ASTROBIOLOGY
Volume 9, Number 6, 2009
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DOI: 10.1089/ast.2008.0238

Research Article

Astrobiological Implications of Rock Varnish in Tibet

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Abstract

The study of terrestrial geomicrobiology and its relationship to rock weathering processes is an essential tool in developing analogues for similar processes that may have occurred on Mars. Most studies of manganese-enhanced rock varnish have focused on samples taken from warm arid desert regions. Here, we examine samples obtained from eolian-abraded lava flows of the 4700–4800 m high Ashikule Basin in Tibet. Because it receives approximately 300 mm of precipitation annually, this site is nowhere near as dry as Atacama Desert locales. However, the dusty, sulfate-rich, high-altitude and high-UV flux environment of the Tibetan locale offers new insight into rock varnish formation processes in a terrestrial environment that displays some attributes similar to those expected on early Mars. Microprobe measurements reveal that Mn enhancements in varnish are two orders of magnitude above the dust source, but Fe is only enhanced by a factor of three. Manganese-enhancing bacterial forms are not abundant but are still approximately 3 times more common than in Mojave and Sonoran Desert varnishes. In addition to its occurrence in subaerial positions, Tibetan varnish also occurs in micron-scale "pods" enveloped by silica glaze and as remobilized constituents that have migrated into the underlying weathering rind. A lack of surficial Mn-rich varnish, therefore, might not imply the absence of varnish. In contrast to suggestions that silica glaze might be a good source of microbial fossils and a key to varnish formation, we did not observe any clear microfossil forms entombed in silica glaze; further, there is no gradation between varnish and silica glaze but only distinct contacts. Key Words: Analogue—Astrobiology—Bacteria—Biomineralization—Desert varnish—Geomicrobiology—Life on Mars—Manganese enhancement—Rock coating—Rock varnish—Microstromatolite—Tibet—Weathering. Astrobiology 9, 551–562.

Introduction

Ever since shiny dark coatings were first imaged by cameras on board the 1976 Viking landers (Moore *et al.*, 1987), questions about whether these rock coatings (Fig. 1) could be similar to rock varnish coatings on Earth have arisen (El-Baz and Prestel, 1980; Burns, 1986; Greeley, 1987; Guiness, 1997; Israel *et al.*, 1997; Dorn, 1998, 2007b; Krinsley and Rusk, 2000; Allen *et al.*, 2001, 2004; Johnson *et al.*, 2001; Landis, 2001; DiGregorio, 2002; Mancinelli *et al.*, 2002; Perry *et al.*, 2002, 2007; Wierzchos *et al.*, 2003; Kerr, 2004; Murchie *et al.*, 2004; Warren-Rhodes *et al.*, 2005; Perry and Sephton, 2006; Mahaney *et al.*, 2008; Spilde *et al.*, 2008). The possible astrobiological implications for rock varnish on Mars are that it may hold the key to knowing whether this Mn-enhancement system has occurred in Mars' distant past or whether it still possibly continues today. Rock varnish is sometimes incorrectly referred to as desert varnish because the majority of it studied thus far has been from arid desert regions (Dorn, 2007b). However, rock varnish is also found in almost every other terrestrial weathering environment of our planet (von Humboldt, 1812; Lucas, 1905; Linck, 1930; Klute and Krasser, 1940; Longwell *et al.*, 1950; Glazovskaya, 1958, 1971; Khak-mun, 1973; Glazovskiy, 1985).

Rock varnish is composed of a complex combination of Fe oxides, clays, and Mn oxides (Potter and Rossman, 1977; Krinsley, 1998; McKeown and Post, 2001; Garvie *et al.*, 2008). Manganese-oxidizing bacteria are often cited in the literature as mediating the dark Mn oxides associated with rock varnish (Krumbein, 1969; Dorn and Oberlander, 1982; Taylor-George *et al.*, 1983; Palmer *et al.*, 1985; Hungate *et al.*, 1987; Staley *et al.*, 1991; Adams *et al.*, 1992; Grote and Krumbein, 1992; Eppard *et al.*, 1996; Sterflinger *et al.*, 1999; Perry *et al.*, 2004a; Boston *et al.*, 2008; Spilde *et al.*, 2008). This association seems natural, considering that Mn-oxidizing microorganisms are ubiquitous on Earth and commonly found accumulating on substrates in wet environments, such as deep-sea nodules; oceanic crusts; stones; pebbles in creek beds, rivers, and lakes; and in municipal water supply pipes. They have even been found in dark caves (Boston *et al.*,

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FIG. 1. Potential rock varnish site on Mars. This image was taken by Spirit on Sol 601 September 11, 2006 (Sol 601A, Seq P2567, LTST: 13:11:59). Processing of the image is by Olivier De Goursac NASA/JPL. This particular location shows greater potential for Mn-rich varnish, as evidenced by the split rock in the middle rear. Since rock surfaces on Mars experience eolian abrasion that can remove varnish, the type of varnish that originates in rock fissures might survive. The inside of the fractured rock shows the same fissuresol color pattern that exists commonly in terrestrial settings (Villa *et al.*, 1995; Cerveny *et al.*, 2006). Color images available online at www.liebertonline.com/ast.

2008). While many in the scientific community have implicated Mn-oxidizing microorganisms (Fig. 2) in the formation of terrestrial rock varnish, debate regarding its origin (*i.e.*, biological or abiotic) remains open, largely because arid varnishes grow at a slow pace of a few micrometers per thousand years (Dorn, 1998; Liu and Broecker, 2000). Over this period of time, the evidence for bacterial casts or fossils is mostly obliterated (Jones, 1991; Dorn and Meek, 1995; Dorn, 1998; Krinsley, 1998; Probst *et al.*, 2002; Perry *et al.*, 2004a) by ongoing nanoscale disequilibrium of Mn-Fe oxides (Krinsley, 1998; McKeown and Post, 2001; Dorn, 2007b). Given that low-temperature Mn-oxide deposits can form through either abiotic (Krauskopf, 1957; Madden and Hochella, 2005) or biologically enhanced deposition, the need to determine whether the role of these processes can be differentiated with regard to rock varnish remains a high scientific goal. A priority for astrobiology would be to demonstrate exactly what type of remains in varnish could be used to prove microbial input was essential in formation of the deposit. Determination of the biosignatures produced only by Mn-oxidizing microbes during the production of Mn-oxide coatings, which may include their fossilized remains, is a key endeavor.

We present results of the first study of rock varnish in a setting that is a heretofore unexplored possible terrestrial analogue: the Ashikule Basin of Tibet. This locale hosts eolian abrasion of lava flows in a cold, sulfate-rich, high-elevation, low-pressure, and very dusty environment, which thus displays some similarities to the martian surface. However, what little climatological data exist for the region suggest that the area experiences about 300 mm of annual precipitation (Dorn, 1998). This is too wet to reflect desert terrestrial sites traditionally used as analogues; at the same time, many warm desert collection sites that dominate the literature on possible terrestrial analogues for Mars receive almost as much precipitation (Perry *et al.*, 2004a, 2004b, 2006; Perry and Kolb, 2004; Kuhlman *et al.*, 2005, 2006; Perry and Lynne, 2006; Kuhlman and Abrecht, 2008).

Study Site

The Ashikule Basin is in one of the driest sections of the Tibetan Plateau and is situated on the north side of the western Kunlun Mountains (Fig. 3). Several different types of rock coatings are found in abundance in the Ashikule Basin, Tibet (see Chapter 15 in Dorn, 1998). Sulfates typically attributed to the physical weathering of lava flows are found on the sides of fractures. Gypsum spreads in fractures and precipitates along joint surfaces. Carbonates are also found along rock fractures, sometimes precipitated on top of gypsum. Oxalates occur as well. Rock varnish is also found commonly along the fracture walls. Silica glaze is the most common rock coating seen on the lava-flow surfaces.

A number of geographic features also make the region a Mars analogue, though the composition of the volcanic rocks differs from that on Mars. The Akesu volcanic field in the Ashikule Basin is comprised of trachyandesite lava flows sourced from the Ashishan Volcano (Wei *et al.*, 2003). Two playas, Ashikule and Urukele, deflate carbonate and sulfate mineral dust. Like Mars, dust dominates, and there is field evidence of eolian abrasion. Loess mantles all lava flows. In the Ashikule Basin, samples were collected from ventifacted lava flows and volcanic bombs of the Ashisan volcano between 4700 and 4800 meters in elevation.

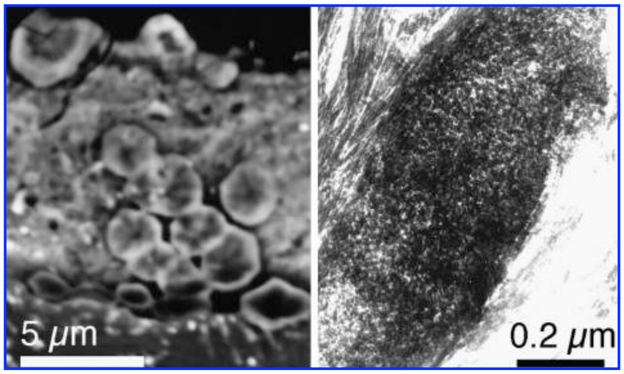

FIG. 2. Back-scattered (left) and transmission electron (right) photomicrographs of cocci-like forms encrusted with Mn in a rock varnish collected from Marie Byrd Land, Antarctica (Dorn, 1998; Krinsley, 1998). The underlying rock is quartz. The high *Z*-contrast particles observed in backscatter could be the type of granular Mn-Fe deposits seen in the transmission electron microscope photomicrograph (same sample, but not the same thin section as the back-scattered SEM photomicrograph).

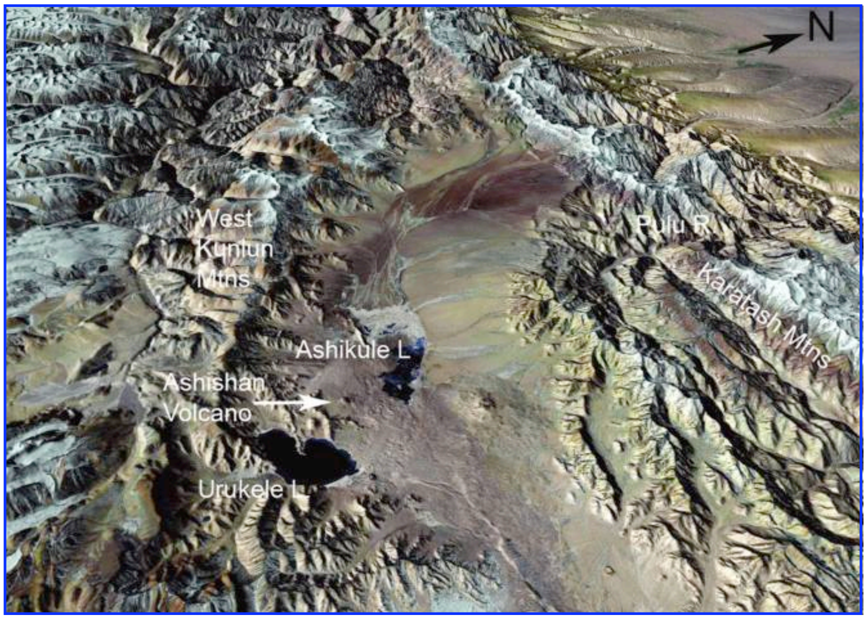

FIG. 3. Ashishan Volcano study site in the Ashikule Basin, Tibet at 35.69885 N, 81.57623 E. The length of Urukele Lake is about 7 km, Ashikule Lake about 5.5 km, and the width of the graben at the location of the Ashishan Volcano is about 22 km. The image is courtesy of William Bowen. Color images available online at www.liebertonline.com/ast.

Methods

Results reported here are based on different electron microscope methods. The cross-section results were taken via a FEI Quanta 200 scanning electron microscope (SEM) that features a high-sensitivity 18 m diameter back-scattered detector for atomic number contrast. The resolution of back-scattered images is 10 nm or less. We used an HV of 10kV and a working distance between 10 and 15 mm.

In addition, a lower-resolution SEM was used with its energy-dispersive X-ray (EDX) unit to examine bacterial forms on the surfaces of varnishes and, in cross sections, for evidence of *in situ* Mn and Fe enhancement. We scanned approximately $8.0\times10^7\,\mu\text{m}^2$ of subaerial Tibetan varnish and approximately $3.2\times10^5\,\mu\text{m}^2$ of cross-section Tibetan varnish. The initial scan was carried out at a magnification where every cocci-shaped, filamentous-shaped, or rod-shaped form could be identified. Then, each form was assessed for Mn enhancement by comparing the spectrum produced by a focused beam ($\sim$1 μm) spot on the bacteria with the spectrum produced by a defocused beam ($\sim$30 μm) centered on the same locale as the focused spot in order to average elemental chemistry spatially.

Thirty samples of dust on rock varnishes from the Ahikule Basin were homogenized in a flux of lithium metaborate. The resultant homogenized beads were mounted in epoxy, polished, and carbon coated. Microprobe measurements were also made on silica glaze coatings and rock varnish coatings. Composition was analyzed by a wavelength-dispersive JEOL Superprobe electron microscope. We used an accelerating voltage of 15 kV and beam currents of between 10 to 20 nanoamps. Dust samples were analyzed by counting for 100 seconds with a 100 μm spot size, whereas silica glaze and varnish analyses were made with counting times of 60 seconds and a focused beam spot size of about 1 μm.

Results

The subaerial rock varnish formed on Ashikule lava flows merges laterally and intercalates vertically with many of the other rock coatings, for example, phosphate films and gypsum. However, the most common rock coating that intercalates with varnish is silica glaze (Fig. 4). At all spatial scales we observed, there is no gradation between rock varnish and silica glaze as argued by others (Perry *et al.,* 2006). Instead, we observed only very clear and distinct boundaries. In the Ashikule samples, we observed a texture not previously reported in the literature: rock varnish in distinct oval-shaped accretions of varnish completely surrounded by silica glaze, which we informally call "pods" (Fig. 4). Pod lengths ranged from tens of micrometers long down to a few nanometers.

Deflation from the shorelines of Urukele and Ahikule dry lakes provides the raw ingredients for the varnish. Electron microprobe analyses of 30 samples of dust collected from rock varnish revealed average (and ± standard deviation) concentrations of 0.11% ± 0.08% MnO with FeO values of 5.13% ± 2.52%. Comparisons of the electron microprobe measurements made from cross sections of rock varnish with those from the dust samples scraped off the rock varnish (Table 1) revealed that Mn enhancements in varnish are 2 orders of magnitude above the dust source. However, Fe enhancements in varnish are only on the order of a factor of 3. Microprobe measurements of silica glaze coatings revealed similar chemistries to those reported elsewhere (see review in Chapter 13 in Dorn, 1998).

Examination of subaerial varnishes with secondary electrons, in tandem with the acquisition of EDX spectroscopy, revealed only a few examples of bacterial forms (Fig. 5) associated with higher concentrations of Mn. These forms were either clusters of cocci forms (Fig. 5A), filamentous hyphae forms (Fig. 5B), or bacterial cocci forms that appear to emerge from hyphae forms (Fig. 5C). In each example in Fig. 5, the Mn enhancement revealed by the focused beam ($\sim$1 μm) spot on the bacteria demonstrates enrichment in both Mn and Fe over the adjacent varnish measured in a defocused beam ($\sim$30 μm) centered on the same locale as the focused beam measurement.

Although the paucity of microbial forms on the outermost surface of the samples could be the result of coating the

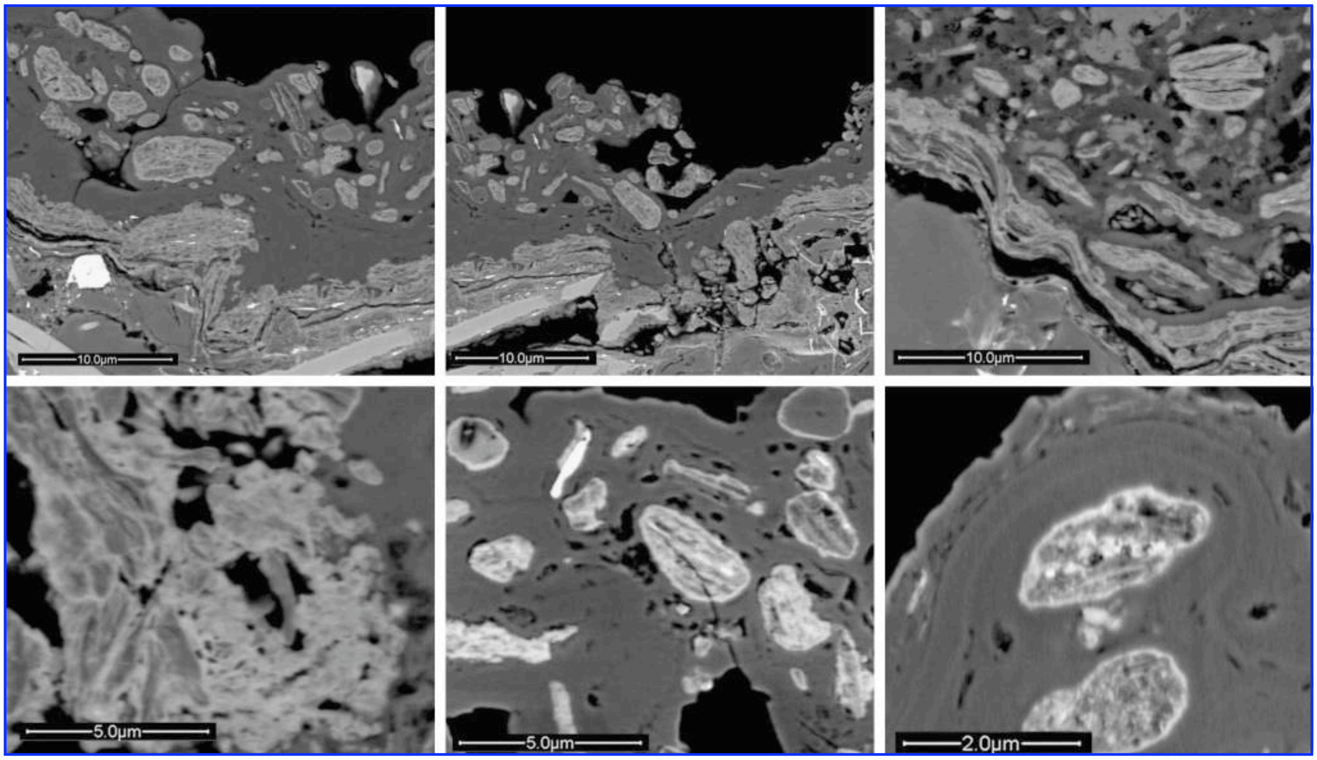

FIG. 4. Intercalation of silica glaze (darker coating) and rock varnish (brighter material) as seen in cross section in backscattered electron microscope photomicrographs. The composition of these distinct and separate rock coatings was assessed through EDX analyses and electron microprobe measurements (Table 1). This style of varnish growth in oval-shaped "pods" is not uncommon in the Ahikule coatings.

TABLE 1. WAVELENGTH-DISPERSIVE ELECTRON MICROPROBE ANALYSES OF ROCK VARNISH AND SILICA GLAZE FROM THE ASHIKULE BASIN, TIBET

Rock varnish

Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	SO_3	K_2O	CaO	TiO_2	MnO	FeO	BaO	Total
0.00	2.69	11.98	25.89	7.03	0.22	1.75	8.70	0.42	11.26	14.54	1.62	86.10
0.05	3.02	10.52	20.97	1.70	0.02	1.48	3.65	0.38	18.76	17.86	2.05	80.46
0.16	2.84	10.90	19.83	1.92	0.00	1.53	3.79	0.32	20.25	17.19	2.32	81.05
0.07	2.95	10.32	20.62	1.74	0.12	1.55	4.04	0.50	19.70	17.67	2.37	81.65
0.12	3.05	11.15	20.60	2.41	0.07	1.69	4.58	0.77	17.64	19.03	2.17	83.28
0.20	3.28	11.90	24.67	1.79	0.40	1.86	3.65	0.42	15.71	18.52	1.92	84.32
0.20	3.12	11.79	22.89	2.25	0.45	1.69	4.06	0.32	16.35	18.37	2.18	83.67
0.00	3.02	11.41	22.19	2.41	0.12	1.66	4.46	0.70	15.70	19.64	2.39	83.70
0.16	2.55	11.19	21.80	2.98	0.15	1.73	4.45	0.53	13.76	24.68	2.22	86.20

Silica glaze

Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	SO_3	K_2O	CaO	TiO_2	MnO	FeO	BaO	Total
0.67	3.64	16.18	49.88	0.19	0.71	2.44	3.03	0.14	0.00	6.45	0.67	84.00
0.55	2.94	17.00	47.26	0.31	0.83	2.16	3.14	0.23	0.00	4.14	0.70	79.26
0.94	2.07	16.18	45.99	0.31	1.06	2.16	3.55	0.21	0.00	7.19	0.83	80.49
0.18	0.45	5.22	62.07	1.52	0.33	0.24	2.17	0.10	0.22	1.51	0.20	74.21
0.33	0.60	5.70	63.49	1.62	0.29	0.18	1.96	0.13	0.24	1.30	0.19	76.03
0.19	1.17	7.30	59.83	1.49	0.18	0.25	1.80	0.09	0.20	1.97	0.15	74.62
0.07	5.46	1.44	60.53	0.16	1.07	0.05	15.53	0.03	0.00	0.01	0.00	84.35
1.23	4.74	7.90	45.97	0.09	1.25	0.46	20.67	0.03	0.00	0.09	0.00	82.43

Values are in oxide weight percent. Totals do not reach 100% due to porosity, water, and content of organic matter.

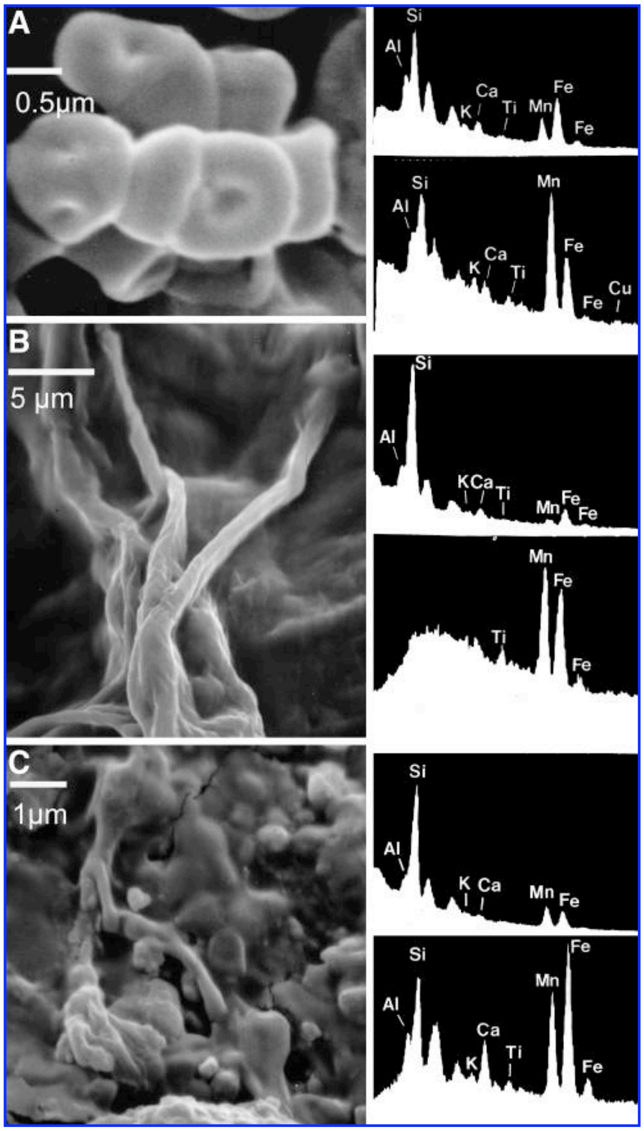

FIG. 5. Manganese-enhancing bacterial forms on the surface of Ashishan Volcano varnish. Three different bacterial-sized morphologies were associated with Mn-enhancement: (**A**) clusters of cocci-appearing forms; (**B**) filamentous hyphae forms with bacteria widths; and (**C**) budding bacterial forms that appear to emerge from submicron-scale hyphae-like forms. The adjacent EDX analyses show the strong spike in Mn in the focused beam (lower analysis in each pair of spectra), as compared to the lower abundance of Mn in the defocused beam spectra obtained by analyzing primarily the surrounding varnish (upper analysis in each pair).

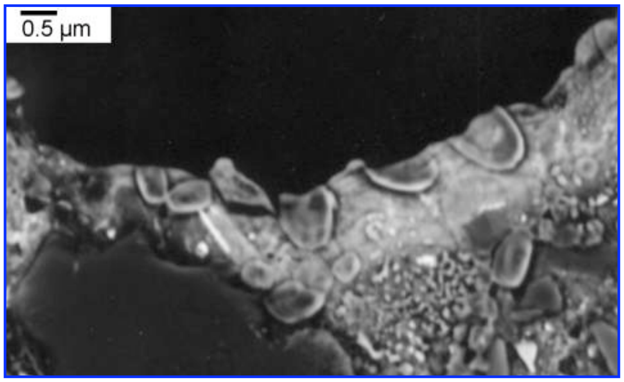

FIG. 6. Bacterial-sized fossil-like morphologies revealed with the use of back-scattered electron imaging conditions in rock varnish samples cut in cross section. Note that eolian abrasion has truncated some of the cocci-like forms.

samples without preparing the samples to preserve and avoid dislodging cells, microfossil-like objects were similarly rare inside the varnish. Bacterial-sized morphologies are unusual in cross sections made normal to the varnish surface, though, as shown in Fig. 6, bacteria-like objects were found in some locations of the Mn-enriched pods (Fig. 6). In another area of the sample, a group of rod-shaped forms in dust that was lightly cemented onto the surface of a varnish was observed (Fig. 7). In this latter case, the sample was not a cross section normal to the surface but rather a very lightly polished section oriented parallel to and just a few micrometers beneath the varnish surface.

The hypothesis that silica glaze might be a good place to detect microbial fossils (Perry *et al.*, 2006) could not be verified in the Ashikule samples, since we did not observe any clear microfossil forms entombed in the silica glaze. We did not observe microfossil-like objects in sulfate crusts or carbonate rock coatings, either. However, some microfossils were found embedded in calcium-oxalate coatings (data not shown).

In scanning approximately $8.0\times10^7\,\mu m^2$ of subaerial Tibetan varnish and approximately $3.2\times10^5\,\mu m^2$ of cross-section Tibetan varnish, subjectively "obvious" Mn-enhanced (determined by EDX analysis) bacterial forms had a density of approximately 1.8×10^6 forms/μm^2 on the surface and approximately 1.3×10^4 forms/μm^2 in cross section. These concentrations are similar to previously studied Antarctic varnishes (Dorn *et al.*, 1992) and approximately 3 times higher than what we have found for Sonoran and Mojave Desert samples (Dorn, 1998).

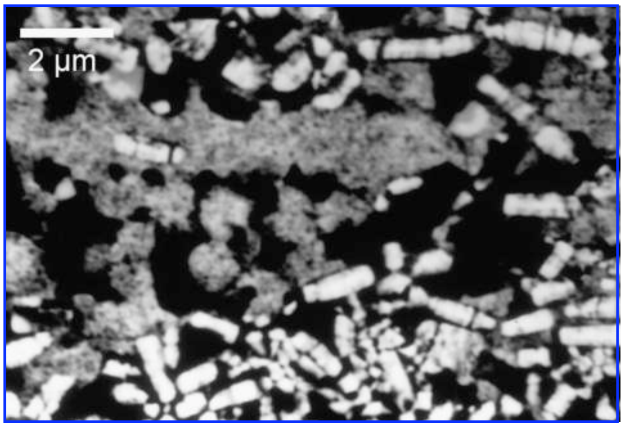

FIG. 7. Bacterial-sized, Mn-rich forms seen in the upper micrometer of varnish, imaged with back-scattered electrons. This sample was mounted in epoxy and polished down until the view was planimetric for the upper few micrometers of the varnish. The rod-shaped forms are brighter because of their higher concentration of Mn and Fe. The higher concentration of Mn and Fe was measured qualitatively with focused-beam ($\sim1\,\mu m$) EDX analyses.

Much more common than Mn-enhanced microbial forms are discontinuous submicron-sized bright fragments (Fig. 8). Though high-resolution transmission electron microscopy (HRTEM) analysis confirmed that some of these structures are mineral fragments, most likely detrital fragments of Mn-Fe incorporated into the varnish, many appear to have the nanometer-scale granular texture associated with the obvious microbial forms observed by Krinsley (1998). However, it was not possible to demonstrate that these latter high atomic number–contrast (high *Z*-contrast) objects are bacterial remains because of the inherent instability of the Mn-Fe objects due to observed nanoscale disequilibrium in Mn-Fe oxyhydroxides (Krinsley *et al.,* 1990, 1995; Dorn, 1998, 2007b; Krinsley, 1998; McKeown and Post, 2001). These objects are common in the Ashikule samples (Fig. 9).

We did observe a few areas of finely layered fabrics in the Tibetan samples (Fig. 10), though they were not common. Sometimes, the microlaminations occurred in isolation. In a few cases, they occurred in groups of 2 to 20 rounded forms (Fig. 10). Micromounds ranged from 10 μm in diameter to less than 700 nm.

The varnish-rock boundary was quite distinct in most of the cross sections we examined (Figs. 4, 6, 8, 10). However, the underlying weathering rind, in some cases, had developed pores from dissolution and other weathering processes. Figure 11 shows two examples where varnish extends down in the weathering rind, which contributes to the process of case hardening the surface (Dorn, 2004). This type of varnish-rock boundary has also been observed in the silica glaze. The downward movement and reprecipitation of silica in the underlying rock is attributed with causing this type of boundary feature (Gordon and Dorn, 2005a, 2005b).

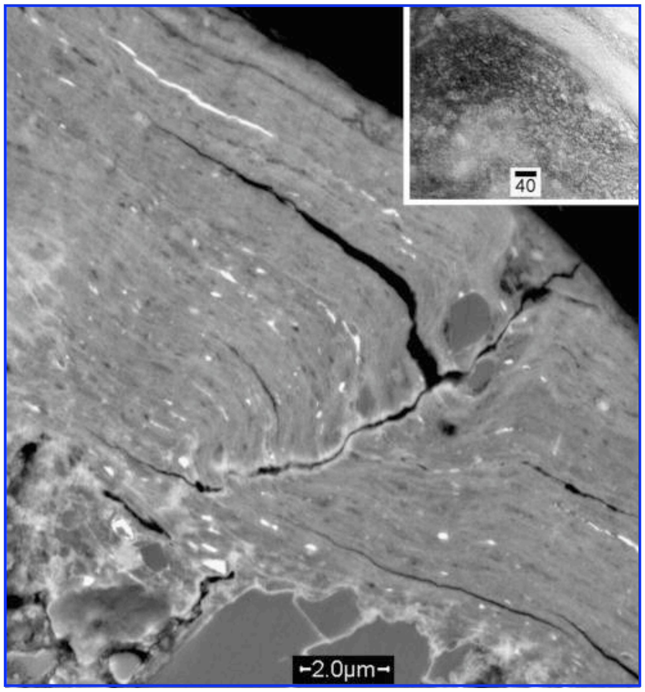

FIG. 8. Subaerial rock varnish that displays submicrometer-sized bits of high *Z*-contrast minerals in back-scattered SEM photomicrographs. One highly speculative interpretation is that these are partial fragments of Mn-encrusted cells. This image also illustrates the evidence for mobilization and reprecipitation of varnish in fractures—features commonly seen in other varnishes (Krinsley *et al.,* 1990; Garvie *et al.,* 2008). The image inset into the upper right with a 40 nanometer scale bar is a HRTEM image of the granular texture seen when bacterial forms undergo post-depositional diagenesis (Krinsley, 1998).

Discussion and Conclusions

Soils used as martian analogues are hyperarid; they experience a high ultraviolet flux, host very low bacterial levels, and exhibit a very low organic content (Navarro-González *et al.,* 2003). Rock coatings, however, develop in a very different biogeochemical environment than soils for a host of reasons, including the nature of the coating itself, its microtopographic position shedding rather than collecting aerosols and moisture, and its constant subaerial exposure until buried by a new accretionary layer (Dorn, 1998).

The rock coatings of the Ashikule Basin, Tibet, are different from other rock coating sites that have been posited as possible terrestrial analogues for Mars. With approximately 300 mm of precipitation, the study area is far wetter than hyperarid warm deserts. Samples from Antarctica (Dorn *et al.,* 1992) and Tibet, however, may actually be more appropriate Mars analogues than those in warm deserts, especially for ancient Mars. The altitudes of our sites in Tibet are ~4700 m; hence, the collected varnish derives from the lowest air pressure yet studied on Earth. The host rock is lava from the Ashishan Volcano, which has experienced eolian abrasion. Dust dominates in the Ashikule Basin. Sulfates are common in dust and as coatings on rocks. Although we did not measure UV flux, previous research in the region revealed very high dose rates at this altitude, sometimes exceeding UV indices of 15 at noon (Dahlback *et al.,* 2007). For the above reasons, our samples from the dry Tibetan Plateau offer important insights into the potential for rock varnish to retain evidence of geomicrobial input with regard to its formation.

Microfossils were not common in the Tibetan samples we examined, though there were some bacteria-sized forms that consisted of Mn and Fe (Figs. 5, 6, 7). If such objects were the remains of encrusted bacteria, our observations would be similar to those of previous studies, which have shown that microfossils are not terribly common in rock varnish outside of very unusual microenvironments (Jones, 1991; Dorn and Meek, 1995; Dorn, 1998; Krinsley, 1998; Krinsley and Rusk, 2000; Probst *et al.,* 2002; Perry *et al.,* 2004a). It is worth noting, however, that our previous studies of Antarctic rock varnishes (Dorn *et al.,* 1992; Krinsley, 1998), along with our current work with Tibetan varnishes, have shown that cold desert sites host approximately 3 times more Mn-encrusted bacteria-like forms than warm desert samples collected from the Mojave and Sonoran Deserts. We speculate that this greater abundance could be due to different preservation potential. More-detailed studies will be required to confirm our initial estimate of a 3-fold greater abundance. It is worth noting that we did not observe any microfossil-like objects or *bona fide* microfossils entombed in Ashikule silica glazes, unlike observations made in samples of rock varnish from Antarctica (Friedmann and Weed, 1987).

The general paucity of microfossils in our Tibetan varnishes is consistent with the polygenetic model of varnish

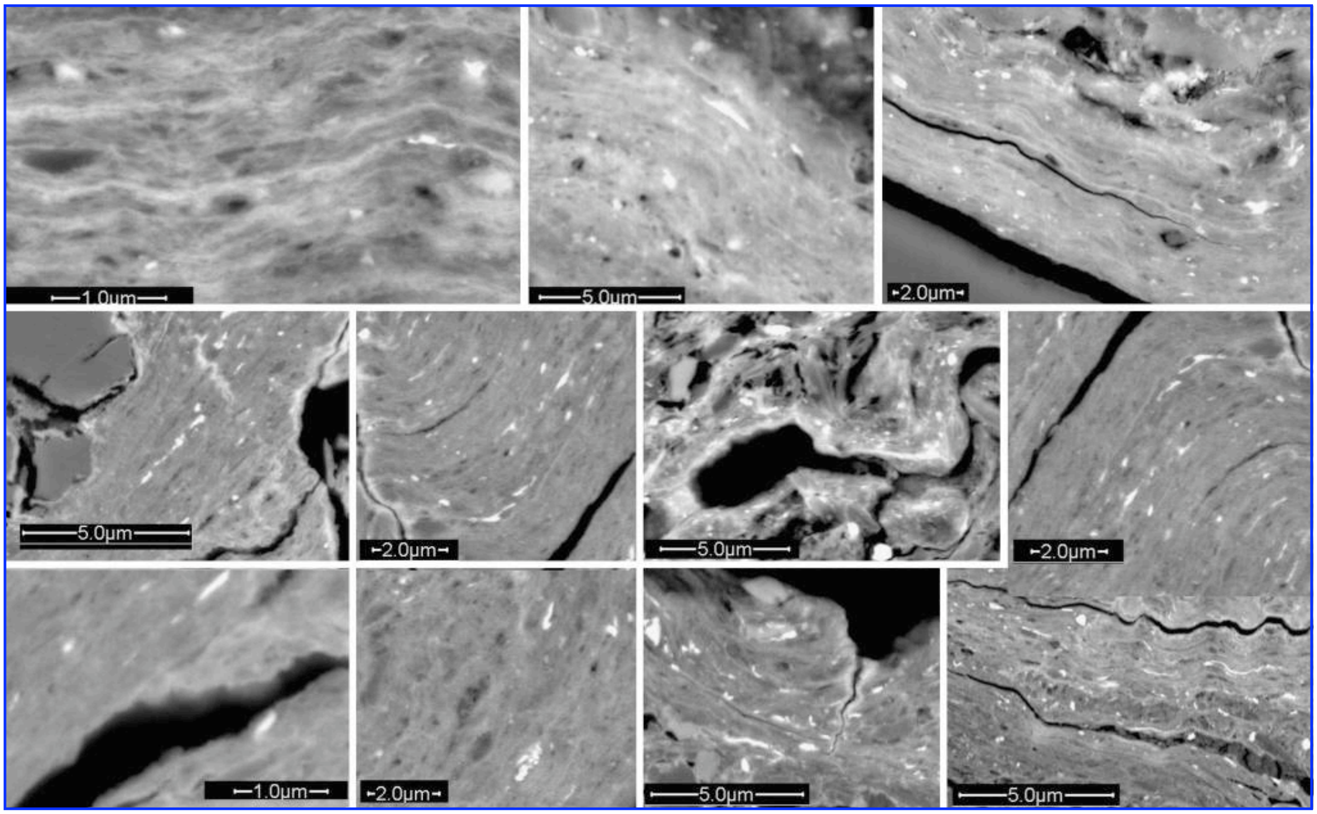

FIG. 9. This back-scattered SEM photomicrograph shows different views of possible bacterial remains seen in cross section. Submicrometer high Z-contrast objects documented in back-scattered SEM images generally trend with the varnish bedding planes. These objects are very common in some Tibetan samples, and though some represent Mn-Fe minerals, some may be pieces of Mn-Fe casts of bacteria. Note how these bits tend to be elongated in the bedding direction. Given that the organic material in bacterial cells can be expected to last for only a short length of time, the bacterial forms compress and lose Mn-Fe to ongoing nanoscale disequilibrium-driven phase transformations (Krinsley *et al.*, 1995; Krinsley, 1998; McKeown and Post, 2001; Dorn, 2007b).

formation that depends on a lack of equilibrium in the Mn precipitated by microbes (Potter, 1979; Krinsley, 1998; McKeown and Post, 2001; Dorn, 2007b). With the use of HRTEM imagery, it appears that Mn and Fe become remobilized from bacterial casts and reprecipitate on the "feathered" edges of clay minerals (Dorn, 1998, 2007b; Krinsley, 1998). As McKeown and Post (2001, p 712) explained: "common elements of these structures enable them to easily intergrow with and transform with one another. Furthermore, many of the phases, particularly the layered structures, readily exchange interlayer cations in response to even slight changes in chemistry on a microscale." The Mn-Fe phases associated with encrusted microbes should be broken down continuously into nanometer-scale granules, similar to the phases observed by Krinsley (1998) in HRTEM images (see also Fig. 2), which can then migrate and move into clay minerals. The expectation of finding microfossils preserved in any single random ("grab bag" sample) terrestrial or martian varnish, therefore, should be expected to be low.

We stress that the absence of *bona fide* Mn-encrusted microfossils is fully consistent with a key role for microorganisms in the polygenetic model of varnish formation (Potter, 1979; Krinsley, 1998; McKeown and Post, 2001; Dorn, 2007b). The extremely slow rate of varnish formation, on the scale of microns per millennia (Liu and Broecker, 2000), means that there is a key rate-limiting step of varnish formation (Dorn, 2007b). Although abiotic processes enhance the Mn found in varnish (Krauskopf, 1957; Madden and Hochella, 2005), rates of accretion would be much faster if abiotic processes actually formed varnish. In contrast, finding the observed low abundance of Mn-encrusted microfossils would be consistent with observed varnish formation rates that require an additional extreme rate-limiting step.

If the prediction that "silica glazes will probably be found on Mars" (Dorn, 1998, p 321) is true, then the presence of varnish "pods" enveloped by silica glaze (Fig. 4) in Ashikule samples raises the possibility that spatially discontinuous pods of varnish might be a part of the mixtures of weathering rinds and rock coatings found on Mars (McAdam *et al.*, 2002; Kerr, 2004; Klingelhöfer, 2004; Haskin *et al.*, 2005; Klingelhöfer *et al.*, 2005). The lack of continuous Mn-rich coatings on a rock's surface, therefore, need not indicate the lack of geomicrobiologically initiated varnish. Mineralogical or elemental analyses of the surface of martian rocks could reveal the existence of entombed varnish pods.

In almost every cross-sectional view of Tibetan varnishes, clear boundaries exist between the accreting varnish and the underlying host mineral (Figs. 4, 6, 8, 10). This observation is consistent with previous secondary (Potter and Rossman,

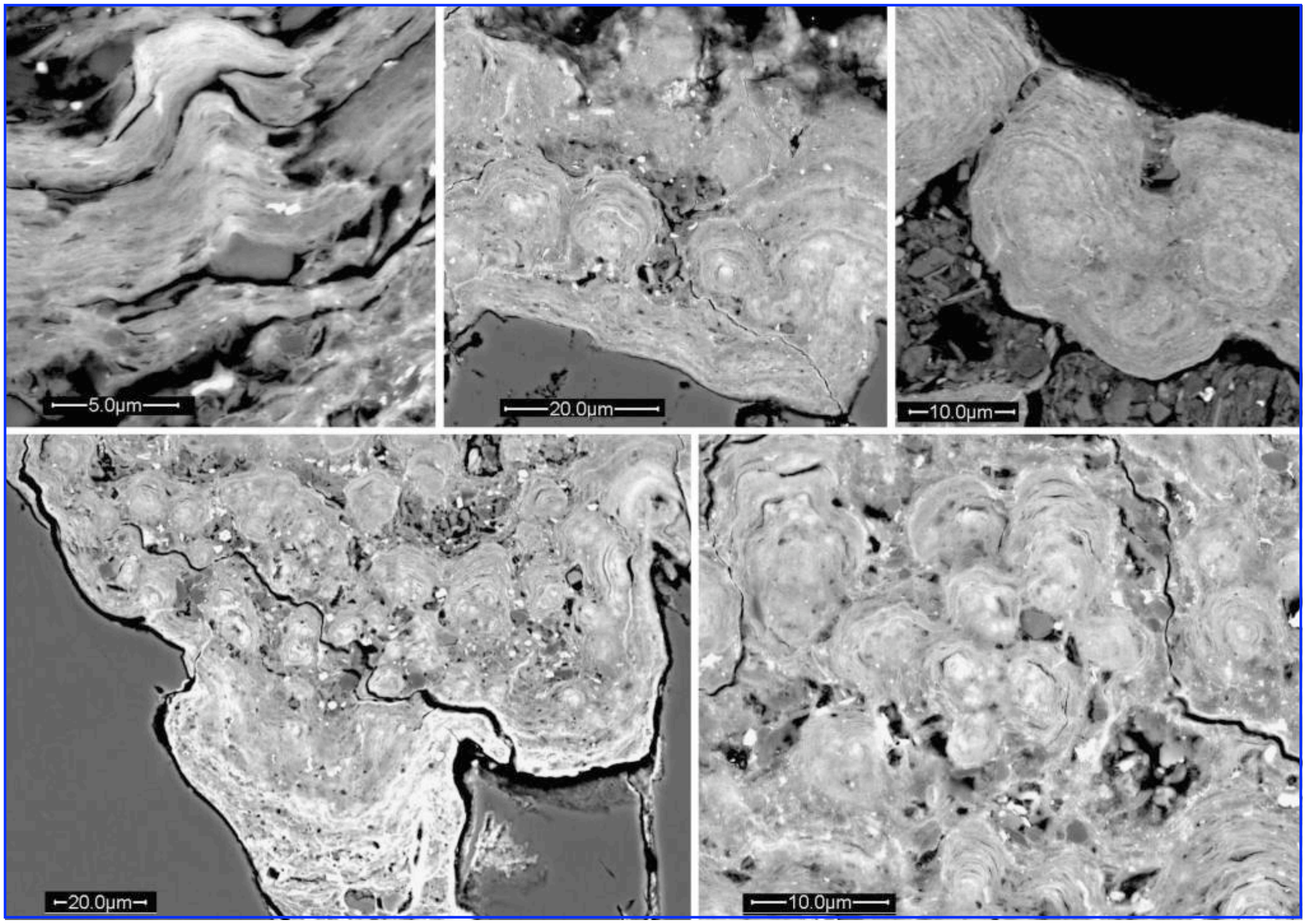

FIG. 10. Botryoidal stromatolitic-like structures visualized in different samples in cross section reveal the presence of microlamination, as viewed by back-scattered electron microscopy.

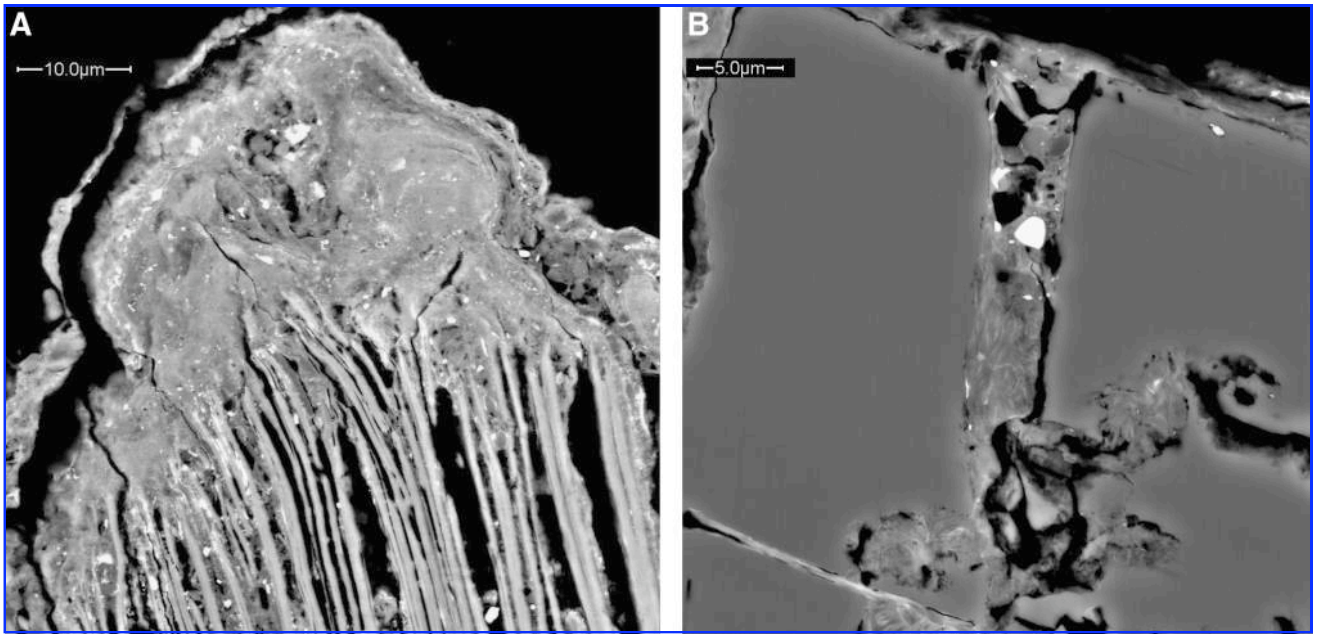

FIG. 11. Just as martian rock coatings appear to be a mix of accretionary coating and weathering rind (McAdam *et al.*, 2002; Haskin *et al.*, 2005), we observed similar combinations of such features in many Tibetan samples. Back-scattered electron microscope photomicrograph 11A shows varnish that has remobilized downward as the underlying mica has gradually opened. Back-scattered electron microscope photomicrograph 11B reveals varnish that has reprecipitated into pore spaces of an underlying silicate.

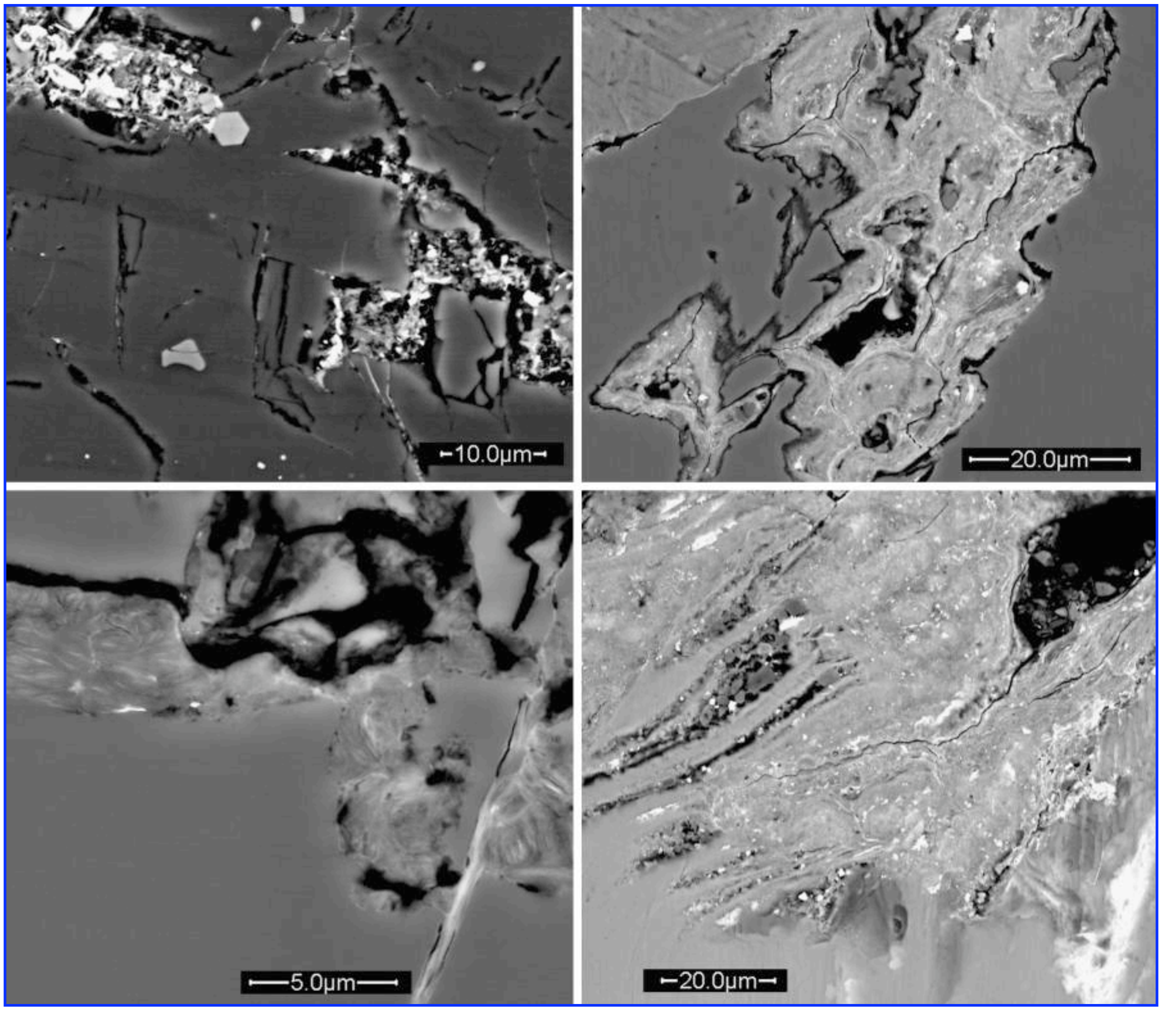

FIG. 12. The infills of remobilized varnish in the Tibetan samples display very different fabrics. Since eolian abrasion of Mars varnish is a distinct possibility, a fruitful line of terrestrial investigation would be the study of these reprecipitates in weathering rinds. In Ashikule lava flow samples, some textures are speckled with bacterial-sized Mn-rich fragments (upper left); some are more laminated (upper right). Other textures show a swirling pattern not reported previously (lower left). Others have bright nodes of more Mn-Fe concentration (lower right). These anecdotal observations point to the possibility that a thorough study of these infillings might provide valuable clues to the sorts of martian deposits found beneath an abraded rock surface.

1977; Dorn and Oberlander, 1982), back-scattered (Krinsley and Dorn, 1991), and high-resolution transmission (Krinsley *et al.*, 1995) electron microscope images, which indicate that varnish is a rock coating and not a weathering rind.

We did, however, observe clear examples of varnish that had presumably been remobilized from the coating into the underlying weathering rind (Fig. 11). In other locations on Earth, the remobilization of primary mineral constituents can lead to case hardening of the outer shell of a rock coated with varnish (Dorn, 1998, 2004; Tratebas *et al.*, 2004). We interpret the features shown in Fig. 11A as evidence of a two-step process, by which varnish first starts to accrete on unweathered biotite. Then, as the underlying biotite "feathers," the varnish migrates downward into the pore spaces. In Fig. 11B, the underlying dissolution pores, in turn, are the location of reprecipitation of varnish.

Since some martian rock surfaces appear to be a mixture of rock coatings and weathering rinds (Kerr, 2004; Klingelhöfer, 2004; Haskin *et al.*, 2005; Klingelhöfer *et al.*, 2005), varnish, if it exists on Mars, might be present inside weathering rinds—that is, long after the varnish formed in a wetter period and long after the original surficial varnish was blasted by eolian abrasion. Weathering-rind infills of remobilized varnish constituents (Fig. 12) might store the remains of ancient varnish.

Microstromatolitic forms seen in varnish cross sections have been used to infer a biological origin (Raymond *et al.*, 1992). We did see a few examples (Fig. 10), but they are not common in examined Tibetan samples. In varnishes from regions with less of a dominant eolian influence, authors Krinsley and Dorn have seen botryoidal stromatolite-like forms comprised of microlaminations in much greater abundance. The initiation point for botryoidal morphologies are often bacterial-sized forms (Dorn, 1986); thus the common speculation is that the genesis of stromatolitic forms in varnish could require microbial seeding (Raymond *et al.*, 1992;

Krinsley, 1998). This speculation, however, has been contested by investigators whose experimental results suggest that stromatolitic forms, such as those seen in varnish, could be produced by abiotic processes (McLoughlin *et al.,* 2008).

There is one last issue that looms over our analysis of Tibetan varnish. As Dorn (2007b) indicated, "the real key in astrobiology research as it relates to varnish, however, does not rest in understanding terrestrial varnish; the biggest gap is the need to falsify abiotic explanations of Mn enhancement on Mars." Even on Earth, where abiotic processes of Mn enhancement are suspect for varnish because of a host of geographical, rate-based considerations, and geochemical factors reviewed elsewhere (Dorn, 2007b, 2007a), no one has been able to falsify the original abiotic conceptual model of varnish formation (Linck, 1900; Krauskopf, 1957; Engel and Sharp, 1958). Further, new nanoscale insights have provided support for abiotic mechanisms to enhance Mn (Madden and Hochella, 2005) that might be present on Mars. Until abiotic enhancement of Mn varnish can be falsified for terrestrial samples, simply finding Mn-rich coatings (or pods or weathering-rind deposits) on Mars will not prove the presence of microorganisms unless atomic force microscopy on the Phoenix lander (Foucher *et al.,* 2008) links fossilized bacterial forms with Mn enhancement.

Acknowledgments

Thanks to Tanzhuo Liu for field collection of varnish in Tibet, suggestions from C. McKay and an anonymous reviewer, and sabbatical support for R.I. Dorn.

Abbreviations

EDX, energy-dispersive X-ray; HRTEM, high-resolution transmission electron microscope; SEM, scanning electron microscope; *Z*, atomic number.

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