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Research Paper

On Biogenicity Criteria for Endolithic Microborings on Early Earth and Beyond

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ABSTRACT

Micron-sized cavities created by the actions of rock-etching microorganisms known as euendoliths are explored as a biosignature for life on early Earth and perhaps Mars. Rock-dwelling organisms can tolerate extreme environmental stresses and are excellent candidates for the colonization of early Earth and planetary surfaces. Here, we give a brief overview of the fossil record of euendoliths in both sedimentary and volcanic rocks. We then review the current understanding of the controls upon the distribution of euendolithic microborings and use these to propose three lines of approach for testing their biogenicity: first, a geological setting that demonstrates a syngenetic origin for the euendolithic microborings; second, microboring morphologies and distributions that are suggestive of biogenic behavior and distinct from ambient inclusion trails; and third, elemental and isotopic evidence suggestive of biological processing. We use these criteria and the fossil record of terrestrial euendoliths to outline potential environments and techniques to search for endolithic microborings on Mars.
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INTRODUCTION

ENDOLITHS ARE macro- and microorganisms that live within rocks. Many microbial endoliths can tolerate extreme environmental stresses, including repeat desiccation, intense ultraviolet irradiation, oligotrophy, and temperature extremes that make them strong candidates for the colonization of early Earth and planetary surfaces (*e.g.*, Friedmann and Koriem, 1989). Endoliths that inhabit fissures and cracks in rocks are known as *chasmoendoliths*, those that dwell within pore spaces as *cryptoendoliths*, and those that actively bore into rock substrates and create microtubular cavities as *euendoliths* (Golubic *et al.*, 1981). The same microorganism can adopt these various modes of life at different stages in its life cycle or in response to a changing external environment (*e.g.*, De los Ríos *et al.*, 2005). This article focuses upon intragranular microborings,

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which can be termed trace fossils, as they record the behavior of organisms. Euendolithic microborings are found in a range of environments that include marine carbonates (*e.g.*, Campbell, 1982) and volcanic glasses (*e.g.*, Thorseth *et al.*, 1991, 1992; Fisk *et al.*, 1998). Euendoliths have a higher preservation potential compared to non-boring crypto- and chasmo-endoliths or surface and mat-dwelling microorganisms, which require early mineralization to be preserved. The recent discoveries of euendoliths in modern *in situ* oceanic crust and putative fossilized equivalents from ophiolites and greenstone belts have greatly expanded the range of environments in which microbial endoliths are sought and increased their potential as planetary biosignatures (*e.g.*, Furnes *et al.*, 1996; Fisk *et al.*, 1998; Furnes and Muehlenbachs, 2003, and references therein). Herein, we review the nature and distribution of euendoliths in the terrestrial rock record and propose the first synthesis of criteria for testing their biogenicity. We then discuss approaches to searching for and testing the biogenicity of candidate microborings on Mars.

TERRESTRIAL HABITATS AND FOSSIL RECORD OF EUENDOLITHS

Microorganisms that adopt a euendolithic mode of life include archaea, bacteria, algae, and fungi. The metabolic strategies employed range from photosynthesis in the shallow subsurface (*e.g.*, cyanobacteria living beneath translucent quartz) to chemolithoautotrophs in the deep biosphere. Such subsurface chemolithoautotrophs utilize H_2, H_2S, S, CH_4, CO, Fe^{2+}, or Mn^{2+} as potential electron donors, and CO_2, Fe^{3+}, and Mn^{4+} in rocks or $SO_2{}^{-4}$ and O_2 in circulating fluids as electron acceptors (*e.g.*, Stevens and McKinley 1995). Endolithic microborings have traditionally been studied in near-surface sedimentary rocks and biological substrates as environmental and ecological indicators. In carbonate environments, for example, microborings are used as paleo-depth indicators. Cyanobacterial borings dominate intertidal and shallow marine carbonate assemblages, whereas red and green algae are more abundant in deeper euphotic waters, and fungal borings occur beneath the photic zone (*e.g.*, Glaub *et al.*, 2001). The presence of microborings is also used in seafloor hardgrounds to confirm hiatuses in sedimentation and in shell and bone substrates to understand predation patterns (*e.g.*, Kowalewski *et al.*, 1989).

More recently, it has been discovered that euendoliths inhabit volcanic rocks deep beneath the seafloor, at depths of up to 4,000 feet (*e.g.*, Fisk *et al.*, 2003, and references therein), and these findings have generated many questions regarding the metabolic strategies of these "volcanic" dwelling euendoliths. Molecular profiling work suggests that iron and manganese cycling may be among the metabolic strategies employed (Thorseth *et al.*, 2001; Lysnes *et al.*, 2004). Molecular profiling also suggests that autotrophic microbes dominate the early colonizing communities in modern seafloor volcanics, followed by heterotrophs in older, more altered samples (Thorseth *et al.*, 2001).

The distribution of euendolithic microborings in sedimentary and volcanic substrata is controlled by a number of variables. Light levels and hence changes in paleo-depths are a key control on the distribution of euendoliths in carbonate substrates that are well documented. Other important controls are thought to include the concentration and availability of essential nutrients in the rock matrix, the density of physical weaknesses such as microfractures and fluid inclusion trails, the transparency of the rock matrix in the case of photosynthetic euendoliths, and the volume, E_h, pH, salinity, and nutrient content of circulating fluids. In the case of "volcanic" euendoliths in particular, these controlling variables are yet to be fully and systematically documented.

Euendolithic microorganisms have been shown by experimental studies to erode by congruent and incongruent dissolution of the rock through local changes in pH (*e.g.*, Thorseth *et al.*, 1995; Staudigel *et al.*, 1998). Siliceous substrates, for instance, exhibit a solubility minimum at circum-neutral pH and become more soluble below pH 4 and above pH 8—some endoliths exploit this, by eroding by either a process of bioalkalization (*e.g.*, Büdel *et al.*, 2004) or producing organic acids (*e.g.*, Callot *et al.*, 1987). In carbonate and calcophosphatic substrates, acidification is also widely suggested as the mechanism of microboring, *e.g.*, Golubic *et al.* (1984). However, Garcia-Pichel (2006) has recently pointed out that oxygenic photosynthesis causes alkalization that can lead to carbonate precipitation, and this is exactly the opposite effect to microboring by a process of acidification and dissolution that is widely envisaged. It is suggested that this apparent paradox may be resolved by temporal or spatial separation of photosynthesis and respiration by euendolithic microorganisms in carbonates (Garcia-Pichel, 2006). Support for spatial separation comes from the observation that some euendolithic cyanobacteria can bore on their lower surface while encrusting calcite around their exposed filaments above the substrate (Kobluk and Rick, 1977). Indeed, a constructive role is played by euendoliths in the lithification of Bahamian carbonate stromatolites. Here, multicyclic boring by the coccoid cyanobacterium *Solentia* sp. and concurrent infilling of these borings by carbonate precipitation welds together the carbonate grains, creating lithified stromatolite laminae (Macintyre *et al*., 2000).

A review of the terrestrial rock record of euendolithic microborings provides some signposts for those engaged in the search for biosignatures beyond Earth. This paper does not aim to provide an exhaustive review of the terrestrial microboring fossil record, but rather summarizes some of the major examples discussed below (see Fig. 1). Reviews of marine bioerosion can be found elsewhere (*e.g.*, Bromley, 2004), and summaries of candidate microborings described from volcanic glasses and silicate minerals are given by Furnes *et al*. (2007) and Fisk *et al*. (2006), respectively. In Fig. 1, fossil microborings are classified by substratum type, and current estimates of the time ranges of likely constructing organisms are also

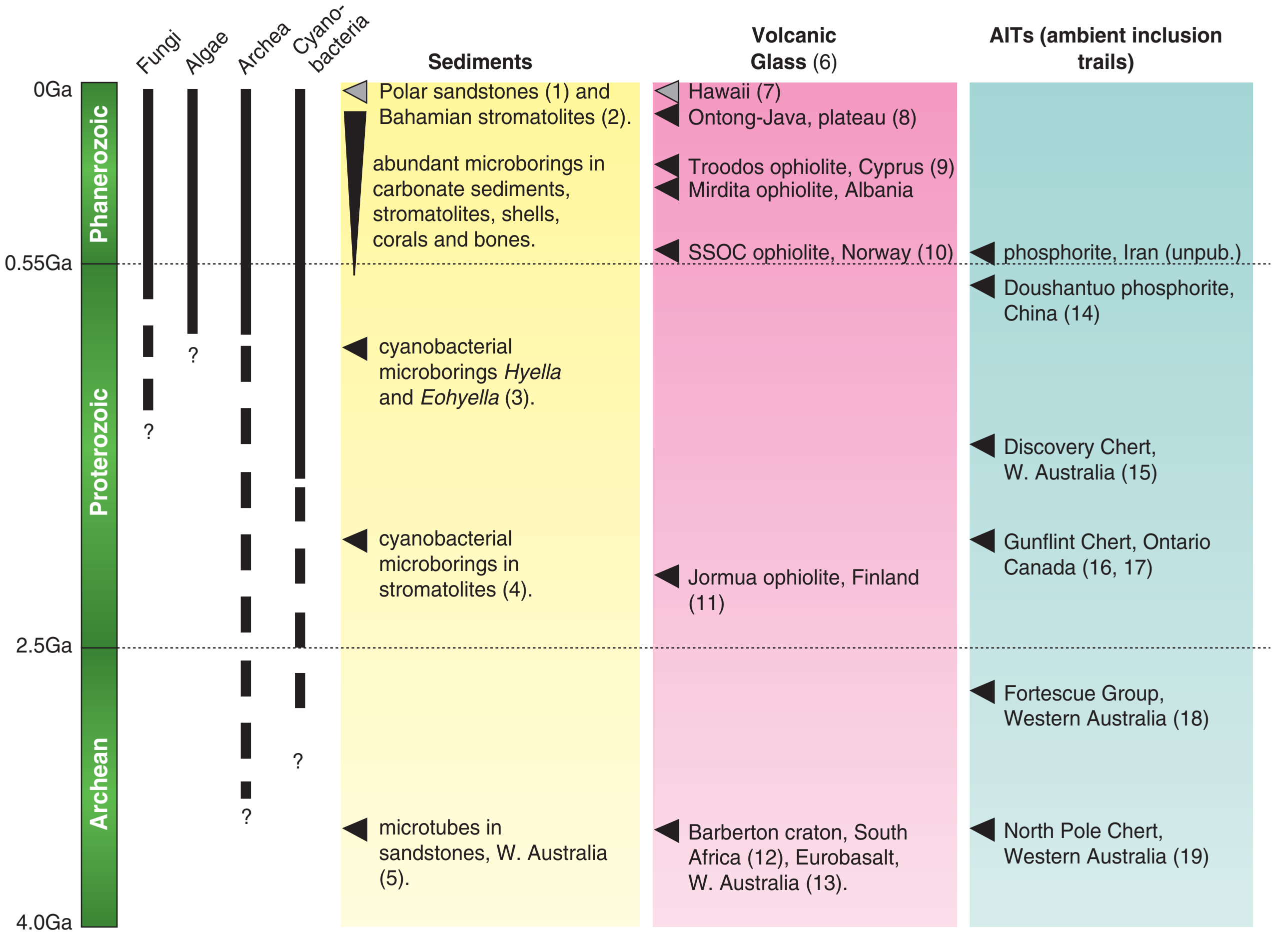

FIG. 1. Summary of the fossil record of putative endolithic microborings classified by substratum type. Column 1 shows the fossil record (solid line) and inferred time ranges (dashed line) of microorganisms that may adopt an endolithic mode of life; column 2 shows selected examples of endolithic microborings for sediments (predominantly silicified carbonate); column 3 shows examples from volcanic glasses; and column 4 shows examples of microtube mimics known as AITs. References: 1, Friedmann and Weed (1987); 2, Macintyre *et al*. (2000); 3, Green *et al*. (1988); 4, Zhang and Goloubic (1987); 5, Brasier *et al.* (2006); Wacey *et al*. (2007); 6, Furnes and Muehlenbachs (2003); 7, Fisk *et al*. (2003); 8, Banerjee and Muehlenbachs (2003); 9, Furnes *et al*. (2001b); 10, Furnes *et al*. (2002b); 11, Furnes *et al*. (2005); 12, Furnes *et al*. (2004); 13, Banerjee *et al*. (2007); 14, Xiao and Knoll (1999); 15, Grey (1986); 16, Tyler and Barghoorn (1963); 17, Knoll and Barghoorn (1974); 18, Knoll and Barghoorn (1974); 19, Awramik *et al*. (1983).

shown. The record of Precambrian euendoliths is relatively sparse, though reports of "volcanic" microborings have increased greatly in recent years (Fig. 1, column 2). Extensive studies of Proterozoic microbial mats and stromatolites have yielded surprisingly few examples of endolithic microborings, perhaps because of their different taphonomic pathways. Meanwhile, recent reports of microtubular structures in a silicified Archean sandstone have led to the suggestion that these may also be profitable facies within which to search for candidate euendoliths (Brasier *et al*., 2006; Wacey *et al*., 2007). Figure 1 shows that all Precambrian microborings recognized thus far from sedimentary substrates have been silicified. This is because silicification enhances their morphological preservation by minimizing post-depositional dissolution and aids the preservation of decayed organic remains (cf. Walker *et al*., 2005). A review of these terrestrial examples suggests that the most promising candidate environments for the preservation of endolithic microborings on early Earth and perhaps Mars are silicified carbonates and sandstones, iron-magnesium silicate minerals (especially olivine and pyroxene), and volcanic glasses and perhaps also impact glasses.

FORMULATING BIOGENICITY CRITERIA

To test the biogenicity of candidate endolithic microborings from the early Earth and perhaps even Mars requires criteria that are distinct from those applied to putative carbonaceous microfossils (cf. Buick, 1990; Brasier *et al*., 2004). The most robust claims for ancient or extraterrestrial life require three interdependent lines of evidence. Summarizing Rose *et al*. (2006), these are: (1) a geological context that demonstrates the syngenicity and antiquity of the putative biological remains; (2) evidence of biogenic morphology and behavior; and (3) geochemical evidence for biological processing.

These three criteria are developed below for endolithic microborings and are summarized in Fig. 2. Previous work has only attempted to list such criteria on a case-by-case basis and has also been largely substratum specific. While the following section aims to provide a first general syn-

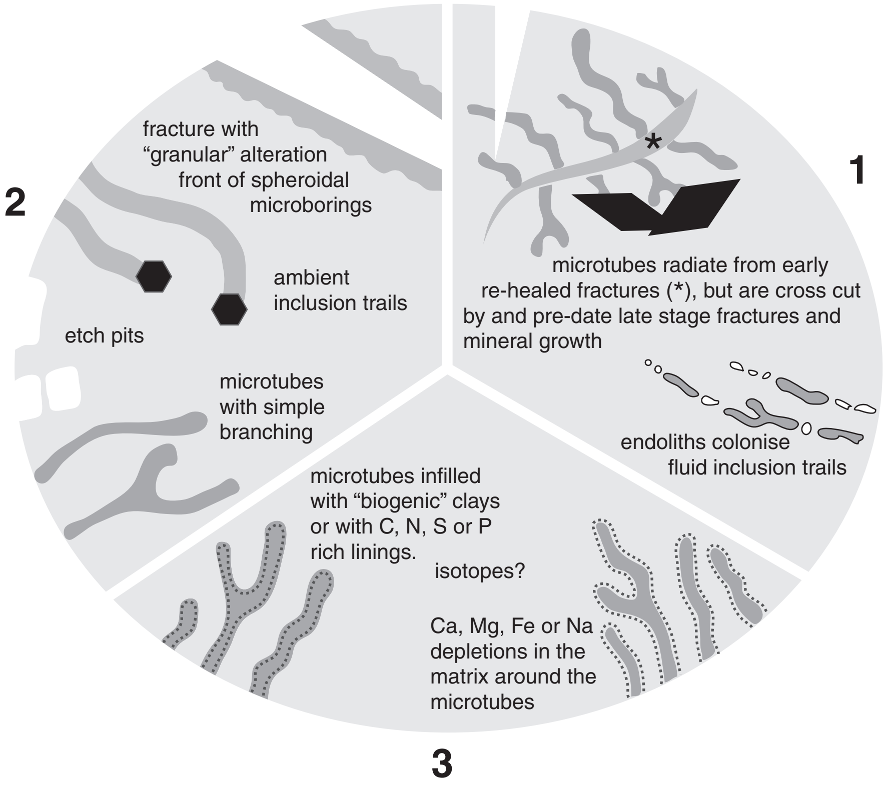

FIG. 2. Schematic summary of the criteria required to demonstrate the biogenicity of a putative euendolithic microboring: (1) syngenicity and geological context conducive for life; (2) a uniquely biogenic morphology and behavior; and (3) geochemical evidence for biological processing. These three criteria are explained in the text.

thesis of the criteria that may be used to test the biogenicity of ancient microborings, it is acknowledged that this is not yet an exhaustive list. We begin this discussion with an exploration of criteria needed to establish the syngenicity of putative euendoliths, given that an investigation of their morphology and geochemistry is contingent upon a full understanding of their age. We then go on to explore the second and third criteria, bearing in mind their interdependence.

A PRIMARY GEOLOGICAL CONTEXT

Euendoliths may colonize a rock substrate at many points throughout geological time and are a well-documented source of recent contamination (*e.g.*, Westall and Folk, 2003). Demonstration of their syngenicity, *i.e.*, primary depositional age, relies largely upon evidence obtained by detailed optical and scanning electron microscopy. In sedimentary rocks, this involves documenting the distribution of the microtubes and their cross-cutting relationships with depositional features such as clast margins and sedimentary laminae. It also requires the recognition and mapping of diagenetic features such as cement phases, resealed fractures, pressure solution fronts, late-stage veins, and fractures. A complex picture can emerge, particularly in shallow marine carbonates where microborings created by pioneering euendoliths can be occupied by later generations of cryptoendoliths, or may be infilled, cemented, and then reworked by subsequent generations of euendoliths. These overprints can be distinguished in environments where diagenesis and extensive microboring have not completely homogenized the sediment. For example, the syngenicity of euendolithic microborings from the ~700 Ma Eleonore Bay Group was confirmed by the observation that they are not restricted to the matrix and intergranular pore spaces, but also bored into silicified carbonate grains cross-cutting the primary concentric oolite laminae, and are independent of the distribution of post-depositional veins and fractures (see Green *et al.*, 1988, Fig. 3). Another illustrative example is the fabric context of microtubular structures in an Archean sandstone from the Kelly Group of Western Australia (Brasier *et al.*, 2006). These microtubular structures are truncated at clast margins by pressure solution fronts and quartz overgrowths. They are also cross-cut by metamorphic mineral growths and early-stage fractures, which suggests a syn-depositional to early diagenetic origin for at least some of the microtubes (Brasier *et al.*, 2006, Fig. 5; Wacey *et al.*, 2007). In these sediments, fluid inclusions are currently being explored to better constrain the diagenetic history of the microtubes.

In volcanic rocks such as basalts and hyaloclastites, *i.e.*, brecciated volcanic glass, it is necessary to investigate the distribution of microtubes relative to clast margins and fractures that may have acted as conduits for younger fluids and endoliths. In Fig. 3a, for example, the majority of microtubes originate at the clast margins. These microborings can be distinguished from abiotic glass–palagonite alteration fronts because the latter are much smoother, lack microtubes, and are symmetric about the fractures from which they develop (Furnes *et al.*, 1996, 2002a, Fig. 2; Fisk *et al.*, 1998, Fig. 1A). In ancient volcanic glasses, obtaining an age estimate for these biotic alteration textures can be difficult. However, the presence of titanite ($CaTiSiO_5$) in putative tubular microborings from the Euro Basalt of the Warrawoona Group, Western Australia, has enabled direct $^{206}Pb/^{238}U$ dating using multicollector-inductively coupled plasma mass spectrometry by Banerjee *et al.* (2007). In the case of the ~3.5 Ga Barberton microtubes, the overlap in radiometric ages obtained from metamorphic minerals that overprint the microtubes and igneous minerals in the host pillow basalts supports a syn-eruptive, early Archean age for these putative endolithic microborings (Furnes *et al.*, 2004; Banerjee *et al.*, 2006). In contrast, no examples of radiometric dating of phases associated with fossilized euendoliths in sedimentary rocks have yet been reported.

We note that, in meteorite samples such as those recently reported by Fisk *et al.* (2006), McKay *et al.* (2006), and Gibson *et al.* (2006), the presence of candidate euendoliths is extremely exciting, and it is not their primary depositional age that needs to be established, but, rather, whether the candidate microborings are extraterrestrial in origin and predate transport to the Earth. To establish an indigenous origin for candidate euendoliths in meteoritic samples requires application of the same general principles that were outlined for terrestrial samples above. With regard to the martian Nakhla meteorite, carbonaceous vein-filling materials with micron-sized tubular projections and bleb-shaped mor-

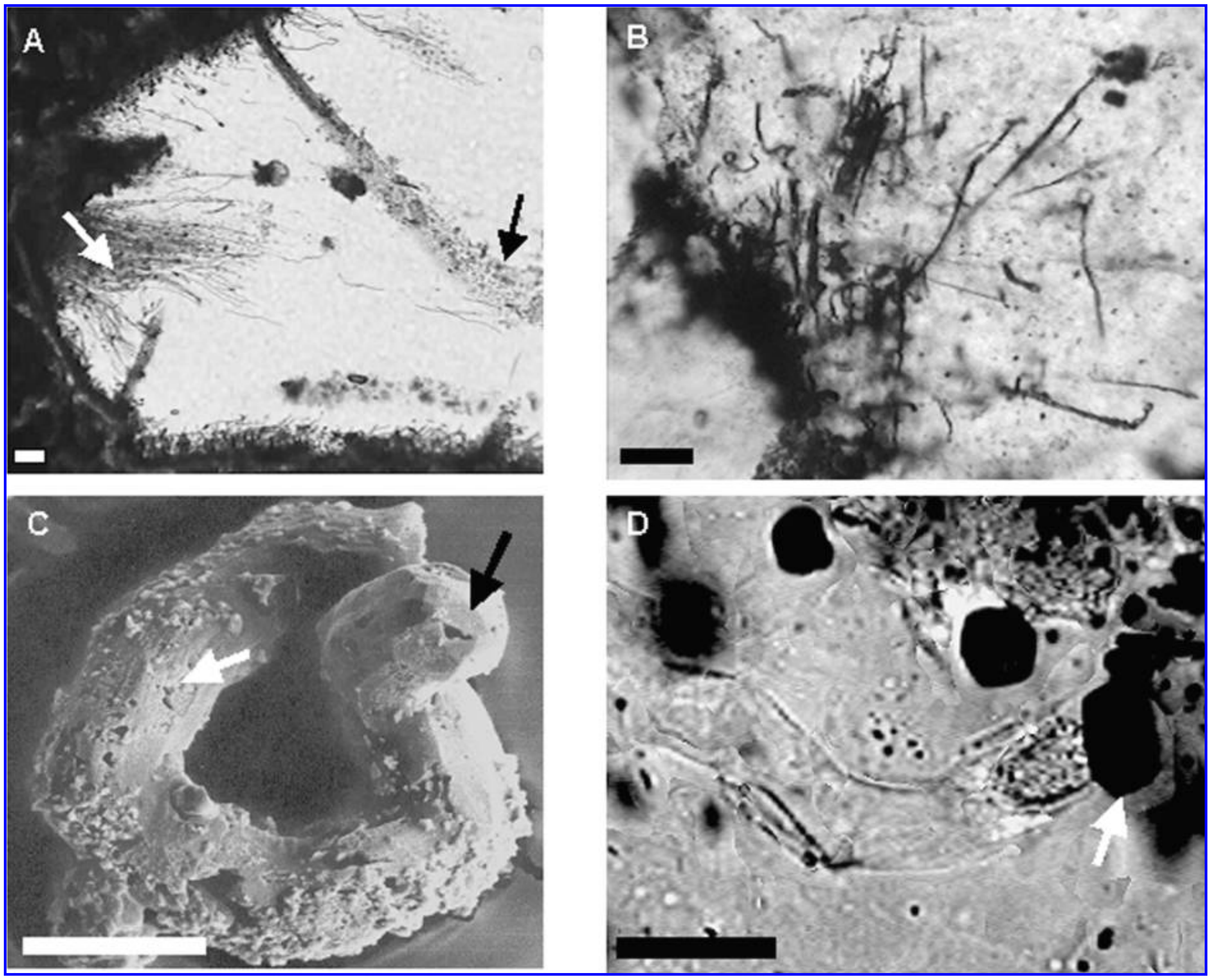

FIG. 3. Optical images of: (a) volcanic microborings in glass clasts within a volcanic breccia from the Ontong-Java plateau (white arrow shows tubular microborings; black arrow shows granular alteration textures); (b) comparable microtubular structures in an Archean sandstone clasts from Western Australia; (c) a secondary electron image of an AIT preserved in phosphorite from Iran (I. Ogilvie, personal communication) (black arrow shows the terminal crystal; white arrow shows the longitudinal striae); and (d) optical image of an AIT from an Archean chert (N.McL., unpublished data). Scale bars = 50 μm in (a) and (b); 10 μm in (c) and (d).

phologies have been recently described by McKay *et al*. (2006) and Gibson *et al*. (2006), along with tunnels and galleries in olivine and pyroxene crystals (Fisk *et al*., 2006), all of which have been tentatively compared to "volcanic" microborings. This secondary vein-filling material is argued to be a product of preterrestrial alteration, *i.e.*, is indigenous, because the host veins show evidence of increased devolatization and eventual annealing in proximity to the fusion crusts (Gooding *et al*., 1991; Wentworth *et al*., 2004, Fig. 1a). Also, ^{40}Ar/^{39}Ar radiometric dating has provide an upper age estimate for the vein-filling phases of ~650 Ma, which is much older than the observed fall-date of the Nakhla meteorite in 1911 (Swindle and Olson, 2002), although undoubtedly the Nakhla-type meteorites have experienced several phases of aqueous alteration. Further support for an indigenous origin of this dendritic vein-filling material is provided by geochemical evidence that is discussed below.

Once the geological context has been shown to support the syngenicity of the microboring, the next question to ask is whether the primary geological context was conducive to life? This is a non-trivial question because the limits of extremophile life are continually being expanded by scientific research. In the case of "volcanic" euendoliths, it has been suggested that ambient temperatures soon after eruption of <113°C would be conducive to hyperthermophilic life (Stetter *et al.*, 1990) and volcanic glass in contact with seawater would be colonized under these conditions (cf. Thorseth *et al.*, 2001). With regard to cyanobacteria-like microborings (see below), one might look for evidence consistent with deposition in the photic zone (*e.g.*, wave ripples, beach deposits, fluvial intercalations) or the shallow, approximately <1 cm subsurface of translucent sediment. The problem here is that sediment grains often undergo down-slope transport and reworking. Cyanobacterial euendoliths also require locally oxygenic conditions that would be indicated by primary oxide minerals. However, the oxidized surface zone of the cyanobacterial community is invariably overprinted by anaerobic conditions during early burial and diagenesis. Thus, it is perhaps more useful to rephrase the question and explore whether the distribution of the candidate microborings is consistent with biological control and can be used as a proxy for biological gradients. For instance, a positive correlation has been observed between the density of volcanic microborings and mechanical weaknesses such as microfractures (Furnes *et al.*, 2001a). Similarly, it has been shown that euendoliths preferentially exploit fluid inclusion trails within quartz grains from Antarctic dry valley sandstones (Parnell *et al.*, 2005). Likewise, chemical parameters can control their distribution, and experimental studies have shown that endoliths preferentially colonize silicate fragments rich in Fe and P (Roberts-Rogers and Bennett, 2004). These types of relationships are yet to be systematically documented for "volcanic" microborings but may be useful proxies for investigating the penetration of oxygen and nutrient-rich fluids in the early oceanic crust (cf. Furnes and Muehlenbachs, 2003). Also, if those datasets are mutually consistent, *i.e.*, microboring morphologies and densities are correlated with independently determined biogenic controls, such as fluid temperatures inferred from accessory-phase mineralogy or fluid inclusions where preserved, then this would be consistent with their biogenicity. This approach has been used, for example, to support the biogenicity of putative microbial mat remains from the ~3.5 Ga Buck Reef Chert of South Africa, where the distribution of laminar, carbonaceous remains is controlled by paleo-water depth inferred from trace metal content and sedimentary structures (Tice and Lowe, 2004).

BIOGENIC MORPHOLOGY AND BEHAVIOR

Information concerning the geological context of putative euendoliths needs to be combined with evidence for biogenic morphology and behavior. The morphology of candidate microfossils can be a notoriously ambiguous indicator of their biogenicity, as summarized by García Ruiz *et al*. (2002), Cady *et al*. (2003), Brasier *et al*. (2006), and others. The interpretation of the morphology of candidate euendoliths is subject to many of the same problems, though these can be ameliorated to some degree, first by an understanding and falsification of abiotic mechanisms of microtube formation and second by investigating the interaction of the microtubes with the rock substrate to test for biogenic behavior. We will explain this twofold approach to investigating the morphology of candidate euendoliths below, but first it is instructive to briefly summarize their morphological diversity.

Endolithic microborings described from sediments are linear to curvilinear, may show simple or complex branching often with swelling at the nodes, and may taper toward their ends and apical cells. The reasons why microborings branch are not well understood. Microboring dimensions reflect the size of the constructing organism, and identification of the trace maker is sometimes possible, as explained by Bromley (2004). Algal borings are among the largest, bacterial borings tend to be smaller (with the exception of some large, modern cyanobacteria), and "nanobacterial" borings are less than 1 μm across (cf. Folk and Rasbury, 2002). Many ichno- or trace fossil taxa are identified on the basis of microboring morphology in sedimentary and biological substrates and are reviewed by Bromley (2004). In comparison, euendoliths described from volcanic glasses appear to produce two main morphotypes: tubular and granular (Furnes *et al.*, 2001a). The tubular microborings are typically 1–5 μm wide, have lengths up to 100 μm, and may be twisted, coiled, and/or branched (*e.g.*, Fig. 3a, black arrow), sometimes with segmentation. The granular microborings occur as masses of spherical-shaped microborings, are 0.1–1.5 μm in diameter, and occur in bands or zones (*e.g.*, Fig. 3a, white arrow). More detailed size distribution data measured from granular and tubular microborings from both *in situ* oceanic glasses and ancient volcanic glasses are presented by Furnes *et al.* (2007). The factors that determine whether granular or tubular morphologies dominate an assemblage are unknown. In recent volcanic glasses from *in situ* oceanic crust, granular microborings are the most abundant and account for up to 80% of the alteration observed (Furnes and Staudigel, 1999). In contrast, microtubular morphologies are more easily recognized in the ancient rock record and apparently more abundant, possibly because of their better preservation potential (H. Furnes, personal communication). Further, appreciable morphological variation, with regard to these euendolith morphotypes, can arise through diagenetic modification. In carbonate rocks, for example, linear, curved, and branched arrays of tubular fluid inclusions can form along the original microboring by calcite partitions that are precipitated during diagenesis (*e.g.*, Buijs *et al.*, 2004).

Closely related morphological phenomena to endolithic microborings that deserve discussion here are etch pits. These are depressions that form on the outer surfaces of rocks and minerals with shapes and dimensions that match those produced by ambient microbes (*e.g.*, Thorseth *et al.*, 1992, 1995, 2003, Fig. 3g–i; Bennett *et al.*, 1996) and, less frequently, by larger organisms such as diatoms (*e.g.*, Brehm *et al.*, 2005, Fig. 3). Microbial etch pits may be aligned along specific crystallographic axes (*e.g.*, Longazo *et al.*, 2002) or preferentially occur in regions with more abundant structural defects or chemical heterogeneities (*e.g.*, Bennett *et al.*, 1996). It is believed that they form by the corrosive activities of microorganisms that reside on the mineral surface, and some show "growth rings," which suggests that they may develop into microtubular structures (Thorseth *et al.*, 1992). The potential of these structures to provide biosignatures in the ancient rock record is rather limited, however, by their morphological simplicity and susceptibility to modification. In some instances, this morphological simplicity may lead to confusion between microbial etch pits and abiotic weathering textures. Thus, it would be timely to conduct further abiotic laboratory experiments to discern whether there are localized environmental conditions, perhaps extreme under which microtubular or granular weathering textures can be produced.

A significant mechanism that may create microtubular cavities in rock substrates and needs to be distinguished from euendolithic microborings is the formation of ambient inclusion trails (AITs). AITs (Tyler and Barghoorn, 1963) are thought to be created when mineral grains such as iron sulfide are driven by high fluid pressures through materials like cryptocrystalline chert or phosphorite, leaving behind a hollow tubular trail that may remain empty or be infilled by a secondary mineral phase. AITs can be distinguished morphologically from endolithic microborings by (1) the presence of a mineral grain (*e.g.*, a metal sulfide or oxide) at the end of a microtube, which may be pseudomorphed by later minerals (*e.g.*, silica, metallic oxide or phosphate), (2) longitudinal striations created by the facets of the propelled mineral grain, which would be absent from endolithic microborings and are perpendicular to any annulations that might reflect cell septation, though both may be obscured by later mineral infill, (3) angular cross-section and curved or twisted paths, particularly toward their ends, arguably due to the increasing impedance of the host grain, and (4) a tendency for the microtubes to crosscut or branch, *i.e.*, where the impacting mineral splits or a second grain is intercepted (these branches may show abrupt changes in diameter and lack the nodal swelling sometimes seen in microborings).

Numerous examples of AITs, including some that were mistaken for microfossils, *e.g.*, Gruner (1923), are recognized from the geological record and summarized in Fig. 1. The critical question here is whether biological processing and/or primary, bioorganic compounds are prerequisite for AIT formation. In their initial description, Tyler and Barghoorn (1963) hypothesized that AITs were "almost certainly an inorganic process" formed by pressure solution ahead of pyrite grains that were propelled by the force of crystallization in the trails, or so-called "appendages" behind them. Subsequent work by Knoll and Barghorn (1974) suggested that metamorphic heating of biogenic carbon would produce H_2S, CO_2, and, at higher temperatures, CH_4, which would drive this pressure solution. If this is so, then at least some, and possibly the majority of, AITs might involve a biological component, and just such a scenario is being explored for microtubular structures found in a ~3.4 Ga sandstone from the Kelly Group of Western Australia (Brasier *et al.*, 2006). Furthermore, Knoll and Barghoorn (1974) identified two AIT size classes in their study of cherts from the Fortescue Group of Western Australia, the larger of which they attributed to the decomposition of cyanobacterial cells. The smaller AIT size class, comprising radial clusters or "starburst" arrangements of AITs, was attributed to the decay of bacterial colonies. This discussion highlights the need for experimental work to test the various hypothesized roles biology may play in AIT formation. Lithologies that are likely to host AITs include those that contain appreciable organic material and those that may permit flow and internal movement over geological time scales, such as cherts, devitrified glasses, and sedimentary phosphorites.

Support for biological behavior can be inferred from the distribution and orientation of microtubular cavities. We suggested above that euendoliths may preferentially exploit horizons with favorable lithological compositions, perhaps those that are rich in trace metals utilized by their metabolisms or those that contain structural defects and weaknesses that facilitate microboring. For example, it has been observed that, in some volcanic rocks, candidate euendolithic microborings tend to migrate toward olivine crystals and away from plagioclase, which may reflect an euendolith preference for metabolically important metals. There exists significant scope for more experimental studies to document these types of relationships surrounding phenocrysts, vesicles, and varioles. Further illustration of behavioral information is provided by the oldest yet reported sedimentary euendoliths found in ~1.7 Ga silicified stromatolites (Zhang and Goloubic, 1987). These were distinguished from the contemporaneous mat-building microorganisms by their downward, or inverted, growth position and branching patterns (Zhang and Goloubic, 1987, Fig. 1). This so-called reverse polarity was also used in the ~700 Ma Eleonore Bay Group to distinguish true endoliths, which bore into silicified carbonate grains and cross-cut the concentric laminae, from epiliths (surface-dwelling microorganisms that grow parallel to the laminae) (Green *et al.*, 1988, Fig. 3).

GEOCHEMICAL EVIDENCE FOR BIOLOGICAL PROCESSING

If the geological context and morphology of a putative microboring are suggestive of a biotic origin, further support can be sought from investigation of elemental and isotopic variations in and around the microtubes. These are perhaps among the most exciting biogenic indicators, with advances in high-resolution analytical techniques rapidly expanding their scope. If the phases that infill a putative microboring can be shown to be syn-depositional or early diagenetic in age, then they may also preserve geochemical signatures of euendolithic activity. An ancient age for these infilling phases is supported if they have experienced the same degree of metamorphism as the host rock, if they are cross-cut by later diagenetic features, and in some instances if they can be directly dated. In recent volcanic microtubes, the occurrence of nucleic acids—bacterial and archeal DNA localized within the microtubes as spherical chains and concentrated in the tips of tubular bodies—is a major line of evidence in support of biogenicity (*e.g.*, Torsvik *et al.*, 1998; Thorseth *et al.*, 2001, and references therein). Such genetic material is unlikely to survive in the early rock record, and so "organominerals," which form in environments that are modified or controlled by microbial metabolism, can be used to test the biogenicity of euendoliths (*e.g.*, Perry *et al.*, 2007). For example, principal component analysis has shown that clay-mineral compositions in recent volcanic glasses that contain microtubes are distinct from clay minerals in abiotically altered zones that lack microtubes and those in unaltered zones (Storrie-Lombardi and Fisk, 2004). Similarly, clay minerals and iron oxyhydroxides found in polar sandstone inhabited by crypto- and chasmoendoliths are being explored as potential biosignatures and must be carefully distinguished from abiotic weathering crusts (*e.g.*, Wierzchos *et al.*, 2003, 2005; Blackhurst *et al.*, 2004). The challenge to this approach, however, is to establish whether such mineralogical signatures can be extended to the ancient rock record. It should be noted that supporting morphological and contextural information is required to make the distinction between biogeochemical signatures and the complex geochemical signatures that can result from abiotic weathering.

Much more informative are high-resolution compositional variations in the phases that infill putative microborings, which have been used to advance a biogenic origin for microtubular structures (*e.g.*, Banerjee and Muehlenbachs, 2003). Thin, <1-μm-wide linings of C, N, and P have been detected by electron probe analysis of modern and ancient putative volcanic microborings, which some take to represent decayed cellular remains (*e.g.*, Giovannoni *et al.*, 1996; Furnes and Muehlenbachs, 2003). Accompanying elemental depletions in the rock matrix that surround putative microborings have also been reported, and may provide further support for the growth and metabolism of euendoliths that drew nutrients and trace metals from the host rock. For example, depletion in Mg, Fe, Ca, and Na has been described from across zones <0.3 μm wide that surround putative microborings in ~6 Ma seafloor volcanic glasses (Alt and Mata, 2000). If such signatures can be confirmed as primary in origin, and not the products of abiotic water–rock interactions, then they are valuable indicators of biological processing. The transmission electron microscopy (TEM) technique used in this and a small number of other studies allows for a better understanding of the nanoscale chemistry of fossil euendoliths (cf. Alt and Matar, 2000; Benzerara *et al.*, 2005). Figure 4 illustrates a dataset obtained from the TEM analysis of a 100-nm wafer cut using a focused ion beam normal to a microtube from the Ontong Java plateau (cf. Banerjee and Muehlenbachs, 2003). The image shows three distinct zones: an altered zone in the matrix that surrounds the tube, a discrete curvilinear band that defines the margin of the microtube, and globular structures in the phases that infill the microtube (R.S. Perry *et al.*, unpublished data). Ongoing analysis by energy-dispersive X-ray and energy electron loss spectroscopy will help to elucidate compositional differences among these three zones and perhaps also constrain metabolisms that might have been involved (see the section Areas for Future Research). Comparable TEM analyses of recent mineralized cryptoendoliths from the Antarctic dry valley sandstones are also being undertaken by other investigators to explore their taphonomy and potential as fossil biosignatures (*e.g.*, Wierzchos *et al.*, 2003, 2005).

High-resolution compositional data can also be coupled to isotopic evidence to help test the biogenicity of candidate euendoliths. For example, disseminated carbonate preserved within unaltered pillow interiors exhibits carbon isotopic values between δ^{13}C $+0.7$‰ and -6.9‰ that are similar to mantle values, whereas altered pillow basalt rims exhibit a wider range in carbon isotopic values, from δ^{13}C $+3.9$‰ to -16.4‰ and this difference has been taken to support bioalteration of the pillow rims; further explanation of the possible microbial metabolisms involved can be found in Furnes *et al.* (2002a, Fig. 9); Banerjee *et al.* (2006), and references therein. Corroboration of such an interpretation may be possible by direct *in situ* analysis of the carbon lining the microtubes using nanoscale secondary ion mass spectrometry (nanoSIMS). This state-of-the-art technique has never been used to study recent or ancient endolithic microborings, and there are only a small number of preliminary reports in-

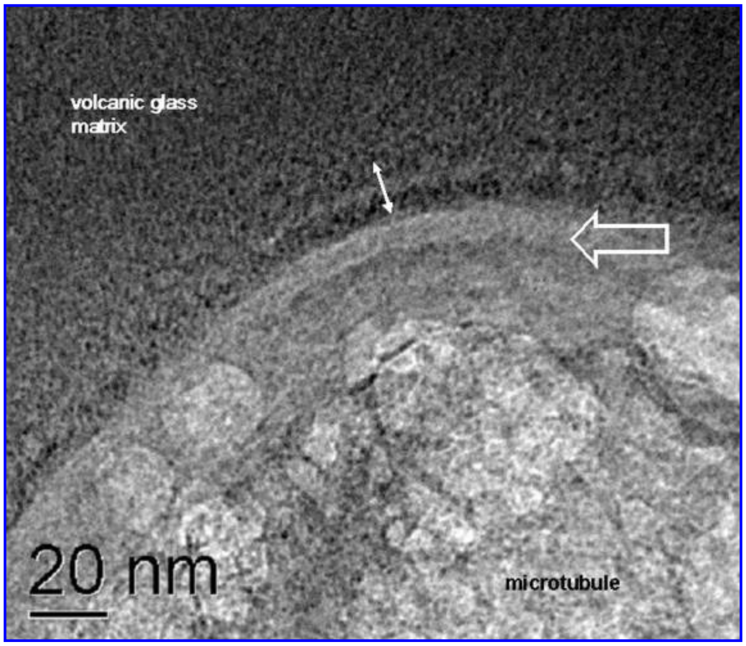

FIG. 4. TEM image of the margin of a microtube in volcanic glass fragments from the Ontong Java plateau, showing a depletion zone in the surrounding matrix (white double-headed arrow) and a curvilinear mineral band defining the margin of the microtube (open white arrow). Sample 192-1184A, 13R-3, 145–148 cm was kindly loaned by N. Banerjee for this study. Further description of this sample and evidence of euendolithic microborings from the Ontong Java Plateau are given in Banerjee and Muehlenbachs (2003).

vestigating Precambrian microfossils (*e.g.*, Roberts *et al.*, 2005; Oehler *et al.*, 2006). NanoSIMS is being utilized with some success to measure the C isotopic composition of organic carbon phases that are found to line the microtubes previously mentioned from the ~3.4 Ga Kelly Group of Western Australia (Kilburn *et al.*, 2005; Wacey *et al.*, 2007). Such detailed spatial and contextual exploration of microtube morphologies and infilling phases will help to investigate putative metabolisms as well.

In the absence of such compositional or isotopic variations within candidate endolithic microtubes, their biogenicity will be more difficult to establish and can only be tentatively inferred by morphological comparison with other fossil or extant euendoliths. Unfavorable taphonomic conditions where this is likely to be the case include (1) high-grade metamorphism, which is particularly abundant in Archean terrains and causes modification of the textural and compositional signatures, and (2) lower-grade metamorphism, which may also result in recrystallization of the host rock and change or destroy the euendolithic remains. In addition, subsequent physical processes such as erosion and fluid circulation or biological processes that include further bioerosion can obliterate the geochemical signatures. Under such circumstances, great caution must be taken when inferring biogenicity.

APPLICATIONS TO ASTROBIOLOGY

Many hypotheses for the colonization of planetary surfaces by microbial life involve endoliths (*e.g.*, Friedmann and Koriem, 1989; McKay *et al.*, 1992). Considerable attention has been focused on the cryptoendoliths of Antarctic dry valley sandstones as potential analogues for martian biota (*e.g.*, Friedmann and Weed, 1987; Wierzchos and Ascaso, 2002), and it has also been suggested that volcanic dwelling euendoliths may be found on Mars (Fisk and Giovannoni, 1999a; Banerjee *et al.*, 2004). The recent discovery in the Nakhla meteorite of carbonaceous, tubular, and bleb-shaped microstructures that show some similarities to volcanic microborings has re-invigorated these hypotheses (Fisk *et al.*, 2006; Gibson *et al.*, 2006; McKay *et al.*, 2006). It is envisaged that the intense ultraviolet flux, absence of liquid water, and freezing temperatures on the outer surface of Mars may encourage an endolithic mode of life. Simulation experiments also suggest that the morphological remains of endolithic organisms may persist under these conditions (Cockell *et al.*, 2005). Moreover, the extremely slow metabolic rates observed in polar cryptoendoliths and their ability to survive under conditions far below their physiological optima have led to the suggestion that they may have persisted on Mars well after the disappearance of more equable conditions (Friedmann and Koriem, 1989). Alternatively, it has been proposed that life, if ever present on Mars, may have been relegated to the deep subsurface where, it has been postulated, the redox gradients, temperatures, and nutrient and water content within the silicate rocks are suitable for chemolithoautotrophic metabolisms like those thought to be adopted by some euendoliths in volcanic rocks (Stevens and McKinley, 1995; Fisk and Giovannoni, 1999b).

The detection of extinct or perhaps extant endoliths on Mars will require detailed mapping of their geological context coupled to *in situ* microscopic analysis of the rock textures and high-resolution geochemical analyses of C-, N-, P-, O-, Fe-, S-, Si-, Mg-, Ca-, and Na-bearing phases as reviewed above for terrestrial examples. The selection of target sites could be remotely guided by the identification of favorable rock types such as carbonates, sandstones, volcanic or impact glasses, iron-magnesium silicate minerals, or clay-palagonite mineral assemblages that are associated with microborings here on Earth (cf. Storrie-Lombardi and Fisk, 2004). The detection of extant euendoliths, if they exist, might also be possible using Raman spectroscopy to locate protective hematite crusts and ultraviolet shielding pigments, though distinguishing these from abiotic weathering crusts could prove challenging (Jorge Villar *et al.*, 2005). In all likelihood, positive identification of endolithic microborings by these criteria will have to rely upon sample return missions, except in the case of meteorite samples. The latter possibility has recently been raised by the discovery of an indigenous, carbon-bearing, vein-filling phase in the Nakhla meteorite that exhibits tubular and bleb-like morphologies (McKay *et al.*, 2006). Laser Raman analysis has shown that these dendritic vein fills are composed of a complex mix of carbonaceous compounds, which have a distinct CN^- signature identified by nanoSIMS analysis and a C isotopic composition measured *in situ* of $-18‰$ to $-20‰$ (Gibson *et al.*, 2006). Furthermore, this C-bearing phase is distinct from a later terrestrial C-bearing contaminant phase that is released at lower temperatures during stepped combustion, static mass spectrometry of the meteorite samples (Gibson *et al.*, 2006). In light of this evidence, it has been suggested that the tubular carbonaceous phase is either (1) derived from an impact on Mars that also produced the fractures and veins in the Nakhla meteorite or (2) the products of martian euendoliths that may have been similar to terrestrial, volcanic microborings (Gibson *et al.*, 2006). Another team has also recently described microtubular tunnels in olivines and pyroxenes from the same class of meteorites that are remarkably similar to bioweathering textures found in terrestrial iron-magnesium silicates and volcanic glasses (Fisk *et al.*, 2006). Fisk *et al.* (2006) also made cautious conclusions about their microtubular weathering textures: "though the tunnels found in Nakhla are similar to the biosignatures found in terrestrial minerals, their presence cannot be used to prove that the martian alteration features had a biogenic origin." This position underscores the need for further investigation into biotic and abiotic mechanisms of microtube formation and additional development with regard to the biogenicity criteria proposed herein. Toward this end, we now make some suggestions for future research directions.

AREAS FOR FUTURE RESEARCH

In the course of this review, we have identified five outstanding questions that relate to endolithic microborings and their application as biosignatures on early Earth and beyond.

1. *What metabolic pathways do euendoliths in recent volcanic glasses utilize?* To date, molecular profiling studies and enrichment cultures from a small, but increasing, number of oceanic sites suggest that several bacterial groups and a single Crenarcheaota group that utilize Fe and Mn cycling are the dominant organisms associated with microborings within *in situ* volcanic glasses (*e.g.*, Thorseth *et al.*, 2001; Lysnes *et al.*, 2004). It has not yet been possible, however, to identify specific microbes or metabolic pathways that are responsible for the tubular and granular microborings discussed herein, and, therefore, some still view "volcanic" microborings with caution and perhaps even skepticism with regard to their biogenicity. There is a clear need for further characterization of endolithic microbial communities within different rock types and at various depths within recent seafloor volcanic glasses. Investigations such as those of Giovannoni *et al.* (1996), Thorseth *et al.* (2001), and Lysnes *et al.* (2004) have taken great care to try and minimize contamination of the microbial euendolith community by microbes from younger circulating seawater, but this will always remain a concern. We suggest that nutrient injection and utilization experiments at drill sites within *in situ* seafloor volcanics may help to constrain the metabolisms of "volcanic" euendoliths. Perhaps, resin fixing of the samples at depth and confocal laser scanning microscopy at the surface may also enable investigation of the euendoliths in their lithic context while attempting to minimizing contamination (cf. Ascaso and Wierzchos, 2002). These types of studies will help improve our understanding of how and why euendolithic microorganisms inhabit seafloor volcanic rocks.
2. *What mechanisms do oxygenic, photosynthetic euendoliths use to bore into carbonate substrates?* In a recent review, Garcia-Pichel (2006) highlighted the significant gap in our understanding of how euendoliths that utilize oxygenic photosynthesis bore into carbonate substrates, given that alkalization rather than acidification is the predicted consequence of their metabolisms. Considering the widespread occurrence of such organisms in modern shallow-water carbonates and their potential as planetary biomarkers, this apparent paradox needs to be resolved (Garcia-Pichel, 2006). In this study, Garcia-Pichel outlined three possible models for the spatial and temporal separation of photosynthesis and respiration in such microorganisms that may enable microboring. Laboratory experiments are required to investigate these mechanisms and might help focus the search for environments where bioerosion of carbonates is most likely to be found.
3. *Which taphonomic pathways preserve morphological and chemical signatures in euendolithic microborings?* To help refine attempts to search for and interpret the morphological and chemical signatures preserved in euendolithic microborings, an increased understanding of their taphonomy would be very instructive. It has been observed, for example, that fossil euendoliths in ancient volcanics show appreciable size variation (*e.g.*, Furnes *et al.*, 2007, Figs. 6 and 7), some of which may be attributed to taphonomic processes. Work to date also sug-

gests that tubular microborings have a more extensive fossil record than granular textures, and this may be due to their higher preservation potential. No comparable size distribution data are available for microborings in sedimentary rocks, and the consequence of their widespread silicification is not understood in detail. Systematic laboratory experiments akin to those that have been conducted on carbonaceous microfossils could be designed to investigate these taphonomic processes (cf. Toporski *et al*., 2002) and explore, in particular, the consequences for C- and N-rich linings preserved within microtubes (see point 4 below). In addition, we have also identified the need to investigate experimentally the hypothesized mechanisms of AIT formation to help distinguish ancient AITs and euendolithic microborings.

4. *What are the fine-scale mineralogical and isotopic signatures that are unique to euendoliths and can survive in the rock record for millions of years?* This article reviews the current range of compositional and isotopic signatures that are used to help identify euendoliths. There exists great scope for high-resolution techniques such as focused ion beam TEM for further exploration of the fine-scale chemical, mineralogical, and morphological characteristics of euendolithic microborings, perhaps in conjunction with energy electron loss spectroscopy to investigate the redox states of the infilling phase and possible metabolic scenarios (cf. Alt and Mata, 2000; Wierzchos *et al*., 2003, 2005; Benzerara *et al*., 2005) (Fig. 4). Fine-scale isotopic signatures could also be measured, *in situ*, at the scale of the microtubes using emerging nanoSIMS techniques (*e.g.*, Kilburn *et al*., 2005).
5. *What are the macroscale controls on the distribution of endolithic microborings in the fossil record, and do these encode information about biological behavior?* There currently exists only sparse data on the outcrop to thin section scale, *i.e.*, decimeter to micrometer density and distribution of endolithic microborings in ancient volcanic glasses and, to a lesser extent, sediments. We posit that these may reflect biologically significant variables such as fluid flux and oxygenation, nutrient supply, fracture density, and thermal gradients, and should thus be explored to document ancient ecosystem gradients. For example, do microtubes in volcanic breccias show a preference for specific clast compositions and, thus, reveal clues having to do with their putative metabolisms?

CONCLUSION

If we are to test the hypothesis that euendolithic organisms have inhabited or may still inhabit the subsurface of Mars, we need to be able to identify endolithic organism remains here on Earth. Toward this goal, this article reviews studies of recent and ancient endolithic organisms in volcanic glasses, silicate minerals, silicified carbonates, and sandstones in an attempt to formulate the following criteria for establishing their biogenicity:

1. *Age and syngenicity.* The relative age of candidate, endolithic microborings in sedimentary substrates can be estimated by placing them within a diagenetic and depositional framework. Advances in the absolute dating of mineral phases preserved within "volcanic" microborings are also explained.
2. *Biogenic morphology and behavior.* The range of morphologies displayed by sedimentary and "volcanic" euendoliths is compared, and criteria are offered to distinguish them from AITs. Hypotheses are developed to explore how the distribution of euendolithic microborings might reflect biological behavior and be controlled by variables such as fluid flow and oxygenation, nutrient content, and integrity of the substrate.
3. *Geochemical processing.* Examples are explained to illustrate how *in situ* analytical techniques, including scanning electron microscopy-energy-dispersive X-ray, focused ion beam TEM, and nanoSIMS, can be used to document fine-scale compositional and isotopic variations within and around microtubes to test their biogenicity.

Research is continuing apace to obtain a more complete fossil record of euendolithic microorganisms on Earth. Some critical terrestrial intervals that should be targeted include 3.8–3.0 Ga and the early evolution of life, as well as 2.4–2.0 Ga and the oxygenation of the Earth's surface. Initial reports of tubular textures in martian meteorites are extremely intriguing and will greatly benefit from further comparisons with terrestrial equivalents.

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ABBREVIATIONS

AIT, ambient inclusion trail; nanoSIMS, nanoscale secondary ion mass spectrometry; TEM, transmission electron microscopy.

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