

The Dry Valley Lakes, Antarctica: A key to evolutionary biomarkers on Europa and elsewhere

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ABSTRACT

Most organisms dwell in what we consider to be “normal” environments, while others, which are called extremophiles, may thrive in harsher conditions. These living organisms are mainly of unicellular (both prokaryotes and, to a lesser extent, there are some eukaryotes) But the extremophiles also include multicellular organisms, including worms, insects and crustaceans. In the present work we survey specific extremophiles in some detail. Astrobiology is concerned with all of these extremophiles, as they may be models for extant life in similar environments elsewhere in the universe. In the more restricted search for life through exploration of the Solar System, the main focus is on the preparation of suites of experiments that may attempt to discover the habitability of planets and their satellites. In this context we ask ourselves: What biosignatures can facilitate life detection, both unicellular and multicellular, in extreme environments? The environments that are within reach of present and future space missions include the Jupiter satellite Europa. The ice-covered lakes of Antarctica’s McMurdo Dry Valleys have long been of interest to astrobiology. These environments harbor unique microbial ecosystems that could orient us how to plan our experiments on Europa.

Keywords: astrobiology, Europa, terrestrial analogues, extremophiles,

1. INTRODUCTION

Microbial life constitutes a substantial fraction of life on our planet. They are found in ecosystems organized in microbial mats and sometimes into biofilms (Tewari and Seckbach, 2011). For this reason the description of different aspects of microbial mats are fundamental for our deeper understanding of astrobiology. (We refer the reader to a published review for more details, on which Secs. 1-3 of this paper is based, Chela-Flores, 2011.) Amongst the multiple implications of the study of microbial mats emerges the understanding of the early Earth, before multicellularity evolved. Microbial mats may help us to understand the possibility of life elsewhere in the Solar System. Microbial mats are ubiquitous in extreme environments: at high and low temperatures, in hypersaline bodies of water such as the Dead Sea, in hot springs where they not only survive, but they even thrive, being present even in hydrothermal vents on the ocean floor. Other environments suitable for microbial mats are deserts and, specifically the Dry Valleys of Antarctica in the McMurdo region. Sir Robert Scott discovered the Dry Valleys in 1905 (cf., Table 1 taken from Doran *et al.*, 1994; Wharton *et al.*, 1983; Parker *et al.*, 1982).

Some of the most interesting lakes in this region are permanently covered by ice. These extraordinary environments present us with an ideal window to glance at significant events that are relevant for ancient life, and even for paleolimnology that is suggestive of the possible perseverance of life on Mars in an earlier Eden-like epoch. The ice-covered lakes of Antarctica’s McMurdo Dry Valleys have long been of interest to astrobiology (Doran *et al.*, 2010).

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Lake or pond	Maximum depth (meters)	Elevation (meters above sea level)	Lake type
Lake Hoare	34	73	Perennial ice cover; liquid water
Lake Vanda	69	123	Perennial ice cover; liquid water
Lake Joyce	37	1677	Perennial ice cover; liquid water

These environments harbor unique microbial ecosystems that could orient us how to plan our experiments on Europa. Lake Joyce is of special interest to NASA, as it is ice covered year-round: Its icy surface is 6 meters deep. Yet, even the few percent of light that penetrates through the ice is enough to support an algal ecosystem in the lake. Many of the structures on the lake bottom look like what we see in the Archean rock record from about 3 Gyr before the present (BP), because its waters harbor carbonate structures known as microbialites. These unique structures are formed with layers of cyanobacteria.

The research team is interested in how these organisms are able to grow in the dark, cold waters of Lake Joyce: In these environments the extremophiles that are trapped in microbial mats may also be living under the Taylor Glacier in the Taylor Valley. These microbes probably lived in the ocean at one time, but when the floor of the Dry Valleys rose more than a million years ago, the glacier covered seawater when it advanced and trapped the microorganisms in pockets of water (cf., Table 2).

Table 2. Microbial life in the Dry Valleys lakes, Antarctica.

Organism	Domain	Habitat
Cyanobacteria	Bacteria	Lakes Chad, Fryxell and Vanda
<i>Leptothrix</i>	Bacteria	Lakes Fryxell and Hoare
<i>Achronema</i>	Bacteria	Lakes Fryxell and Hoare
<i>Clostridium</i>	Bacteria	Lakes Fryxell and Hoare
<i>Chlamydomonas subcaudata</i> (Phylum Chlorophyta)	Eucarya	Lakes Bonney (east lobe) and Hoare
Diatoms (Phylum Bacillariophyta)	Eucarya	Lakes Bonney, Chad, Fryxell, Hoare and Vanda
<i>Bryum</i> (a moss)	Eucarya	Lake Vanda

An intriguing feature—Blood Falls—suggests the presence of microbial mats underneath the Taylor glacier. The name is due to the resemblance with a blood-red color waterfall at the glacier's extreme end. Isotopic measurements of sulfate, water, carbonate, ferrous iron and gene analyses imply that a microbial consortium facilitates a catalytic sulfur cycle analogous to the metabolic events that may sustain life elsewhere in the Solar System (Mikucki *et al.*, 2009). This is especially relevant to the icy satellites of the outer Solar System, including Europa, where the Galileo Mission discovered sulfur patches (1995-2003). These stains on the icy surface of the Jovian satellite are suggestive of chemosynthetic products of metabolism (Chela-Flores, 2010)

From the point of view of geology and microbiology, some of the best studied frozen lakes are in the Taylor Valley, (Chad, Fryxell and Hoare. Amongst the microbial mats that are permanently thriving in the frozen lakes there are examples of both prokaryotes and eukaryotes. Besides, some of the most interesting geologic paleoindicators for reconstructing the history of these lakes are stromatolites. In the Dry Valleys these structures consist of various species of cyanobacteria, such as *Phormidium frigidum*, a prokaryote that forms the matrix of most mat types (Wharton *et al.*,

1983). Modern organisms analogous to ancient life are to be found in the Dry Valley lakes. What is most significant is that single-celled eukaryotes are amply represented in this Antarctic biota (cf., Table 3).

Table 3. A few examples of eukaryotes present in Antarctica.

Organism	Domain	Habitat
Diatom shells	Eucarya (Bacillariophyta)	Lake Vostok (ice core, at depth of 2375m)
<i>Caloneis ventricosa</i>	Eucarya (Bacillariophyta)	Lakes Chad, Fryxell, Hoare and Vanda
<i>Navicula cryptocephala</i>	Eucarya (Bacillariophyta)	Lakes Bonney, Fryxell, Hoare and Vanda
<i>Chlamydomonas subcaudata</i>	Eucarya (Chlorophyta)	Lakes Bonney and Hoare
<i>Tetracystis</i> sp.	Eucarya (Chlorophyta)	Lakes Fryxell, Hoare and Vanda
Yeast	Eucarya (Ascomycota)	Lake Vostok (ice core)

Amongst the related paleoindicators that have been found are diatom frustules, cyst-like structures, most likely of crysophycean origin have also been identified. These intriguing lakes contain various taxa of planktonic and benthic microorganisms. These environments are dominated by lower life forms inviting us to search for biomarkers of an earlier biota since grazing, for instance, is totally absent (Doran *et al.*, 1994). Microbial mats in lake Bonney, Chad, Fryxell, Hoare and Vanda have been thoroughly documented, especially since the 1980s. For instance, in these environments microbial mats are known to include heterotrophic bacteria, eukaryotic algae (mainly diatoms) and fungi (Baublis *et al.*, 1991) besides the above-mentioned cyanobacteria. There are some dinoflagellates *Gymnodinium* and *Glenodinium* in Lake Fryxell, where in addition protozoan taxa are associated with the algal mats (Cathey *et al.*, 1981). The existence of these permanently frozen lakes adds an extra bonus to our model of the European Ocean. Modern organisms analogous to the early Earth biota are found in the Dry Valley lakes. Single celled eukaryotes are represented. In Tables 2 and 3 we have summarized the names of some of the organisms that inhabit in these lakes (Doran *et al.*, 1994, 2010; Wharton *et al.*, 1983; Parker *et al.*, 1981, 1982; Ellis-Evans and Wynn-Williams, 1996).

2. A SOUTH POLE ANALOG TO EUROPA

From the point of view of the possibility of the existence of life on Europa, we should consider a lake called Vostok, which is the largest of about 80 subglacial lakes in Antarctica (Siegert *et al.*, 2005). Its surface is of approximately 14,000 km² and its volume is 1,800 km³. Indeed this Ontario-sized lake in Eastern Antarctica is also deep, with a maximum depth of 670 m. On the other hand, from the point of view of microbiology, the habitat provided by Lake Vostok presents us an analogue for the Europa environment. The ice above the lake has been cored to a depth of over 3,600 m, stopping just over 100 m over the surface of the lake itself. This work has revealed great diversity of single-celled organisms: yeast, actinomycetes, mycelian fungi (which remain viable for almost 40,000 years), the alga *Crucigenia tetrapodia*, diatoms, and most interestingly, 200,000 year old bacteria. Besides it appears that in the lake water temperatures do not drop too far below zero centigrade, with the possibility of geothermal heating raising the temperatures above this level. Extrapolation of data retrieved from work deep in the ice core to the lake itself, implies that Lake Vostok may support a microbial population, in spite of the fact that that large volume of water has been isolated from the atmosphere for over one million years.

Bacterial density is found to be two to seven-fold higher in accretion ice than in the overlying glacial ice. This implies that Lake Vostok is a source of bacterial carbon beneath the ice sheath. Phylogenetic analysis of the amplified small subunit ribosomal ribonucleic acid (rRNA) gene sequences in this accretion ice has revealed the presence of *alphaproteobacteria*, *betaproteobacteria* and *gammaproteobacteria* (Christner *et al.*, 2006). Lake Vostok and its relevance for astrobiology have been extensively reviewed (Christner *et al.*, 2006; Priscu *et al.*, 2003). It has been estimated that the youngest water is at least 400,000 years old. It is a window into life forms and climates of primordial eras. The zone of ice layer up to 3,309 m (referred to as I), and the layer between 3,310 to 3,509 m (zone II) provide detailed information about the paleoclimate record spanning during the last 420,000 years.

The basal portion of the ice core from 3,539 to 3,623 m has many features differing from overlying glacial ice and its geochemical composition indicates that it represents actual lake water that has accreted (i.e., frozen) to the underneath of ice sheet. In spite of extremely cold air temperatures above the ice (an average of -55°C), liquid water is stable in the lake owing to the combined effect of background geothermal heating, together with the insulating properties of the overlying icy sheet, (Siebert *et al.*, 2003).

Lake Vostok appears to be harboring hydrothermal vents beneath the water surface. This is suggestive of what may be occurring on Europa. The circulation of pure water in Lake Vostok will be driven by the differences between the density of meltwater and lake water. Geothermal heating will warm the bottom water to a temperature higher than that of the upper layers. The water density will decrease with increasing temperature resulting in an unstable water column. This leads to vertical convective circulation in the lake, in which cold meltwater sinks down the water column and water warmed by geothermal heat ascends up the water column (Siebert *et al.*, 2001). Similarly, Europa may also have geothermally-heated warm water under its ice-crust. Processes of the type that occur in Lake Vostok may be taking place on Europa, where sulfate-reducing microbes may be processing sulfur originating from hydrothermal vents. Such isotopically modified sulfur may be reaching the surface.

In the near future, scientists from the Vostok Base plan to enter the lake (Schiermeier, 2011). This would be done by sending what we once called a "hydrobot" into the lake to collect water samples and sediments from the bottom. However, even though Lake Vostok is what we have called in this section a terrestrial analog to Europa, within the foreseeable future the concept of a hydrobot is suitable for the search of life on Earth—in Lake Vostok—but the costs would be prohibitive in the Europa Ocean itself.

3. A CANADIAN ARCTIC ANALOG TO EUROPA

Besides the biogenic sulfur-related traffic through the icy surfaces of the lakes that is well understood in the dry valley lakes lying on the western shore of McMurdo Sound (Chela-Flores, 2006), there is another major European analog in North America. Once again, we are dealing with a valley that has been given the name of "Borup Fiord Pass", where minerals accumulate on glacial ice. This site is a geologically relevant feature that lies on Ellesmere Island in northern Canada. The valley lies over saline springs that are rich in sulfide and sulfate that make their way all the way to the icy patchy surface. This additional traffic of surficial sulfur provides a terrestrial laboratory to test the instrumentation that may be used later on a future mission to Europa (Gowen *et al.*, 2010).

The biosignatures that have been studied in this environment are not related to the sulfur isotopes, as we have done in our above-mentioned work of 2006. Rather, what has been followed up in Ellesmere Island is the process of biomineralization (Gleeson *et al.*, 2010). This interesting biogenic process takes place when biominerals are generated as a result of interactions of microbial life and its environment. In chemosynthesis this is a well-understood process (Konhauser, 2007).

4. PROBING FOR THE FIRST HINTS OF MULTICELLULARITY IN THE SOLAR SYSTEM

The first steps in the evolution of multicellularity remain unknown. Nevertheless, it is evident that evolutionary pressures encourage cells with genomes codifying for cell signalling, as well as for proteins that favour the formation of tissues of primitive multicellular organisms (Chela-Flores, 2000). A point worth emphasising is that higher organisms are not only characterised by the eukaryotic blueprint, but by multicellularity. This second feature evolved gradually from microbial life. An advantage of cellular aggregation is its adaptive value. This follows from direct observation of contemporary colonies of the bacterium *Myxococcus xanthus* (Farmer, 1992). This bacterium forms spherical colonies that increase their ability as predators. The unicellular aggregation lets the prey enter the sphere through gaps, which are able to retain digestive enzymes that are produced by the bacteria. The prokaryotic blueprint is a consequence of chemical evolution. In a geologic time scale, unlike eukaryotes, the prokaryotic blue-print emerges almost instantaneously. It follows from these considerations that multicellularity is also a consequence of chemical and biological evolution in a terrestrial-like environment, but on a longer time scales than the emergence of the eukaryotes.

The next stage in evolution that would be necessary to test in Solar System exploration, if the underlying technology were feasible, is undoubtedly the transition from single-cell microorganisms to multicellularity. Two paths are open for testing. The most evident one would be to test directly for the lowest lying metazoan body plans that are known to us: either cnidarians, or sponges, regardless of which was the earlier to emerge, both are important from the point of view of astrobiology, since in both cases the first stages of the development of neurons have taken place generating action potentials (Villegas *et al.*, 2000; Miller, 2009). When the glass sponge gets a stimulus, it takes its nourishment, a voltage

change is triggered in its body, and the cilia that are used for taking water close down. This action potential lasts thousands of times longer than action potentials in neurons in animals and plants (as long as 5 secs.), but the coordinated response to an external input is the characterising feature of an eventual nervous system (Leys and Mackie, 2007). Similar physiological responses are observed in cnidarians, for instance in the case of the jellyfish *Aglantha digitale*, where Ca- and Na-dependent channels have been identified (for a list of references, we refer to the earlier exhaustive review of Villegas *et al.*, 2000). Taken together, these remarks richly illustrate how the early stages of evolution are truly significant for laying out the bases for a deeper understanding of the eventual evolution of intelligence (the SETI project's main objective for the last half a century) in our local environment (both on Earth, and eventually, elsewhere in the Solar System). Imaging techniques may suffice for a lander placed at an appropriate surface location in case of direct confrontation with a living organism, or a collective ensemble of microorganisms (for instance with a microbial mat).

5. WILL IT BE POSSIBLE TO PROBE FOR EARLY STAGES OF MULTICELLULARITY?

One of the most striking—and biologically unusual—properties is a question that has been raised (Sun *et al.*, 2004): *Are there universal signals for interspecies communication?* The most difficult task to assign a robotic search for higher stages of evolution, beyond the simplest and most often discussed single-cell prokaryoticity would be to target with given experimental miniaturized technique for a well defined molecular species that could be considered an evolutionary biomarker.

Laser-induced breakdown spectroscopy (LIBS) is a technique in which firstly we vaporize small quantities of material of the order of micrograms to nanograms (including biological matter) with the use of highly energetic laser pulses. Secondly, by thermally exciting the resulting material into plasma, we can proceed to a spectroscopic analysis of the light emitted by the atoms. This has been a technique for the analysis of elements by retrieving a unique elemental fingerprint spectrum. Since chemical elements are known to emit light of a given frequency when excited to sufficiently high temperatures, LIBS suggests itself as a technique by means of which we could detect all elements in a given target. LIBS can be used to distinguish bacteria with fewer constraints that were previously thought to be possible. There are advantages when planning the exploration of the Solar System. For example, LIBS shows potential for development instrumentation with characteristics typical of LIBS, but in addition rapid *in situ* analysis is possible with little or no sample preparation and the feasibility of automated spectroscopic analysis (Multari *et al.*, 2010). MALDI, matrix-assisted laser desorption/ionization, is likewise a relevant technique that has also been appropriately miniaturized and already tested in space missions (Tulej, 2011). However, whether there is a detectable universal signal of multicellular organisms that could be targeted with LIBS, MALDI, or other experimental techniques remains as a challenge. It is encouraging that the miniaturisation of additional relevant equipment is proceeding at a fast pace (Tulej *et al.*, 2011).

The case for identifying the transition from prokaryotes to eukaryotes is conceptually a relatively simpler question to face. It was discussed in the past (Chela-Flores, 1998). But at present we can add that the fossil evidence suggests that biomarkers typical of eukaryotes (and, hence, of the rising presence of an oxygenic atmosphere) are already present in the 2.7 Ga shales in Western Australia—the Pilbara Block's Fortescue Group (Brocks *et al.*, 1999). If we focus on the transition between prokaryotic microorganisms to eukaryotes, then the problem facing a penetrator search on the icy surface of Europa becomes a valuable analogy with the search for the earliest prokaryotes in the late Archean and we can profit from it: The question of identifying the evolutionary transition prokaryote/eukaryote, rather than attempting to identify the transition microorganism/multicellularity is simpler than the one we have discussed in Sec. 5. Eukaryote biomarkers are radically different from any prokaryotic biomarkers that have been identified. Specific sterols used in eukaryotic membranes is a well discussed example (Konhauser, 2007), as the only prokaryotes that are known to synthesize sterols lead to different structural isomers. (We should underline that oxygen is needed for biosynthesizing sterols.) As a consequence of these remarks, the discovery of such biomarkers on the surficial stains of Europa would, in addition, imply that there would be some dissolved oxygen in the European ocean.

6. DISCUSSION

With the help of the instrumentation that is now available, we can face one of the most significant questions in astrobiology, namely the discovery of a habitable ecosystem in our own Solar System. Such discovery would constrain further two of the main research programs in the least developed sector of astrobiology, namely the study of the distribution of life in the universe. These two programmes are the SETI program, and the direct exploration of the Solar System, especially the Jovian system with Europa, its intriguing icy moon.

Retrieving the relevant data *in situ* in the 2020s was a tantalizing possibility with the 'Europa Jupiter System

Mission' (EJSM), in which the main partners were the American Space Agency, the National Aeronautics and Space Administration (NASA), and the European Space Agency (ESA). The mission consists of two flight elements operating in the Jovian System: the NASA-led Jupiter Europa Orbiter (JEO), and the ESA-led Jupiter Ganymede Orbiter (JGO) (Grassett *et al.*, 2009). The JEO and the JGO would have explored Europa and Ganymede, respectively.

However the EJSM mission has to be reformulated due to the lack of funds to support the Europa orbiter JEO (Dougherty, *et al.*, 2011). Fortunately, the new project JUICE (JUPiter ICy moon Explorer) taking advantage of the JGO has left the following possibility as an attractive alternative to be followed up: to concentrate on ocean research at Europa and Ganymede. This option is full of promise from the point of view of the interpretation of the stains that we have discussed throughout the present paper (Chela-Flores, 2010).

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