Geomicrobiology Journal

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ugmb20

Silicified Biota in High-Altitude, Geothermally Influenced Ignimbrites at El Tatio Geyser Field, Andean Cordillera (Chile)

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Accepted author version posted online: 29 Oct 2013. Published online: 05 Jun 2014.

To cite this article: Roberto Barbieri, Barbara Cavalazzi, Nunzia Stivaletta & Purificación López-García (2014) Silicified Biota in High-Altitude, Geothermally Influenced Ignimbrites at El Tatio Geyser Field, Andean Cordillera (Chile), Geomicrobiology Journal, 31:6, 493-508, DOI: 10.1080/01490451.2013.836691

To link to this article: http://dx.doi.org/10.1080/01490451.2013.836691

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Silicified Biota in High-Altitude, Geothermally Influenced Ignimbrites at El Tatio Geyser Field, Andean Cordillera (Chile)

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Received March 2013, Accepted August 2013

Biofilms and filamentous communities provided favorable sites for silica precipitation on deeply weathered ignimbrites that make up the substrate at the hydrothermal field of El Tatio (Andean Cordillera, Chile). The amorphous silica encrustation enabled the preservation of a variety of biotic and abiotic features. An integrated study based on optical/scanning electron microscopy and molecular methods of totally to partially silicified microbial communities and biofilms allowed a comparative evaluation of the microfacies and the microbial diversity in the siliceous sinters produced by the digression of a little braided stream departing from a hot spring pool. This study showed useful convergent identifications of certain groups of microbes, such as filamentous cyanobacteria attributed to the genera *Phormidium* and *Rivularia*. Together with these microbes, other presumably initial colonizers, such as the halophilic and thermophilic pennate diatoms *Nitzschia* and *Synedra*, were widely present and could have contributed to the formation of biofilms and mucus that, as potential home to early silicification, could have contributed to the preservation of microbiologically derived morphologies.

**Keywords:** El Tatio geyser field, geyserite, ignimbrites, microbial diversity

**Introduction**

The El Tatio geothermal field is located in the northern Chilean part of the Andean Cordillera (Figure 1) and represents the largest geyser field in the southern hemisphere. It is located within the Atacama region, which is a unique region of South America because of its combination of severe environmental conditions that in many respects can be considered a terrestrial model of a Martian environment (e.g., Azua-Bustos et al. 2012; Navarro-González et al. 2003). At El Tatio, where geysers erupt hot and salty water in a volcanic substrate, specific limiting factors for life depend on the co-occurrence of altitude (approx. 4,300 m above mean sea level), water temperature (up to 87°C, the boiling point at El Tatio spring sources) and chemistry (abundance of arsenic and antimony), high daily thermal oscillation, and atmospheric dryness (Cortecci et al. 2005; Glennon and Pfaff 2003; Healy and Hochstein 1973; Lahsen and Trujillo 1976; Phoenix et al. 2006).

All the above environmental parameters make the El Tatio geothermal field a natural laboratory in which a relatively easy location enables field observation and samplings in a variety of geyser subenvironments, including hundreds of spouters, hot pools, streams, mudpots, sulfataras, and fumaroles. The alkali-chloride (thermal) waters of the El Tatio geothermal system (Cortecci et al. 2005; Garcia-Valles et al. 2008) contain dissolved silica in a sufficiently high concentration to allow, wherever discharging water cools down, the precipitation of remarkable amounts of (noncrystalline opal-A) silica sinter in subaqueous and subaerial settings.

Commonly, the formation of amorphous silica deposits (geyserite) in the geothermal fields seems to be largely dependent on abiotic factors (e.g., Bennett and Omelon 2011; Fournier 1985; Rimstidt and Cole 1983), in particular the evaporation and the rapid cooling of the hot spring water, that may reach (and exceed) the local boiling temperature, and pH change. Biotic factors, such as a direct microbial participation, do not appear to play a significant role in the silicification (e.g., Cady and Farmer 1996; Guidry and Chafetz 2003; Konhauser 2007; Konhauser et al. 2004; Orange et al. 2013a; Yee et al. 2003): microorganisms and biofilms appear rather passively involved, and their contribution seems to rely...
on the production of different morphological patterns (e.g., the type of lamination) that then depends on their cell shape, size, colony growth, and organization.

In favorable conditions and moderately high water temperatures, microbes and their biofilms can act as templates for amorphous silica precipitation (see spectacular examples in Jones et al. 1997; Jones et al. 2003; Konhauser et al. 2001). In a recent investigation the silicification textures of the sheathed cyanobacteria *Calothrix* were correlated with specific environmental variables and microhabitats (Hugo et al. 2011). Thus, hot spring deposits can potentially preserve information of extreme environmental conditions and habitats even in very ancient periods of the geological past (e.g., Lowe 1983; Schopf and Packer 1987). Overall, there is now wide documentation, in both laboratory and natural settings, suggesting that microorganisms can influence some silica precipitation in geothermal hot spring environments (see review in Benning et al. 2005). It has also been demonstrated that, at high silica concentration in modern hot springs, certain thermophilic bacteria show biofilm overproduction (Lalonde et al. 2005).

This increased production could preserve the cells themselves from very early silicification and, at the same time, promote the siliceous sinter formation. A further interesting result in hot spring environments is that presented by Ferris and Magalhaes (2008) who described a difference in size for silica particles nucleated on a cell surface compared to those nucleated away from bacterial cells. Despite what still appears to be a lack of active role in silicification, sometimes microbes

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**Fig. 1.** Location of the study area in northern Chile. The white star (in A and B) locates the El Tatio Geyser Field. C: Location of the Upper (U-GB), Middle (M-GB) and Lower (L-GB) Geyser Basins; the white star locates the sampled sites in the Middle Geyser Basin. D: The two sampled sites (black arrows) in the bed and at the edge of a runoff stream are distant from each other about 3 m. The white spots in the area are produced by the evaporation of salt.
Silicified Biota in High-Altitude, Geothermally Influenced Ignimbrites

495

The amorphous silica precipitation at El Tatio leads to the preservation of biological features including filamentous and coccoid microbes embedded in laminated and oncoid morphologies, that have been described in terraces and sinter aprons in the northern part of the geothermal area (Jones and Renaut 1997), as well as microbial fingerprints described from the silica deposits around hot springs and geysers (Fernandez-Turiel et al. 2005; Phoenix et al. 2006). Because of its high UV flux environment, El Tatio makes possible the investigation of the UV shielding characteristics of silica in natural settings (Phoenix et al. 2006). An investigation in a natural setting on microbial mats at El Tatio by using high-resolution thermal infrared imaging (Dunckel et al. 2009) has also expanded the understanding on the effects of temperature gradients on the communities of microbial thermophiles.

Based on the above-described extreme conditions for life, the El Tatio area can be regarded as a good analog for Earth and Mars's primordial conditions (e.g., Farmer 1996, 1998, 2000; Schulze-Makuch et al. 2007; Skok et al. 2010). Also, because of its long-term hydrothermal activity, El Tatio allows combining the study of fully active ecosystems and their preserved remains in the silicous sinters. This information may be valuable to interpret fossil remains within silicified settings through the geologic record.

This study aims at providing a microfacies and microbiological analysis of the amorphous silica sinters that precipitated in a runoff stream of the Middle Geyser Basins (Figure 1) flowing on altered rhyolitic ignimbrites that represent most of the geological substrate in the El Tatio area. In these volcanic rocks the rapid sinter precipitation enabled the preservation of a variety of biotic components in the amorphous silica groundmass, and it also helped to keep together the various elements of a profoundly altered rock substrate. The microbial diversity associated to these substrates based on the amplification of small subunit ribosomal (SSU) rRNA of Bacteria, Archaea and Eucarya, in order to link it to data on the microbial remains and microfacies preserved in geyserite deposit, was also explored. This combination of physical and microbiological data may provide useful information in still poorly understood natural systems, such as the ones centered on the interactions between colloidal silica and microbes.

El Tatio Geyser Field

The geothermal region of El Tatio consists of three distinct areas, the Upper, Middle, and Lower Geyser Basins (Figure 1C) (for details see Glennon and Pfaff (2003). The Middle Basin, from which come the studied samples, consists of pools, fountain-type geysers, and runoff streams departing from hot springs and pools.

The substrate of the three different geyser basins mostly consists of Pliocene and Pleistocene pyroclastic deposits (De Silva and Francis 1991; Lucchi et al. 2009). These volcanic products extensively crop out in the region and are locally covered by the sinter deposits of the geysers system. The Tatio, Puripicar, and Rio Salado pyroclastic Formations, which consist of thickly bedded, multicolored, and compositionally heterogeneous ignimbrite deposits, have been described in the El Tatio area (Lucchi et al. 2009).

Study Area, Materials and Methods

Overall Description of the Study Area

In the ignimbrites substrate of the Middle Geyser Basin, sinter deposits accumulated with different thickness, depending on the recurring digressions of the runoff streams departing from hot spring pools. These deposits mostly consist of light-colored amorphous silica.

The first set of samples was collected in the bed of a braided, runoff stream (Figure 1D) departing from a subrounded hot pool, approximately 3 m across, fed by a permanent spring. Because of the fast water temperature drop from the boiling pool (measured boiling water temperature: 87°C) and along the stream, the chromatic variation of biofacies associated with pigmented microbial communities change rapidly from bright yellow-red orange (measured water temperature exceeding 50°C) to green-black (measured water temperature of less than 50°C).

At the sampled location of the stream bed, near-neutral pH winters (measured pH: 6.95) had a measured temperature of 32°C (brown-pigmented microbial communities). At the time of collection, the sampled location was partially emerged, with some episodic submersion by flowing water depending on the fast changes of intensity of the pool discharge. Samples were therefore collected with some moisture content, and they represent the common opaline silica that precipitate in this area of El Tatio site, where thin halite crusts formed by evaporation locally whiten the surface of these silica deposits (Figure 2A). Silica encased plant stems and remnants of plant tissues, and produced a partially permineralized local litter that was observable at the mesoscale of rock exposure.

The second set of samples was collected in the dry, right (eastern) side of the runoff stream, approximately 3 m distance from the former sampling site (Figure 1D). The rock exposure at this sampled site exhibits a light brown, irregular surface covered by brittle, whitish, millimetric crusts (Figure 2B). Similarly to those along the bed of the stream, these thin crusts mostly consist of evaporitic halite. The “granular” appearance of the rock surface (Figure 2B) along the eastern side of the stream is the product of the surficial alteration of the ignimbrite rock that makes up the substrate in this part of the geothermal field. As a result, the rock and mineral grains of ignimbritic origin locally appear as loose or poorly cemented sand. These non-coherent materials, however, underwent the adhesive activity of prokaryotic and eukaryotic communities, the silica sinter, and other mineral (e.g., limonite) precipitation.

Samples

Rock samples were collected from the Middle Geyser Basin and the coordinates of the sampling location, at an elevation...
of 4,285 meters above mean sea level, were 22° 20’ 36.54’’ S, 68° 00’ 41.58’’ W (Figure 1C). Five samples were collected from the air-rock interface down to 5–8 cm below the surface in each of two nearby areas. For comparative purposes, several closely spaced samples were collected in two sites at a distance from each other of about 3 m. The first sampling site was along the permanently dry eastern edge of a runoff stream (samples label: ETE), whereas the second site was in a temporarily not submerged sector in the bed of the same stream (samples label: ETS) (Figure 1D).

In both sites the siliceous sinter precipitation occurred on the dacitic to rhyolitic ignimbrites that make up the rock sub-strate of the Middle Geyser Basin. The samples were carefully handled (e.g., sterile forceps and gloves) during collection, then placed in sterile plastic boxes, and stored on ice before and during transport. The ETS samples, from which a pristine fragment of surface crust was obtained for microbiological analysis, were shipped, in ice, at the laboratory immediately after their collection and rapidly analysed.

**Physical Analyses**

The petrographic and microfacies-based investigations of these sintered deposits were performed through uncovered petrographic thin sections (45 x 60 mm² surface area, 30-μm foil thickness). Thin sections were examined with a transmitted and reflected Zeiss Axioplan binocular optical microscope equipped with an Olympus DP12 digital microscope camera. The general composition of the sinter deposits was obtained through uncovered X-ray diffraction (XRD) of powdered samples using a Philips PW 1480 X-ray diffractometer, and a scanning electron microscopy and (qualitative) energy-dispersive X-ray microanalyses (SEM-EDX).

Scanning electron microscope (SEM)-EDX analyses were performed on selected areas of thin and polished cross-sections, and freshly broken sample surfaces using a JEOL 5600 (Japan) equipped with an Oxford Instruments (UK) INCA 350 EDX system. The SEM-EDX observations in imaging and analyzing mode were conducted on Au-coated samples, using accelerating voltages of 10–12 for imaging, and 20 kV for elemental analyses. A conductive carbon paint was spread sparingly around the perimeter of the sample prior to SEM analysis to reduce surface charging. The Environmental Scanning Electron Microscopy (ESEM) observations were performed using an FEI (USA) Quanta 200 using an acceleration voltage of 20–25 kV, available at the Centro Interdipartimen-tale Grandi Strumenti, University of Modena and Reggio Emilia.

**DNA Extraction, PCR Amplification, Cloning, and Sequencing**

A pristine fragment of surficial crust coming from the set of samples collected in the bed of the stream (sample label: ETS, Figure 2A) was ground on a sterile agatha mortar and approximately 200 μl of the powder generated was used for nucleic acid purification. Nucleic acids were extracted using the PowerSoil DNA isolation kit (MoBio Laboratories Inc., California, USA) with minor modifications (longer incubation time at 65°C and elution of DNA was resuspended in 50 μl of sterile 10 mM Tris/HCl, pH 8.5), and stored at −20°C. Bacterial small subunit (SSU) rRNA genes were amplified by polymerase chain reaction (PCR) using different the bacterial-specific primers: B-27F (5′-AGAGTTTGATCCTGGCTCAG) and the prokaryotic reverse primer 1492R (5′-GGTTACCTTGTGACTACAG). To amplify archaean SSU rRNA genes, combinations of the primers A-21F (5′-TTCCGATTGATCCTGGCGGA), AN-MEF (5′-GGCTCAGTAAACACGTGGA) with the prokaryotic 1492R primer were tried. Eukaryotic SSU rRNA genes were amplified with the eukaryotic specific primers 82F (GAAACTGCGAATGGCTC) and 1498R (CACCTACG-GAAACCGTTTACACTTT).

Polymerase chain reactions (PCR) were performed under the following general conditions: 30 cycles (denaturation at 94°C for 15 sec, annealing at 50°C or 55°C for 30 sec, extension at 72°C for 2 min) preceded by 2 min denaturation at 94°C, and followed by 7 min extension at
72°C. Dimethyl sulfoxide was added to a final concentration of 3–5% to the PCR reaction mix and, in the case of archaea, seminested PCR reactions (amplifications with primers ANMEF+1492R using as template DNA PCR products obtained with 21F+1492R) were also performed. SSU rDNA clone libraries were constructed using the Topo TA Cloning system (Invitrogen) following the instructions provided by the manufacturers. Two bacterial gene libraries were constructed and 48 to 96 clones were screened for each library. Inserts of the expected size were partially sequenced (Cogenics, France) with the reverse primers yielding sequences of 800–1000 bp. A total of 90 bacterial and 21 eukaryotic sequences were determined. Archaea were not amplified from this sample.

**Phylogenetic Analyses**

Environmental sequences from the El Tatio crust sample were compared with sequences in the database GenBank (http://www.ncbi.nlm.nih.gov/) by BLAST (Altschul 1997). Our sequences were aligned with their closest relatives in the database and with reference sequences from the taxonomic groups identified using MAFFT (Katoh 2005). Ambiguously aligned positions and gaps were eliminated using Gblocks (http://molevol.cmima.csic.es/castresana/Gblocks.html) and the resulting alignment used as input to build initial phylogenetic trees by approximate maximum likelihood using Fasttree (Price et al. 2010) with a General Time Reversible (GTR) model of sequence evolution. The final phylogenetic trees were then reconstructed using final datasets by maximum likelihood (ML) using TREEFINDER (Jobb et al. 2004) applying a GTR model of sequence evolution, and taking among-site rate variation into account by using a four-category discrete approximation of a Γ distribution. ML bootstrap proportions were inferred using 1,000 replicates. Phylogenetic trees were visualized using the program FIGTREE (http://tree.bio.ed.ac.uk/software/figtree/). The sequences reported in this study have been deposited in GenBank with accession numbers KF057198–KF057289.

**Results**

The microstratigraphy and texture investigations performed through polished cross-sections revealed an intense alteration that is particularly striking in the rock samples collected along the edge of the channel (ETE samples, Figures 3A–C), with a marked granular nature and a random distribution of coarse to fine-grained minerals of ignimbrites. Altered ignimbrites appear not completely lithified by silica and characterized by widespread vugs and pores (Figures 3A, B). In the two sampled areas, compact rock surfaces and thin planar laminations were clearly recognizable by bright color variations that suggested silicification of microbial mats and other biocomponents.

Amorphous silica (opal-A), which is by far the major component, was identified via X-ray diffraction and the microscopy observations of the silica beads that abound in all the samples investigated. Together with the pervasive opaline silica, the X-ray diffraction, also allowed the recognition of thin Martian crusts of halite, and limonitized zones. In addition, a number of elements, such as arsenic, chlorine, iron, magnesium, potassium, sodium, and sulfur, were recognized through SEM-EDX analysis and they are consistent with the composition of both the El Tatio ignimbrites (Lucchi et al. 2009) and waters (Cortecci et al. 2005).

Distinct microfacies were recognized in the silica sinter deposits collected along the eastern edge and in the bed of the stream (ETE and ETS samples, respectively). Collectively, they can be summarized as: i) laminated microfacies with different combinations of microbial-derived morphologies, and ii) “potpourri,” composite microfacies in which biologically derived features, such as oncoids, filamentous tufts, biofilms, microbial and hypha-like morphologies, remains of higher plants, and

![Fig. 3. Middle Geyser Basin, polished surfaces in ETE samples (edge of the stream) with normal stratigraphic orientation. The incomplete nature of cementation by the siliceous sinter deposit, that holds together the different components of a rock consisting of altered rhyolitic ignimbrite, is documented by numerous vugs and pores (A, B). The brown to yellow coloration (arrow in A) may presumably derive from the formation of limonite, whereas the red color (arrow in C) in the silica sinter could be the product of carotenoid pigments by prokaryotic microorganisms or eukaryotic algae, such as Dunaliella, a genus recognized in the molecular survey of ETS samples (bed of the stream).](image-url)
Diatom communities are associated with altered ignimbritic components and opal-A fragments. This last, composite microfacies represents the most common type in the two sampled sites, both on the surface and below the surface. Similarly, the laminated microfacies have been recovered in the two sampled sites, although with some different degree of silicification.

The following descriptions combine observations from both light and scanning electron (SEM and ESEM) microscopies.

**Laminated Microfacies with Combined Microbial-Derived Morphologies**

Microbial colonies are usually associated with almost superficial to superficial, flat and laminated sinter crusts and coatings recovered in both the sampled sites. In the stream bed (ETS samples) the thickness of these fragile crusts range approx. from 50 μm to 1 mm and consist of thinly laminated intervals that also alternate with non-laminated ones (Figures 4A, B). In places, clusters of coalescent, whitish silica microspheres are developed on top of dark, organic-rich horizons. These microspheres have an average diameter ranging between 50 and 100 μm, and contain a wealth of microbial morphologies represented by radially arranged filamentous tufts (Figure 4C). Thin superficial horizons contain arrays of filaments that are arranged vertically, straight to slightly curved, and locally interwoven (Figures 4A, B). Tufts of iso-oriented filaments are commonly set above structureless horizons and they predominantly exhibit the typical phototactic (perpendicular to lamination) orientation (Figure 4B).

Less commonly, tufts are arranged almost parallel to lamination (Figure 4D). All observed filaments attain at least several tens of μm in length. Each filament consists of a hollow core (from less than 2 μm to approx. 5 μm diameter), that was the space originally occupied by the actual microbe (see also examples in Jones et al. 1998), bounded by a dark and thin wall (less than 1 μm thick) that is in turn surrounded by a sleeve of a much greater thickness (1 to 5 μm thick) (Figure 4E), which can be double to quadruple the total thickness of the filament, and is the expected product of silicification.

Different degrees of silicification are suggested by the prevailing amber coloration in the halo of the filamentous microbes recovered from the ETS (stream bed) samples compared to those located in the ETE (stream edge) samples that exhibit a more homogeneous light gray coloration. Moreover, apart from the sampling site, filamentous tufts of the deeper laminae appear almost totally cemented, whereas those from more superficial horizons display fenestral fabrics indicative of a less pervasive mineralization.

Diatoms exhibit dense accumulations embedded in the amorphous silica in both the sampled sites (Figures 5A–C),

**Fig. 4.** Middle Geyser Basin, transmitted light micrographs of petrographic thin sections from ETS samples (bed of the stream) with normal stratigraphic orientation. A, B: laminated sinter crust where filamentous microbes exhibit a phototactic orientation (arrows in A and B). C: clusters of coalescent, precipitated amorphous silica globules; the cross-section of radially arranged filamentous tufts appear as a series of dots. D: filamentous microbes arranged almost parallel to lamination. E: cross-section of filamentous microbes, note their hollow core, the inner wall of dark color (white arrow), and a much thicker surrounding sleeve (black arrow). 4F, G: longitudinal section of filamentous microbes associated with diatom frustules (dark spots) possibly belonging to the epiphytic genus *Synedra*. Note the degree of trichome tapering, the sheath remains (arrows in F), and the traces of septa (arrow in G) interpreted as belonging to the cyanobacterial genus *Rivularia*. 
Fig. 5. Middle Geyser Basin, SEM micrographs from freshly cut surfaces of ETE (A, B) and ETS (C) samples of dense accumulations of randomly organized pennate diatoms encrusted with clusters of silica beads with different diameters.

where single frustules may also be heavily encrusted by the silica precipitation (Figure 5B) making difficult their proper identification. Superficial crusts also incorporate diatom frustules (skeletons) that concentrate in sectors of the crust's surface, such as the cold water, halophile Nitzschia (Figure 5C), a genus that has also been identified in the molecular study performed in ETS samples, and along the filamentous microbes, where specimens identified as the epiphytic genus Synedra adhere in dense communities around cyanobacterial filaments (Figures 4F, G).

Because of the frequent heavy encrustation by bead-like spherical particles (average bead diameter is between tens of nanometers to few micrometers) of opaline silica, the internal diameter of certain filaments can hardly be properly measured (Figures 6A-D). The encrustation by anastomosing spherical particles may also fill inter-filament free areas (Figures 6A, C) and, because of a strongly irregular rate of silica precipitation and the different growth of the silica spheres, residual irregular porosity is common. In general, however, silicification occurred early, relative to the process of microbial degradation, leading to the preservation of mineralized microbial remains.

Filamentous morphologies, similar to the ones previously reported from the stream bed (Figure 4), also occur in the “potpourri” microfacies described from the eastern edge of the stream, where they are present in isolated grains or as loose shrubs within oncoid fabrics that are common components of these microfacies. In these oncoids (Figure 7A), filamentous morphologies have approx. 5-μm diameter, are commonly arranged perpendicularly to their growth direction (Figures 7B, C), and are associated with abundant diatom frustules.

Summarizing, both the sampled sites exhibit dark and crudely laminated superficial crusts with closely-packed palisades of filamentous microbes in a prevailing vertical (phototactic) alignment. The internal diameter of these mineralized filaments can reach 5 to 10 μm, and they do not appear heavily encased by silica precipitation in the inter-filament spaces (Figure 6). Samples collected in the bed of the stream (ETS samples) display both vertically and almost horizontally aligned filamentous microbes (Figures 4B, D) with a cumulative diameter (hollow core and silicified wall) ranging from 5 to 10 μm, and with tapering (Figure 4F) and traces of septa (Figure 4G) observable in longitudinal section. Both sampled sites also contain dense tufts of radiating filaments organized as hemispherical colonies (Figures 7D–F) in which every single filament exhibits an internal diameter of 5 to 10 μm, and walls that reach a 5-μm thickness. In cross-section, walls consist of a dark, inner ring that might be the original filament wall, and a light gray, outer ring as likely result of the progress in the silica encrustation (Figure 7F).

SEM observation of the cross-sections provided a better resolution of these filament walls (Figures 6B, D): whereas the outer wall consists of coalesced silica particles (up to 2–4 μm in diameter), the inner wall consists of tight aggregates of minute silica beads (average diameter 0.5 to 2 μm), which testify that in spite of its dark color, as observed by a binocular microscope (Figure 7F), the inner wall is already a silicified feature. These last filamentous colonies exhibit the largest total diameter for a single filament (up to approximately 15 μm: internal diameter and walls) among those encountered from both the bed and the edge of the stream. They represent an example of how the encrustation by the amorphous silica beads may condition a proper measurement of the original filament wall and the hollow core.

“Potpourri” Microfacies

The most common rock at the surface of the Middle Geyser Basin—and, therefore, in both sampled sites—consists of a porous, altered and sintered ignimbrite that assembles different biotic and abiotic components with the result of
producing a composite microfacies. In the two study sites, this multi-component, “potpourri” microfacies is found to consist of the following components: i) silica oncoids and oncid clusters, and other laminated elements with occasional filamentous morphologies housed in their inner core; ii) single grains consisting of filamentous tufts with a radial organization; iii) silicified land plant remains (culms and pollens); iv) silica grains with abundance of diatom frustules; v) bacterial filaments and fungal-like morphologies in complex biofilm settings; vi) massive, structureless opaline silica. The high heterogeneity of this microfacies does not allow a reconstruction of reliable quantitative relationships between its various components, however, especially in the ETE samples oncoids make up the bulk of the rock (Figures 7A–C).

Oncoids and other laminations
Laminated morphologies occur either parallel to bedding or as oncoids. Parallel laminations have fairly regular spacing (from 10 to 20 μm), may locally anastomose, and clear microbial morphologies rarely occur. Depending on the amount of the silica precipitated, the porosities between adjacent laminae have various filling levels. Oncoids (Figure 7A) represent common elements in the ETE sampled site, where they may be single or coalesce forming clusters partially cemented by amorphous silica. Crudely laminated oncoids consist of undulating, continuous, light and dark-colored concentric laminae; they represent a typical feature of the microfacies sampled at the edge of the stream and resemble the amorphous silica structures described from other geothermal sites, such as in the shallow pools described at the Orakeikorako geothermal site, New Zealand (Renaut et al. 1996). With the exception of filaments (Figures 7B, C), oncoids commonly lack microbial-derived morphologies.

Tufts
Radially arranged filamentous tufts of the “potpourri” microfacies display the largest microbial colonies recovered (Figures 7E–G, described in the previous section) and characterize both sampled sites.

Silicified land plant remains
Structures referable to culms and other land plant remains (Figures 8A–C) and pollen grains (Figure 8D) are common in the sinter samples collected from the bed and the edge of the stream as a presumable result of early permineralization processes. Cylindrical culm (stem) morphologies are circular to elliptical in cross-section, ranging in thickness from ~0.3 to 0.5 mm, with complex internal structures (Figures 8A, B). Silicification processes can be observed in peripheral sectors (outer cortex) of stem remains, where transverse sections reveal the typical surface (silica beads) of the opaline silica precipitation (Figure 8C).
Silicified Biota in High-Altitude, Geothermally Influenced Ignimbrites

Fig. 7. Middle Geyser Basin, transmitted light micrographs of petrographic thin sections from ETE samples (edge of the stream). A: oncoloid morphologies (arrows) as they commonly appear in the “potpourri” microfacies (see text for further explanation). B: dense accumulation of filaments forming distinct palisade and iso-oriented tufted morphologies within the oncocoids are the only microbial-derived morphologies. Details in C (boxed area). C: filaments (arrows) arranged perpendicularly to the growth direction of the oncocoids. D–F: tufts of filaments arranged radially and organized as hemispherical colonies; note the inner wall of dark color (white arrow) and a thicker surrounding sleeve (black arrow). On the basis of morphology, organization and habitat these communities have been tentatively assigned to *Phormidium*.

Silica grains with diatom frustules
Pennate diatoms are abundant biotic components; some of them, such as the genera identified as *Nitzschia* (Figure 5C) and *Synedra*, display dense and irregular accumulations of their lanceolate frustules in the opaline silica deposited in the two sampled sites.

Bacterial- and hyphal-like filaments, amorphous surfaces (Figures 9A–C)
Filaments having bacterial and/or fungal origin can be easily found associated in different environments, for example in the case of bacterial colonization of larger fungal hyphae. The “potpourri” microfacies displays examples of remnants of silicified surfaces in which large and thin filaments were interpreted as remains of cyanobacteria (or other trichomic prokaryotes) and fungal hyphae associated with morphologies interpreted as derived from microbial polysaccharides (Figures 9A, B). Other amorphous silica membranes of presumable inorganic origin are fairly abundant in the sintered deposits of the ETE sample site (Figure 9C).

Massive opaline silica
In spite of the strongly porous appearance of the sintered ignimbrite in the sampled areas of El Tatio, at the microscopic scale the opaline silica appears a pervasive feature. Pore spaces and their distribution provide information on the silica deposition process, and reflect the variations in the timing and intensity of the silicification. This is also suggested by the filamentous morphologies, because their different degrees of silicification result in different fillings of the inner part of the filament (Figures 6A–D).

Microbial Diversity Associated to Crust Samples
To characterize the diversity of living microorganisms potentially belonging to the three domains of life associated to the rock samples, which likely correspond to those progressively incorporated into the deposits of silica sinter, we used molecular methods based on the amplification, cloning and sequencing of SSU rRNA genes. In this way, the microbial diversity associated to the deposit collected from a partially emerged (partially dry) sector of the stream bed (sample ETS, Figure 2A) was analyzed.

Despite the use of various specific primer sets and PCR conditions, it was not possible to amplify archaeal genes, which suggests that archaea are not dominant in the superficial sinter deposits and/or are heterogeneously distributed. We obtained PCR amplification products with archaeal primers using less stringent PCR conditions but, after cloning and sequencing, the amplified genes turned out to be bacterial ones.
Fig. 8. Middle Geyser Basin, ESEM (A, C) and SEM (B, D) micrographs of silicified land plant remains (ETS samples) and pollen grains (ETE sample) from freshly cut surfaces. A–C: transverse section of culms (stems) with their complex internal structures; note the partial collapse of the outer cortex (arrows in A) and the typical surface (silica beads) of the opaline silica precipitation from a transverse tissue section (in C). D: pollen grains immersed in siliceous sinter deposit.

(clones labeled ETS_3A). Interestingly, several of them belonged to the rare candidate divisions NKB19 and SBR1093 (Figures 10 and 11), suggesting that primer bias may affect the detection of members of those clades in other environments. By contrast, the amplification of both bacterial and eukaryotic SSU rRNA genes was successful, indicating the presence of living microorganisms in the sampled dry deposit.

As expected, bacteria were more diverse than eukaryotes. Gamma- and Alphaproteobacteria (around 25 and 23%, respectively) accounted for about half of the diversity identified, followed by Deltaproteobacteria, Cyanobacteria and Bacteroidetes (12% each) and, in less important proportions, Verrucomicrobia, Planctomycetes, and the uncultured Candidate Divisions NKB19 and SBR1093 (Figure 10). The latter divisions have been seldom detected in most environments, and their sequences in the database are relatively scarce. Interestingly, we also detected a very divergent clone, ETS-1B-68, which formed a clade with a restricted group of sequences retrieved from hypersaline microbial mats in the Candelaria lagoon of Puerto Rico (Isenbarger et al. 2008) and in Guerrero Negro (Kirk Harris et al. 2013).

Many of the detected bacterial clones had as closest relatives other environmental sequences retrieved from microbialites, notably from the high altitude, saline Lake Alchichica, Mexico (Couradeau et al. 2011), but they were also detected at Shark Bay and in Bahamian stromatolites (Allen et al. 2009; Myshrall et al. 2010; Papineau et al. 2005), as well as from hypersaline mats, anoxic sediments, and mud volcanoes (Figure 11). Many close relatives of the above sequences correspond to environmental sequences from an independent study of El Tatio dry surface mats deposited in GenBank (Figure 11), which indicate the consistent presence of the identified lineages in this area and type of samples. Within cyanobacteria, the identified sequences related both to filamentous cyanobacteria (Oscillatoriales, Nostocales), including typically endolithic genera (e.g., *Rivularia*), and coccoid cyanobacteria (e.g., Chroococcales). Among the Gammaproteobacteria, several ETS sequences appeared to originate from
Silicified Biota in High-Altitude, Geothermally Influenced Ignimbrites

Fig. 9. Middle Geyser Basin, SEM micrographs from freshly cut surfaces (ETE samples). A, B: mature biofilm of presumable cyanobacterial origin in which filaments (cf) are associated with morphologies derived from microbial polysaccharides (arrows). C: amorphous, presumably inorganic silica membranes cover clusters of silica beads that are similar to biofilms (arrows).

ETS - Bacteria

ETS - Eukaryotes

Fig. 10. Relative proportions of bacteria and eukaryotes in SSU rDNAs gene libraries.

sulfur-metabolizing organisms, e.g., related to *Sulfitobacter*, a genus of sulfite-oxidizing bacteria (Figure 11).

The eukaryotic sequences detected distributed in three different high-rank taxa at roughly equal proportions: Chrysophyta and diatoms (Bacillariophyta) within the Stramenopiles, and green algae (Chlorophyta) within the Archaeplastida (Plantae) (Figure 10). The diversity within these groups was limited, with only one operational taxonomic unit (OTU) identified per clade: an OTU belonging to *Dunaliella*, a genus of halotolerant green algae, a diatom OTU related to *Nitzschia*, in agreement with microscopy observations, and a chrysophyte OTU related to *Spumella* (Figure 12). Although *Spumella* belongs to a group containing photosynthetic members (chrysophytes or golden algae), it is a genus of colorless flagellate grazers.

Discussion

The array of biotic morphologies in the sintered ignimbrites of the Middle Geyser Basin of El Tatio documents of a good preservation potential and, although not yet fully understood, this potential is the expected result of the interactions between the abiological silica precipitation from supersaturated solutions and the role (if any) played by microorganisms during
Fig. 11. Maximum-likelihood phylogenetic tree of bacterial SSU rRNA genes detected in El Tatio silica (ETS) samples. A total of 664 non-ambiguously aligned positions were used for the analysis. Sequences retrieved in our study are indicated in bold. Bootstrap values higher than 50% are shown at nodes.
the process of silicification. In the present investigation an evidence that might suggest that the silicification is largely a nonbiological process is the density of the amorphous silica beads that concentrate (with similar size and morphology) both in the rock porosities and in the empty spaces of biological structures (including filaments). Examples suggesting that microorganisms may have had some active role during early stages of the silicification process, however, do exist.

This is shown in ETS samples (bed of the stream) where cylindrical filaments with a two-layered morphology of the

Fig. 12. Maximum-likelihood phylogenetic tree of eukaryotic SSU rRNA genes detected in El Tatio silica (ETS) samples. A total of 949 non-ambiguously aligned positions were used for the analysis. Sequences retrieved in our study are indicated in bold. Bootstrap values higher than 50% are shown at nodes.
The morphology and size (the diameter can reach 10 μm) of the (partially) silicified filaments and the colony organization, these communities have been tentatively attributed to *Phormidium*, a mat-forming cyanobacterium that is able to produce tufts and fibrous palisades in mid-temperature geyser basins (Cady and Farmer 1996; Estep 1984; Fernandez-Turiel et al. 2005; Lau et al. 2005), or to a morphologically analogous genus. Certain filamentous microbes collected from the bed of the stream (ETS samples) resemble the size and shape of the ones collected along its edge (ETE samples); they can, however, be distinguished because of their tapering that is recognizable when filaments are longitudinally sectioned (Figure 4F).

Together with a trichome tapering and traces of seaptate organization (Figure 4G), some sheath remains appear still recognizable (Figure 4F). Data of DNA sequencing from the same material revealed the presence of *Rivularia*, a genus taxonomically close to *Calothrix*, two cyanobacteria of the order Nostocales that appear morphologically indistinguishable once isolated from their natural settings (Berrendero et al. 2008). Because *Rivularia*-related sequences were identified in the molecular analyses (Figure 11) these tapered filaments were interpreted as belonging to this cyanobacterial genus.

Together with filamentous morphologies, the microbial communities of the thermal springs also include rod and coccoid bacteria (e.g., Ward and Castenholz 2002; Wickstrom and Castenholz 1985). For example, the small coccoid cells of *Synechococcus* and similar morphologies, abound in hot springs near their thermal maximum (Brock 1967; Miller and Castenholz 2000), and have been widely observed in endolithic communities in Yellowstone, where they may become silicified as well (Norris and Castenholz 2006; Walker et al. 2005). *Synechococcus* is a paralyphyletic cyanobacterium, i.e., it does not correspond to a single monophyletic clade and it is scattered in various cyanobacterial orders, needing taxonomic reassessment based on molecular markers. Sequences related to some *Synechococcus* lineages were identified during the molecular survey from ETS samples. Their recognition based on morphology is, however, made difficult (see also Orange et al. 2013b) owing to the abundance of small (few micrometers in size), amorphous silica beads that encrust biotic and non-biotic surfaces (Figure 9).

The abundance of pennate diatom frustules (Figures 4F, G; 5)—a common feature of the geyser ecosystems—at El Tatio is in accordance with i) its being a suitable habitat for the development of halophilic and thermophilic genera, such as *Nitzschia* and *Synechococcus* (indeed, *Nitzschia*-related sequences were identified in the molecular studies; Figure 12); ii) the abundance of filamentous, sheathed cyanobacteria, such as *Phormidium* (or a morphologically analogous genus) and *Rivularia*, and their biofilms (Figures 9A, B), that can produce adhesive surfaces able to facilitate frustule concentrations; iii) the contribution of the biofilm mats and mucus strands secreted by diatoms and their (helpful) role in the fossil preservation (see also O’Brien et al. 2008); iv) the rapid and early silica precipitation that enabled the preservation of microbial and eukaryote (diatom) communities, together with some of their metabolic products.

A further reason that makes ubiquitous microbes (including cyanobacteria) and diatoms in the El Tatio sinters is their ecological role as initial colonizers of a given surface (e.g., Giller and Malmqvist 1998); this can explain their widespread distribution in this complex of environmental extremes. In addition, many of these microorganisms have an endolithic mode of life, being adapted to grow within the rock surface, where they can find protection against the harshness of surface environment.

In a number of instances, morphologies that could apparently be assigned to a biological origin may in fact be nonbiological. Laminations in the oncoids, as well as the amorphous, silicified membranes described from different microfacies represent presumably non-biological morphologies for which a distinction with genuine microbial structures is problematic even in case of their proper contextualization. An example are the amorphous silica films (Figure 9C) that, although similar to mineralized biofilms and associated with microbial morphologies, display extension and distribution that rather suggest an origin due to late draping of inorganic precipitated silica.

The convergent identification both by morphology and molecular data of particular groups of microorganisms (e.g., certain cyanobacteria, diatoms, and chrysophytes) makes of El Tatio a convenient playground for the study of fossilization processes in the natural environment and, therefore, to potentially define reliable biosignatures for the search of bona-fide microfossils in older silicified environments.

**Acknowledgments**

Thanks are due to Piermaria Luigi Rossi, with whom El Tatio was first visited in 2007. Thanks are also due to Giorgio Gasparotto for his help during EDS analysis and to Maria Cristina Bonci for her help in the diatoms identification. Special thanks to Yerko Felix Anza Colamar and the Comunidade de Caspana.
Silicified Biota in High-Altitude, Geothermally Influenced Ignimbrites

y Toconce de la Comuna de Calama for the warm welcome given at El Tatio.

Funding

Work financially supported by Progetto Strategico Atacama of the University of Bologna and NASA Astrobiology Institute/American Philosophical Society – Lewis and Clark Fund for Exploration and Field Research.

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Barbieri et al.


