

Rapid Communication

Discovery of a New Chert-Permineralized Microbiota in the Proterozoic Buxa Formation of the Ranjit Window, Sikkim, Northeast India, and Its Astrobiological Implications

J. William Schopf,^{1,2} Vinod C. Tewari,³ and Anatoliy B. Kudryavtsev^{2,4}

Abstract

For the foreseeable future, the search for evidence of past life in rocks acquired from other planets will be constrained by the amount of sample available and by the fidelity of preservation of any fossils present. What amount of rock is needed to establish the existence of past life? To address this question, we studied a minute amount of rock collected from cherty dolomites of the Proterozoic Buxa Formation in the metamorphically altered tectonically active northeastern Himalaya. In particular, we investigated 2 small petrographic thin sections—one from each of 2 bedded chert horizons exposed in the Ranjit River stratigraphic section northwest of Rishi, Sikkim, India—that together comprise an area of $\sim 5 \text{ cm}^2$ (about the size of a US postage stamp) and have a total rock weight of $\sim 0.1 \text{ g}$. Optical microscopy, confocal laser scanning microscopy, and Raman spectroscopy and imagery demonstrate that each of the thin sections contains a rich assemblage of 3-dimensionally permineralized organic-walled microfossils. This study, the first report of Proterozoic microfossils in units of the Ranjit tectonic window, demonstrates that firm evidence of early life can be adduced from even a minuscule amount of fossil-bearing ancient rock. **Key Words:** Buxa Formation—Ranjit tectonic window—Precambrian microfossils—Mars Sample Return mission—Raman spectroscopy—Confocal laser scanning microscopy. *Astrobiology* 8, 735–746.

Introduction

AS THE FIELD OF ASTROBIOLOGY HAS EMERGED in recent years, it has become widely accepted that the search for life elsewhere in the Cosmos should center on a search for evidence of microbe-level living systems—whether ancient or extant—in part because such “simple” forms can be expected to exist even if more advanced life has subsequently arisen, but largely because microbe-level life has been ubiquitous, abundant, metabolically diverse, and, for the earliest (Precambrian) 4 billion years of Earth’s history, biotically predominant. In the search for life elsewhere, the prokaryote-dominated world of the Precambrian is the best analogue we know.

Missions are now planned to sample the surface of Mars and deliver the regolith and rocks acquired to Earth for scientific study. For the first several such missions, however, the amount of material to be returned seems likely to be minimal, a few hundred grams at most, its weight limited chiefly by the payload that can be carried by the returning vehicles. Moreover, much and perhaps all of the surficial debris and pebble-sized rocks thus returned are likely to be geologically ancient, having experienced over their protracted history the fossil-degrading and ultimately obliterating effects of the heat and pressure of volcanism, bolide impacts, and the like. Given such constraints, is it plausible to imagine that such missions might yield definitive evidence of past life?

¹Department of Earth and Space Sciences, Institute of Geophysics and Planetary Physics (Center for the Study of Evolution and the Origin of Life), Molecular Biology Institute, and ²NASA Astrobiology Institute, University of California, Los Angeles, California.

³Wadia Institute of Himalayan Geology, Dehradun, Uttarakhand, India.

⁴Institute of Geophysics and Planetary Physics (Center for the Study of Evolution and the Origin of Life) and ²NASA Astrobiology Institute, University of California, Los Angeles, California.

A first step toward answering that question is provided by the results reported here from studies of an exceptionally small sample of Precambrian rock obtained from a tectonically and metamorphically altered terrain. In particular, slivers of rock were collected from the Proterozoic Buxa Formation in the Ranjit tectonic window—a region from which cellularly preserved Precambrian fossils have not previously been reported—which is situated in northeastern-most India in the tectonically active northeast Himalaya. To maximize the potential for the discovery of ancient microbial fossils, (1) a stratigraphic section known to contain carbonate stromatolites was selected for sampling, and (2) two small pieces of chert (a rock type that, though very commonly not fossil-bearing, provides potential for the cellular preservation of minute microbes) were collected for study, both of which are dark gray to black in color (an indication of the presence of opaque minerals, such as pyrite, and of the possible presence of carbonaceous matter such as that comprising chert-permineralized organic-walled fossils). Although the co-occurrence of stromatolites and black cherts in this stratigraphic section prompted its selection for sampling, the bedded cherts themselves were not obviously stromatolitic, either in outcrop or in hand specimens. Only 2 petrographic thin sections, 1 from each of the 2 rock specimens, were available for study. The sections were notably small (each ~ 2.5 cm², about half the size of a US postage stamp), and together they comprised a minuscule amount of rock (~ 0.04 cm³, less than half the volume of a grain of rice), having a total rock weight of ~ 0.1 g.

As is documented below, each of these 2 samples of cherts from the Buxa Formation—despite their minute size and occurrence in a tectonically active metamorphosed terrain—contain definitive evidence of ancient life. If discovered in a sample returned from Mars, the abundant and diverse assemblage of organic-walled fossil microorganisms reported here would, for most scientists, constitute firm evidence of the past existence of life on the Red Planet.

Materials and Methods

Materials studied

The permineralized microfossils reported here were studied in petrographic thin sections BR-1 and BR-2, which were prepared from rocks collected (by V.C.T.) from outcrops of 10–12 cm thick chert beds, BR1 and BR2 situated, respectively, ~ 70 and 100 m stratigraphically above the base of the predominantly dolomitic Proterozoic Buxa Formation in the Ranjit tectonic window, Sikkim, northeastern India (Fig. 1). The locality sampled is ~ 10 km northwest of Namchi and ~ 2 km northwest of Rishi, Sikkim, at 27°15'N latitude and 88°18'E and longitude, in the northeast Lesser Himalaya (Fig. 1b, 1c). The 2 thin sections (BR-1: $\sim 1.15 \times 2.0$ cm, ~ 50 μ m thick; and BR-2: $\sim 1.35 \times 2$ cm, ~ 110 μ m thick) have a total area of ~ 5 cm² and a combined volume of ~ 0.04 cm³ that, given the density of silica of ~ 2.6 g/cm³, represents a total rock weight of ~ 0.1 g. The fossils were located by optical microscopy and documented by photomicrography; selected specimens were subsequently imaged by confocal laser scanning microscopy (Schopf *et al.*, 2006) or were analyzed by Raman spectroscopy or imagery (Schopf *et al.*, 2005a, 2005b). Some specimens were examined via both techniques.

Optical microscopy

Studied in the unpolished petrographic thin sections specified above (each finished by use of a slurry of 600 mesh carborundum and covered by a ~ 1 μ m thick veneer of Type FF, fluorescence-free, microscopy immersion oil; R.P. Cargille Laboratories, Inc., Cedar Grove, NJ), transmitted-light optical photomicrographs of the specimens were obtained by use of UCLA #0026535 Leitz Orthoplan 2 microscope (Leitz, Wetzlar, Germany) equipped with an Olympus DP12 Microscope Digital Camera (Olympus, Melville, NY). Microscope stage coordinates for specimens illustrated here from thin sections BR-1 and BR-2 (oriented with the thin section label to the right) are noted in the captions for Figs. 3–7.

Confocal laser scanning microscopy

Three-dimensional confocal fluorescence images were obtained by use of an Olympus FluoView 300 confocal laser scanning biological microscope system equipped with 2 Melles Griot lasers, a 488 nm 20 mW output ion argon laser, and a 633 nm 10 mW output ion helium-neon laser (Melles Griot, Carlsbad, CA). Confocal laser scanning microscopy (CLSM) images were acquired via a 60 \times oil-immersion objective (numerical aperture = 1.4), with the use of the fluorescence-free microscopy immersion oil noted above and filters in the light-path of the system that permitted transmission to its detector only of wavelengths >510 nm (for 488 nm laser excitation) and >660 nm (for 633 nm laser excitation) from the kerogen-derived fluorescence emitted by the specimens.

Raman spectroscopy and imagery

Raman spectra of the kerogen that comprises the permineralized fossils were obtained by use of a T64000 (JY Horiba, Edison NJ) triple-stage laser-Raman system having macro-Raman and confocal micro-Raman capabilities. This system permitted acquisition both of individual point spectra and of Raman images that display the 2-dimensional spatial distribution of molecular-structural components of the specimens and their associated mineral matrices, with the varying intensities in such images corresponding to the relative concentrations of the molecular structures detected. Due to the confocal capability of the system, use of a 50 \times objective (having an extended working distance of 10.6 mm and a numerical aperture of 0.5) provided a horizontal resolution of ~ 1.5 μ m and a vertical resolution of 2–3 μ m. Use in this system of a Coherent Innova (Santa Clara, CA) argon ion laser to provide excitation at 457.9 nm permitted measurements to be made over a range from <500 cm⁻¹ to >3100 cm⁻¹ by use of a single spectral window centered at 1800 cm⁻¹. Spectra were acquired simultaneously of the major bands (at ~ 1365 and ~ 1604 cm⁻¹) and the second-order band (at ~ 2800 cm⁻¹) of the kerogen that comprises the fossils as well as of the major band of the permineralizing quartz (at ~ 465 cm⁻¹) and that of subsidiary calcite (at 1087 cm⁻¹).

For imaging, the region of a thin section containing a specimen to be analyzed was covered by a thin-veneer fluorescence-free microscopy immersion oil [the presence of which has been shown to have no discernable effect on the Raman spectra acquired; (Schopf *et al.*, 2005a)], and the area of the fossil studied was centered in the path of the laser beam pro-

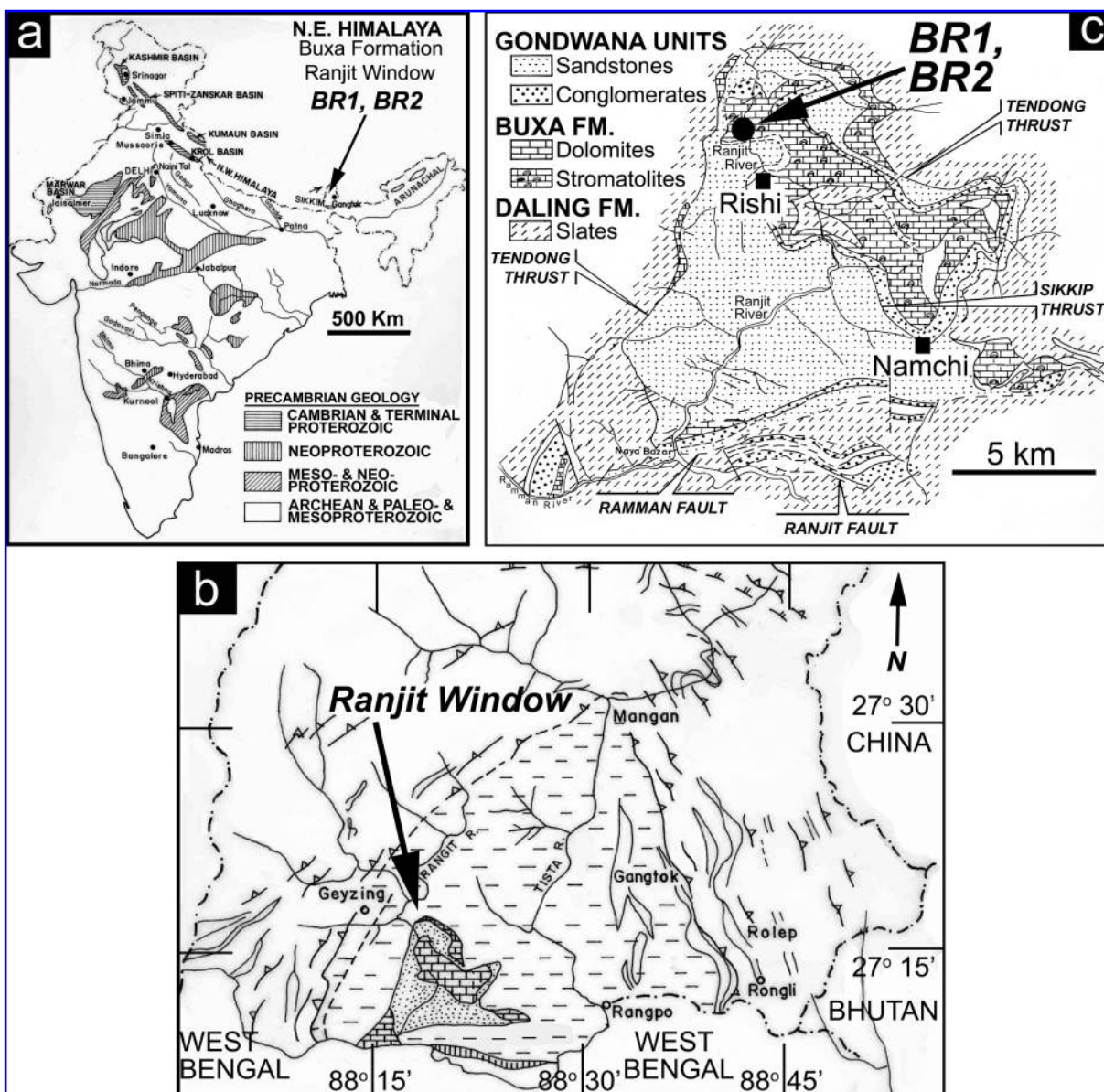


FIG. 1. Geologic maps showing the location of the microfossiliferous carbonaceous cherts of the Proterozoic Buxa Formation in northeastern India. (a) Sketched map of the Indian subcontinent showing the major Precambrian outcrop areas, the geochronologic relations within many of which (e.g., the Vindhyan Basin of north-central India) are poorly constrained, and the location of fossil-bearing samples *BR1* and *BR2* (Buxa Formation, Ranjit Window, chert beds 1 and 2) in the northeast Himalaya. (b) Location of the Ranjit Tectonic Window in the Indian state of Sikkim, north of the state of West Bengal and west of Bhutan. (c) Proterozoic strata of the Ranjit Window (the Buxa Formation and immediately underlying Daling Formation) surrounded by Phanerozoic-age Gondwana units (after Raina, 1976). As is indicated, fossil-bearing samples *BR1* and *BR2* were collected from outcrops situated about 10 km northwest of Namchi and about 2 km northwest of Rishi, Sikkim, at 27°15'N latitude, 88°18'E longitude.

jected through the system's microscope. The laser power used was ~1–8 mW over a ~1 μ m spot, an instrumental configuration well below the threshold that results in radiation damage to specimens such as those here studied (Schopf *et al.*, 2005a).

Geologic Setting

Situated in the northeastern Lesser Himalaya in Sikkim, a state of India that shares boundaries with the state of West

Bengal to the south and with Bhutan and China to the east (Fig. 1a, 1b), the Proterozoic Buxa Formation is predominantly dolomitic (Fig. 2). As is shown in Fig. 1c, the sampled locality occurs in the Ranjit tectonic window, a surviving remnant of Precambrian strata that is surrounded by much younger, Permian-age, tectonically folded Gondwana units. Within this window of Proterozoic deposits, the Buxa Formation, underlain by the Daling Formation (Fig. 1c), is the youngest unit of the Daling Group. Table 1 summarizes the stratigraphic succession within the Ranjit tectonic window

(Fig. 1c) and the position of the Buxa Formation within this sequence.

Lithology and stromatolites of the Buxa Formation

As is summarized in Fig. 2, the Buxa Formation, which extends eastward from Sikkim past Bhutan into the Indian state of Arunachal, is composed of cherty stromatolitic, oolitic, and intraclastic dolomites; limestones; calcareous quartzites; and black shales, slates, and phyllites (Acharyya, 1974; Raina, 1976; Sinha Roy, 1980; Tewari, 2001, 2003). Within the Ranjit tectonic window, the ~800 m thick Buxa Formation has been measured stratigraphically (by V.C.T.) along the Ranjit River from the locality sampled here to the village of Rishi, some 2 km to the southeast (Fig. 1c). The lower part of the formation, the source of the cherts studied here, is composed of a ~250 m thick sequence of well-bedded grey cherty dolomite (e.g., Fig. 3a) that contains stromatolites of the groups *Colonnella*, *Conophyton*, *Kussiella*, *Nucleella*, and *Stratifera* (Fig. 2). Two lenses of well-bedded carbonaceous chert within this sequence (BR1 and BR2, Fig. 2) were sampled for the present study. The middle third of the formation, stratigraphically above the sampled horizons, consists of a 300 m thick succession of finely laminated silicified grey dolomites, which contains thin bands of black chert and olive grey shales. The upper third of the Buxa Formation, its uppermost 250 m, is composed of interbedded dolomites and subsidiary

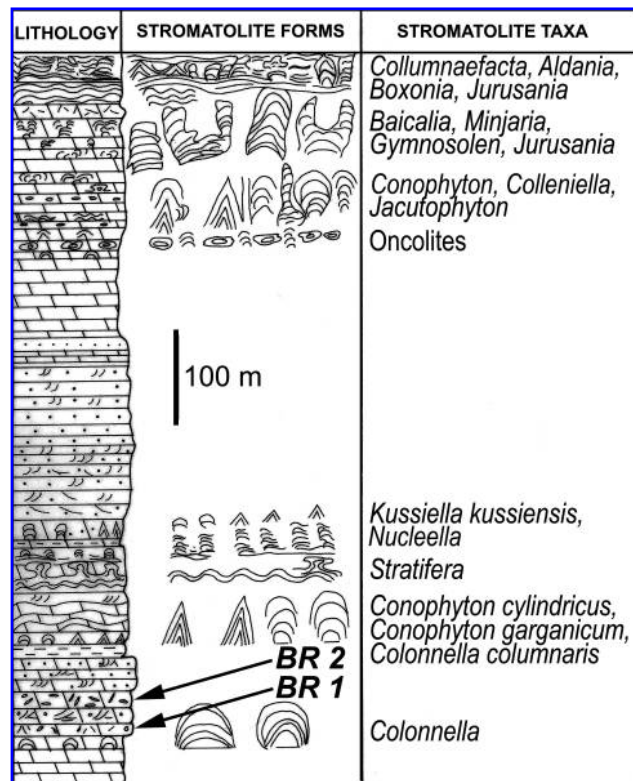


FIG. 2. Measured stratigraphic section of the dolomitc Buxa Formation along the Ranjit River, immediately northwest of Rishi, Sikkim (Fig. 1c), showing the stratigraphic distribution of stromatolite forms and named stromatolitic taxa and the position within the lowermost 100 m of the formation of fossiliferous samples BR1 and BR2.

TABLE 1. TECHNOSTRATIGRAPHY OF THE RANJIT TECTONIC WINDOW REGION, SIKKIM, INDIA*

PRECAMBRIAN (Paleoproterozoic)

SIKKIM/DARJEELING GROUP

Chungthang Formation

Gneisses, quartzites, amphibolites

—Main Central Thrust—

PALEOZOIC (Permian Gondwana Units)

RANJIT GROUP

Damuda (Namchi) Formation

Sandstones, black shales, coals

Ranjit (Tatapani) Pebble Slate Formation

—Unconformity/Tectonic Contact—

PRECAMBRIAN (Meso- to Neoproterozoic)

DALING GROUP

→ Buxa Formation

Stromatolitic cherty dolomites, pyritic black slates

Daling (Reyang) Formation

Quartzites, slates, cherts, carbonates, conglomerates

Gorubathan Formation

Volcanoclastics, metasediments

—Main Boundary Thrust—

CENOZOIC (Tertiary)

Paleogene-Neogene sediments

*Ranging geographically from the Sikkim/Darjeeling Group and the Main Central Thrust in the north to Paleogene-Neogene sediments and the Main Boundary Thrust in the south; arrow denotes the position of the microfossil-bearing Buxa Formation studied here. Simplified after Acharyya (1974), Raina (1976), Sinha Roy (1980), and Tewari (2001, 2003).

dark gray shales, the dolomitc beds of which contain oncolites as well as stromatolites of the groups *Aldania*, *Baicalia*, *Boxonia*, *Colleniella*, *Collumnaefacta*, *Conophyton*, *Gymnosolen*, *Jurusania*, and *Minjaria* (Fig. 2).

Age of the Buxa Formation

The radiometric age of the Buxa Formation is yet to be determined. Earlier workers, on the bases of lithostratigraphy and the presence of the stromatolite assemblages noted above, suggested ages that range from Late Precambrian to Late Paleozoic (Acharyya, 1974; Raina, 1976; Sinha Roy, 1980; Valdiya, 1980). Though neither of these lines of evidence provided firm evidence of the age of the unit, more recent geologic studies and the findings of ministromatolites (reported also as "microstromatolites") and of characteristically Neoproterozoic organic-walled microfossils in the Buxa Formation in Arunachal state (Tewari, 2001, 2003; Shukla *et al.*, 2006), some 500 km to the east of the locality sampled here, have established the Precambrian age of the unit. The lithology and stromatolite assemblages of the Buxa Formation in the Ranjit tectonic window (northeast Lesser Himalaya) are notably similar to those of dolomites of the Meso-Neoproterozoic Shali-Deoban-Gangolihat carbonate belt of the northwest Lesser Himalaya, a comparability regarded as suggesting that units of the 2 regions may be stratigraphically correlative (Tewari, 1989). The assemblage of Neoproterozoic microfossils recently reported from the Buxa For-

mation in Arunachal (Shukla *et al.*, 2006) and from the north-west Lesser Himalaya (Tewari, 2004, and references therein) are consistent with this possibility, as is the composition of the biota recorded here, including the occurrence in such assemblages of *Obruchevella*, a helically coiled fossil cyanobacterium similar to the modern oscillatoriacean *Spirulina* and a taxon that is particularly widespread in latest Proterozoic to earliest Cambrian strata.

Microscopic Fossils

Given the miniscule amount of rock studied here, 2 thin sections having a total area of $\sim 5 \text{ cm}^2$ and a total rock weight of $\sim 0.1 \text{ g}$, it would be inappropriate to use this preliminary investigation as a basis for the formal monographic treatment that is merited by the Buxa microbiota of the Ranjit window or to attempt its comprehensive comparison with the diverse fossil assemblage known from the formation in Arunachal state (Shukla *et al.*, 2006). Rather, our more modest goal is to record the most abundant components of the assemblage and document their biogenicity.

Petrology of the fossil-bearing cherts

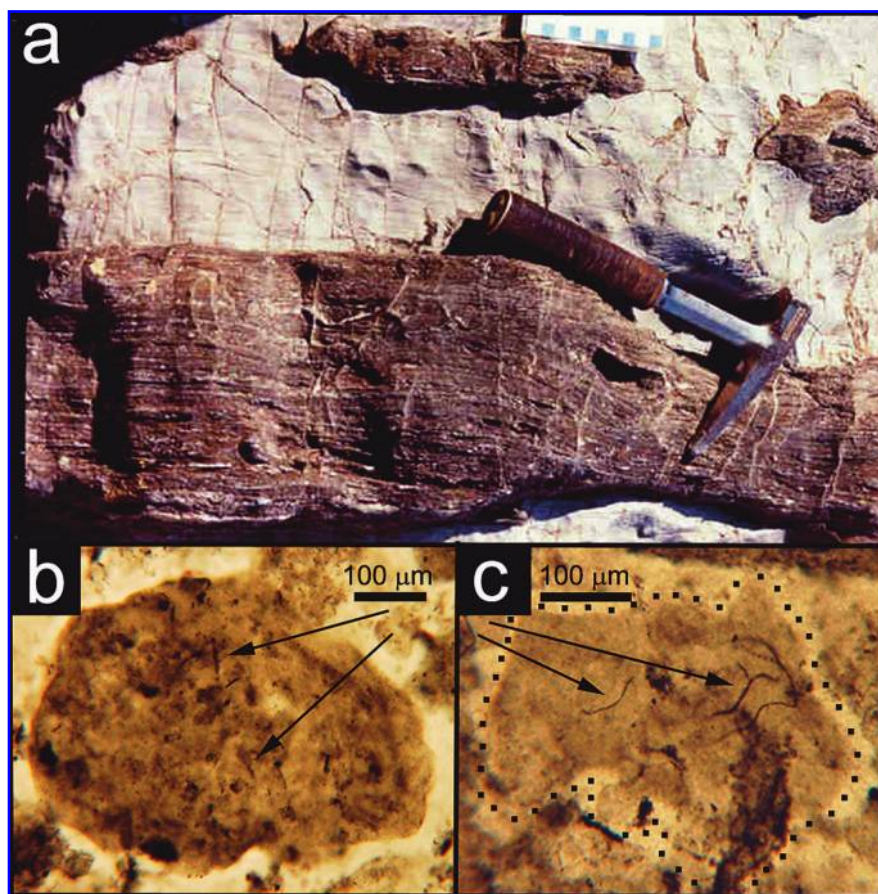
Studied in thin sections, both of the chert samples were seen to be composed of interlocking mosaics of fine-grained quartz in which occur closely packed, millimeter-sized, sub-angular to rounded silicified fossil-bearing detrital clasts and subsidiary, euhedral, carbonate rhombs ~ 20 to $40 \mu\text{m}$ in long

dimension. Figure 3b and 3c show examples of 2 such clasts, virtually all of which appear to be fragments of originally coherent, mucilage-embedded microbial mats that have been removed from their site of formation, redeposited, and then silicified to form the bedded cherts. The quartz grains in which the fossils are permineralized are generally $10\text{--}15 \mu\text{m}$ in size, with some grains as much as $40 \mu\text{m}$ in long dimension. The maximum size of these evidently somewhat recrystallized grains—appreciably larger than the $6\text{--}12 \mu\text{m}$ sized quartz grains typical of fossil-bearing unmetamorphosed Neoproterozoic cherts [*e.g.*, those of the Bitter Springs Formation of central Australia (Schopf, 1968; Schopf and Blacic, 1971)]—is consistent with the tectonic setting in which the Buxa cherts occur. Similarly, the geochemically rather mature state of the kerogen that comprises the fossils (discussed below), which is indistinguishable from that of particulate kerogen in their surrounding matrices, reflects preservation in a moderately metamorphosed terrain.

Abundance of fossils

As viewed in the 2 petrographic thin sections, the fossil-bearing detrital clasts of the Buxa cherts range from small ovate bodies that average about 0.5 mm in diameter (in thin section BR-1, ranging from $\sim 0.4 \text{ mm}$ to 1.8 mm ; and in BR-2, ~ 0.1 to 2 mm) to larger elongate shards, shreds, and pieces of originally mucilage-bound microbial mats (in BR-1, ~ 0.3 to 2 mm broad and ~ 1 to 8 mm long; and in BR-2, ~ 0.1 to 0.3 mm broad and ~ 1 to 4 mm long). Each of the thin sec-

FIG. 3. Field photo (a) of bedded cherts of the Proterozoic Buxa Formation in the Ranjit River stratigraphic section (*cf.* Figs. 1c and 2) and optical photomicrographs of fossil-bearing clasts. Rounded (b) and sub-angular (c) detrital clasts in chert thin section BR-1 [(b) microscope stage coordinates 49.6×96.4 ; (c) 47.7×102.5]. Arrows point to filamentous microfossils; the dotted line in (c) outlines a fossiliferous clast.



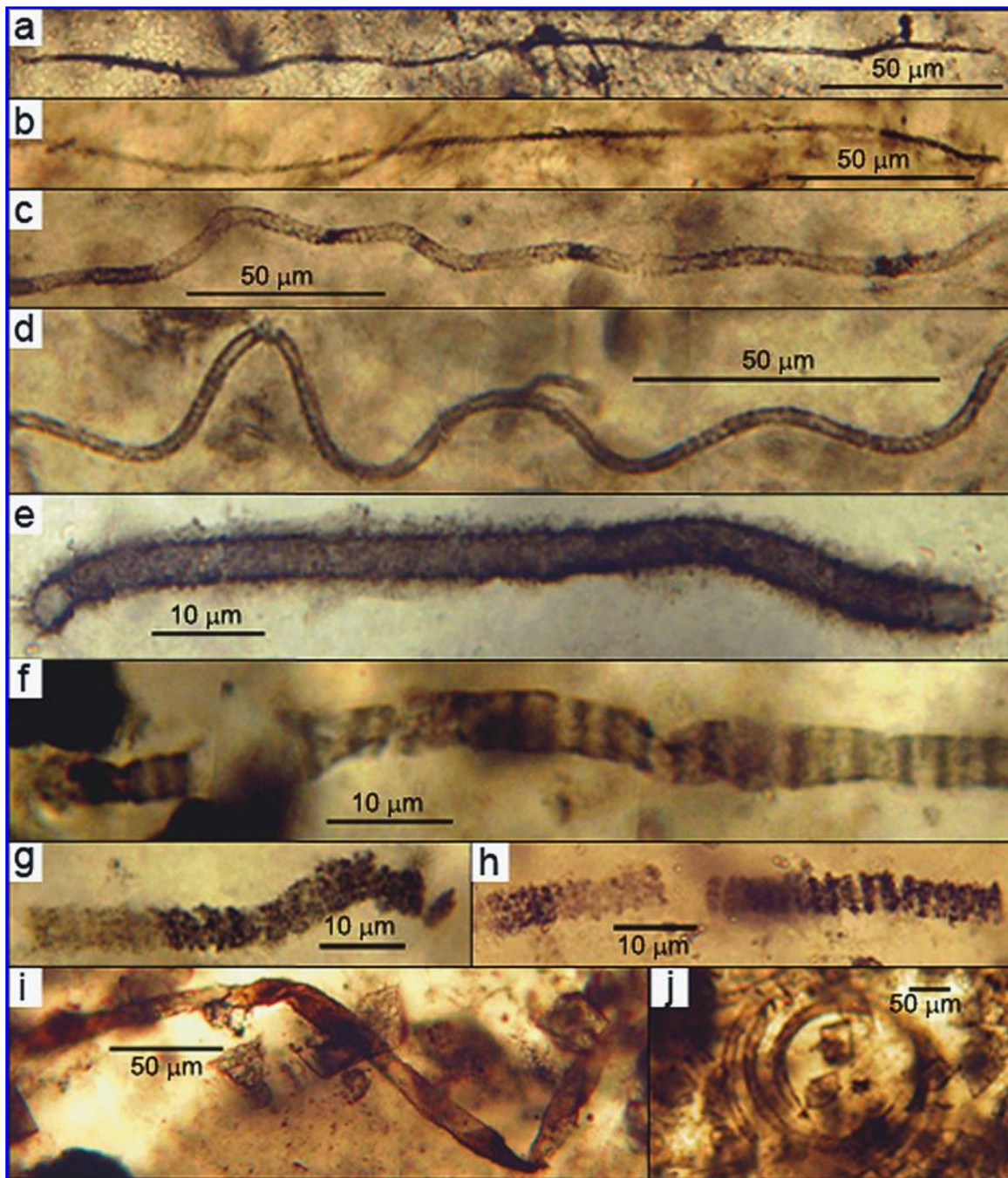


FIG. 4. Optical photomicrographs showing filamentous chert-permineralized microfossils in petrographic thin sections of the Proterozoic Buxa Formation of the Ranjit Window, Sikkim, northeastern India. Parts (a–i) show photomontages, necessitated by the 3-dimensional sinuosity of the figured specimens. (a) and (b) Narrow (~ 1 to $3\ \mu\text{m}$ diameter) bacterial and/or cyanobacterial filaments, cf. *Archaeotrichion* [(a) thin section BR-1, microscope stage coordinates 49.5×99.9 ; (b) section BR-1, 35.6×94.9]. (c–e) Tubular ~ 3 to $5\ \mu\text{m}$ diameter filaments (cf. *Eomycetopsis*), the cylindrical extracellular sheaths of oscillatoriacean cyanobacteria [(c) section BR-1, 42.5×100.5 ; (d) section BR-1, 35.0×92.8 ; (e) section BR-2, 34.4×103.7]. (f) Septate ~ 3 to $5\ \mu\text{m}$ diameter oscillatoriacean trichome (cf. *Cephalophytarion*) composed of box-shaped cells (section BR-2, 33.5×96.5). (g) and (h) Septate $\sim 5\ \mu\text{m}$ diameter oscillatoriacean trichomes (cf. *Oscillatorioopsis*) composed of disc-shaped cells (both specimens are from the same area of section BR-2, 31.6×97.8). (i) Broad ($\sim 10\ \mu\text{m}$ diameter) ribbon-like filament (cf. *Siphonophycus*), the flattened originally tubular extracellular sheath of an oscillatoriacean cyanobacterium (section BR-2, 32.9×102.6). (j) Helically coiled ~ 15 to $20\ \mu\text{m}$ diameter filament, cf. *Obruchevella*, a fossil oscillatoriacean similar to modern *Spirulina* (section BR-2, 41.0×99.4).

tions contains ~200 such clasts, which comprise 70–80% of their total area, the remainder being composed of interstitial fine-grained quartz in which occur abundant carbonate rhombs.

In each of the 2 thin sections, the abundance of identifiable fossils varies markedly both among and within their component clasts. In thin section BR-1, ~60% of such clasts are fossil bearing, whereas less than 20% of the generally smaller clasts in section BR-2 are fossiliferous; both sections contain clasts that, though fossil-bearing in one region, are devoid of fossils elsewhere (e.g., Fig. 3c). As is typical of Precambrian microbial communities, the concentration of structurally preserved microbes in the clasts is patchy. Quantified in terms of thin section area, some clasts are completely devoid of fossils or include only 1–2 fossils/mm² (equivalent to ~10⁸ μm³), whereas others contain localized packets of narrow, 1–3 μm diameter filaments (e.g., Fig. 4a, 4b), which are densely interlaced and present in concentrations of a few to several hundred fossils per mm². Numerous clasts contain entangled networks of subparallel ~3 to 5 μm diameter filaments (e.g., Fig. 4c–e) that have a density of tens to more than a hundred specimens/mm². In general, filamentous fossils tend to be particularly abundant in the elongate clasts, whereas the relatively rare, smaller-celled, coccoidal fossils (e.g., Figs. 6, 7a–m) typically occur in localized regions, whether in ovate or elongate clasts, where they are present singly, in small clusters of a few to ~30 cells, or in larger colonies composed of a few hundred cells (e.g., Fig. 6a, 6t).

It should be noted that the foregoing data regarding fossil abundance, despite being based on systematic measurements of clast sizes and fossil concentrations, are approximations. The patchy millimetric-scale distribution of the Buxa fossils is typical both of fossilized microbial communities and of their modern equivalents (where it is a result of founder effects—e.g., colonization by a particularly rapidly proliferating species—and of local environmental conditions such as the availability of light and nutrients, initial growth conditions, competition with near neighbors, and so forth). Moreover, for fossilized communities, assessment of “microbial density” can be based only on morphologically identifiable fossil remnants, which because of post-mortem degradation represent just a fraction of the assemblage originally present. Such assessment cannot take into account organic debris that does not exhibit identifiable evidence of its biologic origin (for the Buxa cherts, like that of all such deposits, comprising ≥95% of the organic matter preserved), nor can it include the tiny bits and pieces of cellular debris—true fossils, but optically nondescript—that occur in such deposits.

Illustrated morphotypes

As is typical of permineralized Neoproterozoic microbial assemblages, the Buxa cherts studied here contain both filamentous and coccoidal microfossils. The filamentous forms include narrow specimens, 1–3 μm in diameter, that are similar to fossils of the genus *Archaeotrichion* of bacterial or cyanobacterial affinity (Fig. 4a, 4b); tubular 3–5 μm diameter filaments, cf. *Eomycetopsis*, a fossil taxon composed of the cylindrical extracellular sheaths of oscillatoriacean cyanobacteria (Fig. 4c–e); and similarly sized septate filaments that closely resemble species of *Cephalophytarion* (Fig. 4f) and *O-*

cillatoriopsis (Fig. 4g, h), taxa of fossilized cellular oscillatoriacean trichomes. Also present are broad, 10 μm diameter non-septate filaments, the encompassing sheaths of relatively large-diameter oscillatoriaceans (Fig. 4i), and 15–20 μm diameter helically coiled specimens, cf. *Obruchevella* (Fig. 4j), a fossil taxon composed of specimens similar to the mod-

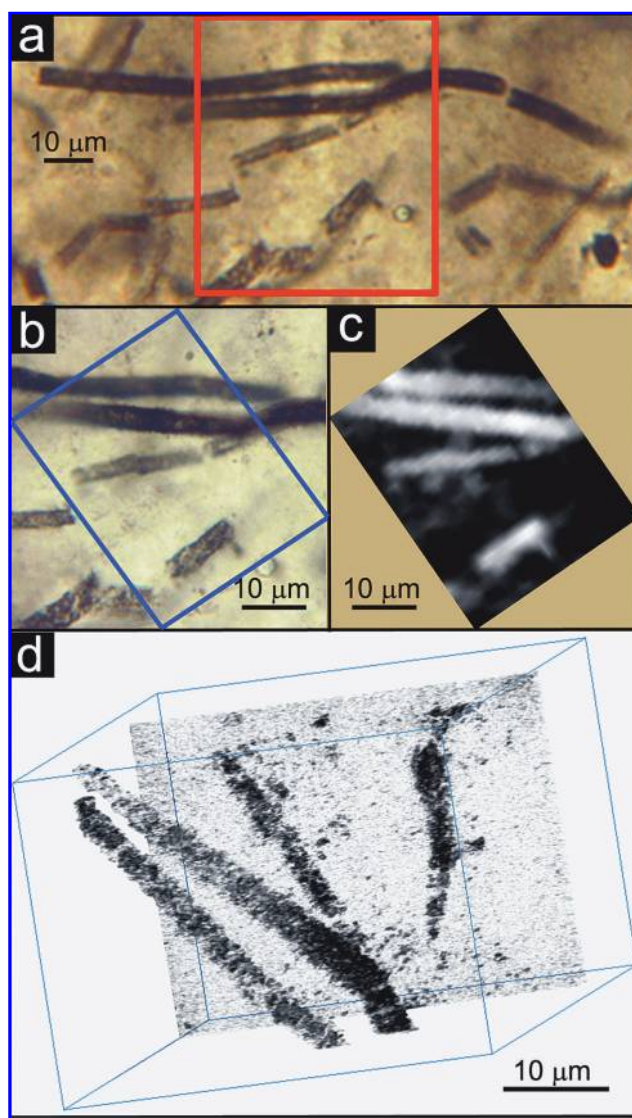


FIG. 5. Optical photomicrographs, Raman image, and CLSM image of chert-permineralized tubular cyanobacterial (oscillatoriacean) sheaths in a petrographic thin section of the Proterozoic Buxa Formation of the Ranjit Window, Sikkim, northeastern India. (a) and (b) Photomicrographs of the specimens (*Eomycetopsis* sp.) with the red rectangle in (a) denoting the area shown in (b) and (d), and the blue rectangle in (b) denoting the area shown in (c). (c) Raman image acquired in a spectral window centered at the ~1600 cm⁻¹ band of kerogen that documents the carbonaceous composition of the fossils. (d) CLSM image of the fossils, rotated to show the specimens from the underside of the thin section (the gray focal plane at the back of the figure being situated toward the top of the section) and a perspective for viewing of the fossils uniquely provided by CLSM, demonstrating the 3-dimensionality of the filaments.

ern oscillatoriacean *Spirulina*. The uniseriate nature of the cellular trichomes (Fig. 4f–h) evidences their formation by repeated cell divisions perpendicular to the long axes of the filaments, whereas CLSM studies of such filaments, results of a representative example of which are shown in Fig. 5d, demonstrate their 3-dimensional form. Of the various morphotypes detected—all of which are well known from numerous Neoproterozoic assemblages (e.g., Schopf, 1992a,

1992b)—the *Eomycetopsis*-like extracellular sheaths are particularly abundant, whereas well-preserved cellular trichomes, such as that shown in Fig. 4f, are rare.

Included among the coccoidal fossils of the assemblage are small, 3–5 μm diameter, originally mucilaginous envelope-enclosed colonial coccoids that closely resemble those of the fossil chroococcacean cyanobacterium *Palaeoanacystis* (Fig. 6a–i) and dyads and tetrads composed of 5–7 μm diameter

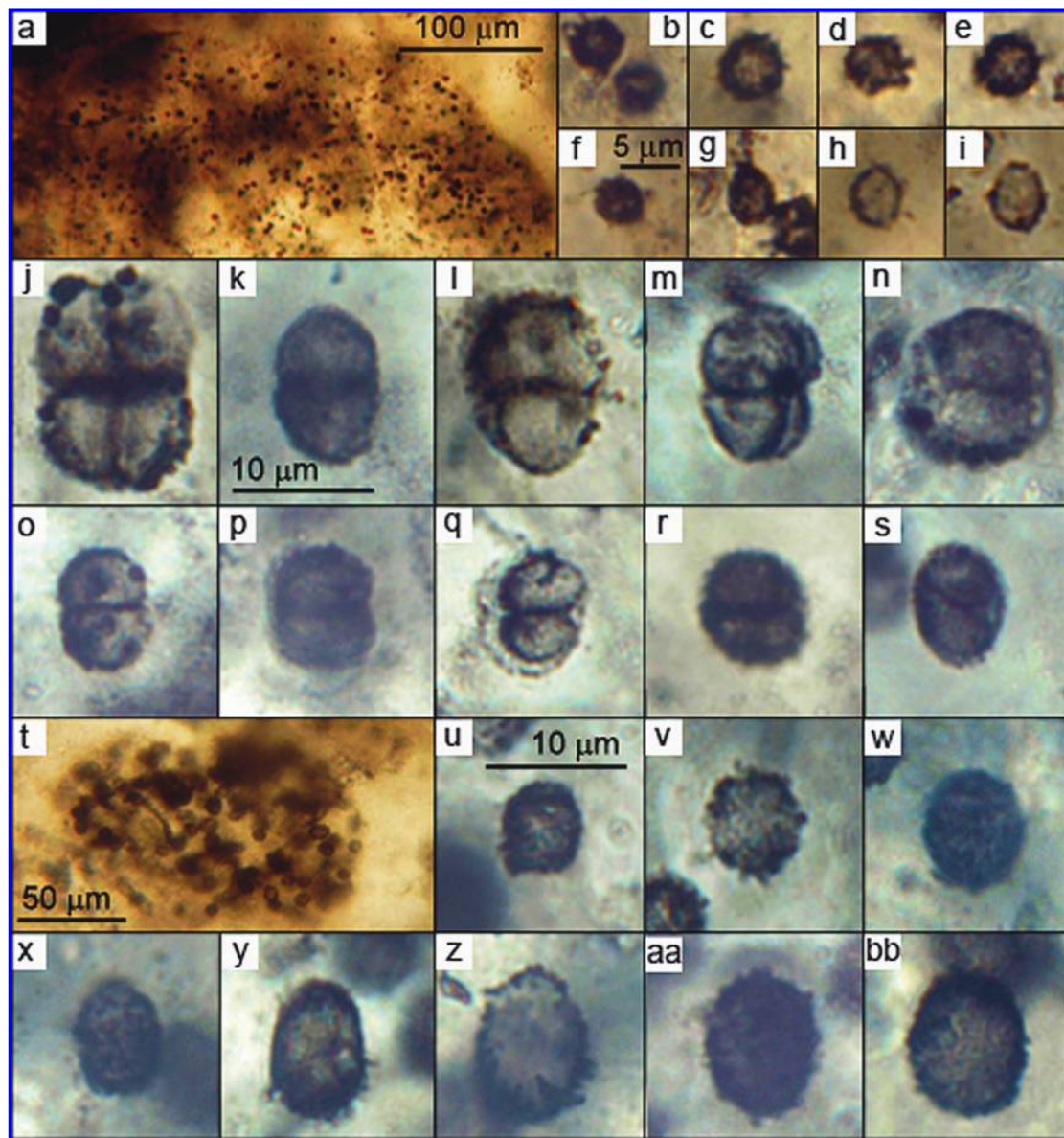


FIG. 6. Optical photomicrographs showing chert-permineralized colonial coccoidal microfossils in petrographic thin sections of the Proterozoic Buxa Formation of the Ranjit Window, Sikkim, northeastern India. (a–i) Colonial ~3 to 5 μm diameter chroococcacean cyanobacterial coccoidal cells (cf. *Palaeoanacystis*) irregularly distributed within an originally mucilaginous envelope. Scale in (f) applies to (b–i); all specimens are from the same area of thin section BR-2, microscope stage coordinates 42.2×103.5 . (j–s) Dyads and tetrads [in (j) shown in face view, in (m) shown at an oblique angle] of ~5 to 7 μm diameter “lima bean-shaped” chroococcacean coccoids, cf. *Eogloeocapsa*. Scale in (k) applies to (j–s); all specimens are from section BR-1 [(j) 46.0×98.6 ; (k) 47.6×101.8 ; (l) 46.1×98.7 ; (m) 46.2×99.4 ; (n) 48.4×101.8 ; (o) 46.3×99.5 ; (p) 46.3×99.6 ; (q) 46.3×99.4 ; (r) 46.7×101.7 ; (s) 47.0×101.7]. (t–bb) Colonial ~5 to 10 μm diameter chroococcacean coccoids (cf. *Myxococcoides*) irregularly distributed within an originally mucilaginous envelope; scale in (u) applies to (u–bb); all specimens are from the same area of section BR-2, 37.2×101.0 .

“lima bean-shaped” cells similar to those of the fossil chroococcacean taxon *Eogloeocapsa* (Fig. 6j–s). The assemblage also includes colonial, 5–10 μm diameter (Fig. 6t–bb), 10–15 μm diameter (Fig. 7a–i), and 15–18 μm diameter (Fig. 7f–m) chroococcaceans that resemble species of the fossil genus *Myxococcoides*; larger ellipsoidal solitary unicells, 25–30 μm in size (Fig. 7n); and large sphaeromorph

acritarchs $\sim 95 \mu\text{m}$ in breadth (Fig. 7o, 7p). The occurrence of such coccoids irregularly distributed in envelope-enclosed colonies (Fig. 6a, 6t) or in close-packed colonial aggregates (Fig. 7a, 7f), as well as their presence in dyads (Fig. 6k–s) and tetrads (Fig. 6j, 6m), demonstrates that they were produced by the standard processes of cell division. Like the filamentous forms noted above, all of these coccoidal morphotypes

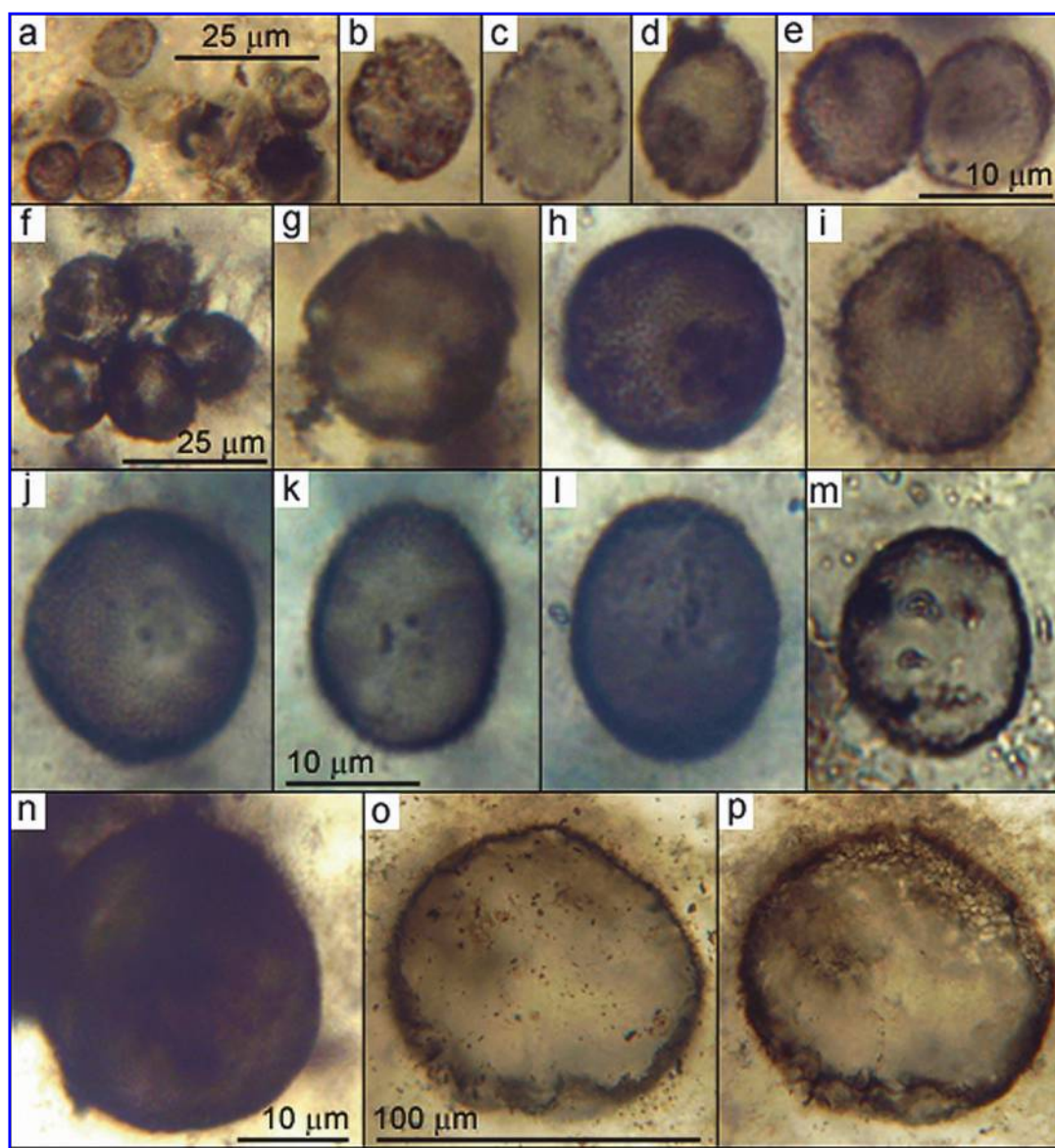


FIG. 7. Optical photomicrographs showing chert-permineralized colonial coccoidal microfossils and solitary sphaeromorph acritarchs in petrographic thin sections of the Proterozoic Buxa Formation of the Ranjit Window, Sikkim, northeastern India. (a–e) Spheroidal to ellipsoidal ~ 10 to $15 \mu\text{m}$ diameter colonial chroococcacean cyanobacterial cells (cf. *Myxococcoides*) irregularly distributed within an originally mucilaginous envelope. Scale in (e) applies to (b–e); all specimens are from the same area of thin section BR-1, microscope stage coordinates 46.0×106.0 . (f–m) Colonial (f) and solitary spheroidal to ellipsoidal ~ 15 to $18 \mu\text{m}$ diameter chroococcaceans, cf. *Myxococcoides*. Scale in (k) applies to (g–m). Specimens in (j–l) are from the same area of section BR-2, 37.3×101.4 ; all other specimens are from section BR-1 [(f) 46.0×103.7 ; (g) 46.0×103.4 ; (h) 36.5×104.7 ; (i) 34.9×99.8 ; (m) 36.6×104.6]. (n) Unicellular coccoidal cyanobacterium or sphaeromorph acritarch $\sim 27 \mu\text{m}$ in diameter (section BR-1, 36.6×104.6). (o) and (p) Sphaeromorph acritarch $\sim 95 \mu\text{m}$ in diameter, shown in (o) in an equatorial view of the specimen, where it transects the upper surface of the thin section, and in (p) at a lower focal plane that illustrates the diagenetically produced reticulate texture of the sphaeromorph cell wall (section BR-2, 46.2×104.4).

are well known from other Neoproterozoic cherts and, with the exception of the phytoplanktonic acritarchs, all appear to have been benthic members of mat-building microbial communities.

Carbonaceous composition of the Buxa fossils

The molecular-structural composition of the carbonaceous kerogen that comprises the Buxa fossils has been documented by Raman spectroscopy. As is shown by the representative example illustrated in Fig. 5c, two-dimensional Raman imagery demonstrates that such specimens (in this example, *Eomycetopsis*-like tubular cyanobacterial sheaths) are composed of kerogen. Moreover, the Raman spectra of the carbonaceous matter acquired in such studies can themselves be analyzed to yield the Raman Index of Preservation (RIP) of such materials (Schopf *et al.*, 2005a), RIP values that provide an established basis for assessment of organic geochemical maturity (Schopf *et al.*, 2005a). In Fig. 8 are shown 7 Raman spectra acquired from organic-walled Neoproterozoic microfossils preserved at various stages of geochemical maturation (Schopf *et al.*, 2005a). As is there illustrated, the 2 major Raman bands of kerogen change markedly as a function of increasing geochemical alteration: the left-most "D" band becomes increasingly more peaked (and, correspondingly, less broad and "bumpy") as the right-most "G" band becomes increasingly narrow and ultimately bifurcated. Such data, obtainable from organic-walled fossils permineralized in rocks subjected even to greenschist facies metamorphism (Schopf *et al.*, 2002, 2005a), can provide definitive evidence of the fidelity of organic preservation that is unavailable by any other means. As is shown in Fig. 8, Buxa fossils from the Ranjit window, which have an RIP value of 5.1, exhibit an intermediate grade of organic maturation, being not as well preserved as fossils of the Bitter Springs (the uppermost spectrum in Fig. 8) and other relatively little-altered Precambrian units but exhibiting an appreciably greater fidelity of geochemical preservation than fossils preserved in more metamorphosed Neoproterozoic deposits.

Discussion

As is documented above, both of the Buxa thin sections studied here contain diverse organic-walled microscopic fossils, the first such Precambrian fossils to be reported from the Ranjit tectonic window of Sikkim, northeastern India. Despite the rather poor state of cellular preservation of some of these chert-permineralized microbes and the moderately advanced geochemical maturity of the kerogen of which they are composed (Fig. 8), they are, in fact, *bona fide* fossils. Primary among the numerous lines of evidence that establish biogenicity are (1) their morphology and carbonaceous composition, (2) the diversity of the fossils detected, (3) the presence of many examples of the various morphotypes (exhibiting varying stages of cellular degradation and, in some instances, evidence of cell division), and (4) their close similarity both to fossils known from other Proterozoic deposits and to microorganisms living today. Indeed, the evidence of life contained in these 2 tiny thin sections is so voluminous and compelling that, had only one of the sections been available for study—decreasing by half the source of the evidence presented here—the conclusion drawn would have been the

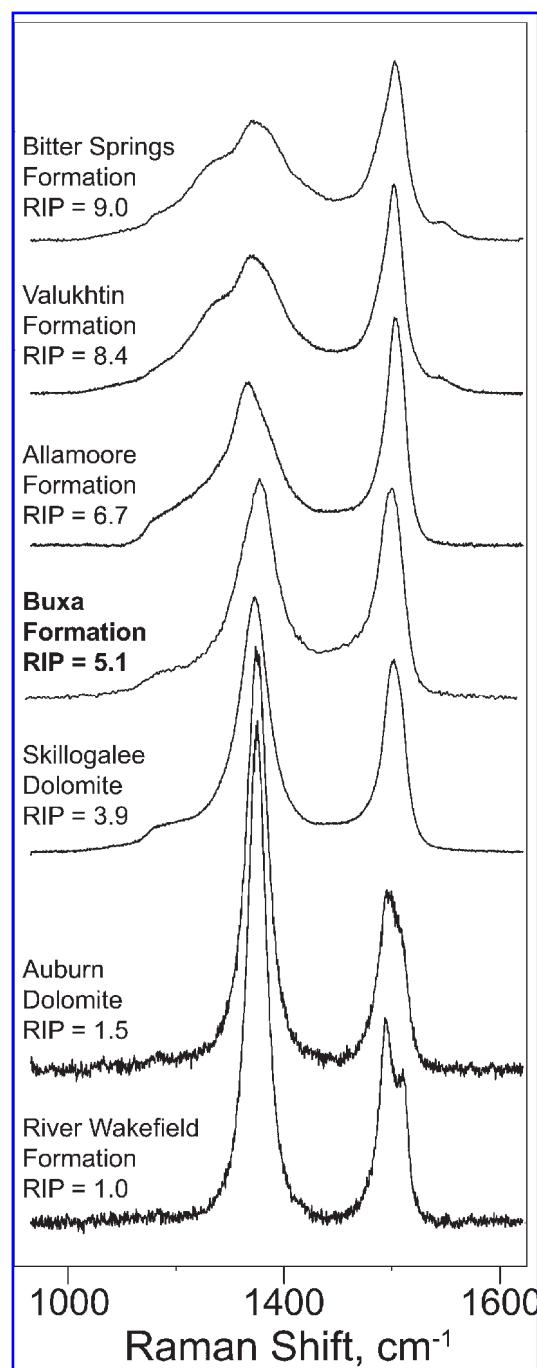


FIG. 8. Raman spectra of chert-permineralized Neoproterozoic carbonaceous microfossils. Ordered by their RIP values (Schopf *et al.*, 2005a) from less (top) to more (bottom) geochemically mature. The spectra shown are those of the kerogenous walls of fossils preserved in cherts of the ~750 Ma old Bitter Springs Formation, the ~1,050 Ma old Valukhtin and Allamoore formations, the ~760 Ma old Skilogalee and ~720 Ma old Auburn dolomites, and the ~775 Ma old River Wakefield Formation (Schopf *et al.*, 2005a), compared with that of a representative fossil filament (*Eomycetopsis*, cf. Figs. 4c–e and 5) from the Buxa Formation.

same: microbial life was present and abundant when and where these rocks were formed.

It is notable—and to some perhaps surprising—that in this study it has proven possible to establish the existence of past

life on the basis of such a minuscule amount (~0.1 g) of rock material. In fact, however, comparable results might have been obtained from such studies of small fossil-bearing samples of numerous other Precambrian deposits [including virtually all the 25 Proterozoic cherty units from which fossils are illustrated in Schopf (1992b)]. Because the microorganisms of microbial assemblages are so minute, commonly only a few microns to less than a micron in size, and because they typically are preserved in clumped or intertwined masses composed of prodigious numbers of individuals, even small slivers of rock can contain huge numbers of specimens. Nevertheless, the preservation of intact fossil microbes in such deposits, even in rocks of the same mineralogy from a single locality and stratigraphic horizon, can vary greatly. In this respect, the success of this study of the Buxa cherts was fortuitous. After this study was completed, 2 additional samples of chert collected from stratigraphically higher strata of the Buxa Formation in the Ranjit River section were examined; although each was highly carbonaceous, both were found to be barren of fossils. Even in this stromatolite-rich stratigraphic section, now demonstrated to be assuredly microfossiliferous, not all bedded cherts at all horizons contain cellularly identifiable remnants of life.

Implications

Given the foregoing, it would be unwarranted and naive to suggest that results of the current study should in any way be regarded as a reliable predictor of those to be obtained from Mars surface sample return missions. Interpretation of the evidence of life afforded by ancient rock samples on Earth is based on half a century of experience and the understanding of the early fossil record thus produced, as well as on deep knowledge of modern microbes, the communities they comprise, and the understanding of microbial evolution provided by molecular biology. Such data, of course, are unavailable for any other planet. Nevertheless, were the evidence presented here to have come from studies of a returned martian sample, most knowledgeable scientists would be convinced of the presence of past life on Mars. Other workers, however, would no doubt retain a degree of skepticism, perhaps calling for a search for nonbiological means by which to explain the seemingly compelling biological signals. And all could be expected to clamor for additional rock samples hosting additional examples of fossil microbes to be returned from Mars in order to build a body of paleobiologic data from that planet like that available for Precambrian Earth. Whether ultimately relevant to Mars samples or not, what this study does show is that under appropriate circumstances firm evidence of early life on Earth can be adduced from even a minuscule amount of fossil-bearing ancient rock—in this instance, the first Precambrian microfossiliferous samples to be discovered in the Ranjit tectonic window of the northeastern Himalaya.

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Abbreviations

BR, Buxa Formation, Ranjit tectonic window; CLSM, confocal laser scanning microscopy; RIP, Raman Index of Preservation.

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Address reprint requests to:
J.W. Schopf
Center for the Study of Evolution and the Origin of Life
Geology Building
University of California, Los Angeles
Los Angeles, CA 90095

E-mail: Schopf@ess.ucla.edu