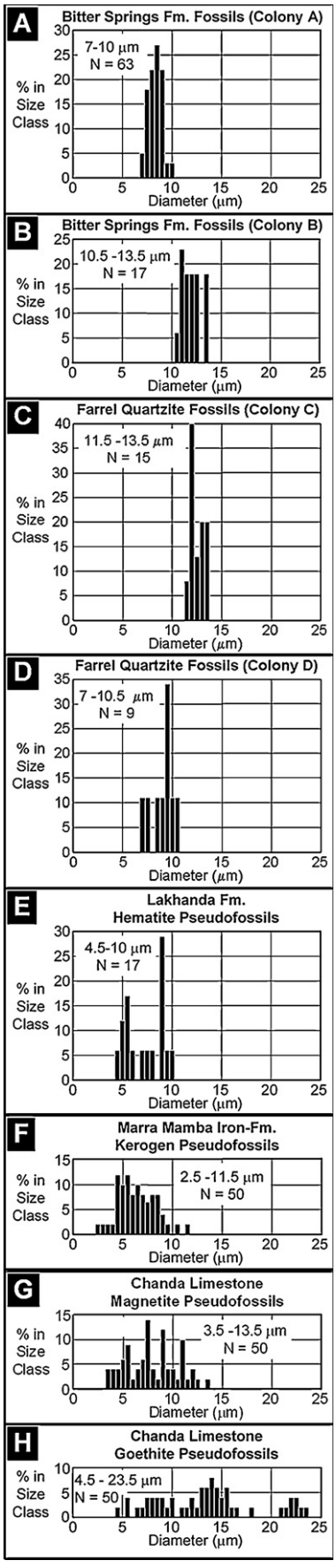

Fig. 4. Size distributions of *bona fide* microfossils measured in petrographic thin sections of cherts from the ~800 Ma Bitter Springs Formation of central Australia (A and B) and the ~3100 Ma Farrel Quartzite of northwestern Australia (C and D) for comparison with those of similarly sized thin section-embedded spheroidal pseudofossils from a siltstone of the ~1020 Ma Lakhanda Formation of southeastern Siberia (E) and from cherts of the ~2629 Ma Marra Mamba Iron Formation of northwestern Australia (F) and the ~770 Ma Chanda Limestone of southern India (G and H). (A, B) Unimodal size distributions of the kerogenous unicells that comprise Bitter Springs Colony A (Fig. 1A through F) and, in (B), Colony B (Fig. 1G and H). (C, D) Unimodal size distributions of the kerogenous unicells that comprise Farrel Quartzite Colony C (Fig. 1I through M) and, in (D), Colony D (Fig. 1N through P). (E–H) Polymodal size distributions of microscopic coccoidal pseudofossils: in (E), hematite spheroids (Fig. 2A through C); in (F), kerogenous interlinked spherulites (Fig. 2 M through T); in (G), magnetite spheroids and ellipsoids (Fig. 2H through L); and in (H), goethite spheroids and ellipsoids (Fig. 2D through G).

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 μm, a DDI of 2 ("Colony D": Fig. 1N–P and Fig. 4D);
- they are colonial, occurring in close-packed groups that are embedded in a carbonaceous (originally mucilaginous) diffuse diaphanous matrix (e.g., Fig. 1A, G, L, M);
- they are represented by numerous specimens – some 80 cells for Bitter Springs colonies "A" and "B," and 24 cells for Far-

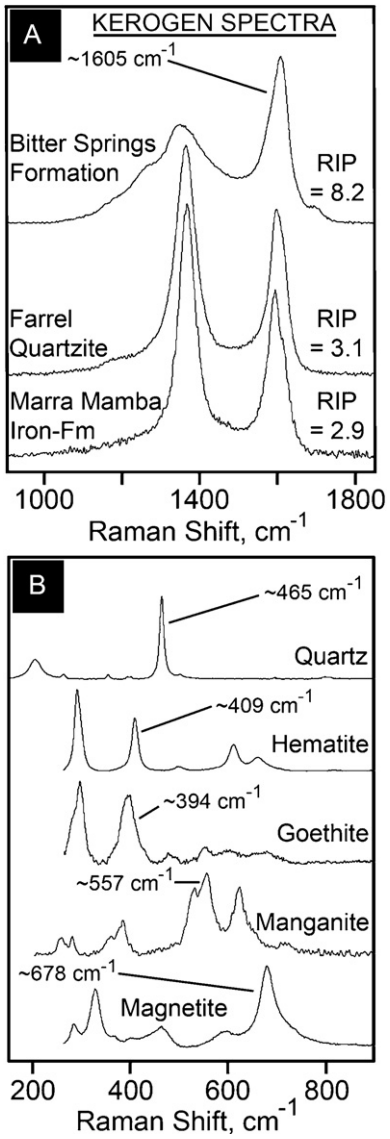

Fig. 5. (A) Raman spectra of the kerogen that comprises *bona fide* fossils of the ~800 Ma Bitter Springs Formation of central Australia (Figs. 1A through H, 3A and B, and 4A and B) and the ~3050 Ma Farrel Quartzite of northwestern Australia (Figs. 1I through P, and 4C and D), and of the partially graphitized kerogen that comprises the interlinked spherulitic pseudofossils of the ~2629 Ma Marra Mamba Iron Formation of northwestern Australia (Figs. 2 M through T, and 4F); listed for each kerogen is its Raman Index of Preservation (RIP) value, a quantitative measure of its geochemical maturity (see text), the 8.2 value for kerogen of the Bitter Springs Formation indicating that it is much less altered than the mature kerogens of the Farrel Quartzite (RIP = 3.1) and Marra Mamba Iron Formation (RIP = 2.9). (B) Raman spectra of the minerals that comprise the pseudofossils illustrated in Figs. 2 and 3: quartz (SiO_2), comprising the filamentous pseudofossils of the ~1700 Vempalle Formation of central India (Fig. 3F) and the ~800 Ma Myrtle Springs Formation of South Australia (Fig. 3G through M); hematite (Fe_2O_3), comprising the spheroidal pseudofossils of the ~1020 Ma Lakhanda Formation of southeastern Siberia (Fig. 2A through C, and 4E); and iron and manganese oxides that comprise the spheroidal, ellipsoidal or filamentous pseudofossils of the ~770 Ma Chanda Limestone of southern India – goethite ($HFeO_2$; Figs. 2D through G, and 4H), manganite (MnO[OH]; Fig. 3C through E), and magnetite (Fe_3O_4; Figs. 2H through L, and 4G).

rel Quartzite colonies "C" and "D" – such cells in each colony exhibiting the same limited range of morphological characters with various specimens exhibiting differing states of preservation; for example, compare the well-preserved Bitter Springs cells in Fig. 1A and D with the more deformed cells in Fig. 1G, and the Farrel Quartzite cells in Fig. 1L with the more altered examples in Fig. 1O;

- they are part of multicomponent microbial communities, well-documented both for the Bitter Springs (Schopf, 1968; Schopf and Blacic, 1971) and Farrel Quartzite fossils (Sugitani et al., 2007, 2009; Sugahara et al., 2009; Grey and Sugitani, 2009; Oehler et al., 2009); and
- the kerogenous composition of their colonial cells is shown by Raman-overlays (Fig. 1B, C, H, K, P) that document the spatial correlation of their biological morphology and biologically derived carbonaceous chemistry.

The color of organic-walled fossils – ranging from yellow to brown to jet black, a sequence of increasingly darker color that corresponds to increasing, geologically induced thermal alteration (e.g., Schopf et al., 2005) – has long been used to assess the geochemical maturity of the kerogen of which such fossils are composed and is a standard tool used in the petroleum industry (Peters et al., 1977; Traverse, 1988). Thus, compared with that of microfossils permineralized in many other Precambrian cherts (Schopf et al., 2002, 2005), the amber-brown color of the Bitter Springs cells (Fig. 1A, G, D) reflects the relatively well-preserved (geochemically less altered) state of their organic constituents, whereas the dark brown to black color of the Farrel Quartzite specimens (Fig. 1I, J, L, M–O) indicates that their kerogenous cell walls are geochemically more mature.

Though this color-based assessment of the fidelity of organic preservation is useful (and illustrates why color photomicrographs of such fossils are to be preferred in scientific publications), it is both qualitative and subjective. A more precise, quantitative and analytically objective method by which to determine the geochemical maturity of such kerogen is provided by measurement of its Raman Index of Preservation (Schopf et al., 2005). The "RIP" values thus produced, calculated directly from the kerogen Raman spectra of permineralized fossils, track the sequential changes in the molecular structure of originally disordered rock-embedded kerogen that occur as it progresses along a geochemical pathway toward graphite (Schopf et al., 2005). Thus, for example, the two major Raman bands of the kerogen that comprises the Bitter Springs fossils, the left-most "D" band and the right-most "G" band shown in Fig. 5A, are broad, indicating that the Bitter Springs kerogen (having a relatively high RIP value of 8.2, like that of kerogens comprising fossils of other well-preserved Precambrian microbial communities; Schopf et al., 2005) is molecularly disordered. During the geochemical maturation of such kerogen, as its molecular structure is altered, primarily by heat, and the kerogen becomes increasingly more ordered and graphitized, the shape of these two major bands changes: the "D" band becomes increasingly narrow and much more peaked while the "G" band gradually narrows and, in partially graphitized kerogen, ultimately bifurcates (Schopf et al., 2005). The Raman spectrum of the kerogen comprising the Farrel Quartzite cells (having a low RIP value of 3.1; Fig. 5A) evidences such changes.

Comparison of RIP values shows that although the kerogen of the ~3050 Ma Farrel Quartzite fossils is less well-preserved than that of the Bitter Springs fossils, it is appreciably less altered than kerogen comprising cellularly permineralized fossils of the very much younger (~750 Ma) Auburn Dolomite (RIP = 1.5) and River Wakefield Formation (RIP = 1.0) of South Australia (Schopf et al., 2005). Now used increasingly in studies of ancient permineralized carbonaceous fossils (e.g., Chen et al., 2007; Schopf et al., 2008; Schopf and Kudryavtsev, 2009; Igisu et al., 2009), such RIP values are highly reproducible and easily calculated (Schopf et al., 2005).

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. 2A–C) and of cherts from the ~770 Ma Chanda Limestone of southern India (Fig. 2D–L) and the ~2629 Ma Marra Mamba Iron Formation of northwestern Australia (Fig. 2M–T). We interpret these objects to be pseudofossils.

Unlike the *bona fide* coccoidal fossils of the Bitter Springs and Farrel Quartzite, the microscopic fossil-like spheroids of the Lakhanda Formation (Fig. 2A–C) – being solid; exhibiting no evidence of defining cell walls; and having a bimodal, rather than unimodal, size distribution (Fig. 4E) – do not exhibit the biological morphology of authentic fossils. Raman spectroscopy (Figs. 2C and 5B) shows them to be composed of hematite, not biogenic kerogen. Because these mineral spheroids have an acceptably biologic-like size distribution (ranging from ~4.5 to ~10 μm, a DDI of 4; Fig. 5B) and in black and white images (Fig. 2A and C) have the appearance of authentic coccoidal fossil microbes, they are particularly instructive, showing that neither a biologically plausible DDI nor black and white photomicrographs, alone, can be relied on to provide firm evidence of biogenicity. Instead, full-color images, in this case documenting the hematitic red color of the "fossil-like" objects (Fig. 2B), combined with their lack of the characteristics of biologic morphology and of biologically derived chemistry listed in Table 1, indicates to us that they are mineral pseudofossils.

In Fig. 2D through L are shown microscopic objects in cherts of the Chanda Limestone that have been interpreted to be Neoproterozoic microfossils (Bandopadhyay, 2007). Like the hematite spheroids of the Lakhanda Formation, we regard these objects to be mineral pseudofossils. The spheroidal to ellipsoidal to irregularly shaped specimens shown in Fig. 2D through G are composed of goethite (Fig. 5B), rather than kerogen, and those in Fig. 2H through L, of magnetite (Fig. 5B). Not only are these putative fossils mineralic, but also they are solid, rather than "hollow"; are not defined by carbonaceous cell walls; and are morphologically too variable to be credibly biogenic. Their polymodal, rather than unimodal size distributions – for the goethite specimens, 4.5–23.5 μm, a DDI of 8 (Fig. 4H); and for the magnetite specimens, 3.5–13.5 μm, a DDI of 6 (Fig. 4G) – differ decidedly from those typical of authentic fossils. Moreover, their abundance and spatial distribution in the Chanda chert are biologically implausible. They are unlikely to be remnants of colonial microorganisms: rather than being clustered in discrete closely packed groups of a few to many individuals (like the cellular colonies of the Bitter Springs Formation and Farrel Quartzite, for example), the Chanda objects are randomly distributed and represented by huge numbers, literally tens of thousands of specimens (Fig. 2D and H). Similarly, it is implausible for them to be fossilized planktonic unicells that settled from an overlying water column onto a substrate – they are far too abundant and occur throughout an appreciable volume of the chert rather than being concentrated on bedding surfaces. These Chanda objects exhibit neither the biological morphology and spatial distribution nor the biologically derived chemistry expected of *bona fide* fossil microbes (Table 1). We interpret them to be goethite and magnetite microscopic pseudofossils (Fig. 5B).

Fig. 2M through T shows one additional example of microscopic coccoidal pseudofossils, from chert of the ~2629 Ma Marra Mamba Iron Formation of northwestern Australia, important because it shows that biologically derived chemistry, by itself, is not sufficient to establish biogenicity. Originally reported by LaBerge (as "Type C" and "Type D" structures, shown in pl. 3, Figs 1–6 and 8 of LaBerge, 1967) and later described as "dubiomicrofossils" (Schopf and Walter, 1983), these specimens are demonstrably composed of kerogen (Figs. 2O, R, T and 5A). Nevertheless, like the mineralic pseudofossils of the Lakhanda Formation and the Chanda Limestone, the Marra Mamba spheroids are solid and are not defined by distinct cell walls (Fig. 2N, O, Q–T). Their broad polymodal size distribution – ranging from 2.5 to 11.5 μm, a DDI of 8 (Fig. 4F) – is unlike that of *bona fide* fossil microbes. Like the Chanda pseudofossils, the Marra Mamba spheroids occur in a biologically non-plausible profusion and are not concentrated on bedding surfaces (Fig. 2 M and P); for reasons noted above for the Chanda microstructures, they are therefore unlikely to be remnants either of fossilized benthic colonies or of planktonic unicells. Similarly, they differ significantly from fossils of filamentous microbes: although some specimens are interconnected (Fig. 2S) or appear to be linked by kerogenous strands into filament-like chains (Fig. 2N, O, S, T), they also occur as isolated individuals (Fig. 2N, Q) and their linked aggregates commonly exhibit decidedly nonbiologic branching patterns (e.g., Fig. 2S; LaBerge, 1967, pl. 3, Fig. 8; Schopf and Walter, 1983, Photo 9-3H-K).

Although the chert-embedded Marra Mamba microstructures seem not to be fossils, as shown by their lack of concordance with the criteria expected of authentic fossil microbes listed in Table 1, they are nevertheless composed of kerogen. Given their lack of biological morphology, what can account for this presence of biologically derived chemistry? Experimental studies of the artificial permineralization of microbes in silica suggest an answer. During the laboratory crystallization of silica designed to mimic the processes involved in the preservation of microorganisms in Precambrian cherts, large numbers of microscopic spherulitic quartz grains are formed that are of the same size and shape as the Marra Mamba pseudofossils and that, like these kerogenous microstructures, occur in close-packed massive aggregations (Oehler and Schopf, 1971). Such spherulites form in 2–4 weeks at 150°–300 °C (Oehler and Schopf, 1971; Oehler, 1976, Fig. 8), temperatures consistent with the low RIP value of the Marra Mamba kerogen of 2.9 and the bifurcated "G" band of its Raman spectrum (Fig. 5A) that evidence its thermally induced partial graphitization (Schopf et al., 2005). Thus, as previously suggested (Schopf and Walter, 1983, p. 232; Walter and Hofmann, 1983, p. 376–377), the spheroidal Marra Mamba pseudofossils seem likely to have formed when biogenic kerogen was incorporated into gelatinous (colloidal) silica during the spherulitic stages of quartz-formation (as described by Oehler, 1976), its thermal alteration occurring during subsequent diagenesis.

4.2. Filamentous microscopic fossil-like objects

Although assemblages of nonbiological mineralic or carbonaceous structures that mimic convincingly the morphological characteristics of a diverse community of threadlike microbial filaments are unknown, several types of "fossil-like" mineralic filaments (e.g., mineral-filled veins and trails of ambient pyrite grains) have been mistaken for authentic microorganisms (for a listing of examples, see Hofmann and Schopf, 1983; Schopf and Walter, 1983; Mendelson and Schopf, 1992).

4.2.1. Bona fide filamentous fossils

Shown in Fig. 3A and B is an optical photomicrograph and a CLSM image of an elongate microscopic filament permineralized in chert of the ~800 Ma Bitter Springs Formation of central Australia. This threadlike specimen is a *bona fide* microbial (cyanobacterial) trichome. Consistent with the criteria enumerated in Table 1:

- it is "hollow" (quartz-infilled) and cylindrical, as is shown both by optical microscopic study of the thin section-embedded specimen and by rotation of the three-dimensional CLSM image (Fig. 3B);

- 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. 3A, B, at arrows), incipient cell walls that evidence cell formation by binary division (Schopf and Kudryavtsev, 2009);
- the dimensions of its component cells, ~5 μm broad and ~3 μm long, are identical to those of numerous species of modern and Proterozoic oscillatoriacean cyanobacteria (Schopf, 1992b, Fig. 5.4.33B, D);
- it is a member of a well-documented multicomponent microbial community that contains many other specimens having this same morphology (Schopf, 1968; Schopf and Blacic, 1971); and
- it is composed of kerogen, identified by Raman spectroscopy (Fig. 5A) and evidenced also by the CLSM-detectable fluorescence of its lateral and transverse cell walls (Fig. 3B).

4.2.2. Filamentous pseudofossils

In Fig. 3 are also shown filamentous microscopic fossil-like objects in petrographic thin sections of cherts from the ~770 Ma Chanda Limestone of southern India (Fig. 3C–E), ~1700 Ma Vempalle Formation of central India (Fig. 3F), and the ~800 Ma Myrtle Springs Formation of South Australia (Fig. 3G–M). We interpret these objects to be pseudofossils.

Originally considered to be a filamentous microfossil (Bandopadhyay, 2007), the specimen shown in Fig. 3C through E appears, instead, to be a mineralic pseudofossil. In contrast with authentic chert-permineralized filamentous microbes (Table 1), this Chanda specimen is straight, rather than sinuous; is much longer and somewhat broader than typical microbial fossil filaments; is not "hollow"; and exhibits no evidence of cellularity. Most importantly, it is planar, a fossil-like microstructure shown by optical microscopy to be a ~30-μm-thick mineral vein oriented approximately perpendicular to the upper surface of the thin section. Despite its carbonaceous-like brown color (Fig. 3C and D), Raman analyses show it to be composed of manganite (Fig. 5B), not kerogen. We therefore regard this object to be a cross-cutting vein filled by manganite derived from the "manganese oxide ore/manganiferous shale" of interbedded geological strata (Bandopadhyay, 2007).

Other fossil-like mineral veins, all oriented more or less perpendicular to the upper surface of the chert thin section in which they occur, are shown in Fig. 3F though I. Unlike the broad, opaque, manganiferous vein of the Chanda chert, these veinlets have microbe-like narrow widths (≤10 μm) and are quartz-filled and in places translucent, their "life-like" appearance evidently produced by included opaque minerals that in black and white photomicrographs, as shown here, have the appearance of cell-defining transverse septa (Fig. 3F, I, at arrows). Nevertheless, because such chert-cutting veinlets can be shown by optical, CLSM, and/or petrographic microscopy to be planar structures and to be composed of relatively large, angular, vein-filling quartz crystals (rather than the interlocking mosaic of cryptocrystalline quartz grains in which microbes are typically permineralized), such veinlets have only rarely been confused with authentic fossil microbes.

In Fig. 3J through M are shown examples of one additional type of filamentous pseudofossil, narrow elongate chert-embedded microstructures of the type discussed above as having been mistaken for oscillatoriacean "blue-green algae" (cyanobacteria) by Gruner (1923, 1924), Tilden (1935), and Vologdin and Korde (1965). As would be expected of such cyanobacteria, these specimens are of an appropriate width (~10 μm in diameter), have variable lengths, range from straight to gently curved to sinuous, and are more or less cylindrical in cross section. Despite these "life-like" characteristics, such microstructures – all of which have nonbiologic crystalline striated surfaces, rather than smooth microbe-like exteriors; lack biologically distinctive lateral walls and cell-defining transverse septa; and are devoid of kerogenous organic matter – have been shown to be nonbiologic threadlike trails produced by minute ambient pyrite grains as they moved through an encompassing chert matrix (Tyler and Barghoorn, 1963; Knoll and Barghoorn, 1974; Hofmann and Schopf, 1983; Grey, 1986; Mendelson and Schopf, 1992). Not only does the morphology and chemistry of these distinctive pseudofossils differ from that of *bona fide* microbial fossils (Table 1), but their nonbiologic origin is well established.

5. Summary and conclusions

A long history of the sporadic misintepretation of Precambrian microscopic pseudofossils, coupled with evident differences between the relatively well-documented Proterozoic fossil record and that of more ancient, Archean-age deposits, has raised doubt about early evidence of life, for some workers including all evidence older than ~1900 Ma (Moorbath, 2005). Such misgivings are unfounded. Not only are microbially laminated stromatolites known from some 50 Archean geological units, *bona fide* microbial microfossils from 40 such units, and evidence of biological carbon isotopic fractionation from hundreds of Archean deposits (Strauss and Moore, 1992; Hofmann, 2000; Schopf, 2004, 2006a,b; Allwood et al., 2006, 2007; Sugitani et al., 2007, 2009; Van Kranendonk et al., 2008; Oehler et al., 2009; Sugahara et al., 2009; Waldbauer et al., 2009), but recent studies have confirmed the biological origin of the fossil microbes of the ~3465 Ma Apex chert of northwestern Australia (Schopf et al., 2002, 2007; De Gregorio et al., 2009) – the oldest diverse assemblage of microfossils known (Schopf, 1992a, 1993) – cellularly preserved microorganisms that exhibit all of the characteristics of *bona fide* filamentous prokaryotic fossils listed in Table 1. Microbial life was extant and flourishing at least as early as ~3500 Ma.

Nevertheless, and though errors in the interpretation of putative Precambrian fossil microbes have diminished greatly over recent years, mistakes continue to be made. In contrast with the other four criteria to be satisfied by authentic fossils – the *provenance* and *age* of the rock from which they are reported, and their *indigenousness* to and *syngeneticity* with the formation of such host rocks – the fifth criterion, establishment of their *biogenicity*, has proved particularly difficult to meet.

Demonstration of the biogenicity of such microscopic objects is crucial to deciphering early records of life on Earth and may prove equally critical to analyses of any fossil-like objects detected in rocks to be acquired from Mars. For example, use of criteria such as those listed in Table 1 have shown that microfossil-like objects reported from Martian meteorite ALH84001 (McKay et al., 1996) are of nonbiologic origin, narrow elongate microstructures that lack evidence of cell walls; are evidently solid, rather than enclosing identifiable remnants of cell cavities; and are of composed of pyroxene or carbonate, rather than of carbonaceous matter (Bradley et al., 1997; Schopf, 1999b). Interpretation of other mineralic structures known as "biomorphs" – were they to be found in nature – might be more problematic. These crystalline structures, synthesized in laboratory experiments by the coprecipitation of barium carbonate (witherite) and silica from alkaline media, exhibit a broad array of micron- to millimeter-sized "life-like" morphologies, including globular, filamentous, spiral, and radial forms that resemble microorganisms (García-Ruiz et al., 2002, 2003, 2009). Composed of solid nanocrystallites, they can be enveloped by a silica skin (Kunz and Kellermeir, 2009) shown to absorb organic compounds (García-Ruiz et al., 2003). Although such structures are

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 like those of other microbes, both modern and fossil; and they are represented by numerous specimens, exhibit varying states of preservation, and are members of well-documented biologically diverse microbial communities. All of these specimens also exhibit the biologically derived chemistry expected of authentic organic-walled fossils: Raman spectroscopy shows them to be composed of biogenic kerogen (for the geochemically relatively unaltered Bitter Springs specimens, a result backed by their CLSM-detectable laser-induced fluorescence).

In contrast with these *bona fide* Precambrian microbial fossils, optical microscopy and Raman analyses of coccoidal and filamentous fossil-like objects in the other five geological units indicates that they are most probably microscopic pseudofossils: for the coccoids, hematite spherules (Lakhanda Formation), goethite and magnetite grains (Chanda Formation), and quartz-kerogen spherulites (Marra Mamba Iron Formation); and for the filaments, a manganite vein filling (Chanda Formation), quartz-filled veinlets (Myrtle Springs and Vempalle Formations), and trails of ambient pyrite grains (Myrtle Springs Formation). These apparent pseudofossils lack all or many of the characters of biological morphology expected of authentic microbial fossils (Table 1) and only one, the Marra Mamba spherulites, exhibits biologically derived chemistry.

Though only one of these varied types of putative pseudofossils exhibits biologically derived chemistry, this exception illustrates a cardinal lesson, namely, that for establishment of biogenicity, there is no single "smoking gun." No one criterion is sufficient to establish the biological origin of microscopic fossil-like objects. Not only is this true of a kerogenous composition (cf. Pasteris and Wopenka, 2003), but it applies also to the numerous characteristics that evidence biological morphology: for example, the quartz-filled veinlets of the Myrtle Springs and Vempalle cherts, interpreted here as pseudofossils, have microbe-like widths and a superfically cell-like appearance; and though the trails of ambient pryite grains in the Myrtle Springs cherts have microbe-like sizes and shapes, they, too, seem assuredly nonbiologic.

To resolve the question of the biogenicity of putative Precambrian microfossils, we therefore suggest the use of mutually reinforcing characters that, taken together, can document the presence both of biological morphology and of biologically derived chemistry. We recognize that certain of the techniques used here to evaluate such objects – viz., CLSM and Raman imagery, and color photomicrography – are not currently available to all workers, worldwide. Nevertheless, it seems to us that their use can provide the level of rigorous analysis and data-presentation needed to resolve this question, an interdisciplinary approach illustrated here that combines the data and techniques of geology, biology, and chemistry.

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