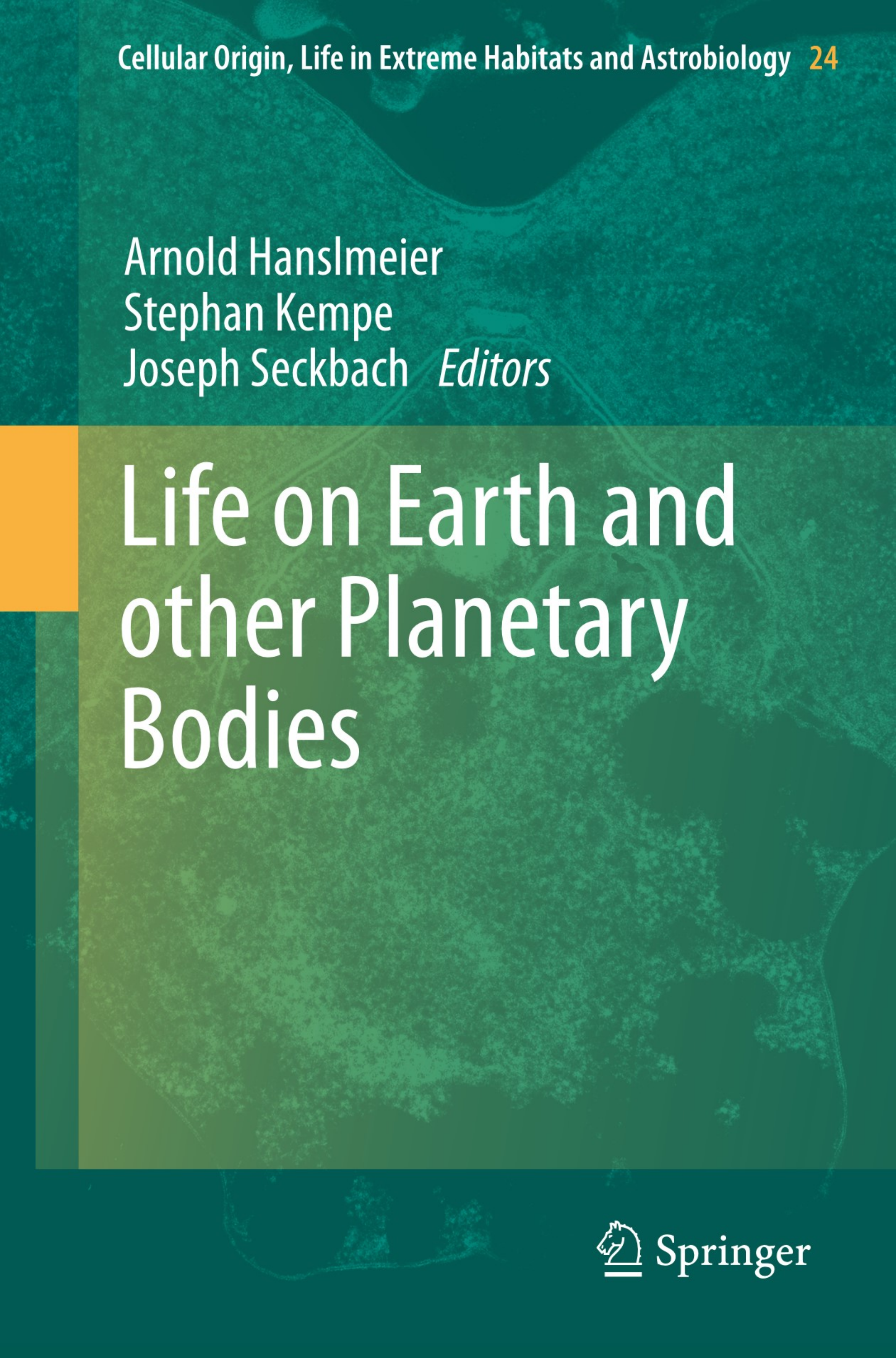

Cellular Origin, Life in Extreme Habitats and Astrobiology 24

Arnold Hanslmeier
Stephan Kempe
Joseph Seckbach *Editors*

Life on Earth and other Planetary Bodies

Springer

LIFE ON EARTH AND OTHER PLANETARY BODIES

Cellular Origin, Life in Extreme Habitats and Astrobiology

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Biodata of **Joseph Seckbach**, coeditor of this volume and author of "***Pathways to Life on Earth and Other Planetary Bodies*** (**EPLA**)."

Professor Joseph Seckbach is the founder and chief editor of ***Cellular Origins, Life in Extreme Habitats and Astrobiology*** **("COLE")** book series. He has coedited other volumes, such as the *Proceeding of Endocytobiology VII Conference* (Freiburg, Germany) and the *Proceedings of Algae and Extreme Environments meeting* (Trebon, Czech Republic). See http://www.schweizerbart.de/pubs/books/bo/novahedwig-051012300-desc.ht. His edited the recent volume (with Richard Gordon) entitled *Divine Action and Natural Selection: Science, Faith, and Evolution* published by World Scientific Publishing Company (2009). Beyond editing academic volumes, he has published scientific articles, edited and translated several popular books. Dr. Seckbach is the coauthor, with R. Ikan, of the Hebrew-language *Chemistry Lexicon* (Deveer publisher, Jerusalem).

Dr. Seckbach earned his Ph.D. from the University of Chicago (1965) and did a postdoctorate in the Division of Biology at Caltech, in Pasadena, CA. He was appointed to the faculty of the Hebrew University (Jerusalem, Israel). Dr. Seckbach spent sabbaticals at UCLA and Harvard University, and DAAD-sponsored periods in Tübingen, Germany, and at LMU, Munich. Professor Seckbach served at Louisiana State University, Baton Rouge, as the first chair for the Louisiana Sea Grant and Technology transfer. His recent interest is in extremophiles and Astrobiology.

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PATHWAYS TO LIFE ON EARTH AND OTHER PLANETARY BODIES

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1. Introduction

"Are we alone?" or "Is Anybody Out There?" have been permanent quandaries among mankind from the ancient times to now. In this book, we are discussing the diversity of life on Earth and possibly on other celestial bodies. We always consider the phenomena of life as we *know* it on Earth, with the requirements for it being liquid water and carbon-based biochemistry, plus other nutrients and energy sources. In this presentation, we precluded the possibility of life as we do *not* know it. It is a formidable challenge to find other habitable planets or satellites, which might contain signatures of life (de Vera and Seckbach, 2013). In addition, of finding water in the search for life, we have also to search for biomolecules in our galaxy. Indeed, astrobiologists have discovered regions in our galaxy that might have *the potential for producing very complex organic molecules* and the starting point for the development of life. Let us examine life on Earth and compare it with some extraterrestrial conditions.

2. Ubiquity of Life on Earth

Some scientists argue that life may have developed first in hydrogen-rich environments around warm ocean vents, while others claim that it originated in cold areas. There is also a hypothesis from a smaller group of scientists who believe that life on Earth originated via Panspermia (McNichol and Gordon, 2012; Wickramasinghe, 2012). These proponents claim that life existed throughout the Universe and was distributed on meteoroids, asteroids, and planetoids and finally harbored on Earth. Life is almost everywhere on Earth; however, to date now it has not been detected in some parts of it, such as in sections of the Atacama Desert in Chile (personal communication from Chris McKay). We know most organisms dwell under ambient "normal" conditions, as defined with respect to an anthropocentric point of view. Such common organisms exist under environments of a pressure of 1 atm of aerobic environment, temperatures around 25–40°C, and pH~6–7.

There are also, however, on or below Earth's surface, organisms dwelling at the edge of the normal limits for life under very harsh environments. They are called *extremophiles* (see Rothschild and Mancinelli, 2001; Seckbach, 1999, 2000, 2007). There are *polyextremophiles* (Seckbach et al., 2013), which are able to withstand environments of multi-extreme conditions, such as hyperthermophiles and high acidity, or hypersalinity and radiation levels, high pressures, and low/high temperatures. Let us look briefly at the organisms living in these severe conditions.

3. The Extremophiles

In this category, we include organisms, both autotrophic and heterotrophic microorganisms, living at high or low pH, or both scales of temperature ranges, high salinity, alkaline waters, acidic sulfur, high pressures (subsurface living organisms or those in depths of oceans), and alkaline soil. Among these organisms dwelling at the limit of life are prokaryotes and to a lesser extent eukaryote representatives; they include the bacteria; Achaea; algae; other protists, unicellular and multicellular; lichens; plants; and invertebrate animals. These organisms can tolerate very severe conditions such as:

- High temperature (thermophiles to hyperthermophiles, 40–115°C and higher) or very low temperature, −20°C (cryophiles/psychrophiles)
- A large range of pH (0–4 pH the acidophiles vs. pH 8–14 scale of the alkaliphiles)
- High salt concentration (halophiles) and up to saturated salt solutions (hyperhalophiles)
- High hydrostatic pressures (Barophiles/Piezophiles) ~ 1,000 atm
- Uptaking toxic chemicals, such as arsenic solutions (Wolfe-Simon et al., 2011) and other toxic metals, such as copper II, Zn II, and Cd II

Moreover, some bacteria are able to metabolize fuel from oil spills. Not only prokaryotes but also several eukaryotic microbes can live under anoxia (Altenbach et al., 2012) and utilize anaerobic metabolism. Also, some algae are able to thrive under various gaseous atmospheres, such as CO_2 (Seckbach, 1994), ammonia, methane, and under a dormant desiccated status.

The *polyextremophiles* (Seckbach et al., 2013) may dwell in snow and ice, as in the Siberian permafrost, Antarctica, and the Arctic zones. Others live in places like the arid Atacama Desert (Chile), haloalkaline soda lakes, geysers, and deep sea hydrothermal vents; in hot springs; and in hypersaline lakes, such as the Dead Sea, Israel (Oren and Seckbach, 2001). The barophiles thrive at the bottom of the oceans, or in subterranean environments. Among these harsh living organisms, we find prokaryotes, Archaea, bacteria, and eukaryotes. The cyanobacterium *Chroococcidiopsis* survives in extreme dry, cold, and salty environments. The unicellular acido-thermophilic alga *Cyanidium caldarium*, a red alga (Rhodophyta), appears as green spherical cells (Seckbach, 1994) and thrives in pure CO_2 (Seckbach et al., 1970), at elevated temperatures, and in very acidic solutions (pH 0–4). *Cyanidium* even tolerates 1N H_2SO_4. One genus of this family (Cyanidiaceae) *Galdieria* thrives in autotrophic and/or heterotrophic conditions with supply of sugars (Seckbach, 1994). There are also the snow algae, such as polyextremophiles, that appear as green, orange, or red coloration and have carotenoids during some periods. Other algae dwell in additional multi-extreme conditions.

Each lichen cell is a symbiotic association between a fungus and cyanobacteria, or alga. These could survive in very severe environments on Earth and in the unprotected conditions of space (exposed to space conditions of vacuum, ultraviolet radiation, and severe cold of ~ −20°C). They have survived also under simulated conditions of space (deVera et al., 2003, 2004; Raggio et al., 2011).

Among the polyextremophilic invertebrates we find the water bears—the *Tardigrates*—segmented, multicellular organisms that are mainly aqueous animals. They survive high doses of ionization and UV radiation, as well as an extremely wide range of temperatures, vacuum atmosphere, and high pressures in anhydrobiosis (Horikawa, 2011). They are small-size (<1 mm long) animals and may be considered as analogues for candidate for extraterrestrial living. These animals have indeed tolerated the conditions of space.

4. Astrobiology

The search for life beyond Earth is in fact essentially the search for habitability on other worlds. There have been some attempts to discover traces of fossil nanobacteria from a Martian meteorite (McKay et al., 1996) that fell in Antarctica (ALH84001). However, opponents rejected these observations and claimed that the illustrations are artifacts. Recently, Hoover (2011) has discussed some evidence for traces of microfossils of cyanobacteria and algae in a meteorite, which caused some subsequent discussions. Other chemical biomarkers were also observed in Achaean rock and on carbonaceous meteorites.

In the past decades, some effort has been invested to find terrestrial life forms on other planets. The comprehensive new book *Astrobiology* (Chela-Flores, 2011) discusses the new interdisciplinary field's concern with all of these extremophiles, as they may be models, or analogues for survivors in similar extraterrestrial environments in the Universe. A substantial amount of data about extraterrestrial bodies, mainly from the Solar System, has come from a variety of sources (surface rovers, flyby space crafts, and other means).

In the Solar System and beyond, there might be some habitable lands for organisms. Among them, near planet Earth, are some more promising lands for finding life such as on Mars, or in Europa (a Jupiter satellite). Saturn's satellites Titan (which contains lakes of methane and ethane) and Enceladus might resemble early Earth. Mars, with its sterile land, until now lacks organic matter; no life has been detected of its surface. Mars possibly contains liquid water in spite of its harsh surface temperature. It has been predicted that Europa has an internal salty ocean that may contain living species.

A similar subsurface ocean is located in Antarctica (at Lake Vostok station, located 4 km under the ice layer and containing salty liquid water, where microbes were observed deep in the icy layers). Furthermore, views of Mars' surface reveal that in the past it was a water world and warmer than it is today. The photos show contours of dry river lines, canyons, lakes, ancient oceans, and waterfalls. So there is a good sign that in the past there were living organisms. Spores from such ancient microorganisms may be waiting dormant under the surface of this planet also analogous to bacteria that were found on Earth after millions years of dormancy and then were revived (Vreeland et al., 2000). Photosynthesis pigments have already been detected in Galactic ecosystem. An ancient ecosystem discovered beneath Antarctic glaciers at "Blood Falls" may also show how aliens might live in icy worlds.

If micro-life could stay dormant in our local planet for millions of years and then wake up, why could not the same phenomenon take place in other extraterrestrial bodies. It is to be hoped that further away from the Solar System are more biosignatures of life in habitable extraterrestrial locations.

5. Summary of EPLA

This volume has gathered 68 expert authors from around the world to discuss questions of life on Earth and elsewhere. We thank Mrs. Christine Prietl for helping to compile the manuscript.

Their chapters deal with primeval seas, the origin of the genetic code, panspermia, and terrestrial habitability. The *extremophiles* section includes the halophiles, the polar cyanobacteria, and life without water, as well as microorganisms tolerating, surviving, and flourishing in severe environments. The extremophiles are important for practical uses (enzyme production) and extraction of special proteins. In the *Extraterrestrial Life* section of this volume, there are discussions about the search for extraterrestrial intelligent life, terrestrial analogues for planetary oceans, life in Earth lava-caves, as implications for life detection on other planets; habitability of Earth-like exoplanets; Mars water and polar dunes; Antarctica as a model for life on Europa; Saturn and its moons; astrobiology of Titan; habitability of extrasolar planets; and cosmic catastrophes.

This volume is number 24 of the *Cellular Origin, Life in Extreme Habitats and Astrobiology (COLE)* series (J. Seckbach (editor) 1999–2013, www.springer.com/series/5775). This book complements previous books of this series discussing also topics associated with this volume, namely, the Science of Astrobiology (2011), Stromatolites (2011), Symbioses and Stress (2010), Algae and Cyanobacteria in Extreme Environments (2007), and Enigmatic Microorganisms and Life in Extreme Environments (1999). The target audience for this new book comprises scientists, microbiologists working with extremophiles, biology, geology students, teachers, and general readers.

6. Acknowledgements

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INTRODUCTION

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Astrobiology, as a scientific viable discipline, is a fast growing and emerging science. This is so because of the fantastic increase in technological means and methods to discover extra solar planets. Our space age, which provides us with incredible and powerful satellites like CoRot and Kepler, allows the detection of planetary systems in which not only giant gaseous planets revolve around a central star but also Earth-like planets (ELPs) covered with a significant fraction of their area with liquid (not necessarily water). On one hand, several planetary systems containing few planets were discovered. On the other hand, over a thousand planets discovered so far, some tens appear to be ELPs and several systems with a number of planets were discovered. What can we expect from such planets? What kind of worlds there are? In particular, can we find planets the temperature and atmospheric pressure on which are close to the triple point of a certain molecule to facilitate unfamiliar and may be never speculated forms of life?

The discovery of any form of life on an extraterrestrial planet will be one of the most fantastic and extraordinary discoveries of mankind and is bound to have profound impact on all facets of life as we know them on our planet. The search for life on ELPs and the evolution of ELPs toward the formation of habitable zones (HZ) is what this compilation of papers is all about. The collection spans from the discovery of ELPs to esoteric forms of life, found under extreme conditions, to the structure of young planets and the planetary evolution toward the creation of a HZ and its eventual disappearance.

While the list of covered topics is long, it may not be everything relevant and the complexity of the problem may still be beyond us. For example, the role of the Greenhouse Effect, greenhouse gases emitted naturally, etc., are still not clear. Why Venus is so hot? Long time evolution of planets insolated by main sequence stars of different types, etc., to name just few examples.

Astrobiology and the search for extraterrestrial life may sound as subjects detached from our daily life here on Mother Earth. This is a very wrong perception. It is during these times that we witness how whatever goes on the Earth is intimately connected to astronomical phenomena, the sun (which does not serve only as an energy source), and the galaxy at large. Understanding our evolution on a timescale of decades and potential fate on much longer timescales is closely related to the questions posed in this compilation of papers. Where will global warming bring mankind? How global warming is connected and affected by cosmic phenomena and what mankind can and should do to preserve life on our planet in this corner of the Milky Way galaxy.

Biodata of **Roberto Barbieri** and **Nunzia Stivaletta**, authors of the chapter "***Halophiles, Continental Evaporites and the Search for Biosignatures in Environmental Analogues for Mars***."

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HALOPHILES, CONTINENTAL EVAPORITES AND THE SEARCH FOR BIOSIGNATURES IN ENVIRONMENTAL ANALOGUES FOR MARS

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1. Introduction

In the current decade, two Martian missions are planned by NASA and ESA having as primary target the search for possible signs of past or present life: Mars Science Laboratory (MSL), which is part of NASA's Mars Exploration Program, and the ExoMars of ESA's Aurora Programme. The reasons that make Mars of special interest from an astrobiological perspective include its nearness, some of the present-day physical characteristics of the planet's surface, and its geological history. Mars seems to have experienced earthlike conditions in its geological past, with lots of liquid water (Squyres and Kasting, 1994; Hynek and Phillips, 2003; Baker, 2006) that was able to produce the depositional processes and the erosional features described in different regions. Recently formed water features have also been described (e.g., gullies on cliffs and crater walls, Malin and Edgett, 2000), and they suggest that near surface, liquid water may episodically be present currently. Other conditions suitable for life (for example: a warmer climate) likely characterized the planet during the earlier phases of its geological history. Periods with a possible robust greenhouse warming may have taken place in the early Mars, during the Late Noachian-Hesperian period, through the combined effect in the atmosphere of gases, such as CO_2, NH_3, and CH_4, which might have maintained a surface temperature above the freezing point of water (Beaty et al., 2005). The finding – by rovers and, remotely, by orbiters and spectrometers – of salt- (especially hydrated sulfate) rich deposits in different areas of the Martian surface (Squyres et al., 2004; Vaniman et al., 2004; Gendrin et al., 2005; Langevin et al., 2005) is a further indication of past aqueous processes.

Processes related to the evaporite deposition from arid saline lakes (such as continental sabkha/playa or equivalents) have been proposed for explaining some of the sulfate-rich deposits of Mars (Gendrin et al., 2005; Murchie et al., 2009). Because evaporites formed in continental lacustrine/playa settings may preserve a rich record of processes related to water and biology, they might have obvious implications for the search of Martian life. In evaporite ecosystems, local microbial colonizers can be either involved in specific biochemical processes, such as the oxidation of sulfides to sulfates, or they may just use the evaporite crusts and minerals as physical habitats (Rothschild, 1990; Rothschild et al., 1994; Martinez-Frias et al., 2006); in both cases, they can leave a permanent record.

Investigation of Earth environments that are thought to closely resemble sectors of the Martian surface is a useful tool until real Martian environments can be thoroughly studied. As far as evaporites are concerned, terrestrial analogue environments should primarily be centered on the understanding of the multiple microbe-mineral interactions, how the microbial communities may influence the mineral and textural features, and the ways for distinguishing biological- from nonbiological-derived features permanently delivered to the fossil record. This is a necessary prerequisite to allow in situ work by the science payload carried by ESA's ExoMars and NASA's MSL rover missions in case of the selection of evaporite-rich terrains as landing sites. Since the objectives of the ExoMars mission include the search for shallow subsurface biosignatures, which will be investigated through a drilling equipment that is able to collect samples down to about 2 m (http://sci.esa.int/science-e/www/object/index.cfm?fobjectid=47089), we point out here a further potential for the evaporitic environments: the possibility to host endolithic microbial communities (Oren et al., 1995; Stivaletta and Barbieri, 2008). Their ability to be protected by surface hostile factors (e.g., ultraviolet radiations and surface oxidation processes) improves the chances of finding traces of life.

Here we will briefly consider saline hot and dry deserts, in which a combination of limiting environmental factors – including high-energy electromagnetic radiation, low water availability, oxidizing chemistry, and soil salinity – make these environments suitable to be considered Martian analogues.

2. Microorganisms in Evaporite Environments

Halophilic microbes have been described from a diversity of lowlatitude continental/lagoon settings and artificial salterns (Madigan et al., 2009). Regardless of their location, modern hypersaline environments share several physical characters, such as flat surface morphologies (in sabkhas and salt lakes) and strong evaporation processes that favor the development of surface to shallow subsurface hard evaporite crusts and horizons (Warren, 2006). The layering, composition, and texture of the salt crusts depend on a number of factors, such as recurrent phases of crust erosion and precipitation (from rainfall, flooding, and the evaporation of groundwater), amount of siliciclastics (clay, silt, and sand), and chemical composition of groundwater. Although most evaporite rocks in the geological record have a marine origin, paralic and continental environments display the best examples of present-day evaporitic settings. Together with the classical coastal sabkhas, having their type-locality in the Persian Gulf (Alsharhan and Kendall, 2003), inland hypersaline lakes have been investigated in recent years. Some of the best examples include the alkaline lakes in the Wadi El Natrun depression, Egypt (Taher, 1999); the continental sabkhas of the southern Tunisia chotts (Bryant et al., 1994; Drake et al., 1994); the *salares* of the Atacama region, Chile (Stoertz and Ericksen, 1974), and Death Valley, California (Crowley and

Hook, 1996). In the above examples, salt flats and crusts are derived from concentration through solar evaporation. Non-marine evaporites display ranges of mineral composition higher than in the evaporites of marine origin (Kendall, 1992). Compared with the hyperarid coastal sabkhas, salterns, and other salt environments in permanent aqueous media, the continental evaporites (and the associated microbial habitats) differ remarkably. First of all, they are only temporary aqueous environments, since water depends on ephemeral events, such as floods, changes of the groundwater level, atmospheric moisture, etc. In addition, because mineral crusts precipitate at or near Earth's surface, microbial communities can be exposed to environmental damages that are not only limited to dryness and include high solar ultraviolet radiations, seasonal (and daily) thermic excursions, and strongly oxidative processes. Altogether, these conditions make the Earth similar in some respects to Mars surface and make certain terrestrial regions an effective Martian analogue. Hyperarid saline areas, such as the Salar Grande (Garcia-Veigas et al., 1996) – the driest part of the Atacama region, where the only moisture comes from the early morning fog of the Pacific coast – and the Qaidam Basin (Wang and Zheng, 2009; Zheng et al., 2009), in the Tibetan Plateau – with an average elevation of about 4,500 m – are ideal locations for detecting the limits of microbial densities. Rather than sterility by itself, which is extremely rare on Earth, the density gradient might be an important biological information in an astrobiological perspective. Microbial densities, their limits, and the search for useful criteria for their detection should be considered in astrobiology missions, where the investigation should likely be conducted in sterile or extremely rarefied biological components. Because evaporite environments display microbial population abundances that are among the lowest on Earth, they should be ideal for conducting in the field experiments of density evaluation. The available data, however, indicate a different answer by the microbial communities with increasing salinity: whereas bacteria decrease their abundance, archaea increase and become predominant at high salt concentrations (Maturrano et al., 2006; Oren, 2008).

Since on Earth life processes use water as solvents, the presence of liquid water is the most readily known criterion to evaluate whether life is (or was) hosted in a given environment. In strongly desiccated environments – however, certain extremophiles can survive and spend prolonged periods in anhydrobiosis state (Billi and Potts, 2002). At hypersaline environments, water may be substantially limited by salt concentration, and halophiles meet physiological constraints in the osmotic stress conditions derived by high solute concentrations (Grant, 2004). At extremely high salt concentrations (salinities from about 30% up to saturation), most of microbes are haloarchaea belonging to the family Halobacteriaceae, and their ability to colonize environments with an extreme range of salt concentrations, as in sabkhas/playas that alternate flooding-evaporation phases, seems to be dependent on efficient mechanisms for haloarchaeal osmoadaptation (Soppa, 2006; Oren, 2008). Haloarchaea would, therefore, represent model organisms living under high salt conditions.

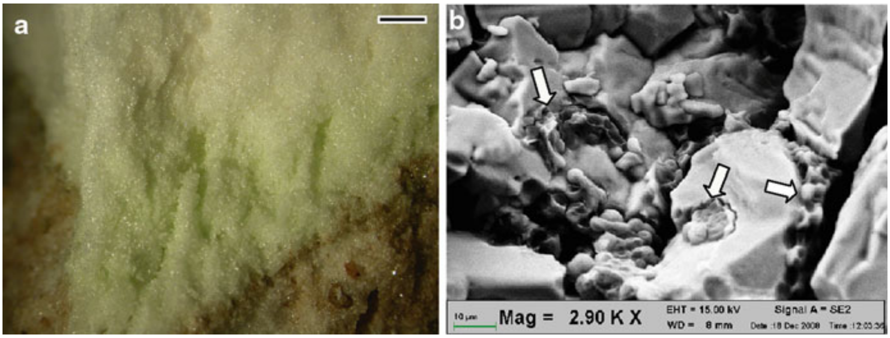

Figure 1. Detail of halite crust from the Salar Grande of the Coastal Cordillera (Atacama Desert). (**a**) The *pale green* color is imparted to the finely crystalline halite by the coccoid (Chroococcales) cyanobacteria colonies. Scale bar: 5 mm. (**b**) Environmental SEM micrograph of the coccoid cyanobacteria (*white arrows*) (Image reprinted with permission from Barbieri et al., 2009) (Color figure online).

In spite of their being listed among the extreme environments, there are several physical conditions that make salt environments suitable for specific components of the microbial communities. Evaporite minerals have generally transparent or light coloration: gypsum, for example, consists of clear, translucent crystals, whereas halite (the most common evaporite mineral) is an aggregate of light-colored to colorless (depending on impurities) cubic crystals. Certain endolithic communities, such as cyanobacteria, may benefit from living within light-colored evaporite crusts through the double advantage of sufficient light for photosynthesis and protection from exposure to strong ultraviolet light that typify salt environments of arid regions (Rothschild, 1990; Krumbein et al., 2004; Stivaletta and Barbieri, 2009; Stivaletta et al., 2010). A further advantage of the endolithic mode of life lies in the hygroscopy of evaporite minerals, especially halite (Davila et al., 2008). This enables some relative humidity to be maintained especially in hyperarid environments, such as the Atacama Desert (Wierzchos et al., 2006).

The intense colors of the salt crusts in evaporite environments are caused by the pigments of the populations of halophilic microorganisms. Whereas red and bright orange coloration depends on the carotenoids produced by green algae (especially *Dunaliella*) and purple bacteria, such as Chromatiaceae, a green color is imparted by coccoid and filamentous cyanobacteria (Stivaletta et al., 2009, 2011). Colonies of the order Chroococcales, one of the most extremophilic cyanobacteria, have been detected in the hyperarid salt environments of the Atacama and Sahara Deserts (Wierzchos et al., 2006; Stivaletta and Barbieri, 2009; Stivaletta et al., 2011). Figure 1 shows the physical relationships between Chroococcales colonies, likely belonging to the genus *Chroococcidiopsis*, and the salt (halite) deposit of the Salar Grande, in the most arid sector of the Atacama region, where they established an endoevaporitic mode of life.

In hyperarid environments with harmful ultraviolet and ionizing radiation, the recovery of the genus *Deinococcus* provides evidence of its twofold resistance to desiccation and radiation (Battista, 1997; Stivaletta et al., 2010) and makes this bacterium one of the most resistant to the harshest of environments.

3. Evaporites and Microbial Preservation

Non-altered evaporite deposits have been documented in the fossil record since the Neoproterozoic (Warren, 2006). Because of their rapid precipitation and sealing properties, salts accumulated in evaporite environments may represent useful storage places for biologically derived morphologies and compounds, especially with the absence of surface hydrological cycles, as is the case on present-day Mars. Primary fluid inclusions, in particular, are frequent in halite, where prokaryote cells have long been recognized (Dombrowski, 1963); traces of microbial life preserved in ancient halite have a likely high potential in the search for signs of life on Martian salt environments (Schubert et al., 2009). As the dormant stage of bacteria, spores have been frequently found in very ancient deposits. In 250-million-year-old salt crystals, for example, strains of halophilic archaea have been detected and isolated (Satterfield et al., 2005; Vreeland et al., 2006), and the recent finding of the world's oldest known cyanobacterial DNA isolated (Panieri et al., 2010) comes from primary evaporites (gypsum). In spite of the quick DNA decay, the discovery of the segments of haloarchaeal DNA sequences, extracted from 419-million-year-old salts (Park et al., 2009), suggests that the salt potential in terms of microbial preservation is far from exhausted. Other useful, potentially preservable biomarkers include complex organic molecules whose presence is correlatable with specific microbial components. Ongoing investigation of modern, subfossil, and fossil sulfate (gypsum) deposits reveals that evaporitic sulfates can be considered prime targets in the search of biologically derived organics. From the set of potential Martian analogue environments, a recent example is to be found in the gypsum mounds of continental sabkhas of the Sahara Desert (Stivaletta et al., 2010), which enabled the detection of endolithic prokaryote communities through preserved chemical biomarkers such as pigments and DNA molecules. Another example comes from laboratory experiments of identification tests via Raman spectroscopy on ß-carotene pigments from evaporitic matrices (Vitek et al., 2009). In a variety of sulfate minerals of North America, from modern to fossil (40 Ma old), the preservation of amino acids and other organic compounds (Aubrey et al., 2006) may provide an effective tool in the search for preserved (fossil) biological signatures. Although amino acid synthesis can be nonbiological, in case of the recognition of homochirality, a common structural feature of biomolecules in nature (Blackmond, 2010) is an unambiguous property indicating their origin via biological processes.

Biologically induced features in evaporites include mineral biosignatures, which may be delivered and permanently preserved in the fossil record (Konhauser, 2007).

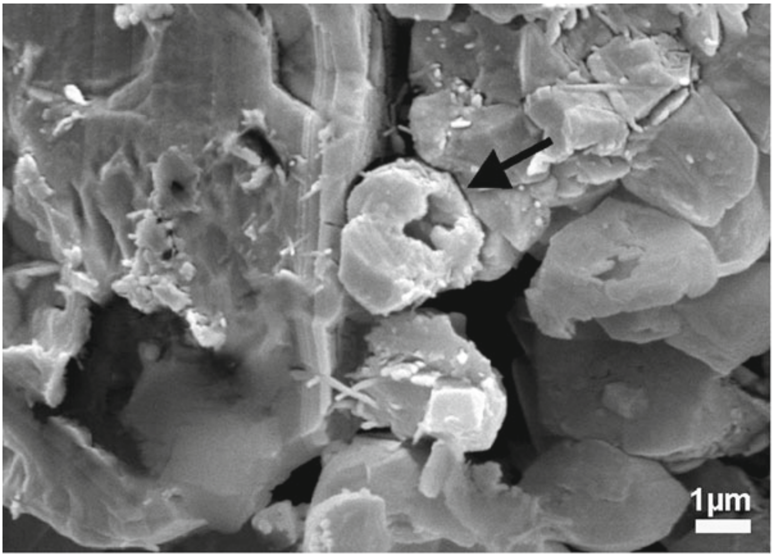

Figure 2. Fossil evaporite (sabkha) deposits of the Chott el Gharsa (Tunisian Sahara): SEM micrograph of a dumbbell shape (*black arrow*) from the hollow core of a dolomite crystal (Image reprinted with permission from Barbieri et al., 2006) (Color figure online).

Because in hypersaline settings microorganisms play primary roles in establishing the environmental conditions and mineral kinetics, they may determine the precipitation of specific mineral species, which may therefore become inorganic fingerprints of life in evaporites. Examples in present-day settings include the records of rosickyite and bassanite, which are reported as bio-induced minerals from evaporitic layers colonized by dense colonies of endolithic cyanobacteria in the Death Valley (Douglas and Yang, 2002). In laboratory experiments with simulated conditions similar to the ones occurring in the hypersaline lakes of south Australia (Wright and Wacey, 2005), a successful dolomite precipitation has been attributed to kinetically favorable conditions produced by bacterial sulfate reduction associated to the degradation of organic compounds. Dolomite precipitation was also recovered in a fossil (upper Pleistocene) continental evaporite, as the Chott el Gharsa of southern Tunisia (Barbieri et al., 2006). At the Chott el Gharsa dolomite crystals often have a dumbbell shape to their hollow cores (Fig. 2), which is a bacterial-related morphology (Buczynski and Chafetz, 1991) driven by sulfate-reducing bacteria in both natural and laboratory conditions (Warthmann et al., 2000; van Lith et al., 2003).

Textures that may be produced biogenically and preserved in the fossil record of hypersaline environments may serve as reliable biosignatures (Barbieri et al., 2006; Douglas et al., 2008). Microbial remains, biofilm, and microbialites, for example, may gain a high fossilization potential once early mineralization provides the twofold advantage of keeping a biological-related morphology and a potential permanent delivery to the fossil record.

4. Terrestrial Analogues of Martian Evaporites

Recent research on the mineral composition of the Mars surface suggest the presence of liquid water during the Noachian period on a global scale (Murchie et al., 2009; Carter et al., 2010), and this has important implications in the search for life and habitable environments. Extensive evidence of polyhydrated sulfate minerals – including epsomite, gypsum, bassanite, hexahydrate, jarosite, and kieserite – has also documented aqueous processes. However, they provided further and different environmental information. Jarosite, a potassium iron sulfate, for example, can precipitate via bacterial oxidation to convert ferrous sulfate to ferric sulfate in low pH conditions (Grishin et al., 1988). This mineral – which has also been detected in Rio Tinto, Spain (Fernandez-Remolar et al., 2005) at an extremely acidic site, as well as at Meridiani Planum by the rover Opportunity (Klingelhoefer et al., 2004) and other Martian sites – indicates that the ambient water formation could be acidic and highly saline. Although jarosite can be the product of the weathering of basaltic rocks on Mars (Elwood Madden et al., 2004; Squyres and Knoll, 2005) in oxidizing and acidic environments, the overall data on the Martian sulfate diversity, distribution, and the layered organization of some of the sulfate deposits (Gendrin et al., 2005; Murchie et al., 2009) are consistent with environments where minerals such as epsomite and gypsum would be formed from evaporation. Of astrobiological significance is the recent evaluation of salt composition at the Phoenix landing sites (Kounaves et al., 2010), which indicates that the local water activity at gypsum and epsomite brines would be tolerated by halophilic microbes. This is a significant point that emphasizes the evaporite environments as potential locations for extraterrestrial life.

Terrestrial evaporite ecosystems located in areas that are similar in some respects to Martian conditions can be useful environmental analogues that can elucidate the interactions between physical evaporite environments and the biogeochemical/metabolic processes governed by halotolerant microorganisms. Such examples include the few-100-m sized sinkholes with steep edges (Fig. 3a), locally called *puquio* (Pueyo et al., 2001), in the Salar de Llamara of the Atacama Desert, where ongoing research (N. Stivaletta and others, unpublished data) has documented a high diversity for the local sulfate-rich deposits derived from evaporation processes (Fig. 3b). At least five different sulfate minerals – bloedite, gypsum, glauberite, kainite, and leonite – have been recognized in the salt crusts that precipitate in these modern evaporite environments where visually observed microbial colonies (Fig. 3c) undergo extreme stress conditions (Demergasso et al., 2003). Such an association of evaporite minerals has been described from different Martian sites as product of evaporite processes. The endoevaporitic microbial colonizers make these slightly alkaline, spatially confined habitats suitable for a

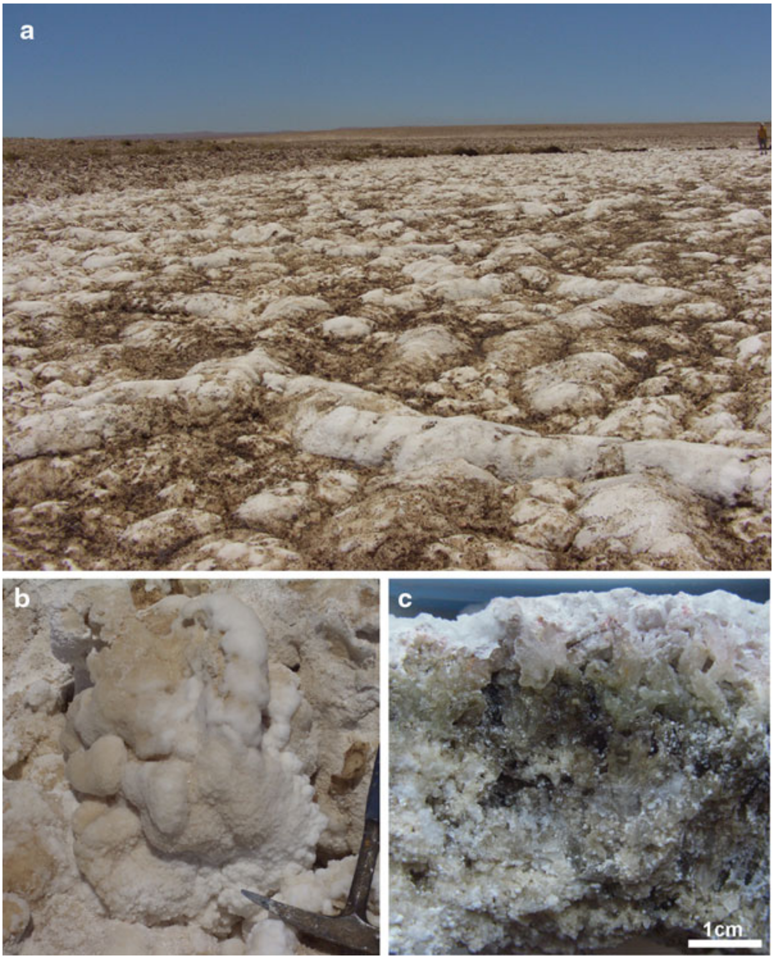

Figure 3. Salar de Llamara (Atacama Desert). (**a**) Evaporite (sulfate) filling of a sinkhole. (**b**) Detail of the powdered, sulfate crust of a sinkhole. (**c**) Cross section of a crystalline gypsum deposit, the *dark color* is imparted by the degrading microbial communities (Image reprinted with permission from Barbieri and Stivaletta, 2011) (Color figure online).

thorough investigation, in the context of the near-future planetary missions, with the primary target of the question of life on Mars. They may also enable an in-depth analysis for a better understanding of the preservation of the biological signatures and the ways for their detection.

The evaporitic environments do not exhaust the overall context of the Mars analogue settings, which also consider phyllosilicate-, hematite-, and carbonate-rich (among others) materials. Experience gained during the study of evaporites on Earth, however, provides evidence on the uniqueness of such environments, both in modern and fossil settings. The ability of salt brines and crystals to keep living materials, either trapped in crystals or within fluid inclusions (Norton and Grant, 1988), is largely documented on Earth, in which this property would be a primary advantage in the search for evidence of the Martian origin of life (an origin dependent or independent from Earth), if any. For this recognition, the recovery of biological material is a bare necessity (McKay, 2010).

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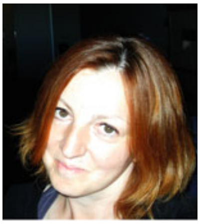

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VESICULAR BASALTS AS A NICHE FOR MICROBIAL LIFE

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1. Introduction

The presence of microbial communities in nearly every type of extreme environment where liquid water and metabolically suitable carbon, energy, and nutrient sources exist suggests that unrecognized vestiges of microbial ecosystems and habitats remain to be discovered (Rothschild and Mancinelli, 2001). Their paleobiological repositories, if biosignatures of the communities were preserved in the geological record, also likely await discovery (Westall and Cavalazzi, 2011).

The hypothesized extensive deep terrestrial and oceanic subsurface biosphere has been the focus of study for over a decade (e.g., Onstott et al., 1997; Torsvik et al., 1998; Pedersen, 2000; Amend and Teske, 2005; Peckmann et al., 2008; Heim, 2011; Stan-Lotter and Fendrihan, 2011). Microbes characterized from sedimentary and volcanic/hydrothermal environments in ocean floor sediments and in the Earth's crust comprise some of the most physiologically and phylogenetically rich and complex microhabitats discovered to date (e.g., Monty et al., 1991; Fisk et al., 1998; Furnes and Staudigel, 1999; Amend and Teske, 2005; Mason et al., 2007; Peckmann et al., 2008; Santelli et al., 2008). For example, microorganisms and evidence of their activities are found at depths >3,000 m within the continental crust in the deep gold mines of the Witwatersrand Basin in South Africa (Onstott et al., 1997; Lippmann-Pipke et al., 2011) and associated with basalts in all ocean basins (e.g., Furnes and Staudigel, 1999; Santelli et al., 2008; Staudigel et al., 2008 for a review).

The deep biosphere is estimated to contain more than 50% of Earth's biomass and contribute significantly to the diversity of life on Earth. The largest portion of the deep (prokaryotic) biomass is found in the sub-seafloor biosphere (Whitman et al., 1998; D'Hondt et al., 2002; Parkes et al., 2005). Oceanic crust, which constitutes 60–70% of the Earth's exposed lithosphere, consists mainly of basaltic igneous rock and pillow lava. The approximately 20 km^3 of basalt produced annually at mid-ocean ridges provides an important potential habitat for microbial life (Parsons, 1981; Fisk et al., 1998; Furnes and Staudigel, 1999; Mason et al., 2007, 2009; Santelli et al., 2008; Staudigel et al., 2008; Nielsen and Fisk, 2010). Microorganisms and their metabolic products, as well as microfossils and other traces of microbial activity, occupy all oceanic crustal habitats (e.g., Furnes and Staudigel, 1999; Furnes et al., 2004; McLoughlin et al., 2007; Staudigel et al., 2008).

Microbial ecosystems in oceanic crustal habitats play a crucial role in balancing chemical exchange between the lithosphere, hydrosphere, and atmosphere. They affect the transformation rates, mechanisms, and pathways of exchange of many chemical elements that contribute significantly to biogeochemical cycling (Thorseth et al., 1995; Furnes and Staudigel, 1999) and may even impact the magnetization of young oceanic basalts (Carlut et al., 2007). The primary glassy rinds of basalts provide a habitat for microbes that actively bore into rock surfaces and create microtubular cavities. Recent findings demonstrate, however, that microbial activity in sub-seafloor volcanic rocks is not limited to the glassy rinds of crystalline volcanic rocks. Vesicular basalts also support microbial ecosystems whose communities may leave traces in the geological record. Here, we present a brief review of the present state of knowledge with regard to epi- and endolithic microorganisms that colonize volcanic basaltic rocks with an emphasis on the relatively poorly known vesicular basalts that host microbial ecosystems. These types of habitats are of particular interest in the search for life on rocky water-bearing planets, such as early Mars, Europa, Enceladus, and possibly Titan.

2. Overview

After their formation, oceanic basalts undergo weathering (abiotic alteration) via reaction with seawater and hydrothermal fluids while exposed at the ocean floor and through fluid–rock interaction beneath the seafloor. The palagonitization of basaltic rocks and volcanic glass (cf. Stroncik and Schmincke, 2001), a low-temperature hydrolytic process in which mafic glass is altered by seawater, produces amorphous or microcrystalline secondary hydrous minerals such as Fe hydroxides, Fe and Ti oxides, authigenic clay minerals (e.g., zeolites), and micas (Honnorez, 1981; Alt, 1995; Storrie-Lombardi and Fisk, 2004; Banerjee et al., 2006; Staudigel et al., 2008).

Phylogenetic analysis of microbial communities associated with marine basalts and rocks collected from the deep-ocean seafloor has demonstrated that they are colonized by dense endemic microbial communities (Edwards et al., 2004; Mason et al., 2007; Santelli et al., 2008). Edwards and coauthors (2005) argue that the chemical energy released by seawater-basaltic rock reactions support the metabolic growth of a variety of microorganisms. In spite of these considerations, our understanding of the microbial communities that inhabit sub-seafloor environments and exploit metabolically these chemically favorable conditions is limited.

A wide variety of signatures indicative of the bioalteration of modern and ancient oceanic volcanic glass, which include fossilized microbes and their geochemical and molecular traces (e.g., pits and globular, tubular or channel-like structures, depleted $\delta^{13}C$ values, relatively high concentrations of C, P, N, and S, and traces of DNA/RNA; Furnes et al., 2007; McLoughlin et al., 2007, 2009; Staudigel et al., 2008; Thorseth, 2011 for a review), underscore the importance of these types of habitats. Thorseth et al. (1992) first described microbial activity in hyaloclastite formed in an Icelandic subglacial eruption. When the temperature of freshly formed ocean crust drops to values that become tolerable for life,[1] volcanic surfaces can be colonized by microbial communities that utilize the inorganic elements and compounds of the rocky substratum as energy and nutrient sources. These same surfaces thus undergo subsequent biological and hydrothermal alteration processes. Bioalteration of the glassy basalts is accompanied by the formation of authigenic phases, such as smectite and phillipsite, in the alteration zone (for a schematic model see Figure 1 in Staudigel et al., 2008). Microorganisms have known affinities for specific, bioessential elements (e.g., Mg, Ca, Fe, P, Mn, S, C) that are present in silicate glass and minerals found in basalts, which include olivine, pyroxene, feldspar, and hornblende (Thorseth et al., 2003; Fisk et al., 2006; Furnes et al., 2007). However, despite the ubiquity and diverse nature of life associated with the bioalteration of oceanic basalts and basaltic glass, their biochemical pathways and role in geomicrobial processes remain poorly understood (e.g., Edwards et al., 2005).

Endoliths, organisms that reside in the interior of rocks, and euendoliths, microbes that actively bore into rock substratums and create microtubular cavities (Golubic et al., 1981), are known to exploit the habitat of primary glassy rinds of seafloor basalts and extrusive volcanic rocks (McLoughlin et al., 2007). Moreover, the chemical alteration of these rocks increases the range of microenvironments that could support such microbial communities. Communities of endoliths, including epilithic microorganisms that attach themselves to the external surfaces of rocks, chasmoendoliths that inhabit rock fissures and cracks, and cryptoendoliths that tend to colonize rocks with preexisting interstices and porous structures, also populate basaltic habitats. Recent studies have revealed that chasmoendolithic and cryptoendolithic prokaryotes and eukaryotes inhabit the primary and alteration rinds of crystalline volcanic rocks. Living and fossil cryptoendoliths and chasmoliths have been identified in vesicles, vacuoles, cracks, and veins/fractures within volcanic rocks in terrestrial and marine environments (Schumann et al., 2004; Jorge Villar et al., 2006; Cavalazzi et al., 2008, 2011; Ivarsson et al., 2008a, b; Peckmann et al., 2008; Eickmann et al., 2009). Examples include fossil chasmoendoliths in

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[1] At present, the highest temperature known to permit growth and life is less than 113–121 °C (Takai et al., 2001; Kashefi and Lovley, 2003); however, higher temperatures may be tolerated during short-term survival.

zeolite-filled veins associated with sub-seafloor basalts sampled at the Emperor Seamounts in the Pacific Ocean (Ivarsson et al., 2008a, b) and putative fossilized cryptoendolithic microorganisms found in the vesicles of deep oceanic basalt from a site in the North Pacific, first described by Schumann et al. (2004).

3. Vesicular Basalts as a Cryptic Habitat: The State of the Art

Vesicles in volcanic rocks are primary textures, also known as segregation structures that represent the fossilized vestiges of bubbles that formed during the extrusion of magma and decompression of gas from a volatile-rich melt during solidification of the rock (Peck, 1978). Amygdales or amygdaloids are relict vesicles that have been infilled with secondary minerals that precipitated after the rock formed, long after the melt source cooled.

A wide variety of vesicle types is known, and different mechanisms have been invoked to explain the origin of segregation structures. Among the most common are spherical segregation vesicles that are ubiquitous throughout an entire basalt flow and attributed to either the shrinkage of gas during cooling (Smith, 1967) or the escape of gas to form microvesicle chains (Bideau and Hékinian, 1984). Pipe vesicles are elongated bubbles that occur near the base of basalt flows or in the interior part of pillow lavas (Fig. 1). Vesicles range in shape from spherical to elliptical, elongated or flattened, and in size from ~1 mm to more than 1 cm. Basalts (e.g., pillow basalt and hyaloclastite) are most commonly vesicular. The types of vesicles and their distribution provide evidence of emplacement and flow mechanisms as well as rates of effusion of the lavas and their cooling history. The degree of vesiculation that lava undergoes during eruption under water or ice and where subaerial flows reach the sea or other bodies of water is controlled primarily by the volatile content of the lava at the time of eruption and by the confining pressure. Thus, vesicularity can theoretically be related to the rock chemistry and water depth at the time of eruption (Jones, 1969; Moore, 1970).

Secondary minerals most commonly found in vesicles and amygdales include Ca carbonate (e.g., calcite), crystalline and cryptocrystalline varieties of quartz (SiO_2, e.g., chalcedony or agate), clay minerals (e.g., chlorite and smectite groups), and authigenic silicates (e.g., phillipsite and other zeolites) (Fig. 2). They are mainly associated with water–rock interaction processes, such as low-temperature hydrothermal alteration processes of basalt. These processes depend on the groundwater chemistry and local physical conditions (Honnorez, 1981). Calcite ($CaCO_3$) and phillipsite ($(Ca,Na_2,K_2)_3(Al_6Si_{10})(O_{32}12H_2O)$) are among the most common secondary minerals found in vesicles (Fig. 2a, c), and zeolites, including phillipsite, are the most common secondary phases found in sub-seafloor environments (Honnorez, 1978).

After cooling and as soon as the temperature of the host basalt becomes tolerable for life, vesicles are likely to be colonized by microorganisms (cf. Sect. 2)

Figure 1. Base of basalt flow with distinctive pipe vesicles, c. 3.0 Ga Agatha Formation, Nzuse Group, White Umfolozi Inlier, South Africa.

as seawater and hydrothermal fluids circulate through cracks and fractures in oceanic basalts. Reduced chemicals in basaltic rocks, such as iron and sulfur (Edwards et al., 2005), represent a metabolic energy source for cryptoendolithic and chasmoendolithic microorganisms that can exploit protected niches in the rock. Recently described cryptoendoliths discovered within amygdales in the alteration rinds of oceanic basalts (Schumann et al., 2004; Cavalazzi et al., 2008, 2011; Peckmann et al., 2008; Eickmann et al., 2009) reflect such behavior (Figs. 2, 3, and 4). Furthermore, the potential for the fossilization of endolithic microorganisms that inhabit the margins, walls, and pore spaces of fluid-filled vesicles of basaltic rocks by authigenic minerals, such as carbonates, clay minerals and zeolites, is excellent (Peckmann et al., 2008; Cavalazzi et al., 2011).

Fungi have also been discovered in the primary and alteration rinds of crystalline volcanic rocks. Schumann and coauthors (2004) reported putative fossil cryptoendolithic filamentous microorganisms (5–10 μm diameter and brown in color) in small carbonate-filled vesicles in an oceanic pillow basalt from Eocene-age, upper oceanic crust collected at the ODP Site 1224 in the North

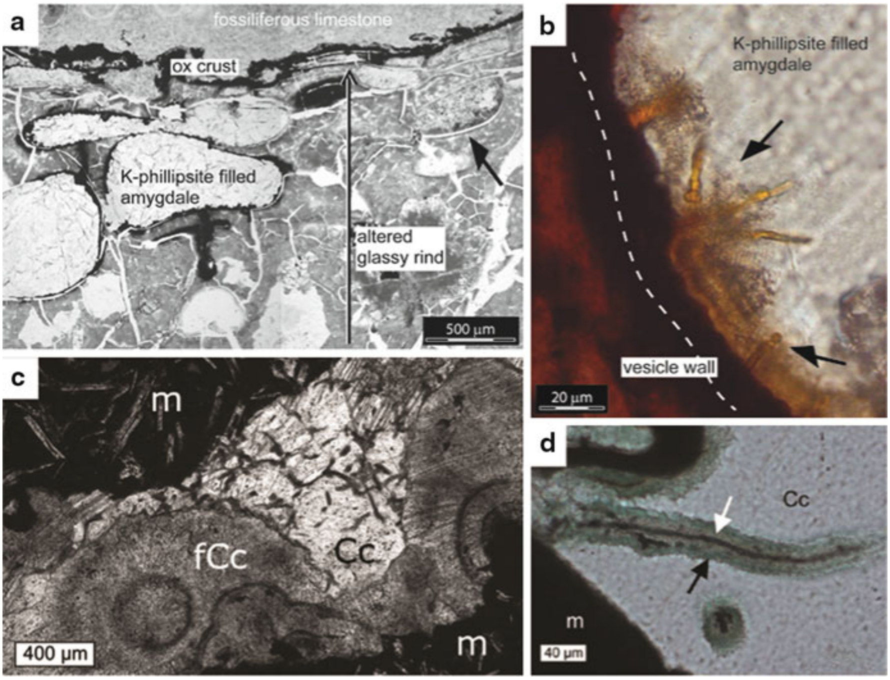

Figure 2. Transmitted-light photomicrographs of petrographic thin sections of vesicular basalts. (**a**) Vesicles within the altered, palagonitized glass of pillow lava rinds from Ampère–Coral Patch Ridge Seamounts in the eastern North Atlantic are partially or entirely filled with K-rich phillipsite. Some amygdales (*black arrow*) are filled with granular K-phillipsite that contains disseminated Fe–Ti oxides and microfossil-like structures (detail in **b**). An oxide-rich crust separates the outer edge of the pillow basalt from the encrusting fossiliferous limestone. (**b**) Detail of microfossil-like structures within amygdales (*arrows*). Note the filamentous structure attached to the vesicle wall and embedded in K-phillipsite. (**c**) Carbonate-filled vesicle in pillow basalts from the Frankenwald and Thüringer Wald within the Saxothuringian zone in Germany. Note the abundant putative biogenic filaments in the vesicles (see detail in **d**). (**d**) Mineralized filament within the Thüringer Wald vesicles. The filament is mineralized by chamosite/illite (*central portion*), chamosite/glauconite (*white arrow*), and illite/glauconite (*black arrow*). *m* basaltic matrix, *fCc* fibrous rim cement, *Cc* equant calcite spar (Images **a**, **c**–**d** are modified and reprinted with permission from Cavalazzi et al., 2011, and Eickmann et al., 2009, respectively) (Color figure online).

Pacific (Fig. 3). The fossilized filamentous structures were interpreted as marine fungal vegetative filaments (hyphae) growing in anaerobic conditions. Epilithic fungi have been discovered on the surfaces of young basalts that formed at Vailulu'u Seamount, Samoa, an active deep-sea volcano (Connell et al., 2009). Since fungi have historically been studied mainly in terrestrial environments, little is known about their distribution, diversity, taxonomic identity, and the mechanisms by which they could penetrate rock substrates in the oceanic realm (e.g., Verrecchia, 2000; Golubic et al., 2005).

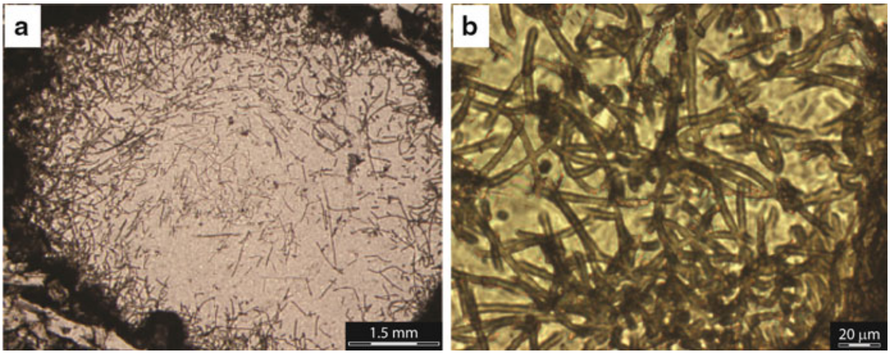

Figure 3. Fossil fungal filaments in an amygdule in pillow basalt from the North Pacific (Reprinted with permission from Schumann et al., 2004) (Color figure online).

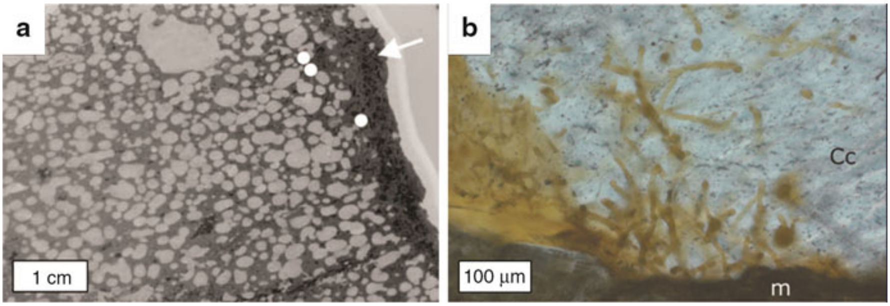

Figure 4. Fossilized microbial structures in a Middle Devonian pillow basalt from the Rheinisches Schiefergebirge zone, Germany. (**a**) Scanned thin section of the vesicular basalt shows the co-occurrence of mineralized filaments within the vesicles (*white dots*). (**b**) Transmitted light photomicrograph of the fossilized filaments. *m* volcanic matrix, *Cc* equant calcite spar (Modified and reprinted with permission from Peckmann et al., 2008) (Color figure online).

Peckmann and coauthors (2008) described putative marine cryptoendolithic filamentous prokaryotes (Fig. 3) within Ca-carbonate amygdales from a Middle Devonian (Givetian) pillow basalt of the Rheinisches Schiefergebirge zone in Germany (Fig. 4). Late Devonian (Frasnian) pillow basalts from the Frankenwald and Thüringer Wald within the Saxothuringian zone, also in Germany, have similar features (Eickmann et al., 2009) (Fig. 1c, d). In both cases, filamentous structures were observed within amygdales located in the external margin of pillow basalts. The filaments, which consist of clay minerals (Fe-illite surrounded by chamosite) associated sometimes with Ti-bearing phases (titanite and Ti oxides) (Fig. 1d), protrude inward from the vesicle walls. The cryptoendolithic behavior of the filaments, preserved in the Ca-carbonate cement of the amygdales, provides definitive evidence of their syngenicity during authigenic mineral precipitation

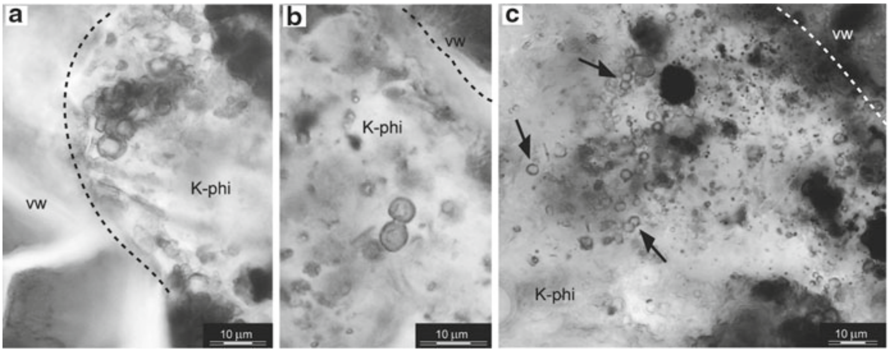

Figure 5. Thin section photomicrographs (*transmitted light*) of large and small (*black arrows*) spherical microbial structures embedded in phillipsite-filled amygdales from the altered glassy rind of a pillow basalt collected from the Ampère–Coral Patch Ridge Seamounts, Gulf of Cadiz, eastern North Atlantic Ocean. *vw* vesicle wall, *K-phi* K-phillipsite (Modified and reprinted with permission from Cavalazzi et al., 2011).

(see the schematic paragenetic model of the growth of such structures in Figure 10, page 132, in Peckmann et al., 2008). The putative microfossils are interpreted as fossil prokaryotes, based on similar mineralization processes that preserve evidence of recent prokaryotes (Konhauser and Urrutia, 1999), and due to the associated high concentration of iron, which is an indication of preferential microbial selectivity of the metal ion in spite of the fact that the concentration of other cations in solution would have been higher (Konhauser and Ferris, 1996).

Putative fossil microbial endoliths have recently been described from potassium-rich, phillipsite-filled amygdales discovered in the chilled rinds of a Paleocene basalt pillow lava from the Ampère–Coral Patch Ridge Seamounts, Gulf of Cadiz, eastern North Atlantic (Fig. 2a, b) (Cavalazzi et al., 2008, 2011). The filaments, which protrude inward from the walls of the vesicles (Fig. 1a, b), consist of K-phillipsite tubules surrounded by an Fe-oxyhydroxide (lepidocrocite)-rich membranous structure (Cavalazzi et al., 2011). Spherical structures (2–4 μm in diameter) (Fig. 5) comprised of carbon-bearing Ti oxide (anatase) are also associated with these amygdales. These spherical structures were interpreted as the fossilized remains of microorganisms because they display a suite of characteristics consistent with those of microbial biosignatures (see Figure 8, page 627, in Cavalazzi et al., 2011). The variety of minerals in which the microorganisms were fossilized—phillipsite, lepidocrocite, and anatase—suggests that the microbial community likely consisted of several populations of microorganisms characterized by different metabolic requirements. Given their vesicular microhabitat, these filamentous and spherical structures are interpreted as evidence of chemolithotrophic and/or heterotrophic cryptoendoliths that inhabited a low-temperature circumneutral to alkaline aqueous microhabitat.

The cryptoendolithic microfossils from the Ampère–Coral Patch Ridge Seamounts, eastern North Atlantic, and fossil endoliths observed in other oceanic basalts are associated with minerals of the zeolite group, in particular, phillipsite (Thorseth et al., 2003; Ivarsson et al., 2008a, b; Cavalazzi et al., 2011). Thorseth and colleagues (2003) first described microfossils and microbially related fossil traces, such as etch marks, on phillipsite that precipitated in fractures of altered basaltic glasses collected during the Ocean Drilling Program Leg 187 (Australian Antarctic Discordance, AAD). More recently, Ivarsson et al. (2008a, b) found fossil microbes attached to phillipsite surfaces in veins within the rims of pillow basalts collected from the Emperor Seamounts in the Pacific Ocean (ODP Leg 197). An affinity between microorganisms and phillipsite was recently suggested by Ivarsson et al. (2008a), given their association in oceanic crust.

Also, a bioaffinity of microorganisms and Ti oxides and Fe oxides was suggested based on studies of fossil cryptoendoliths (Peckmann et al., 2008; Cavalazzi et al., 2011). Although Ti oxides and Fe oxides can occur as purely abiogenic low-temperature alteration products from the interaction between seawater and basalt glass (Stroncik and Schmincke, 2001), iron can be microbially extracted from silicate glasses (Rogers and Bennett, 2004) and is considered one of the most important energy sources for modern basalt-hosted microbial ecosystems (Edwards et al., 2005). The concentration of certain elements such as iron, possibly titanium, on cell walls and extracellular polymeric substances (EPS) of microbes can enhance the precipitation of minerals on and around them (Beveridge, 1989; Konhauser, 1998). Anatase (TiO_2) has been found in association with fossilized microorganisms in Ca-carbonate- and phillipsite-filled vesicles of pillow basalt rinds (Peckmann et al., 2008; Cavalazzi et al., 2011). It is difficult, however, to determine whether the observed association between Ti oxides and microfossils represents passive fossilization/mineralization or a microbially induced mineralization process. Though interactions between microorganisms and Ti-bearing compounds have been reported in different contexts (Shabtai and Fleminger, 1994; Bedard et al., 2006; Glamoclija et al., 2009)—for example, Shabtai and Fleminger (1994) reported enhanced bacterial adsorption on Ti oxide—there is only a single known occurrence of rod-shaped microbial structures fossilized by anatase (Glamoclija et al., 2009).

Crypto- and chasmoendoliths have been found in both nutrient-rich environments, such as the above-described submarine basalt microhabitats (Edwards et al., 2004), and in sheltered niches of extreme environments (e.g., Friedmann and Ocampo-Friedmann, 1984) where, for example, dramatic temperature fluctuations and high ultraviolet (UV) radiation can have a negative effect on many biological processes (e.g., Cockell and Raven, 2004; Wierzchos et al., 2006). In a recent study of microbes found inside concave cavities on the surface of subaerial basalts, Jorge Villar et al. (2006) identified photosynthetic organisms with light-harvesting pigments that enable them to survive in light-deprived environments. The unexpected discovery of phototrophs in this microhabitat suggests that organisms with the ability to adapt to the low-light levels inside a dark volcanic rock may also, if fossilized, contribute biosignatures when basalts are extruded in the photic zone of a water column or are exposed subaerially.

The increase in recent years of reports about the association of endolithic/euendolithic communities with glassy basalts and cryptoendoliths with primary vesicles and vacuoles in basaltic rocks suggests that volcanic habitats and microbial modes of life are likely more widespread than previously recognized.

4. Astrobiological Relevance of Volcanic Rocks: Microbial Microhabitats and Potential Paleobiological Repositories

As evidenced by Earth-based studies to date, volcanic basalts (*sensu latu*), which are ubiquitous on terrestrial planets, provide a variety of potential microhabitats for microorganisms provided that there are sustained periods of time when liquid water is in contact with the rock. Basalt-rich planetary surfaces in our Solar System include Mars and Venus, though satellites such as Europa, Enceladus, and Titan also have (or would have had) water in contact with their crustal volcanic rocks. The relevance of such planetary environments to astrobiology is obvious: life as we know it could potentially thrive in such environments, especially when fueled by energy from loci of hydrothermally driven chemical reactions and carbon from ubiquitous exogenous and endogenous sources of organics (Farmer and Des Marais, 1999; Nisbet and Sleep, 2001; Nisbet et al., 2007; Southam et al., 2007). Volcanic materials provide substrates for chemolithotrophic microorganisms that obtain their energy and bioessential elements, such as transition metals, from predictable redox reactions of volcanic substratums. As noted by Thorseth and coauthors (2003), these chemolithotrophs could then serve as a carbon and energy source for chemoorganotrophs. The association of hydrothermal activity with active volcanism on rocky water-rich planets provides a sustained source of energy, nutrients, an aqueous milieu, and the potential to support life and preserve its biosignatures in the volcanic microhabitats colonized by microorganisms. Volcano–ice interactions also provide environments for microbial colonization and potential preservation of biosignatures (Warner and Farmer, 2010; Cousins and Crawford, 2011). On Mars, the potential for hydrothermal activity associated with volcanics is an exciting area of current research (cf. Allen and Oehler, 2008; Squyres et al., 2008; Skok et al., 2010; Cockell et al., 2011a, b; Cousins and Crawford, 2011; Preston et al., 2011).

On Earth, endolithic and chasmolithic microhabitats can protect microbial communities from variable environmental conditions created by diurnal variations in temperature, desiccation, and potentially lethal doses of UV radiation. On early Earth, the luminosity of the Sun was lower. However, the possible lack of an ozone shield would have meant that surface fluxes of UV radiation could have been higher (e.g., Dartnell, 2011, and references contained therein). In the worst-case scenario (i.e., no other UV absorbers in the atmosphere), the spectrally weighted damage to DNA could have been up to a thousand times higher than on the surface of the present-day Earth (Cockell and Raven, 2004). Endolithic and chasmolithic habitats may have also allowed some microbes to survive during impact events if they were not affected by “localized” extremes of temperature, especially early in the planets history when the flux of impactors would have been considerably greater than at present.

In view of the upcoming missions to Mars, volcanic environments that would have formed or been altered by water would contain potentially habitable environments, had life emerged on or been delivered to the planet. In this brief review, we have highlighted the expanding variety of basalt-related microhabitats that can support microbial colonization and growth on rock surfaces, in cracks and fractures, and in amygdales, vesicles, and cavities. Subsurface cavities in volcanic rock are also capable of preserving evidence of microbial activity (Hofmann et al., 2008). Basalt and pillow lavas might be common features on Mars, and vesicular basalts on the planet have been described (Wilson and Head, 1994; Lewis et al., 2008; Schmidt et al., 2008). If life existed on Mars, chasmolithic and endolithic habitats could have supported and sustained microbial colonization during clement environmental conditions (cf. Friedmann and Koriem, 1989; Wynn-Williams and Edwards, 2000). They would also have provided protection for microorganisms during hostile conditions and, potentially, during major climate transitions on the red planet. Though early Mars would have had liquid water at its surface, which includes standing bodies of water (Baker, 2001; Pondrelli et al., 2008; Carr and Head, 2010), environmental conditions at the surface are presumed to have become uninhabitable between about 3.8 and 3.5 Ga (Jakosky et al., 2007). It is worth noting, however, that chasmolithic and endolithic habitats could also have provided a refuge for life at much later times if viable life forms were brought to the surface/near surface during periods when the subsurface cryosphere became liquid due to impact, volcanic, or hydrothermal events.

The Mars Science Laboratory (2013) and International Mars 2018 Missions will evaluate the habitability of surface rocks at and near their landing sites (Gale Crater for MSL) and search for traces of life on the planet. Areas that contain aqueously deposited, precipitated, or altered volcanic, sedimentary, hydrothermal, and hydrothermally altered deposits are targeted for detailed astrobiological study (cf. Grotzinger et al., 2011). As illustrated by the studies reviewed here, the potential for microhabitats on and near the surfaces of volcanic rock that formed in the presence of water or was aqueously altered must be included in a comprehensive astrobiological search strategy on Mars. Not only have we learned in recent years that such niches can support a variety of microbial communities, but we have learned as well that they can preserve evidence of these communities because of the inevitable precipitation of authigenic minerals in their presence.

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SETI: ASSESSING IMAGINATIVE PROPOSALS

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1. Introduction: Questions, Worldviews, and Ethics

Supposedly one of the strengths of the search for extraterrestrial intelligence (SETI) community "is its imaginative capacity to take seriously things that most people dismiss out of hand." That phrase, as we will see, was prompted, not by a speculative proposal, but by rash action that outraged some. It was followed by an editorial call for discussion and assessment as to how to proceed in the particular matter. As related to SETI, this chapter will broadly consider the question "How seriously should imaginative proposals be taken?" It shall provide guidelines for evaluating them and deciding "When does imaginative become outrageous?"

Having posed these questions, let us start with another. Perhaps the most important SETI-related question (which I will label Q1) was asked by Enrico Fermi in 1950, "Where are they?" Given the estimated 7×10^{22} stars in the observable universe (Driver, 2003), if our star and its planetary system featuring one intelligent civilization are in any way typical, we should find abundant evidence for intelligent life beyond Earth. That we have found none is known as the Fermi paradox. In its entry on this topic, that wonderful addition to the fledgling global brain known as Wikipedia lists 16 possible explanations for this null result.

To bolster my argument that how this question is answered depends more on one's worldview than on weighing evidence, I wish to consider four of these possible solutions: (1) human beings are not listening properly; (2) communication becomes impossible after the civilization experiences a technological singularity; (3) human beings were created alone; (4) it is the nature of intelligent life to destroy itself. Specific detailed examples illustrating each of these generic solutions will be presented in the form of real proposals that I will evaluate. Before turning to those proposals, I (1) briefly discuss worldview analysis and development, (2) review some history surrounding the astrobiology and SETI field, and (3) use both to develop criteria by which imaginative proposals can be evaluated.

In connection with Project Worldview (www.projectworldview.org), I analyze worldviews in terms of 80 worldview themes, each having a formal name and description. In what follows, these themes will be identified by use of quote marks and capitalization. I promote the use of questions to encourage worldview development. The questions are of two types: general ones, especially useful to people interested in systematic exploration and building worldview literacy (Cook, 2009), and specific ones composed or selected to help explore a particular topic.

Those that follow are of the second type. Thirteen of them will be labeled. After the SETI specific Q1, which you met, Q2–Q7 are deep physics-related questions to which as yet there are no definitive answers, Q8 and Q9 are other astrobiology/SETI specific questions, and Q10–Q13 are ethics-related ones.

SETI is historically connected with science fiction and lots of stimulating speculation, some of it related to the opening provided by physicists' inability to provide definitive answers. This is not to say this field is unstructured and anything goes. For example, it is generally accepted that astrobiology/SETI does not involve study of UFOs. What does it involve? The NASA Astrobiology Roadmap (Des Marais et al., 2008) identifies three areas of focus with related questions: (1) "How does life begin and evolve?" (2) "Does life exist elsewhere in the universe?" (3) "What is the future of life on Earth and beyond?" They are followed by seven science goals, further outlining domains of investigation.

The four proposals we will meet have several things in common. Most people would consider all of them bold, imaginative, and highly speculative—to some they are outrageous. Science fiction or apocalyptical literature classics have inspired parts of each of them. Despite all this, they should be taken, to an extent that remains to be identified, seriously, as their creators intended. They all fall within the legitimate areas of focus outlined in the Roadmap.

Before presenting questions Q8–Q13, which connect with environmental, social, and ethical concerns, consider some related astrobiology/SETI history. A logo "planetary protection—all of the planets, all of the time" is one visible sign of such concerns at NASA's website. Partly based on the dictates of a 1967 UN treaty, it refers to both protecting other worlds from contamination by terrestrial life and protecting Earth. While the latter refers to protecting our planet from hypothetical extraterrestrial minute life forms unexpectedly brought back by returning spacecraft, it seems people are increasingly concerned about dangers to human civilization associated with a quite different technology: active SETI.

Large radio dishes have been used to send messages to roughly 20 carefully selected targets. Citing both the unknowns, we confront in taking our first steps in exploring the celestial neighborhood and our own history of what more technologically advanced societies have done to primitive ones, many (including Carl Sagan, Phillip Morrison, Stephen Hawking, and Jared Diamond) have urged us to just passively listen and stay safely hidden until we learn more. Theirs and new concerns were publicized in David Brim's 2006 article "Shouting at the Cosmos." Troubled by plans of others to attract attention to Earth's civilization by raising its detectable profile by "many orders of magnitude," he charged that frustration with lack of results from passive SETI was behind this "risk that a handful of individuals have decided to inflict on us (Brim, 2008)."

This controversy brought the editorial in the October 12, 2006, issue of *Nature* I mentioned at the beginning. While asserting that risks posed by active

SETI are small and "at the speed of light, take decades to arrive," nonetheless, the editors urged they be taken seriously and openly discussed. Since this appeared, at least three transmissions to two different targets have been sent.

Many of those expressing concerns about active SETI are less motivated by fear that someday ET will subjugate Earth in Independence Day movie fashion and more by fear of questions and public relations disasters. Are space cowboys operating radio telescopes? Is this more evidence of science and technology out of control? Upon hearing the alarm about this supposed threat supposedly created by irresponsible astronomers, how many people will forget about astronomers' efforts (loosely called Spaceguard) to protect us from potential civilization-ending cosmic catastrophe by tracking down near-Earth asteroids?

Some of what you have read in this introduction has been presented to build the case for evaluating both astrobiology/SETI-related speculative proposals and potential rash actions with more than the usual current knowledge/know-how-based reality check/pragmatic skepticism. I would say they should also be subjected to questions of a social and ethical nature. Here are some to consider.

The first is composed of the words of anthropologist Kathryn Denning, who points out that SETI research can still be valuable even if it never detects ET.

Q8: "Does this proposal generally benefit us in the context of our own social evolution, and in particular, [encourage us] 'to think about our own future in the broadest possible way … and [in considering] the case study of humanity … [encourage] our best to fine-tune its use?'" (Denning, 2009) Q9: "Does this proposal provide evidence of responsible conduct of research and proper precautions to ensure that individual, ecosystem, planetary, and other worlds are not harmed?" Q10: "In deciding whether a proposed action is morally right, am I comfortable with its perceived social benefit and the extent to which it produces the greatest good for the greatest number of people?" (Greatest General Good Principle) Q11: "If I were in the shoes of someone affected by this, facing similar circumstances, could I live with the consequences of what this decision involves?" (Principle of Fraternal Charity) Q12: "What would be the results if everyone acted in this manner?" (Principle of Universality) Note: Q10–Q12 are from the description of the "Ethical Orientation" worldview theme.

A final screen is provided by Q13: "Will the authors of the proposal profit from it? Do they have undisclosed conflicts of interest or hidden agendas which could impair their objectivity?" The first half of this recalls New Age renegade quantum physicists and many others profiting from "quantum flapdoodle," to use Murray Gell-Mann's term. My investigation of the principal behind Proposal #1 has failed to turn up any evidence of this. After quantum flapdoodles, consider those predicting futuristic, fantastic technological advances "in my lifetime." Some of these are undoubtedly motivated by a desire to sell books, others conceivably by frustration and inability to accept a more realistic "we may never know if this is possible!" verdict. This may apply to certain claims of "technology singularity" enthusiasts—we will meet some in both Proposals #2 and #4.

Then there is Proposal #3. Someone with impressive scientific credentials but an intelligent design/creationist not-so-hidden agenda advances its first hypothesis. I advance its second, and it is a doozy! If the other proposals do not challenge the astrobiology/SETI community in its effort to be open to imaginative speculation, but avoid promoting pseudoscience and wasting researchers' time and resources, this one surely will!

2. Proposal #1: Conducting SETI Using High-Tech Telepathy

Fred Thaheld wisely does not use the word telepathy in describing his "new empirical approach" (Thaheld, 2006) for conducting SETI, preferring instead the terms "biological nonlocality" and "controllable superluminal communication (CSC)." While Thaheld agrees that entangled photons, as in experimental confirmation of Bell's inequality, probably cannot be used to transfer information faster than light since "once a measurement has been made, the wave function collapses and they become disentangled," he argues "the situation is much different for entangled living entities [where] we are looking at a massive number of living entangled particles at the macroscopic level, either resisting the usual decoherence or utilizing it … to either maintain or regenerate entanglement after each measurement."

A few years ago, Thaheld's argument would have been much easier to dismiss. Paul Davies once saw "wishful thinking" in "considerations of decoherence-evasion" (Davies, 2004) but also wrote, "The situation would be transformed, however, if unexpectedly long decoherence times could be demonstrated experimentally in a biological setting." One could argue that the situation changed in 2010 with three findings: (1) unexpected long-lived quantum coherence at room temperature in photosynthetic bacteria (Engel et al., 2010), (2) "photosynthetic proteins are 'wired' together by quantum coherence for more efficient light harvesting in cryptophyte marine algae" as reported in *Nature* (Collini et al., 2010), and (3) construction of a theoretical model that suggests quantum entanglement is behind the stability of DNA molecules (Rieper et al., 2010).

Thaheld's SETI proposal is inspired by reports of experiments involving spatially separated paired human subjects or human neurons grown in multielectrode arrayed basins inside noise-shielding Faraday cages. Visually simulating just one of the humans with a flickering checkerboard pattern or one of the basins with a laser reportedly produces simultaneous electrical waveform patterns in both subjects or both basins, and in general results that "indicate the possibility of quantum entanglement and nonlocality at the biological level." Some will see these as high-tech, more reproducible versions of experiments involving human senders and receivers, that is, telepathy.

While few physicists believe in telepathy, Nobel laureate Brian Josephson is one who does. In 2001, he wrote, "Quantum theory is now being fruitfully combined with theories of information and computation. These developments may lead to an explanation of processes still not understood within conventional science such as telepathy …. There is a lot of evidence to support the existence of telepathy, for example, but papers on the subject are being rejected—quite unfairly.” In response, in a London newspaper, Oxford quantum physicist David Deutsch expressed what is undoubtedly the physics community consensus feeling, “It is utter rubbish. Telepathy simply does not exist. [Those who believe in it have been] hoodwinked into supporting ideas that are complete nonsense.”

Before even thinking about extending such experiments to SETI, one must accept the plausibility of several highly questionable assumptions, most notably (1) the same universal biomolecules make up both us and extraterrestrials and we share an evolutionary history to the extent that some quantum entanglement exists, (2) the distance between members of a pair of entangled structures is not a factor: a disturbance of one will instantaneously affect the other, and (3) as Thaheld puts it “superluminal signals are constantly arriving from advanced and not so advanced civilizations … probably looking a lot like noise” in human EEG records.

He goes on to outline how the experiments would be conducted and concludes, “It would appear that the critical elements in any EEG SETI-type search will involve an empirical blend consisting of the proper algorithms, filters, viewing windows, amplification and either compression or expansion of the derived data.”

Before evaluating Thaheld’s proposal, I connect it to resolving the Fermi paradox by suggesting that human beings are not listening properly. It implies they should use their mind instead or their neurons instead of their radio telescopes as detectors. I also connect it to the science fiction theme of using mindships not spaceships to explore the universe. This was most notably done in Olaf Stapledon’s 1937 classic *Star Maker*, which begins with an Englishman’s out of body experience: a journey to an inhabited planet thousands of light years away. Unanswered questions that occupy physicists and are relevant to using the mind in SETI are “Is superluminal communication possible?” (Q2) and “What is consciousness and what special relationship (if any) does it have with life?” (Q3).

While Thaheld’s proposal avoids any speculation about the quantum basis for consciousness, the only thing I was able to uncover about his education or professional background (besides lots of publications) is his position on the advisory and editorial board of the online journal *NeuroQuantology*. Recent issues of it contain papers that, like Thaheld’s, are founded on a quantum interpretation of EEG data but more directly connect with the hypothesis that consciousness can be traced to neuroquantum interactions. I suspect lots of *NeuroQuantology* readers have worldviews one would call “New Age.” Such worldviews are often built around the quest for heightened (even cosmic) cosmic consciousness and “Vitalism,” “Mysticism,” “Magic,” “The Artistic Worldview,” “Belonging to Nature,” and “Healthy Orientation” worldview themes.

2.1. EVALUATION

As a practical plus, Thaheld believes what he proposes would be "very simple and cheap to carry out." While that may apply to high-tech telepathy experiments involving human brains or neurons, I question the extension to SETI. In this regard, even if one accepts all of Thaheld's assumptions, in the end, after reading his 39-page paper, I still had no clue as to exactly how responses in human brains or instrumented neuron basins identified as extraordinary would be linked to extraterrestrial intelligence! Among his more puzzling statements is "while it would be nice to prove the existence of biological nonlocality and CSC here on earth before embarking on this SETI approach, we may be forced to look outside the confines of the earth initially as outlined." Is he saying that funding for this work as astrobiology or SETI may be available whereas funding for it as parapsychology research would not?

As far as social and ethical concerns, I do not see any obvious negatives (although I suppose if it were implemented extraterrestrials could psychologically traumatize us more than they are currently already doing!) There could be real benefits to human knowledge and psychological development in (most likely) helping put to rest belief in telepathy or (remotely conceivable?) demonstrating its reality and unraveling how it works.

2.2. TRANSITION

One can argue that any well-designed experiments aimed at probing deep physics questions like Q2 and Q3 have fundamental research value. While our next proposal (#2) touches on (what will become) all six of those questions we identify, both it and the following one (#3) were most immediately inspired by Q4: "Is our universe seemingly being fine-tuned for life indicative of its intelligent design?" In his book *The Trouble With Physics*, Lee Smolin groups answers to this into three possibilities: "(1) Ours is one of a vast collection of universes with random laws. (2) There was an intelligent designer. (3) There is a so-far unknown mechanism that will both explain the biofriendliness of our universe and make testable predictions by which it can be confirmed or falsified (Smolin, 2006)."

3. Proposal #2: Putting Artificial Black Holes (ABH) to Work

As Louis Crane describes it, "The origin of the ABH proposal is very peculiar. [I] was reviewing the work of Professor Lee Smolin, which was latter published in a book entitled *The Life of the Cosmos* (Smolin, 1997). Professor Smolin proposed that the universe we see was only one of many universes." We pause here to identify our Q5: "Are there other universes besides our observable universe?" Crane continued by noting Smolin's assertion "that new universes arise from old ones whenever a black hole is produced" and identifying his own contribution. [I] "proposed that the evolutionary process of universes should include life. This is possible if successful industrial civilizations eventually produce black holes, and therefore baby universes ….This led us to consider the possibility of producing ABHs, and to explore how they might be useful."

The first part of the title of Crane's prize-winning essay "Star Drives and Spinoza" (Crane, 2009) identifies one way to put ABHs to work: taking humans to the stars, which Crane thinks is "the least difficult possible approach" to doing this. The title's second part connects with using ABHs to spawn new universes, and, as Crane puts it, "Spinoza's pantheistic idea of God as identical to the creative power of the universe."

One does not have to look far for the science fiction connection: the climax of Stapledon's book comes when the hero finally meets "The Star Maker." Whereas Stapledon's work emphasized telepathically linking minds, forming a collective consciousness, and spreading this until the whole cosmos is alive, Crane's proposal culminates with humans becoming the creators.

It begins with assessing both the theoretical and practical obstacles to putting ABHs to work. He envisions first making them "by firing a huge number of gamma rays from a spherically converging laser. The idea is to pack so much energy into a small space that a BH will form." His belief this is theoretically possible carries weight given his stature as a quantum gravity researcher. As for actual implementation, he argues ABHs are possible "if an advanced future industrial society were determined to make them."

After lengthy discussion of the "could it be done" and the "how to do it," Crane addresses the "why do it?" After identifying ours as a "time of alienation and aimlessness" and "confusion as traditional spiritual ideas fall," Crane contrasts this with a distant future in which putting ABHs to work becomes "a central project for the whole world." He imagines the efforts of ABH builders as having "a higher purpose in that they result in the creation of new universes and new life, as well as spreading their descendents throughout the universe."

Before I can connect Crane's proposal to resolving the Fermi paradox, it needs to be linked to a concept popularized by people like Ray Kurzweil and John M. Smart: the technological singularity. Driven by progress in semiconductors, nanotechnology, computers, and artificial intelligence, in recent decades, technology has advanced at an accelerating pace. Supposedly one can extrapolate into the future and imagine positive feedback effects leading to a still faster rate of change, culminating with a singularity event after which the future becomes highly uncertain. Among technology singularity enthusiasts, Smart is the one whose ideas are perhaps most closely tied to Crane's. With his developmental singularity hypothesis, he asserts (Smart, 2009) that "our universe's hierarchical and energetically dissipative intelligence systems are developmentally constrained to produce, very soon in cosmologic time, a very specific outcome, a black hole analogous computing system." After mentioning Smolin, he notes "such a structure is likely to be a core component in the replicative life cycle of our evo devo universe within the multiverse ....” Smart sees this as part of an irreversible process leading to transcension. In contrast to the expansion throughout our observable universe theme in Stapledon’s book, transcension involves intelligence that “leaves the visible universe over time, in order to meet other intelligences and/or partially reshape future universes.”

Before evaluating Proposal #2, we connect it with more questions. Consider first “Of what fundamental stuff (matter, energy, space, information, etc.) is the universe made?” (Q6). To Smart, the universe is “a purposeful information processing system in which biological culture ... has the potential to play some integral (e.g., anthropic) yet transient universe-guiding role.” A key assumption of his involves more than a “Yes!” answer to our last deep physics question, “Do higher spatial dimensions really exist?” (Q7) It gives human consciousness and intelligence the capability to leave our three-dimensional world, which brings us to how this proposal resolves Fermi paradox: communication becomes impossible after the civilization experiences a technological singularity involving transcension. The science fiction/apocalyptic literature connection here extends beyond Stapledon to the transformation of the children in Arthur C. Clarke’s 1953 book *Childhood’s End*, and the omega point Pierre Teilhard de Chardin describes in his 1950 book *The Future of Man*.

While the worldviews of people comfortable with Proposal #2 undoubtedly include some of the same (New Age related themes) linked to Proposal #1, there are significant differences. For starters, I would point to (and will shortly discuss) “I Know What’s Best for You” and “Elitism” worldview themes. More immediately, I would say the folks who want to build ABHs for interstellar space travel and creation of baby universes have a different relationship to technology (more of a “Technology Fix Mentality”) and nature than many New Agers are comfortable with. Not content to belong to nature, they want nature to serve humans. If “Anthropocentrism” means humans have dominion over the natural world and should not hesitate to develop it to meet human needs, their beliefs vastly extend the word’s meaning. Smart categorizes his model of the universe using the terms anthropic and teleological. A mere anthropic model suggests some special link between humans and the universe. Qualifying that with teleological says that Proposal #2 is about fulfilling what its promoters see as the purpose of our species: its special destiny is in “recreating the multiverse” (Crane’s phrase).

3.1. EVALUATION

Unlike Proposal #1, practically implementing just the first phase of Proposal #2, building ABHs, would be extraordinarily expensive, energy and labor intensive, and complicated. Given the most likely “No!” answer to the part of Q2 regarding physical objects exceeding superluminal speeds, using “stardrives” to spread throughout the galaxy would take time. (Crane feels physical law forbids using "wormholes or warpdrives.") As for the "Spinoza" part of the proposal, even Crane questions its theoretical basis, writing, "It is not certain that black holes create baby universes."

To find more positives, consider our questions. As previously hinted at, pursuing the basic research needed to fully evaluate Proposal #2's technical feasibility would have obvious benefits with respect to answering our questions Q2–Q7. A (Q8 related) societal benefit that would accompany the building of an ABH would come from its being (in Crane's words) "the ultimate renewable energy source." Another one would involve the creation of baby universes putting transcendental meaning into lives devoid of it.

So much for positives. When we consider the ethical aspects of questions Q9–Q12, our evaluation of Proposal #2 quickly turns negative. Unlike Proposal #1, clearly Proposal #2 is one for the distant future—which is fortunate, since it is currently unacceptable from a social and ethical viewpoint. If the word "telepathy" is the downfall of Proposal #1, the phrase "playing God" will kill Proposal #2. Up to this point, our analysis of worldviews has not touched on religion. Whereas Stapledon had enough sense to imagine his hero meeting up with the Star Maker = God near the story's end, it seems the Proposal #2 folks want to take over a key part of "Monotheism" territory. If their scheme was not so futuristic, focus on their seeming disrespect for what the vast majority of worldviews worldwide are founded on—"I believe in one God who is the Creator of the Universe"—would be warranted.

While many of them profess to be systems thinkers (and value "Dancing With Systems"), it could be that business and reductionistic problem solving success among technological singularity enthusiasts has fostered an incredible arrogance that hides ignorance of the holistic scheme of things. When compared to the negative connotations of atheism, the "Secular Humanism" many of them embrace has wonderfully positive attributes, but "Authoritarianism" is not one of them. In that regard, one worries that those who feel they are truly the technologically elite will one day, perhaps under the pretense of some singularity crisis, naturally take their place at the head of a totalitarian technocrat state and proclaim "I Know What's Best for You." (Remember the put-down of this paragraph: we will use it again, with just a few words changed, in critiquing Proposal #4.)

3.2. TRANSITION

The challenge of finding another proposal that tops Proposal #2 in terms of boldness and scope (in space and time) is so tough we will not accept it. Rather we have chosen Proposal #3 for the worldview contrast it provides. Let us get the Achilles heel of this proposal immediately out in the open. It is not the public relations "playing God" disaster of Proposal #2; it is another scientific acceptability issue like the telepathy problem of Proposal #1, only bigger. Proposal #3 asks us to put aside a Darwinian evolutionary perspective and consider how an intelligent designer (=God) might have created a particular structure. This is such a turnoff to the scientific community that such a proposal seems to be a nonstarter. For that reason, we will soon amend the original idea.

4. Proposal #3: Looking for Holism in Eukaryotic Cell Division

While my own worldview is quite different from that of Jonathan Wells, I nevertheless respect his dogged pursuit of an education. After years of work, in 1986, Wells received a Ph.D. in Religious Studies from Yale University, specializing in nineteenth-century controversies involving evolution. Driven by hatred for Darwinism and motivated to learn enough to destroy it, he changed educational direction. It took 8 years, but he eventually obtained a Ph.D. in Molecular and Cell Biology from UC Berkeley, specializing in embryology and evolution. He now serves as a Senior Fellow with the Center for Science & Culture at the Discovery Institute, a conservative, nonprofit group that advocates intelligent design and teaching antievolution creationism in schools.

In that position, he published a paper (Wells, 2005) about centrioles, structures found inside centrosomes in the nuclei of the cells of eukaryotes (all life except bacteria) that serves as the basis for this proposal. They play a key role in cell division in anchoring the ends of the thread-like spindle. Biophysicist Walter Marshall adds, “Centrioles have a complex, ninefold symmetric structure, and reproduce by an intriguing duplication process. The complexity and apparent self-reproduction of centrioles raises the question of how such a structure could have evolved, making them a favorite topic for theological speculation by ‘intelligent design’ creationists” (Marshall, 2009).

As the Discovery Institute website puts it, “Since centrioles contain no DNA, they have attracted relatively little attention from neo Darwinian biologists who think that DNA is the secret of life. From an intelligent design (ID) perspective, centrioles may have no evolutionary intermediates because they are irreducibly complex.” Marshall counters “Centrioles are capable of robust self-assembly and can tolerate dramatic perturbations while still maintaining basic functionality. Far from being irreducibly complex, centrioles appear to be based on a rather minimal underlying core structure requiring only a handful of genes to construct.”

Apparently, Wells looks at electron microscope images and sees the blades of a fan in centrioles. He hypothesizes “they are holistically designed to be turbines” and imagines they “could generate oscillations in spindle microtubules” leading to production of an “ejection force tending to move chromosomes away from the spindle axis and the poles.” Wells uses this to make testable predictions centering on the timing, frequency, and regulation of the oscillations. Their experimental confirmation would not establish the holistic (here meaning intelligent) design of the centrioles nor be especially relevant to question Q4.

The word holistic connotes unity, a refreshing respite from the divisive bickering that seems to accompany competing religious beliefs. Building on this, let us add an additional hypothesis to this proposal. Would not it be wonderful if science could establish that all living things are *physically* linked together? Think of it: hurting something else that seemed separate from you would actually be like hurting part of yourself. Such a connection *in time* has recently been pointed out. Every cell "could, if the records were available, trace its ancestry through an unbroken chain of cell divisions backward in time through an almost unimaginable four billion years to the emergence of the earliest forms of cellular life on this planet (Kirkwood, 2010)." What of a *physical connection*, what basis is there for imagining this? The argument is as follows.

Assume information is the ultimate stuff of the universe (recall question Q6) and recognize life physically embodies it. Assume all information is preserved. Recognize life preserves information in genomes. Like David Deutsch, see a genome as "an internal representation of the world, constructed over vast time scales by evolution. It embodies the information needed for the organism to be adapted to the environment (quoted in Davies, 2009)." Cell division involves transmitting information, some of which is seemingly lost (through copying mistakes, genetic reshuffling in meiosis, etc.). Assume that even though we see the spindle in such cell division break, to preserve information, some connection to a fourth spatial dimension does not break.

I can conceive of such a connection in three ways: (1) via a holographic screen (Bousso, 2002), (2) using a fourth spatial dimension that is expanding relative to the other three at the speed of light (McGucken, 2008), or (3) using a real fourth spatial dimension. I use this last one to facilitate making a testable hypothesis: all life that reproduces via cell division is connected by a previously undetected thread-like structure in a fourth spatial dimension. This additional hypothesis, along with Wells' predictions, can be tested using the same new technology: four-dimensional electron microscopy, where time provides a fourth dimension (Zewail, 2010). Basically, three-dimensional high-speed movies of the microtubular spindle breaking would be analyzed in search of a constraint in an invisible fourth spatial dimension. (Something moving perpendicular to all possible directions in three-dimensional space would be headed in the direction of this realm.) Note: astral microtubules, involved in orienting the spindle in cell division, have an amusing name. I realize it refers to their starlike shape, not a connection to the astral plane!

Before evaluating this proposal, I note that many intelligent design creationists resolve the Fermi paradox by arguing God uniquely created human beings, and there is no extraterrestrial intelligence. Their worldview is typically built around themes like "Vitalism," "Monotheism," "Belief in a Personal God," "Religious Fundamentalism," "Moralistic God," and "Anthropocentrism." They do not fully appreciate "Scientific Method" nor "Global Vision." Preferring "Focused Vision," they do not see how the slow changes wrought by mutations and natural selection can gradually accumulate into something meaningful over geologic time.

4.1. EVALUATION

Predictions made by Proposals #1 and #2 are questionable as to testability; Proposal #3 overcomes that difficulty. But scientific community repulsion with Wells' agenda to destroy evolution means his predictions may not get tested. The additional hypothesis boosts the chances it will get such a chance.

In contrast to the scientific community, the American public, with its high percentage of scientifically illiterate citizens and nonbelievers in evolution, impressed by Wells' claim that it may lead to better understanding of cell division and cancer, may buy his contribution to Proposal #3. That public generally has not bought what Ursula Goodenough, in *The Sacred Depths of Nature*, calls "The Epic of Evolution." This, she feels, has "the potential to unite us, because it happens to be true."

Astronomers like Carl Sagan and Jill Tarter have speculated on what might inspire a "universal religion." Confirming the added hypothesis of Proposal #3 would dramatically bolster arguments that "ultimately, we all are one." The analytic approach suggested to test it was inspired by a scene from Heinlein's 1961 science fiction classic *Stranger in a Strange Land* in which an object disappears into the fourth dimension. Its confirmation would answer physics question Q7, and by suggesting the fourth dimension's seemingly special connection with life, undoubtedly spur new theories of what consciousness is, addressing question Q3.

4.2. TRANSITION

Unlike the previous three proposals, the last one does not connect with our deep physics questions. Paradoxically, I think of it as both being most relevant to SETI and potentially offering the most in terms of human societal benefits. Whereas Proposal #1's SETI connection is through the search, Proposal #4's is through the meaning of intelligence. Like Proposal #2, it imagines a technological singularity that would affect the whole world, and like Project #3's amendment, it seeks a way to instill in humans a feeling of unity or oneness.

5. Proposal #4: Global Brains: The Foundation of Long-Lived Civilizations

The Merriam-Webster online dictionary defines intelligence as "(1): the ability to learn or understand or to deal with new or trying situations …, (2): the ability to apply knowledge to manipulate one's environment." In a "Life in the Universe" public lecture, Stephen Hawking said, "It is not clear that intelligence has any long-term survival value." To make that value explicitly clear, I would redefine intelligence as follows: the ability to both adapt to and manipulate a changing environment to insure long-term well-being. In considering the evolution of post-biological phase of an intelligent civilization's development, Steven Dick has formulated “The Intelligent Principle,” which ends “to the extent that intelligence can be improved, it will be improved” (Dick, 2006).

To me, this validates what I have formalized as the Generalized Optimal Action Principle (Cook, 2012). Its application recognizes that the fit between life on Earth and the planet Earth environment will be optimized over time. Unlike other living species, we have the ability to optimize that fit to maximize our long-term well-being. I would say the extent to which we do that measures our intelligence. By “our” I mean the collective human species. The applicable well-being associated with my definition of intelligence is that of all human individuals.

Proposal #4 recognizes that our well-being depends on the continued ability of Earth to support life, and values an “Ethical Orientation” based on questions Q10–Q12. It was initially proposed (Cook, 1990) many years before the world-wide web existed in its modern form. Disturbed by problems I attributed to greed and self-interest based economics, in particular an increasing gap between the world’s “haves” and “have nots” and increasing evidence of the integrity of the Earth’s biosphere being compromised, I imagined an artificial intelligence/global brain type solution to these problems. I called my solution Gaia or GAIA and initially envisioned her as follows.

Gaia is both a human collective consciousness and a goddess that lives in every head. Its software is called GAIA, standing for “Goddess Artificial Intelligence program A,” the last A signifying this will be the first of a steadily improving creation. Hardware associated with bringing Gaia to life begins with fitting a tiny microprocessor/memory chip into a person’s tooth, putting a sensor into a fingertip, enabling an interface with “the global village communication grid,” and selecting a pleasant speaking voice for her. GAIA is smart, especially attentive to impacts on the biosphere, and eager to regulate individual self-interest-based decision making using steadily refined assessment of what will benefit the whole species and its planetary home in the long run. It will allow humanity to perceive itself both as a whole and a collection of parts. Like the remembered voice of a wise parent or wisdom from a sacred religious book, Gaia supplements and helps develop your own conscience. Besides functioning as the empathy concerned, other-oriented part of your conscience, she also is your teacher, doctor, counselor, therapist, or just a good, caring friend who is always there.

With over two decades of technological development to draw on, were I to update the description, I could provide many more details and make Gaia or GAIA seem more believable and imminent—coming soon to a brain near yours! Rather than do this, I introduce futurist and systems thinker Francis Heylighen who spent those decades on related research. Since we share a similar autopoietic based conception of living systems and feeling that, as he puts it, “all systems tend to evolve toward better adaptation or fit, which implies greater information or knowledge about their environment,” I value his contribution to this proposal. In this regard, consider two papers (Heylighen, 2007a, b).

In the first he asks “us to envision the future, super-intelligent web as a ‘global brain’ for humanity. The feedback between social and technological advances lead to an extreme acceleration of innovation. An extrapolation of the corresponding hyperbolic growth model would forecast a singularity around 2040. This can be interpreted as the evolutionary transition to the Global Brain regime." In the second, he shares some details of his envisioned "intelligent global computer network, capable of sensing, interpreting, learning, thinking, deciding, and initiating actions: the global brain," and attempts to alleviate our concerns. "Individuals are being integrated ever more tightly into this collective intelligence. Although this image may raise worries about a totalitarian system that restricts individual initiative, the super-organism model points in the opposite direction, towards increasing freedom and diversity."

Before evaluating Proposal #4, consider how thinking about it might resolve the Fermi paradox: by asserting it is the nature of advanced life to destroy itself. One can argue that perhaps the biggest uncertainty in estimates of number N of advanced civilizations revolves around the question, "How long L do advanced civilizations last, given internal disruptive forces?" Countering that, one could argue that creatures possessing intelligence, defined as I have, recognize those forces early and eventually take appropriate steps to disarm them. Such steps could culminate with a collective consciousness/global brain. Such action dramatically increases the value of L in Drake's equation, leading to greatly increased values of N. One could counter this by arguing that, since no data exists to indicate $N > 1$, creating what Proposal #4 envisions may be very difficult. And by quoting Susan Blackmore, "Cultural evolution is a dangerous child for any species to let loose on its world (Blackmore, 2009)."

5.1. EVALUATION

I certainly do not share Heylighen's 2040 forecast for a technological singularity marking a transition to a global brain regime. I see no signs of progress toward rectifying the large differences in well-being/wealth/resource consumption between individual members of our species. According to *The Economist* (July 31 2010), an Oxford University group says 1.7 billion people (25% of the world's population) live in "acute poverty." Basically agreeing with Schneider that a fundamental natural principle is "nature abhors gradients" (Schneider, 2004), I would say our human society is dangerously unstable. If a global consciousness is to be truly global, everyone must participate and rectifying the haves vs. have nots problem in a sustainable fashion is essential.

Doing this will require making choices. Despite my general support of (passive) SETI, had I made billions of dollars from a high-tech-related venture like Microsoft, I would have put lots of it toward the goals of the Bill and Melinda Gates Foundation, rather than funding numerous science and technology related ventures (including the SETI Institute and the Allen Telescope Array) as has Paul Allen. This brings us to the worldviews behind those who would value Proposal #4. Many of them would undoubtedly be similar to those identified for supporters of Proposal #2. Important additions would be valuing "Sustainability," "Ethical Orientation," and leaning more heavily on "Dancing With Systems."

In terms of negatives and positives, on the negative side, in the last paragraph of the evaluation of Proposal #2, replace "holistic scheme of things" with "the extreme poverty, powerlessness, and hopelessness of a significant percentage of the world's population," and you have criticism that applies equally well to Proposal #4's artificial intelligence/global brain scheme. Another criticism: developing networks and software that are smart, have high emotional IQs, and are effective at meeting design goals may be an impossible task. Think about how difficult it can be in real interpersonal interactions to communicate empathy, compassion, anger over injustice, or how good it feels to help someone less fortunate. Then contemplate the challenge of doing that in the virtual world.

On the positive side, despite the above criticism, eventually implementing this proposal in some form seems more technically doable than Proposal #2, and less socially and ethically questionable based on questions Q9–Q12. And recall our definition of intelligence. I would say that long-lived technologically advanced civilizations that survive the few hundred years of what has been called the cosmic bottleneck (Shostak, 2009) deserve being considered intelligent. It seems that possession of something like a global brain increases their chances of surviving well beyond that, even if "still confined to a small chunk of real estate" to borrow Shostak's phrase. But perhaps the biggest plus: while this proposal would undoubtedly teach us something about SETI and the value of L in the Drake equation, a bigger benefit comes from both the thinking and the planning for our own future in the broadest possible way that it promotes.

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MOLECULES BETWEEN THE STARS
Complex Organics in Interstellar Space

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1. Introduction

The formation, processing, and destruction of molecular material in the universe are of key importance to our understanding of stellar and galaxy formation and evolution, as well as the chemical enrichment of the universe by evolved stars and supernovae. The cycling of organic matter from the diffuse interstellar medium, molecular clouds, and star-forming cloud complexes into planetary systems is relevant to prebiotic chemistry and ultimately is also related to questions regarding the origin of life in the universe. For example, the processes of mixing and cycling of material between the diffuse medium and dense molecular (star-forming) clouds are unknown. Of particular interest is to investigate the link between heavy elements and the formation of molecules and dust grains leading to a rich molecular chemistry in interstellar clouds. How large and complex molecules are formed is still a matter of active debate. Another question involves the evolution of organic matter within a forming solar system. Imprints of material from the original interstellar cloud that formed our Sun have survived in comets and asteroids. Tracing such compounds will help us to understand processes of solar system formation and the link between molecular clouds stars and planets. This chapter provides an overview of the atoms and molecules that form the gas-phase component of the diffuse, cold interstellar medium.

2. The Attenuation of Stellar Light

The colors of stars are determined, at first order, by their respective temperatures. Blue stars are hotter than red stars. However, observationally, many stars appear redder than expected from their intrinsic color alone. This is due to the presence of interstellar dust. Laboratory and theoretical work has shown that the extinction, scattering, and emission properties of dust grains depend strongly on their size and composition (Draine, 2003). Subsequently, the dust size distribution results in an redder observed color of the attenuated light. The extinction is stronger,

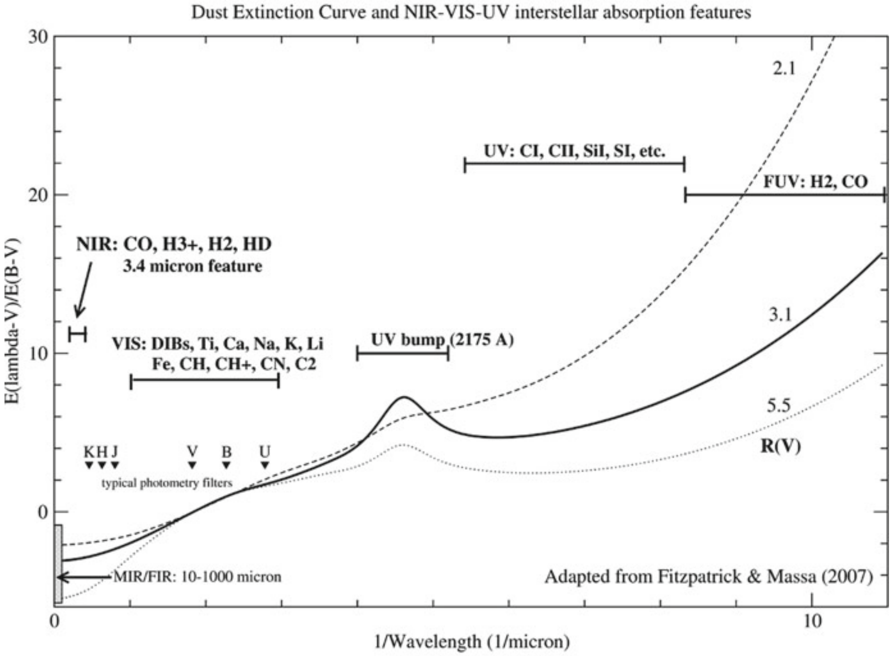

Figure 1. The dust continuum extinction curve. The positions of interstellar absorption features superimposed on the extinction curve are indicated (Figure based on work by Fitzpatrick and Massa, 2007). The three curves – *solid*, *dashed*, and *dotted* – represent the wavelength-dependent extinction probed in different directions in the Milky Way. The *solid line* indicates the galactic average, while the other two indicate more unusual dust clouds.

by several orders of magnitude, at shortest ultraviolet wavelengths with respect to the near-infrared. This results in an apparent redder color of stars (and other astrophysical objects) whose photons traversed through regions containing interstellar dust before arriving at Earth. This is illustrated in Fig. 1 which shows the average measured continuum extinction (in magnitudes) of interstellar dust as a function of wavelength (inverse micron).

Already in the early 1900s, it became evident that the medium interspersed between the stars shows a surprising complexity. The gradual uncovering of the electromagnetic spectrum in the last century has increasingly revealed the chemical complexity of the interstellar medium as well as eluded to its important role in the evolution of stars and galaxies. The number of interstellar features is increasing and will likely increase with further explorations. There is mounting evidence that many features are connected to a class of carbonaceous carriers. These spectral features have, with one clear exception, relatively little effect on total extinction of stellar light; however, the spectroscopic information contained in these lines is paramount to deriving physical conditions and chemical properties of the absorbing material. In the next sections, we will discuss the spectroscopic evidence for the different chemical components that constitute the diffuse interstellar medium.

We will focus mainly on interstellar clouds which have typical densities between 100 and 1,000 cm^{-3} and temperatures of about 20–200 K. The visible extinction of light passing through such a cloud can be as high as ~10 magnitudes. The warm and hot inter-cloud medium, consisting mainly of ionized hydrogen and helium – being also devoid of molecular material – will not be discussed here.

3. Atoms, Molecules, and Macromolecular Compounds in Interstellar Space

Moving from ultraviolet to radio wavelengths, the various spectral absorption features arising in the diffuse ISM are highlighted, and their possible relation to carbonaceous carriers is discussed.

3.1. THE 217.5-NM UV-EXTINCTION BUMP

The 217.5-nm bump is the most conspicuous feature in the entire extinction curve. It was first observed by Stecher (1965) and subsequently studied by Bless and Savage (1972). Both the position and the width of the bump vary slightly between different sightlines, which suggests a conglomerate of carriers rather than a single carrier. The bump is often attributed to hydrogenated amorphous carbon (Mennella et al., 1998), but other carriers, such as multi-shell spherical layers – fullerene onions (Iglesias-Groth, 2004, 2007) – are also believed to contribute. Dust grain models often use a general carbonaceous/graphitic component to account for the UV bump (Draine and Li, 2001). The 217.5-nm bump is absent in interstellar regions where the abundance of carbon and other heavy elements is severely depleted with respect to values for the solar neighborhood.

3.2. ATOMS AND MOLECULES IN THE UV-VISUAL

At the beginning of the twentieth century, stationary absorption lines were discovered toward spectroscopic binaries which initiated the postulation and study of the interstellar medium (e.g., ionized calcium, Ca *II,* by Hartmann, 1904 and neutral sodium, Na *I,* by Heger, 1919). Subsequently, several other interstellar atomic absorption lines – electronic transitions – such as K *I*, Ca *I* (Dunham, 1937), and Ti *II* (Adams and Dunham, 1938), were discovered and identified. Important contributions to this new field of the interstellar medium were made by renowned astronomers such as Plaskett (1924), Eddington (1926), and Struve (1927, 1930). Shortly after the discovery of sodium and calcium, also the first interstellar molecules (CH and CN) were detected and identified. Only with more recent (1970s and onward) advances in balloon-borne and space experiments were additional atomic and molecular transitions detected in the ultraviolet toward diffuse sightlines (notable examples are HI, C, N, O, H_2, C_2, and CO). Atomic and molecular UV/visual transitions are also indicated in Fig. 1.

The C_2 molecule was already predicted to be present in the ISM by Swings in 1937. And thanks to laboratory studies of C_2 in the near-infrared light by Philips (1948a, b) and advances in instrument and telescope sensitivities, it was eventually possible to detect this molecule in translucent clouds (Souza and Lutz, 1977; Hobbs, 1979). Later, Lambert et al. (1995) also detected the UV transitions of C_2. Even more recent is the discovery of C_3, though subsequent searches for larger carbon chains (e.g., C_5 and C_7) have not yet been successful (Maier et al., 2001, 2002; Galazutdinov et al., 2002; Oka et al., 2003).

3.3. THE DIFFUSE INTERSTELLAR BANDS

Several broad interstellar absorption bands were first noticed by Heger (1922) due to their stationary behavior with respect to stellar lines in a binary system. These lines, however, were clearly broader by a few tenths of nm (i.e., diffuser on a photographic plate) than the stationary lines of sodium and calcium. Only a handful of these so-called diffuse interstellar bands (DIBs) were known (with rest wavelengths at 5,780, 5,797, 6,270, and 6,283 Å) until the study by Herbig (1975) which listed 39 DIBs for 57 reddened sightlines. Nowadays, there are about 380 known diffuse bands (Hobbs et al., 2008) of which dozens are observed ubiquitously toward reddened stars in our galaxy and beyond. Russell (1935) and Swings (1937) postulated, independently, that DIBs are in fact molecular bands (either rotational or electronic oscillations) of large molecules. Merrill and Wilson (1938) on the other hand proposed small solid particles (i.e., grains) as the culprits. This started a discussion on the possible molecular or grain-based nature of the DIB carrier which continues until this day, though many other, some exotic, carriers have been proposed in the past century (see Herbig, 1995 and Sarre, 2006 for overviews of proposals on DIB carrier candidates). The mysterious elusive carriers of the DIBs will be discussed in more detail in the next section.

3.4. NEAR- AND MID-INFRARED ABSORPTION LINES

A number of molecules have transitions in the near-infrared. H_2 has a vibrational band near 2.0 μm, and CO has several electronic/rotational transitions around 2.35 and 4.7 μm. H_3^+, an important species for many chemical models, has a near-IR doublet transition at about 3.7 μm. Note that in PDRs (warmer than diffuse clouds), H_2 and HD show up also in emission in the mid-infrared, between 10 and 30 μm. More complex molecules, such as CH_4, C_2H_2, CO_2, and C_6H_6, also have transitions in the near-infrared, but again these have not yet been detected in diffuse clouds.

The broad 3.4 μm infrared absorption feature was first detected toward the Galactic Center (Butchart et al., 1986; McFadzean et al., 1989) as well as the highly extincted sightlines toward Cyg OB12 (Adamson et al., 1990). Similar to the UV bump, this broad feature cannot be due to a single molecular carrier. The 3.4 μm band is now attributed to C–H stretching vibrations of aliphatic hydrocarbons (e.g., Pendleton et al., 1994. Sandford et al., 1995). The carrier is widespread in diffuse dust, while it disappears in denser clouds, where another water-ice related feature appears at 3.47 μm.

3.5. (UNIDENTIFIED) EMISSION BANDS

These features, though obviously not in absorption, are of potentially great importance to understanding the previous features (i.e., UV bump, DIBs, 3.4 μm band), and thus, we mention them here as well. The infrared emission bands at 3.3, 6.2, 7.7, 8.6, 11.2, 12.7, and 16.4 μm were discovered in the mid-1970s by ground-based and airborne studies. Space missions, in particular ISO and Spitzer, showed the enormous richness of the mid-IR wavelength window. These IR emission bands are observed in many different astrophysical environments, such as star-forming regions, H *II* regions, circumstellar envelopes, and protoplanetary disks as well as the diffuse ISM. Evidence indicates that these are infrared fluorescence lines of far-UV pumped polycyclic aromatic hydrocarbon (PAH) molecules. The different – aromatic – bands correspond then to C–H and C–C stretch and C–H bending modes of PAHs or derivative molecules such as PANHs. However, not one such aromatic molecule has been uniquely identified. An excellent review of the aromatic bands was recently presented by Tielens (2008). Recently, a set of emission bands observed toward both an evolved star and an H *II* region were identified as originating from neutral fullerenes, C_{60} and C_{70} (Cami et al., 2010; Sellgren et al., 2010). Other features observed in the mid-infrared spectra are forbidden emission lines of atoms and also broad solid-state features that correspond to different types of silicate incorporated in dust grains.

3.6. (SUB) MILLIMETER MOLECULAR ROTATIONAL LINES

Radio astronomy has vastly increased the number of known molecules in the universe, though few have been detected in the diffuse ISM (most are detected in hot molecular cores and around evolved stars; both environments that show a very rich gas-phase grain-surface induced chemistry; Herbst and Van Dishoeck, 2009). A complete and up-to-date list of molecules detected in space is maintained at www.astrochemistry.net.

In recent years, Liszt, Lucas, and coworkers have also observed a surprisingly complex chemistry in diffuse clouds through observations of rotational mm/cm lines of molecules such as HCO^+, C_2H, NH_3, C_3H_2, H_2CO, etc. in absorption against the strong radio continuum of bright background sources (e.g., Liszt et al., 2006, 2008 and references therein). The (relatively) high abundance of some of these molecules in diffuse clouds (only a few sightlines have been studied so far) is not understood, and their detection challenges current chemical models and indicates more complex formation processes or feedback with the dense medium than expected from traditional UV/visible studies of diffuse clouds. Using the far-infrared heterodyne receiver HIFI on board of the Herschel Space Observatory, different teams are now discovering the presence of many more simple polyatomic molecules in the diffuse ISM as probed in absorption toward bright continuum sources (see special A&A issue on HIFI results with Herschel). Observational efforts have also been initiated to find prebiological important molecules such as pyrimidine, aziridine, and glycine. Searches are conducted in particular regions such as dusty envelopes around evolved stars and hot molecular cloud cores where high abundances are predicted (Kuan et al., 2004; Charnley et al., 2005). The low-column densities expected currently preclude the detection of these molecules in the diffuse, cold interstellar medium.

4. The Diffuse Interstellar Bands: Direct Evidence for Large Organic Molecules?

DIBs are ubiquitously present in the diffuse ISM. Uncovering the identity of their carriers will be essential to understand their impact on the cosmic carbon budget, interstellar chemical models, heating and cooling of the ISM, the overall enrichment of galaxies, and the formation of molecules related to the origin of life. The DIB carrier puzzle is the oldest standing spectroscopic problem in astronomy. As noted before, 380 DIBs are currently found of which only a dozen or so have depths of more than 10% per unit reddening (Herbig, 1995). Until now, at most, a few hundred individual sightlines, mostly toward early-type stars, have been studied for DIBs. Nonetheless, these include many different environments both inside the Milky Way as well as in other galaxies, such as our nearest neighbor galaxies, the Large and Small Magellanic Clouds. The number of interstellar clouds harboring DIBs will change drastically with the new European astrometry mission, GAIA, which will observe, as a serendipitous bonus inherent to the design of its low-resolution spectrograph, one diffuse band, at 862.1 nm, toward hundreds of thousands of stars.

4.1. PROPERTIES AND PROFILES OF DIBS

To illustrate the different distinct spectral shapes of the DIBs, we show in Fig. 2 the spectrum of HD183143 as observed by Merrill in 1938 and by the author in 2005 (the latter spectrum was taken with ESpaDonS at CFHT). It can be seen that already in the early days of DIB research, it was clear that the carriers could not

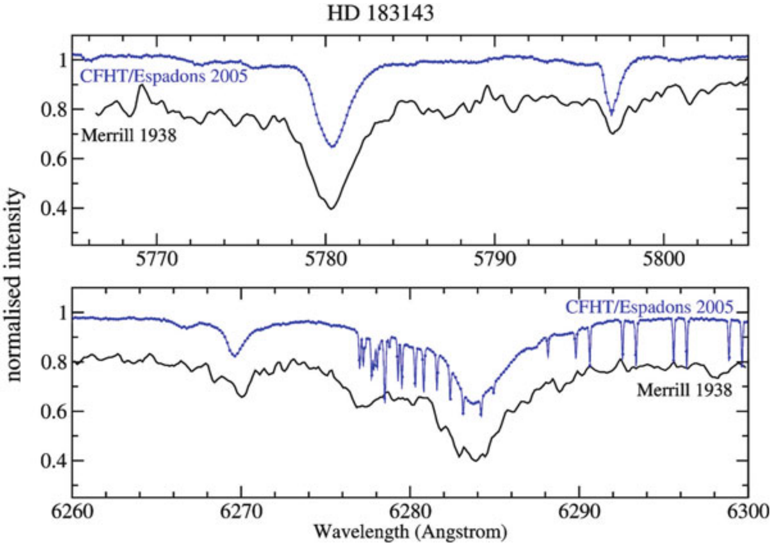

Figure 2. Four strong diffuse bands are shown. Upper spectrum (in both panels) is obtained with modern day equipment, while the lower trace was obtained in the 1930s by Merrill. The latter has been reproduced from Merrill (1934). The modern spectra allow for resolving substructure in individual bands as well as accurate removal of atmospheric water and oxygen absorption lines (these are the very narrow features in the upper trace in the lower panel).

be atoms or diatomics in the gas phase. The features are clearly much broader (50–1,000 km/s) and shallower than those expected and observed for electronic transitions of simple diatomic molecules (FWHM < 10 km/s)!

From this limited view, it was already shown that DIBs show variations in band shape (i.e., in width, strength, asymmetry). The new observations confirm that DIBs are not unresolved narrow lines, though high-resolution spectroscopy has revealed that some DIBs show band substructure (Sarre et al., 1995b; Ehrenfreund and Foing, 1996; Galazutdinov et al., 2003). For example, the 6613 DIB shows a clear three-peak structure, and the 5,797 Å DIB shows a two-peak structure with superimposed a very narrow strong peak that is only revealed at R ~ 200,000 (Kerr et al., 1996; Cami et al., 2004). The broad DIBs at 5,780 and 6,283 Å on the other hand show no such substructure, but do show slight asymmetry in their profile. One suggestive idea is that in fact the diffuse bands are band-heads (so-called P, Q, and R branches) of large molecules whose electronic transitions have rotational levels that overlap (e.g., Russell, 1935; Swings, 1937; Edwards and Leach, 1993; Ehrenfreund and Foing, 1996; Kerr et al., 1996). Simple contour calculations show that these substructures are consistent with medium-size organic molecules (consisting of about 20–40 carbon atoms). The substructure of DIB profiles is not seen to vary in time, though they do differ between lines-of-sight.

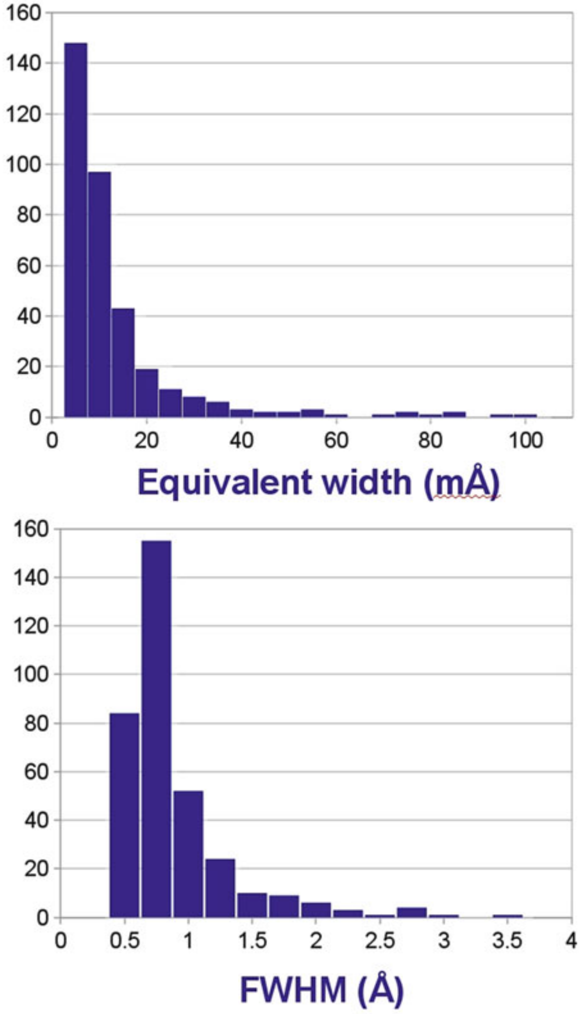

Figure 3. Histogram of DIB equivalent widths (*top*) and full width at half maximum (*bottom*). There are 9 DIBs with FWHM between 5 and 25 Å, and there are 8 (other) DIBs with equivalent widths > 100 mÅ. Histograms are based on the DIB survey of HD204827 by Hobbs et al. (2008). Note that atoms and diatomic molecules have FWHM < 0.1 Å.

A study of the six strongest DIBs revealed no linear line polarization at levels expected for a grain-related carrier (Cox et al., 2007). Figure 3 shows the strength (expressed as equivalent width) and full width at half maximum (FWHM) of the 300+ DIBs detected toward HD204827 by Hobbs et al. (2008). This work confirms the previous results which show that there are only about ten strong diffuse bands (with strength > 100 mÅ). Most DIBs are weak (<20 mÅ) and/or are narrow (FWHM ~ 1 Å).

4.2. RELATION BETWEEN DIFFUSE BANDS AND DUST?

The first detailed multi-sightline studies by Merrill (1934; Merrill et al., 1937) revealed a strong relation between band strength and reddening (commonly denoted E(B-V), the color excess between the B and V magnitudes arising from dust absorption). In subsequent studies, it was also shown that DIB strengths correlate roughly with the presence of (neutral) gas (i.e., H *I* and Na *I*, e.g., Herbig, 1993). Radial velocity studies of atomic and molecular lines and DIBs show also that all are spatially colocated. There are small velocity differences between these different atoms, molecules, and DIBs that constitute the diffuse ISM (e.g., Sonnentrucker et al., 1999). Observationally, it is found that not all DIBs respond equally to increases in the amount of dust or gas in the line of sight (in other words, DIBs are not observed with the same strength ratios between different sightlines). And since not any two DIBs correlate perfectly with each other, this suggests each DIB is due to a unique carrier, although some correlation between different bands may indicate that carriers of these bands have properties that respond similarly, though not equally to interstellar conditions (Krelowski and Walker, 1987; Krelowski and Westerlund, 1988; Cami et al., 1997). A simple explanation for this behavior is the attenuation of the interstellar radiation field as a function of the cloud's optical depth which results in a different chemical balance (e.g., ionization state) for different interstellar species as a function of distance from the edge to the center of the cloud. This could mean that different carriers, with for example a different ionization potentials, give rise to different DIBs. Alternatively, the relative strengths of individual lines in a vibrational progression band would change due to changes in the local environmental conditions (i.e., temperature and density). Recent works show clearly that environmental conditions of the ISM are important to the strength of the DIBs (Vos et al., 2011). DIBs show variations in strength on scales of hundreds of astronomical units (1 AU is 150 million km) as seen from observations of multiple star systems (Cordiner, 2005; Smith, 2006).

4.3. DIB CARRIERS IN OTHER ASTROPHYSICAL ENVIRONMENTS

Evolved stars are known to produce copious amounts of carbonaceous dust and are considered to be one of the main sources of chemical replenishment and enrichment of the ISM. Frenklach and Feigelson (1989) proposed formation routes of PAHs in circumstellar envelopes, and indeed, many complex molecules have been detected in/toward these stars at radio wavelengths as well as the aromatic emission bands.

However, all searches for transitions of DIB carriers in the (extended) envelopes of these stars have been negative (see Luna et al., 2008 and references therein).

Also, the atmospheres of cool stars show no such diffuse *circumstellar* bands (Destree et al., 2007). From this, we can conclude that the carriers responsible for the DIBs are not formed (at least not in the right state) in circumstellar environments of evolved stars. On the other hand, their progenitors – either the small building blocks or large parent conglomerates – might be detectable through more detailed spectroscopic studies. Neither have similar optical absorption features been reported for H *II* regions, protoplanetary disks, molecular clouds, etc. One possible exception is the Red Rectangle whose narrow optical emission bands approach the rest wavelengths of some DIBs with increasing distance from the central star suggesting a direct common link between their carriers (Sarre et al., 1995a; Van Winckel et al., 2002).

5. PAH-DIB Hypothesis

From the aromatic infrared emission bands, there is mounting evidence for the ubiquitous presence of aromatic molecules, such as PAHs, in the universe. Shortly after the PAH hypothesis for the IR bands was proposed, it was realized that the same molecules would absorb at visible wavelengths (Van der Zwet and Allamandola, 1985; Leger and d'Hendecourt, 1985; Crawford et al., 1985). Small PAHs (up to 20–40 C-atoms) absorb mainly in the UV, below ~4,000 Å. Cations or anions absorb above 4,000 Å where transitions shift toward the NIR with increasing size (number of C-atoms). Also, specific geometries can give rise to unusually strong transitions. It is clear that also other organic molecular structures (e.g., carbon chains, carbon rings, fulleranes, and fullerenes) can give rise to absorption lines in the visual-to-near-infrared wavelength range (see Fig. 4 for an illustration on possible PAHs/fullerenes present in the ISM).

Furthermore, once PAHs reach a certain size (20–30 carbon atoms, depending on geometry), they become very photoresistant. The exact transition frequencies depend on several molecular (size & symmetry) and environmental (ionization & hydrogenation state) properties. Ruiterkamp et al. (2005) compared the diffuse bands with the electronic transitions of a selection of 70 neutral PAHs and their respective anions and cations. Despite intensive efforts and several tentative attributions of molecules to DIBs (e.g., C_{60}^{+}; Foing and Ehrenfreund, 1994 and coronene cation; Duley, 2006), no unambiguous identification of any DIB has been accepted yet. The recent detection of C_{60} emission bands (see above) opens a promising new avenue to link these new emission bands to this diffuse band. Iglesias-Groth et al. (2008, 2010) recently reported the possible detection of new optical features corresponding to laboratory transitions of naphthalene and anthracene in a sightline toward a region with strong anomalous far-infrared emission. How and where these complex molecules are formed (e.g., carbon-rich stars or in the diffuse ISM) is also not known yet. Calculations of formation and destruction mechanisms and rates do not favor PAH with less than 20 carbon atoms (see, e.g., Duley and Williams, 1986; Le Page et al., 2003) which constrains

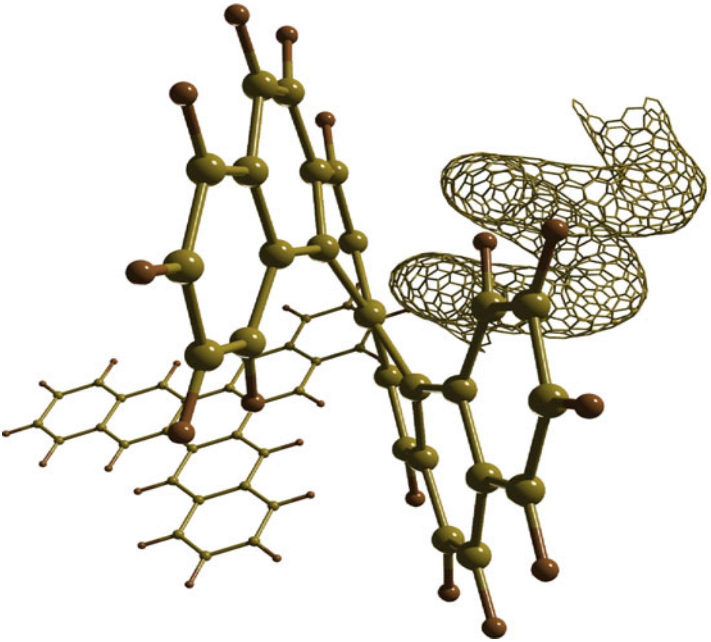

Figure 4. Illustration of different aromatic structures. From *left* to *right*, we show trinaphthylene, hexahelicene, and helical nanotube.

the PAH-DIB model. Detailed considerations and consequences of this hypothesis were reviewed by Salama et al. (1996).

6. Can We Expect Further Identification of Large Molecules in Space?

Recent studies show that the behavior of DIBs (and thus likely the formation and destruction balance of their carriers) depends on the local physical and chemical conditions of the environment they reside in. This balance is maintained in different galaxies, nearby and faraway. Metallicity, star formation (i.e., UV radiation field), and dust size distribution (i.e., extinction curve) are the most important global differences between Local Group galaxies (Welty et al., 2006; Cox et al., 2006; Cordiner et al., 2008a, b). It is important to stipulate that DIB carriers (e.g., large organics) are present, and are therefore efficiently formed, excited, and protected (stable), throughout the universe. The spectroscopic features arising in the diffuse ISM share that most are thought to have a carbonaceous nature which in turn leads to an interrelation of carriers. A positive link between the presence of the 217.5-nm bump (due to ensemble of carbonaceous material) and DIB carriers (organic molecules) is already hinted at, though it needs further observational support. Furthermore, carbon is the third most abundant element (after hydrogen and helium), and it is known to easily form complex structures including also other abundant elements, such as nitrogen and oxygen. Whether it is possible to create stable PAHs in the diffuse medium, either by top-down destruction of carbonaceous aggregates or by bottom-up from smaller building blocks, is an open issue. Another possibility may be to form PAHs in envelopes of evolved stars that are expelled rapidly into the ISM and/or in dense cloud cores from which this complex matter is recycled back into the diffuse ISM. Any identification of a DIB carrier or a new feature will rely on an unambiguous match between astronomical observations and laboratory measurements (under similar – low-temperature, gas-phase – conditions) or detailed theoretical calculations. However, the pool of potential candidates is enormous, if not infinite, and the most likely candidates need to be identified to optimize laboratory experimental efforts. This is one area where observational studies can contribute as well.

The Herschel Space Observatory will also provide new windows on the universe. Large PAHs, such as coronene and bigger, give rise to vibrational band emission at FIR wavelengths due to skeletal modes that involve the whole molecules and are therefore unique for each molecule (note that the mid-IR modes are believed to be due to bending or stretching modes of functional groups in PAHs). The unveiling of the FIR window may lead to a direct detection of a PAH molecule which can subsequently be used to directly test the PAH-DIB hypothesis.

Analysis of interstellar gas and dust allows us to unravel the complex inventory of materials, molecules, etc. This provides list of molecules, complex organics, that are potentially present at the stage of planetary system formation and that can be incorporated in asteroids, comets, and planets.

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PROKARYOTIC COMMUNITIES BELOW PLANETARY SURFACES AND THEIR INVOLVEMENT IN THE NITROGEN CYCLE

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1. Introduction

Nitrogen is essential to the chemistry of all living organisms. While NASA's plans for the search for life in the universe focus mainly on the detection of water. Capone et al. (2006) suggested to look for the presence of nitrogen, since its detection would be an important clue for potential life. Even the absence of extinct life on Mars could possibly be declared, if only abiotic nitrate deposits were found there (Capone et al., 2006). Microorganisms are the principal participants in the terrestrial global nitrogen cycle, and several surprising discoveries during the last years have changed the understanding of their involvement (Ward et al., 2007). For instance, ammonia oxidation had long been thought to be performed only by chemolithoautotrophic bacteria, but our studies (see below) as well as those of others suggested strongly the occurrence of possibly widespread archaeal ammonia oxidation. The first hints came from metagenomic studies of Crenarchaeota (Schleper et al., 2005); subsequently, marine and freshwater environments, various soils, hot springs, and wastewaters were found to contain ammonia-oxidizing archaea (You et al., 2009). Here, a little explored subsurface ecosystem will be considered in more detail with respect to a potential nitrogen cycle.

Thermal springs, which deliver their water from deep reservoirs, are in contact with the rock-dwelling subsurface biosphere and can transport members from these environments to the surface. They are thus a link between surface and subsurface, providing access to the hidden communities underground. Bad Gastein, a spa in the Central Alps near Salzburg, Austria, is known for its numerous thermal mineral springs, which contain slightly radioactive water with temperatures of up to 47°C and an outflow of about 4–5 million liters of water per day (Zötl, 1995; Weidler et al., 2007). Very little information about the microorganisms of these thermal springs and their sources of energy and carbon was available until recently. From gene analysis (Weidler et al., 2007, 2008) as well as microcosm studies, which were prepared with samples from the springs, evidence for the involvement of crenarchaeal representatives in the global nitrogen cycle was obtained. Further studies involving scanning electron microscopy and stable-isotope probing (SIP) were carried out, and identification of the phylogeny of strains, which may be involved in the nitrogen cycle, and the elucidation of possible energy source for the subsurface community were attempted. We present and discuss some novel data, which should be of interest not only to the growing awareness of archaeal involvement in the nitrogen cycle but also for the search for potential extraterrestrial biosignatures in subsurface environments, where nitrogenous compounds may exist and would be considered an exciting finding (Capone et al., 2006).

2. Thermal Springs as Subsurface Environment for Microorganisms

Subsurface environments are generally limited with respect to nutrients and oxygen (Ghiorse and Wilson, 1988; Stevens and McKinley, 1995; Pedersen, 2000) and therefore provide a niche for microorganisms which are able to tolerate these environmental conditions. Stevens (1997) discussed two different types of ecosystems; in the first one, the primary production is carried out only by photosynthetic organisms at the Earth's surface. All other organisms would act as consumers of the products of photosynthesis, as secondary consumers, or as recyclers of photosynthetic energy. He suggested that if the microbial metabolism in subsurface were only heterotrophic, such a system would break down, since with increasing time the organic matter would decline. The alternative must be ecosystems in subsurface environments in which primary production occurs, using *in situ* energy sources of geological origin, which implies that primary production has to be based on chemolithoautotropy. In other words, both the energy source and the electron sink must be inorganic compounds, and inorganic carbon is metabolized into organic carbon (Stevens, 1997).

The thermal springs of Bad Gastein contain radium and radon, which make them slightly radioactive and might be important, besides the other mineral constituents, for the healthy effects on humans in the spas of the village (Zötl, 1995). Four to five million liters of thermal water are delivered per day from a cluster of 17 major springs which are located between 962 and 1,029 m above sea level. One of the major springs, called "Franz-Josef-Quelle" (FJQ), is located at the end of a gallery, horizontally driven about 150 m into the rock, and consists in total of 27 single water discharges, which arise directly from rock fissures, delivering 295-m^3 thermal water per day. Based on results of studies on microbial diversity in the spring (Weidler et al., 2007, 2008) and considering that the thermal mineral water of the FJQ contains inorganic nitrogen compounds (see chemical analysis below), the question arose which energy source certain microbial inhabitants of the thermal spring may use. In the study described here, we focused on the identification of microorganisms, which use nitrogen compounds as electron donors (nitrifiers) as well as electron acceptors (denitrifiers).

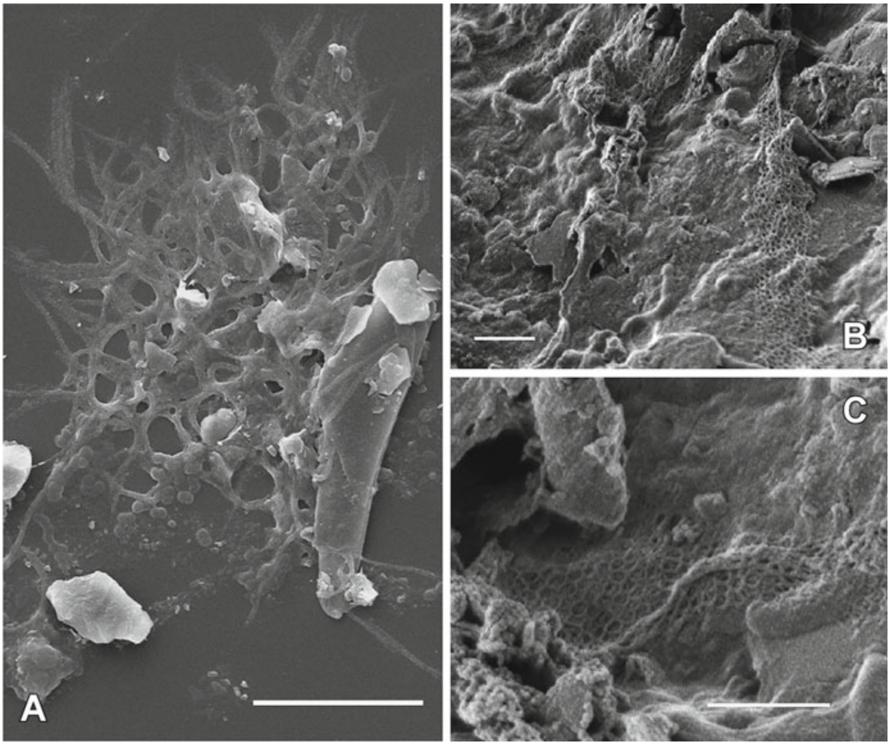

Figure 1. Examples of scanning electron micrographs of biofilm grown in the thermal FJS spring (Bad Gastein, Austria). Images show microbial structures that are attached to mineral matter. Samples were air dried prior to coating with gold-palladium. (**a**). Biofilm with filamentous structures and coccoid cells; bar, 20 µm. (**b**) and (**c**), net-like structures in biofilms as observed by the late Dr. Wolfgang Heinen (see Dornmayr-Pfaffenhuemer et al., 2010); bar, 1 µm.

Chemical analysis of the thermal mineral water of the FJQ showed the following cations (in brackets: mg/liter of thermal water): Na^+ (76), Ca^{2+} (19.6), NH_4^+ (up to 0.02), and Mn^{2+} (traces) and anions, respectively: SO_4^{2-} (130), HCO_3^- (60.8), Cl^- (26), NO_3^- (up to 0.5), NO_2^- (up to 0.003), and F^- (traces). In situ measurements of the O_2 content revealed up to 1.9 mg/l oxygen dissolved in the thermal water of the FJQ.

Scanning electron micrographs were obtained from biofilms growing in the springs, which showed a diversity of morphological forms, such as filaments and coccoid cells, both of various diameters (Fig. 1a). In addition, unusual net-like structures were present (Fig. 1b, c), which had already been observed by Wolfgang Heinen and coworkers (http://www.vcbio.science.ru.nl/en/fesem/applets/nanobes/), and were confirmed recently by Dornmayr-Pfaffenhuemer et al. (2010). Quite similar structures were published by Melim et al. (2008), who suggested to call them "reticulated filaments" and discussed the possibility that they derived from so far unknown cave microorganisms.

3. Estimation of Microbiological Diversity by Molecular Techniques

Molecular techniques like the analysis of phylogenetic characteristics such as the 16S rRNA gene sequences became a widespread tool to obtain knowledge about the microbial inhabitants of an environment of interest. Weidler et al. (2007) performed a study on the microbial diversity in the FJQ and obtained 132 bacterial 16S rRNA sequences, which were affiliated with 14 operational taxonomic units (OTUs). OTUs are defined as clusters of related organisms and as such provide a certain statistical measure of the similarities of communities. Sequences, which were related to the *Proteobacteria* (alpha, beta, gamma, and delta groups), *Bacteroidetes*, and *Planctomyces* as well as to the *Nitrospira*, were detected in the FJQ (see Table 1). In detail, eubacteria which cluster together with members of the *Nitrospirae and Nitrospina* (delta proteobacteria) as well as *Burkholderiales* or with *Thiobacillus denitrificans* were identified by their partial 16S rRNA sequences. Database searches revealed similarities to the closest database hits of 87% for *Nitrospina*, 98% for *Nitrospirae*, 92% to *Burkholderiales*, and 97% for *Thiobacillus denitrificans*.

Only four OTUs were assigned to a total of 283 archaeal 16S rRNA sequences in the FJQ. These sequences belonged to the groups of the *Crenarchaeota*, which were officially numbered 1.1b and 1.3b (sequence similarities up to 99%; see Weidler et al., 2007, for references), or to uncultivated *Euryarchaeota*. Some members of the *Crenarchaeota* were suggested to oxidize ammonia and convert it to nitrite (Treusch et al., 2005; Leininger et al., 2006; Hatzenpichler et al., 2008). The key functional enzyme for this process is the ammonia monooxygenase, which contains an alpha(3)beta(3)gamma subunit structure (for a review of the bacterial enzyme, see Arp et al., 2002). Genes related to subunit A of the enzyme, which is encoded by the *amo*A gene, were identified first in Crenarchaeota of soil communities (Treusch et al., 2005) and later in marine environments, thermal springs, wastewater, sediments, and in association with corals (for a recent review, see Prosser and Nicol, 2008). In the FJQ crenarchaeal *amoA*, related genes were detected (Weidler et al., 2007, 2008). The dominating group of the *amoA* genes clustered to genes belonging to water column/sediment clones, including the *amoA* gene of *Candidatus Nitrosopumilus maritimus*, a marine crenarchaeon (Könneke et al., 2005). This latter strain was the first cultivated isolate representing an autotrophic, free-living ammonia oxidizing marine crenarchaeon. Sequences of its *amoA* gene were 77–81% similar to several clones from the FJQ spring (Weidler et al., 2007).

In summary, the results disclosed evidence for the occurrence of archaeal and bacterial microorganisms in the FJQ, all of which may be involved in a putative nitrogen cycle in this subterranean environment.

Table 1. Most similar database sequences of archaeal (see Fig. 4) and bacterial (see Fig. 5) 16S rRNA genes, associated with phylotypes, which were recovered from microcosms following stable-isotope probing and incubation at 40°C.

Phylogenetic relationship					
OUT	**Phytotype**	**Closest database sequence**	**Acc. Nr.**	**Origin of database sequence**	**% of similarity**
Archaea					
FGA1	FG34A3	Uncultured crenarchaeote FJQFA2	AM039531	Subsurface thermal spring, Austria	99.9
		Candidatus *Nitrososphaera gargensis*	EU281336	Garga hot spring, Russia	97.6
FGA2	FG34A37	Uncultured crenarchaeote FJQFA13	AM039534	Subsurface thermal spring, Austria	94.4
		Candidatus *Nitrososphaera gargensis*	EU281336	Garga hot spring, Russia	98.5
FGA3	FG35A23	Uncultured methanogenic archaeon RC-1 complete genome	AM114193	Rice paddies	59.4
		Marine metagenome, clone 1061006184749	ER088556	Global-Ocean-Sampling_ GS-33-01-01-1P3-1P8KB	58.5
Bacteria					
FGB1	FG34B1	*Nitrospira moscoviensis*	X82558	Iron pipe of a heating system in Moscow, Russia	97.7
FGB2	FG34B2	Bacterium SG-3	AF548381	Pond water	93.6
FGB3	FG34B6	*Curvibacter gracilis*	AB109889	Well water, Japan	96.4
FGB4	FG34B8	*Thiobacter subterraneus*	AB180657	Terrestrial subsurface hot aquifer	92.7
FGB5	FG34B14	*Nitrospira moscoviensis*	X82558	Iron pipe of a heating system in Moscow, Russia	92.1

(continued)

Table 1. (continued)

Phylogenetic relationship					
OUT	**Phytotype**	**Closest database sequence**	**Acc. Nr.**	**Origin of database sequence**	**% of similarity**
FGB6	FG34B34	Uncultered bacterium, clone SK27B-27	AB300065	Holocene mud sediment, Japan	86.8
FGB7	FG35B9	*Lysobacter brunescens*	AB161360	Anaerobic sludge blanket reactor	93.6
FGB8	FG35B38	Alpha proteobacterium CRIB-02	DQ123619	Hospital water network	96.9
FGB9	FG35B49	*Leptothrix* sp. MBIC3364	AB015048	Hot spring, Japan	99.8
FGB10	FG35B32	Unc. proteobacterium, clone HAuD-LB/2-36	AB176699	Subsurface geothermal water, Japanese gold mine	99.6
FGB11	FG35B34	*Thiobacter subterraneus*	AB180657	Terrestrial subsurface hot aquifer	92.7

4. Stable-Isotope Probing (SIP) and Microcosm Studies

Stable-isotope probing has been established as an experimental tool to link the taxonomic affiliation of inhabitants of certain habitats and their function in these ecosystems (Radajewski et al., 2000, 2003; Dumont and Murrell, 2005; Friedrich, 2006). This method is essentially based on the experiment of Meselson and Stahl (1958), who demonstrated the incorporation of labeled nitrogen into the DNA of *Escherichia coli* during growth on ^{15}N-containing ammonium salts and the separation of ^{15}N-labeled DNA from unlabeled DNA during density gradient centrifugation.

For the current study, microcosm experiments in 5 l glass flasks were carried out to enrich and identify microorganisms, which are able to metabolize nitrogen compounds and use them as energy sources. Microcosm experiments were performed by using two different media, two different temperatures of incubation (30 and 40°C), and two different types of samples (microorganisms filtered from the springwater and microbial biofilms). The first medium was prepared in accordance with the media described by Wuchter et al. (2006) with slight modifications. Two and a half liters of sterile-filtered thermal mineral springwater from the FJQ were supplemented with 0.5 mM $^{15}NH_4Cl$, 100 mM $NaNO_3$, 100 mM NaH_2PO_4, 100 mM $NaHCO_3$, and trace elements and vitamins were added as described in Weidler (2006). The second medium was developed in this study, consisted of 2.5 l of water as mentioned above, and supplemented with various minerals (Gerbl, unpublished), trace elements, and vitamins as in Weidler (2006). As inocula, either microorganisms filtered from 25 l of thermal water from the FJQ or 35 ml of biofilm, which was collected from the surrounding of a rock fissure, were used. Microcosms were incubated as batch cultures in the dark for 8 weeks, and DNA was extracted after 3, 5, and 8 weeks of incubation. The pH was adjusted every second day to 7.5–7.7; nitrite and nitrate were measured three times a week. Figure 2 shows the continuous decrease of ammonium during incubation of a microcosm while nitrite and nitrate increased concomitantly. Control microcosms with the same composition as described above, but without inocula, showed neither an alteration of the initial ammonium content nor the appearance of nitrite or nitrate during the period of incubation. Substrates labeled with stable isotopes ($^{15}NH_4SO_4$, $^{15}NH_4Cl$) were added to environmental microcosms. During metabolic activity, the label will become incorporated into the biomass (e.g., DNA) of the ammonium-/nitrite-oxidizing microbes, increasing its density. DNA was extracted at the end of the incubations according to Hurt et al. (2001) and loaded onto a cesium chloride gradient for isopycnic centrifugation as described by Neufeld et al. (2007).

The result of an isopycnic centrifugation is shown in Fig. 3. A distinct shift in buoyant density of microbial DNA extracted from microcosms supplied with labeled nitrogen compounds is visible. Furthermore, this image indicates the high resolution of this technique with respect to DNA composition, since putatively A+T-rich DNA from complex microbial communities could be separated from G+C-rich DNA (Cadisch et al., 2005; Buckley et al., 2007).

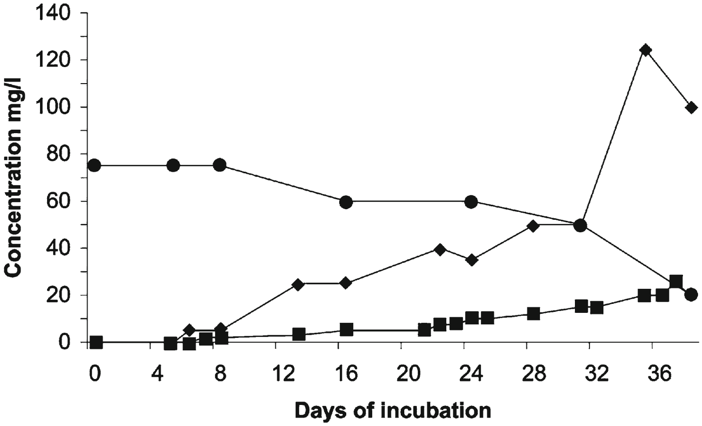

Figure 2. Oxidation of ammonium and formation of nitrite and nitrate during incubation of microcosms. The inoculum consisted of the prokaryotic planktonic community of the FJQ, which was concentrated by filtration. Incubation was carried at 40°C, and the conditions were as described in the text with the exception that the incubation time was shortened to 38 days. λ, ammonium; ν, nitrite; υ, nitrate (From Weidler et al., 2008, with permission).

5. Phylogenetic Diversity Within Microcosms Following SIP

Following separation on CsCl-gradients, DNA was purified and precipitated in accordance to Neufeld et al. (2007), to remove ethidium bromide and cesium chloride. Subsequently, 16S rRNA genes were amplified using specific primers for Archaea (DeLong, 1992; Massana et al., 1997). Bacterial 16S rRNA genes were amplified using universal primers: E27F (AGA GTT TGA TC(AC) TGG CTC AG) modified from DeLong (1992) and U1390R (GAC GGG CGG TGT GTA CAA) modified from Zheng et al. (1996). Primers which are specific for certain phyla were used to search for *Planctomycetes*, which are known to be ubiquitously distributed in the environment and were also reported to convert nitrogen compounds (Bock and Wagner, 2006). Cloning of PCR products and further processing were done as described previously (Weidler et al., 2007). ARDRA (amplified rDNA restriction analysis) patterns were used to group the clones into OTU. Each OTU is represented by a phylotype, which is constituted by two randomly chosen 16S rRNA clones (if OTUs had more than one representative), which were sequenced. Chimeric sequences were not detected in either archaeal or bacterial sequences, using the chimera check program of the Bellerophon server (Huber et al., 2004). Phylogenetic analysis was done by using distance-based maximum parsimony and maximum likelihood methods as described (Weidler et al., 2007).

The results for the database searches for archaeal and bacterial relatives to the 16S rRNA clones obtained from microcosms are visualized as a phylogenetic

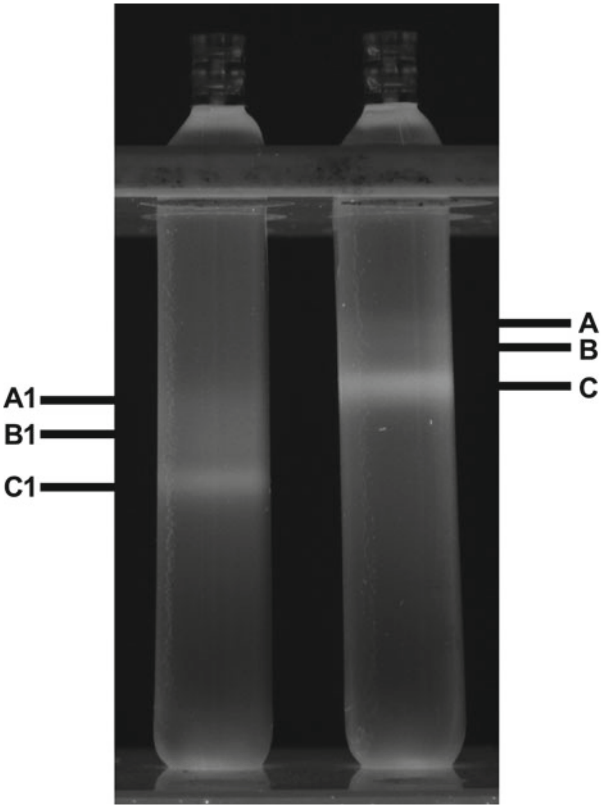

Figure 3. Density gradients of DNA extracted from microcosms. DNA was stained with ethidium bromide and illuminated with UV light. *Left tube*: DNA from microcosm with labeled ^{15}N compounds; *right tube*: DNA from a control microcosm supplemented with unlabeled nitrogen compounds. A/A1 indicate regions of the tubes containing putatively A+T-rich DNA, B/B1 mark regions of DNA with lower A+T content, and C/C1 indicate bands made up of DNA with a high G+C content.

tree in Figs. 4 and 5, respectively. Only three OTUs of archaeal origin could be detected (FGA1–FGA3; A for *Archaea*), whereas the *Bacteria* comprised 11 OTUs (FGB1–FGB11; B for *Bacteria*). Clones from the microcosms were designated as FG34 or FG35, respectively. Clones belonging to FG34 were isolated from microcosms which had been inoculated with microorganisms filtered from the thermal springwater. Clones with the designation FG35 were derived from microcosms containing microorganisms from biofilm. High similarities (94.4–99.9%) were found between the two archaeal OTUs FGA1 and FGA2 to thermophilic ammonia-oxidizing Crenarchaeota (Avrahami and Conrad, 2003); less similarity (ca. 59%) was seen between OTU FGA3 and potential methanogenic archaea (Erkel et al., 2006; Parthasarathy et al., 2007; Table 1, upper part).

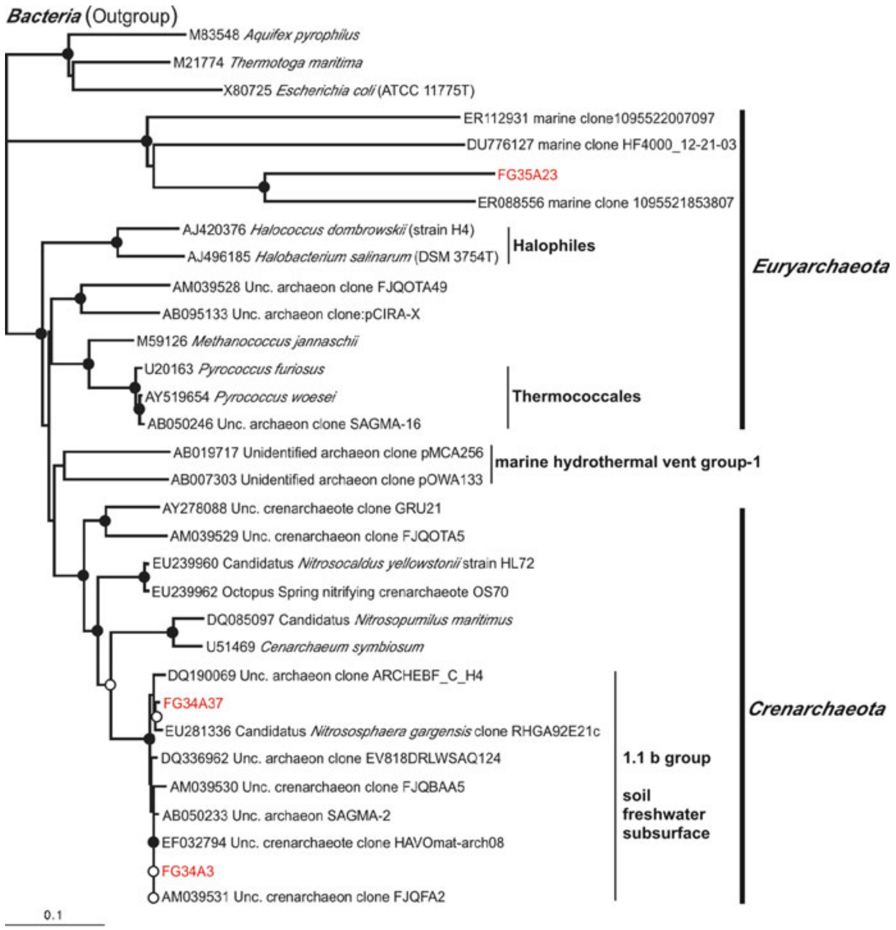

Figure 4. Archaeal phylogenetic tree, based on 16S rRNA gene sequences, depicting three OTUs (*red lettering*) detected by stable-isotope probing in microcosms. Scale bar indicates 10% nucleotide sequence difference. Symbols on the branches indicate bootstrap confidence values as follows: λ, >90 %; O, >75 %. Three sequences from species of *Bacteria* constituted the outgroup.

Each of the bacterial OTUs (FGB1–FGB11) showed high similarity (92.7–99.8%) to isolates, or uncultured bacteria thought to be involved in oxidation of ammonia and/or nitrite (Table 1, lower part).

The results of the database searches for closest relatives in accordance with the 16S rRNA sequences obtained with specific primers for the group *Planctomycetales* are summarized in Table 2. This bacterial order contains anaerobes, some of which have been implied in anaerobic ammonium oxidation (ANAMMOX), a process, which occurs in environments worldwide and is of

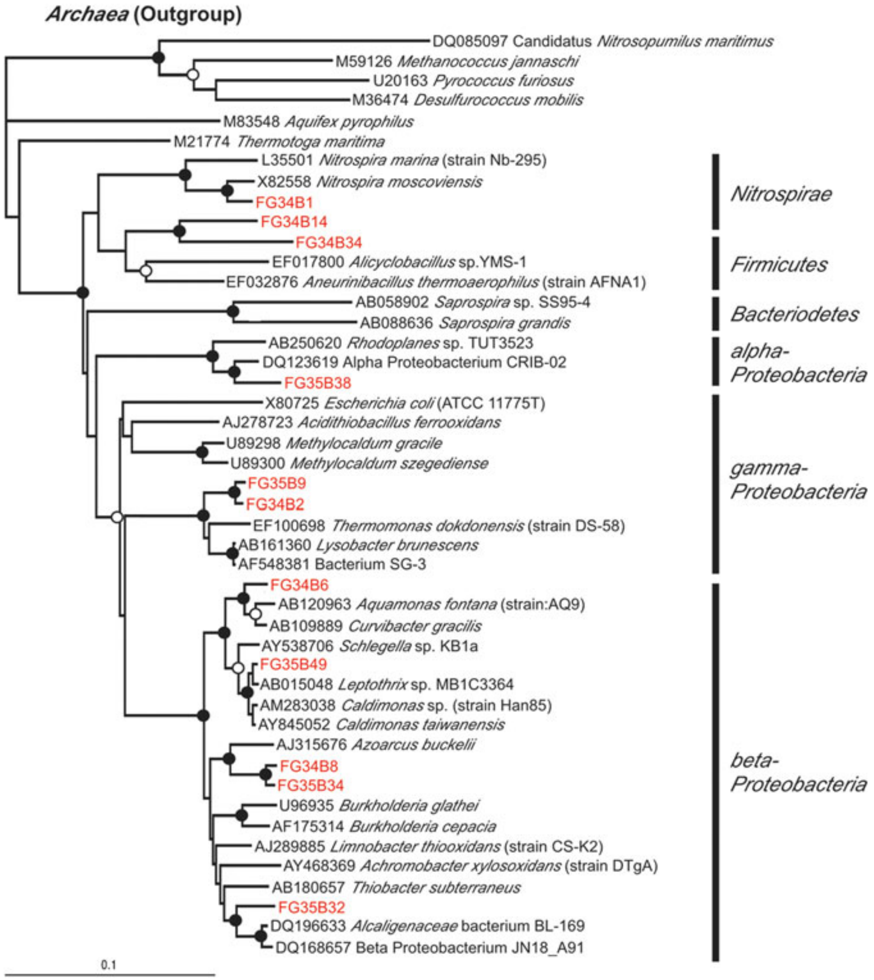

Figure 5. Phylogenetic tree of *Bacteria*, based on 16S rRNA gene sequences, including sequences of 11 OTUs from the FJQ thermal spring detected by stable-isotope probing in microcosms (*red lettering*) and their closest relatives obtained from databases. The scale bar represents 10 % nucleotide sequence difference. Symbols on the branches indicate bootstrap confidence values as follows: λ, >90 %; O, >75 %. Four sequences from species of *Archaea* constituted the outgroup.

particular interest for wastewater treatment. Evidence for members of the *Chloroflexi* and *Thermaceae* (see Table 2) has repeatedly been found in cave environments (Spear et al., 2007), but their function in the nitrogen cycle has not been established. OTUs FGP1-8 from the FJQ showed similarities in the range of 77.7–99.9% to sequences from the *Planctomycetales* and 94.8–98.4% to those from the *Chloroflexi* and *Thermaceae.*

Table 2. Nearest database hits for 16S RNA sequences for clones obtained from microcosms, which were affiliated to the orders *Planctomycetales* and *Thermaceae* as well as the phylum of the *Chloroflexi.*

Phylogenetic relationship					
OTU	**Phylotype**	**Closest database sequence**	**Acc. Nr.**	**Origin of database sequence**	**% of similarity**
Planctomycetales					
FGP1	FG34P21	Uncultured bacterium DSSD101	AY328798	Drinking water distribution system simulator	98.7
		Planctomyces maris (strain DSM 8797^{T})	AJ231184		86.3
FGP2	FG34P22	Uncultured planctomycete	EU044298	Subtropical dune fields	94.1
FGP3	FG34P23	Uncultured planctomycete	AM902608	Subsurface thermal spring	99.9
FGP4	FG34P26	Uncultured planctomycete	AY963410	Trembling aspen rhizosphere	91.7
FGP5	FG34P28	Uncultured bacterium clone F	EU135205	Soil	96.1
FGP6	FG34P33	Uncultured planctomycete	AM902622	Subsurface thermal spring	99.9
FGP7	FG34P36	Uncultured organism	EU245616	Hypersaline microbial mat	87.0
FGP8	FG34P44	Uncultured organism	EU245616	Hypersaline microbial mat	87.2
		Candidatus *Jettenia asiatica*	DQ301513	Anaerobic ammonium-oxidizing (ANAMMOX) reactor	77.7
Chloroflexi, Deinococcus-Thermus					
FGP10	FG34P31	Uncultured *Chloroflexi bacterium*	AY922047	Farm soil	95.8
FGP11	FG34P35	Uncultured bacterium	DQ906807	Subsurface soil	94.8
FGP13	FG34P32	*Meiothermus timidus*	AJ871168	Biofilm in hot spring	98.4

6. Discussion and Conclusions

The prokaryotic communities of a subsurface thermal spring in the Alps have been studied by culture-independent molecular analysis, based on sequence analyses of the 16S rRNA gene, and revealed the presence of bacterial and archaeal groups. Most of the bacteria were related to *Proteobacteria*, *Bacteriodetes*, and *Planctomycetes*. The archaeal community consisted mainly of *Crenarchaeota*. The roles and possible functions of subsurface microorganisms remain still enigmatic, but recent insights into the involvement of microorganisms in the geochemical cycles have shed some light on these issues. For example, from metagenomic analyses and a few cultivated isolates, it became clear that the old notion of considering the involvement of only bacteria in the oxidation of ammonia as the first step of nitrification had to be revised. The presence of ammonia-oxidizing archaea was identified in terrestrial and marine ecosystems (Könneke et al., 2005; Schleper et al., 2005; Hallam et al., 2006). Probing for the key enzyme of the process, ammonia monooxygenase, homologues to the bacterial subunit A (*amoA*) gene of the enzyme, were detected, together with crenarchaeal 16S rRNA genes (Schleper et al., 2005; Treusch et al., 2005; Hallam et al., 2006). Attempts of quantification of the *amoA* genes in various environments led to discussions on the dominance of archaea or bacteria and their relative contributions to the nitrification process (Adair and Schwartz, 2008; Agogue et al., 2008; Jia and Conrad, 2009; Wells et al., 2009). However, evidence for the mere presence of the key enzymes alone cannot be considered proof of activity, and detailed assessments of substrate incorporation, influences of environmental parameters, and cultivation studies are therefore needed (for reviews, see Prosser and Nicol, 2008; Erguder et al., 2009; Junier et al., 2010).

Probing for the presence of the ammonia, monooxygenase subunit A (*amoA*) gene in the thermal spring FJQ indicated sequences which were related to crenarchaeotic *amoA* genes from marine and soil habitats, suggesting an involvement of archaea in the oxidation of ammonia in this environment (Weidler et al., 2007, 2008). To explore this notion further, microcosm experiments of up to 8 weeks duration were set up, using biofilms and concentrated planktonic microorganisms from the spring as inocula. Semiquantitative measurements showed a decline of ammonium which correlated with an increase of nitrite and nitrate (see Fig. 2). Crenarchaeal genes were present in the microcosms, while bacterial *amoA* genes were not found; fluorescence *in situ* hybridization (FISH) analysis of biofilms had indicated the presence of archaeal cells with an abundance of 5.3% (± 4.5%) in the total community (Weidler et al., 2008). The data suggested that ammonia-oxidizing archaea (AOA) perform the first step of nitrification in this subsurface environment (Weidler et al., 2008).

All aquatic ammonia-oxidizing archaea, which were isolated so far, were shown to be autotrophs (Könneke et al., 2005; de la Torre et al., 2008; Hatzenpichler et al., 2008), although heterotrophs or mixotrophs may also be involved (Jia and Conrad, 2009). As far as it is known, all ammonia-oxidizing bacteria use nitrogen compounds as energy sources (Klotz et al., 2006; Koops et al., 2006); if this applies also to ammonia-oxidizing archaea, remains to be established. For a more detailed analysis of the microbial communities in the FJQ spring with respect to their involvement in the nitrogen cycle, stable-isotope probing (SIP) was applied with microcosms. This method allows generally the coupling of molecular biological methods with stable-isotope abundance originating from biomarkers. Thus, a cultivation-independent method of linking the identity of prokaryotes with their function in the environment is possible. Here, ^{15}N-containing salts ($^{15}NH_4Cl$, $^{15}NH_4SO_4$,) were added to the microcosms which, upon incorporation, resulted in heavier ^{15}N-containing DNA, compared to the usual ^{14}N-containing DNA of the microbial community. Density gradient centrifugation of extracted DNA resolved the two populations of DNA; subsequent gene probing and sequence analysis was used for characterization of the microbial groups. The microcosm studies, together with SIP, suggested the presence of autotrophic members in the microbial community, which would probably utilize ammonium compounds as energy sources, probably via oxidation to hydroxylamine (see Bock and Wagner, 2006). Some evidence for (bacterial) anaerobic representatives was also obtained (Table 2). The similarities of 16S rRNA genes to those from known strains were generally ranging from 87 to 98%, with a few exceptions (60–77 and 99.9%, respectively); these data suggested that novel strains are to be expected in the FJQ spring communities. Morphological data (Fig. 1; Weidler et al., 2008; Dornmayr-Pfaffenhuemer et al., 2010) supported this notion.

Much of the prokaryotic life on Earth is adapted to a subterranean lifestyle. This suggests that planets or moons with apparently lifeless surfaces may well harbor life in their deeper regions, provided liquid water, an energy and carbon source, as well as nitrogen compounds are present.

7. Summary

Thermal springs which deliver water from deep reservoirs, such as the FJQ spring in the Central Alps, can be considered to be directly in contact with the rock-dwelling biosphere of the subsurface. Stable-isotopic probing with incorporation of ^{15}N revealed a community which contained presumed novel archaeal and bacterial representatives, capable of metabolizing nitrogenous compounds. In addition, morphological studies indicated novel features of some members of the communities. The exploration of terrestrial subsurface microorganisms involved in the nitrogen cycle could be informative for planning future extraterrestrial searches for subsurface life with a requirement for nitrogen.

8. Acknowledgements

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EXTRASOLAR PLANETS

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1. Early Years and First Discoveries

Although the detection of extrasolar planets was impossible until the end of the twentieth century, the idea that such objects exist was discussed since the dawn of modern astronomy. For example, the book "*Entretiens sur la pluralité des mondes*" (dialogue about the plurality of the worlds) written by Bernard le Bovier de Fontenelle in 1686 became very popular. Also, Isaac Newton (1643–1727) and Immanuel Kant (1724–1804) discussed the idea of extrasolar planets. It was also realized that the direct detection of extrasolar planets would be very difficult because of the huge brightness contrast between a planet and a star. Today, we know that even a giant planet like Jupiter still is 10^9 times fainter than the Sun in the optical regime. In previous centuries, it was thus entirely impossible to detect extrasolar planets directly. However, already in the nineteenth century, scientists were aware of the fact that the indirect detection of planets would be much easier. The gravitational pull of a planet induces radial velocity (RV) variations as well as variations in the position of the star in the sky (astrometric method). In the case of the astrometric method, it is easier to detect a planet with a long orbital period than a planet with a short one. In contrast to the astrometric method, the RV method is more sensitive for short-period planets. Initial astrometric search programmes thus aimed for detecting planets with very long orbital periods.

Probably the first person who claimed the detection of an extrasolar planet using the astrometric method was See (1896) (see also Reuyl and Holmberg, 1943). The claimed discovery was very disputed. Only much later, it was proven that this planet does not exist. In the 1960s, van de Kamp (1963) claimed the discovery of several planets orbiting Barnard's Star. For this analysis, he used photographic plates taken over a time span of 40 years. It was thus difficult to prove or disprove these results. In 1973, Gatewood and Eichhorn finally proved that also these planets do not exist. Quite surprisingly, Struve wrote already in 1952 a very visionary article in which he argued that planets with orbital periods as short as 1 day might exist and that such planets would induce RV variations of the host of up to ±200 m/s. This chapter is particularly interesting, because Griffin pointed out in 1973 that it is possible to achieve an accuracy of 10 m/s with the technology of the time. In the 1980s and 1990s, Gatewood carried out another astrometric survey for massive planets of M-stars using the Alegenny refractor and a precision astrometric device called MAP. In 1996, Gatewood came to the conclusion that he had detected a planet of Lalande 21185 but again also this planet does not exist.

While the detection of extrasolar planets proved to be rather difficult, the discovery of circumstellar discs of young stars in the 1980s gave a first hint that such objects should exist. The presence of discs was initially inferred indirectly from observations of β Pic. Additional observations in the 1990s showed that discs are quite common for stars with the mass of the Sun at an age of a few million years (Strom and Strom, 1994). The discs of young stars were immediately related to the formation of planets (Smith and Terrile, 1984). Theoretical studies showed furthermore that protoplanetary discs are the natural outcome of the star-formation process. The masses of the discs were found to be in the range between 0.005 and 0.2 M_{Sun} (Beckwith and Sargent, 1993). The disc masses are thus comparable to the "minimum-mass solar nebula", which is about 0.02 M_{Sun}. The minimum-mass solar nebula is defined by the mass of solid material contained in the planets of our solar system adding the hydrogen and helium to its corresponding cosmic abundance. Observations of regions with different ages gave a first estimate of the lifetime of the discs, roughly 10 Myrs. Multiwavelength observations furthermore showed that the inner parts of the discs are hot and the outer parts cold, as expected. With Very Large Telescope Interferometer (VLTI), it became possible to study the inner disc within two astronomical units of the star. The VLTI observations showed that silicates crystallize before any terrestrial planets form, consistent with the composition of meteorites in the solar system (van Boekel et al., 2004). Simulations finally showed that it is possible to form planets in such discs.

However, up to the last decade of the twentieth century, it was generally believed that giant planets form at distances of several AU from the host star and would also stay there. It was thus expected that the orbital periods of giant planets had to be decades and the amplitudes of the RV variations had to be of the order of 10 m/s. Given these constraints and given that the astrometric search programmes turned up nothing, it was not surprising that only very few RV surveys aimed in the detection of extrasolar planets prior to 1995. One of the few groups that carried out an RV survey during this time was Walker with his team (Walker et al., 1992). In order to obtain the required accuracy, they used an HF absorption cell. By measuring the position of photospheric lines in respect to the lines produced by the cell, they reached an accuracy that would have allowed them to detect a planet like Jupiter. In total, Walker et al. observed 16 stars and found periodic RV variations for γ Cephei. Unfortunately, they convinced themselves that the RV variations are caused by active regions on the surface of the star rather than a planet. It took another 10 years until Hatzes et al. (2003) finally showed that γ Cephei has a planet. The first extrasolar planet that was generally accepted was found by Wolszczan and Frail in 1992, but this planet does not orbit a main-sequence star, it orbits a pulsar. Three years later, Michel Mayor and Didier Queloz (1995) discovered 51 Peg b, an extrasolar planet orbiting a main-sequence star. For their observations, they used the 1.93-m telescope at the

Figure 1. The 1.93-m telescope of the Observatoire de Haute-Provence (*OHP*) with which the planet of 51 Peg was discovered. This discovery led to a tremendous increase of the activity in the extrasolar planet research.

OHP (Fig. 1). This planet has an orbital period of 4.3 days, which is much shorter than any of the planets in our solar system. The discovery of 51 Peg b led to a tremendous increase of activity in exoplanet research. It is interesting to note that Latham et al. found in 1989 an object with a minimum mass of 11 M_{Jup}, which could be either a planet or a brown dwarf.

Today, more than 500 extrasolar planets have been discovered. The next section describes the methods of how these objects were detected. An overview of the results obtained is given in the third section. Given that more than 7,750 articles on extrasolar planets have been published up to now, it is impossible to give a complete overview of this field of research. Quite naturally, my overview thus has a personal bias.

2. Techniques for Detecting and Studying Extrasolar Planets

2.1. THE TIMING METHOD

Applying the timing method to a pulsar, Wolszczan and Frail discovered the first extrasolar planet in 1992. Pulsars emit radio waves that are extremely regular. Because the signal is so regular, slight anomalies in the timing caused by an orbiting planet can be detected. To date, this method has revealed planets with masses as small as 0.02 M_{Earth}. However, planets were found in only 2 out of 2,000 known pulsars. This type of planets thus is very rare. Whether the pulsar planets survived the supernova explosion or whether they formed after the explosion from the remnants, is still being discussed. The timing method has also been used to detect a planet of the sdB V391 Peg (Silvotti et al., 2007). The interesting aspect of this discovery is that this planet must have survived the common envelope phase. The timing method was also used in order to detect a planet of the white dwarf GD 66 (Mullally et al., 2009).

2.2. RADIAL VELOCITY MEASUREMENTS

By far, most of the extrasolar planets have been found with the RV method. RV measurements thus provide us with an overview of the statistical properties of extrasolar planets. The method allows to determine the orbital period, the eccentricity, Ω, and also the minimum mass of the planet (*m sin i*).

At first glance, the fact that only the minimum mass (*m sin i*) can be determined with the RV method looks like a serious draw back. One might think that it is possible that an object with a small *m sin i* is in reality a binary orbiting in the plane of the sky and not a planet. However, a number of tests can be made which allow to exclude this possibility. Let us take as an example a binary consisting of a G-type primary and an M-star companion. The M-star companion can be excluded either by taking a single high-resolution IR spectrum, by measuring the RVs using a G-star and an M-star template (differences in the amplitudes would indicate a binary or a stellar spot), or by measuring the line asymmetry (bisector) in the red and in the blue part of the spectrum. Since there is also a brown dwarf (BD) desert, it is not very likely that objects with a small minimum mass are BDs orbiting in almost in the plane of the sky (Mazeh et al., 1998; Grether and Lineweaver, 2006). In many cases, the binary star hypothesis can be excluded by using astrometric measurements obtained by the Hipparcos satellite. Certainly, GAIA will be a big leap forward in this respect. The discovery of transiting planets definitely rules out that all RV planets are binaries. Statistically, the true masses of a planet is given by $m_{true} = 1.27 \times (m\ sin\ i)$.

A problem of larger concern is periodic RV variations caused by stellar oscillations and surface features. In order to remove these false positives, a number of tests have been developed: (1) The amplitude, phase, and period of the RV variations of the planet candidate have to remain constant in time, unless there are other massive bodies in the system. (2) The RV variations caused by the planet have to be independent of wavelength. (3) The RV variations caused by a planet have to be independent of the spectral lines used. (4) The orbital period of the star and its harmonics have to be different from the period of the planet. (It is thus very difficult to detect a planet that has the same orbital period as the rotation period of the star.) (5) There should be no correlation between the activity indicators (bisector, FWHM of lines, CaII H, K-index, etc.) and the RV signal caused by the planet. (6) It has to be excluded that there are several stars within the point spread function (PSF) of the telescope.

There are two basic methods that allow to achieve high-precision RV measurements: The absorption cell method and the emission line method. In the first case, an absorption cell is placed in front of the slit of the spectrograph, which creates a dense forest of absorption lines in the spectrum of the star. Since the lines from the cell are recorded simultaneously with the spectrum of the star, instrumental wavelength shifts can be removed (see Marcy and Butler, 1992 for details). In the optical regime, usually an I_2 cell is used. The second possibility is to use a fibre-fed spectrograph with two fibres. One fibre is used for the starlight, the other for the wavelength reference. Usually, a Thorium-Argon hollow-cathode lamp is used as wavelength reference. The planed E-ELT instrument CODEX is designed to achieve an accuracy of up to 2 cm s^{-1}, using a laser comb as wavelength reference (Pasquini et al., 2008). For comparison, the (semi-) amplitude of the RV variation for planet like the Earth is 9 cms^{-1} sin i. If all instrumental effects are removed, the accuracy is given by the signal-to-noise (S/N), resolving power (R), and wavelength coverage (B in Å):

$$\sigma \approx 1.45\times 10^{9} \bullet (\mathrm{S/N})^{--1} \bullet R^{-1} \bullet B^{-1/2} \bullet \left(\mathrm{m\ s}^{-1}\right) \text{ (Hatzes and Cochran 1992)}$$

2.3. ASTROMETRIC MEASUREMENTS

This method consists of precisely measuring a star’s position in the sky and observing how it changes in time. Using the Hubble Space Telescope Fine Guidance Sensor, Benedict et al. (2002, 2006, 2010) have determined the true masses of HD 38529 c, ε Eri b, and Gliese 876b. In all cases, the planets were initially discovered with the RV method. The advantage of the astrometric method is that it is sensitive for planets of long orbital period, less affected by stellar activity, and allows measuring the true masses of the planets. In the future, GAIA will allow detecting Jupiter-like planets with orbital periods of ≤5 years of stars out to about 200 pc (Sozzetti et al., 2001). It is estimated that GAIA may discover 1,000 planets or more.

2.4. TRANSITS

A planet passing in front of a star occults a small part of its surface. A distant observer will thus observe a dimming of the starlight. If the planet paths completely in front of the star, and if we furthermore assume that the face of the star has a constant surface brightness, then the depth of the transit is simply given by $\Delta I/I = (R_{\mathrm{planet}}/R_{\mathrm{star}})^2$. Transit observations allow us to determine the radius of the planet, if the radius of the star is known (Mandel and Agol, 2002, review by Deming and Seager, 2009). The depth of the transit for a Jupiter-like planet orbiting a solar-like star is 10^{-2}. For a planet like the Earth, it is 8×10^{-5}.

Since a detailed analysis of the light curve gives also the exact inclination of the orbit, the density of the planet can be determined by combing the transit and the RV method. In principle, even the atmospheres of planets can be detected by observing the transit in suitable spectral lines. Observations of the secondary transit in the optical and in the infrared regime allow to measure the amount of reflected light and the thermal emission of the planets (Charbonneau et al., 2005; Snellen et al., 2009). The two most successful ground-based planet search programmes are SuperWASP and HATNet. These surveys are limited to short-period planets and to planets which have roughly the size of Jupiter. The transit search programme MEarth monitors 2000 nearby M-stars and has detected a planet with 2.7 R_{Earth} (Charbonneau et al., 2009). Another method is to observe stars with planets that have been found with the RV method and just see if they transit or not. In this way, Moutou et al. (2009) found a transiting planet with an orbital period of 111 days in a highly eccentric orbit. The space-based transit search programmes CoRoT and Kepler allow to detect planets with radii down to one or two R_{Earth} orbiting solar-like stars. CoRoT has already detected a planet of 1.68 ± 0.09 R_{Earth} (Léger et al., 2009; Fig. 2). Recently, the Kepler team has announced the discovery of a candidate with 1.4 R_{Earth} (Holman et al., 2010). A big leap forward will be obtained with the PLATO (if selected by ESA). PLATO will be able to find and study extrasolar planets down to the size of the Earth and simultaneously determine the properties of the host stars, including their ages (Catala et al., 2010). In this way, it will be possible to study the evolution of planets. Because the stars observed by PLATO are relatively bright, it will also be possible to study the atmospheres of some of their planets with the JWST and the E-ELT.

2.5. DIRECT IMAGING

In the view of the general public, the direct imaging technique is the most interesting one because a picture of an extrasolar planet is taken. Technically, it is presumably the most challenging one. The first extrasolar planet discovered in this way was 2 M1207 b (Chauvin et al., 2004). The planet has about 4 M_{Jup} and is orbiting a relatively young brown dwarf at a distance of 46 AU. Exciting as it this discovery is, it also illustrates how difficult it is to prove that such an object is a planet.

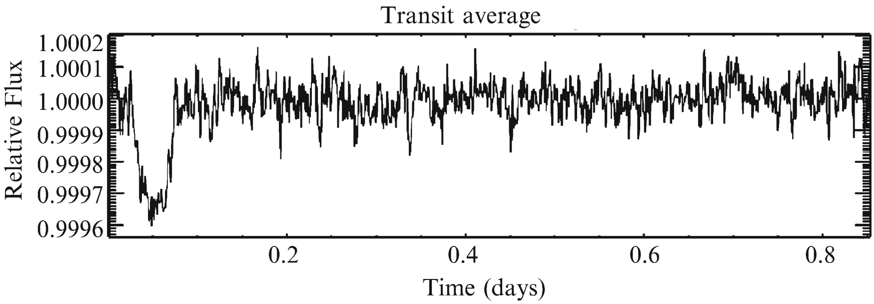

Figure 2. Light curve of CoRoT-7. CoRoT-7b is the first rocky planet detected outside the solar system (Taken from Léger et al., 2009).

Because the orbital period is likely to be larger than a 1,000 years, it is impossible to obtain the dynamical masses of the planet. Thus, theoretical evolutionary tracks have to be used to estimate its mass. Future instruments like EPICS on the E-ELT may allow detecting planets down to the size of Neptune in the habitable zone (Kasper et al., 2008). A number of space missions have been proposed for observing extrasolar planets directly (SPICES, Boccaletti and Schneider, 2010, EPIC, Clampin, 2009; ACCESS, Trauger et al., 2008; PECO, Cahoy et al., 2009, TPF-C, Traub et al., 2006; TPF-O, Heap, 2007).

Unfortunately, up to now, many planets discovered by direct imaging have orbital periods that are too long for the full orbit to be observed. In these cases, it can just be shown that the candidate has the same proper motion as a nearby star, not that it is actually orbiting this star. There is thus a gradual transition between long-period planets and free-floating objects with the mass of a planet. A number of such free-floating objects have been discovered. The first one was detected by Zapatero Osorio et al. (2000).

2.6. MICROLENSING

Microlensing is based on the gravitational lens effect. A massive object (the lens) will bend the light of a bright background object (the source). This can generate multiple distorted, magnified, and brightened images of the background source. With microlensing, the lens mass is too small (mass of a planet) for the displacement of light to be observed, but the apparent brightening of the source can still be detected. In such a situation, the lens will pass by the source in a few hours or days. The analysis of the light curve allows to constrain the properties of the lens and source. Up to now, ten planets have been detected with this method. These planets have semimajor axis in the range between 0.6 and 4.5 AU and are typically orbiting low-mass stars (0.3–0.7 M_{Sun}). Microlensing surveys thus cover a different parameter space than RV surveys. With microlensing, it is possible to probe the entire mass range of planets down to the mass of the Earth. The planets with the lowest masses yet found are OGLE-05-390L and MOA-2007-BLG-192-L. OGLE-05-390L has a mass of 0.017 M_{Jup} or 5.5 M_{Earth} (Beaulieu et al., 2006). MOA-2007-BLG-192-L is a planet of $0.01^{+0.015}_{-0.005}$ M_{Jup}, but it orbits a very low-mass star or even a brown dwarf (Bennett et al., 2008).

2.7. RADIO EMISSION

Since Jupiter is the brightest source in the solar systems at metre wavelength, some extrasolar planets might also emit in this wavelength regime. Calculations by Grießmeier et al. (2007) show that instruments like LOFAR have a chance of detecting low-frequency radio emission from planets.

3. Properties of the Planets

3.1. HOT PLANETS ($a < 0.1$ AU)

About 36% of the known extrasolar planets have semimajor axis of less than 0.1 AU (orbital periods shorter than 10 days). Such objects are commonly referred as “hot Jupiters”, or “Pegasides” after the first object of this kind discovered. Because transit and RV search programmes are biased towards planets of short orbital periods, the true fraction of such planets is much smaller. It is estimated that only 1.2 ± 0.2 % of the solar-like stars have a hot Jupiter (Marcy et al., 2005). About 100 transiting planets have already been found in this category. We thus know more about these species of planets than of any other one.

The discovery of these close-in and massive planets was very surprising, and their formation is still an active field of research. One possibility for the formation of gas giants is the core-accretion scenario. In this scenario, a core of 5–10 M_{Earth} has to form first, which then accretes matter (mostly hydrogen and helium) from the disc (e.g. Hubickyj et al., 2005; D’Angelo et al., 2010). Given the lack of material in the inner part of the disc, it is unlikely that hot Jupiters can form in situ. At distances larger than about 4 AU, ices condense out, and the surface density of the disc increases by a factor three. Because a larger surface density helps the formation of the core, it is generally assumed that the cores of gas giants form beyond the ice line. Giant planets thus presumably form beyond the ice line and then migrate inwards. The critical step in this process is the formation of the core, which has to form within the live time of the disc. Simulations by Alibert et al. (2005) show that the formation time is more than an order of magnitude shorter if migration of the protoplanets is taken into account. The formation time problem can thus be solved in this way. Observationally, the best evidence for migration is that there are systems of planets in resonances. Because it is very unlikely that they formed in a resonance, at least these planets must form have migrated (Rein et al., 2010). An alternative scenario for the formation of gas giants is that they form via an instability of the disc (Boss, 2009). In this scenario, the planet forms in a very short time, but a very massive disc is required.

Most hot Jupiters have orbits of low eccentricity. This is not surprising since the tidal interaction between the planet and the star is so strong that even a highly eccentric orbit will relatively quickly become circular. As pointed out by Eggenberger et al. (2004), planets with a minimum mass larger than 2 M_{Jup} are rare amongst the objects with orbital period of less than 30 days. This result cannot be a selection effect, since it would be easy to detect a massive, close-in planet. The only massive, short-period planets known are all in binary systems. Pätzold and Rauer (2002) explain the lack of close-in massive planets by tidal interaction between the star and the planet, which causes the planet to spiral into the host star.

Recent observational results point in a new direction how close-in planets form: For transiting planets, it is possible to determine the relative angle between the spin axis of the star and the spin axis of the orbit of the planet (λ) by measuring the RV of the star during the transit (Rossiter-McLaughlin effect). In our solar system, the spin vector of the Sun is inclined by 7.2° to the normal of the ecliptic and only 1.3° to the normal of the plane of the angular momentum of the solar system (Hutchison et al., 2001). In sharp contrast to this, 45–85 % of the extrasolar planets are misaligned, and some even orbit retrograde (Triaud et al., 2010). Triaud et al. (2010) also show that the distribution of λ can best be reproduced with a model in which the orbit of the planet is changed by interaction with a massive body. These discoveries lead to a new scenario: The idea is that several planets form in a closely packed configuration. Once the disc is dispersed, the eccentricity of the orbits grow, resulting in close encounters between planets, which then lead to the observed distribution of eccentricities, misalignments, and orbital periods (Chatterjee et al., 2007). Moutou presented an alternative scenario that would explain the misalignment in 2010: In this scenario, the orientation of the spin axis of the star is changed by the gravitational interaction between the star and the planet.

3.1.1. The Density and the Evaporation of Hot Jupiters

If the transit method is combined with RV measurements, the radius, mass, and the density of the planets are obtained. Although planets with the mass of Jupiter are expected to consist mainly of hydrogen and helium, they show a surprisingly large spread in density. In other words, two planets of the same mass can have very different radii (Fig. 3). Planets of high density are explained easily by the assumption that they have a large core. Finding an explanation for the low-density planets is more difficult. In order to explain the low density of these planets, it is not enough to just assume that they consist exclusively of hydrogen and helium. An additional factor is needed. One possibility is that the planets are inflated due to the heating of the star by radiation and tidal forces. Unfortunately, even if we take these two effects into account, it is still not enough.

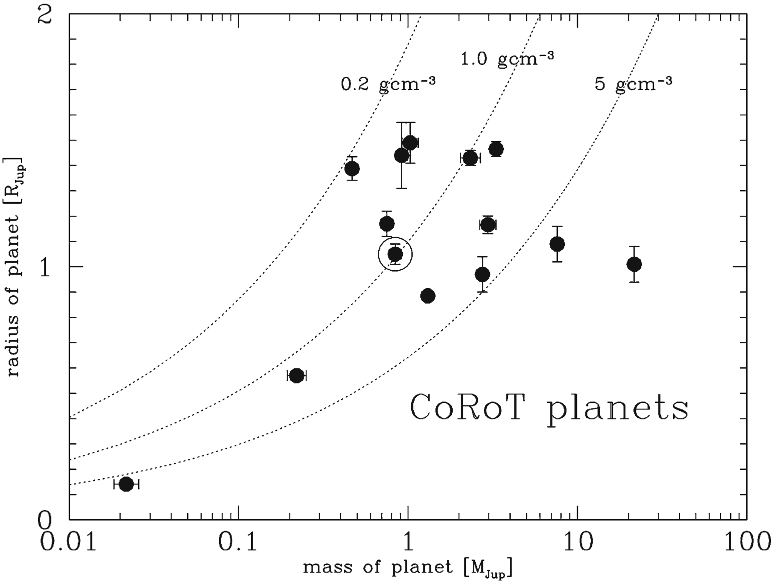

Figure 3. Mass/density diagram for the planets found by CoRoT. Into the realm of low-mass planets.

3.1.2. The Properties of the Atmospheres of Gas-Giant Planets

By observing the secondary transit in the optical and in the infrared, it is possible to determine the amount of reflected light and the thermal emission of a planet. The first detection of the reflected light of an extrasolar planet was obtained by the CoRoT satellite (Snellen et al., 2009). In good agreement with the expectation, hot Jupiters are very dark. The reflectivity is smaller than 20 %. The first observation of the thermal emission of an extrasolar planet was obtained with the Spitzer satellite (Charbonneau et al., 2005). Because all planets studied so far orbit close to their host stars, their temperatures are correspondingly high. Temperatures in the range between 1,000 and 3,000 K are typical (e.g. Charbonneau et al., 2005; Deming et al., 2005; Croll et al., 2010; de Mooij and Snellen, 2009; Rogers et al., 2009; Lopez-Morales et al., 2010).

Two transiting planets with masses below 10 M_{Earth} have been found: CoRoT-7b (Léger et al., 2009) and GJ1214b (Charbonneau et al., 2009). The radii of these two planets are 1.68 ± 0.09 and 2.68 ± 0.13 R_{Earth}, respectively. The difference between CoRoT-7b and GJ1214b is that CoRoT-7b orbits a solar-like star and GJ1214b an M-star. With 1.87 ± 0.40 g cm^{-3}, GJ1214b has a rather low density. The density of CoRoT-7b is with 5.6 ± 1.3 g cm^{-3} comparable to the terrestrial planets in our solar system (Queloz et al., 2009). Models by Valencia et al. (2010) show that the mass and radius can be reproduced assuming a rocky planet with the same composition as the Earth. Thus, CoRoT-7b is the first confirmed rocky planet outside the solar system. Marcy (2009) suggests that

GJ1214b could be a waterworld, a planet that contains a large quantity of water. The possible existence of ocean planets was already discussed before the discovery of GJ1214b (Selsis et al., 2007a). The temperatures of the side facing the star of GJ1214b and CoRoT7b are very high, 390–560 K for GJ1214b and 1,800–2,600 K for CoRoT-7b.

RV surveys now turn up more and more systems containing low-mass planets. Already 20 planets with $m \sin i$ below 10 M_{Earth} are known. The current record holder is the system of planets orbiting HD10180. Five of them have masses in the range from 13 to 25 M_{Earth}, another one seems to have only 1.4 M_{Earth}, and there seems to be also a brown dwarf companion (Lovis et al., 2010). We can therefore be confident that there is a large population of planets with less than 10 M_{Earth}. The interesting question is whether these are mostly rocky or ocean planets.

Terrestrial planets form in three steps: Initially, dust settles into the mid-plane of the disc and is accreted to form planetesimals. Then, planetary embryos form by the collisional accretion of planetesimals. The growth of the embryos occurs initially via runaway growth (the increasing geometric cross section and gravitational field allow for the accretion of an ever increasing number of planetesimals) before transitioning to oligarchic growth (neighbouring embryos grow at similar rates). Finally, as numbers of planetesimals decrease, the interaction between embryos becomes the dominant factor as they perturb each other on to crossing orbits, leading to accretion via violent collisions (see review by Chambers, 2001).

3.2. TEMPERATE PLANETS ($0.1 < a < 2.0$ AU)

About 48 % of the known planets belong to this class. This region is particular interesting because for solar-like stars it includes the so-called habitable zone. The habitable zone is defined as the region in which the equivalent temperature allows liquid water to exist on the surface of a low-mass planet (Kasting and Catling, 2003). In the case of a solar-like star, this region is at a distance between 0.7 and 1.3 AU from the star. However, whether liquid water really exist depends also on the composition of the atmosphere, particularly the greenhouse effect. Therefore, not every planet (or satellite) in this region that has the mass of the Earth will be habitable. In a review article, Lammer et al. (2009) summarizes all possibilities for the habitability of planets. Class I habitats represent bodies on which stellar and geophysical conditions allow Earth-analogue planets to have multicellular life forms. The detection of transiting planets down to the size of the Earth in this region is the aim of the Kepler and PLATO missions. These missions will also have the capability to detect Earth-like satellites of gas giants in the habitable zone.

For M-stars, the habitable zone is at a distance of about 0.1 AU. However, planets at this distance are tidally locked. One side is exposed to X-ray and XUV radiation from the star (Tian, 2009). Whether the M-star planets Gl 571c and Gl 571d are habitable is intensively discussed (Selsis et al., 2007b).

An important factor for the habitability of planets is the eccentricities of the orbits, which are on average 0.30 in this region. Only 19 % of the planets have orbits with eccentricities smaller than 0.1. The relatively large eccentricities imply that that has to be a lot of interaction between planets and other bodies in the first 100 Myr.

Not too much is known about the properties of the planets. Only two transiting planets have been found which have semimajor axis larger than 0.2 AU: HD 80606 b (Moutou et al., 2010) and CoRoT-9b (Deeg et al., 2010). HD 80606 b has an eccentricity of 0.934 ± 0.003 and CoRoT-9b one of 0.11 ± 0.04. The fact that CoRoT-9b is not inflated supports the idea that inflation is caused by the interaction between stars and planets. The temperature of CoRoT-9b is estimated to be between 250 and 430 K. Since moons of giant planets may also be habitable (Williams et al., 1997; Kaltenegger, 2010), this planet would be a good target in order to search for a habitable moon. Large moons can be detected via transit-time variations (Sartoretti and Schneider, 1999).

3.3. THE SNOW LINE AND BEYOND ($a > 2.0$ AU)

In Hayashi's (1981) original model, ice condenses out when the temperature in the protoplanetary disc drops below 170 K (the so-called ice or the snow line). In the original model, the ice line was located at 2.7 AU. Newer calculations place it closer to the star (Lecar et al., 2006). In the core-accretion scenario, giant planets form in this region and then migrate inwards. Giant planets in the outer region are important for the habitability of planets in the temperate region because they play a fundamental role in shaping the orbital architecture of planetary systems and because they affect the delivery of volatile materials to the terrestrial planets. At later evolutionary stages, they also protect inner terrestrial planets from life-inhibiting impacts (Wetherill, 1995).

The RV method is less sensitive for planets of larger orbital period because $K \sim P^{-1/3}$ (with P the orbital period and K the velocity semi-amplitude). Nevertheless, 23 % of all known planets belong to this class. Observations of microlensing events show that even M-stars have planets at distances of several AU. The orbits in the outer region are as eccentric as the temperate planets. The average eccentricity is 0.34. Only 23 % of these planets have orbits with eccentricities smaller than 0.1.

Using direct imaging methods, a number of planet candidates have been found. Up to now, the method is limited to young planets because young planets are much brighter than older ones at infrared wavelength. Because young planets contract, they are bright in the infrared. Possibly the most interesting object detected so far is β Pic b. This planet has a mass of 7–10 M_{Jup}, an age of 12 Myr, a temperature of 1,700 K, and its orbital period is possibly not much longer than a few decades (Lagrange, 2010; Quanz et al., 2010). Three planets were found for HR8799 (Marois et al., 2008). β Pic and HR8799 are both A-stars, and both have debris disc. More than 20 stars with debris disc are now known. Since the lifetime of dust grains due to collisions is much smaller than the age of the host stars, there has to be a source for the grains. The most plausible source is planetesimals. Thus, in β Pic and HR8799, we can study the planets together with the seeds of planet formation.

Most other planet candidates have projected distances of the order of 100 AU or more. In this respect, it is very interesting that S Ori 68, a free-floating object with the mass of a planet (FFPMO), is located at a projected distance of only 1,700 ± 300 AU from a brown dwarf ($m = 45\ M_{Jup}$), which also has the same age and distance from the Earth as the FFPMO (Caballero et al., 2006). This object thus is not really free floating, which means that there is a gradual transition between planets of very long orbital period and free-floating objects. Planets that can be observed directly have the advantage that their masses can be determined spectroscopically if spectra of sufficient quality are available. Future telescopes as powerful as the E-ELT will allow studying the atmospheres of planets as small as Neptune.

Moons of such long-period planets may even harbour life, if they are tidally heated to the right amount. Such life would then be similar to the life forms which are discussed for Europa (moon of Jupiter) and Enceladus (moon of Saturn) (Abel, 2007; Lammer et al., 2009).

4. The Properties of Planet-Hosting Stars

The environment of the planets certainly plays an important role for their formation, evolution and for their habitability. The host star's EUV and X-ray radiation is the key property determining the temperature of the exosphere, which in turn governs the evaporation rate of the atmosphere. Evaporation of the atmosphere has already been observed for the transiting planet HD209458b (Vidal-Madjar et al., 2003). Coronal mass ejections and X-rays emitted by flares from the host star may also have a negative effect for the habitability of planets. Schaefer et al. (2000) identified nine superflares with energies of 10^{33}–10^{38} ergs which were observed on otherwise normal Sun-like stars. They estimate that a normal solar-like star produces such a flare every 600 years. In this context is the discovery made by Apollo 11 particularly interesting. The astronauts from Apollo 11 discovered small lunar craters that frequently contained lumps of soil whose upper surfaces are coated with glassy material. It is interesting to note that the glassy material cannot be older than about 20,000 years. Gold (1969) and Zook et al. (1977) suggested that the glassy material has been created by a giant solar flare. If true, it must have been a flare which was large enough to heat up the lunar surface to its melting point. This would mean that giant flares are not that critical for the habitability of planets. Cuntz et al. (2000) pointed out close-in planet may also induce stellar activity.

A very important parameter for the formation and evolution of planets is the mass of the central star. Kennedy and Kenyon (2008) developed a model in which they take into account that the snow line moves inwards in the disc while the planet is forming. From their model, they derive a frequency of gas giants of 1, 6, and 10 % for stars of 0.4, 1.0 and 1.5 M_{Sun}, respectively. The theoretical prediction is in excellent agreement with the observations (Johnson et al., 2010). Stars with a higher abundance in heavy elements also have a higher frequency of gas giants. Both observations support the core-accretion scenario for the formation of massive planets. The picture is however slightly different for planets of lower mass, which do not show the same trend (Ghezzi et al., 2010). According to Israelian et al. (2009), planet-bearing Solar-like stars have less than one per cent of the primordial Li abundance, while about 50 % of the Solar analogues without detected planets have on average ten times more Li.

González et al. (2001) introduced the concept of the galactic habitable zone. By assuming that the metallicity of the star-forming region is important for the formation of terrestrial planets, and assuming that the relative abundances of Si, Mg, and Fe are important (Because they affect the mass ratio of the core to the mantle), and assuming furthermore that the amount of the radioisotopes ^{40}K, ^{235,238}U, and ^{232}Th is critical (in order to sustain plate tectonics), the authors concluded that habitable planets can only exist in very few regions of our galaxy. Because most galaxies are less luminous and thus have a lower abundance in heavy elements than the Milky Way, this would mean that habitable planets are very rare.

Whether or not our Sun is special has also been discussed intensively. In contrast to older results, a recent study by González et al. (2010) shows that the abundance patterns observed for the Sun are not related to the presence of terrestrial planets. It is interesting to note that the Sun's orbit around the galactic centre is less eccentric than 93 ± 1% of the F, G, and K stars in our neighbourhood (Robles et al., 2008). The Sun also orbits close to the corotation circle, which minimizes the number of spiral-arm crossings (Mishurov and Zenina, 1999). Whether these properties of the Sun are important for the formation and evolution of life is an open issue.

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"LIFE WITHOUT WATER": THE SLEEPING CHIRONOMID AND OTHER ANHYDROBIOTIC INVERTEBRATES AND THEIR UTILIZATION IN ASTROBIOLOGY

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1. Introduction: Anhydrobiosis in Living Organisms

An increasing number of terrestrial organisms are becoming recognized for their ability to withstand various extreme abiotic stresses. Understanding the limits of such resistance is fundamental in assessing the possibility of interplanetary transfer of life and also to determine a search strategy for signs of life during future interplanetary missions.

Among the organisms successfully adapted to severe environmental stresses, those resistant to water deficit are of special interest as promising models for astrobiological experiments, since general effect of space vacuum on living cells is extreme dehydration. While most living organisms have limited ability to resist water loss, many species from certain taxonomic groups have evolved the ability to inhibit this. However, there are also organisms which can resist extended period of complete dehydration, entering a particular state termed *anhydrobiosis* (Watanabe, 2006; Crowe and Crowe, 1992).

It is widely accepted that anhydrobiosis had evolved independently several times, but all anhydrobionts share key features: in a water-free state, they lack all dynamic features of a living organism due to the absence of metabolism but preserve biomolecules and are able to restore biological activity upon rehydration (Crowe et al., 2002; Crowe, 2002; Wolkers et al., 2002; Goyal et al., 2005b). While there are many anhydrobiotic species among the bacteria, the protists, and the plants, among the metazoans, anhydrobiosis is found only in several groups of invertebrates. The invertebrates, being the highest and most complex anhydrobionts with a developed system of tissues, the genetic system close to the mammalians and a highly developed host-symbiont interactions, are probably the most attractive group for application in astrobiological studies. In this short review, we outline the key features of the main groups of anhydrobiotic invertebrates that are already utilized or are being proposed for future application in real astrobiological and space life sciences programs both inside of the spacecraft and in the outer space.

2. The Holo-Anhydrobiotic and Developmental Stage-Restricted Anhydrobiotic Invertebrates

The term “holo-anhydrobiotic” is used to refer to the groups of invertebrates which can enter an anhydrobiotic state at any stage of their life cycle (Jonsson, 2005). This group consists of taxa with direct development, and the ability to undergo anhydrobiosis often extends to the egg stage and includes three types of metazoan invertebrates: the rotifers, the tardigrades, and some nematodes (Rebecchi et al., 2007).

The bdelloid rotifers are small ciliated invertebrates abundant mostly in the freshwater (Caprioli and Ricci, 2001). The ability of most bdelloid species to resist complete desiccation made this group ubiquitous, inhabiting even temporally hydrated environment (Marotta et al., 2010; Denekamp et al., 2010). Intensive studies during last several decades revealed that high resistance of the rotifers to complete desiccation can be explained to a large extent by the combination of several key biochemical, physiological, and genetic mechanisms, including the evolving of specialized groups of proteins, increasing the number of copies of the protective genes in the genome, and successfully coping with dehydration-induced damaging factors (Pouchkina-Stantcheva and Tunnacliffe, 2004; Tunnacliffe and Lapinski, 2003; Ricci and Caprioli, 2005).

The tardigrades are arthropod-like creatures, initially discovered by Leeuwenhoek in 1702 together with rotifers in rehydrated sediments (Wright, 2001). Due to remarkable ecological plasticity, this group successfully exploited most of the terrestrial environments, from dry regions to oceans substrate. While marine species of tardigrades are non-desiccation resistant, many terrestrial tardigrades are truly anhydrobiotic. Similar to rotifers, upon dehydration the tardigrade forms a “tun,” a morphological form with decreased body water content down to 2–5% of the hydrated state. In anhydrobiotic form, tardigrades show high cross-resistance to external stresses and can be stored for many years (Crowe and Madin, 1974; Hengherr et al., 2008; Schill, 2010; Somme, 1996).

The soil nematodes are the third widely distributed group of metazoan holo-anhydrobiotic invertebrates, which can successfully withstand extreme conditions such as hot and cold deserts and temporarily desiccated habitats (Crowe and Madin, 1974; Womersley, 1988). In anhydrobiosis, the nematodes lose more than 90 % of their body water and cease all metabolic activity, but unlike the rotifers and the tardigrades do not form tuns (Nicholas and Stewart, 1989; Riley et al., 2001; Wharton and Barclay, 1993; Womersley, 1988). Nematodes show diverse patterns of desiccation resistance-related biochemical and physiological mechanisms, and there is a “slow dehydration” (for survival under relatively high rates of dehydration) and “quick dehydration” (possess adaptations that slow down the rate of water loss) strategies (Crowe and Crowe, 1992; Goyal et al., 2005a; Riley et al., 2001; Chen and Glazer, 2004). The true anhydrobiotic species are found among animal and plant-parasitic, entomopathogenic, and saprophytic nematodes

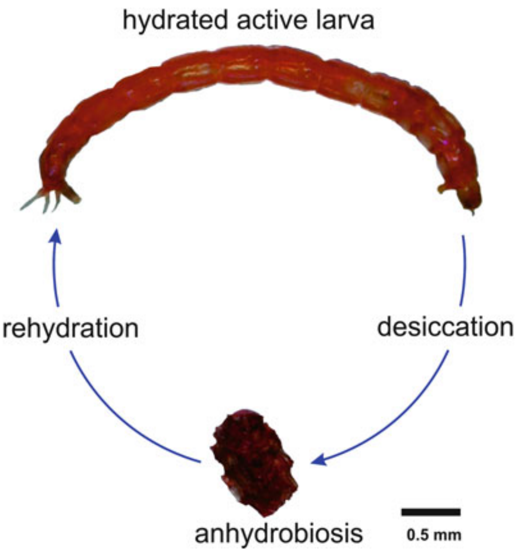

Figure 1. Anhydrobiosis in the sleeping chironomid *Polypedilum vanderplanki.*

(Crowe and Madin, 1975; Crowe et al., 1977; Darby and Neher, 2006; Saeed and Roessner, 1984).

The second group of anhydrobiotic invertebrates includes species in which their ability to anhydrobiosis is restricted to early developmental stages. The most studied examples of this group are the dormant eggs and the cysts of lower crustaceans and the larvae of an African chironomid *Polypedilum vanderplanki* (Fig. 1).

Anhydrobiosis in the lower crustaceans is an extreme form of diapause: endogenously controlled form of dormancy that is characterized by developmental arrest (Alekseev, 2007). The brine shrimp, *Artemia*, is an anostracan crustacean known to inhabit hypersaline water bodies (Watanabe, 2006). The females of this genus respond to changes in the photoperiodicity, the population density, and/or the salinity by switching from ovoviparous to oviparous reproduction of encysted embryo (Crowe and Crowe, 1992; Glasheen and Hand, 1988; Clegg, 2007). The diapausing cysts have greatly reduced metabolism, show high stress tolerance due to rigid shell, and decreased water content, associated with accumulation of nonreducing disaccharide trehalose and molecular chaperons, preventing irreversible protein denaturation and inhibiting apoptosis (Clegg, 2007; Qiu and MacRae, 2010). The similar, yet less studied, adaptations are present in freshwater tadpole shrimps *Triops* and water flea *Daphnia* (Vanvlasselaer and De Meester, 2010; Shinokawa, 1997).

The African sleeping chironomid *Polypedilum vanderplanki* is the only known true anhydrobiotic insect (Watanabe et al., 2002). Many insects are resistant to water loss, but the majority cannot tolerate desiccation beyond a certain threshold. However, the larvae of *P. vanderplanki* inhabiting the temporal water pools in semiarid areas of Africa get completely dehydrated upon seasonal evaporation, yet they are able to restore metabolism upon rehydration and continue ontogenesis (Fig. 1). Interestingly, the sleeping chironomid is also a single anhydrobiotic species in a large *Polypedilum* (chironomid) genus, consisting of more than 100 species. The sleeping chironomids are able to undergo anhydrobiosis only during the larval stage, which lasts 2–3 weeks out of approximately a month long life cycle (Watanabe, 2006; Watanabe et al., 2003, 2005).

Recent studies suggest this species, similar to other invertebrate anhydrobionts, tolerate desiccation by replacing its water by trehalose and through the activity of specialized proteins including LEA and chaperons (Kikawada et al., 2006; Wolkers et al., 2002; Wright, 2001; Tunnacliffe et al., 2010; Gusev et al., 2010). The ability of anhydrobiosis in this species is likely to have evolved independently. In the anhydrobiotic form, the larvae can be preserved for an extended period of time and exhibit a high cross-tolerance to external stresses, including ionizing radiation (Watanabe, 2006; Watanabe et al., 2006b).

3. Resistance of Anhydrobiotic Invertebrates to Excessive Abiotic Stresses of Space Environment

Modern space technology enables the transportation of terrestrial organic matter or living organisms beyond the protective shield of the Earth's atmosphere, which allows the actual study of their response to a spaceflight environment. In the case of manned spaceflight, biological systems, including humans, are protected from most of the hostile parameters of space by containment within a space capsule which is a pressurized module with an efficient life support system. If living organisms were exposed to space without any protection, they are confronted with an extremely hostile environment, characterized by a high vacuum, an intense radiation field of solar and galactic origin, and high temperature fluctuations (Horneck, 1982, 2003; Horneck and Bucker, 1986; Horneck et al., 1984). This environment, or the selected parameters of it, is the test bed for astrobiological investigations, thereby exposing biological systems to selected parameters of outer space or defined combinations of them. In contrast to unicellular organisms (mostly bacteria) intensively used both in laboratory and real spaceflight studies, higher multicellular organisms received less attention (Brack et al., 2001; Meyer et al., 2008; Stoffler et al., 2007).

Anhydrobiotic invertebrates, as complex multicellular organisms possessing high resistance to environmental stresses, are the promising model for studies focused to understanding of limits of resistance of terrestrial organisms to the harsh conditions of space. The results of such studies would include estimations on the upper limits of the biosphere; assess the possibility of interplanetary

Table 1. Longevity records of anhydrobiotic invertebrates.

Taxa	Species	Longevity/cryptobiotic form
Insecta	*Polypedilum vanderplanki*	17 years (larva)
Crustacea	*Branchinecta packardi*	16 years (encysted embryo)
Crustacea	*Artemia franciscana*	15 years (encysted embryo)
Crustacea	*Streptocephalus sealii*	15 years (encysted embryo)
Rotifera	*Mniobia* sp.	9 years (eggs)
Rotifera	*Mniobia magna*	2.5 years (adult)
Rotifera	*Macrotrachela musculosa*	2–3 months (adult)
Nematoda	*Tylenchus polyhypnus*	39 years (adult)
Nematoda	*Anguina tritici*	32 years (adult)
Nematoda	*Ditylenchus dipsaci*	23 years (adult)
Tardigrada	*Ramazzottius oberhaeuseri*	9 years (eggs) 4.4 years (adult)
Tardigrada	*Echiniscu* spp.	3 years
Tardigrada	*Adorybiotus coronifer*	8 months

Modified from Watanabe (2006).

transfer of living organisms and defining the limiting factors of panspermia (a hypothesis of the distribution of life beyond its planet of origin); and, finally, provide understanding of the relevance of extraterrestrial organic molecules to the delivery of the building blocks of life on Earth or on other space bodies. Here, we describe the present knowledge on resistance of anhydrobiotic invertebrates to the key parameters which limit the distribution of majority of living organisms in the outer space: continuous preservation underwater-free conditions, resistance to high and low temperatures, and different types of radiation.

3.1. LONGEVITY IN ANHYDROBIOSIS

The ability of anhydrobiotic invertebrates to survive under lack of water for periods comparable with durations of interplanetary flight is one of the most attractive features for astrobiological studies. In principle, under a water-free condition, oxygen metabolism as well as all biochemical processes should be almost completely terminated. Although the life cycles of active invertebrates are relatively short, in anhydrobiotic form they able to maintain viability for decades and even hundreds of years. Among the records of the "long runners," there are descriptions of rotifers and tardigrades which revived after 120 years of dry storage on a moss in a museum and viable copepod embryos found in 40–323-year-old anoxic sediments. Table 1 represents some results of more detailed laboratory studies on longevity of anhydrobiotic invertebrates recently summarized by Watanabe (2006).

The longest records of recovery from the anhydrobiotic state are 39-year survival in nematodes, 17 years in insects, 16 years in crustaceans, and 9 years in tardigrades and rotifers. In some nematodes, the revival rate decreases with increase of the anhydrobiotic period: the maximum period for which the species can survive may be 10 years (Keilin, 1959). The variation in survival period between anhydrobiotic animals seems to depend more on the rate of water loss they can tolerate than on the absolute degree of desiccation stress they can survive (Wharton et al., 2002). As we see, from the perspective of astrobiology, the anhydrobiotic invertebrates appear to have the potential for resisting long-term ametabolic preservation during a continuous space flight. Furthermore, studies conducted on cysts of crustaceans, tardigrades, rotifers, and chironomid larvae provide evidence that in anhydrobiotic form they can successfully withstand a prolonged exposure to vacuum. In some cases, preservation under vacuum conditions even increased survivability, probably, due to the absence of oxidative damage, proposed to be one of the factors that limit the longevity of anhydrobionts (Franca et al., 2007; Neumann et al., 2009; Hengherr et al., 2008; McLennan, 2009b).

3.2. RESISTANCE TO TEMPERATURE EXTREMES

In space, the temperature of a body depends on its position with respect to Sun and other orbiting object, as well as on its surface, size, mass, and reflectivity. In low Earth orbit (LEO), the main energy sources would be solar radiation, the Earth's reflectivity, and the terrestrial radiation. Due to 90-min orbit lasting, a spacecraft in LEO is exposed to the sun for about 60 min and shadowed by the Earth for the next 30 min. So, even in LEO, a body temperature can potentially reach both extremely high and extremely low limits. For example, the International Space Station (ISS) expected possible fluctuation of the temperature to be within the range of +120 to −120°C(Baglioni et al., 2008).

The given range of possible temperature fluctuation in outer space and extreme heating of a space body upon entering the planetary atmosphere would be one of the most critical limiting factors that determine survivability of life form in a spaceflight without additional protection. In contrast to prokaryotic extremophiles, some of which are successfully adapted to life under high temperatures, higher metazoans have very limited capacity to cope with excessive temperature regime, especially on its higher values. Surprisingly, under ametabolic dehydrated state, the anhydrobiotic invertebrates gain surprising cross-resistance to both high and lower temperatures, beyond the extremes under which active ones would survive(Watanabe, 2006; Hengherr et al., 2009).

In anhydrobiotic stage, both tardigrades and rotifers were reported to successfully resist a wide range of temperatures from continuous freezing at −273 °C to short-term overheating at +151°C. Encysted embryos of branchiopods and cysts of *Daphnia* and *Artemia* can continue development after heating at 60–80 °C (Clegg et al., 2001). Desiccated larvae of the sleeping chironomid revive after exposure to −270 to 103°C, although the survivability drastically decreases with an increasing time of heat stress (Watanabe, 2006; Grewal et al., 2006). While the exact mechanism of the heat tolerance of anhydrobionts is not fully understood, it is generally accepted that glass state formed by the molecular conservants replacing water (such as nonreducing saccharides, thermostable LEA proteins, and sHSP) also prevents general proteins from heat-induced denaturation (Gusev et al., 2010; Tunnacliffe et al., 2010; Hengherr et al., 2009).

3.3. RADIORESISTANCE OF ANHYDROBIONTS

Planets and other space bodies of solar system are continuously exposed to a complex radiation field of galactic and solar origin. The surface of the Earth is largely protected from cosmic radiations due to the deflecting effect of the Earth's magnetic field and atmospheric shield. Thus, terrestrial annual effective dose from combination of cosmic rays (including galactic cosmic radiation and solar cosmic radiations) is at least 100 timers lower than that in LEO. In addition, depending on orbit parameters and flight data, a space body, spacecraft, or exposed living organism would receive an additional dose of up to 20 mSv per month due to the interaction of the cosmic rays with the Earth's magnetic field (effect of radiation belts). Finally, of special interest to astrobiology is the resistance of terrestrial life forms to solar ultraviolet (UV). Stratospheric ozone layer of the Earth absorbs UV radiation at wavelengths shorter than 290 nm. Based on the calculations and direct measurements, solar UV is divided into three ranges of spectrums: UV-C (200–280 nm), UV-B (280–315 nm), and UV-A (315–400 nm). Among them, UV-C and UV-B, which contribute only about 2 % of the entire solar radiation, are most harmful to a living organism due to the absorption characteristics of DNA (with absorption maximum in the UV-C range) which is the target for inactivation and induction of mutations (Baglioni et al., 2008).

In contrast to other abiotic stresses, it is unlikely that anhydrobiotic invertebrates would experience selective pressure based on ability to resist to high doses of radiation during evolution. Yet, surprisingly, anhydrobionts often show extremely high tolerance to different types of ionizing radiation. Encysted dry embryos of *Artemia* can hatch with the median inhibitory dose (ID50) of 5,000 Gy of gamma rays, and there is only little effect on survivability of the cysts irradiated up to 1,000 Gy (Iwasaki, 1964). A tardigrade, *Macrobiotus areolatus*, can survive at least one hour after irradiation with 5,000 Gy of X-rays (May et al., 1964). Furthermore, desiccated as well as hydrated tardigrades *Richtersius coronifer* irradiated with 500 and 1000 Gy did not deviate in survival from non-irradiated specimens (Jonsson et al., 2005). For another tardigrade species *Milnesium tardigradum*, median lethal doses of 5,000 Gy (gamma rays) and 6,200 Gy (high-linear energy transfer (LET) heavy ions) in hydrated tardigrades and 4,400 Gy (gamma rays) and 5,200 Gy (LET) heavy ions in anhydrobiotic tardigrades at 48 h after irradiation have been calculated(Horikawa et al., 2006).

Hydrated and dried tardigrade *R. varieornatus* survive after irradiation with 4,000 Gy of heavy ions(Horikawa et al., 2008). Recently, the radioresistance of anhydrobiotic rotifers was demonstrated to be higher than for non-anhydrobiotic species in a series of experiments focused on analyzing stability of their reproduction system after exposure to doses of ionizing radiation up to 1,200 Gy (Gladyshev and Meselson, 2008). Anhydrobiotic nematode *Pratylenchus thornei* survives gamma ray irradiations of up to 5,000 Gy (Thompson, 1990). Hydrated active larvae of the sleeping chironomid *P. vanderplanki* survive for at least 48 h after gamma irradiation up to 4,000 Gy or heavy ions irradiation up to 3,500 Gy. In anhydrobiotic form, the larvae are even more radioresistant and tolerate gamma irradiation up to 9000 Gy or He ions irradiation up to 5500 Gy (Watanabe et al., 2006a, c, 2007).

It is known that insects, and invertebrates in general, have much higher radiation tolerance than vertebrates. In addition, anhydrobiotic metazoans in many cases are characterized by higher resistance to different types of radiations compared with their close non-anhydrobiotic relatives (Watanabe et al., 2006c; Gladyshev and Meselson, 2008). Functional and evolutionary relationship between the ability to anhydrobiosis and enhanced radioresistance is still subject of controversy. One possible explanation for the higher resistance of dried anhydrobiotic species is the shielding and ROS-scavenging effect of nonreducing sugars and other molecular protectants which replace the water in the body of invertebrates (Watanabe et al., 2006a; Yoshinaga et al., 1997). Another hypothesis is that DNA repair capacity is higher in anhydrobionts in general which is further increased during anhydrobiosis (Franca et al., 2007; Rebecchi et al., 2009; Schill, 2010; McLennan, 2009b). Recent studies using anhydrobiotic rotifers suggest that there would be desiccation-induced DNA damage associated with every cycle of anhydrobiosis. It is generally accepted that anhydrobiosis-induced fragmentation of genome DNA would be one of the reasons for massive horizontal gene transfer patterns, as well as specific patterns in evolution of stress response genes (Gladyshev and Meselson, 2008; Gladyshev et al., 2008). While desiccation itself causes only little modification to integrity of genome DNA of tardigrades and encapsulated cysts of *Artemia*, DNA damage accumulates in the tuns and the cysts proportionally to the duration of anhydrobiotic state (Neumann et al., 2009; Rebecchi et al., 2009; McLennan, 2009a). Dehydration of the larvae of the sleeping chironomid is associated with drastic accumulation of heat shock proteins, antioxidants, and increased expression DNA reparation enzymes, which most probably reflects desiccation stress-associated modifications of genome. While further detailed studies on the maintenance of genome integrity are yet to be conducted, it is obvious that evolution of anhydrobiotic invertebrates proceeded under strong pressure of harmful effect of desiccation on nucleic acids and resulted in improving DNA repair and protection machinery that would be one of the main factors explaining extraordinary resistance of this group of organisms to the effects of radiation.

4. Astrobiological Experiments of Exposure of Anhydrobiotic Invertebrates to Space Environment

Anhydrobiotic invertebrates can express extremely high tolerance against various abiotic stresses and their combinations, comparable with severe conditions of outer space. At the same time, for a long time, possibility of transfer of living organisms (especially higher multicellular forms) through the space ("panspermia" theory) has been criticized using several key arguments including lack of platforms for experimental testing and impossibility for terrestrial organisms to survive continuous exposure to the combination of harmful factors of space environment, especially space vacuum and radiation (Horneck, 2002; Horneck and Brack, 1992). However, in a sequence of unique experiments, it has been shown that at least bacterial and fungal spores can survive in both short-term (up to 10 days) unprotected and more than five years (if embedded in chemical protectant) exposure to space vacuum in a real spaceflight (de Vera et al., 2009; Baglioni et al., 2008; Stoffler et al., 2007).

Today, inspired by promising results from space experiments with prokaryotes, an increasing number of multicellular organisms are being actively utilized in astrobiological studies (Brack et al., 2001). We are now only at the starting point of the road leading to understanding the borders of resistance in higher terrestrial eukaryotes to space environment. Table 2 summarizes the chronology space experiments on anhydrobiotic invertebrates which have already been conducted and those which are waiting to be done in near future.

Series of LEO flight experiments using cysts of *Artemia* in the frame of Apollo and Cosmos biosatellites programs conducted in 1973–1990 provided the first evidence that such complex organisms as invertebrates can successfully survive short-term exposure to space environment in containers both inside and outside of a spacecraft (Planel et al., 1994; Gaubin et al., 1986). Two groups of the cysts (one of which was placed inside the spacecraft and another on the outer side to be exposed to outer space) successfully survived the spaceflights and showed no significant difference in the rate of hatching (Planel et al., 1989).

Further space experiments using preflight and on-flight irradiation of the cysts with gamma rays with the doses ranging from 50 to 500 Gy revealed that even such combination of stresses had little effect on survivability of the cysts. It was concluded that under conditions of ambient temperature and protection from space ultraviolet, encapsulated embryos of the crustaceans can resist harmful effects of space vacuum and excessive radiation level during a short-term spaceflight. New insights on the resistance of *Artemia* came from the results of unexpectedly prolonged Long Duration Exposure Facility (LDEF) space experiment. Under condition of protection from the space vacuum and ultraviolet, a part of encysted *Artemia* embryos were able to hatch after nearly six-year spaceflight (Gaubin et al., 1996).

Table 2. Astrobiological experiments using dormant stages of invertebrates.

Year	Spacecraft/facility	Test objects	Duration	Type of exposure
1973–1987	Bion, Salyut, Apollo/Biostack, Biobloc, Exobloc experiment series	Artemia cysts	6–22 days	Inside of spacecraft Partial exposure to outer space
1984–1990	LDEF	Artemia cysts	6 years	Inside of sealed container
1994	Foton-9/Biopan-1	Artemia cysts	10 days	Inside of spacecraft
1997	Foton-11/Biopan-2	Artemia cysts	11 days	Inside of spacecraft
2007	Foton-M3/Biopan-6	Tardigrades	14 days	Outer space
2007–2010	Biorisk-MSN	Crustacean cysts Insect larvae	2.5 years	Outer space
2009–2010	EXPOSE-R	Insect larvae	1.5 years	Outer space
2011–future	Phobos-Grunt/BioPhobos	Crustacean cysts Insect larvae Tardigrades	Several years	Inside of the sealed container
2011–future	EXPOSE-R2	Crustacean cysts Insect larvae	1–1.5 years	Outer space/Mars-like environment
2011–future	Biorisk-MSN2	Crustacean cysts Insect larvae	Up to several years	Outer space

Figure 2. Facilities for long-duration astrobiological experiments in outer space. (**a**): LDEF (1984–1990); (**b**): EXPOSE-R (2009–2010); (**c**): Biorisk-MSN (2007–2010) (Credits: NASA, Roscosmos).

In 2007, two tardigrade species were exposed to outer space environment in Bion-6 mission on LEO for 12 days. In addition to space vacuum and space radiation, the anhydrobiotic tardigrades were irradiated with whole specter of UV using artificial source onboard the spacecraft. While ultraviolet was lethal factor for most of the exposed tuns, the tardigrades that were not irradiated successfully resisted outer space conditions(Jonsson et al., 2008).

Another important step in understanding the cross-resistance of anhydrobiotic invertebrates to combination of stresses of outer space was made in "Biorisk" experiment. The Biorisk hardware (Fig. 2b) consists of three containers, each containing 24 petri dishes 65 mm in diameter. Every plastic dish containing various anhydrobiotic samples, including cysts of crustaceans and chironomid larvae, was equipped with a filter permeable to air. The loaded containers were transferred to RKK Energia and delivered to the ISS onboard Soyuz-TMA-10 in April 2007. On June 6, 2007, the Russian cosmonauts performed EVA, which included Biorisk hardware installation. The Biorisk hardware was placed on a support platform and attached to the outer wall of the PIRS docking node (Fig. 2).

Prior to the placement, lids of each container were opened. Due to changes in overall EVA schedules, the first container was removed 13 months later (rather than 6 months, as initially expected), the second one was retrieved after 18 months, and the final, third container was removed from the platform after 31 months of exposure to outer space. "Biorisk" was the first space experiment focused on exposure of higher eukaryotic organisms to outer space for duration comparable with that of interplanetary flight. In addition, due to the experimental design, no temperature control was conducted, and the biological objects experienced severe fluctuations in temperature with the highest values exceeding +90 °C (some of the plastic cases had melted). Surprisingly, most of the crustacean species as well as the sleeping chironomid larvae survived more than 1.5 years flight, showing that even terrestrial invertebrates can resist exposure to outer space comparable in duration to return mission to Mars (Novikova et al.). How the anhydrobiotic invertebrates have resisted high temperature during the exposure is yet to be explained in details, but laboratory experiments suggest that space vacuum, harmful for an ordinary living organism, might have acted as a thermo-insulator in outer space, preventing the biological objects from overheating, while the temperature of the containers increased (Baranov et al., 2009).

Taking together the results from both laboratory and real spaceflight experiments have shown that under conditions of protection from solar ultraviolet, anhydrobiotic invertebrates can fully resist combinations of harmful factors during a continuous spaceflight. Thus, data on resistance to solar UV in a real spaceflight would be of great interest in order to elucidate limits of resistance of this group of organisms to outer space. Since 2009, anhydrobiotic larvae of the sleeping chironomid together with other biological objects (mostly bacteria and biochemical components) are being exposed to different levels of solar UV radiation in outer space in a framework of EXPOSE-R jointly managed by ESA and Russian Space Agency. Design of EXPOSE-R hardware allows exposing the samples to different levels of solar UV by protecting them by different filters. The hardware is due to return to the Earth at the end of 2010 or early 2011, and analysis of the effect of different modes of exposure to UV on biological samples would provide long-awaited information on limits of resistance of toughest terrestrial organisms to complete combination of harsh factors of the outer space (Baglioni et al., 2008).

So far space experiments with living organisms were limited to LEO there is no evidence that our present knowledge about resistance of cryptobiotic organisms to space environment can be extrapolated to real interplanetary flights. Finally, there is even more exciting opportunity to extend our knowledge about the possibility to transfer living organisms through the space environment in real interplanetary mission. In “BioPhobos/LIFE” experiment in the frame of Phobos-Grunt mission to Mars’s moon, Phobos aimed to collect Phobos’s soil samples and return them back to Earth; a capsule with anhydrobiotic organisms will be installed in returning space module (Shishov, 2008). Scheduled for launch in 2011 and return in 2014, it will be the third actual experiment testing the ability of terrestrial life to survive in real interplanetary flight without specific life support system. At least two species of anhydrobiotic invertebrates, larvae of the sleeping chironomid and tardigrades, will be used as model organisms in this experiment, providing opportunity to get new exciting updates of our present knowledge about limits in adaptation of the toughest and most complex terrestrial survivors to space environment.

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HABITABILITY AND COSMIC CATASTROPHES

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1. Introduction

During the solar system's history, several catastrophic events occurred. Many anomalies of planetary parameters can be only explained by catastrophic events. To mention a few of them: slow and retrograde rotation of Venus, the formation of the Moon, large climate variation on Mars, etc. In this chapter, we define a cosmic catastrophe by an event or influence that is not related with the evolution of the planet itself and that considerably changes physical parameters of that planet (like orbital parameters, rotation rates, climate, etc.). These cosmic catastrophes can be further divided into events that are related to the special properties of the planetary system itself. The second class of cosmic catastrophes comes from the activity of their respective host stars, and the third class of catastrophes comes from outside the solar (planetary) system.

At the time of writing this chapter, more than 1000 extrasolar planets are known, and the study of cosmic catastrophes will be extended to some of these systems. It is of main interest however whether such catastrophes can influence on the habitability of the planets.

In the first section, we will discuss different catastrophes that can occur in a planetary system and give a comparison to what is known in the solar system. The final aim is then to estimate the significance of the different threads to life in the solar system and to estimate what could be the greatest thread to life therein. Finally, this will be extended to habitable zones around stars in general.

2. Cosmic Catastrophes in the Early Solar System

2.1. THE FORMATION OF THE MOON

The Earth moon's formation has long been a puzzle to science. The first explanation for the formation of the solar system dates back about 350 years (Descartes). Today, it seems well established that planetary systems form in an environment that is similar to that which exists throughout the galaxy and we even can observe processes of star and planet formation. Considering our solar system, there exist some facts that must be explained by any theory of its formation: the Sun contains most of the solar system's mass, but the largest part of the angular momentum is distributed in the motion of the planets about the Sun, the orbits of the planets is quite regular (they move in the same direction, coplanar nearly circular orbits), there exist two types of planets (terrestrial and gas planets), same sense of rotation and orbital motion of the planets (two exceptions: Venus and Uranus), belt of small asteroids, and the Earth has an unusual large satellite, the Moon. A review paper on theories of the solar system formation was given by Brush (1990).

In 1878, G. H. Darwin (Darwin, 1878) (the son of Ch. Darwin) proposed the fission theory for the origin of the Moon. The Moon had been once part of the Earth and was ejected from it by an instability triggered by the action of the Sun's tidal force. The scar left by the separation of the Moon could be the Pacific Ocean. There appeared several objections to this theory. For example, it was estimated that before the Moon separated from Earth, the Earth's rotation would have been around 5 h. This value is too small to explain fission. Even if such an event has occurred, the result would not be a single body since the material was inside the Roche limit. Roche proposed in 1873 that the Moon was formed from a nebula like the Earth and other planets. Therefore, the Moon was also called the sister to Earth. In 1909, See proposed that the Moon was formed elsewhere in the solar system and was captured by the Earth. The average density of the Moon is significantly lower than that of the Earth. The fission theory would predict that the Moon should be similar to the Earth's mantle. In 1965, Hartman estimated the age of the lunar maria at about 3.5 billion years. In July 1969, Apollo 11 returned the first lunar rock samples from the Mare Tranquillitatis. In 1974, W. K. Hartmann (see Hartmann and Davis, 1975) presented a hypothesis that the Moon was formed from material ejected into circumterrestrial disk by a large (>1,000 km) body that struck the early Earth. Differences in the chemical composition between Earth and Moon can be explained by the fact that most of the material that formed the Moon might have come from the impactor.

The Rb-Sr age of the moon is 4.48 (uncertainty plus minus 0.02) Gyr. Therefore, the collision occurred 70–110 Myr after the formation of the solar system (see also, e.g., Der-Chuen et al., 1997).

The Moon-forming impact had severe consequences to the Earth: it obtained a hot silicate atmosphere that cooled and condensed over about 1,000 years, and during this cooling, the Earth degassed its volatiles into the atmosphere. The magma ocean on the surface froze within about 2 Myr. The tidal heating by the new Moon was a major energy source to the magma ocean. As soon as a solid mantle was formed, a steam atmosphere rich in carbon dioxide was formed (about 100 bar about 500 K). A further cooling required a reduction of carbont dioxide. This removal could have happened by subduction within about 10 million years. Therefore, efficient subduction in combination with a faint young Sun leads to a cold early Earth with frozen oceans. In the case of an inefficient subduction rate, the carbon dioxide would have stayed long in the atmosphere, and therefore, the Earth's surface temperature was kept near 500 K for many tens of millions of years.

If carbonates in oceanic crust subducted as quickly as they formed, Earth could have been habitable as early as 10–20 Myrs after the Moon-forming catastrophe (Zahnle et al., 2007).

2.2. OTHER CATASTROPHES IN THE EARLY SOLAR SYSTEM

There are indications that several other catastrophes, mainly impacts, occurred in the early solar system. The rotation of Venus is in the opposite direction to its revolution about the Sun; therefore, it is called retrograde and it is extremely slow (sidereal period about 243 Earth days). The tilt of the rotation axis is 177° (Earth: 23.5°), and the nature of the retrograde rotation of Venus is still not clearly understood but could also have been caused by a major impact event (see, e.g., the simulation given by Castillo et al., 1998). Core-mantle friction inside the planet, together with atmospheric tides, could also have resulted in such an abnormal behavior. Correia and Laskar (2001) have shown that terrestrial planets with dense atmospheres can evolve into four different spin states; the behavior is rather chaotic, strongly depending on the initial conditions.

The ice giants Uranus and Neptune might have formed closer to the Sun because at their present location there would not have been enough time for their formation. Maybe they were scattered to their present orbits by multiple gravitational interactions with the gas giant planets, Jupiter and Saturn.

2.3. CHANGES OF ORBITAL PARAMETERS THAT INFLUENCE HABITABILITY

Let us first consider the orbital parameters of the Earth. These are not constant values because of the perturbations by the other planets in the solar system; thus, the stability of orbital parameters of a planet in a system strongly depends on the configuration of the objects therein.

In the case of the Earth, the following variations lead to the so-called Milankovitch cycle which triggered the onset of glaciation/deglaciation.

- Orbital eccentricity: varies between 0 and 0.06, variation ~100 kyr
- Axial tilt, obliquity: varies between 22.1 and 24.5°, variation ~41 kyr
- Precession: variation ~23 kyr

The Milankovitch cycles are generally acknowledged to have been the dominant factor governing the ice ages of the last several million years. The main driver for the eccentricity variation of the Earth's orbit is the eccentricity of Jupiter (0.04, a small value but very important). If Jupiter's eccentricity were greater, the amplitude of the earth's eccentricity variations would also be larger. Generalized Milankovitch cycles and long-term climatic habitability were discussed by Spiegel et al. (2010). They showed that the presence of giant planets in a planetary system can trigger eccentricity variations of habitable terrestrial planets there.

Many of the known extrasolar planets have masses comparable to Jupiter. About 20% of the known exoplanets have eccentricities >0.4; some have extreme values like the object HD 20782b with $e = 0.97$ (O'Toole et al., 2009). Miguel and Bruini (2010) developed a simple model of planetary formation and investigated planets with less than 10 Earth mass and considered the first 20 million years of the system's evolution. They simulated 1,000 different disks and found rotation periods between 10 and 10,000 h and a large population of planets similar to terrestrial planets in the solar system. Collisions between the objects play an important role, and it can be assumed that such collisions occur when their orbital distance Δa becomes less than 3.5 Hill radii. Let us assume a smaller body (e.g., Earth) with mass m that orbits a more massive body (e.g., Sun) of mass M with a semimajor axis a and eccentricity e; then the radius of the Hill sphere for the smaller body is

$$r \approx a(1 - e)\sqrt[3]{\frac{m}{3M}}.$$

Habitability on an Earth-like planet depends on the perturbation of giant planets beyond the Hill sphere for close encounters. A terrestrial planet may reach the zone outside habitability because of secular eccentricity variations and also strong motion resonances. In a study about habitability in known systems, Menou and Tabachnik (2003) concluded that about half of the systems might not harbor habitable Earth-like planets because of the perturbing influence of distant, eccentric giant planets. Systems with close-in giant planets could have a dynamically stable habitable zone (in their study, it was ¼ of the systems studied).

The planet's (semimajor axis a, eccentricity e, mass Mp) gravitational zone of influence is three times its Hill radius which now can be defined (M_* Mass of the central star):

$$R \approx a\left(\frac{M_{\mathrm{P}}}{M_*}\right)^{\frac{1}{3}}.$$

Within three Hill radii, collisions with the planet occur or with the central star or even ejections from the system. Let us consider a planet with eccentricity e; then the extreme distances from its host star are $(1-e)a$ and $(1+e)a$. Thus, the entire region of influence around that planet becomes

$$R_{\mathrm{in}} = (1 - e)a - 3R$$
$$R_{\mathrm{out}} = (1 + e)a + 3R.$$

For further details and a study applied to some extrasolar planetary systems, see Menou and Tabachnik (2003).

2.4. THE EARLY SUN

The early Sun was different from the present day. It was more active because of faster rotation and the activity occurred at higher amplitudes. Gough (1981) gave a formula for the past overall solar luminosity variation.

The radiation in the X-ray und UV domain was more intense during the formation of the planetary system by a factor of 100 for UV and 1,000 for X-rays. This contributed, e.g., to the photolysis of water vapor in the early Venusian atmosphere. However, the total luminosity of the Sun L was probably only about 70 % of its present value (Gough, 1981). There is no geologic evidence on earth that due to the lower solar luminosity, a global glaciation has occurred. This is also known as the faint young Sun paradox. Many solutions have been suggested such as a higher concentration of certain greenhouse gases in the Earth's atmosphere like ammonia, NH_3, and methane CH_4. But these are photochemically unstable. Measurements of CO_2 concentration (deduced from iron-rich minerals like siderite, $FeCO_3$, or magnetite, Fe_3O_4) showed that the CO_2 concentration was too low to resolve the paradox. Archaean CO_2 concentrations were only a factor of three higher than today, but the models require en enhancement of CO_2 by a factor of 70 to keep water above the freezing point in a low solar luminosity situation. Another solution might be a different albedo of the early Earth. There were less continents, and maybe the droplets of the water clouds were of different size because of the different atmospheric composition. A recent review on that problem was given by Kasting (2010).

The influence of solar evolution, especially the increase in luminosity on planetary atmospheres, was first studied by Sagan and Mullen (1972).

3. Definitions of Habitability

3.1. HABITABLE ZONES IN A PLANETARY SYSTEM

Various definitions of habitability have been given in the past. Now, it is generally agreed that the development of a carbon-based life requires (a) liquid water in the atmosphere and (b) must be stable over geologic timescales. This definition is mainly a requirement for the atmosphere of a terrestrial-like planet. It has to be taken into account that of course there are atmospheric feedbacks of such a planet that regulate its temperature. The luminosity of the host star also varies over long timescales. Hart (1978) constructed such a model of planetary habitability that accounted for the increasing solar luminosity and included the runaway greenhouse effect and the planetary glaciations effect. Kasting et al. (1993) showed that the habitable zone could be extended if one accounts for the positive feedback mechanism: the greenhouse gas CO_2 is released during volcanic activity, and this may happen even after planetary glaciation occurs. The main factors that regulate the temperature in the atmosphere of a terrestrial planet are H_2O and CO_2 clouds.

Another class of habitable objects in a planetary system is the moons. Let us first consider moons around terrestrial planets. In that case, the moons must be massive enough (>0.2 Earth mass) to (1) keep a stable atmosphere over long timescales and (2) sustain tectonic activity which is important to the carbon-silicate cycle. Such a moon must also possess a strong enough magnetic field to protect its atmosphere against the bombardment of energetic ions. If the moon is around a giant planet, the eccentricity of the giant's planet orbit is also important because large variations of the insolation flux can be expected in the case of a high eccentricity. Thus, there could exist habitable moons around giant planets that are located in the habitable zone around a central star, the planets themselves being not habitable. Moons of more distant giant planets can become habitable because of tidal heating (this could be the case for the Jupiter's satellite Europa).

3.2. THE GALACTIC HABITABLE ZONE

The extension from a circumstellar habitable zone to a galactic habitable zone was made first by Gonzales et al. (2001).

The galactic habitable zone is defined as an annular region lying in the plane of the galactic disk. This region contains the heavy elements necessary to form terrestrial planets and a stability (dynamical, evolutionary) over several billion years to allow the chemical evolution of complex multicellular life. The extension of this region can be estimated by combining the metallicity map of a galaxy with the constraints derived from extrasolar planet data. There exists possibly a correlation between large close-orbiting massive planets and high metallicity of the host star.

A probability $P_{\mathrm{GHZ}}(r,t)$ can be written as (GHZ denotes habitable zone, t means time, r the location inside a galaxy):

$$P_{\mathrm{GHZ}} = \mathrm{SFR} \cdot P_{\mathrm{metals}} \cdot P_{\mathrm{evol}} \cdot P_{\mathrm{SN}}.$$

With too little metallicity, Earth-like planets are unable to form; with too high metallicity, giant planets destroy Earth-like planets because they preferably then form near their host stars. The evolution time can be only deduced from the Earth's value: about 4 billion years for more complex life. Finally, in the equation above enters the number of supernova explosions due to the death of massive stars (>8 solar masses). A supernova triggers a blast wave and releases cosmic rays and X-rays which can be fatal to life on nearby Earth-mass planets. Another factor is the star formation rate, SFR. With a higher SFR, the supernova danger increases and therefore P_{SN} decreases. Intense star formation occurred in the inner galaxy; the heavier elements for life were provided; however, the high frequency of supernovae remained a danger for life. The GHZ emerged about 8 Gyr ago in

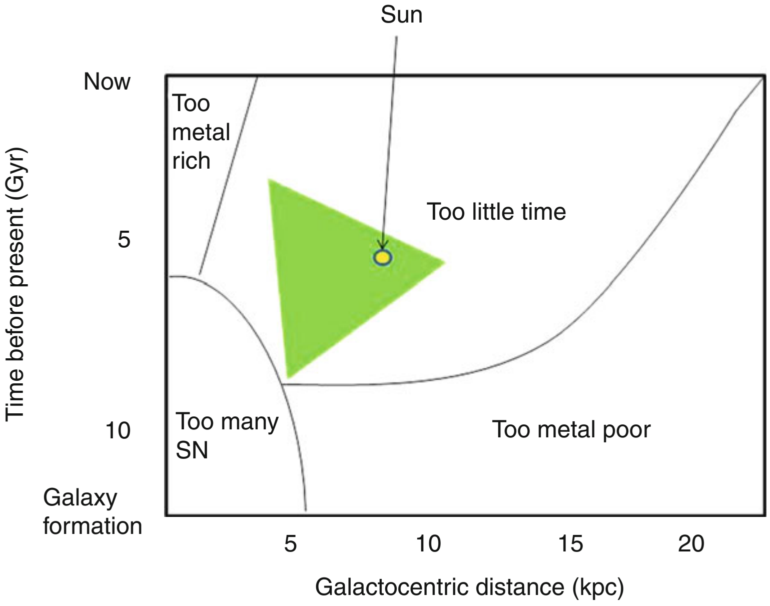

Figure 1. The galactic habitable zone (GHZ).

the outer regions and is expanding outward, as metallicity spread outward and the supernova rate decreased. From that, it follows that about 75% of the stars that could harbor complex life are older than the Sun, and their average is about 1 Gyr older than the Sun.

Summarizing, the GHZ in a galaxy is restricted to a certain region: (1) too near to the center there occur too many supernovae, and (2) too far from the center the stars are metal poor. More details about the GHZ can be found, e.g., in Lineweaver et al. (2004).

In Fig. 1, the galactic habitable zone is given for our galaxy. The situation will be quite similar for other spiral galaxies. The concept of a GHZ is criticized by Prantzos (2008) who argues that many physical parameters are still poorly understood and that maybe the whole galaxy could be habitable.

4. Impacts and Supernovae

4.1. IMPACTS ON EARTH

Large-body impacts may be the primary cause of mass extinctions of life on the Earth. Impacts can be assumed when layers with high Ir content, shocked minerals, and microtektites are found, and at least six cases of such catastrophic impacts that happened during the Earth's history seem to be well established.

Table 1. Periods of mass extinction during the Earth's history.

Period	Time (BP)	Percentage
Ordovician	444 million	85
Devonian	370 million	83
Permian	250 million	95
Triassic	210 million	80
Cretaceous	65 million	76

In Table 1, some known mass extinctions in Earth's history are listed. Percentage means the percentage of species extinct. Table 1 shows that five major known mass extinctions occurred during the past 500 million years and that in each case more than ¾ of all species became extinct.

The largest known craters are Manicouagan (about 100 km diameter) close to the end-Triassic, Doulon (China, about 80 km) near the Jurassic/Cretaceous boundary, Popigai (100 km) near the Eocene/Oligocene transition, and Chicxulub (>200 km) at the KT boundary. These events are strongly correlated with mass extinctions on Earth. There seems to be no correlation between impacts and mass extinctions for craters less than 50 km. A large impact is the structure detected on the Falkland Plateau (350 km) which may correlate with the end-Permian extinction (250 Myr) which was the most severe in the last 540 Myr. Large impacts create earthquakes with magnitudes >12, a deep fracturing of the crust results, and lava can penetrate to the surface (flood basalt events, Rampino, 1994).

In Fig. 2, the energy of an impact is plotted vs. the frequency of the occurrence. It is seen that a global catastrophic event is likely to occur every 10^5–10^6 year; however, a mass extinction event like the KT impact occurs at intervals of about 10^8 year. The solar system is surrounded by a cloud of cometary-like particles, the Oort cloud. The objects therein get disturbed by nearby passing stars and enter the planetary system, thus increasing the impact hazard there. It has been speculated that due to the passage of the solar system through the galactic plane, such events could become triggered because of the higher stellar density there. This however is still under question (for a summary and further references, see Hanslmeier, 2009).

4.2. IMPACTS IN EXTRASOLAR PLANETARY SYSTEMS

As it was discussed, most catastrophic events occurred during the formation phase of the solar system when, e.g., the Moon was created. Are such catastrophic collisions a general phenomenon that can be also observed in extrasolar planetary systems? What is the amount of energy released during such a collision and will such an event be observable? Studies about the stability of extrasolar planetary systems (i.e., the orbits of their planets) were made, e.g., by Sandor et al. (2007). They investigated 86 extrasolar planetary systems and found that 60% of them

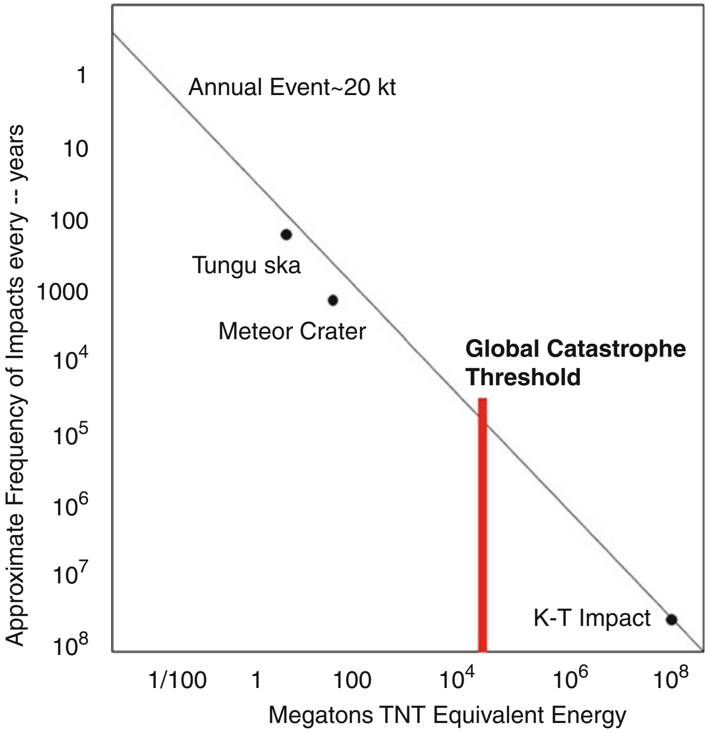

Figure 2. Energy of an impact on Earth and frequency of occurrence.

could harbor habitable Earth-like planets on stable orbits. In 18 cases, the conditions even seem to be better than in our solar system.

Let us assume that an Earth-sized object collides with a Jupiter-sized planet. Then, two effects can be observed, an immediate EUV to soft X-ray flash which lasts for several hours. This makes the detection probability extremely low. The second effect however is more easy to observe: an IR afterglow that will last for several thousand years. Zhang and Sigurdson (2003) studied how such signals could be observed. It is found by these authors that nearby effects (within 3 kpc) should be seen from radio observations. More simple observations can be made by photometrically monitoring a huge number of stars over long timescales. The future GAIA mission will monitor a sample of one billion stars for several years and maybe detect several of such collisions. Small body collisions can also be observed.

4.3. SUPERNOVA EXPLOSION

Stars with masses larger than about 8 solar masses end up as a supernova, leaving behind a neutron star or a black hole. On the average, a supernova occurs every

Table 2. Possible supernovae candidates in the solar neighborhood.

Star	Constellation	Distance (Lyr)	Type
Scheat	Pegasus	200 Lyr	Red giant
Mira	Cetus	230	Red giant
Betelgeuse	Orion	1,400	Red supergiant
Antares	Scorpius	470	Red supergiant
Ras Algethi	Hercules	550	Red supergiant
Sher 25		25,000	Red supergiant
Eta Carinae		7,500	LBV

50 years in a galaxy. During the event, the energy of $1\text{–}2 \times 10^{44}$ J is set free within a few seconds, and a shock wave propagates outward, and the velocity is in the range of 50,000–200,000 km/s. There are estimates that a near-Earth supernova within a distance of less than 100 Lyr would have severe effects on the biosphere. The most dangerous is the emitted gamma rays which react with molecular N in the upper atmosphere. The resulting N atoms combines with an oxygen atom, and nitrogen oxides are formed causing a depletion of the ozone layer. Possible supernovae candidates are listed in Table 2. According to Melott et al. (2004), an increase by 10–30% of UV radiation in the range 315–200 nm will have lethal consequences for life on Earth. Phytoplankton which is at the base of the food chain will be mostly affected. The effect of a nearby supernova can be treated in two parts: (1) short-lived gamma ray blast (300 days), 2×10^{47} erg, and (2) longer-lived cosmic ray component (20 year, 4×10^{49} erg). A supernova nearer than 8 pc could half the Earth's ozone column density (Gehrels et al., 2002). For our neighborhood on the average, such an event has to be expected once every 1.5 Gyr. Ellis and Schramm (1995) gave a higher probability (once every several 10^8 years) and estimated that the ozone layer might be strongly perturbed for hundreds of years. The most probable candidate for a supernova explosion within the next 10,000–20,000 years is Eta Carinae (Table 2).

Gamma ray bursts are hypernova explosions. They were detected by US military satellites in the late 1960s that watched for Soviet nuclear testings. These satellites carried gamma ray detectors on board because these rays are produced during nuclear tests.

The NASA Compton Gamma Ray Observatory with the BATSE instrument was launched in April 1991 and detected more than 2,700 gamma ray bursts (GRBs) distributed uniformly over the sky which proves their extragalactic origin. It can be estimated that once in the last Gyr, the Earth has been irradiated by a GRB that occurred in our galaxy at a distance lower than 2 kpc. Thomas et al. (2007) calculated that such an event would have produced a global ozone depletion by 35 %, and a significant decrease of ozone (about 50%) might have occurred for 5 years. The Earth's surface would have received three times the present UVB radiation. One hypothesis to explain mass extinction in the late Ordovician (445 My BP) is such a GRB event.

4.4. SHIELDING EFFECTS

As it has been discussed already, several shielding effects are provided by the Earth and the solar system environment. The galactic environment of the solar system changes due to (1) its rotation about the galactic center and (2) its motion perpendicular through the galactic plane. The Earth lies within the heliosphere that is a result of the solar wind and the solar magnetic field and provides a shielding against cosmic radiation. Gas planets outside a habitable zone play an important role. In the solar system due to the gravitational attraction of Jupiter, many cometary-like bodies are deflected, and therefore, the thread of a collision with planets in the habitable zone becomes considerably smaller.

The greatest threat to a habitable planet may come from large impacts. For the Earth, an impact rate of 1.31×10^{-15} km^{-2} year^{-1} can be given. The atmosphere of a planet provides a shielding against radiation hazards. For Mars due its extremely thin atmosphere, such a shielding does not exist, but it is conceivable that bacteria like the *Deinococcus radiodurans* may survive the harsh environment there. A planetary magnetic field provides a shielding against energetic particles that are mainly emitted by the host star. As has been stated already also the icy satellites of giant planets have to be considered as possibly habitable beneath a shielding ocean.

There are still other factors to consider like active phases of a galactic nucleus. This will enhance radiation and particle fluxes; however, at a certain distance from a galaxy (see chapter about galactic habitable zone), such effects will not play a dominant role. Collisions between galaxies are observed quite often. During such collisions, the density of stars and interstellar matter increases and therefore the perturbations in the Oort cloud, resulting in considerably large impact rates in the inner planetary system. In about 3 billion years, the galaxy will collide with the Andromeda galaxy. At that time however, the Earth would be no longer habitable since the solar luminosity will have increased. Maybe Mars would become a habitable planet. Another effect that threats habitability will be a strong increase in star formation.

5. Solar and Stellar Activity

5.1. SOLAR OUTBURSTS AS A PROXY

In this section, we discuss the variability of the host star and the consequences for planets around them. The influence of solar events on the habitability in the solar system can be summarized as space weather or space climate. The major disturbances from the Sun are (1) solar flares, (2) coronal mass ejections (CMEs), (3) the solar wind, and (4) radiation. We will discuss these briefly.

Solar Flares

Flares produce a burst of radiation, mainly in the short wavelength range, and they are classified according to their X-ray brightness in the wavelength range from 0.1 to 0.8 nm. If I denotes the intensity, then the following classes are used:

1. X-class flare, $I > 10^{-4}$ W/m^2, they trigger planet-wide radio blackouts on Earth and influence on the ionization of the upper Earth's atmosphere.
2. M-class flares, $10^{-5} < I < 10^{-4}$, medium sized can cause brief radio blackouts affecting mainly the polar regions.
3. C-class flares, $10^{-6} < I < 10^{-5}$, small flares, no consequences on Earth.

One of the most powerful events was the so-called Bastille Day Event (14th July, 2000). About 15 min after, the observed eruption energetic protons from the flares arrived at Earth, and besides damage in satellite detectors and solar panels on Earth, travelers at high latitude were exposed to radiation that is equivalent of a brief chest X-ray. It was estimated that 10^9 t of solar plasma was ejected at a speed between 1,300 and 1,800 km/s toward Earth. These particles hit the magnetosphere where they are deflected. Due to the compression of the magnetosphere, magnetic storms evolved which induced electric currents that interfered with electric power transmission on the surface. Thus, such events can have severe effects on civilizations at a high technical level. The global response of the low-latitude to mid-latitude ionosphere was studied by Huba et al. (2005). Aurora light shows were seen as far south as El Paso, Texas. Power companies suffered geomagnetically induced currents that tripped capacitors and damaged at least one transformer. Global positioning system (GPS) accuracy was degraded for several hours.

The Bastille Day flare coincided with a coronal mass ejection. About 31 h after the flare, an intense geomagnetic storm was observed (Fig. 3).

The amount of energy released (about 6×10^{25} J which is about 1/6 of the total energy output of the Sun each second) is the equivalent of millions of 100-megaton hydrogen bombs exploding at the same time. Flares are produced by the rearrangement of magnetic lines of force when two oppositely directed magnetic fields are squeezed together. Then, a sudden release of energy stored in the original oppositely directed fields occurs. This is called magnetic reconnection (Fig. 4).

Other extreme solar events that were studied in detail include the Halloween events around end of October 2003. During these extreme solar events, the stratospheric ozone decreases resulting in an increase of UV radiation at the surface. It was estimated that a 1% loss of stratospheric ozone results in a 2% increase in UV on the Earth's surface, and this could lead to about one million extra human skin cancers per year (Cunningham et al., 2005).

Coronal mass ejections are also caused by magnetic reconnection, but this occurs higher in the outer solar atmosphere. In principle, a CME is a massive solar wind bursts. Very often CMEs are associated with flares, and they occur during solar maxima and minima (but with a reduced frequency). The solar wind is a stream of charged particles ejected from the upper atmosphere from the Sun, and it consists mainly of electrons and protons. There exist two components, the slow speed solar wind (400 km/s, composition matches the corona, originates from regions around the solar equator, coronal streamers) and the fast speed wind

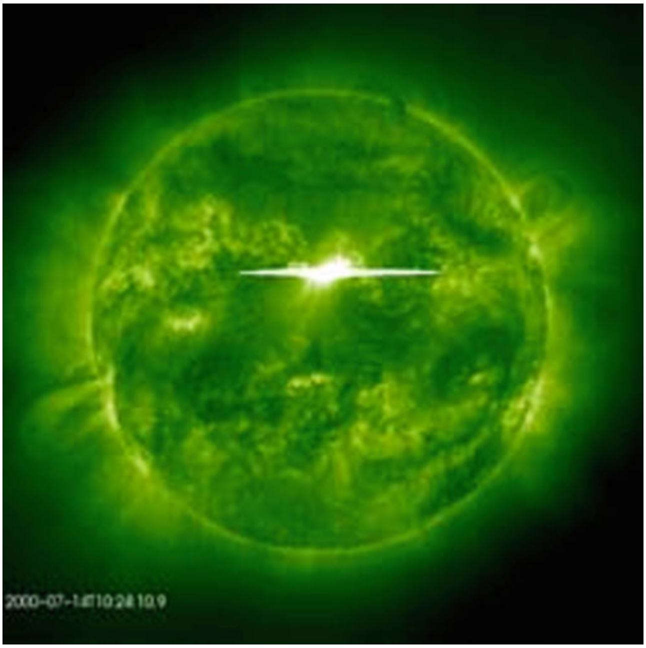

Figure 3. The Sun during the Bastille Day Event (Credit: ESA/NASA).

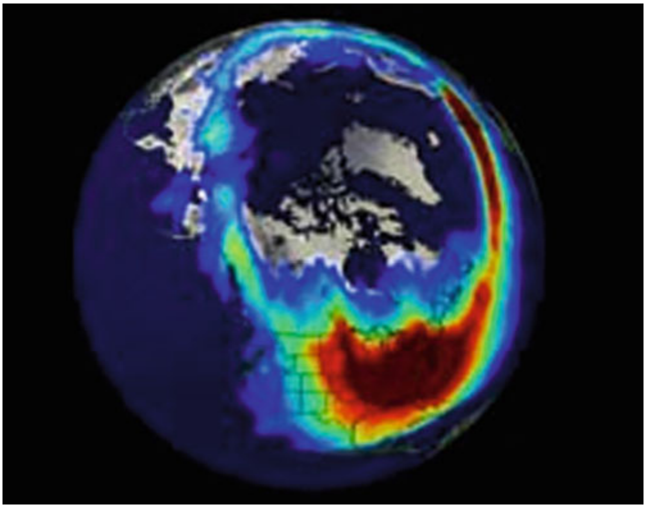

Figure 4. The Polar satellite (launched 1996) measured the auroral activity during the Bastille Day Event. *Black-red* areas in the image are the most intense auroral activity. The large area over the United States extends as far south as Florida (Credit: NASA and the University of Iowa).

(originates from coronal holes which denote open field lines, speed is around 750 km/s, composition is similar to the deeper solar photosphere). The total mass loss of the Sun is about 2–3 × 10^{-14} solar mass per year.

Thus, solar flares and CMEs influence on the state of the ionosphere, trigger geomagnetic disturbances and even change the composition of the atmosphere above the troposphere by ozone depletion. However, the amplitude of activity of the present Sun might not cause real catastrophic events on Earth. We must on the other hand also take into account that the shielding against charged particles is not at a constant level because the Earth's magnetic field varies and even changes polarity at quite irregular intervals. During such field reversal, the protection against charged solar particles becomes considerably weaker.

The influence of the solar wind and flares and CMEs on other planets becomes more severe when they have no magnetic field or atmosphere. For Venus, at solar maximum the pressure in the ionospheric plasma is sufficient to hold off the solar wind at an altitude of 400 km above the surface at the subsolar point and 1,000 km above the terminators. The deflection of the solar wind occurs through the formation of a magnetic barrier on the inner edge of the magnetosheath or shocked solar wind. Under typical solar wind conditions, the timescale for diffusion of the magnetic field into the ionosphere is so long that the ionosphere remains field free and the barrier deflects almost all the incoming solar wind (Russell et al., 2006). So Venus does not possess the magnetic shielding, but due to the above mentioned processes, a shielding against the solar wind particles is provided. Mercury possesses only a transient atmosphere and has a dense atmosphere but no shielding magnetosphere; therefore, atmospheric stripping by the solar wind occurs. This effect might have also occurred on Mars; about 1/3 of its original atmosphere has stripped away (Nilsson et al., 2010).

5.2. THE RADIATIVE ENVIRONMENT OF COOL STARS

In Fig. 5, the number of so far detected exoplanets as a function of the surface temperature of their host stars is given. Note that most exoplanets detected are found for host stars with surface temperatures between 5,000 and 6,500 K. Among this sample, there are 75 F stars, 198 G stars, 120 K stars, and 23 M stars.

In Table 3, we give some properties and the extension of the circumstellar habitable zone for these stars.

From this table, the following trends can be seen:

The HZ comes closer to the host star for later spectral types resp. lower surface temperature of the stars, and the extension of the HZ becomes smaller. Late M stars become more and more unstable with deeply penetrating convection zones. This causes large irregularities, and during eruptions of flares, the amount of radiation and particles will have extreme effects on planets in the habitable zones which lie at close distance around them. Many dMe stars emit large amount of UV radiation during flares. M stars are much smaller in mass than the Sun

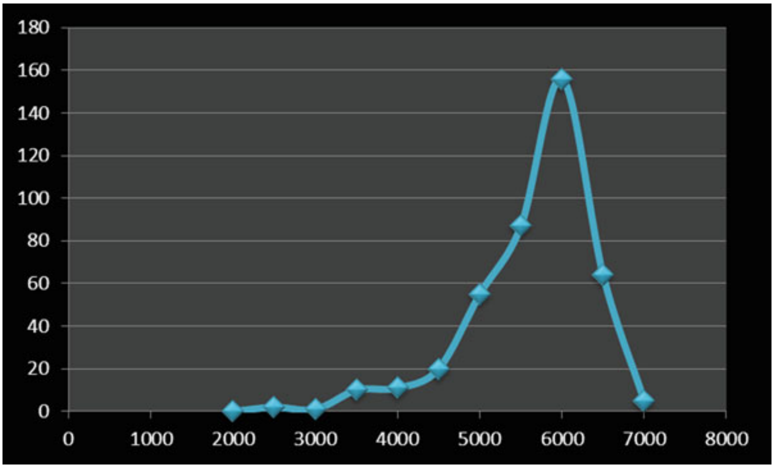

Figure 5. The number of detected exoplanets as function of the surface temperature of their host stars.

Table 3. Properties of late-type stars and their habitable zones (HZ).

Spectral type	Surface temp. (K).	Main sequence age (year)	Abundance %	HZ (AU)
F5V	6,400	4×10^{9}	4	1.3–2.5
G5V	5,800	2×10^{10}	9	0.7–1.4
K5V	4,400	7×10^{10}	14	0.3–0.5
M5V	3,200	3×10^{11}	72	0.07–0.15

(0.08–0.5 Mo). In the visible range, a M0 V spectrum represents 1.9% of the solar flux in the infrared 16%. On M stars, huge starspots cause strong variations of their luminosity on timescales of a few days, depending on their rotation rates. Planets in the habitable zone are mostly tidally locked because of their small distance. Atmospheric heat transport can prevent freezing on the dark side of the planet. A 1 bar pressure atmosphere would be sufficient to compensate for the stellar variations (Joshi et al., 1997). Stars of type dMe have strong flares. Scalo et al. (2007) studied the influence of such events on the atmospheric composition and chemistry. Typical stars of this type are AD Leo (M 3.5 Ve) and EV Lac (M4.5 Ve). During quiet conditions. the habitable zone for AD Leo is about 0.03 AU; in the case of a strong flare, it lies around 0.15 AU. For the emergence of life, a certain level of UV radiation seems to be necessary further details can be found in Buccino et al. (2007). Mechanisms of DNA repair could operate in the time between damaging flares. In consequence, the intermittent variation of UV radiation emitted by flares could be a source of higher mutation nrates. This, in turn, would imply higher microbial biodiversity and faster adaptation to the changing UV environmental conditions.

6. Conclusions

The influence of cosmic catastrophes on habitability of planetary systems can be seen in two respects. It can lead to mass extinction or even total extinction of life on a planet; it can, however, also trigger the evolution by mutation. For solar-like stars, the most probable cosmic catastrophe scenario is the impact by a large asteroid. This has occurred on Earth several times, and as it was shown, smaller than planet-sized bodies may be quite common in extrasolar planetary systems. In our solar system, the occurrence rate of such an impact is about once every 50 million years. Catastrophes by nearby supernova explosions are not very probable in the present solar neighborhood but may have occurred also in the past. Planetary systems around M stars suffer from the strong variability of the star and violent flares that occur at much higher frequencies than for the Sun.

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Biodata of **Joop M. Houtkooper**, author of "***Glaciopanspermia: Seeding the Terrestrial Planets with Life?***"

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GLACIOPANSPERMIA: SEEDING THE TERRESTRIAL PLANETS WITH LIFE?

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1. Introduction

The question of where and when life originated in our solar system centers on Earth, as this is the only planetary body of which we are certain that life exists. Therefore, it is our prevalent perspective to consider the place of origin of life to be Earth itself. It harbors the only life we know, while creation myths invariably place the origin of life on our own planet. The question is, is our prominent perspective based on a myopic preconception?

Several circumstances shed doubt on the Earth as the only place of origin of life as we know it. First, it has been established that it is possible that spores of life have been transported between planetary bodies, for instance, from Mars to Earth or vice versa (Horneck et al., 2008). Second, the Earth has not always been the benign environment for numerous species of organisms as it is now. In the early stages of the history of the solar system, the Earth may well have been sterilized. Third, other planetary bodies in our solar system may well have harbored, or still harbor, life. The overarching question is how likely it is that life originates on a planetary body where circumstances are more or less suitable for life.

Basic requirements for life are the presence of a source of energy or entropy, the availability of carbon compounds which constitute the building blocks for more complex compounds, and the availability of liquid water, temperature and pressure conditions which do not preclude the formation of more complex carbon compounds. According to these criteria, any planetary body which has at one time contained a substantial amount of liquid water—an ocean—is a possible candidate for the origin of life.

An examination of the possibility of the origin of life in different bodies of the solar system, and the likelihood that this life has been ancestral to life as we know it on Earth, including the possibilities of the transport to Earth, and to Mars and Venus, is the subject of this chapter.

2. Formation of the Solar System and the Occurrence of Life

2.1. THE FORMATION OF THE SOLAR SYSTEM

The solar system started to condense out of an interstellar cloud of gas and dust 4.566 Ga. That was the time the first mineral grains and CAIs, as preserved in meteorites and crystallized. In a few millions of years after that, protoplanets formed, including the proto-Earth. Further collisions added matter, until a giant impact occurred with a Mars-sized body, which resulted in the formation of the Earth-Moon system (e.g., Canup and Asphaug, 2001). The time of this Giant Impact can be dated at 4.527 (±0.010) Ga or 30–50 Ma after the beginning of the solar system (Kleine et al., 2005). Using similar dating methods, Yin et al. (2002) arrive at a date for the Giant Impact of 25–30 Ma after the beginning of the solar system, while Touboul et al. (2007) date the Giant Impact at 62 (−10/+90) Ma after the beginning of the solar system.

Although these authors arrive at a range of dates, it can be stated that the Giant Impact occurred significantly later than the main growth stage of terrestrial planet formation, which is estimated at ca. 10 Ma, with the smaller bodies forming more rapidly (Kleine et al., 2002). For instance, the core of the asteroid 4 Vesta, in all likelihood being the parent body of the eucrite meteorites, has formed in ca. 3 Ma (Yin et al., 2002).

2.2. THE HABITABILITY OF THE EARLY EARTH

If the early Earth had been habitable before, this habitability was thoroughly destroyed by the Giant Impact. But after the Earth cooled and became habitable after this late stage of Earth's accretion, its habitability has also been affected by the Late Heavy Bombardment (LHB). Apparent from a cataclysmic spike in the cratering rate on the Moon, the LHB must also have impacted on the Earth and other bodies in the inner solar system. The LHB is usually dated at 4.1–3.8 Ga or about 700 Ma after the formation of the planets; at which time, it may well have been caused by the migration and resonance of the giant planets (Gomes et al., 2005). The time of the LHB is close to the earliest indications of life on Earth, at 3.85–3.65 Ga.

The question is whether the Earth was sterilized during the LHB (Sleep et al., 1989; Hazen, 2005, p. 38). However, this depends on the size of the biggest of the asteroidal impacts and the maximum intensity of the LHB. The hyperthermophiles on the bottom of the oceans and the microbes kilometers deep in the crust were in good positions to survive periods of extremely hot conditions on the surface, short of the existence of a magma ocean (Abramov and Mojzsis, 2009).

Therefore, while it is possible that life has only been continuously present on Earth since the LHB, on the other hand, it may have been present before the LHB but after the Giant Impact which created the Moon. In both cases, life may have originated earlier elsewhere in the solar system, even if this depended on unlikely chance conditions, because we still only have evidence of life having originated once.

The evidence is that soon after the last impacts of the LHB, life occurred on Earth. This means that life either rapidly developed de novo, or that it rapidly arrived from elsewhere.

2.3. ORIGIN OF LIFE

The origin of life in a planetary body requires suitable conditions, liquid water, organic compounds, and a usable source of energy, such as chemical disequilibrium or solar radiation. Under those conditions, the origin of life is a process which is still not well understood. Therefore, the time it takes for self-reproducing, metabolizing organisms to appear is widely unknown. On the one hand, it may depend on a series of unlikely chance events in order for a macromolecule such as RNA to occur spontaneously from simple compounds, whereas, on the other hand, this may be an inevitable process, like the meandering of a river when flowing over loosely packed substrate matter. (In this analogy, life is regarded as a by-product of the increase in entropy involved in the conversion of solar photons of the visible spectrum to thermal IR photons, whereas the water in the river increases in entropy by flowing to a lower gravitational potential. In both cases, the entropy takes a more circuitous route than what seems the simplest route.) The emergence of complex patterns seems to be associated with dissipative processes (Hazen, 2005) so that life might be considered as the manifestation of meandering entropy. The biologist De Duve (1995) is even more outspoken in his "life as a cosmic imperative."

We may assume that when suitable conditions occur, life appears quickly on a geological time scale. At least, the oldest sedimentary rocks of 3.85 Ga already revealed signs of the presence of life, but whether life appears quickly de novo, or whether it is imported and therefore appears quickly, remains the question. The presence of life on Earth shortly after the Giant Impact cannot be excluded but no records of that period remain, except perhaps as terrestrial meteorites on the Moon or on asteroids.

Whereas life may also occur in exotic forms (e.g., see Schulze-Makuch and Irwin, 2004), life as we know it may well have started in the chemical disequilibrium conditions at hydrothermal vents on an ocean bottom.

The hypothesis that life may have started more than once has been put forward—for example, by Cleland and Copley (2005), who propose to look for relics of it on Earth. However, once a habitat has been invaded by organisms, these tend to use available resources to exhaustion. This makes it hard for latecomers to find a niche. Although life could have originated on different planetary bodies independently, the possibility of lithopanspermia may have caused the life on different bodies of the solar system to have a single origin. The crucial variables are the time it takes for life to develop de novo versus the time it takes for life to arrive by lithopanspermia. This is a trade-off with, at present, too many unknowns to obtain a theoretical estimate. The observational result would be many origins versus a single origin of the life on different planetary bodies.

2.4. CRADLE OF LIFE: WHERE?

The putative conditions for the origin of life were present in the planetary bodies which at one time in their history contained an ocean. These are the terrestrial planets, Venus, Earth, and Mars, and the larger icy bodies of the solar system—large enough to develop hydrothermal vents on the bottom of their oceans. We consider here particularly some of the moons of Jupiter, Saturn, and Neptune: Europa, Ganymede, Enceladus, Titan, and Triton, and the dwarf planets Ceres and Pluto.

As we have seen, the smaller bodies had at an earlier stage completed their accretion so that these stand a good chance of being the place of origin of life found elsewhere. Moreover, the smaller bodies have lower escape velocities, which make these a more proficient source of impact ejecta. The asteroids had their impacts from the LHB, but apart from a smaller cross section, many asteroids must have been fragmented, as is shown by 4 Vesta and its major impact crater at its south pole. However, Ceres survived the LHB relatively unscathed.

3. Lithopanspermia

The seeding of life, particularly of microbial spores, by meteorites has been shown to be a feasible process (Horneck et al., 2008). Lithopanspermia depends, first, on the departure of spores inside pieces of rock, ejected with more than escape velocity by a massive impact on the parent body. Second, the spores have to survive interplanetary transfer, which may take as long as thousands of even millions of years, by a random orbit which happens to intersect the receiving planetary body. Third, the arrival of the spore-bearing rock has to be survivable for the spores present.

The mechanics of lithopanspermia entail the problems of impact ejection, preservation during transfer, and arrival. The ejection of pieces of the surface into space requires achieving at least the escape velocity of the parent body. Calculations have shown that the shock wave pattern created by a large impact is able to accelerate pieces of the planetary crust to escape velocity. Horneck et al. (2008) have shown experimentally that viable microorganisms may well survive the pressures and temperatures involved.

Preservation during travel from the parent body to the seeded “child body” appears to be a lesser problem. The evidence that viable organisms may survive millions of years in fluid inclusions in halite crystals (Vreeland et al., 2000) bodes well for the survival of endolithic spores in meteorites.

The arrival of spore-bearing meteorites is a more severe problem for airless bodies like the Moon because of the shock upon arrival than for the terrestrial planets where meteorites may survive through aerobraking. This stage of lithopanspermia has been studied experimentally by the STONE experiments (Foucher et al., 2010).

The question which remains is, if life did spread from a single source to different bodies of the solar system, which are the likely candidates for the originating parent body?

3.1. SOURCES OF LITHOPANSPERMIA

Possible sources of life for lithopanspermia include Earth itself (before the LHB or even before the Giant Impact), Mars, Venus (if it had a more benign climate than today), and the icy bodies further out in the solar system.

Indeed, Earth may have "reseeded" itself after the Giant Impact or the LHB; that is, pieces of the Earth's crust ejected before, or by, a cataclysmic event, return to Earth after some time has passed and surface conditions have become benign again. This scenario is less likely because of the necessary time interval and the difficulty of ejection from Earth, see Table, and the escape of ejecta through the atmosphere (the same problem occurs with ejecta from Venus). Furthermore, reseeding only displaces the problem where life first came from. If life was present before the Giant Impact on the proto-Earth or its impactor, which likely will remain unknown, the question still is where that life might have come from.

The first step of lithopanspermia is the ejection of fragments of the surface into space. As the velocity distribution of impact ejecta falls off steeply, attention is drawn to bodies with lower escape velocities. For icy moons, the ejecta have to escape not only the moon but also the gravitational well of their planet (viz. Europa). Moreover, the ejecta have to reach the target planet, possible by gravitational slingshot effects, but more likely with an extra velocity increase sufficient for a Hohmann transfer orbit. Possible parent bodies for lithopanspermia are listed in Table 1: The velocity increase Δv is the minimum amount necessary to achieve a direct transfer orbit, assuming that the velocity vector of the ejecta points in the right direction. For the planetary satellites, this means that also the orbital velocity vector has to point in the optimum direction at the time of impact.

In the table, the Δv necessary for a Hohmann transfer orbit is given for each of the transfers to the three terrestrial planets. It turns out that moons deep in the gravitational well of their planet, like Europa, are at a disadvantage: Ejecta from Ganymede require less Δv, despite its larger escape velocity, as can be seen in the table. If we disregard far-out bodies like the KBOs, which we will discuss later, a likely parent body for lithopanspermia appears to be the dwarf planet Ceres.

As we consider icy bodies as the source of lithopanspermia, it may be useful to apply the term "glaciopanspermia" to this special case with its particular properties.

Table 1. Transfer of impact ejecta from ocean-bearing planetary bodies via Hohmann ellipse to Venus, Earth, or Mars.

Originating planetary body	In orbit around	Escape velocity (km/s)	Total Δv (km/s) for transfer to Venus	Earth	Mars
Venus	Sun	10.46	0	10.80	11.94
Earth	Sun	11.19	11.46	0	11.57
Mars	Sun	5.03	6.93	5.68	0
Ceres	Sun	0.51	6.40	4.88	2.86
Vesta	Sun	0.35	6.12	4.44	2.24
Europa	Jupiter	2.03	8.95	8.27	7.40
Ganymede	Jupiter	2.74	8.45	7.72	6.79
Enceladus	Saturn	0.24	7.98	7.55	6.96
Titan	Saturn	2.64	6.96	6.47	5.77
Triton	Neptune	1.46	4.85	4.67	4.41
Pluto	Sun	1.20	4.02	3.88	3.65

3.2. CERES

The dwarf planet Ceres has an average diameter of 935 (±5) km. It is a differentiated body with an average density of 2.21 (±0.04) g cm^{-3}. It likely consists of a rocky core and an icy mantle of more than 100 km thickness, possibly still partly liquid (Carry et al., 2008; Castillo-Rogez et al., 2007). Ceres cooled down early in the history of the solar system, and it probably has or at least has had hydrothermal vents on the bottom of its ocean (Castillo et al., 2008), and thus, life may have evolved early. Ceres has not suffered devastating impacts during the LHB; otherwise, its icy mantle would have evaporated and escaped. Life may well have survived minor impacts on the bottom of the 100-km-deep ocean.

The surface composition of Ceres has been affected by impact gardening and addition of chondritic material. The gradual freezing of the ocean and thickening of the icy crust has probably led to icy volcanism with material from the ocean being deposited on the surface. The spectroscopic detection of carbonates and phyllosilicates seems to confirm this (Rivkin et al., 2006).

3.3. CERES COMPARED WITH OTHER ICY BODIES

Vesta is not an icy body, but it is the second most massive asteroid after Ceres, and although it appears to be solid rock (density 3.4 g cm^{-3}), Vesta may have had an icy mantle before a major impact occurred (Thomas et al., 1997).

The moons of Jupiter and Saturn are situated in the gravitational potential well of their planet and to escape it extra energy is needed, see Table 1. The moons of Uranus, Titania and Oberon, are sizable icy bodies (Miner and Stone, 1988).

We know little of their internal structure, but the plane of their orbits is almost perpendicular to the ecliptic, so their orbital velocity does generally not contribute to the velocity change, necessary for impact ejecta to reach the terrestrial planets.

Plutoids, and also Triton, deliver ejecta which, coming from the outer reaches of the solar system, involve a relatively high inclination and a high relative speed when reaching Earth or Mars. Thus, the ejecta from KBOs have a relatively low probability of reaching the surface of one of the inner planets intact, as compared with ejecta from main-belt asteroids.

4. Glaciopanspermia

Impact ejecta from Ceres, for a substantial part consisting of ice—hence glaciopanspermia—will be subject to evaporation of volatiles, like comets are (see, e.g., de Pater and Lissauer, 2001, p. 373), although the material may well have a more compacted structure than that of a comet. The evaporation process will have the effect of a relatively short lifetime (Schorghofer, 2008), dependent on the size and structure of the ejecta. The other side of the same coin is that spores may be loosened by the evaporation and enter the atmospheres of the terrestrial planets as micrometeorites. Micrometeorites tend to enter the atmosphere, remaining quite intact (e.g., pictured by Duprat et al., 2010), which may well mean that glaciopanspermia is an effective way of transporting life.

5. Testing the "Ceres Origin" Hypothesis?

The Dawn mission will visit Vesta in 2011 and Ceres in 2015. Our knowledge of Ceres will greatly increase: its composition, internal structure, and geology, including past and possibly present cryovolcanism. As Ceres cools down, more of its water freezes and thereby expands: The increased volume of ice puts the ocean below under pressure. Biomarkers may be found in ejecting water vapor, of which signs have been found by A'Hearn and Feldman (1992), but this has not been confirmed by others (Rousselot et al., 2008), of course at another point in time. More of these observations are called for.

Corroboration of the Ceres origin hypothesis could come from commonalities with life or its biomarkers found elsewhere. Such biomarkers might be found in the ices at the Moon's poles, as there the arrival of pieces of Ceres' crust may have taken place at relatively low velocity. The conditions at the ever-dark crater floors at the Moon's poles are suitable for the preservation of organic material (Houtkooper and Schulze-Makuch, 2008). Other places where ejecta from Ceres may be found are the surfaces of other main-belt asteroids, especially the Gefion family of asteroids, as these have orbital parameters close to those of Ceres (de Pater and Lissauer, 2001).

5.1. POSSIBLE EXTANT CEREAN LIFE

Extant life on Ceres would be possible: The surface temperature of Ceres is in spots as high as 239 K, sufficient for life in brine-filled channels in its dirty-ice crust to survive even until today. Such life might employ photosynthesis or compounds such as oxidants created by radiation for energy and possibly hydrogen peroxide as an antifreeze (Houtkooper and Schulze-Makuch, 2007; Lipps and Schulze-Makuch, 2008). The life in the ocean might still survive by using serpentinization as a source of energy (Vance and Pappalardo, 2008).

The detection of life in the surface layers of Ceres would support the hypothesis. If so, a commonality of Cerean life with Terran and possible Martian life (Levin and Straat, 1979; Levin, 2007; Houtkooper and Schulze-Makuch, 2007) could provide a strong corroboration of the "Ceres origin" hypothesis. Also, Schulze-Makuch et al. (2004) have put forward the possibility of a habitable zone in the atmosphere of Venus so that also the putative Venusian life may have resulted from the same common origin.

6. Conclusion

Ceres, which formed at in early stage in the history of the solar system, (1) is a differentiated body with a rocky core and an icy mantle, possibly still partly liquid, with a depth of more than 100 km; (2) may have or have had hydrothermal vents at its rocky core and thus life may have evolved early; (3) has not been damaged much during the LHB, as it kept its icy mantle; (4) its impact ejecta need only a small Δv to transfer to Earth or Mars; and (5) is relatively accessible to further research. Other sources of life which originated elsewhere have been proposed, such as in comets (Sheldon and Hoover, 2007). Of course, the transport of life via lithopanspermia between Earth and Mars remains also a possibility. Moreover, the reseeding of Earth by its own ejecta has to be considered (Wells et al., 2003). Nevertheless, life may have originated earlier on Ceres and may have spread from there by glaciopanspermia.

The seeding of the terrestrial planets from Ceres is a hypothesis that can be tested by, first, the detection of life in the crustal layers of Ceres and, secondly, a commonality of Cerean life with Terran and possible Martian and Venusian life. Third, spores of life from the Cerean ocean may be, or have been, ejected by icy volcanism. Fourth, biomarkers of Cerean life might be found in the ices at the Moon's poles and on the surface of other main-belt asteroids, as there the arrival of ejecta from Ceres may have taken place at low velocity. The future exploration of the Moon's polar regions may shed more light on this, while the Dawn mission may give indications of the past and present habitability of Ceres. Icy volcanism may still occur on Ceres, as the continuing freezing of the ocean increases the total volume.

Further exploration of Ceres is called for, pending the 2015 Dawn mission results, to search on the surface of Ceres, particularly close to locations of icy volcanism, for organics, the spectrum of organic macromolecules (McKay, 2004) and for biogenic hydrogen peroxide.

Although the focus here has been on Ceres, the observations by the Dawn mission may point to remnants of an icy mantle on Vesta. By the same token, further research on 24 Themis, on which water ice and organics have been found (Rivkin and Emery, 2010), and other members of its Hirayama family of asteroids might provide relevant and interesting data.

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CHEMICAL EVOLUTION IN PRIMEVAL SEAS

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1. Chemical Evolution in Primeval Sea

During the last few decades, experimental evidence and theoretical investigation have led us to believe that life on earth must have evolved as a result of several complicated reactions that occurred among the primitive substances present on the earth's surface. The complexity of the reactions increased stepwise, and more and more complex molecules were generated which ultimately took shape of a living cell. This idea was first proposed by the Russian scientist A. I. Oparin in 1924, and since then researchers have been trying to find out more about it.

The problem of origin of life is interdisciplinary, and knowledge of biology, chemistry, geology, astronomy, etc., is required. Therefore, in recent years, the study of chemical evolution and origin of life has attracted researchers from different disciplines.

In the beginning of the twentieth century, S. Arrhenius, a Swedish chemist, propounded the theory of "panspermia," which proclaimed that life did not originate on earth but was brought in from outside. This theory was discarded on the basis of the fact that living organism could not have survived on exposure to strong ultraviolet radiation during the long course of travel through space. During recent years, evidence has been obtained by radio-astronomers for the presence of a variety of simple and complex ions, radicals, and molecules in the interstellar medium. The total number of species characterized so for exceeds hundred. Some of the recent studies on meteorites are even more significant and revealing. More excitingly, McKay et al. (1996) hypothesized the presence of "nanobacteria" in the Mars meteorite collected from Antarctica in 1984. Recent spectroscopic observations through different expeditions have provided us a wealth of information related to the possibility of existence of life in our solar system. Among them, information on Mars, Europa, Titan, and Enceladus is important. Formation of "tholin" in the simulated experiment of Titan's atmosphere is being studied (Sagan and Khare, 1979; Raulin et al., 1982). Although one can neither prove nor disprove the theory of panspermia, it is certain that if life did not originate on earth, it must have evolved at a place with conditions not much different from those present on the primitive earth. Thus, it must have been very conducive for the growth of cells free from oxygenic atmosphere, since oxygen molecules are dangerous and corrosive to carbonaceous substances.

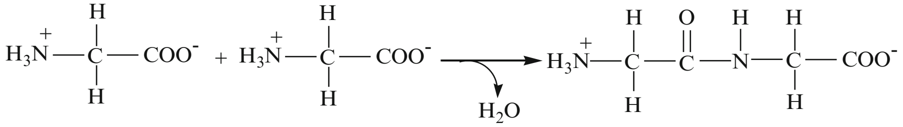
On the basis of the various radioactive isotope tracer techniques of dating, life has been estimated to have evolved some 3.5 billion years ago on earth while the earth itself was formed some 4.6 billion years ago. This means that it took about 1.1 billion years for life to evolve on earth through a series of chemical reactions leading to the first living organism. The process by which all these chemical reactions ultimately gave rise to life is known as chemical evolution.

Since long, living systems were thought to have arisen from organic matters which in turn have been derived from living organism. This means, organic substances would not have been synthesized before the origin of life on the earth, or in other words, only inorganic substances existed on the earth before the origin of life. This implies that "the first organism born on the earth must be organism able to grow ingesting exclusively inorganic substances," and that is what we call autotrophic organism. This was a generally accepted scientific concept till the beginning of the twentieth century. Oparin (1924) raised a fundamental contradiction to the above concept. How could the first individual of autotrophic organism have obtained organic substances for its own construction? And this phenomenon cannot be explained to have occurred just by a chance. Oparin proposed that the origin of life is a step of evolution of materials on the earth. Before the origin of life, there existed the abiotic synthesis of organic materials. Further abiotic organic synthetic activity was more pronounced before the origin of life but decreased when the living organisms themselves started vigorous synthesis of organic materials on the earth. He also recognized that present autotrophs have morphologically, physiologically, and biochemically advanced characters which are a consequence of biological evolution and hence cannot be regarded as the most primitive one.

The impact of Oparin's work was profound. He was the first to set out a series of hypothetical steps by which life might have emerged, and the possible duplication of these steps in the laboratory offered an experimental proof of the origin of life. Chemists could now investigate each stage of the hypothesis.

Based upon the present-day knowledge of biochemistry, one can define a living system as one having the ability to carry out energy transfer reactions, substrate metabolism, and information transfer ability, and all these characteristic functions are being mediated through proteins and nucleic acids in the cell. The most fundamental reactions necessary for life could be:

1. Formations of proteins – the formation of the peptide bond through interaction of the carboxyl function of an amino acid with the amino group of another amino acid, with the production of one molecule of water (Fig. 1)
2. Nucleic acid synthesis – the end result of a number of reactions:
   (a) Nucleoside synthesis through the reaction of a purine or pyrimidine ribose or deoxyribose to form nucleoside (Fig. 2)
   (b) Phosphorylation of nucleoside leading to the synthesis of a nucleotide (Fig. 3)
   (c) Polymerization of nucleotides through formation of ester bonds between the phosphate residue of one nucleotide and hydroxyl group of the pentose residue (Fig. 4)



$$H_3\overset{+}{N}-\overset{H}{\underset{H}{C}}-COO^- + H_3\overset{+}{N}-\overset{H}{\underset{H}{C}}-COO^- \xrightarrow[H_2O]{} H_3\overset{+}{N}-\overset{H}{\underset{H}{C}}-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-\overset{H}{\underset{H}{C}}-COO^-$$

Figure 1. Peptide bond formation.

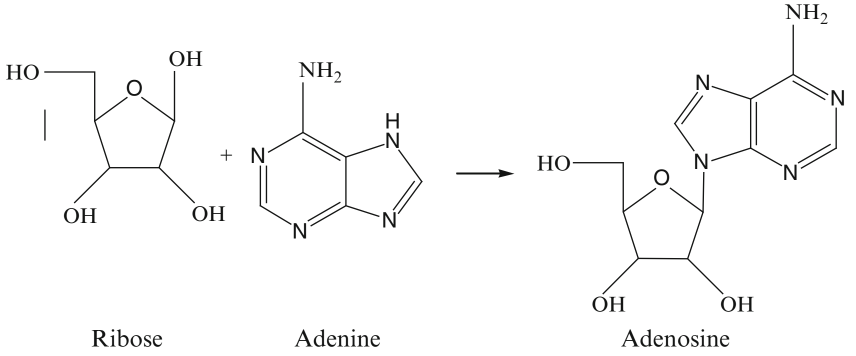


Figure 2. Adenosine formation.

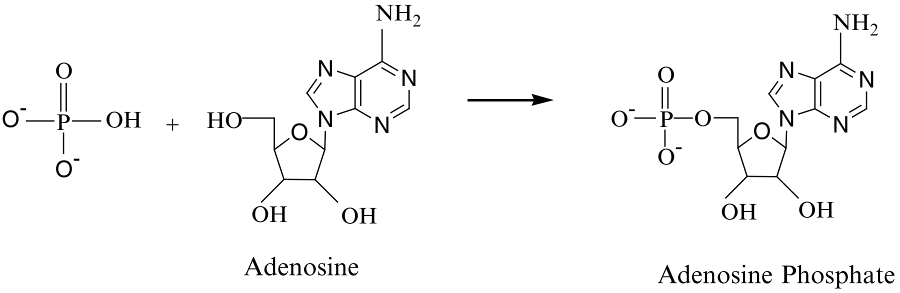


Figure 3. Nucleotide formation.

At present, it is widely believed that the first living organism had the same composition as the contemporary ones because amino acids, sugars, purines, and pyrimidines are major products of reactions carried out by different researchers under prebiotic conditions. Of course, these are the major products, not the exclusive products. The simple reason that amino acids and sugars were the constituents of the first living organism could be that these were abundant on the primitive earth. A gradual development from simple molecules to the emergence of life occurred successively, probably through the following events:

1. The synthesis of the small molecules (e.g., amino acids, monosaccharides, organic bases)

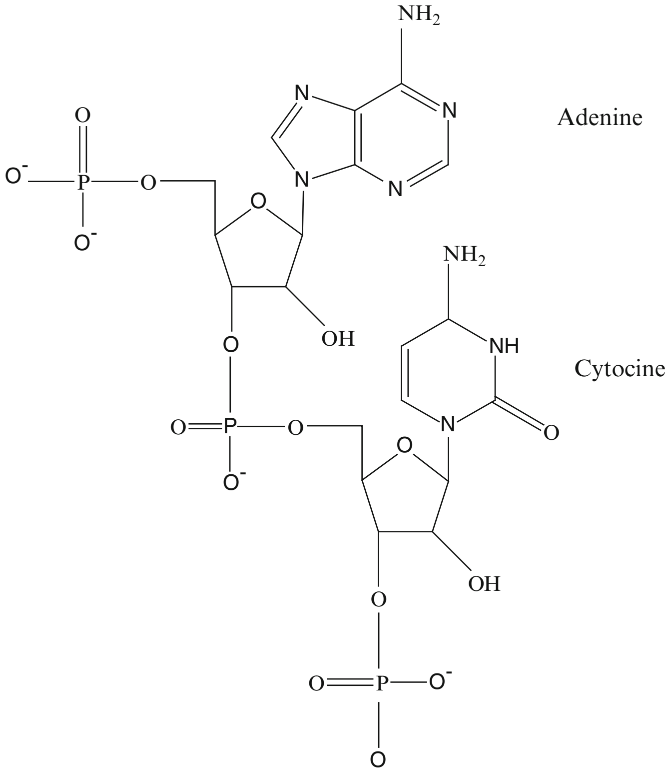


Figure 4. Formation of dinucleotide.

2. The condensation of these small molecules into biomacromolecules – especially proteins and nucleic acids
3. The organization of the macromolecules into a system of increasing complexity
4. The emergence of life

The initial step, the synthesis of the small organic molecules, has been extensively studied. Many of the small molecules necessary for life have been synthesized under presumably prebiotic conditions. An experimental approach to the origin of life on the earth was made by Miller (1953), assuming that primeval atmosphere was highly reducing. Miller chose methane as carbon source and ammonia as nitrogen source, and he obtained various amino acids, from a mixture of CH_4, NH_3, and H_2 by electric discharge on a water phase. Following the pioneering work of Miller, several amino acids have been synthesized by bubbling methane through aqueous ammoniacal solution and by passing the mixed gases through solid silica gel, quartz sand in a glass reaction tube heated to 900–1,100°C (Fox and Harada, 1961). Energy from electric discharge was also used (Ponnamperuma et al., 1963) for the synthesis of many biologically important species from methane, ammonia, and water vapors under reducing atmosphere. Monosaccharides were synthesized from formaldehyde (Gabel and Ponnamperuma, 1967). Purines and pyrimidines were synthesized by plausible prebiotic reactions of alkaline HCN (Oró and Kimbal, 1962). In all, such preliminary experiments in reducing atmosphere were considered as the most probable for chemical evolution. However, strong doubts have been raised for the existence of such a highly reducing atmosphere especially after the Viking mission to Mars. Probably, less reducing atmosphere should now be taken into consideration.

In the second step, condensation-polymerization products, prototypes of proteins, nucleic acids, polysaccharides, and lipids were produced. Then, through interaction among these macromolecules and by natural selection process, the products approached more and more in the form of a "living cell." It is easy to postulate the hypothesis given above, but we have very little experimental facts to support this stage in chemical evolution, viz., the formation of macromolecules, put the researchers in search of the driving force which made these monomers come together and create complex molecules.

What followed could be this:

Clays present on the seashore first adsorbed the monomers which then detached in activated form and combined together leading to the formation of macromolecules. Synthesis of a polypeptide containing a maximum of eight amino acid molecules, using different clays was achieved (Lawless, 1979).

Condensation reactions have been carried out with several prebiotic condensing agents (Fig. 5).

The surprising thing is that most of the condensing agents were derived from HCN. Oró (1961) was first to synthesize adenine from HCN alone. It is now generally believed that HCN is the precursor of early life. The reason lies in the following facts:

1. In almost all the simulated experiments carried out so far, hydrogen cyanide has been confirmed as one of the products.
2. Hydrogen cyanide seems to be the most primitive and basic molecule of biological significance as it is the simplest molecule containing a nitrogen and a carbon atom.
3. The carbon nitrogen triple bond probably provides a lot of energy source for its polymerization or as an active reaction site for other molecules present around.

After the historic synthesis of adenine from an alkaline solution of HCN, there has been a flurry of activity. Many papers have appeared showing the formation of several amino acids, peptides, and organic bases (purines and pyrimidines).

Among others, Ferris and coworkers (Ferris et al., 1978) have shown that in their simulated experiments on the primitive earth, a dilute aqueous solution (0.1 M) of HCN standing in dark at room temperature at pH 9.2 for 0.5–1.5 h produced a low-molecular-weight oligomeric species. The substances released were

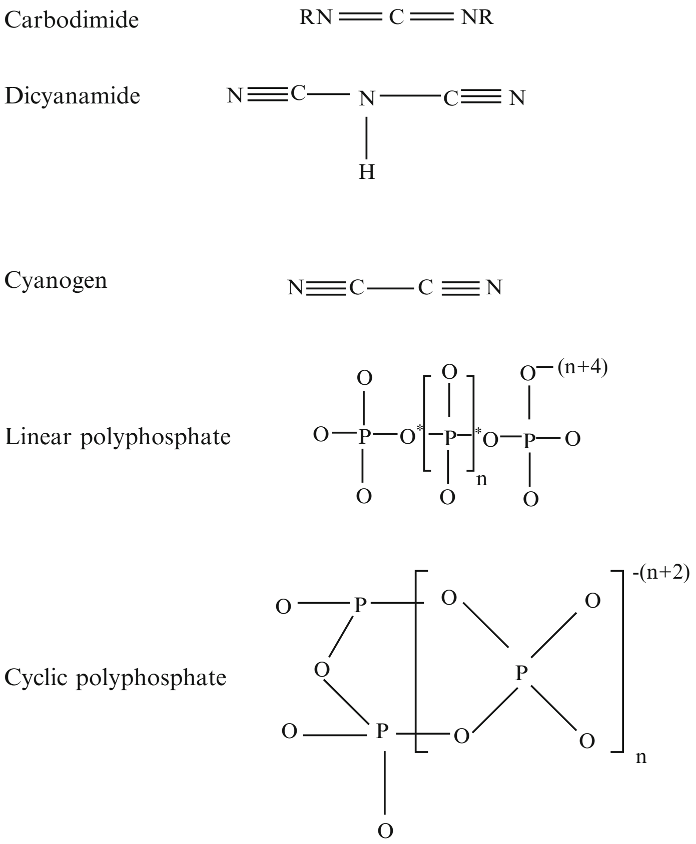


Figure 5. Prebiotic condensing agents.

purines, pyrimidines, and amino acids on heating in aqueous (pH 8.5) solution at 110°C and thus demonstrated that all the varied building blocks for life such as purines, pyrimidines, and amino acids might have formed from the same starting material, HCN. Once these monomers are formed, subsequent condensation may successfully be achieved by several dehydrating agents generated from cyanide.

It has now been generally accepted that chemical evolution and origin of life took place in the primeval seas on earth irrespective of the starting materials. The most accepted hypothesis for the conditions under which prebiotic reaction took place is one extended by Egami. Egami (1974) reported that a good correlation exists between the biological behavior of minor elements, such as molybdenum, zinc, and iron, and their present concentration in the seawater and extended a hypothesis on the evolution of biometals.

2. Evolution of Biometals

Egami (1974) hypothesized that in the course of early evolution of microorganisms in the sea, elements became bioelements when (1) their properties were advantageous to biological systems and (2) their concentration in the primitive sea was sufficiently high for organisms to utilize them. All the elements having total dissolved concentration in seawater higher than 10^6 nM (H, O, Na, Cl, Mg, S, K, Ca, C, N) became bioelements. Conversely, the elements present in concentration less than about 1–5 nM in seawater could not be utilized.

Among the minor elements present in concentrations exceeding 1–5 nM in seawater, some (the potentially useful ones) became bioelements while others became neutral elements, without marked toxicity at the concentration which occurs in natural conditions. Thus, organisms evolved to utilize elements such as iron (6×10 nM), copper (1×10 nM), molybdenum (1×10^2 nM), manganese (7 nM), and zinc (8×10 nM) and to tolerate elements such as strontium (9×10^4 nM), aluminum (4×10 nM), rubidium (1×10^3 nM), lithium (2×10^4 nM), and barium (2×10^3 nM), i.e., neutral elements. Elements present in concentrations in seawater less than about 1–5 nM could not influence evolutionary process. In other words, organisms have evolved independently of these low-occurrence elements, gaining neither the capacity to use them as bioelements nor the capacity to tolerate them as neutral elements. Consequently, low-occurrence elements of high reactivity, such as mercury (7×10^{-1} nM) and lead (1×10^{-1} nM), became toxic elements.

A hypothesis regarding the composition of the primeval seawater at the time of the early evolution is available. According to this hypothesis, iron, molybdenum, and zinc are the most abundant transition elements in seawater and are found to be essential components of the enzymes in microorganisms, including clostridium, which is regarded as the most primitive existing organism. These transition elements presumably complexed with the compounds accumulated in the primeval sea in the course of chemical evolution, forming compounds which subsequently evolved to form protoenzymes with low activity and broad specificity (Egami, 1975).

3. Evolution of Metalloenzymes

The specificity and the reactivity of metalloenzymes are due to the nature and the kind of metal ions present as well as due to the complex nature of ligands. Though it is very difficult to find out how the complex enzymic system is evolved, many workers put forward various theories considering different chemical and biological factors leading to perfection and high reactivity of enzymes.

Transition elements in the primeval sea presumably formed complexes with compounds readily available to them, and further in the course of evolution, the ligands of the complexes were replaced by biologically important ligands, forming proenzymes. These early enzyme systems formed during chemical evolution kept on getting modified with respect to the replacement of ligands by increasing appropriate biologically important macromolecules reaching their present-day form.

Environmental conditions at very early stages of evolution and relative abundance of transition metals in the sea were considered to be responsible for biochemical evolution of metalloenzymes. Initially, metals were present in the low oxidation state due to the reducing nature of atmosphere, the main constituents of primitive atmosphere being N_2, H_2, CO, H_2O, NH_3, and CH_4. From these basic components and in the presence of different forms of energy sources like ultraviolet light, heat, lightning, and cosmic radiations, a number of simple reactive components such as HCN, $(CN)_2$, NH_3, HC CH, HCl, and H_2S were formed (Beck, 1978). These basic components served as ligands to metals at the initial stage of chemical evolution. On the basis of their potential and the ease of forming complexes with metal ions, various ligands such as CO, CN^-, $(CN)_2$, N_2, and CO_2 were assumed as primary ligands. Formation of such complexes has been considered to be the preliminary stage of evolution of metalloenzymes. Further, in the process of evolution, compounds like amino acids, carboxylic acids, and macrocyclic components were formed which subsequently replaced the primary ligands and termed them secondary ligands (Shemin, 1956). When these changes were taking place, the reducing atmosphere was slowly changing to an oxidizing atmosphere, mainly due to the development of photosynthetic system, and it has been assumed that chemical evolution and biological evolution are correlated to each other.

CN^- is a primary ligand of importance, and due to its high solubility, small size, and easy formation under prebiotic conditions, it is considered that CN^- might have formed stable complexes with transition metals. Orgel (1974) also supported the possibility of formation of cyano complexes and thought that these complexes could have played a very important role in biochemical evolution. Probability of formation of mixed ligand complexes has also been taken into account (Beck, 1978). In particular, diatomic isoelectronic ligands such as CN^-, CO, C_2^{2-}, and N_2 could have formed mixed ligand complexes with transition metal ions at early stages of chemical evolution. Although these ligands have similar electronic structure, they differ in reactivity and ability to form complexes. Analogous to the above two theories and on the basis of experimental evidence, Nina (1984) analyzed the possible role of metal ions in transformation of systems from prebiological to biological in view of some important evolutionary aspects. It was assumed that the functional characteristics of metal ions were of prime importance. On the basis of available data, a few conclusions have been drawn regarding the role of metal ions in the synthesis of organic substances at prebiological stage as well as in the initial integration of molecules: (1) A large number of metal ions possess functional prototypes of factors similar to a

Table 1. Important amino acids involved in metal protein binding.

Amino acid residue	Possible role of protein, enzyme structure, function
1. Lysyl	Hydrophilic, electrostatic interactions, attachment of prosthesis group or cofactor in amino bond, interacting to form Schiff base, ligand to metal ion
2. Histidyl	Hydrophilic or hydrophobic (depending on ionization) electrostatic interactions, proton transfer, ligand to metal ion, hydrogen bonding, acceptor in transfer reactions
3. Glutamyl, aspartyl	Hydrophilic, electrostatic interactions proton transfer, ligand to metal ion, covalent linking in ester of amide through carboxyl
4. Cysteinyl	Nucleophile, acyl acceptor, hydrogen bonding, ligand to metal ion
5. Methionyl	Hydrophobic, hydrogen bonding to S. ligand to metal ion

number of functional characteristics of biomolecules, viz., metal-dependent synthesis of amino acids (Khenokh and Lapinskaya, 1974; Hatanaka and Egami, 1977), participation of metal ions in primary formation of polyphosphates (Handschuth et al., 1973; Kulaev, 1973) in synthesis of polypeptides (Choughuley et al., 1972), and participation of metal ions in a biotic synthesis of porphyrins (Ryan and Fox, 1973). (2) At early stage of biological evolution when there was transition from nonliving to living matter, metal ions precisely changed their most primitive natural catalytic function to more complex biochemical functions, thus achieving continuity in the development of the catalytic functions in the two stages of the systems. (3) During the later stages of evolution when the functional units were complex enough and consisted of biological macromolecules and macromolecular complexes, modification, specification, and integration of function took place. For example, in many cases, the changes in isoenzymes spectrum of peroxidase are connected with adaptive processes in plant organism.

It is well known that the high specificity and reactivity of metalloenzymes are due to their complex structure and mode of coordination of metal ions to the amino acid residues. Of the twenty amino acids commonly occurring in proteins, only a few participate actively in the mechanism of enzyme catalysis. Those with appropriate side chains are involved in proton transfer, as group acceptor, nucleophiles or electrophiles. Some other amino acids are effective in determining the tertiary and quaternary structure of proteins, binding to substrates, and in forming large complex structures. Therefore, the possible role of each amino acid should be considered. Table 1 lists the amino acids involved in specific enzymic reactions. Here, only those amino acids are included which are responsible for metal protein binding in the case of metalloenzymes.

In biological evolution, metal ions could have influenced the functional evolution at different levels. This influence became more profound when there was interaction between metal ions and biological molecules particularly proteins,

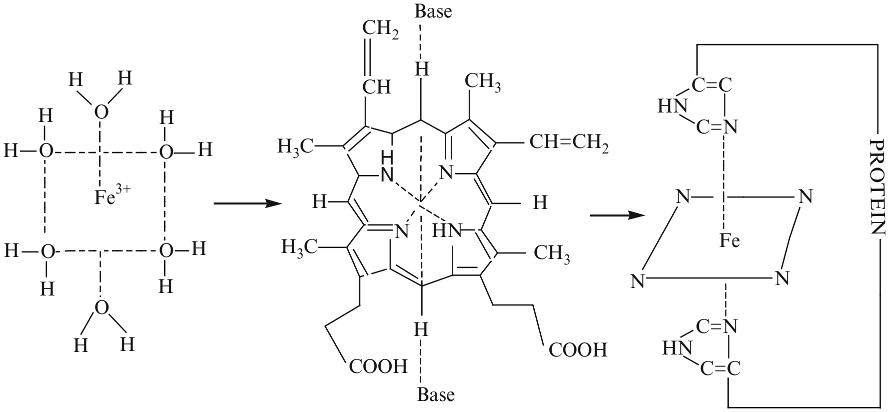


Figure 6. Evolutionary steps for catalase.

and then further, additional interaction with other type and greater number of metal ions introduced something new in the manifestation of function thus increasing the general functional activity of the systems as a whole.

Comparing the sequence of amino acids and properties of enzymes in various organisms, a speculative study has been made to trace the evolutionary path of enzymes (Smith, 1970). Some general conclusions have also been drawn in the context with protein evolution which may be outlined as follows: (i) those proteins which serve the same function in all organisms that frequently manifest strong homology in structure but with some modification in amino acid sequence, e.g., hemoglobins, insulins, and cytochrome C; (ii) some enzymes present in lower systems (organisms) have disappeared from higher organisms, e.g., enzymes in synthetic pathway for certain vitamins and essential amino acids; and (iii) many new proteins (enzymes) have appeared in higher organisms.

Calvin (1959) suggested that enzymes themselves were developed in autocatalytic processes. For example, when necessity of the decomposition of hydrogen peroxide emerged, the Fe^{2+}/Fe^{3+} redox couple could serve as catalyst. However, its efficiency is very small. On the other hand, iron could promote the formation of tetrapyrrole ring. The formation of porphyrin from pyrrole and formaldehyde needs an oxidation by six equivalents. This oxidation step is assisted by hydrogen peroxide and iron ions, and the resulting iron porphyrin complex is a much more efficient catalyst for the decomposition of hydrogen peroxide. The catalytic effect dramatically increases when the complex is incorporated to a suitable protein. The progressive increase in catalytic ability is from 10^{-5} mol L^{-1} S^{-1} for the aqueous ferric ion to 10^{-2} mol L^{-1} S^{-1} for heme and to 10^{5} mol L^{-1} S^{-1} for the complete enzyme (catalase) (Fig. 6).

Although this example is an excellent one, it cannot be taken as a general case. Further, the oxidation state of iron in this example is three, which shows that these evolutionary processes might have occurred at a later stage of the evolution when redox potential of the atmosphere was considerably high.

Studies on the evolution of iron-containing enzymes at an early stage of evolution when iron was present in its lower oxidation state have been investigated by us (Kamaluddin et al., 1986, 1988). Experimental observations suggest that mixed ligand complexes of iron (II) are able to catalyze a class of redox reaction, i.e., decomposition of H_2O_2 and dehydrogenation of NADH and ascorbic acid both coupled with reduction of methylene blue. Further, the catalytic activity of the complexes increased when primary ligands like CN^- were partially substituted in the complex by amino acid and peptide molecules, respectively. It was concluded from the observations that increase in catalytic activity of complexes was quite appreciable and might indicate pathways leading to the evolution of enzymes containing iron. A tentative pathway for the evolution of iron-containing enzymes is proposed as follows:

> iron (II) iron (II) cyanide complexes mixed ligand iron (II) cyanide and amino acid complexes iron (II) complexes of macromolecule proenzymes of early enzymes containing iron.

The proposed pathway for the evolution of iron-containing enzymes is not contradictory to one proposed by Calvin. The only difference lies in the fact that we have considered a much earlier stage of evolution where iron was present in its lower oxidation state. Cyanide complexes of iron have been chosen because cyanide is very good complexing agent and is believed to have been present in primordial soup in large concentration.

4. Chemical Evolution in Modified Sea Medium

It is generally believed that the first living organism evolved in the primeval seas. Different reactions could have occurred under water in depth of 3–4 ft in order to protect the molecules from degradation due to high energy UV radiation coming from the sun. To have an experimental test of this, an artificial sea medium was constructed. Ventilla and Egami (1977) named it as standard modified sea medium(SMS) in which the concentration of six metal ions known as biometals was kept high up to 10^3–10^4 times more than these in contemporary seas. The composition of SMS is shown in Table 2. A mixture of 0.5 M each of HCHO and NH_2OH in SMS medium was kept in a sterilized glass tube. The solution was degassed by freeze and thaw method, sealed, and then kept at 105°C in a dry block for some time. In the system, the atmosphere was water vapor above the modified sea medium. Keeping the system at 105°C excluded development of any bacterial growth. The products of the reaction between HCHO and NH_2OH in SMS medium

Table 2. Composition of modified sea medium.

Composition	Concentration (M)
HPO_4^{2-}, SO_4^{2-}, Mg^{2+}, Ca^{2+}	0.01
Na^+	0.15
K^+	0.563
Cl^-	0.519
NO_3^-	0.0005
Zn^{2+}, MoO_4^{2-}, Fe^{3+}, Cu^{2+}, Co^{2+}, Mn^{2+}	0.0001
pH	5.5

Conc^n. of NaCl is decreased.
Conc^n. of metal ions are increased by 10^3–10^4.

after 20 days were analyzed, and it was found that glycine, serine, aspartic acid, and β-alanine were formed in larger amount than any other amino acids. Further, cyanide, glycine nitrile, glycinamide, and larger amount of urea were also produced. The formation of glycine nitrile and glycinamide and the presence of cyanide in the reaction mixture suggest that the formation of glycine took place through Strecker synthesis; the same mechanism was also proposed as given below:

$$\begin{aligned}
&HCHO + NH_2OH && \rightarrow && CH_2NOH + H_2O \\
&CH_2NOH && \rightarrow && HCN + H_2O \\
&HCHO + NH_3 + HCN && \rightarrow && CH_2(NH_2)CN + H_2O \\
&CH_2(NH_2)CN + H_2O && \rightarrow && CH_2(NH_2)CONH_2 \\
&CH_2(NH_2)CONH_2 + H_2O && \rightarrow && CH_2(NH_2)COOH + NH_3
\end{aligned}$$

Once glycine is formed in the reaction of formaldehyde and hydroxylamine, other amino acids might be produced by addition of certain aldehydes to glycine molecule and their modification in aqueous solution. Formation of serine by the reaction of formaldehyde and free glycine in solution on the primitive earth has been suggested, and this process is believed to have been a significant source of serine. Formation of serine may also have occurred by the same pathway in our reaction mixture. Moreover, aspartic acid might have formed by the reaction of formaldehyde dimer and glycine. The above reaction pathway for the production of serine and aspartic acid is supported by the observation that when glycine was allowed to react with formaldehyde in the modified sea medium, serine and aspartic acid were produced (Kamaluddin et al., 1979).

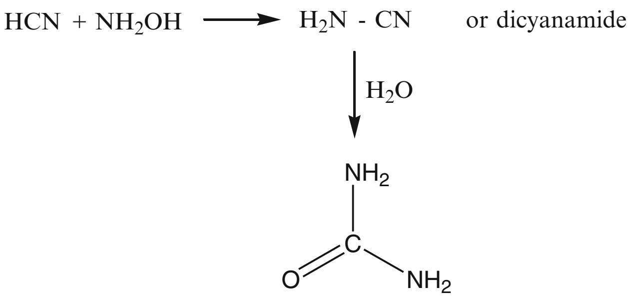
Formation of cyanide was confirmed, and in the course of the reaction of formaldehyde and hydroxylamine at 105°C, cyanamide formation was also

Table 3. Synthesis of peptides in aminoacetonitrile mediated modified sea medium.

	Reaction time, days					
	3	**10**	**15**	**25**	**35**	**50**
Peptides	**(mmol/l)**					
Glycylglycine	0.34	0.73	1.05	1.12	0.72	063
Glycylserine	0.23	0.30	0.34	0.48	0.43	0.40
Serylglycine	0.07	0.33	0.24	0.20	0.18	0.19
Total	0.64	1.36	1.63	1.80	1.33 1.22	

Reaction conditions: HCHO, 0.5 M; NH_2OH, 0.5 M; glycine, 0.08 M; aminoacetonitrile, 0.08 M; Mg^{2+}, 0.01 M; SO_4^{2-}, 0.26 M; K^+, 0.13 M; HPO_4^{2-}, 0.01 M; Zn^{2+}, MoO_4^{2-}, Fe^{3+}, Mn^{2+}, & Co^{2+} 0.00012 M; pH, 5.47; T, 105 °C.

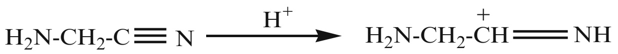
possible. Cyanamide is very unstable under our reaction conditions, so its rapid reaction pathway for the synthesis of urea may be written as follows:



The most exciting feature of the modified sea medium seems to be the feasibility of dehydration processes even in aqueous solutions.

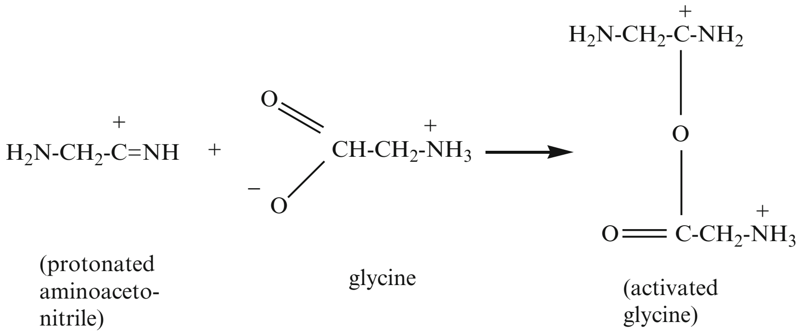
Later, it was discovered that glycine nitrile, an intermediate in the reaction and a higher homologue of a condensing agent (cyanamide), was working as condensing agents to afford peptide linkage (Table 3). Addition of glycine nitrile in the reaction of HCHO and NH_2OH in SMS further increased the yield of diglycine in the reaction and yielded many other peptides (Kamaluddin et al., 1981).

On the basis of the mechanism proposed for cyanamide as condensing agent, a similar mechanism may be proposed for aminoacetonitrile as condensing agent as follows:



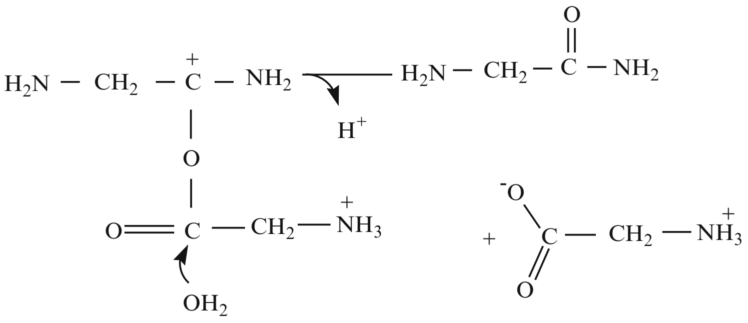
$$H_2N\text{-}CH_2\text{-}C{\equiv}N \xrightarrow{H^+} H_2N\text{-}CH_2\text{-}\overset{+}{C}H{=}NH$$

which reacts with the glycine molecule as follows:



The activated glycine may have two fates:

1. Water hydrolysis



Nucleophilic attack by water may also occur on the carbonium carbon; however, the products will be the same as shown.

2. Nucleophilic attack by the lone pair of electrons on the amino group of another amino acid, resulting in the desired dipeptide and the hydrolyzed condensing agent.

Nucleophilic attack of the amino nitrogen in glycine on the carbonium carbon may lead to a side product; however, glycylglycine will also be formed by the above mechanism.

5. Role of Clays in Chemical Evolution

Clay minerals are hydrous aluminum phyllosilicates and are typically formed over long periods of time by the gradual chemical weathering of rocks by low concentrated carbonic acids or other diluted acidic solvents.

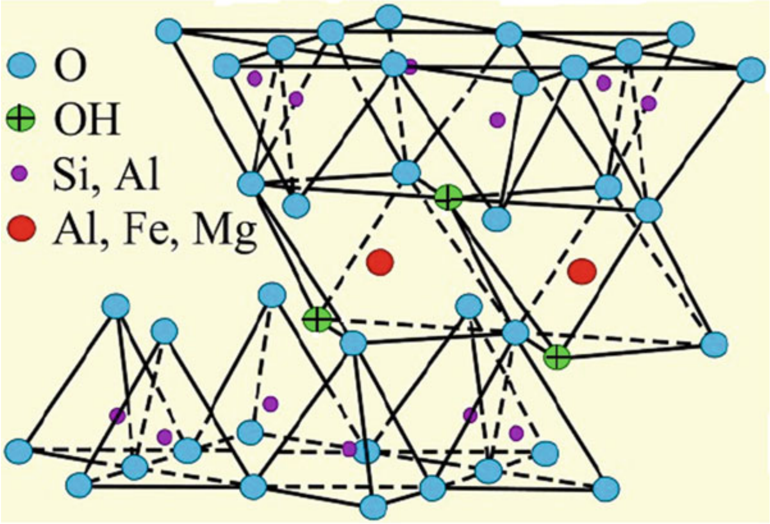


Figure 7. Structure of montmorillonite clay.

Bernal (1951) proposed that clay minerals may have provided active sites for monomers to get concentrated through adsorption and were thus protected from degradation. Also, clays might have catalyzed polymerization of biomonomers.

Clays are inorganic minerals with excess of surface charges, acting as natural ion exchangers. Geologists have classified many different kinds of clays, some containing iron and magnesium, but all are oxides of silicon and aluminum and share a basic-layered molecular structure. The aluminum (III) cations are bonded to an octahedrally arranged oxygen anions. Repetition of these AlO_6 units in two-dimensional forms as octahedral layer. Likewise, a tetrahedral layer is formed from SiO_4 silicate units. Clays are classified according to the relative number of tetrahedral and octahedral layers. Montmorillonite clays, which have been used in organic chemical applications, have an octahedral layer sandwiched between two tetrahedral layers (Fig. 7). The diversity of clays arises from deviations with respect to this idealized description. Aluminum and phosphorous could be substituted for silicon in the tetrahedral layer. A number of metallic cations, usually divalent ones such as Mg^{3+}, Fe^{2+}, Mg^{3+}, Zn^{2+}, and Ni^{2+}, substitute for Al^{3+} in the octahedral layer. Such isomorphous substitution produces additional chemical diversity. Replacement of the tetravalent silicon with Al^{3+} or of the trivalent aluminum with a divalent cation results in a net negative charge. In order to maintain electroneutrality, exchangeable cations come to the interstellar space. In natural clays, these are typically Na^+, K^+, Ca^{2+}, Mg^{2+}, and similar ions.

A remarkable property of clays is their surface property. With overall dimensions below 2 μm, clay particles show larger specific surfaces, as measured with adsorption of dinitrogen in the standard BET method. Specific areas are

15–20 m^2/g for kaolinite, a 1:1 phyllosilicate; they are 80–90 m^2/g for illite. Montmorillonite has specific areas of the order of 500 m^2/g, sometimes as high as 760 m^2/g. By comparison, alumina has a specific area of 70–200 m^2/g, while values range between 400 and 600 m^2/g for silica gel.

In montmorillonite and micas, the excess negative charge is smeared over all the superficial oxygen sites of tetrahedral on octahedral sheet. High acidity has been attributed to the presence of exchangeable cations. The cations polarize coordinated water molecules and induce their dissociation. This consequently makes hydrogen of water molecule more acidic.

The Bernal hypothesis was seminal. Many prebiotic scenarios involving clays have been written, and likewise, many prebiotic experiments have used clays. Clays have been variously postulated as buffers, adsorbents, catalysts, and templates. A number of prebiotic simulations have used clay surfaces. Many experiments have used clays as catalysts for the formation of biomolecules such as porphyrins (Cady and Pinnavaia, 1978). Montmorillonite has been shown to work as multicatalytic system in performing reactions of prebiotic significance. Sorption of amino acids by copper montmorillonite has been reported (Bodenheimer and Heller, 1967). Detailed studies on adsorption of 15 proteinous amino acids from diluted (10 μM) distilled water solutions onto kaolinite and montmorillonite have been done (Hedges and Hare, 1987). Lawless and Levi (1979) have synthesized polypeptide containing a maximum of eight amino acid molecules through adsorption processes using clays such as montmorillonite, kaolinite, and bentonite coordinated with divalent cations, viz., Zn^{2+}, Cu^{2+}, Mg^{2+}, etc. Ferris and his research group have done a great deal of research on the role of clays in the oligomerization of nucleotide units. Montmorillonite catalyzed polymerization of unblocked nucleotides has been shown to afford 30–40 mers of RNA oligomers. Template-directed synthesis was carried out using heterogeneous mixture of oligo(C)s to determine whether complementary G oligomers would form (Ertem and Ferris, 1996). Weiss (1981) has suggested that clays might have served as templates for the first replicating systems on this planet. He claimed that the irregularities in an aluminosilicate sheet are transferred to the replica layers during an intercalating synthesis. Cairns-Smith's genetic takeover scenario states that clays could very well have served as "low-tech" precursors to the self-replicating biomolecules as known today. According to Cairns-Smith (1992), "a perfect crystal is a blank object." One has to create a pattern of changes, a disorder imposed on the crystal. Disorder is precisely the thing which can hold information.

6. Evolution of Self-Assembly of Probionts

Once small molecules accumulated, aggregation of molecules took place leading to the formation of protocells or protobionts. Protobionts maintain a localized environment separate from the surroundings. They are membrane-surrounded

Figure 8. SEM image of marigranules.

sacs containing genetic material and metabolically active molecules. Protobionts exhibit some properties associated with life like metabolism and show membrane potential. Such structures have been experimentally produced. It is proposed that these protocells (or protobionts) ultimately changed into cells. Formations of some protocells are discussed below.

7. Marigranules of Egami

Taking into consideration the fact that essential transition metal ions had to contribute to chemical evolution in primeval seawater and anticipating the concerted catalytic action of clays and metal ions, Egami and his coworkers (Yanagawa and Egami, 1977; Yanagawa et al., 1980) got a packed organized particle which they called marigranules (Fig. 8). The particles were 0.3–2.5 μm in diameter and were produced from glycine, acidic (Glu, Asp), basic (Arg, Lys, His), and aromatic (Phe, Try, Tyr) amino acids in a modified sea medium containing magnesium sulfate, calcium chloride, dibasic potassium phosphate, and six transition elements (Fe^{++}, Mo^{6+}, Zn^{2+}, Cu^{2+}, Co^{2+}, and Mn^{2+}). The reaction was carried out at 105°C for 8 weeks under nitrogen atmosphere.

The infrared spectrum of the marigranules suggested the presence of peptide bonds showing first amide bond at 1,680–1,610 cm^{-1} and second amide

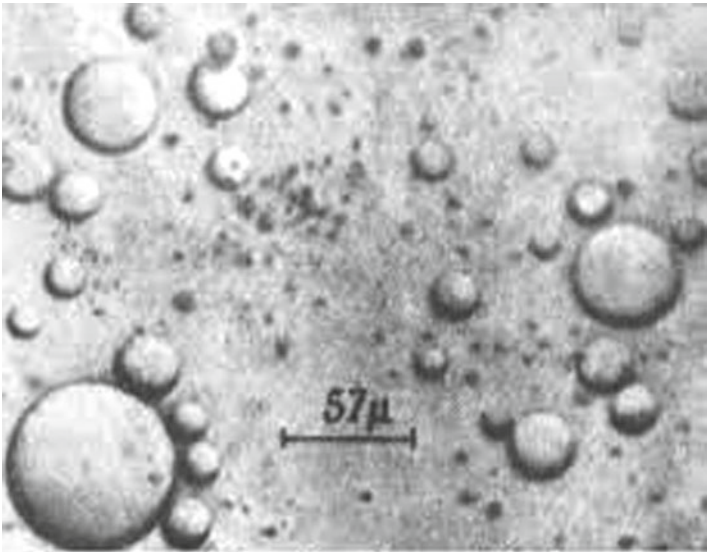


Figure 9. SEM image of coacervates.

bond at 1,550 cm^{-1}. The marigranules were solubilized by treatment with NaOH solution. The solubilized marigranules consisted of polymers with molecular weights of 1,800, 6,900, 10,000, and 82,000 Da. When solubilized marigranules were digested with enzyme elastase, 34 % of total nitrogen was released as primary amino groups. It therefore seems that the marigranules consist of polymers with an elastin-like structure. It is believed that such particles might have been produced in primeval sea before the origin of life.

8. Coacervates of Oparin

The coacervate droplets of Oparin (1961) were obtained from gum arabic and histone (Fig. 9). Oparin regarded it as the simplest model for the flow of materials through living cells as these droplets can incorporate phosphorylase and β-amylase from the surroundings and can perform metabolism. It can also incorporate glucose 1-phosphate from the surroundings and excrete metabolic products, phosphate, and maltose as waste products.

In another reaction, Oparin reported self-growing system in which the coacervate droplets are made from histone and RNA. The enzyme RNA polymerase was introduced into the droplets, and ADP was added to the surrounding medium as “food.” When the ADP enters the droplet, it encounters the RNA polymerase and is polymerized into polyadenylic acid. The droplets grow with

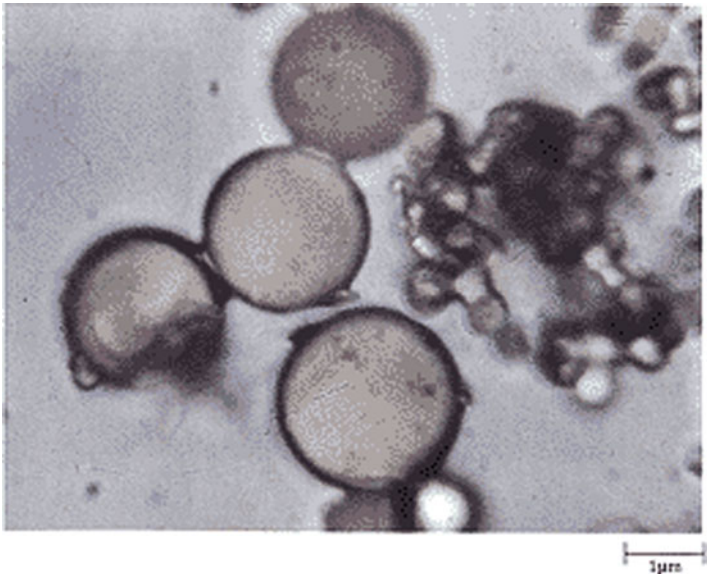


Figure 10. SEM image of proteinoid.

time as the new polyadenylic acid adds to the total RNA in the coacervates. The droplets grow with time and break up into delegate droplets.

Such systems eventually wind down because the supply of enzyme molecules for polymerizing ADP does not increase with the total mass of the coacervate droplets. Nucleic acids, however, can be polymerized nonenzymatically with small, energy-rich coupling agent molecules such as cyanogen. It should be possible to construct coacervate droplets from protein and RNA, to provide them with ADP and the appropriate coupling reagents, and to see them grow and multiply as long as their "nutrients" continue to be supplied.

9. Proteinoid Microspheres of Sidney Fox

Proteinoid microspheres are formed by heating dry mixtures of amino acids to moderate temperature and then cooling (Fig. 10). The micro spheroidal aggregates thus formed under suitable conditions will grow to microspheres with diameter of several micrometers. The proteinoid microsphere seems to have two-layer membranes similar of that in bacteria (Fox and Dose, 1977). Fox came to the conclusion that amino acids under the influence of various factors could have been converted to polypeptides in heated volcanic soils. It is thought that the zones of volcanic activity could have been the site of primary synthesis of proteinoids. Rain washed rivers or seas, where the subsequent processes of formation of more complex proteinoid structures took place.

10. Conclusion

The origin and evolution of the first living cell in the cosmos could have taken place where conditions were not very harsh. Since our earth is the only place where life is known to exist, it could reasonably be thought to have originated under seawater on primitive earth.

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TERRESTRIAL ANALOGUES FOR EARLY PLANETARY OCEANS: NIUAFO‘OU CALDERA LAKES (TONGA) AND THEIR GEOLOGY, WATER CHEMISTRY, AND STROMATOLITES

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1. Introduction

Earth is a unique planet in a special planetary system to have not only made biogenesis possible but also to sustain life’s long-term evolution toward multicellularity and self-recognition. The reconstruction of Earth’s history relies on evidence recovered from its rock record. From this record, we know that life originated prior to 3.8 Ga (e.g., Schidlowski et al., 1979; Mojzsis et al., 1996), that multicellular life arose at around 0.6 Ga, and that biologically controlled, enzymatic biomineralization started with the beginning of the Phanerozoic eon, at around 0.54 Ga ago (e.g., Lowenstam and Margulis, 1980; Simkiss, 1989). Geochemical forcing most probably triggered these events (Kazmierczak and Degens, 1985; Kazmierczak et al., 1985; Kempe and Degens, 1985; Kempe and Kazmierczak, 1994; Kazmierczak and Kempe, 2004; Brennan et al., 2004). The nature of this forcing, however, remains unknown, and one can only formulate working hypotheses. The Earth system is governed by too many parameters, excluding the possibility of building models, which, by representing all the physical interactions, would create life. Therefore, we rather have to ask, what are the necessary environmental conditions under which life could have been initiated and can we find such environments even today in order to study these primordial conditions?

Most researchers agree that life arose in an aqueous solution, most probably in the Hadean ocean, even though special niches such as hydrothermal systems, lagoons, or even fresh water or volcanic lakes cannot be excluded *a priori*. For an overview of the different hypotheses of origin of life, see, for example, Davis and McKay (1996) and Martin and Russell (2003). Also, life could not have arisen in an oxygenated environment in which organic matter would have been oxidized too quickly. We furthermore suggested that life could not arise in a solution rich in Ca^{2+} such as in the present ocean which has a concentration of 10 mmol Ca/l (Kempe and Kazmierczak, 1994; Kazmierczak and Kempe, 2004). Appreciable concentrations of Ca^{2+} or other divalent ions in the cell-fluid prohibit the proper functioning of proteins. In fact, Ca^{2+} concentration cannot rise to higher than 10^{-6} M without damaging the cell proteins irreversibly. It is a well-established fact that for the proper functioning of cells, a Ca^{2+} level of $<10^{-7}$ M needs to be maintained in the cytosol (e.g., Borle, 1981; Campbell, 1983; Marmé, 1985; Carafoli, 1987; Berridge et al., 1998; Jaiswal, 2001; Case et al., 2007). Otherwise, the very low solubility product of $[Ca^{2+}]$ and $[PO_4^{3-}]$ would lead to the precipitation of calcium phosphate which would be deleterious to cell functioning (Lowenstam and Margulis, 1980; Simkiss and Wilbur, 1989). Today, cells regulate their internal Ca concentration by sophisticated proteins, called Ca pumps, situated in the phospholipid cell membranes. These certainly have not been available *ab initio*, excluding, to our understanding, environments high in Ca from the possible choice of biogenesis solutions. In search for likely places for the creation of life, not only on Earth but possibly also on Mars and on Jupiter's moon Europa or Saturn's moon Enceladus, we therefore must look for environments which have very low Ca concentrations by nature (Kazmierczak and Kempe, 2004; Kempe and Kazmierczak, 1997, 2002, 2007).

Examples of such environments are alkaline lakes, particularly soda lakes rich in HCO_3^- and CO_3^{2-} (e.g., Eugster and Hardie, 1978; Kempe and Kazmierczak, 1990a). Due to the relatively low solubility product of Ca, Mg, and Fe carbonates, alkaline solutions are low in these ions. Furthermore, in the absence of silica-utilizing algae and animals, high silica concentrations, as they are typical for alkaline solutions, should have reduced the Ca^{2+} and even more so Mg^{2+} concentrations further. It is therefore very interesting to study modern soda lakes as analogues of a Precambrian Soda Ocean (Kempe and Degens, 1985; Kempe and Kazmierczak, 1990a, b 2003, 2011a, b; Kazmierczak et al., 2004). Such lakes are found today mostly in areas where fresh volcanic rocks are exposed to weathering, that is, along the volcanic plate boundaries or at hotspot volcanic sites. Two such sodium-carbonate lakes, one of them containing only a dilute solution and one containing a highly alkaline solution illustrating the long-term stability of such lakes, provide examples to study the mode and rapidity with which alkaline lakes can form. These are the crater lake of the Nemrut Volcano and Lake Van, both of them situated in Eastern Anatolia, Turkey (Kempe, 1977; Kempe et al., 1991; Reimer, 1995; Landmann, 1996; Kempe and Kazmierczak, 2003; Reimer et al., 2009).

2. Historical Background

Niuafo'ou is the northernmost island of the Kingdom of Tonga (Fig. 1). It was discovered by the Polynesian seafarers in prehistoric time. The name means "many fresh coconuts." European mariners (Dutch explorers Schouten and Lemaire)

Fig. 1. Location of Niuafo‘ou Island in the Tonga Archipelago (*red arrow*) with aerial view of its two biggest caldera lakes: Vai Lahi and Vai Si‘i.

sighted the island as early as 1616 and named it “Good Hope.” To philatelists, Niuafo‘ou is also known as “Tin Can Island.” Formerly, the mail was thrown overboard from the postal steamer in a tin container, and the postman collected it by swimming out. This service was discontinued after the mailman was killed by a shark in 1931, but Niuafo‘ou still retains the right to print own stamps (not sold on the island, however). The island suffers both from tropical storms (one hit before we came, toppling 70% of the coconut palms) and volcanic eruptions. After the last eruption in 1946, which destroyed the main village Angaha, the 1,200 inhabitants were evacuated to Tongatapu and Eua. Only 28 remained at the time (Macdonald, 1948). Since then, many of them have returned. Today, the island has about 1,000 inhabitants. It is still the least populated island of the Tonga Kingdom (Kreisel, 1991).

The island became of scientific interest in 1930, not because of its volcano or lake but because it was situated in the swath of the central shadow of the solar eclipse of October 21st that year. A joint US/New Zealand expedition set up observatories on the island to monitor this event (Marriott, 1931). Among the invited scientists was the famous Pacific volcanologist Thomas Jaggar, founder of the Hawaiian Volcano Observatory. He published three accounts of his visit (Jaggar, 1930, 1931, 1937) and a chapter on the island in one of his books (Jaggar, 1945) plus a National Geographic article (1935) that brought the lake and its carbonates to our attention because it contained a picture showing carbonate-cemented plates along the beach (Fig. 6a). In 1930, he already mentioned (page 4) in a figure caption “singular limey concretions are deposited on these lake beaches, containing tiny crabs a half inch in diameter.” These structures are, however, not visible on the picture.

3. Geographic and Volcanologic Setting

Niuafo‘ou is a spectacular, circular volcanic island 8.1*8.5 km wide and 52.3 km^2 in area, with a central caldera, occupied by a lake measuring 4.6*4.6 km (N-S and E-W, respectively). Within the caldera, tephra cones occur, some of them forming islands in the lake (Fig. 1). The center of the island (Motu Lahi) is at 15°36′00 S/175°38′30 W. Google Earth provides a very good geographical view of the island.

The plate tectonic situation of the island is still an enigma (Nunn, 1994). It rises at the northern end of the Lau-Basin, an actively spreading back-arc basin, at the leading edge of the Indo-Australian Plate, situated west of the Tonga subduction trench (Parson and Tiffin, 1993). To the north runs the transform fault between the Pacific and the Indo-Australian Plates. The volcano sits either on a local spreading ridge extending NE-SW from the Peggy Ridge or on a ridge running west from the Kings triple junction. The existence of this triple junction, situated between Tafahi Island (the northernmost of the island-arc-volcanoes of the Tonga chain proper) and Niuafo‘ou, suggests that the island sits on a separate microplate. More recent investigations (Regelous et al., 2008) suggest that the island is the result of partial mantle melting causing an intra-plate volcano.

Only a few petrographic analyses of Niuafo‘ou rocks have been published. These include Cohen (1880) with an 1867-eruption sample (presumably collected by E. Graeffe in 1867 and deposited at the Godeffroy Museum in Hamburg), Macdonald (1948) with a 1929-eruption specimen (collected by T. Jaggar), and Reay et al. (1974) with 50 samples collected during a soil survey (by P. Whelan in 1971).

All samples are remarkably fresh and show a basaltic composition with an SiO_2 concentration of 49.3% to less than 51%. Plagioclase and, less abundant, olivine phenocrysts occur, giving the rocks a porphyritic or microporphyritic texture. The groundmass is composed of plagioclase, olivine, augite, and Fe/Ti oxides. The $K_2O + Na_2O$ sum is between 2.2 and 3.3%, which is below the field of alkali-basalts. The MgO and CaO concentrations are high (ca. 7 and 12%, respectively) suggesting that the lavas are tholeiitic basalts, that is, of Mid-Ocean Ridge (MOR) characteristics. Compared to MOR-basalt averages, the Niuafo‘ou lavas have a higher FeO*/MgO ratio and slightly lower MgO and higher Na_2O concentrations. Also, the trace elements show only small differences to MOR-basalts, if at all (Reay et al., 1974). Zr and Zr/Y values fall within the MOR-basalt window. Thus, the island – unlike the neighboring islands – offers a rare subaerial view of oceanic crustal rocks.

Jaggar (1931) provided a first geological sketch map (later, reproductions appeared in Nunn (1994) and abstracted versions in Jaggar (1945) and Macdonald (1948)). Taylor (1991) made a color version of it with some amendments available on the net (http://www.volcano.si.edu/volcanoes/region04/tonga/niuafoou/2804niu2.jpg). These maps plus own observations were used to produce a more detailed geological map of the island (Fig. 2).

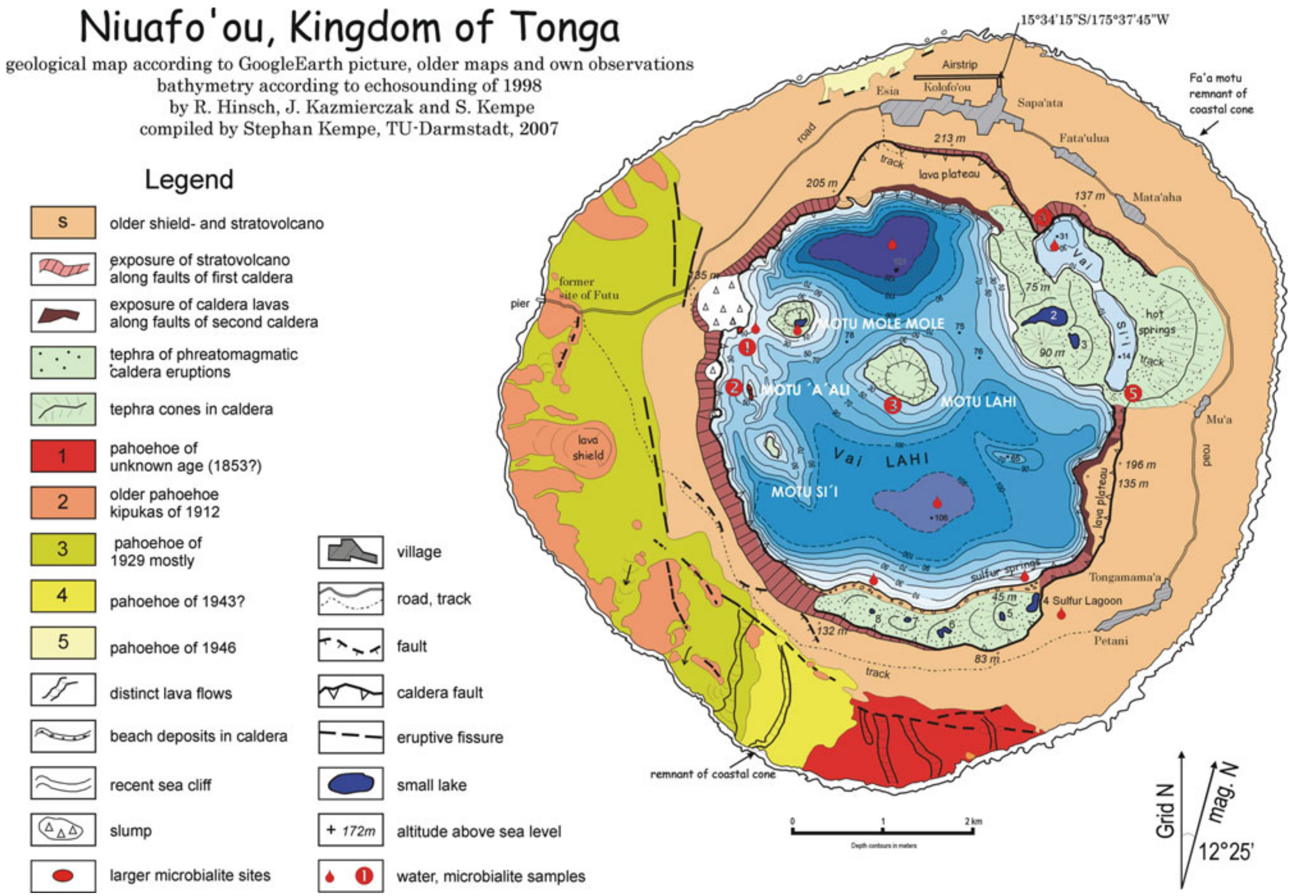


Figure 2. Geological map of Niuafo'ou, drawn after the maps of Jaggar, own observations and data, and by using the Google Earth image of the island.

The following phases can be differentiated in the development of the island:

1. The gas-poor eruption of a large basaltic shield volcano; its lavas form the base of the outer perimeter of the island and most of the northern and eastern coastline.
2. Intrusion of seawater into the magma chamber and the eruption of tephra onto the shield volcano, forming steeper terrain about 1 km inward of the present coast. These tephra layers form the wealth of the island; they sustain agriculture and coconut farms. Macdonald (1948) estimated that the volcano may have been 1 km high.
3. Collapse of the central magma chamber and formation of the first caldera. The remaining ring-like island is only up to 205 m high. Caldera faults expose strata of the volcano all around the central depression, unless covered by later tephra and thick vegetation.
4. Filling of the caldera with horizontally layered lava to an altitude of 100–110 m a.s.l.
5. Second collapse of the caldera, leaving two flat plateaus of the caldera lavas in the north of the caldera and on the SE side of the caldera. Further eruption of lava along the older northern caldera fault may have produced pahoehoe lavas that precipitated also down the inner caldera fault below the highest point of the island. (Jaggar interpreted these plateaus as fault blocks, not as caldera lavas.)
6. Filling of the caldera by a rain-derived lake.
7. Phreatomagmatic eruptions in the center of the caldera lake, forming tephra cones. Motu Molemole and Motu Lahi are the largest of these, separating the lake into a northern and southern basin along an eruptive line trending WNW-ESE. By echo profiling, we found a third cone protruding about 30 m from the lake bottom in continuation of that line to the SE. These tephra cones show clear signs of wave erosion. The cones of Motu Si‘i and Motu A‘ali are already reduced to the level of the lake. The crater of Motu A‘ali is to the east of this island, under water as shown by our echosounding.
8. Phreatomagmatic eruptions along the southern caldera fault, building a series of (five?) low tephra cones. One of them may have erupted last 1814 (Jaggar, 1945). Waves have cut a cliff into their bases; a wide beach of redeposited volcanic sand formed, separating the depression of the Sulfur Lagoon from the main lake.
9. Further phreatomagmatic eruptions occurred in the NE section of the lake. Remnants of a 1.5-km-wide tephra ring straddle the eastern caldera rim north of Mu‘a. Further eruptions following the NE-SW striking fault form a ridge of tephra cones that separated Vai Si‘i from the main lake (Vai Lahi). The last of these eruptions occurred in 1886 at the northern end of this ridge. Wave erosion is less evident along this ridge suggesting that these eruptions are younger than along the southern part of the island.
10. The outer sections of the islands begin to settle causing normal faults to form, specifically along the western shoulder of the island.

11. These faults may have been accompanied by earthquakes that may have caused sections of the caldera wall to collapse. One such prominent collapse formed a peninsula west of Motu Molemole.
12. Along some of these faults, very fluid lavas erupt, building low spatter ramparts. A few of the eruptions form low shields and craters that issued longer-lived lava channels. Most of these lavas form pahoehoe flows. Upon entry to the sea, some of these have formed coastal cones now much eroded. Historic eruptions in 1853, 1912, and 1929 covered substantial areas when lava issued from several N-S striking fissures burying the old village of Futu. The last of these eruptions occurred in 1946, destroying the main village on the island. Coral xenoliths were observed by Jaggar (1931) in some of the ejecta of minor phreatomagmatic explosions of these eruptions, suggesting the existence of a reef understructure of the present shield volcano.

Jaggar (1935) described the 1886 eruption as follows: "*There was no loss of life, for the trade wind forced the huge cauliflower clouds of sand and dust westward away from the settlements. The site of the eruption was near the north east corner of the big lake where large blocks of cliff rock were engulfed and heaps of sand were piled 300 to 400 feet high. Ponds were left where the explosive craterlets had formed. About three feet of ash fell on the settlements. The story goes that an earthquake shook the island with a gentle swaying motion at 7 p.m. on August 31, 1886 and smaller shocks continued till midnight, causing alarm. Then came a detonation, a "rocket" ascended 3000 feet above the lake, and the quaking ceased. Violent thunderstorms developed and lighting struck in many places. The blizzard of black dust and sand weighted down the vegetation during the night of inky darkness. On the leeward side of the island broken fragments of rock and pumice along with sand and fine dust piled 20 feet deep. The eruption continued in spasms, geyserlike for 18 days with recurrences of terrifying clouds of dust that shut off the light of day.*"

On our geological map, we can only roughly correlate the surface flows with certain eruption dates of Table 1. In order to do this correctly, the individual lava flows would have to be survey in the field. Our expedition did not allow for this time.

4. Materials and Methods

We investigated the caldera lakes of Niuafo'ou during an expedition that lasted from May 29 to June 23, 1998. Three persons participated, the authors and, as a diver, Ralf Hinsch from the Geoforschungszentrum in Potsdam. Equipped with a research permit from the Office of the Prime Minister, we arrived on the island by rented plane on June 2 and were kindly housed by Mr. Semisi Halaholo, the Governor of the island. In order to move around on the lake, an open fishing boat with an outboard motor was rented from Mr. Kulima Lama for 50 Tongan $ per day and taken to the lake by tractor. This proved, because of the steep and narrow track, to be quite a remarkable accomplishment in itself. We investigated

Table 1. List of historic eruptions of Niuafo‘ou (Jaggar, 1935; Macdonald, 1948).

Year	Date	Location	Character
ca. 1814		Caldera ?	Explosive
1840		?	Effusive ?
1853		SW flank	Effusive, destruction of Ahau, fatalities
1867	April 12	SW flank	Effusive
1886	August 13	NE-caldera	Explosive, accompanied by subsidence of the border benches of the caldera, eruption cloud rose 1 km above caldera
1912		W flank	Effusive
1929	July 25	W flank	Effusive, destruction of Futu
1935		S flank	Effusive
1943	September	SW flank	Effusive
1946	Sept. 9–17	NW flank	Effusive, destruction of Angaha

Vai Lahi on June 5, 8, 9, 10, 11, and 12 and Vai Si‘i on June 13. On June 6, a storm kept us off the lake, and June 7 and 14 were Sundays that we used for laboratory work. For most of the days, Mr. To‘aho Lama served us as “captain” on the lake.

The bathymetry of the caldera lake was measured with a car battery-powered echograph (Model Furano Fe 606N) and a handheld GPS (Global Positioning System) (Model Garmin 45) to determine the position. Niuafo‘ou’s map datum was established by the eclipse expedition in 1930, and it is one of the few not stored in the Garmin 45’s memory. We therefore had to use “relational positioning.” We established a point (in WGS 84 coordinates) on the landing between the two lakes and used then the “Go to” function of the Garmin to read distance and bearing to this point. Thus, lists of distance, bearing, and depth were established. These data were then used to draw, first, a map of the track courses and then, second, the bathymetric map of the lake (Fig. 2).

The water column was sampled with a stone-weighted handline and a 5-l Niskin bottle, triggered by a messenger weight. Because of Jaggar’s erroneous depth figure, we had brought only 100 m of rope so that we could not sample the lowest 20 m of the northern basin. Due to weight limitations, we could only take 17 water samples, seven from the southern and one from the northern basin of Vai Lahi, two from Vai Si‘i, three shore samples, two from other lakes, and two seawater samples on Tongatapu for comparison. Temperature, pH, Eh, and conductivity were measured in the field with battery-powered handheld instruments. CO_2-pressure (pCO_2) was measured with a handheld infrared probe, screwed into the headspace of a sample bottle that was allowed to equilibrate for about 15 min. This method is very unreliable, and thermodynamically calculated data proved to be more accurate (see below). Alkalinity was measured titrimetrically on fixed samples, and Si-, HS-, PO_4-, NO_2-, NH_4-, Fe-plus Mn-concentrations were measured photometrically with a portable photometer on the same day of the sampling. Five O_2-samples were fixed and titrated (Winkler Method) in Germany. HNO_3-fixed aliquots were taken to Germany for atomic absorption spectroscopy (AAS) of major cations, and $HgCl_2$-fixed samples were used to analyze for anions by ion chromatography (IC) against standards.

We also collected carbonate stromatolites and sediment samples and benthic algae, some of them by diving down to a few meters of depth, and took above-water and underwater photographs. The rock samples were analyzed for mineral and elemental composition, and their microfabrics were inspected using thin sections and a scanning electron microscope (SEM) and energy dispersive X-ray techniques (EDX).

5. The Caldera Lakes of Niuafoʻou

The caldera of Niuafoʻou houses ten lakes. The largest is Vai Lahi ("Water Big"), followed by Vai Siʻi ("Water Small") in size. The other eight lakes are much smaller. On Fig. 2, these are labeled 1–8. Lake 1 is the crater lake of the Molemole cone; lake 2 is a lagoon of Vai Lai on Jaggar's map. It may have been closed off by a newly formed beach bar. Lakes 3–8 are labeled Vai Fo, Vai Xoma (now Sulfur Lagoon), Vai Sulifa, Vai Langita, Vai Pikopiko, and Vai Ahau, respectively. These names were not mentioned to us while on the island. These lakes may have played a larger role in the past than today as emergency freshwater reservoirs.

Jaggar (1935) reported that the crater lake of Vai Lahi is 275 ft (84 m) deep. No further information was available. Thus, we ran 15 echosound tracks across Vai Lahi and six tracks across Vai Siʻi (methods see above) allowing to construct the first bathymetric map of the crater's two big lakes. It turned out that Vai Lahi is significantly deeper than previously reported. The southern basin has a rather flat bottom with a deepest point at 106 m. The sills toward the northern basin are about 20, 78, and 75 m deep (from W to E, respectively). The northern basin levels out at 120 m depth with a deepest point at 121 m. The northern shore is the steepest, soaring over 200 m from the lake bottom to the caldera rim. Vai Siʻi also has two basins: a shallower one, 14 m deep, in the south and a 31 m deep one in the north, separated by a sill at less than 10 m depth. The depths of the other lakes remain unknown. These bathymetric maps allowed calculating the volumes of the lakes (Table 2). Vai Lahi has an area of 13.6 km^2 and a volume of almost 1 km^3. The values for Vai Siʻi are 1.14 km^2 and 0.0115 km^3.

Table 2. Area and volume of the two larger lakes on Niuafoʻou.

Vai Lahi	**0 to −25 m**	**−25 to −50 m**	**−50 to −75 m**	**−75 to −100 m**	**−100 to −121 m**	**−100 to −106 m**	**Total**
km	15.6	Length of perimeter					
km^2	13.63	11.91	9.98	7.99	1.47	2.88	13.63
km^3	0.3191	0.2733	0.2241	0.1519	0.0103	0.0058	0.9844
Vai Siʻi	**0 to −25 m**	**−25 to −31 m**					
km^2	0.813	0.161					1.14
km^3	0.0111	0.0003					0.0115

Jaggar reported that the lake level is 75 ft (23 m) above sea level (a.s.l.). The sea chart of Niuafoʻou shows that the surface of the small, ca. 2-m-high island of Motu Aʻali in Vai Lahi is 2 m a.s.l. Thus, the lake level is within decimeters of mean sea level. The beach along the southern shore of the lake and cliffs on the inner islands show that the water level was higher not so long ago. In 1998, we were favored by a low water level. The Google Earth picture displayed in 2007 clearly shows a higher water level with all of the stromatolite areas of Motu Aʻali under water and the southern beach inundated.

6. Water Chemistry

6.1. HYDROPHYSICAL RESULTS

Hydrophysical data of the water samples (Table 3) show that the lake has a temperature between 26 and 27°C. The coldest water was encountered at depth. This is true for both basins. With our instrumentation, we could not measure temperature precisely enough to establish a more accurate temperature curve. The conductivity changes even less throughout the water column, with just barely detectable higher values in the bottom waters. These small temperature and conductivity differences indicate that the density difference between the bottom and surface waters must also be small and that the stability of the lake against overturn is low.

Nevertheless, the lake shows a distinct stratification in its chemical characteristic. pH and Eh change substantially between 40 and 45 m, separating a wind-mixed, oxygenated surface layer from an anaerobic bottom layer. The $p\mathrm{CO}_2$ increases substantially with depth, illustrating that the bottom waters are not in equilibrium with the atmosphere and that the lake must have been stagnant for some time since its last overturn.

The waters of Vai Siʻi are different than those of Vai Lahi, suggesting that the two lakes have been separated since some time, in spite of the fact, that they are only separated by a low land bridge that could be flooded by an about 2 m lake level increase. The conductivity is much lower, but the pH is higher than in Vai Lahi. Vai Siʻi is also stratified, with a slightly lower bottom temperature and a distinctly higher bottom conductivity. Overall, Vai Siʻi is more firmly stratified than Vai Lahi. This is also illustrated by the fact that pH and Eh are very low in the bottom waters, while the $p\mathrm{CO}_2$ is much higher than in Vai Lahi, indicative of ongoing volcanic CO_2 input.

Of the three shore samples, two (W4 and W7) show much higher temperature and $p\mathrm{CO}_2$ and a lower pH than the Vai Lahi. These waters show influence by hydrothermal activity. Sample W5 was taken at Stromatolite Bay and is very similar to the average water of the surface layer. Temperatures and pH are slightly higher than in the lake surface water.

Table 3. Hydrophysical data of water samples.

Sample	Depth (m)	Temp. (°C)	pCO$_2$ (ppmv)	pH	Eh (mV)	Eh corr.(+206) (mV)	Conductivity (mS/cm)
Vai Lahi	Southern basin						
W12b	0	26.8		8.34	72	278	5.65
W12	−10	26.5	870	8.35	72	278	5.65
W3	−25	27.0	788	8.39	−52	154	5.69
W9	−40	26.6	1,620	8.27	n.d.	n.d.	5.63
W11	−42.5	26.4	1,180	8.13	−68	138	5.67
W10	−45	26.2	2,330	7.67	−151	55	5.68
W2	−50	26.3	2,055	7.73	−181	25	5.69
W1	−100	26.5	1,938	7.50	−210	−4	5.69
Vai Lahi	Northern basin						
W13	−100	26.0	2,013	7.65	n.d.		5.71
Vai Si'i							
W15	−10	26.9	644	8.69	−5	201	3.24
W14	−30	26.5	>6,000	7.25	−412	−206	4.18
Shore samples							
W4	0	33.0	5,025	6.80	−17	189	6.41
W5	0	27.2	449 (air)	8.53	94	300	5.69
W7	0	40.0	>6,000 (608 air)	7.21	−160	46	5.16
Vai Molemole							
W6	0	28.2	560 (air)	7.70	210	416	0.08
Sulfur Lagoon							
W8	0	31.0	>3,800	7.16	39	245	4.21
Seawater samples, Tongatapu							
W16	0	26.1	575	8.59	−38	168	45.65
W17	0	25.3	545	8.38	−23	183	45.65

Table 4. Alkalinity and nutrient data of water samples.

	Alkalinity	**Si**	**PO_4**	**HS**	**O_2**	**NO_3-N**	**NO_2**	**NH_4**	**Mn**
Sample	**meq/l**	**mg/l**	**mg/l**	**mg/l**	**mg/l**	**mg/l**	**mg/l**	**mg/l**	**mg/l**
Vai Lahi	Southern basin								
W12b							0.04	n.d.	
W12	15.70	5.30	0.00	0.00	15.69		0.08	0.00	
W3	16.60	5.54	0.10	0.00		0.41	0.09	0.05	
W9	15.70	5.52	0.10	0.00	12.90		0.17	0.10	
W11	16.60	4.02	0.10	0.00	10.81		0.06	0.10	
W10	16.30	5.36	0.30	0.11	9.00		0.04	0.30	
W2	18.80	5.09	0.20	0.14		0.35	0.02	0.24	0.21
W1	18.60	5.48	0.20	0.31		0.65	0.03	0.25	0.05
Vai Lahi	Northern basin								
W13	15.50	8.95	0.60	0.63		0.00	0.02	0.84	
Vai Si‘i									
W15	6.30	1.88	0.00	0.01	14.54	0.00	0.04	0.11	
W14	10.90	3.95	2.50	9.60		0.00	0.05	0.28	
Shore samples									
W4	22.30	4.28	0.00	0.00		0.49	0.02	0.50	0.24
W5	14.60	7.74	0.10					0.10	
W7	14.00	5.28	0.10	−0.01			(−0.01)	0.10	
Vai Molemole									
W6	0.23	0.64	0.20					0.20	
Sulfur Lagoon									
W8	6.58	5.52	0.10	0.01			0.03	0.10	
Seawater samples, Tongatapu									
W16	2.19	0.05	(−0.10)	0.00			0.01		0
W17	2.2	0.03	(−0.10)	0			0.01		

The water with the lowest salinity was encountered in the small crater lake of Motu Molemole; it contains essentially fresh water, albeit of a doubtful organic quality.

The Sulfur Lagoon is relatively warm, has a conductivity that is somewhat lower than that of Vai Lahi, and has a low pH and an astonishingly high $p\mathrm{CO}_2$.

For comparison, we have also taken two samples of seawater on Tongatapu, characterized by their high conductivity. Their pH is comparable to that of Vai Lahi, and the $p\mathrm{CO}_2$ is low.

6.2. NUTRIENT RESULTS

The concentration of the main nutrients, of HS^-, and of alkalinity measured in the field or in the evening after the recovery is given in Table 4.

Alkalinity (i.e., $[HCO_3^-] + 2[CO_3^{2-}]$) was proved to be very high, much higher than in seawater. This result was one of the big successes of the expedition, as it is in congruence with our soda ocean model. In seawater (W16, W17), the alkalinity reaches just 2.2 meq/l, while we found values of 15.7 meq/l in surface waters in Vai Lahi increasing to 18.8 meq/l at depth. This high alkalinity causes a high pH and probably inhibits the presence of most brackish water organisms which cannot survive under high alkalinity.

Nutrient concentrations show the stratification of the lake much better than the hydrophysical data. Specifically, the reducing condition of the bottom water is illustrated by the existence of HS^- and ammonia. It is astonishing that at a depth of 45 m, oxygen and H_2S apparently coexist. This is not possible for longer times and may indicate a recent mixing event, affecting the upper layers of the bottom water. The presence of H_2S in the bottom waters is significant and readily detectable by its foul smell, but the concentrations are rather small, again showing the young nature of the lake's stagnation.

The phosphate concentration increases significantly with depth, which is normal for stagnant water bodies, because sinking organic matter is remineralized at depth, liberating phosphate.

We could, however, not find a significant structure in the SiO_2 concentration (given as elemental Si). This is an interesting observation because SiO_2 is, in normal waters, a nutrient essential for diatoms. The high concentration in the euphotic surface waters shows the general absence of diatoms in Vai Lahi, and the missing increase in the bottom waters also shows that hardly any diatom frustules sink and become remineralized. The SiO_2 concentrations in the surface water of Vai Si'i are significantly lower than in Vai Lahi, indicating the presence of diatoms in that lake. There, we also found an increase of SiO_2 with depth, typical for ongoing remineralization. These results were also substantiated by the microscopic investigations that showed that benthic diatoms are present in Vai Si'i, but not in Vai Lahi.

The nitrite concentration is a fine indicator for reducing conditions; it peaks at a depth of 40 m (W9), where apparently some of the nitrate is reduced, and decreases below without vanishing, indicating that the reducing conditions never become severe enough to deplete it completely. This is also illustrated by the existence of nitrate, even in the bottom waters where we also find ammonia. Manganese could not be measured regularly enough in the field because of time limitations; it was detectable only in the reducing bottom waters and will be discussed below with the cations measured by AAS.

The concentrations of phosphate, HS^-, and ammonia in the deep water of the northern basin (W13) are higher than those of the southern basin (W1). The waters here become reducing enough to lose the nitrate concentration completely. Also, the SiO_2 is higher. This could be due to enhanced input of hydrothermal water. The alkalinity value is as low as in surface waters, a fact, that we do not understand and could possibly be an analytical error.

The waters of Vai Si'i have a lower alkalinity than those of Vai Lahi, but difference in alkalinity between surface and bottom waters is much higher. The bottom waters are also much higher in HS^- and phosphate than the bottom

Table 5. Cation concentrations.

Sample	Depth (m)	Na (meq/l)	K (meq/l)	Ca (meq/l)	Mg (meq/l)	Sr (meq/l)	Sum of Cations
Vai Lahi	Southern basin						
W12	−10	42.83	0.55	0.71	21.04	0.0046	65.14
W3	−25	45.28	0.58	0.68	20.74	0.0058	67.28
W9	−40	42.66	0.56	0.70	21.04	0.0054	64.96
W11	−42.5	41.67	0.53	0.78	20.85	0.0065	63.84
W10	−45	43.20	0.55	1.06	21.08	0.0081	65.89
W2	−50	45.51	0.56	1.12	21.10	0.0090	68.30
W1	−100	45.48	0.60	1.15	21.15	0.0083	68.38
Vai Lahi	Northern basin						
W13	−100	41.62	0.54	1.09	21.01	0.0074	64.27
Vai Si'i							
W15	−10	25.27	0.34	1.17	9.43	0.0055	36.22
W14	−30	30.64	0.41	2.32	11.99	0.0110	45.38
Shore samples							
W4	0	52.25	0.67	2.08	24.01	0.0123	79.02
W5	0	43.31	0.61	0.68	21.08	0.0052	65.70
W7	0	46.11	0.61	1.53	17.71	0.0083	65.97
Vai Molemole							
W6	0	0.37	0.04	0.12	0.18	0.0009	0.71
Sulfur Lagoon							
W8	0	32.57	0.47	1.72	11.30	0.0089	46.06
Seawater samples, Tongatapu							
W16	0	454.02	9.00	19.97	107.13	0.1137	590.22
W17	0	451.48	8.63	19.85	106.97	0.1131	587.05

waters of Vai Lahi. In case of phosphate, this could be a consequence of the practice to use this lake for laundering.

The three shore samples are quite interesting because they have a varying Si content but low phosphate concentrations. The crater lake of Motu Molemole is low in Si and alkalinity, as would be expected from its low conductivity. The nutrients are low in the Sulfur Lagoon, except for Si and ammonia. The two seawater samples finally have, with our field techniques, undetectable nutrient concentrations, typically for the surface layer of the ocean. The alkalinity amounted to 2.2 meq/l, as expected for surface seawater.

6.3. MAJOR CATIONS AND ANIONS

As shown in Table 5, Na is the main cation in solution, followed by Mg, Ca, K, and Sr. This order is the same as in seawater, but the ratios are much different. The Na/Mg ratio in seawater is 4.2, as compared to 2.0 in Vai Lahi and 2.6 in Vai Si'i, and the Na/K ratio is 50.4 in seawater and 77.9 in Vai Lahi and 74.3 in Vai Si'i. This shows that these waters are not diluted seawater but have evolved by a different process, that is, by rain water and its reaction with the volcanic rocks.

Cation concentrations significantly increase with depth in Vai Lahi in accordance with the slight increase in conductivity with depth. The increases are small except for Ca and Sr, which almost double their concentrations. These two elements are liberated by the dissolution of aragonite. It is interesting to note that the 100-m sample from the northern basin has, overall, the same concentration as the surface water and does not show the observed increases for Na, K, and Mg but those for Ca and Sr.

Vai Si‘i has a lower mineralization for Na, K, and Mg but shows higher concentration for Ca and Sr and the already noticed doubling in concentration with depth, indicative for the congruent dissolution of aragonite.

The shore samples are much higher differentiated than their other data suggest; specifically, the Na and Mg concentrations do not shift in accordance, indicative of individual hydrothermal pathways of volcanic weathering. W5 is essentially lake surface water, but samples W4 and W7 have higher than normal Ca and Sr concentrations. This shows that the hydrothermal waters constitute inputs for Ca into the lake. This also lets us conclude that Vai Si‘i has recently received more weathering solutions than Vai Lahi because of the larger influence of the fresh 1886 ashes.

The lake on Motu Molemole has very low cation concentrations and a lower Mg/Ca ratio (1.5) than that of Vai Lahi (W12: 29.6). This shows that mobilization of Mg and Ca occurs at a much lower ratio and that the Ca concentration is later decreased by a precipitation process removing Ca.

The concentrations of total iron and manganese in Vai Lahi (Table 6) show the typical peaks at the interface of stratified water bodies (Fig. 3).

The concentration of dissolved iron and manganese shows a peak at around −50 m because here they are reduced to the water-soluble ions Mn^{2+} and Fe^{2+}. Actually, the concentrations should increase toward the bottom, but the presence of H_2S causes the formation of MnS and FeS which settles from the water column. The high concentration in the surface waters most probably results from Fe and Mn taken up in the phytoplankton.

Concerning anion concentrations (Table 7), chloride is the main anion in Vai Lahi, followed by alkalinity, sulfate, and bromide, while in seawater the order is chloride, sulfate, alkalinity, and bromide, again showing that Vai Lahi is not just diluted seawater.

Chloride and sulfate show a slight concentration increase with depth in Vai Lahi, alkalinity a noticeable one, and bromide shows a large increase, tripling its amount in the deep water sample of the southern basin, but not in the northern basin.

In Vai Si‘i, we find overall lower concentrations of chloride and alkalinity, but not for bromide and sulfate. From the shore samples, W7 is remarkable for its high sulfate and low bromide content. A very high sulfate concentration is also noticed in the sample of the Sulfur Lagoon. Possibly, the water of it leaks out into

Table 6. Iron and manganese concentrations.

Sample	Depth (m)	Fe (mg/l)	Mn (mg/l)	Fe (mmol/l)	Mn (mmol/l)
Vai Lahi	Southern basin				
W12	−10	0.23	0.028	0.0040	0.0005
W3	−25	0.11	0.012	0.0020	0.0002
W9	−40	0.20	0.024	0.0036	0.0004
W11	−42.5	0.19	0.035	0.0034	0.0006
W10	−45	0.22	0.046	0.0039	0.0008
W2	−50	0.11	0.014	0.0020	0.0003
W1	−100	0.10	0.008	0.0018	0.0001
Vai Lahi	Northern basin				
W13	−100	0.20	0.15	0.0036	0.0028
Vai Si‘i					
W15	−10	0.16	0.043	0.0028	0.0008
W14	−30	0.49	1.09	0.0087	0.0199
Shore samples					
W4	0	1.4381	0.1019	0.0257	0.0019
W5	0	0.1780	0.0149	0.0032	0.0003
W7	0	0.9879	0.1472	0.0177	0.0027
Vai Molemole					
W6	0	0.3114	0.0023	0.0056	0.0000
Sulfur Lagoon					
W8	0	0.1452	0.1552	0.0026	0.0028
Seawater samples, Tongatapu					
W16	0	0.3569	0.0808	0.0064	0.0015
W17	0	0.3503	0.0819	0.0063	0.0015

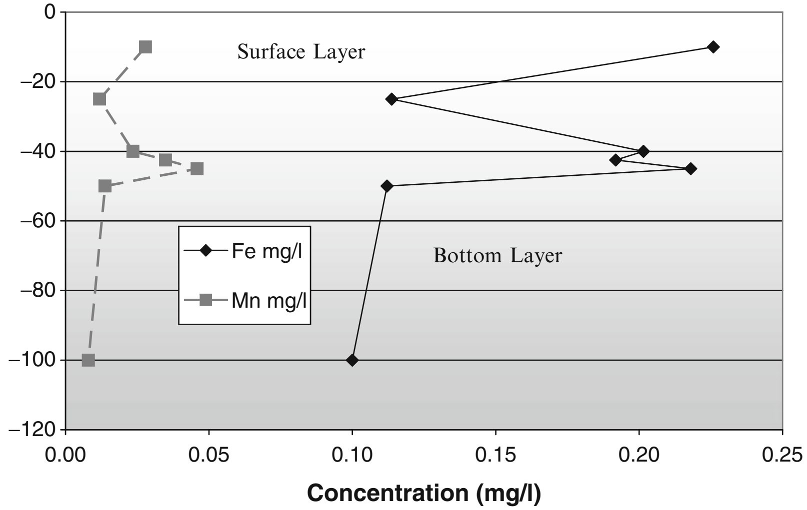


Figure 3. Iron and manganese depth profiles of Vai Lahi.

Table 7. Anion concentrations.

Sample	Depth (m)	Br (meq/l)	Cl (meq/l)	SO$_4$ (meq/l)	HCO$_3$ (meq/l)	Sum of Anions	Cations-Anions (meq/l)
Vai Lahi	Southern basin						
W12	−10	0.0337	53.23	1.63	15.70	70.59	−5.46
W3	−25	0.0127	53.47	1.73	16.60	71.81	−4.54
W9	−40	0.0342	53.30	1.78	15.70	70.81	−5.84
W11	−42.5	0.0482	53.64	1.62	16.60	71.90	−8.06
W10	−45	0.0534	55.59	1.63	16.30	73.57	−7.68
W2	−50	0.0569	55.48	1.75	18.80	76.09	−7.79
W1	−100	0.1091	54.86	1.81	18.60	75.37	−7.00
Vai Lahi	Northern basin						
W13	−100	0.0254	53.62	1.55	15.50	70.69	−6.42
Vai Si‘i							
W15	−10	0.0981	30.33	1.50	6.30	38.23	−2.00
W14	−30	0.0377	37.30	1.39	10.90	49.63	−4.25
Shore samples							
W4	0	0.0477	61.41	1.85	22.30	85.60	−6.59
W5	0	0.0460	54.48	1.78	14.60	70.91	−5.22
W7	0	0.0184	54.93	2.29	14.00	71.23	−5.26
Vai Molemole							
W6	0	0.0000	0.35	0.06	0.23	0.64	0.07
Sulfur Lagoon							
W8	0	0.0407	37.78	4.80	6.58	49.21	−3.15
Seawater samples, Tongatapu							
W16	0	0.4547	529.34[a]	50.94	2.19	582.92	7.31
W17	0	0.3706	526.38[a]	52.08	2.20	581.03	6.02

[a] Calculated by using Na measurement and multiplying it with the Cl/Na ratio of seawater.

Vai Lahi carrying the high sulfate signal with it. The lake on Motu Molemole has overall very low concentrations, but here, the alkalinity concentration is almost as large as the chloride concentration.

6.4. MINERAL EQUILIBRIA AND CARBON ISOTOPES

The concentrations of the cations and anions, pH, and temperature allow calculating the mineral equilibria of important minerals. This was done with the program PHREEQE (Parkhurst et al., 1990). The saturation index (SI) is the logarithm of the ratio between the Ion Activity Product of the free ions in question and the equilibrium constant at the *in situ* temperature. A value of zero means saturation; negative numbers denote undersaturation and positive numbers supersaturation. The CO_2-pressure (pCO_2) is both given as its negative logarithm and as ppmv. Note that the pCO_2 of air was about 365 ppm at time of sampling.

All of the carbonate minerals are highly supersaturated in the surface waters of both Vai Lahi and Vai Si‘i (Table 8). Figure 4 shows their variation with depth.

Table 8. Mineral saturation (SI = Saturation Index) and CO_2 pressure (ppCO$_2$ = neg. logarithm of pCO$_2$).

Sample	Depth (m)	SI calcite	SI aragonite	SI dolomite	ppCO$_2$	pCO$_2$ (ppmv)	δ^{13}C (permil)
Vai Lahi	Southern basin						
W12	−10	0.65	0.50	2.94	2.50	3,180	–
W3	−25	0.69	0.54	3.04	2.51	3,090	0.11
W9	−40	0.57	0.47	2.80	2.41	3,900	–
W11	−42.5	0.52	0.37	2.63	2.24	5,810	–
W10	−45	0.21	0.06	1.87	1.77	17,100	–
W2	−50	0.34	0.20	2.12	1.77	17,100	−0.61
W1	−100	0.13	−0.02	1.68	1.53	29,200	−0.41
Vai Lahi	Northern basin						
W13	−100	0.18	0.03	1.80	1.77	17,030	–
Vai Si‘i							
W15	−10	0.89	0.75	2.87	3.32	480	−1.98
W14	−30	0.04	−0.10	0.95	1.49	32,100	–
Shore samples							
W4	0	−0.17	−0.31	0.94	0.72	192,000	–
W5	0	0.75	0.61	3.18	2.73	1,860	–
W7	0	0.03	−0.10	1.40	1.27	53,300	–
Vai Molemole							
W6	0	−2.06	−2.20	−3.77	3.53	302	–
Sulfur Lagoon							
W8	0	−0.35	−0.45	0.32	1.51	25,600	–
Seawater samples, Tongatapu							
W16	0	0.97	0.83	2.87	3.89	129	–
W17	0	0.82	0.68	2.56	3.61	245	–

Dolomite is supersaturated throughout the water column, but calcite and aragonite decrease so much in supersaturation, that aragonite reaches saturation at depth, making aragonite dissolution possible.

This decrease in supersaturation (Fig. 4) is caused by the large increase of pCO$_2$ toward depth (Fig. 5), which causes the shift of carbonate to bicarbonate ions and, in spite of the increase in Ca and alkalinity with depth, causes a drop in supersaturation. The pCO$_2$ increases to almost 30,000 ppmv, that is, to almost 100 times the partial pressure of CO_2 in air. Furthermore, we notice that even the surface waters of Vai Lahi have a high pCO$_2$, even though the upper layer of the lake should be in equilibrium with the atmosphere. This suggests that the lake currently receives an excess of volcanic CO_2 through hydrothermal springs and that therefore the calcite and aragonite saturation as well as pH in the surface waters should be higher in periods of the volcano history when less volcanic CO_2 is available.

The δ^{13}C was measured on three samples from Vai Lahi and on the deeper sample of Vai Si‘i. The surface sample is measurably heavier than the deep samples. This suggests that part of the alkalinity and pCO$_2$ increase with depth is also due to

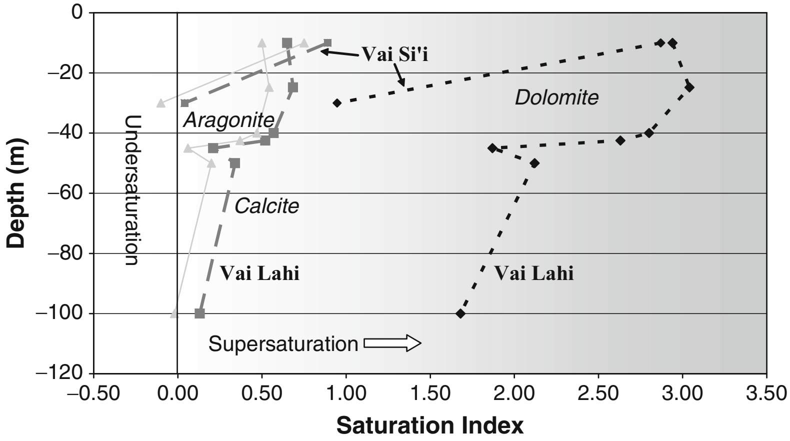


Figure 4. Carbonate mineral saturation in Vai Lahi and Vai Si'i.

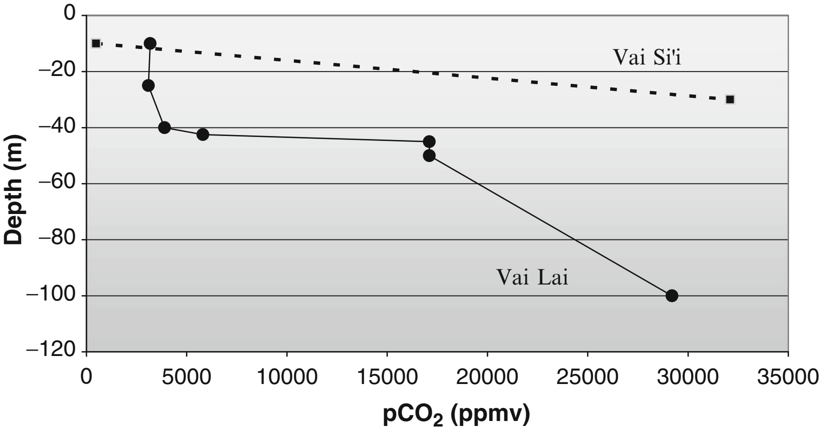


Figure 5. CO_2-pressure increase with depth in Vai Lahi.

respiration of light organic matter. Nevertheless, this increase is very small compared to other anaerobic lakes, like the marine-water-filled lakes of Kaukaho, Molokai, and Satonda, Indonesia (Kempe, 1990, 2002; Kempe and Kazmierczak 1990a, b, 1993), where massive sulfate reduction causes increase in alkalinity and decrease in $\delta^{13}C$.

6.5. TRACE ELEMENTS

The concentration of trace elements was measured on a selected set of samples (W1, W2, W3, W4, W15, W16) by Dr. Jean-Luc Probst, University of Strasbourg with ICP-MS. Results are given in Table 9.

Table 9. Trace element concentrations.

Vai Lahi	Depth (m)	Cr 52 (ppb)	Co 59 (ppb)	Ni 60 (ppb)	Cu 65 (ppb)	Zn 66 (ppb)	Rb 85 (ppb)	Y 89 (ppb)
W3	−25	7.17	0.33	0.58	5.28	31.23	41.99	n.d.
W2	−50	7.59	0.37	4.25	6.04	41.52	43.16	0.0039
W1	−100	7.35	0.63	9.70	6.11	26.52	43.04	0.0097
Vai Si‘i								
W15	−10	6.30	0.41	2.33	5.87	33.76	22.88	0.0268
Shore samples								
W4	0	7.92	0.56	2.74	7.06	36.47	51.46	0.0972
Seawater samples, Tongatapu								
W16	0	82.77	4.80	(79.80)?	83.48	392.61	109.89	0.0460

Vai Lahi	Depth (m)	Cd 111 (ppb)	Ba 137 (ppb)	La 139 (ppb)	Ce 140 (ppb)	Pr 141 (ppb)	Nd 146 (ppb)	Sm 147 (ppb)
W3	−25	0.1696	5.42	0.1747	0.0352	0.0058	0.0084	n.d.
W2	−50	0.1765	8.51	0.0688	0.0322	0.0037	0.0298	n.d.
W1	−100	0.2924	8.87	0.0365	0.0434	0.0138	0.0346	n.d.
Vai Si‘i								
W15	−10	0.1799	3.86	0.0791	0.0362	0.2292	0.0346	0.0040
Shore samples								
W4	0	0.2163	6.97	0.1828	0.0927	0.0563	0.0811	0.0499
Seawater samples, Tongatapu								
W16	0	2.6472	26.12	0.8521	0.6022	0.1340	0.2626	0.0657

Vai Lahi	**Depth (m)**	**Eu 151 (ppb)**	**Gd 157 (ppb)**	**Tb 159 (ppb)**	**Dy 163 (ppb)**	**Ho 165 (ppb)**	**Er 166 (ppb)**	**Tm 169 (ppb)**
W3	−25	0.0000	n.d.	0.0043	0.0040	0.0028	0.0103	n.d.
W2	−50	0.0111	0.0036	0.0010	0.0064	n.d.	0.0169	n.d.
W1	−100	0.0086	0.0218	0.0070	0.0064	0.0024	0.0127	0.0026
Vai Si‘i								
W15	−10	0.0218	0.0085	0.0183	0.0175	0.0153	0.0320	0.0146
Shore samples								
W4	0	0.0542	0.0894	0.0600	0.0469	0.0520	0.0622	0.0341
Seawater, Tongatapu								
W16	0	0.0001	0.2416	0.0449	0.1590	0.0363	0.1509	0.0380

Vai Lahi	**Depth (m)**	**Yb 172 (ppb)**	**Lu 175 (ppb)**	**Pb 208 (ppb)**	**Th 232 (ppb)**	**U 238 (ppb)**
W3	−25	n.d.	n.d.	3.54	0.100	n.d.
W2	−50	n.d.	0.0022	4.18	0.171	0.002
W1	−100	n.d.	0.0052	3.78	0.696	0.214
Vai Si‘i						
W15	−10	0.0000	0.0093	3.40	0.122	0.019
Shore samples						
W4	0	0.0413	0.0516	3.63	0.404	0.032
Seawater sample, Tongatapu						
W16	0	n.d.	0.0434	48.13	1.031	2.795

Note that the detection limit for all samples actually is 0.1 ppb (except for W16 which has a detection limit of 1 ppb) and that the numbers given are approximates only.
Note: number behind element denotes isotope measured.
n.d. not detected.

Trace element concentrations are mostly much lower in Vai Lahi and in Vai Si‘i than in seawater (compare figures with results of W16). Furthermore, some of the metals, that is, Co, Ni, Cd, Ba, Ce, Nd, Gd, Th, and U, show strong increase with depth due to the increasingly reducing conditions. Some of these metals change oxidation state (e.g., Ce); others become more soluble because of changes in the anion composition (Ba, e.g., which may become more soluble as the sulfate concentration decreases). Elements that readily associate with particulate matter may be purged from the top layer rapidly, thereby also showing depth increases. Some elements decrease in concentration with depth, namely, Zn, La, and Pb, most probably because they settle from the water column as particulate sulfides at increasingly higher sulfide concentrations with depth, that is, they behave similar to iron and manganese. Some other metals do not show much concentration changes because they are neither influenced by redox changes nor by solubility changes induced by changes in the anion concentration nor do they easily adsorb to organic matter (e.g., Cr and Rb).

The differences between Vai Lahi and Vai Si‘i (compare samples W3 and W15) are notable and are, for most of the elements, not just a consequence of Vai Si‘i’s lower salinity. Most of the metals have in fact higher concentrations in Vai Si‘i than in the surface layer sample of Vai Lahi; Rb and Pb are the exceptions.

The same comparison holds true for the hot spring shore sample W4. It has, in general, higher trace element concentrations than the surface sample of Vai Lahi and, for some elements, also of the deep water samples. This clearly shows how most of the trace elements get into the lake: They are mobilized by the leaching of the volcanic rock. Once in the lake, most are controlled by removal processes, such as adsorption onto organics, or by settling as sulfides or as other compounds. This prevents the buildup of higher concentrations of trace metals.

6.6. SUSPENDED MATTER

Both Vai Lahi and Vai Si‘i are quite turbid in spite of the fact that in shallow waters, one can see the bottom down to a depth of about 1–1.5 m. In Vai Lahi, the Secchi depth was 3.3 m and for Vai Si‘i 3.0 m. When diving, the water is clear enough for orientation, but it is always somewhat murky with a greenish hue. Nevertheless, there is no plankton visible to the naked eye. Microscopic examination of surface water filtrates shows abundant cyanobacteria and protococcalean green algae (*Nannochloris* sp.). Table 10 gives the results of the few filtrations we were able to make.

Filtration in the field is difficult because of the tendency of suspended matter to settle in the water sample while the water sample is processed. Specifically, with low suspended matter concentrations, a large scatter is expected in the data. This is especially true for samples rich in organic matter, such as the suspended matter in lakes that consist essentially of microphytoplankton and their excretions. This mucus tends to clog especially Nucleopore filters before a reasonable

Table 10. Weight of suspended matter.

	Nucleopore filter	Nucleopore filter	Weight	Suspended matter	Glass fiber filter	Glass fiber filter	Weight	Suspended matter
Sample	**mg new**	**mg filtered**	**mg**	**mg/l**	**mg new**	**ml filtered**	**mg**	**mg/l**
Vai Lahi	Southern basin							
W12b	14.90	500	1.10	2.20	143.30	2,000	4.60	2.30
W12	15.60	500	2.10	4.20	133.10	2,000	4.70	2.35
W10					142.30	2,350	2.50	1.06
W2	16.00	500(?)	1.00	2.00				
W1					136.20	3,000	2.00	0.67
Vai Lahi	Northern basin							
W13					128.50	2,300	1.80	0.78
Vai Si‘i								
W15	15.40	500	1.00	2.00	143.90	1,500	3.70	2.47
W14					140.70	500	7.10	14.20
Vai Molemole								
W6	15.00	250	0.70	2.80	125.60	150	1.20	8.00

Table 11. C, N, and S analysis of suspended matter from Vai Si‘i (depth 10 m).

Glass fiber filter	Amount (mg)	C_{tot} (%)	N_{tot} (%)	S_{tot} (%)	C/N (weight)	C/S (weight)	N/S (weight)
W15	3.7	33.14	8.15	5.66	4.1	5.86	4.07

amount of material has been collected on the filter. In the upper water column between 2.2 and 4.2 mg/l, suspended matter was found. In the deep water samples, where only bacteria and remineralizing organic debris exist, the concentration is lower, between 2 and 0.7 mg/l. In Vai Si‘i, this is different, in as much as the lower sample has a concentration of 14 mg/l, a quite high value. Also, the crater lake of Motu Molemole has relatively high values, even though the glass fiber filter and the Nucleopore filter results differ because they were done from two different shore samples.

We could analyze the elemental composition of the suspended material from one sample only (W15, 10 m Vai Si‘i) which had enough material collected (Table 11). The total C concentration amounts to 33%. Since organic matter weighs roughly 2.5 times its carbon content, we have to conclude that most of the suspended matter is in fact phytoplankton. Its N-concentration is high, and therefore, the C/N weight ratio is low, that is, 4.1. This can be explained by the fact that much of the phytoplankton organisms are cyanobacteria. They can fix nitrogen, yielding a C/N ratio much lower than in normal oceanic phytoplankton which is dominated by eukaryotic organisms. Since nitrogen is available to the cyanobacteria, phosphorus seems to be the limiting nutrient, a conclusion in line with the fact that we could not detect phosphate in the surface of Vai Si‘i.

7. Macrobiota

The macrobiota of Vai Lahi is very species-poor. Formerly, a small crab inhabited the lake in large numbers (Jaggar, 1935). Of it, we only found small carapace pieces cemented in the sediment. It probably is extinct. Apparently, it was never described scientifically. It most probably was destroyed by the introduction of *Tilapia* in the fifties, a fish which preys on almost anything. Furthermore, a small, cerithoid (turret-shaped) gastropod lives in the lake. Its shells are abundant in places, but we only found one living specimen. Again, *Tilapia* may be the reason for its decline. Dr. E. Neubert of the Senckenberg Museum in Frankfurt kindly looked at the gastropod sample. It is most probably a species of the genus *Melanoides*, typical for tropical freshwater lakes. Looking at Internet lists and graphs, it may well be *Melanoides (Thiara) tuberculata* (Müller, 1774) (red-rimmed melania), belonging to the family Thiaridae (superfamily Cerithioidea, order Mesogastropoda). The shell is generally rather smooth; fresh specimens have a dark color. Rarely, we found smaller shells with more marked ribs, possibly a different species or subspecies.

This extreme poverty in macroscopic life in the lake is most probably due to the high alkalinity in the lake. Once the crab became extinct, *Tilapia* has not much to sustain itself from, and the specimens caught were all rather thin. In fact in Vai Si‘i, where *Tilapia* was also introduced, we caught one of them by swimming after it and grabbing it by the tail.

8. Carbonate Sediments

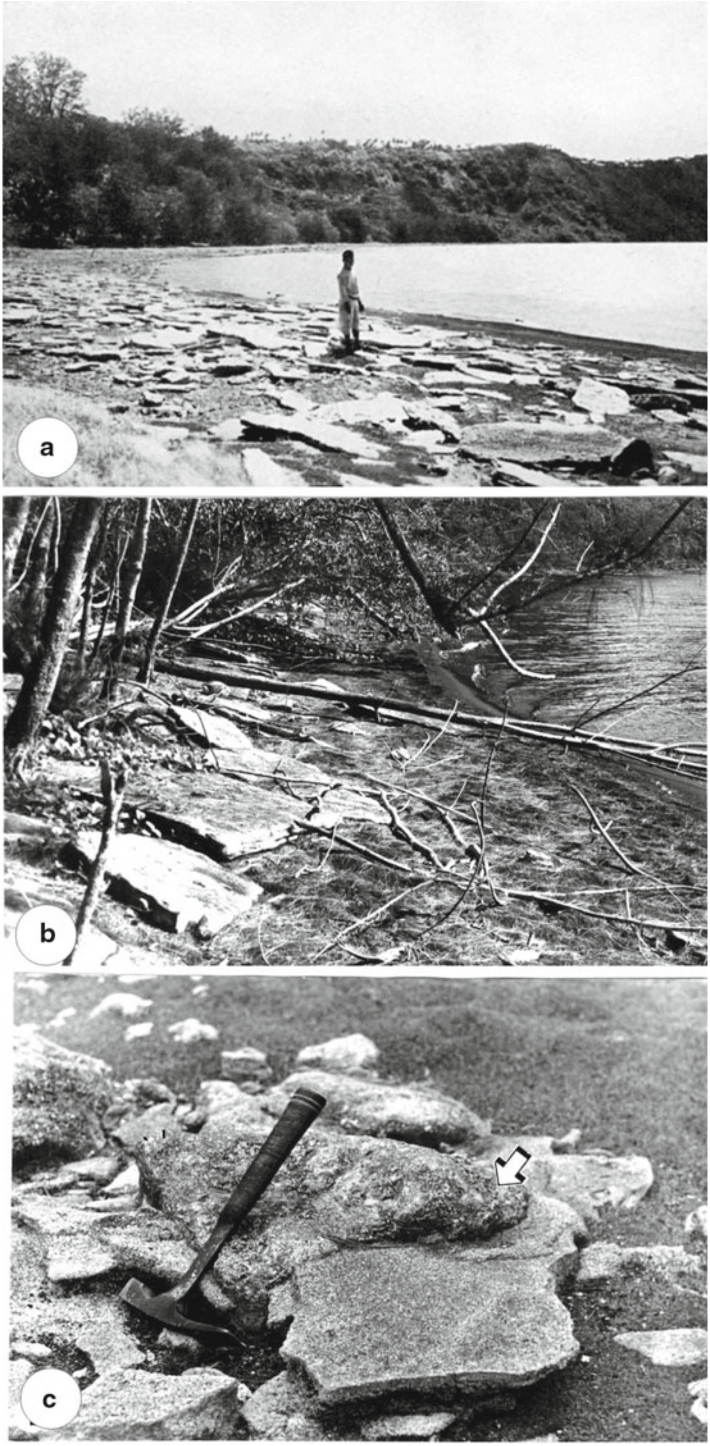
The main biosedimentological features of the lake are the various carbonate deposits. Four types have been identified inshore the caldera lakes. They protrude from the water table or are exposed subaerially along the lake shore. Carbonate structures have been also observed and sampled by free diving to a water depth of about 10 m.

8.1. CALCAREOUS VOLCANOGENIC SANDSTONES

Calcareous volcanogenic sandstones occur as 3–10-cm-thick platy slabs at two localities (loc. 5 and 1) on sandy Vai Lahi shores (Fig. 6b). The poorly sorted volcaniclastic sediment is weakly cemented by acicular and/or micritic aragonite. The platy slabs represent apparently remains of the numerous plates of “limestones” illustrated in Jaggar’s report (1935) (Fig. 6a). Since that time, the lake level has significantly increased flooding the broad beach covered with platy slabs which apparently originated at shallows during times of lake level recession (compare Jaggar, 1935, p. 103, top picture). Most of the platy slabs occur at loc. 5 on the southern shore of Vai Lahi; rare specimens have been found on the eastern beach of Vai Lahi near loc. 1. Part of the volcanogenic sediment is cemented with silica, which replaced the primary fine-grained (micritic) calcareous cement early diagenetically.

8.2. COATED GRAINS OF VOLCANIC GLASS

These bedded deposits have been found only on the western shore of Vai Lahi (loc. 1) where they are associated with calcareous stromatolitic mounds (see below) often forming the bases for their growth (Fig. 6c). The sandstones are composed of grains of volcanic glass weakly cemented with acicular or micritic aragonite (Fig. 7a, b). Most of the grains are coated by a laminated calcareous envelope of varying thickness (Fig. 7c) and can therefore be classified a,s coated grains bearing some similarity to ooids or oncoids (e.g., Tucker and Wright, 1990; Flügel, 2005). The mixture of volcanic glass grains with the coated grains is generally poorly sorted, although size gradation has been observed in some beds. Abraded coated grains occurring loosely in the volcanic beach sand represent most probably remains


a
b
c

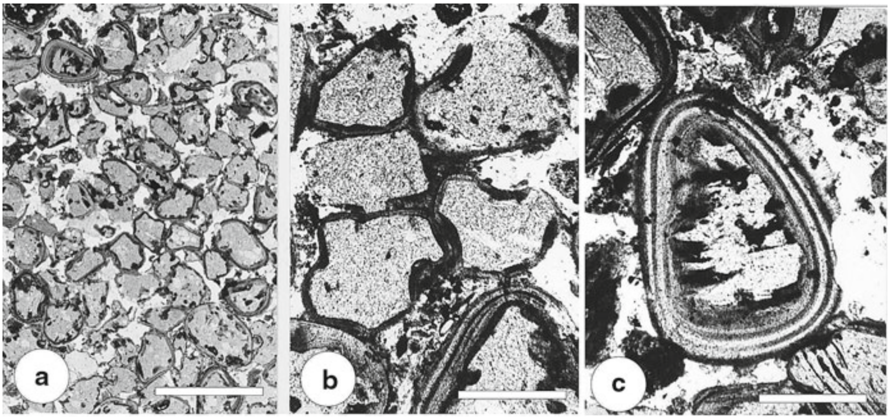


Figure 7. (**a** and **b**) Thin sections of weakly, with $CaCO_3$, cemented volcaniclastic sand with microlaminated coatings over some grains. (**c**) Enlargement of a volcanic glass shard with microlaminated calcium carbonate envelope. Scale bar equals: **a** – 3 mm, **b** and **c** – 250 μm.

of disintegrated beds of weakly cemented volcanogenic sand. The finding of such sediments in Vai Lahi is unexpected. Coated grains similar to ooids are typically interpreted as structures originating in high-energy environments due to inorganic precipitation of $CaCO_3$ on sediment grains during their periodic suspension in water (e.g., Bathurst, 1975; Tucker and Wright, 1990). The distribution of the Vai Lahi coated grains in the volcaniclastic deposit indicates that the accretion of the calcareous (aragonitic) envelopes might have occurred in quiet conditions inside the sediment during periods of increased calcium carbonate supersaturation levels in the lake. Such coated grains closely resemble Proterozoic microlaminated problematic fossil known as *Osagia* Twenhofel (e.g., Twenhofel, 1919; Zhuravleva, 1964; Hofmann, 1969).

8.3. CALCAREOUS STROMATOLITES AND STROMATOLITIC CRUSTS

Large groups of these biosedimentary structures (e.g., Hofmann, 1973; Bathurst, 1975; Kennard and James, 1986; Burne and Moore, 1987; Tucker and Wright, 1990; Fairchild, 1991) have been encountered in three localities: on the islands Motu A‘ali (Fig. 2, loc. 2) and Motu Lahi (Fig. 2, loc. 3) and in the shallow nearshore periphery of the western Vai Lahi (Fig. 2, loc. 1 – see also Kazmierczak and Kempe, 2006; Kremer et al., 2012). Some of the stromatolites protrude above the

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Figure 6. (**a**) Photograph from Jaggar’s (1935) National Geographic article depicting the “carbonate plates” that spurred our curiosity for the chemistry of the caldera lakes of Niuafo‘ou. (**b**) Similar plates observed during our’ 1998 expedition; noteworthy is the presently much narrower Vai Lahi beach due to raised lake water level. (**c**) Calcareous sandstone plates coalesced with a nodular stromatolite (*arrowed*).

water table, some are subaerially exposed on the shore, up to 2–3 m above lake level, and some occur as underwater structures observed by free diving to the depth of several meters. The mounds and heads were founded on an uneven substratum comprised of loose or weakly cemented volcaniclastic material, rarely on small lava blocks. The largest are over 1 m in diameter, up to 0.8 m tall, with a maximal thickness of the laminated calcareous body of ca. 0.3 m. Their surfaces display a characteristic pattern reminiscent of tightly convoluted brains (Fig. 8a, b). Closer examination has revealed that the stromatolites are almost dead structures at present. At the splash zone, their surfaces are covered with a leathery layer of noncalcified filamentous cyanobacteria without growth continuity toward the calcareous stromatolite body. Surfaces of stromatolites located permanently underwater are patchily overgrown with not calcified or only weakly with aragonite permineralized filamentous and coccoid cyanobacteria (Fig. 8c, d), similar to fossilized remnants of the heavily calcified (aragonitic) and/or silicified cyanobacterial microbiota occurring in places within the stromatolite bodies (Fig. 10c–f) (for details see Kremer et al., 2012). In Vai Si‘i, numerous diatoms and ostracods are associated with the living cyanobacterial cover of the microbialites. The cyanobacteria have at present low potential to precipitate actively calcium carbonate compared to the heavily permineralized stromatolite body formed at earlier times.

As shown in our preliminary Niuafo‘ou report (Kazmierczak and Kempe, 2006), the internal structure of the mound- and brain-like stromatolites observed in vertical cross sections (thin sections and polished slabs) shows presence of narrow, dense, laminar zones alternating with broader zones composed of irregular vertical microcolumns separated by irregular voids (Fig. 9a). Judging from the remnants of the fossilized microbiota, the laminar zones were formed predominantly by *in situ* calcified capsular cyanobacteria (*Pleurocapsa* group) (Fig. 10c, d), whereas the microcolumns are built of tube-like filaments of cladophoralean green algae overgrown irregularly by in situ calcified unidentifiable coccoid cyanobacteria. Also, calcified cushion-like colonies of rivulariacean cyanobacteria (*Rivularia* sp. and *Tolypothrix* sp.) occur at certain stromatolite levels (Fig. 10e, f) forming a cystous microfabric (Fig. 9c) similar to those observed in Proterozoic stromatolites (e.g., Bertrand-Sarfati, 1976, Fig. 2a). A special kind of stromatolitic fabric is represented by micro-arborescent structures (Fig. 9d) resembling Proterozoic and Paleozoic fossils described as Frutexites (Maslov, 1960; Horodyski, 1975; Walter and Awramik, 1979; Myrow and Coniglio, 1991). These usually Mn- and F-enriched structures have been often found growing near volcanic hydrothermal vents (Walter and Awramik, 1979).

Flattened or slightly wavy aragonitic (in one case calcitic!) stromatolitic crusts have been encountered on steep rocky shores of Vai Lahi and Vai Si‘i (Fig. 2, loc. 4 and 5). Similarly, as the brain-like stromatolites, these structures are in part exposed subaerially and in part occur under permanent water cover. They have been observed by free diving to a water depth of at least 6 m. The subaerially exposed surfaces of stromatolitic crusts are biologically dead and strongly weathered (leached). The dark green surfaces of submerged crusts are covered in Vai Si‘i by

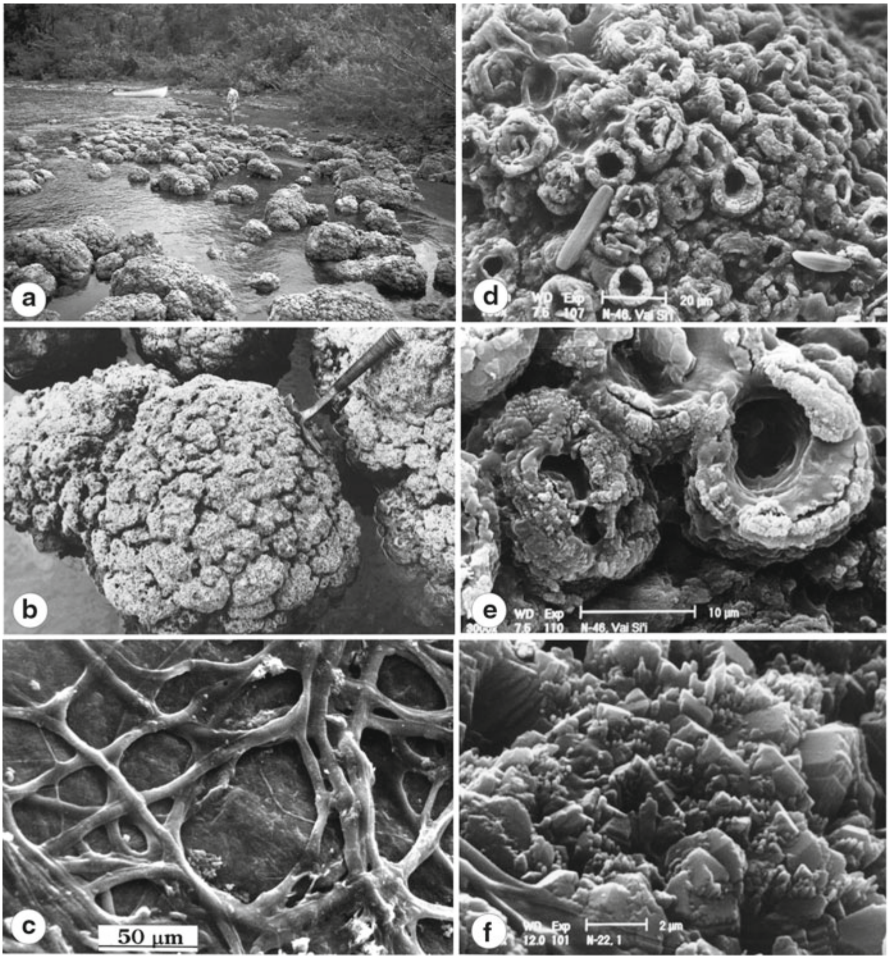

Figure 8. (**a**) View of the Vai Lahi shore with a stromatolite field extending above the lake level. (**b**) A large brain-like Vai Lahi stromatolite. (**c** and **d**) SEM images of air-dried filamentous (**c**) and coccoid (**d**) cyanobacteria growing on underwater surfaces of Vai Lahi and Vai Si‘i stromatolites. (**e**) Precipitation of $CaCO_3$ (aragonite) nanogranules in the mucus enveloping the coccoid cyanobacteria is the main process generating the stromatolites. (**f**) In older parts of the stromatolites, the aragonite nanogranules coalesced and transform into larger microcrysts.

a thin film of living coccoid cyanobacteria (*Pleurocapsa* group), benthic diatoms, ostracods, and cladophoralean green algae. The outer mucopolysaccharide sheaths of the coccoid cyanobacteria are weakly calcified with apparently *in vivo* precipitated nanogranular aragonite (Fig. 8e) which is continuously passing into a more crystalline aragonitic stromatolite body (Fig. 8f). This indicates that the accretion of the stromatolitic crusts is currently proceeding and is particularly well visible in the stromatolitic crusts from Vai Si‘i where the calculated

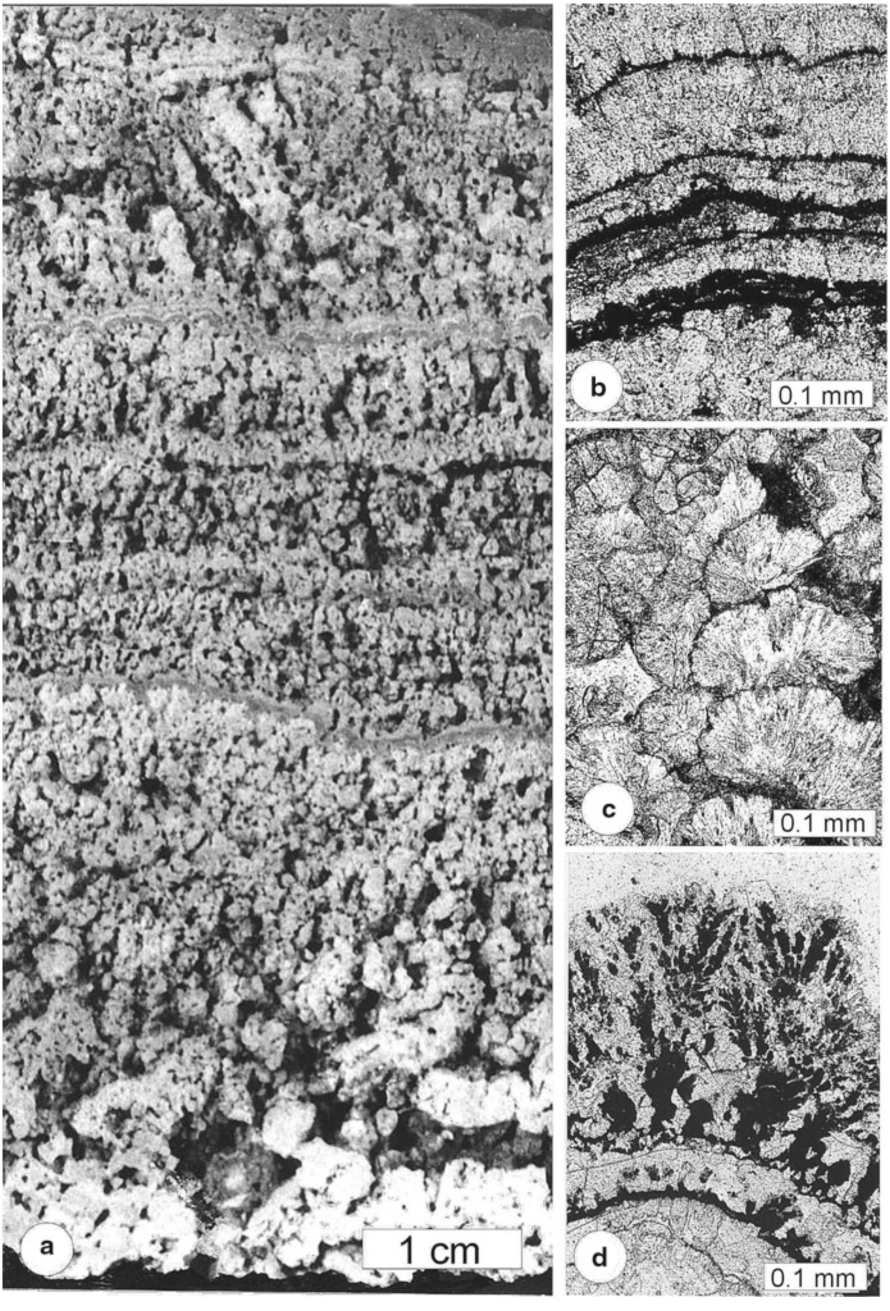

Figure 9. (**a**) Vertical section of a polished slab of Vai Lahi stromatolite to show the variety of its internal structure. (**b**–**d**) Transmitted light micrographs of vertical thin sections showing examples of most typical microstructures (microfabric) identified in Niuafo'ou stromatolites: laminated (**b**), cystous (**c**), and arborescent (**d**).

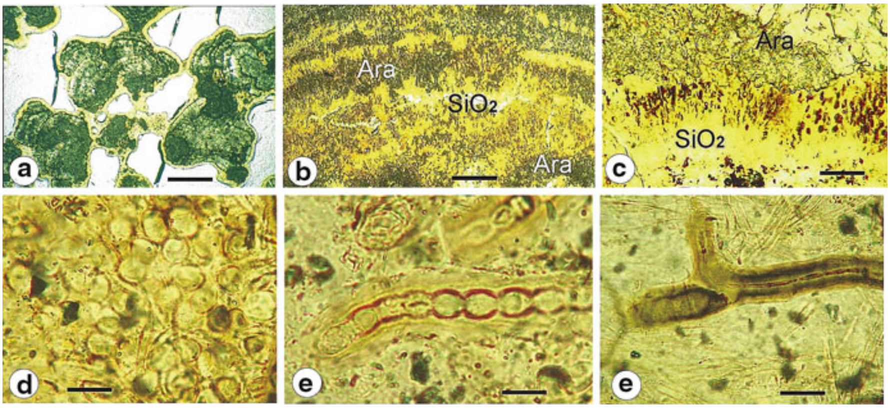

Figure 10. Examples of early diagenetic silicification of Niuafo'ou stromatolites and stromatolite-forming cyanobacterial microbiota. (**a**) An example of silica coatings (*yellow color*) covering the interior of a porous aragonite stromatolite body. (**b**) Vertical thin section showing irregular laminar silicification of originally aragonite stromatolite body. (**c**) Portion of silicified cyanobacterial mat with well-preserved remains of coccoid cells. (**d**–**f**) Examples of early *postmortem* silicified cyanobacteria participating in the formation of Niuafo'ou stromatolites: *Pleurocapsa* sp. (**d**), *Rivularia* sp. (**e**), and *Tolypothrix* sp. (**f**). Scale bar equals: **a** – 500 µm, **b** – 100 µm, **c** – 20 µm, and **d**–**f** – 10 µm.

calcium carbonate saturation level is higher than in Vai Lahi (compare Table 8). In cross sections of stromatolitic crusts, distinct zones can be recognized (Fig. 9). It is noteworthy that relatively heavy $\delta^{13}C$ values characterize the lower zone representing the oldest stromatolitic layers attached to the lava substratum. The internal structure of the stromatolitic crusts is comprised of dense, indistinctly laminated horizontal elements alternating with porous layers built of irregular vertical elements in the form of microlaminated tiny columns (Fig. 9).

8.4. STABLE CARBON AND OXYGEN ISOTOPES OF STROMATOLITES AND STROMATOLITIC CRUSTS

Table 12 lists the carbon and oxygen isotope measurements. All the $\delta^{13}C$ values are positive, that is, slightly heavier than marine carbonates (which range from 0 to +1 permil). Whereas marine skeletal carbonates are formed by enzymatic biomineralization in (relative) equilibrium with the carbon isotope signature of seawater, the Niuafo'ou carbonates form by permineralization and therefore tend to be heavier by 4–6 permil as compared to their source water (which was measured to be around a $\delta^{13}C$ of 0, see Table 8). The $\delta\,^{13}C$ values become heavier, the older the samples appear to be. This indicates that in former times, the dissolved carbonate in the lake must have been heavier than more recently. Such a shift toward presently lighter values could have been accomplished by the input of light volcanic CO_2 into the lake, a process which has already been suggested when the relatively high pCO_2 values in Vai Lahi surface waters were discussed.

Table 12. Stable isotope analyses of calcareous microbialites from caldera lakes on Niuafo‘ou Island.

Sample	$\delta^{13}C$	$\delta^{18}O$
Sample N-2 (contact with lava block), loc. 5	4.458	−3.934
Sample N-2 (control of the above), loc. 5	4.464	−3.964
Sample N-2 (middle), loc. 5	1.641	−4.317
Sample N-2 (upper part), loc. 5	1.667	−4.669
Sample N-5(1), loc. 4	2.733	−1.766
Sample N-5(2), loc. 4	2.386	−1.673
Sample N-5(3), loc. 4	2.301	−2.146
Sample N-13, loc. 1	1.617	−2.691
Sample N-21, loc. 2	2.986	−2.881
Sample N-22, loc. 2	1.692	−5.092
Sample N-29 (contact with lava block), loc. 3	4.240	−4.467
Sample N-29 (upper part), loc. 3	3.111	−3.309
Sample N-41, loc. 1	2.971	−4.477
Sample N-46, loc. 4	1.924	−5.016
Sample N-50 (contact with lava block), loc. 4	2.611	−5.056
Sample N-50 (middle), loc. 4	3.004	−4.018
Sample N-50 (upper part), loc.4	2.471	−3.983

Carbon and oxygen isotopic values lack a specific correlation indicating that the $\delta^{18}O$ values are entirely temperature-dependent, independent of the source of carbon in the lake.

9. Discussion and Conclusions

The importance of Niuafo‘ou caldera lakes is best illustrated when comparing their chemistry to that of other environments. In Table 13, two of the most important parameters of the carbonate system are listed, alkalinity and $\delta^{13}C$ values of dissolved carbonates.

Lowest alkalinity values are found in the current ocean. Its carbonate concentration is controlled by biomineralization, and the $CaCO_3$-saturation of it is therefore kept below the threshold of spontaneous aragonite precipitation. The difference of alkalinity between top and bottom is small, and its downward increase is caused by dissolution of sinking calcite and aragonite:

$$CaCO_3 + CO_2 + H_2O \Rightarrow Ca^{2+} + 2HCO_3^-$$

At the same time, $\delta^{13}C$ decreases downward caused by the respiration of sinking, isotopically light organic carbon produced in the photic zone (e.g., Broecker and Peng, 1982).

In the Black Sea, the largest anaerobic marine water body currently existing on Earth, alkalinity values are much higher than in the open ocean. This is caused

Table 13. Comparison of alkalinity and $\delta^{13}C$ values of various environments.

Site	**Alkalinity meq/l**	**$\delta^{13}C$ (permil)**
Ocean surface	2.35	2.00
Ocean bottom	2.55	0.00
Black Sea Surface	3.31	0.80
Black Sea Bottom	4.53	−6.60
Kauhako 0 m 2001	4.2	0.58
Kauhako 100 m 2001	9.65	−2.01
Satonda 15 m	4.14	−4.16
Satonda bottom	50.43	−19.00
Niuafo‘ou 26 m	16.60	0.11
Niuafo‘ou 50 m	18.60	−0.41
Alchichica (average of water column)	30.90	–
Alchichica 10 m	–	2.37
Alchichica 50 m	–	2.04

Data are from different sources: Ocean: Broecker and Peng (1982); Black Sea, Kempe (1990); Kauhako, Kempe (2002); Niuafo‘ou, this paper; Satonda, Kempe and Kazmierczak (1990b, 1993); and Alchichica, Kazmierczak et al. (2011).
n.d. not determined.

by sulfate reduction in the water column below the pycnocline (Kempe, 1990) according to:

$$C_{106}H_{263}O_{110}N_{16}P + 53\ SO_4^{2-}\ 14H_2O \Rightarrow$$
$$53H_2S + 106HCO_3^{-} + 16NH_4^{+} + HPO_4^{2-} + 14OH$$

In spite of the high alkalinity, the Black Sea does not precipitate aragonite (it has done so 7,000 years ago; Ross and Degens, 1974) because of its salinity, and therefore, its Ca concentration is too low to reach a supersaturation high enough. The $\delta^{13}C$ decreases steeply downward, again a consequence of sulfate reduction that transforms light organic carbon into inorganic carbon. The Black Sea is the best current example of alkalinity control by sulfate reduction, a process termed “alkalinity pump” (Kempe, 1990; Kempe and Kazmierczak, 1994, 2002).

The next data in Table 13 are those of >250-m-deep Kauhako Crater Lake in Molokai, Hawaii (Kempe, 2002). It is filled with a mixture of seawater and ground water and is anaerobic below 4 m of depth. Again, we find sharply increasing alkalinity values downward and decreasing $\delta^{13}C$ values due to respiration of organic carbon. Both parameters again bear the signatures of ongoing sulfate reduction.

The crater lake with the most pronounced influence of sulfate reduction investigated by us as yet is seawater-filled Satonda Cater Lake: There, we found an increase in alkalinity to more than 50 meq/l in the bottom waters, associated with a negative shift of $\delta^{13}C$ by 15 units (Kempe and Kazmierczak, 1990b, 1993; Kempe et al., 1997).

In contrast to the previous example, Niuafo‘ou and Alchichica Crater lakes have very high alkalinities throughout the water column (66-m-deep Alchichica is monomictic, and the parameters of the main ions do not show significant water column variation; see Kazmierczak et al. 2011), and (in case of Niuafo‘ou) the $\delta^{13}C$ difference between top and bottom is very small (−0.5 units only). Moreover, the sulfate concentrations of Niuafo‘ou are very low (between 1 and 2 meq/l, see Table 7) so that the high alkalinity cannot be derived from sulfate reduction alone. In Alchichica, the sulfate concentrations are higher than in Niuafo‘ou (16.73 ± 0.15 meq/l) but still much lower than its very high alkalinity of 30.9 ± 0.35 meq/l. In both cases, alkalinity must have another source and that most probably is silicate weathering (“Urey-Reaction”), such as the disintegration of a plagioclase feldspar according to:

$$\mathrm{Na_{0.62}Ca_{0.38}Al_{1.38}Si_{2.62}O_8 + CO_2 + nH_2O} \Rightarrow$$
$$\mathrm{0.6HCO_3^- + 0.4CO_3^{2-} + 0.62Na^+ + 0.38Ca^{2+} + SiO_2} + \text{kaolinite}$$

Thus, both Niuafo‘ou and Alchichica are prime examples how crater lakes acquire high alkalinities quickly by a process that today does not play an important role in ocean chemistry: CO_2-driven silicate weathering. However, in the early Hadean ocean, when Earth was degassing CO_2 and when impacts and volcanic eruptions provided highly reactive silicate glass blankets, the alkalinity should have been very high. In view of the modern crater lakes, this conclusion is inevitable. Sulfate reduction was a process that could not have governed alkalinity generation in the early ocean because of the lack of oxygen in the atmosphere making the production of sulfate impossible.

Therefore, our studies show that the two largest Niuafo‘ou caldera lakes, Vai Lahi and Vai Si‘i, can be looked at as suitable sites for testing the hypothesis of an early sodic ocean (Kempe and Degens, 1985; Kempe et al., 1989; Kempe and Kazmierczak, 1994, 2002, 2011a, b). In addition, they serve as sites for modern analogues of a variety of Precambrian carbonate sediments (particularly stromatolites) and as a field laboratory for studying early diagenetic silicification of cyanobacterial microbiota enrolled in the stromatolite formation. They may also be model sites for searching deposits potentially analogous to life-associated sediments formed on early Mars (Kempe and Kazmierczak, 1997).

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COMPARISON OF ENVIRONMENT TYPES WITH LIQUID WATER ON MARS

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1. Introduction

Various evidences on the existence of past liquid water on the Martian surface are published in the last years (for comparative review, see Carr and Hear, 2010; Head et al., 2001 and references therein). Based on morphological, mineralogical and theoretical considerations, liquid water could have been on the surface of Mars at several times and at different locations in the past, producing friendly conditions for hypothetical organisms around the edge of the tolerance limit of known extremophiles on Earth (Pikuta et al., 2007).

This work one simple way is presented to characterize and compare wet environments on Mars. The probable periods, durations and volumetric characteristics are summarized where liquid water might be present and available for metabolic activity. The review of the volume and temporal behaviour of wet environments on Mars is an important but only first step towards the detailed analysis of these environments. The reviewed topics in this chapter also form a part of an educational project on the integration of astrobiology and planetary science into the education at university level (Kereszturi and Horvai, 2009; Kereszturi, 2010).

2. Methods

To estimate possible palaeoenvironmental conditions on Mars focusing on the presence of water, the observational evidences (De Hon, 1999) and theoretical assumptions on the stability of liquid phase (Hecht, 2002) should be synthesized. In this work, published data are used to estimate the duration and volumetric characteristics of wet environments, completed with the measurement of depth and volumes of different surface features where it was missing from MOLA (Mars Orbiter Laser Altimeter on Mars Global Surveyor)-based topographic dataset with processing version L (Smith et al., 1999). This data uses IAU2000 planetocentric

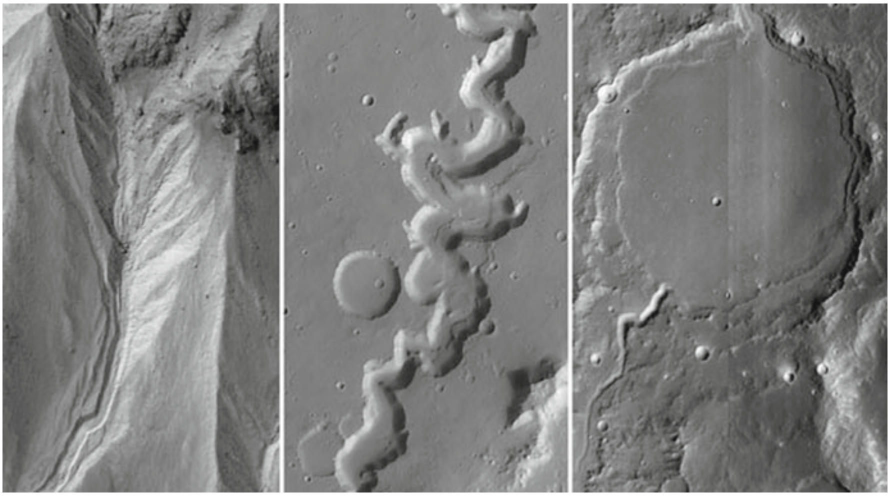

Figure 1. Three examples for different earlier wet environments on Mars (width of the subset images in brackets). From *left* to *right*: gully (200 m), Nanedi Vallis (40 km), and a crater lakebed with terraces, sedimentary infill and an outlet channel at the bottom (50 km) (NASA, ESA, DLR, FU Berlin, G. Neukum).

coordinates, incorporates crossover analysis of individual profiles and is referenced to the latest Mars gravity model.

The volume of liquid water could be estimated by the eroded volume of channels using average estimated discharge and the capacity of lake basins. The duration of these wet periods could be estimated from modelling of the freezing process, accumulation time of fluvial sediments, model-based estimation of cooling rate of magmatic bodies and model-based durations that are favourable for microphysical scale interfacial water layer formation. Chemical-based estimations could also be realized using physicochemical models on the dissolution and alteration of various minerals. All the above-mentioned approaches give us only rough estimations with certain error values. But they are still useful, as the aim here is to characterize different wet environment types and not to gain exact parameters (Fig. 1).

3. Results

In this section, the different types of morphological, physical and chemical indicators are listed that point to ancient wet environments. For each category, the basic parameters, like water volume, duration, reason of melting and the position of certain features in the planetary evolution of Mars, are summarized. Beyond the analysed parameters, other important factors for astrobiology also exist, like area of wetted mineral surfaces, pH, and solved chemical ingredients.

The numbers for the volume of liquid and its duration show the higher and lower ends of the probable range, indicated on the order of magnitude by the power of ten on logarithmic scale in years.

The important features on Mars useful to estimate ancient presence and characteristics of liquid water are the following, listed roughly from the larger towards the smaller liquid volumes:

Seas and Oceans – Liquid water bodies substantially larger than the so-called lakes, unofficially called seas or oceans, could be present on Mars. At the topographic height of −2,540 m (Di Achille and Hynek, 2010), several lakes and deltas were present. This level coincides with the Arabia shoreline level at −2,684 m and theoretical calculations Clifford and Parker (2001) also suggest that in the Noachian the global water level was close to the −2,550 m. The possible volume of the ocean might change as there could be several distinct ocean periods in the earlier Noachian and also by later episodic water outbursts. A rough estimation for the volume of these oceans is in the order of 10^6–10^8 km^3 (Boyce et al., 2005; Carr and Head, 2003). The lifetime of these oceans may depend more than only on simply freezing process, as they influenced the climate (by releasing vapour into the atmosphere and in the case of outflows they might accompanied with volcano-tectonic heat release), and for early oceans, the climate might be substantially warmer too. Based on rough approach, the lifetime of such oceans might be around 10^4–10^6 years (Carr and Head, 2003), or substantially longer in the case of early Noachian.

Outflow Events – Subsurface aquifers broke out at chaotic terrains and faults during volcano-tectonic events, and such great floods also could happen from closed lakes (in Valles Marineris) caused the sudden release of large water mass. These events formed the great outflow channels (Carr, 1987) with duration of fluvial activity around weeks and months (Baker et al., 1991; Kleinhans, 2005) – although the released water could have ponded for extended period, under an ice cover. For example, in the case of Maja Vallis, 4 months are required to empty the source lake existed in Juventae Chasma and another at least 10 months to drain the ponded secondary lake in the northern part of Lunae Planum (de Hon and Pani, 1993). In the case of Ravi Vallis (Harald et al., 2006), calculations based on sediment-carrying capacity and peak discharge values, the estimated duration was between 2 and 10 weeks and the total water amount around 11,000–65,000 km^3. The model for Mangala Vallis (Leask et al., 2007) suggests duration of the flow in 14–46 days and a total volume of 15,000–40,000 km^3.

Lakes – The ancient presence of lakes on Mars is shown by deltas, inflow and outlet fluvial valleys around the basins, sedimentary plains and mineralogical signatures there, as well as polygonal cracks in some cases (Kereszturi, 2011). The lakes on Mars could form by surface run-off and subsurface inflow, and fed by precipitation and ice melting. The valley network-fed lakes' volume was around 10^4–10^5 km^3, values comparable to small seas on Earth, while the smallest Martian lakes could have had 0.02 km^3 volume (Fassett and Head, 2008a, b). Their surface area spanned between 5 and 200,000 km^2. Although most lake signatures display evidence for several lacustrine periods during the earlier part of the planetary evolution, some of them were active in the last 0.5 billion years. The lifetime of a lake depends on the balance between the volume of the water released into the lake (by inflow or by in situ ice melting) and the loss by outflow, or freezing. Using the Antarctic subglacial lakes as analogy, the ice-covered lakes' surface sublimation rate is in the order on cm/years (McKay et al., 1985), suggesting possible 30,000–40,000 year (Moore et al., 1995) lifetimes in the case of lacking continuous water input on Earth. If similar case was present on Mars, the lakes there, several might have lifetime around 10^4–10^5 years (Cabrol and Grin, 2002).

Deltas – At the terminus of several fluvial valleys and channels, deposited sediments are present. Among them, the most interesting ones are those, which resemble to Gilbert-type deltas on the Earth, formed probably by entering the ancient flow into a standing body of water. Deltas could be identified by their characteristic top- and foreset beds, and show the maximal water level of the ancient lake (see the relate parameters in the lake section). Analysing the 150-m-thick lacustrine delta deposit in Holden crater, in the channel feeding the ancient lake around a dozen different avulsion episodes might happen, and suggesting liquid water might be present there for about 150,000 years (Bhattacharya et al., 2005) with volume of several 1,000 km^3.

Valley Networks and Sapping Channels – Excluding the small gullies, these are the most frequent features produced by liquid water on the Martian surface. The characteristics of valley networks suggest that during the Noachian, precipitation-fed surface run-off was present on the planet, while some individual valleys with theatre-shaped heads might form by subsurface sapping. Various estimations were published on the active duration of these fluvial valleys, which were inferred from the estimated erosion or from the accumulation of deposits. In the case of Libya Montes, the fluvial activity (continuous of separated) lasted for about 800 million years until 3.3 Ga (Late Hesperian) (Erkeling et al., 2009) – although probably these ancient rivers were not active continuously at that period. Another work on the valleys of Libya Montes calculated discharges of 15,000–430,000 m^3/s (Jaumann et al., 2010), where in the case of bank-full discharge the formation times are around 50–200 years and suggesting 10^{12} m^3 of water involved in their formation. Terrestrial rivers reach substantial erosion power only during about 20% of their activity, so the above-mentioned durations could be around 250–12,500 years (Kleinhans, 2005). The area and excavated volume of the Libya Montes valleys are around 10^3–10^4 km^2 and 10^2–10^3 km^3, respectively. For the formation of Nili Fossae, ancient crater lake sediment requires about min. 10–20 years (Fasset and Head, 2005) with a water volume of 350 km^3, while in Holden crater the formation of the NE situated deposit also requires about min. 50 years to form (Jerolmack et al., 2004) by 900 km^3 volume of water. As a rough approach, it can be said that formation of valley networks might be in connection with 10^1–10^4 km^3 (10^{10}–10^{13} m^3) volume of liquid water.

Impact Craters and Hydrothermal Systems – The impact process may produce also favourable and unfavourable conditions for possible life forms.

The impact-released heat may melt ice even under current climatic conditions on Mars and also mobilize various chemicals. The impact process produces depression for sediment accumulation and fragments the rocks, increasing reactive mineral surfaces. The impact releases heat by the central uplift and by the melt sheet formation. Several Martian craters show lobate ejecta blanket, suggesting the surface rocks contained water ice during the impact event (Wohletz and Sheridan, 1983). The cumulative impact heat may have exceeded the volcanic heat release during the period of the Late Heavy Bombardment (Kring, 2000). The estimated lifetime of such impact-heat-produced hydrothermal systems for 20–200 km diameter craters is 10^3–10^6 years based on Daubar and Kring (2001) and 10^4–10^6 years for craters with diameter of 30 and 180 km by the calculation of Abramov and Kring (2004). The heated regions may produce a lake in the crater's depression, which resemble to the above-mentioned lakes, although its lifetime is longer as it receives subsurface heat input. Right after the impact, only the regions farther away from the crater show low enough temperature for any kind of living activity, while later the whole terrain may cool down but keeps the water in liquid form for a substantial period. The affected volume of rocks heated above the original temperature is around 10^4 km^3 in the case of a 180 km diameter impact crater on Mars (Abramov and Kring, 2004) and one order smaller for a 30–40-km crater, around the estimated lower limit of impact-induced hydrothermal activity. The volume of liquid water in impact-induced hydrothermal systems is smaller than the heated rock volume. Taking 20% porosity for the regolith, the water volume is around 0.2 times of the above-mentioned values.

Geothermal heat-produced hydrothermal systems – The volcanoes on Mars suggest the presence of various geothermal heat sources during the planetary evolution. Such magmatic and volcanic heat may also produce hydrothermal systems with extended periods of liquid water in the case of available water ice. Beyond theoretical arguments, several volcanoes show channels on their flanks, and in the case of Ceraunius Tholus, one channel finishes its way in a crater and forming depositional deltas (Yamaguchi et al., 2010). On Alba Patera, some channels could be as young as few 100 million years (Gulick and Baker, 1990). Another group of water-related signatures is the hydrothermal-alteration-produced minerals. In the case of Nili Patera, hydrated silica minerals have been found on the flanks of a volcanic cone inside Syrtis Major caldera complex (Skok et al., 2010). In the case of Ascraeus Mons, the observed meltwater channels might be produced by an intrusion of a 10^2 km^3 sill with related activity of 10^2 years (Scott and Wilson, 1998), while in the case of Elysium Mons 30–40 km^3 of fresh lava was produced during one typical eruption (Wilson and Mouginis-Mark, 2001). Magmatic intrusions may support near-surface ice melting for 10^1–10^2 years (Head and Wilson, 2002) on Mars. Assuming resemble eruption characteristics on Mars to that on the Earth (Smellie and Chapman, 2002), one eruption may lasts for 10^0–10^4 years (Robinson et al., 1993), may melt around 10^1–10^3 km^3 of ice and show reoccurring activity on the timescale of 1–10 million years. On Mars, the latest volcanic activity took place not more than 40–100 million years (Hartmann et al., 1999).

Gullies – These fluid erosional features (Reiss et al., 2009) are present in the middle- and high-latitude regions on steep slopes. They probably formed by melting of snow packs accumulated by orbital change forced climate change on tilted slopes with favourable orientation (Christensen, 2003). The melting of these solid H_2O might happen by solar insolation on annual and daily bases. Resemble features that are analysed in the Antarctic Dry Valleys on Earth show melting for 8 h a day in the warm season (Morgan et al., 2010). Using a conservative approach for Mars, taking 1 h around noon during 100 sols in a Martian year when melting occurs, we have a cumulative period of 100 h (1.1×10^{-2} years) for the period of liquid phase annually. Assuming long daylight hours at higher latitude and/or larger tilt of rotational axis, insolation could be favourable for several continuous Martian sols, and the cumulative duration could be 10–50 times higher (1.1–5.5×10^{-1} years) for the liquid presence. The volume of meltwater is equal roughly to the volume of the accumulated snow packs that is in the order of 1,000 m^3 (Malin and Edgett, 2000) for each feature – although the whole volume of "one" ice pack was not in liquid phase at the same time for the above-mentioned period. The involved liquid water is 10^2–10^3 m^3 (or 10^{-8} to 10^{-7} km^3) for one gully. Another approach is to estimate the proposed flow period from the volume and discharge, and it suggests for the duration of liquid phase around the hourly scale (Heldmann et al., 2005).

Altered minerals – Olivine and its alteration products have been identified on Mars and inside Martian meteorites too. For the dissolution of olivine in wet environment, the fayalite- or Fe-rich composition, smaller particle size, higher temperature and acidic pH are favourable. There is a great variety of residence time of water according to the various parameters to produce the observed alterations. For example, above zero Celsius a particle with radius of 0.1 cm could completely dissolve in several 100 years, while under low temperature it might last 10^2–10^4 times longer. In physical contact with low-temperature brines, olivine may retain from solving for several million years in case of neutral to slightly acidic pH – while decomposition in the proposed strongly acidic periods could be faster. The observed survival of olivine minerals at several terrains of Mars suggests that wet periods were short and/or accompanied with low temperature (Stopar et al., 2006). And the available data are not enough to constrain the duration of wet periods based on such chemical analysis, and range for pH and near to zero Celsius is between 10^1 and 10^3 years, and for cold water from 10^5 to 10^6 years. The volume of these liquid water bodies is even more difficult to estimate, as alteration could happen both on microscopic-scale water and inside oceans too. As a result, the duration here shows a wide range from 10^{-9} up to 10^6 km^3.

Microscopic-Scale Liquid Water – On the Martian surface, thin, microscopic liquid water film may exist even today if the temperature is above 180 K, and water ice is in physical contact with mineral surfaces (Möhlmann, 2004). This so-called interfacial water could be liquid by above all van der Waals forces substantially below zero Celsius. Beyond the temperature and areal distribution of H_2O ice on the mineral surfaces, the presence of interfacial water is influenced by various salts too. Using the approximation by Möhlmann (2009, 2010) for the required temperature, the distribution of seasonal water ice cover was measured at the southern hemisphere, analysing in the dark dune spots. Inside these spots, water ice is present without CO_2 ice between Ls = 210 and 220 at 72°S (Kereszturi et al., 2011). Taking an average diameter of 50 m and area of 2,000 m^2 for a spot (Horváth et al., 2009), and a spot density of 10/km^2 on dark dunes that cover roughly 80,000 km^2 in the southern circumpolar region, we have 1,000–2,000 km^2 area with possibly undercooled interfacial water coverage. The later value using 1 nm of liquid layer gives together around m^3 volume. In the northern hemisphere, an H_2O ring follows the receding edge of the seasonal CO_2 cap (Kieffer and Titus, 2001; Bibring et al., 2005; Schmitt et al., 2005). It is present at 65°N, 70°N and 75°N latitude region for 10, 15 and 20° in Ls, respectively (Kuzmin et al., 2009). The area possibly covered by this thin interfacial layer is around roughly 10^6 km^2 for each the dates of Ls = 20, 35 and 50. The water ice layer covers the surface there for periods of about 14, 23 and 30 sols at the above-mentioned latitude bands, respectively. Assuming that the springtime insolation increases the temperature above 180 K in the water ice-covered dark dune spots, under the present climate, 10^6 km^2 area of interfacial water could be present for periods between 14 and 30 Martian sols at north – but the temperature is probably high enough for this only in the warmer part of the day, around 0.5–0.1 times of the above-mentioned duration. Summarizing the estimations, interfacial water may be present for 10^{-4}- or 10^{-2}-year duration, with cumulative volume around 10^0–10^3 m^3 (or 10^{-9} to 10^{-6} km^3) on Mars annually.

4. Discussion

The above-mentioned environment types are the possible locations and environment types where liquid water could have been present on Mars. While the determination of the exact timing of and locations of the occurrence requires much work in the future, the general comparison may give useful information already. In the following table, the environment types are compared regarding the volume (1. column) and duration (2. column) of liquid water, the certain period in the history of planetary evolution (3. column) and some other parameters (4. column).

In the table, the total volume of liquid water is indicated as the maximal volume could be present at a certain time during the representative period for that feature or process. In the cases marked with *, the data are relevant for only one gully or one melting event of magmatic intrusion for geothermal systems, and at the ** mark, the duration of interfacial water is the annual duration in each year under the present climate with cumulative volume values for one hemisphere.

Name	Volume of liquid water in km^3	Duration of liquid phase (years)	Period in planetary evolution	Other characteristics important for astrobiology
Gullies*	10^{-8} to 10^{-7}	10^{-2} to 10^{-1}	Some million years ago	Surface melting, solar insolation, UV radiation, oxidants
Valley networks	10^{1}–10^{4}	10^{1}–10^{5}	Usually more than 3.5 billion years ago	Liquid precipitation and/or ice melting, UV radiation, roughly neutral Ph
Deltas	10^{3}	10^{5}	Most more than 3.5 billion years ago	Early more neutral, later more acidic environments, surface liquids, UV radiation, oxidants
Lakes	10^{-2} to 10^{5}	10^{3}–10^{5}	Most between 4.0 and 3.5 billion years, fewer more recently	Early more neutral, later more acidic environments, surface liquids, low UV in the deeper regions
Outflow channels	10^{4}–10^{5}	10^{-2} to 10^{-1}	Episodically later than 3.5 billion years ago	Cold, acidic water with many sediments, UV shielding surface ice cover
Seas and oceans	10^{6}–10^{8}	10^{4}–10^{6}	Early during planetary evolution, later only episodically by outflows	Surface liquids, strong UV only at their topmost layer, neutral pH in early and acidic in later periods, freezing also from above and below
Large impact craters	10^{2}–10^{3}	10^{3}–10^{6}	Above all in early periods, later episodically	Hydrothermal circulation along faults, no solar insolation, juvenile ingredients
Geothermal heat centres*	10^{1}–10^{3}	10^{0}–10^{4}	Inside volcanoes around magma bodies	Hydrothermal circulation along faults, no solar insolation, juvenile ingredients
Weathered minerals	10^{-9} to 10^{6}	10^{2}–10^{6}	Above all in early periods	On the surface, and subsurface (today exhumed)
Interfacial water**	10^{-9} to 10^{-6}	10^{-4} or 10^{-2}	Observed recently but possibly earlier too	On the surface, UV radiation, it may decompose oxidants

Figure 2 indicates in graphical version the volume and duration of various wet environment types. The size of the boxes does not matter much, as larger the uncertainty, larger the boxes are – but their location in the diagram is more important. The largest uncertainties and/or largest range of values are in the case

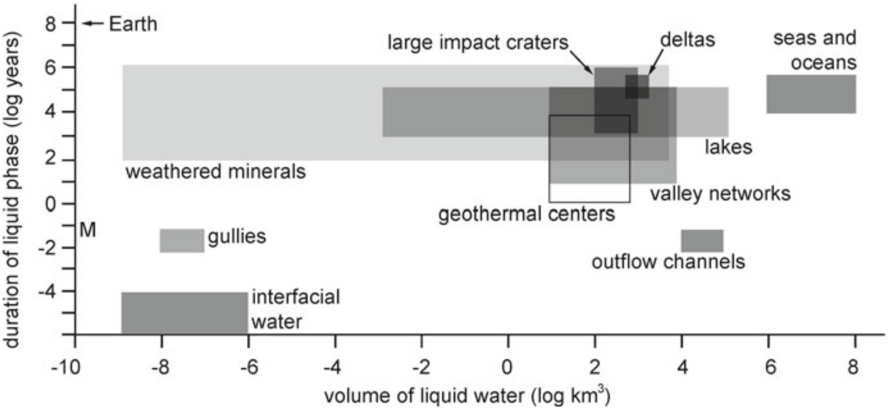

Figure 2. Comparison of volume (*horizontal axis*) and duration (*vertical axis*) range of wet environment types on Mars. The letter "*M*" marks the parameters of Miller's famous experiment, while at the *top left* the "Earth" indicates the maximal duration of the period that the formation of life required on Earth, between the end of Late Heavy Bombardment and the oldest fossils – indicated only to visualize the scales. The different *grey* scaling of the boxes is only to separate them from each other, and the larger size of boxes may reflect only the larger uncertainty of parameters. The box of geothermal centres marked with a *black* outline to separate it from the others boxes in a crowded region of the diagram.

of weathered mineral signatures. This group is probably comprised of different environments with more diverse conditions than outlined here.

Next to the left-hand-side vertical axis, the 400-million-year long period is also indicated with a horizontal arrow and the "Earth" text. It is the maximal period that spans between the end of heavy bombardment (3.9 billion years ago) and one of the earliest fossils from Western Australia (3.5 billion years ago). Miller's first experiment's parameters (Miller, 1953) are also indicated with the letter "M" only for comparison. Its location is really only for the visualization of scales, as the conditions and possible results of prebiotic processes are poorly known today.

5. Conclusion

In this work, ancient and recent wet environment types were classified on Mars according to their temporal and volumetric characteristics. In the following, firstly, the conclusions of the analysed parameters are presented; secondly, other parameters like temperature, radiation and chemical conditions are summarized briefly for these environment types; then, the occurrence and type of these wet environments are put into the context of planetary evolution. Finally, the statements and findings outlined here point to such issues, which require more theoretical work and focused observations and help to direct future inspections.

The smallest (10^{-8} to 10^{-6} km^3) and shortest (10^{-4} to 10^{-1} years/each active period) of liquid phase were found at gullies and interfacial water layer. A large group of wet environments like valley networks, lakes and geothermal- or impact-induced hydrothermal systems show parameters that resemble to each other (10^1–10^5 km^3, 10^4–10^6 years). The largest and longest wet environments could be the ancient northern ocean or episodic seas there (10^6–10^8 km^3, 10^4–10^6 years), while in the case of outflow channels the reasonably large (10^4–10^5 lm^3) water accompanied with short lifetime (10^{-2} to 10^{-1} years).

Regarding the duration of liquid environments, two cases could be separated: the continuous and the cumulative duration of liquid water at any certain location. For prebiotic processes, the possibility for chemical alteration and combination of organic molecules is larger if the cumulative wet period is longer. For possible extremophiles, the cumulative duration is also interesting as the organisms may be able to reactivate themselves fast after even long dormant phase (Garcia-Pichel and Belnap, 1996) and do metabolic activity and cell repair during the short active phase. The produced organic molecules and the organisms themselves have to keep their good shape during inactive periods too. Cumulative UV radiation and cosmic rays may decompose them in the top 1–2-mm- and 1–2-m-thick subsurface layer, respectively. In this aspect, shallow subsurface wet environments (Boynton et al. 2002) are favourable, while possible surface freezing ice layer on the top of liquids may provide shield against the radiation. In the circumpolar region, ephemerally spreading polar ice caps may provide such protection because of the periodic tilt change triggered climate changes on Mars.

Among the environment types reviewed here, the impact-induced hydrothermal systems were activated only once (right after the impact), the outflow channels once or few times, while the lakes, the valley networks and the seas might be active several times during the planet's history. The hydrothermal systems at volcanic centres also might show quasi-periodic activity, as well as the location of gullies, according to the climate changes in the order of million, 10 million years. The interfacial water-wetted surface locations may produce favourable conditions annually. Although recurrent wet periods might be present in the cases of gullies and interfacial water, the organic material or any hypothetic biogen activity there experienced surface oxidants and UV flux and might differ from those locations where liquid periods are longer or even absent.

To characterize various wet environment types on Mars, other basic factors beyond the duration and volume of liquid water should also be analysed, like temperature, radiations and chemical conditions. Using the background knowledge from Earth, the lower limit of sustainable metabolic activity is around −20°C, although there are various models and observations, which suggest the absolute limit may be even lower (Price and Sowers, 2004). To estimate the temperature during the wet periods, the presence of liquid water does not give enough constrain as various melting point decreasers are present on Mars and keep brines in liquid phase down to 205 K (Chevrier and Altheide, 2008). The hottest wet environments are expected to be present at hydrothermal systems, while the other wet environments were probably cold, except at the beginning of the planetary evolution (valley networks, some lakes and oceans). Low-temperature lacustrine environments were present later too but formed probably by ponding of cold waters released suddenly by outflows from subsurface aquifers. Thin interfacial water is/was also present under cold conditions.

Regarding the occurrence of nutrients and chemical catalysers, those conditions might be favourable where many small particles were present with large mineral surfaces in contact with water. Such conditions were present above all in hydrothermal systems, with water circulated along the rock fragments. For early Mars, the best locations were inside the water-laid sediments and shallow subsurface locations where water (from valleys, lakes, oceans) percolated into. Surface water was present during later periods of Mars under cold climates too, but in these cases the ponds were covered by ice layer formed from the top, and an ice layer might also form at their bottom in physical contact with the cold rocks. In these cases, the water kept its liquid phase for the longest period between the top roofing and the bottom lying ice layer, where the possibilities for mineral-liquid contact is the smallest.

The radiation conditions in the analysed wet environments are the worst for gullies and interfacial water layer, while in the case of other liquid bodies the surface metre(s) shielded the deeper layers from the radiation. Regarding the chemical conditions, earlier Noachian wet environments might have roughly neutral pH, while later in the Hesperian and Amazonian, acidic water could be present (Bibring et al., 2005). The average salt content of water also increased along the planetary evolutionary history based on theoretical arguments and supported by the observation of salts on the Martian surface.

The change of wet environments on Mars during the planetary evolution shifted from larger water volumes and wide scattered locations towards smaller volumes and shorter durations. In Fig. 2, the position of wet environments shifted from the top right towards the lower left. The above-presented summary also suggests that regarding the duration and volume of wet environments, hydrothermal systems, valley networks and lakes overlap each other – although they occurred in different periods and under different conditions. Gullies and interfacial water represent the smallest analysed volume and duration that are observable only as recent features – although they might be present on early Mars too.

The results of this work help in the targeting of future research too. More detailed analysis of weathering condition is necessary as the uncertainty in the duration of liquid water is very large in these cases. Long lifetime and reoccurring activity is expected in geothermal centres, but only little research has been done in this field on the observational possibilities, like what kind of surface signatures help to elucidate subsurface conditions. In the case of ancient lakes, the reoccurring ones require more observation, analysing the reason and characteristics of their active periods. Wet environments like valleys, lakes, impact and geothermal hydrothermal systems show resemble liquid water volume and duration parameters, but their characteristic temperature was different. The comparison of chemical alteration products at these sites might provide information on the differences in their evolutionary tracks and point to processes and various issues with astrobiological relevance.

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CRITERIA OF POSSIBLE HABITABILITY OF EARTHLIKE EXOPLANETS

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1. Introduction

By the beginning of 2012 about 700 extrasolar planets had been discovered. Some of them have low masses exceeding the terrestrial mass only severalfold which raises a question of the presence of primitive forms of life on them. Are the advent and evolution of Protozoa (unicellular organisms) determined by numerous physical and chemical factors existing on an extrasolar planet? De Duve (1997) wrote, “Life originated chemically by progressive complexification, probably starting from small molecules such as those generated throughout the cosmos by abiotic chemistry. Due to the fact that the processes involved in this complexification were chemical and comprised a large number of successive steps, they must have been highly *deterministic*, that is, bound to occur under the prevailing conditions. Should the same conditions obtain elsewhere, life would be expected to arise there as well.” Following de Duve’s opinion, the processes of the advent of Protozoa, in principle, may be analyzed and predicted. Just the opposite was Fred Hoyle’s point of view, who compared the probability of a cell to be self-organized with self-organizing montage of an airplane as a result of a storm.

Anyway, the origin of life is one of the greatest secrets of nature. The unique and only known form of life (LANA, amino-nucleic-acid form of life) is based on carbon. Many people believe, this is the only possible form of a life in general. LANA can exist only in a narrow temperature interval, and the Earth is its unique habitat. It is believed that life on Earth has begun, most likely, in shallow warm reservoirs or in oceans, close to underwater volcanoes – the sources of prebiotic compounds. A rise in temperature leads to protein coagulation. At water freezing temperatures, protein reactions slow down, and at temperatures of 90 K typical for Titan, Saturn’s satellite, such reactions virtually stop. Taking it all into account, the assumption of the life advent at temperatures close to 150 K (Martian minimal winter temperature) seem to be rather doubtful. (There is just an opposite view: Simakov, 2010). As for Titan, the process of accumulation of stocks of elementary organic substances took billions of years.

How could the transition of Protozoa into multicellular organisms have occurred? The initiating mechanism of this process remains unknown. Gould (1989) believes that the probability of such transition is considerably lower in comparison to the advent of the Protozoa on a certain planet. Unicellular organisms reigned on Earth over the course of nearly 4/5ths of its history. Commenting on the sudden and mass appearance of multicellular organisms 570 million years ago (known as the "Cambrian Explosion"), Gould writes, "only the last one-sixth of life's time on Earth has included multicellular animals … if you wish nevertheless to view … the transition from symbiosis to integration as predictable in some orderly fashion, then tell me why more than half the history of life passed before the process got started… cosmologists tell us that the Sun is just about at the halfway point of existence in his current state… Since human intelligence arose just a geological second ago, we face the stunning fact that the evolution of self-consciousness required about half of Earth's potential time." Many authors share this opinion, "The fact that it took life on Earth nearly 3 billion years to go from the single-celled to the multi-celled stage implies that this step was very hard" (I. Crawford, *in* Chown, 1996).

In this chapter, the possible constraints on the advent and evolution of life on planets are considered. The majority of these factors, such as photosynthesis, refer to origination of the Protozoa, and only few of them relate to the transition into complicated multicellular organisms, due to the complexity and mysteriousness of the process. The "favorableness" of the physical conditions discussed below is considered to fit our amino-nucleic-acid form of life (LANA) because we still do not know any other forms of life. Silicon life is widely embodied in science fiction; however, it is doubtful due to many limitations for chemical reactions based on silicon in comparison to the endless number of carbon reactions.

Hypothetical planets analogous to terrestrial ones are taken as possible habitats. Some fantastic hypotheses are not considered in earnest. The giant planets (of gas-liquid nature) with their hydrogen-reducing atmospheres do not satisfy the LANA requirements; therefore, they are not discussed here.

A harmonious combination of a number of characteristics of a planet (that are necessary for the advent of the Protozoa) could have been even not this complicated. Spectral and energetic limitations of radiation of the central star are critical for the advent of the Protozoa using photosynthesis. One of the most important factors is the mass of a planet.

As for the search of life on other planets of the Solar system, the only suitable candidate is Mars, where, unfortunately, no positive results have been obtained which should be considered surprising. In the last decades, it has been proved that planets exchange fragmental meteoric material. Meteorites similar to the Martian SNC have arrived not only from Mars to Earth. They have traveled from Earth to Mars, too, carrying a great number of microorganisms inevitably. But if Martian life is not found, the question arises, whether the Earth possesses some exclusive properties which we do not see.

The reader may find new interesting papers on the topic by various authors: Spirin (2009), Zavarzin (2009), Rozanov (2009), Snitnikov (2009), Agol (2009), Chetverin (2009), Zimmer (2009), and many other publications.

In the Solar system, the search of life is possible on the Jovian satellites – Europe and Ganimede, where global subsurface oceans are supposed to be (Fortes, 2000). These oceans are covered by thick icy shells protecting them from the space cold and are warmed up by the energy of tidal dissipation and radioactive decay. Similar conditions can be observed on Triton, the Neptune's satellite (Cruikshank, 2004; Ruiz, 2003).

2. Restrictions on Duration of Existence of Terrestrial Biosphere

An important item is a possible durability of the existence of the already arisen life. It is connected with the physics and evolution of a parent star. Luminosity of the early Sun was approximately 30% less as compared with its contemporary luminosity. It has been concluded on the basis of detailed numerical modeling of the star's evolution. Low luminosity of the young Sun implies, apparently, that surface temperatures of the early Earth and Mars might have been essentially lower than the modern ones. Meanwhile there are some data according to which the early Earth's climate was warm and damp. It was determined by the greenhouse effect and produced by carbon dioxide gas with small impurity of water vapor. The greenhouse effect plays a large role in the modern Earth's climate, maintaining its averaged surface temperature 38° higher than the effective temperature (i.e., corresponding to the balance of the solar incoming and planetary IR leaving radiation). Slight greenhouse effect of about 4° is also observed on contemporary Mars.

The age of the Sun (about 4.5 billion years) corresponds approximately to the middle of its lifetime. The Sun is going to remain a quiet star for about seven billions years. However, for the Earth's biosphere, much more than a half of its comfortable existence, or even of its lifetime, has passed. Further, the density of the solar radiation is going to grow much faster. According to averaged estimations, the Earth's LANA lifetime does not surpass one billion years. The age of the mankind is approximately two million years, and it is rather doubtful, that the remote future after one billion years could be of any practical value for us. But problems may occur much earlier. Solar nuclear reactor which operates in the core of our star is based on the synthesis of a ^{4}He nucleus from four protons. The pressure p in the Sun's core is known to be expressed by the following formula: $p = nkT$, where n – is the number density of particles, k – the Boltzmann constant, and T – the temperature. This pressure is counterbalanced by gravitation. Due to the synthesis, the number n decreases continuously, the core in which the synthesis takes place, shrinks, and its temperature grows, resulting in acceleration of processes of synthesis. Therefore, solar radiation density grows slowly, but steadily, and at accelerated rates. Figure 1 demonstrates how further physical characteristics of the Sun are supposed to change. For the subsequent three billion years, the density of solar radiation will increase by 40%. However, due to the greenhouse effect, the averaged surface temperature of the Earth may increase to 50° in the much less distant future. Once, the last perfect day on Earth will occur (Fig. 2). Next, with the

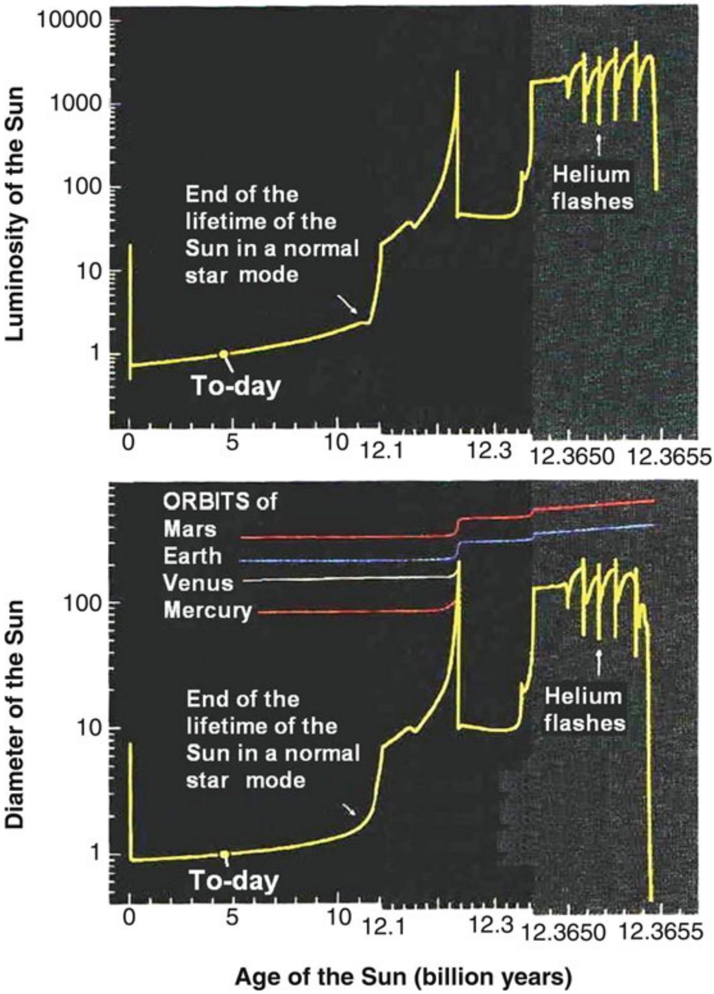

Figure 1. Concerning the lifetime of terrestrial biosphere. Evolution of luminosity and size of the Sun. Vertical scales are logarithmic; the horizontal scale is stretched after the 12.36 billion mark (From Garlick, 2002) Favorable terrestrial conditions are supposed to be maintained on the Earth for no more than 600-700 million years from the point indicated as "to-day".

increase of solar radiation, water vapor begins to initiate the runaway greenhouse effect, and that may lead to vanishing of the oceans and transformation of the Earth into a similarity of the incandescent Venus (Ksanfomality, 1985). Incidentally, it is possible that the state of Venus, in general, may be more frequent than the conditions of the Earth. The question of how the Earth manages to avoid it during 4.5 billion years is another topic. As for LANA, favorable terrestrial conditions in their present state are supposed to be maintained on the Earth for no more than 600–700 million years. There are some ways to prevent the catastrophe by means of celestial mechanics (getting additional orbital moment from a third body).

Figure 2. Once the last perfect day on Earth will occur. Someday the temperatures will never return to the level tolerant for the Earth's biosphere.

3. Factors Critical for the LANA Advent and Existence

The list of main criteria of a planet, critical for the advent and existence of LANA should include many special factors that form a peculiar labyrinth with many impasses. Such a planet should possess the following features (considered in details in the next sections):

- A parent star is of middle spectral type, from the late F to early subtypes of K class (MS) of the Main Sequence.
- An availability of zones with an interval of environmental temperature comfortable for the LANA;
- A planet must possess a rather dense atmosphere (the number density N_a is about 10E19 cm^{-3} close to the surface).
- The photons of energy $E = 1 - 3$ eV must have an access to the surface through the atmosphere; however, EUV and hard radiation must be absorbed by it.
- A sufficient density of radiation of the parent star.
- An availability of other (chemical) sources of energy, for example, an oxidizing medium and oxidized materials.
- A moderate level of planetary gravitation.
- Not-too-long rotational period is supposed.
- An availability of open basins on surface and water vapor in the atmosphere, together with continents or large islands.

- A sufficiently close and massive satellite.
- A position of the habitable zone is determined rigidly by the luminosity of the star.
- The eccentricity of the orbit should not be large, as well as an inclination of the equator whose value with respect to the orbital plane is not too high.
- Volcanism and/or plate tectonics and mild cosmic catastrophes, which can result in renovation of species and the appearance of the most adapted ones.
- The planet should have a rigid constraint on its mass, near 5E24 kg.

4. Contradictory Requests of the Biosphere for Spectrum and Energy of the Stellar Radiation

4.1. PHOTOSYNTHESIS

Photosynthesis is the only process that satisfies all the demands in energy of the Earth's biota. (The minor exception is anaerobic bacteria). In fact, it is a continuously operating chemical factory of global extent. It produces about 2E14 kg of fixed carbon a year with about 80 % of it concentrated in oceanic phytoplankton. Photosynthesis drives global cycles of oxygen, phosphorus, nitrogen, etc. Is the photosynthesis machinery universal? Evidently, it can be considered exceptional in the absence of other mechanisms on Earth comparable with photosynthesis. Therefore, the conditions critical for the existence of photosynthesis should be included in the list of planetary parameters necessary for the onset of life. Photosynthesis is a unique process due to the fact that absorption of light and subsequent generation of organic substances and oxygen in plants and bacteria is realized by the universal mechanism using the green pigment chlorophyll (Kamen, 1963; Bell, 1966; Blankenship, 2008). The mechanism is critical both for the photon energy and the spectral composition of light. The chlorophyll *a* (molecular mass about 890) is a component of chloroplasts' layer-like structure. Chloroplasts are cellular organelles, close to mitochondria in their structure. There are some other pigments (e.g., carotin) used by plants together with chlorophyll. Chlorophyll is close to the pigment of mammalian blood, gem, in its chemical structure.

Simplistically, photosynthesis is a reaction of CO_2 fixation with a release of O_2. The summarizing equation of photosynthesis (Lutsenko, 2002) is:

$$CO_2 + 2H_2O + h\nu \rightarrow C_6H_{12}O_6 + O_2 \qquad (1)$$

consuming the $h\nu$ energy of 5.1 eV for one molecule of CH_2O. Instead of the UV radiation of 240 nm (corresponding to 5.1 eV), the photosynthesis machinery uses the total energy of few photons with the wavelength of 680 nm (the energy of 1.8 eV). Oxygen production is not necessarily realized in the proportion of 1:1 in relation to H_2O. A special feature of photosynthesis is that it uses two spectral bands: 640–680 nm and 410–450 nm, which is a result of the existence of two pigments' system (Bell, 1966). Photosynthesis operates only when there is a radiation in both bands present. However, radiation with the wavelengths shorter than 300 nm *inhibits* the photosynthesis. Starting with 685 nm, there is a cutoff in its efficiency. The final result of the photosynthetic process is accumulation of energy in ATP and pyridine nucleotides (Kamen, 1963).

The conclusion is that contradictory conditions are required for efficient photosynthesis in the Earth's environment: on the one hand, the presence of radiation in the spectral band of 400–680 nm and on the other hand, the absence of UV radiation with the wavelengths shorter than 300–320 nm. These properties are most likely universal for LANA and are provided by the Earth's atmosphere. They are not quite suitable for the Earth and, apparently, indicate the unique features of the photosynthesis machinery once invented by the nature. These features cannot be explained by its cultivated adaptation to the properties of solar radiation. The Sun's efficient temperature is 5,780 K, and the maximum of solar radiation on Earth is $\Lambda_{mx} = 2.89\text{E}6/5{,}780\ \text{K} = 500$ nm, not 680 nm.

The efficiency of the photosynthetic process has been studied by many authors (Blankenship, 2008). It has been found that approximately every 300 chlorophyll molecules are joint in a group with an active functional center. The productivity of photosynthesis linearly depends on illuminance, at low illuminance level. At a higher level, saturation takes place depending on temperature and a number of other conditions. The maximum quantum efficiency is not high and amounts to 4.5–0.6 % (Izakov, 1989) which corresponds to low illuminance level.

4.2. HAZARD OF RADIATION EFFECT ON NUCLEIC ACIDS

The influence of radiation on the complicated structure of a cell may be rather dangerous (Frank-Kamenetskii, 1983). UV-photons constitute a threat to DNA integrity. The energy of a photon, absorbed by adenine, guanine, or cytosine, dissipates without detriment to a cell. However, if the energy is absorbed by thymine, the latter may form a compound with another thymine molecule, damaging the nucleotide sequence and DNA integrity. Wavelengths shorter than 350–380 nm are dangerous for thymine. Despite the fact that the Earth's atmosphere is almost opaque for these wavelengths, nevertheless there is a special cellular mechanism repairing a possible damage.

4.3. QUALITY OF RADIATION

It is often assumed that the density of radiation together with the Bond albedo A_b and the greenhouse effect of a planet define its effective temperature. It is true; however, for the Protozoa using photosynthesis, the temperature is not the only limitation.

No thermodynamical equilibrium is observed on terrestrial planets. These planets are characterized by a quasi-stationary state providing a stable thermal balance. It is easy to comprehend the subject if one understands what mechanism supports the life of the planet, and what significant sources of energy cause powerful dissipative processes, and influence atmospheric and ocean dynamics, heat transfer, and, of course, biosphere existence. The answer seems to be evident: it is the solar energy. However, exactly the same amount of energy got from the Sun is emitted by the Earth to space! Solar energy is transformed in the course of dissipative processes and is stored almost nowhere, with the exception of a small fraction which falls at photosynthesis. Some scientists believe that all the planetary processes are driven by the poles-equator difference in absorption of solar radiation. It is wrong, since in this case the effective temperature of the Earth must have been increasing constantly. The planetary machinery is driven by the flux of negative entropy (negentropy) which is produced due to the difference between the solar entropy and the entropy of the Earth's thermal emission.

This problem was considered by Izakov (1989, 1997) and in a more common sense by Landau and Lifshits (1964), Volkenstein (1986), Nicolis and Prigogine (1989), Prigogine (1997), etc. There is an interesting aspect of the problem, the connection of negentropy and information, considered by Bauer et al. (1997). In this article, we follow the approach of Izakov (1989, 1997).

Taking into account that the endogenic heat flux of 3E13 W (Davis et al., 1991) is neglectable for the Earth in comparison both with the absorbed solar radiation (1.2E17 W) and IR radiation Φ_T emitted by the Earth (which is true for other Earth-group planets), the Earth's thermal balance dE/dt can be expressed in the following way:

$$\frac{\mathrm{d}E}{\mathrm{dt}} = \Phi_\mathrm{O} - \Phi_\mathrm{T} = F_\mathrm{O}\left(1 - A_\mathrm{b}\right)\pi R^2 - 4F_\mathrm{T}\pi R^2. \tag{2}$$

Here, Φ_o, Φ_T, A_b and R – the fluxes per unit area, the Bond albedo, and the planet radius. As numerous measurements prove, dE/dt≅0, with seasonal variations of about 8 %. The planet is an open system, not nearly in a state of thermodynamic equilibrium, but in quasi-stationary conditions with almost constant averaged entropy. In accordance with the fundamental Gibbs formula (Landau and Lifshits, 1964; Volkenstein, 1986; Nicolis and Prigogine, 1989; Prigogine, 1997), changes in the entropy dS/dt within the system with the energy U, pressure p, density ρ, velocity v, and temperature T of stuff, attributed to the volume unit V, with a chemical potential and number density of a k-component, μ_k and n_k, can expressed by the following formula:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{\mathrm{d}U}{\left(T\,\mathrm{d}t\right)} + \frac{p\,\mathrm{d}V}{\left(TV\,\mathrm{d}t\right)} - \sum_\mathrm{k} \frac{\mu_\mathrm{k}\,\mathrm{d}n_\mathrm{k}}{\left(T\,\mathrm{d}t\right)}. \tag{3}$$

How to explain the constancy of the averaged entropy when the entropy dS/dt rises due to dissipative processes converting tremendous amounts of energy and stuff forcing the increase of entropy? As it is known, radiation possesses both energy and temperature. The explanation is that it is compensated by the difference between the entropies of absorbed solar radiation and IR radiation emitted by the Earth (which follows from the Boltzmann general formula). The difference is the negentropy. Considering both the Sun and Earth to be black bodies (with $F = \sigma T^4$ for the Earth, σ is the Stephan-Boltzmann constant), one obtains the entropy flux:

$$J(S) = \left(\frac{4}{3}\right) \sigma\, T^3 = \left(\frac{4}{3}\right) \frac{F_\mathrm{O}}{T_\mathrm{T}}, \tag{4}$$

and for $\Phi_\mathrm{o} = \Phi_\mathrm{T}$, the difference between the entropies of absorbed solar radiation and radiation of the Earth is

$$\Delta S = \left(\frac{4}{3}\right) F_\mathrm{O} \left(\frac{1}{T_\mathrm{O}} - \frac{1}{T_\mathrm{T}}\right). \tag{5}$$

T_o is always greater than T_T. In the Solar system, for the case of the Sun and Earth, $T_\mathrm{o} = 5{,}780$ K, $T_T = 254$ K. The global flux of negentropy is 6.8E14 $\mathrm{W\,K^{-1}}$, or 1.25 $\mathrm{W\,m^{-2}\,K^{-1}}$, which is a significant value. This is the explanation of the energy paradox.

Izakov (1989) underlines the leading role of entropy in nonequilibrium thermodynamics: "…entropy is the only state function that differs for irreversible and reversible processes, increasing in irreversible and constant in reversible… The entropy of a system is not conservative… Expenses of energy are actually the expenses of the negentropy, whereas the energy only changes its form."

The negentropy inflow is distributed on the Earth as follows: 70% supports the planet's thermal condition, including the greenhouse effect (averaged temperature T_o of the Earth surface is 288 K, averaged effective temperature of thermal radiation is 254 K); water evaporation takes 25 % (5E17 kg of water is yearly transferred in a hydrological cycle: evaporation – moisture transfer – water in rainfalls); and 3–4 % support heat and mass transfer in the atmosphere and ocean, including all catastrophic phenomena. The share of photosynthesis constitutes only 0.06 %, that is, 7E-4 $\mathrm{W\,m^{-2}\,K^{-1}}$.

Let's return to the dependence of negentropy on the stellar spectral class (for the Main Sequence stars). When passing from A, F, G to K and M classes, the temperature T_T decreases (Table 1), and the spectral maximum shifts out of the tantalizingly constrained spectral band used by photosynthesis. Besides, the efficiency $\eta = 1 - T_T/T_\mathrm{o}$ significantly decreases with the same decrease in negentropy flux. Whereas the thermal condition favorable for LANA is still possible to be provided at small orbital distances, the decrease in the negentropy and shift of Λ_mx to longer wavelength may be fatal for the photosynthesis efficiency. In other words, photosynthesis could take place even on Pluto if other requests for LANA are provided. However, it could never operate even on Mercury orbital distance if the Sun was replaced by a star of a later spectral type (classes from K5 to M8).

Table 1. Effective temperatures T(K), wavelengths Λ_{mx} (nm), and photon energy (E_p, eV) for the main sequence stars.

Class	A5	F0	F5	G0	G5	K0	K5	M0	M5	M8
T (K)	8,500	7,400	6,580	6,030	5,520	4,900	4,130	3,480	2,800	2,400
Λ_{mx}	350	403	453	495	540	608	722	856	1,064	1,042
E_p	4.23	3.16	2.81	2.57	2.36	2.10	1.77	1.49	1.20	0.975
	∇	∇	⊗	⊗	⊗	⊗	⊗?	□	□	□

Table 1 indicates the temperatures T(K), wavelengths Λ_{mx} (nm), and corresponding photon energy (E_p, eV) for the A5–M8 main sequence stars (independently of the major semiaxis of the planet's orbit). The following symbols are introduced in the table: ⊗-favorable for photosynthesis, □-the main radiation flux is beyond the long side wavelengths of photosynthesis, and ∇-there is a UV radiation hazard.

4.4. CONSTRAINTS ON TEMPERATURE IN THE SYSTEMS OF LATE-TYPE STARS: A SEMIMAJOR AXIS AND ECCENTRICITY OF THE ORBIT, ROTATIONAL PERIOD AND OTHER CONSTRAINTS

The conditions necessary for the advent and the evolution of life are not fulfilled on the hypothetical planetary systems of late-type stars (Ksanfomality, 1998). For the stars of types later than K5, the LANA life should face serious problems because of the low density and low negentropy inflow of useful radiation. For Sun-type (G2) stars, the "comfortable zone" is sufficiently wide, but it is limited. For example, Doyle (1994) gives the following constraints: on the one hand, the existence of liquid water and on the other hand, the condensation of carbon dioxide (which in most cases prevents the development of the greenhouse effect). With regard to the latter, this is an interval of planetary orbits from the level of the "runaway" (self-accelerating) greenhouse effect (the case for Venus) to approximately the orbit of Mars. It is clear that both constraints are essential limitations on the temperature and depend on a star type (Fig. 3). In the comfortable zones, the temperature interval should be considerably narrower. A coagulation of proteins takes place at a temperature above 65°C; therefore, $273 < T < 340$ K. If the star produces a bolometric illumination E_1, and T is treated as an equilibrium temperature of the planet equal to that of Earth (that may be attained by shortening planet's orbit semimajor axis a), the condition that a bolometric constant is equal to that of the Sun (E_o) is the following:

$$a = \left[2.512 \exp\left(M_b - M_O\right)\right]^{1/2}, \tag{6}$$

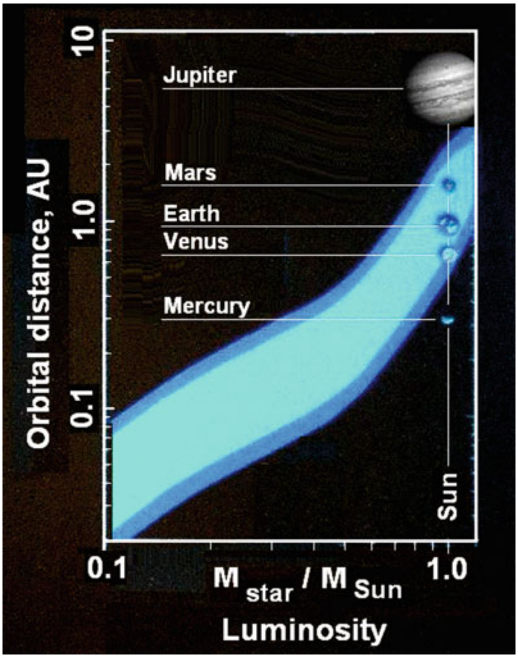

Figure 3. Position of the habitable zone for the Sun and stars of lower luminosity.

where M_b and M_o are the absolute bolometric luminosities of the star and the Sun. Let us assume that the spherical albedos A_b of the Earth and this hypothetical planet are equal. Then at the orbital distance a, the equilibrium temperature of the planet will be equal to that of the Earth's:

$$T = \left[\frac{(1 - A_b)E_1}{(4a^2\sigma)} \right]^{1/4}. \tag{7}$$

If other factors are not taken into account, it appears that it is possible to achieve terrestrial-like conditions on some planets of the late-type stars having low absolute luminosity at the expense of semimajor axis decrease.

Such an assumption was made by Sagan (1975). Ksanfomality (1998) considered the validity of this hypothesis for the stars of the late types, from K4 to M3, and concluded that in all cases, the semimajor axis is found to be less than that of Mercury's orbit (0.39 AU). An exception (0.59 AU) is O^2 Eridanus. For the stars of the M5 and K3 types, the value of the orbital distance a would correspond to only 3–6 million km. The period of revolution P of such a planet depends on the mass of the star, m and, in the cases under consideration, comprises only 3–6 days:

$$P = 2\pi \left(\frac{a^3}{\gamma m} \right)^{-1/2}, \tag{8}$$

(γ is the gravitational constant). At this small a, strong tidal dissipation of energy occurs, and the planet inevitably loses its rotational moment and is caught in a resonant "trap" with synchronization of the rotation and the revolution (Table 2).

Despite the fact that now it is well known that about 25–30 % of extrasolar planets orbit their parent star at such low orbits, the existence of these short periods seemed unbelievable in 1990s. In the solar system, Mercury, Venus, the Moon, and the close satellites of all giant planets are caught in resonance trap under such conditions. Even in the case of indirect resonance (a commensurability), one side of the celestial body caught in the low-orbit "trap" becomes incandescent, whereas the other side remains cold. The advent of life under such conditions is problematical. Mercury, having the temperatures of 670 and 80 K for each side, respectively, is an example. The existence of dense atmosphere (as in the case of Venus) may completely smooth out these differences. If the atmosphere is thin and consists essentially of carbon dioxide (which is typical for the primary planetary atmospheres of the Earth-group planets), it will condense in the trap on the cold side of the planet and may escape from the hot side almost entirely before long. The Martian polar caps are bright examples. The fate of seas and oceans on such a planet should be similar to Mars. The conclusion is the following: for all stars of K5V to late M types, the existence of a planet appropriate for life is doubtful. Shklovskii (1973) wrote that the constraints apparently existed as well for the star classes earlier than G class.

Table 2. The orbital distance a (a.u.) for the planet having temperature similar that of the Earth, for the main sequence stars.

Star	**Class**	M_b	m	R	a **(a.u.)**	a **(10^6 km)**	P **(days)**
Sun	G2	4.75	1	1	1	150	365
Barnard	M5V	13.15	0.14	–	0.020	3.1	2.9
Kruger 60	M3	11.80	0.27	–	0.039	5.8	5.4
η Cas (B)	M0V	8.50	0.52	0.52	0.178	26.7	38
61 Cyg (B)	K7V	8.30	0.60	0.84	0.195	29.2	40.6
61 Cyg (B)	K5V	7.50	0.63	0.86	0.280	43.2	69
O^2 Eri	K1V	5.89	0.80	0.93	0.590	88.7	197

The problems associated with the great inclination of the equator with respect to the orbital plane (the case of Uranus) are similar to the constraints resulting from the commensurabilities (the resonances of orbital rotation). The duration of the polar night on such a planet is up to one half-year, which restricts its suitability for life. A greatly prolonged rotational period also produces limitations, because an extended night, when photosynthesis is off, deprives a weakly protected organism of energy sources. It is known that plants on Earth consume oxygen at night, which points to their continuous needs for energy. However, there has scarcely been any oxygen in the primary atmospheres; therefore, an extended night may be a critical factor for the advent of LANA.

The limits of the "comfortable" zone discussed above narrow down sharply if the orbital eccentricity ε is large; to a first approximation, Δa decreases as $(1-\varepsilon)^2$. This is a more rigid condition than the extended night because the duration of a season that is too cold or too hot comprises a half-year. Therefore, the planets having a large orbital eccentricity are also not suitable for LANA.

4.5. CONSTRAINTS ON MASS OF THE PLANET: PROPERTIES OF THE ATMOSPHERE

According to present notions, life originated in warm shallow basins saturated with prebiotic compounds. Submarine volcanoes and rifts in fault zones, where an extraordinary wealth of plant and animal life was observed under terrestrial conditions, must have primarily been the sources of the prebiotic compounds. The products of accretion of planetesimals and cometary material, without intense thermal treatment in volcanic zones, hardly could provide enough of the important prebiotic materials. In a broad sense, volcanism is a consequence of the thermal processes in the planetary interior. Therefore, such phenomenon as convection within the core and mantle of a planet, is indirectly required for the origin of life.

The development of the convection is, in turn, greatly determined by global geochemical differentiation, with the separation of the core consisting of heavy rocks and the formation of the mantle and the lithosphere enriched with light elements. Radioactive isotopes of potassium, uranium, and thorium, along with the release of energy during the process of geochemical differentiation, are considered the main sources of endogenic heat at the post-accretional stage. Here we face one more constraint: to develop the geochemical differentiation spreading over the entire planet, its mass should be sufficiently large. Moreover, we can suggest its value. So, the global geochemical differentiation on Mars was found to be retarded because of its low mass (6.4E23 kg), although local processes of this type (Tharsis area) left the greatest volcanic structures in the solar system. The interior of Venus (4.9E24 kg) was apparently completely differentiated (Ksanfomality, 1985), although reliable data on the endogenic heat flux on Venus are absent. Finally, the Earth (6E24 kg) possesses all the evidence of intense geochemical differentiation. (Mercury, due to its origin, is not part of this series, its mass being equal to only3.3E23 kg, but definite evidence of geochemical differentiation, such as a magnetic field and a high mean density, is available). Thus, the mass of a terrestrial planet suitable for the advent of LANA should be close to 5E24 kg.

The same value follows from quite a different condition: the planetary gravity should retain the atmosphere. The condition formulated above (the advent of LANA in the shallow basins) implies that the planet has a stable atmosphere (its initial composition is carbon dioxide CO_2 and water vapor). The atmospheres are subjected to thermal (Jeans') and nonthermal dissipation. Being a result of H_2O vapor photolysis, the hydrogen escapes first. The thermal dissipation produces the following flux of hydrogen atoms:

$$\Phi_{\mathrm{H}} = \left(\frac{n_{\mathrm{H}} V_{\mathrm{T}}}{2\sqrt{\pi}}\right) \left(\frac{1+\gamma M m_{\mathrm{H}}}{R_{\mathrm{c}}\, kT_{\mathrm{c}}}\right) \exp\left(\frac{-\gamma M m_{\mathrm{H}}}{R_{\mathrm{c}}\, kT_{\mathrm{c}}}\right), \tag{9}$$

where n_{H} and m_{H} are the numerical concentration and the mass of dissipating atoms, k and γ are the Boltzmann's and gravitational constants, R_{c} and T_{c} are the planetary radius and planetary temperature both determined at a critical level, and V_{T} is the thermal velocity of atoms:

$$V_{\mathrm{T}} = \sqrt{\frac{kT_c}{m_{\mathrm{H}}}}. \tag{10}$$

In this way, Earth loses about 1.0E5 kg of hydrogen daily; however, this constitutes a negligibly small quantity compared to the mass of the ocean (1.45E21 kg).

Along with Jeans' dissipation of the atmosphere, the nonthermal dissipation plays a considerable role. The nature of this phenomenon is connected with the fact that atoms transfer momentum upon collision. As a result, the atoms of heavy gases gain a velocity large enough to escape. An amount of oxygen loss due to this fact is comparable with the amount of hydrogen is lost in thermal dissipation.

One can see from Eq. (9) that the process of escape of the atmosphere exponentially increases with a decrease in the mass of the planet. Only sufficiently massive planets possess an atmosphere that protects their surfaces from hard radiation destroying ribonucleic acid and DNA. However, the photons of energy 1–3 eV needed for photosynthesis cannot reach the surface if the atmosphere is too dense (say, $N_{\mathrm{a}} = 10\mathrm{E}21$ cm^{-3}, the case for Venus), and the supply of energy necessary for the maintenance of life is absent. Finally, oxygen, which along with oxidized materials provides the energy provision for life, is not present in the chemical composition of the primary atmosphere (retained on Mars and Venus) in a significant proportion. (It is known that the oxygen in Earth's atmosphere is of secondary, biogenic origin.)

It is also possible that the spectral transmission of the atmosphere corresponds to the requirements of LANA, but the density of radiation is low. In this case, the constraints on the temperature discussed above mentioned in Doyle's paper are not satisfied. The fact that the Earth avoided the formation of a massive atmosphere, even though the masses of Earth and Venus are nearly the same, apparently points to an important role of Earth's other characteristics. It is possible that Earth's mass slightly exceeds the optimal value for LANA and satisfies its conditions only in a harmonious combination with other factors.

The constraints formulated above also explain the fact that even large satellites cannot be considered possible inhabited bodies: their masses do not allow them to retain an atmosphere and do not satisfy the other conditions listed. Among 60 satellites of the planets in the solar system, Saturn's satellite Titan alone has a dense atmosphere. But this is merely the exception confirming the rule: its atmosphere is retained only due to a very low temperature (about 90 K).

4.6. AVAILABILITY OF OPEN BASINS AND PRESENCE OF WATER VAPOR IN THE ATMOSPHERE: THE ROLE OF THE MASS, PLANETARY SATELLITES, AND OTHER CERTAIN CONSTRAINTS ESSENTIAL FOR THE ORIGINATION OF COMPLICATED FORMS OF LIFE

It is rather difficult to list the critical factors acting at a stage of the advent of multicellular life; therefore, they are discussed below briefly.

By the time of the advent of multicellular organisms, whose demands for energy are sufficiently large, an oxidizing atmosphere should have already existed on the planet (as a result of the activity of innumerable generations of algae). As investigations show, the radical change of ancient forms of living matter and the formation of new organisms often followed giant catastrophes like collisions of large meteorites with Earth, climatic changes, and others. Inhabitation of continents (dry lands) by animals was obviously a great milestone. Complete changes in their way of life apparently prompted evolution to create new forms. Such conditions would not occur on a planet that has no islands and is totally covered by an ocean. It is more difficult to judge about the evolution of life on a planet having only small basins on a continent of global extent.

Tidal phenomena in the ocean shores caused by the periodicity of the gravitational influence of a massive and close planetary satellite (similar to the Moon) should play an important role both at the early stages (unicellular forms) and in the transition from primitive marine species to complex land inhabitants. Shoals become exposed during the tides, the radiation of the star becomes accessible to the Protozoa, and complex species adapt to the future occupation of dry land. Besides, the thermal dissipation of tidal energy in the solid body of the planet apparently accelerates the development of the geochemical differentiation at the early stages of planetary history.

The planetary mass imposes additional restrictions on the transition from Protozoa to multicellular organisms. These constraints are based on the energy demanded and depend on the value of gravitation. On the one hand, small organisms are energy-efficient, since their power-to-weight ratio is inversely related to approximately the square of their characteristic linear dimensions, all other factors being the same. The well-defined mass limit of flying birds is a characteristic restriction. On the other hand, sufficiently complex animals may not be small. However, species that are too large are forced to exist in an evolutionary impasse produced by a positive feedback in the energy-mass-food cycle. The restrictions are apparent, as evidenced by zoology: animals greater than whales and giant reptiles do not and did not exist on Earth. Enormous amounts of food are required for the survival of these animals.

All animals having sufficiently complex behavior appear inevitably to be large in size. Humans are also considered to be among the biggest animals on Earth. It is easy to see this, if the short list of animals bigger than humans is compared with the endless long list of the other inhabitants of Earth. Nevertheless, human sizes for Earth are obviously optimal; this is illustrated if only by the fact that humans, while retaining moderate requirements for food, can compete in speed with most other animals. The role of gravitation (and planetary mass) is obvious here. Thus, planetary mass now serves as the critical factor on a purely biological level of the formation of ecological niches.

5. Conclusion

The data of comparative planetology show that possible ways of planetary evolution are ambiguous and lead to remarkably dissimilar results, even if initial prerequisites were relatively close (similar characteristics of the planets, the case for Earth and Venus). A planet should satisfy a great quantity of requirements, which can be contradictory sometimes, in order to be suitable for the advent and evolution of LANA. In particular, the existence of a comfortable temperature zone is determined by many parameters, including the atmosphere's properties and a rigid constraint on its mass, near 5E24 kg.

The Earth possesses a unique combination of physical properties needed for the evolution of LANA and its transition into multicellular organisms. None of the terrestrial planets have this analogous combination of properties. Nevertheless, the conditions for the advent of unicellular organisms (Protozoa) may occur in many worlds, despite the fact that the main factors of the planet are critical for the existence of the LANA forming a peculiar labyrinth with many impasses. The analysis shows that after the advent of amino-nucleic-acid forms of life, only very narrow intervals of many new parameters and their combinations may provide the conditions necessary for their evolution into sensible beings. The question of possibility of the existence of a similar combination within planetary systems of other stars will not be clear until the planets and systems comparable to the solar system's are actually found.

One may accuse the author of a certain Earth's chauvinism, not admitting the existence of non-LANA living forms. Strictly speaking, there is evidence that LANA would even adapt to the temperatures exceeding the ones in the interval $273 < T < 340$ K and the hard radiation level mentioned above. The known rhizobia use the high energy of π-bonds reaching 10 eV and even more in their metabolism without damaging themselves. If one considers such energies to be a result

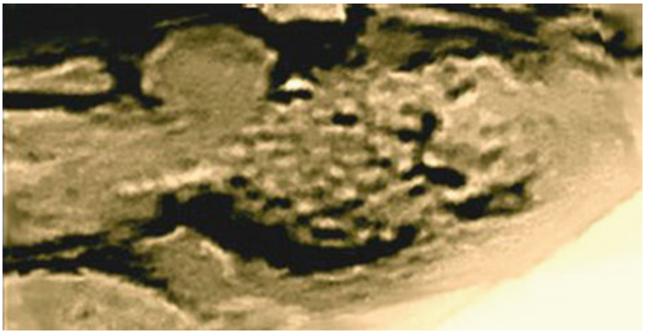

Fig. 4. A birdlike stone on the panorama transmitted by the Venera-9 lander. Its length is about 20 cm.

of equivalent temperatures influence on the action on these bacteria, one may conclude they could exist even on Venus.

A strange stone (or a creature?) which can be seen amid stones (Fig. 4) on the first panorama transmitted by the Venera-9 lander from the Venus surface in October 1975, allowed me to joke, that birds could exist in the dense Venusian atmosphere at the temperatures of Venus of about 740 K (Ksanfomality, 1978). This assumption seems rather fantastic because the conditions formulated above are absent on Venus. Besides, even though such metabolism is possible, the nucleic acids may not resist high temperatures; therefore, the amino-nucleic-acid form of life on Venus is out of the question. Could there actually exist another form of life? (see Ksanfomality, 2012).

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ORIGIN OF THE GENETIC CODE AND ABIOTIC SYNTHESIS OF ORGANIC COMPOUNDS

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1. Origin of the First Organic Molecules

The origin of life on Earth would have needed simple organic molecules, which evolved to more complex structures and then to the first living organisms. However, we are still far from understanding how this process happened.

The idea of synthesizing prebiotic organic compounds relevant from the biological point of view was first proposed by Oparin and Haldane (Oparin, 1924, 1952; Haldane, 1929, 1954). Based on astronomical observations of methane and ammonia present in the atmospheres of Jupiter and Saturn, these authors suggested that the atmosphere of the primitive Earth (4.6–3.8 billion years ago) was reducing and that organic compounds would be synthesized in this type of atmosphere, being later accumulated in a primitive ocean (the so-called primordial soup, Fig. 1). In addition, Urey proposed that the reducing atmosphere of the primitive Earth would have been composed of methane, ammonia, water and molecular hydrogen (Urey, 1952). Based on these ideas, Stanley Miller (who was then a Ph.D. student of Urey) demonstrated experimentally the synthesis of organic compounds (e.g., amino acids) using a spark discharge in a reducing atmosphere (Miller, 1953; Miller and Urey, 1959). He built an instrument in which he simulated the interaction between what was thought to be the primitive atmosphere of the Earth (containing a mixture of methane, ammonia, water, and molecular hydrogen) and a primitive ocean (Fig. 2). However, recent studies (e.g., models of accretion of the Earth, geological evidences, and the present-day composition of volcanic gases) challenge the idea of having a reducing atmosphere in the primitive Earth (Holland, 1984; Walker, 1986; Kasting, 1993; Kasting and Catling, 2003). In these conditions, the synthesis of prebiotic organic compounds in our planet would have been less than favorable (Miller and Schlesinger, 1983; Stribling and Miller, 1987). In the meantime, in the same year that Miller published his results, it was proposed the double helix model of deoxyribonucleic acid (DNA) (Watson and Crick, 1953), which is the molecule that contains the genetic information of each living organism and is responsible for the transmission of hereditary characteristics. All these experiments opened the path for the

Figure 1. Primordial soup of the primitive Earth composed by organic molecules synthesized abiotically on our planet (*left*) or produced in space and brought to Earth (*right*) (Miller and Chyba, 1992).

study of the origin of life on Earth and how the first living organism capable of replication appeared on Earth, between 3.85 and 3.5 billion years ago (e.g., Schopf, 1993; Mojzsis et al., 1996).

Although presently we know that the atmosphere of the primitive Earth was not reducing, the easy way in which amino acids were synthesized in so-called prebiotic conditions (Miller, 1953) led to formulate the hypothesis that the first replication system consisted of polypeptides (e.g., Fox and Dose, 1977; Kauffman, 1986; Wächtershäuser, 1988; De Duve, 1991; Dose, 1994). In this model of "metabolism first," the appearance of a primitive metabolism using peptides is suggested, leading to biochemical reactions and later to the appearance of genes. Alternatively, the hypothesis that the first biological systems based their replication and chemical reactions in ribonucleic acid (RNA) molecules, without the presence of DNA or proteins, is proposed (e.g., Belozerskii, 1959; Brachet, 1959; Oparin, 1961; Rich, 1962; Buchanan, 1965; Haldane, 1965; Woese, 1967; Crick, 1968; Orgel, 1968; Kuhn, 1972; Eigen and Schuster, 1979; White III, 1982; Cech, 1986; Gilbert, 1986; Lazcano, 1986; Joyce, 1991, 2002; Schwartz, 1993; Johnston et al., 2001). In this hypothesis, known as "RNA world," enzymes of RNA (i.e., ribozymes) would have been responsible for both the catalysis of reactions and the storage of genetic information. Although until today the synthesis of RNA under prebiotic conditions was never achieved, the synthesis of RNA from monomers

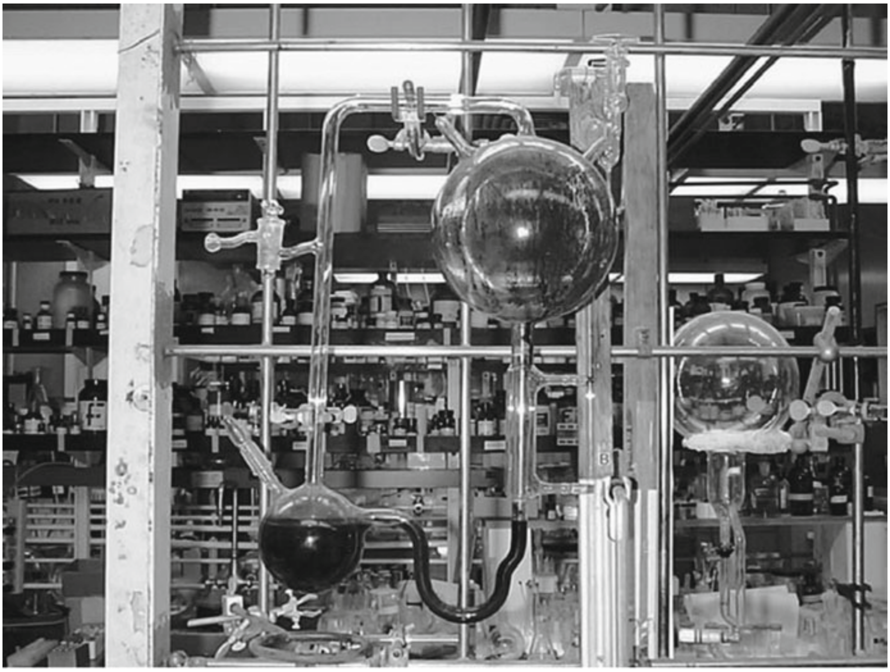

Figure 2. The Miller–Urey experiment used to demonstrate the synthesis of biologically relevant molecules in a reducing and hypothetically primitive atmosphere (Ehrenfreund et al., 2002).

is still the subject of many studies. Additionally, other theories have been proposed, including, for example, the "clay hypothesis" in which the first systems of storage of genetic information would not have been nucleic acids, but instead clay crystals. The reproduction would have been achieved through the growth of these crystals (Cairns-Smith, 1982, 1985). This nongenetic system would have been gradually converted to our current system by a process called "genetic takeover." However, theories such as this one do not have sufficient experimental support (Bullard et al., 2007), therefore not being widely accepted and will not be discussed in this book chapter.

2. Metabolic Model ("Metabolism First")

The metabolic model suggests the appearance of a primitive metabolism in which peptides would have been the first replication system on Earth. Their building blocks, the amino acids, may have been synthesized on the primitive Earth (Johnson et al., 2008), or alternatively outside the Earth (in an extraterrestrial environment), and brought to our planet through comets, meteorites and interplanetary dust particles (IDPs) (Chyba and Sagan, 1992).

2.1. AMINO ACIDS

2.1.1. Prebiotic Synthesis on the Primitive Earth

Although the reducing conditions used in the Miller experiment (Miller, 1953) do not correspond to the ones of the primitive Earth (Holland, 1984; Walker, 1986; Kasting, 1993; Kasting and Catling, 2003), the prebiotic, synthesis of amino acids on our planet may have been possible (Johnson et al., 2008). Besides the famous Miller–Urey experiment, Miller performed another experiment in which he simulated volcanic eruptions on the primitive Earth. Recently, Johnson and collaborators reanalyzed samples of this experiment and detected 22 amino acids (Johnson et al., 2008). These authors suggested that localized prebiotic synthesis may have been effective, and amino acids synthesized in volcanic island systems. The presence of the volcanic gas carbonyl sulfide in these systems may have helped the polymerization of amino acids (Leman et al., 2004).

2.1.2. Abiotic Synthesis in Extraterrestrial Environments

Amino acids have been detected in a variety of extraterrestrial environments including the interstellar medium (ISM), comets, meteorites and IDPs (for a detailed review, see Martins and Sephton, 2009).

Although only glycine was detected in the ISM, amino acids have been proposed to be synthesized on the surface of dust grains, which will then be evaporated by energetic processes (e.g., cosmic rays or UV irradiation), releasing the amino acids into the gas phase (solid-phase reactions) (for a review, see Ehrenfreund et al., 2005; Martins and Sephton, 2009). Alternatively, the gas phase formation of interstellar amino acids may occur via ion–molecule reactions (gas-phase reactions) (Charnley, 1997, 2001; Schutte et al., 1999; Liu, 2001; Blagojevic, 2003). Independent, of the mechanism of synthesis, once formed, the amino acids need to be resistant to space radiation. It was experimentally demonstrated that amino acids do not survive in environments of high UV flux (Ehrenfreund et al., 2001). This does not eliminate the synthesis of amino acids in the ISM, but requires that once formed, they need to be incorporated into UV-shielded environments such as the interior of comets, asteroids, meteorites, and IDPs.

The space missions Giotto and Vega suggested the presence of amino acids in the comet Halley (Kissel and Krueger, 1987), but its presence was not confirmed. Through the use of radio telescopes, maximum abundance of 0.15 of glycine relative to water was determined for comet Hale–Bopp (Crovisier et al., 2004). Glycine was also detected in samples collected from comet Wild 2 (Sandford et al., 2006).

Amino acids have been detected in several meteorites (for a review, see Cronin et al., 1988; Cronin and Chang, 1993; Martins and Sephton, 2009). Murchison is amongst the most analyzed meteorite for amino acids. This meteorite has more than 80 different extraterrestrial amino acids, the majority of which are rare (or inexistent) in the terrestrial biosphere. The abundances and distribution of amino acids in meteorites are very variable. While two Antarctic meteorites,

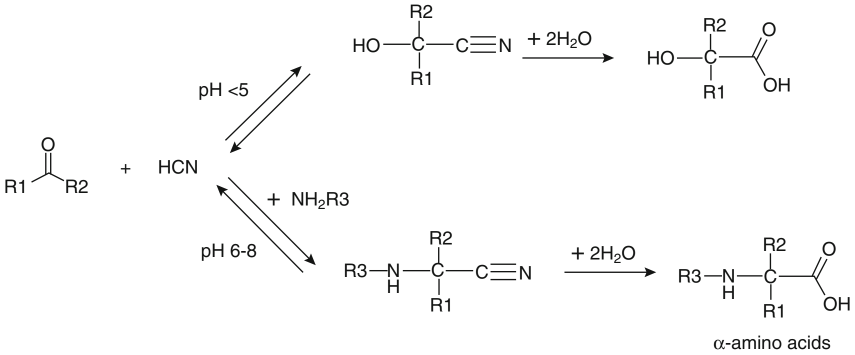

Figure 3. Strecker synthesis for the formation of α-amino acids (Adapted from Peltzer et al., 1984; Botta et al., 2002; Sephton, 2004). R1 and R2 correspond to hydrogen or CnH(2*n* + 1). If R3 corresponds to hydrogen, then α-amino acids are produced.

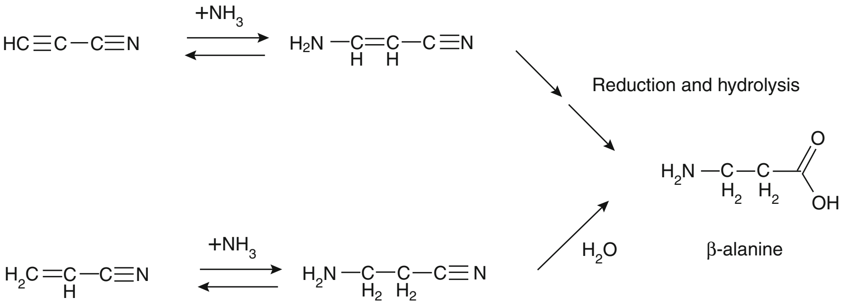

Figure 4. Synthesis of β-amino acids through Michael addition of ammonia to nitriles α,β-insaturated, followed by reduction and hydrolysis (Adapted from Botta et al., 2002).

Elephant Moraine (EET) 92042 and Graves Nunataks (GRA) 95229, have the highest extraterrestrial amino acid content ever detected in a meteorite (Martins et al., 2007), other meteorites such as Taguish Lake (Botta et al., 2002; Kminek et al., 2002) have only residual abundances of amino acids. The synthesis of meteoritic amino acids may occur by several different synthetic pathways. It is suggested that meteoritic α-amino acids are formed by the Strecker reaction (Fig. 3) from carbonyl compounds (aldehydes and ketones), ammonia, and hydrogen cyanide (HCN) present or synthesized in the protosolar nebula and later incorporated in asteroids (the parent body of meteorites) (Cronin and Chang, 1993; Peltzer et al., 1984; Peltzer and Bada, 1978). On the other hand, β-amino acids may be synthesized through the Michael addition of ammonia to nitriles α,β-unsaturated, followed by reduction and hydrolysis (Fig. 4). Other synthetic pathways are possible and were proposed (for a review, see Cronin and Chang, 1993).

For example, the hydrolysis of carboxy lactams and lactams gives origin to dicarboxylic amino acids and β-, γ-, and δ-amino acids, respectively (Cooper and Cronin, 1995).

Micrometeorites and IDPs, which are the remains of comets and asteroids, were also analyzed for amino acids. The majority of these extraterrestrial particles have very small quantities of amino acids (Brinton et al., 1998; Glavin et al., 2004; Matrajt et al., 2004).

2.2. PEPTIDES

2.2.1. Theory of the Iron–Sulfur World and Theory of the Thioester World

The theory of the iron–sulfur world is a hypothesis which proposes that the synthesis of peptides preceded the appearance of genes (Wächtershäuser, 1992, 1997). This would have occurred on the surface of transition metal (such as iron and nickel) sulfides, close to hydrothermal vents at the bottom of the oceans. Peptides would have been formed from the reaction of amino acids in aqueous solution (Huber and Wächtershäuser, 2003, 2006) with hydrogen sulfide and carbon monoxide or carbonyl sulfide (Huber and Wächtershäuser, 1998; Huber et al., 2003; Leman et al., 2004). However, an experimental analysis performed in the laboratory shows that large quantities of initial compounds are necessary (Huber and Wächtershäuser, 1998). In fact, carbon monoxide and carbonyl sulfide occurs in nature in concentration much lower than in those experiments (Symonds et al., 1994). Despite these problems, hydrothermal vents may have been important places for the synthesis of prebiotic compounds (Ferris, 1992; Amend and Shock, 1998).

Another theory in support of the "metabolism first" model is the thioester theory. It suggests that polypeptides may have been synthesized from thioesters of amino acids (De Duve, 1991). These would have been synthesized in a variety of prebiotic environments, such as the primitive atmosphere and oceans, and also through meteorites. According to de Duve (1991), thioesthers of amino acids form peptides spontaneously when in aqueous solution.

3. Genetic Model ("Nucleic Acid First")

3.1. "RNA WORLD" HYPOTHESIS AND THE FIRST MONOMERS

Nucleotides are the monomers of RNA and are composed of sugar molecules (ribose, $C_5H_{10}O_5$), a phosphate group (PO_4^{3-}) and one of four nucleobases (cytosine, uracil, adenine, and guanine; Fig. 5). The possible prebiotic synthesis of RNA on the primitive Earth from nucleotides may have resulted in ribozymes responsible for the storage of the genetic information and for the catalysis of chemical reactions

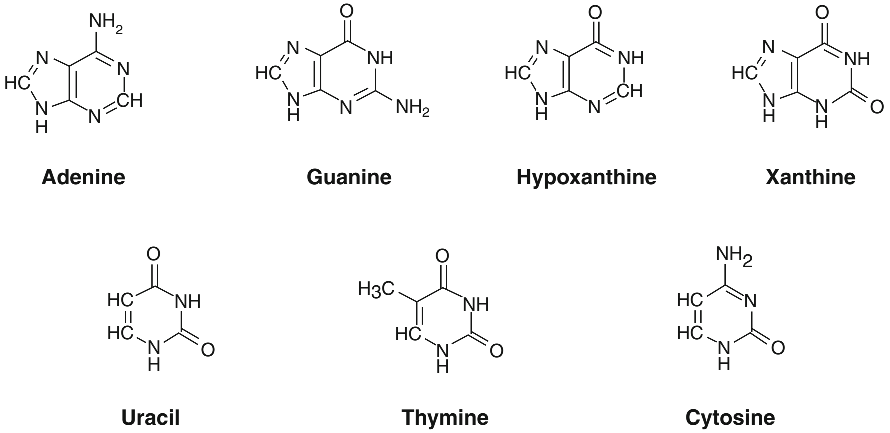

Figure 5. Structure of nucleobases, composed by one aromatic ring (pyrimidines) or two aromatic rings (purines). Adenine, guanine, uracil, and cytosine are part of RNA, while in the case of DNA, thymine substitutes uracil. Hypoxanthine and xanthine are not part of DNA or RNA, but they are important intermediates in the synthesis and degradation of nucleotides.

(RNA world hypothesis). Yet, we need to understand how the nucleotides appeared on the primitive Earth and how their abiotic synthesis may have occurred.

3.1.1. Prebiotic Synthesis of Ribose

The prebiotic synthesis of sugars was first proposed by Butlerow (1861). In this reaction, the polymerization of formaldehyde will lead to a complex mixture of sugars, of which ribose is only obtained in very small quantities (Reid and Orgel, 1967; Zubay, 1998). In order to separate ribose from a prebiotic mixture containing several sugars, Springsteen and Joyce (2004) proposed the “kidnap” of ribose through the reaction with cyanamide. This compound reacts quickly with ribose (but not with other sugars), and forms a stable bicyclical adduct. As an alternative to the polymerization of formaldehyde, the synthesis of a ribose derivative (ribose-2,4-diphosphate) starting from glycolaldehyde monophosphate and formaldehyde (Müller et al., 1990) or starting from glycolaldehyde monophosphate and glyceraldehyde-2-phosphate in the presence of hydroxide minerals was proposed (Pitsch et al., 1995; Krishnamurthy et al., 1999). Another problem with the abiotic synthesis of ribose has to do with the reduced stability of ribose (and other sugars) in strongly acidic or strongly basic solution (Larralde et al., 1995). In addition, ribose rapidly degrades (in a geological timescale) in neutral solutions (ribose has a half-life time of 73 min at pH 7.0 and 100°C, and a half-life of 44 years at pH 7.0 and 0°C). These results suggest that the “skeleton” of the first genetic material did not contain ribose or any other sugar (Larralde et al., 1995). Due to this, it is difficult to explain how ribose may have accumulated (and/or separated from other sugars with abiotic origin) in a prebiotic environment in the primitive Earth (Shapiro, 1988).

3.1.2. *Prebiotic Synthesis of Phosphates*

There is currently no mechanism that can explain efficiently the prebiotic synthesis of phosphates on the primitive Earth (Keefe and Miller, 1995). This is because phosphates are the dominant form of inorganic phosphates on Earth and occur mainly in the insoluble form (i.e., calcium phosphate as apatite). Due to this, several alternatives have been proposed for the presence of phosphates in the origin of life on Earth. These include substitution of the phosphate group in RNA for glyoxylate (Bean et al., 2006), or substitution of the phosphorylated ribose for peptides (ribonucleic peptide, PNA) (Nielsen et al., 1991; Egholm et al., 1992a, b; Nielsen, 1993; Cherny et al., 1993; Nelson et al., 2000). On the other hand, phosphates may have been introduced to the primitive Earth through meteorites (Cooper et al., 1992; Pasek, 2008). Significant concentrations of the methyl- and ethylphosphonic acids were detected in the Murchison meteorites (Cooper et al., 1992), suggesting the synthesis of ribose-2,4-diphosphate from these meteoritic compounds (de Graaf et al., 1997; Schwartz, 1997) .

3.1.3. *Prebiotic Synthesis of Nucleobases*

Several theories have been proposed about the prebiotic synthesis of nucleobases (Fig. 5). These include the abiotic synthesis in the primitive atmosphere and oceans (Ferris and Hagan, 1984; Orgel, 2004) through the polymerization of hydrogen cyanide (HCN) (Oró, 1960, 1961; Oró and Kimball, 1961; Ferris and Orgel, 1965, 1966; Sanchez et al., 1967; Ferris et al., 1978; Voet and Schwartz, 1983; Schwartz and Bakker, 1989; Minard et al., 1998; Levy et al., 1999; Miyakawa et al., 2002), the rapid cooling of a high-temperature plasma of CO-N_2-H_2O (Miyakawa et al., 2000), the reaction of urea with cyanoacetaldehyde in eutectic solution (Nelson et al., 2001) or with cyanoacetylene (Robertson and Miller, 1995; Nelson et al., 2001; Menor-Salván et al., 2009), or the reaction of cyanoacetylene with cyanide in diluted solutions and pH 8 (Ferris et al., 1968). The synthesis on mineral matrixes in the primitive Earth in the presence of inorganic oxides (Saladino et al., 2001) or adsorbed in minerals was also proposed (Winter and Zubay, 1995; Cohn et al., 2001; Sowerby et al., 2001), as some nucleobases are capable of self-association in monolayers (Sowerby and Petersen, 1997, 1999; Sowerby and Heckl, 1998; Sowerby et al., 1998).

Nucleobases may have also been synthesized abiotically in an extraterrestrial environment, and then brought to the primitive Earth by comets and meteorites, between 4.6 and 3.8 billion years ago (Chyba and Sagan, 1992). This synthesis could have occurred in the interstellar medium (ISM). However, the low resistance of nucleobases to UV (Peeters et al., 2003) decreases the changes of successfully finding any nucleobase in the gas phase in the ISM (Kuan et al., 2003). Alternatively, nucleobases may have been formed in the solid phase of cold interstellar grains (Nuevo et al., 2009) or in asteroids (i.e., the parent body of most meteorites). These compounds have been detected in several carbonaceous meteorites (e.g. Martins et al., 2008; Callaham et al., 2011; and references therein), but with no proof they were not terrestrial contamination, until the work of Martins et al. (2008).

These authors have proven that two nucleobases, uracil and xanthine, present in the Murchison meteorite have an extraterrestrial origin. The presence of nucleobases in meteorites may result from processes of synthesis and degradation (i.e., conversion to other nucleobases). As an example, cytosine degrades to uracil with a half-life of 17,000 years, and guanine degrades to xanthine with a half-life of 1.3 million years at 0°C and pH 7 (Levy and Miller, 1998).

3.1.4. Prebiotic Synthesis of RNA from Nucleotides

The prebiotic synthesis of nucleotides may have happened through the phosphorylation of nucleosides (composed by a nucleobase connected to a pentose, such as ribose), or through the insertion of nucleobases in the ribose-5′-phosphate. The prebiotic synthesis of nucleosides has several problems; the reaction of ribose with purines is inefficient and does not work with pyrimidines (Sanchez and Orgel, 1970; Fuller et al., 1972a, b). In addition, nucleobases and ribose could not have been synthesized at the same time in the primitive Earth because their precursor compounds (hydrogen cyanide and formaldehyde, respectively) react with each other, making the synthesis of nucleosides unlikely (Zubay and Mui, 2001). Even if nucleosides were synthesized, they would have been racemic, and therefore polymerization of one enantiomer would have been inhibited by the presence of the other enantiomers (Joyce et al., 1984, 1987). The last step, the phosphorylation of nucleosides, gives origin to a complex mixture of products (Ferris, 1987). On the other hand, the prebiotic synthesis of nucleotides using ribose-5′-phosphate and nucleobases is also a very complex process. Namely, the synthesis starting from cytosine and uracil is difficult because the nucleobases have reduced half-time lives from the geological point of view (Orgel, 1994; Levy and Miller, 1998). Despite all these problems (for a review, see Ferris, 1993), Powner et al. (2009) synthesized pyrimidine nucleotides bypassing ribose and nucleobases, and instead having arabinose amino-oxazoline and anhydronucleoside as intermediates. RNA may then be formed from nucleotides through the use of montmorillonite (clay mineral) as a catalyst (Ferris and Ertem, 1993; Kawamura and Ferris, 1994; Ferris, 2005).

In alternative to RNA as the first nucleic acid, the "pre-RNA world" hypothesis, in which the first self-replicate polymers would have been later replaced for RNA, has been proposed (Nielsen et al., 1991; Joyce, 2000; Nelson et al., 2000; Orgel, 2000; Schoning et al., 2000).

3.2. "PRE-RNA WORLD" HYPOTHESIS

The "pre-RNA world" hypothesis includes polymers capable of self-replication that would later evolve to RNA. Such is the case of pyranosyl-RNA (p-RNA), homo-DNA, threose nucleic acid (TNA), peptide nucleic acid (PNA), or glycol nucleic acid (GNA) (Fig. 6).

Eschenmoser and coworkers (Eschenmoser, 1997, 1999; Pitsch et al., 2003; Schoning et al., 2000) suggested that before RNA, the first nucleic acids would

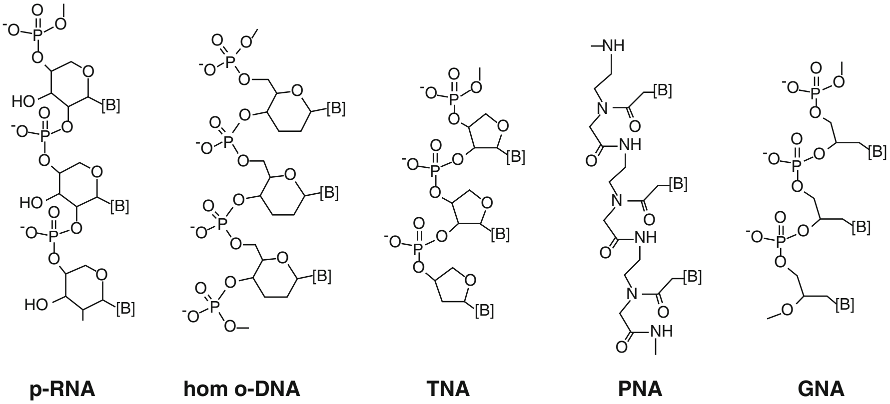

Figure 6. Possible polymers present in a "pre-RNA world": p-RNA, homo-ADN, TNA, PNA, and GNA. [B] represents a nucleobase.

have had ribose substituted by another sugar. Such polymers include p-RNA (composed by ribopyranose), homo-DNA (in which ribose is substituted by 2′-3′-dideoxy-D-glucose, which is a 6-carbon atom sugar), and TNA (composed by threose). The substitution of ribose by glycol propylene, giving origin to GNA (Zhang et al., 2005, 2006), was also suggested.

PNA is another analogue of the nucleic acid that was synthesized by Nielsen and colleagues (Egholm et al., 1992a, b, 1993; Cherny et al., 1993; Nielsen et al., 1991; Nielsen, 1993, 1999), in which the skeleton of phosphorylated ribose is substituted by peptides. In addition, it was demonstrated that genetic information may be transferred from PNA to RNA (and DNA) and vice versa, which supports the hypothesis of transition from the "pre-RNA world" to the "RNA world" (Schmidt et al., 1997a, b).

4. Conclusion

The origin of the genetic code is presently a crucial question for which there is still no answer, despite all the proposed hypotheses. Although the synthesis of RNA performed in the laboratory under prebiotic conditions was still not successfully achieved, the synthesis from its monomers is still currently studied. These prebiotic molecules (i.e., nucleobases, ribose, and phosphates) or their precursors have been detected in a variety of extraterrestrial environments (including the interstellar medium, comets and/or meteorites) and were brought to the Earth and other planets of our Solar System through exogenous sources. On the other hand, the endogenous synthesis of prebiotic compounds may have been constrained by the atmospheric conditions of the primitive Earth. Although we do not know the exact contribution of exogenous sources versus endogenous sources, the study of all these topics gives us a clear picture of the prebiotic environment available for the origin of a genetic code on the primitive Earth, and ultimately the origin of life as we know it.

5. Acknowledgments

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Biodata of **Dr. Elke Pilat-Lohinger** of "***Dynamical Aspects for the Earth's Habitability***."

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DYNAMICAL ASPECTS FOR THE EARTH'S HABITABILITY

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1. Introduction

There is no doubt that stability studies are of great importance in the fascinating extrasolar planetary research, especially due to the growing interest on the search of extraterrestrial life in the universe, where long-term stability of a planetary system is one of the basic requirements for the evolution of life on a terrestrial planet. The numerous extrasolar planetary systems (EPS)[1] allow a classification into three groups:

1. Single-star single-planet systems
2. Single-star multi-planet systems
3. Binary star systems hosting planets

Most EPS belong to the 1st group; for the 2nd and the 3rd group we know about 100 and 60 systems respectively. General stability studies are available for systems of the 1st and 3rd group. The so-called *Exocatalogue* (see Sándor et al., 2007) is an example for such a general study. It provides nearly 100 stability maps covering various mass ratios of a star and a giant planet. The stability maps can be used easily via the www-tool "*ExoStab*" (http://univie.ac.at/adg/exostab). ExoStab asks the user to enter some basic parameters of a planetary system—like the masses of the host star and the known giant planet, the distance between these two bodies, the eccentricity of the giant planet, and its uncertainty. Further input depends on the option chosen by the user, which are (i) stability of an additional planet or (ii) stability of the HZ or (iii) stability of an additional planet with respect to the HZ. According to the input, ExoStab searches the corresponding result of the Exocatalogue and generates a stability map. A detailed description of ExoStab can be found in Pilat-Lohinger et al. (2011).

Taking into account dynamical aspects, the two latter groups (multi-planet systems and planets in binary stars) are certainly more interesting, since planetary motion is restricted to certain regions of the phase space in these systems due to gravitational interactions between the celestial bodies, especially in case of small distances and high orbital eccentricities. However, for planetary motion in a double

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[1] See http://exoplanet.eu/catalog.php or http://exoplanets.org/planets.html

star system, it is possible to verify the stability of the planet via general stability studies like the ones by Rabl and Dvorak (1988), Holman and Wiegert (1999), or Pilat-Lohinger and Dvorak (2002) if there is only one planet. All mentioned studies used the elliptic restricted three-body problem (ER3BP—see Sect. 3) and determined the stable region as a function of the binary's mass ratio and its eccentricity. The eccentricity of the planetary motion was only considered in the paper by Pilat-Lohinger and Dvorak (2002).

This chapter addresses multi-planet systems with two giant planets moving in low-eccentricity orbits near a mean motion resonance (MMR).[2] According to the work by Ferraz-Mello et al. (2005), we can distinguish the following groups of multi-planet systems:

Ia: Planets in MMR
Ib: Low-eccentricity near-resonant planet pairs
II: Nonresonant planets with significant secular dynamics
III: Hierarchical planet pairs

The solar system, which is the reference system for our numerical investigation, belongs to group Ib. By modifying the orbital parameters of Saturn, we generate various fictitious solar system-like configurations and study the influence of the giant planets on the motion of test planets in the habitable zone (HZ). Our calculated stability maps of the HZ show a large region of nearly circular motion and areas perturbed by MMRs or secular resonances (see Sect. 3). The high eccentricity that could occur in the perturbed area would influence the habitability of the Earth in some configurations. This chapter is based on a study published in 2008 (see Pilat-Lohinger et al., 2008a, b).

2. The Habitable Zone

The HZ is the region around a star where liquid water is stable on the surface of an Earthlike planet (Kasting et al., 1993). Planets in the HZ are also presumed to be capable of having an appropriate atmosphere. Kasting et al. (1993) defined the HZ in our solar system for human life from 0.93 to 1.37 AU. They based their work on a planet with a terrestrial ocean of superficial water, the carbonate–silicate cycle which controls the CO_2 level in the atmosphere, and the surface temperature that is above freezing in the HZ. In the outer region, (for semimajor axes $a > 1.37$ AU) CO_2 condensates in the atmosphere producing CO_2 clouds that can affect the temperature–CO_2 coupling significantly. For the inner region ($a < 0.93$ AU), H_2O becomes a major atmospheric compound and is rapidly lost to space after UV photolysis.

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[2] Two celestial bodies are in MMR if the ratio or their orbital periods is a ratio of integers.

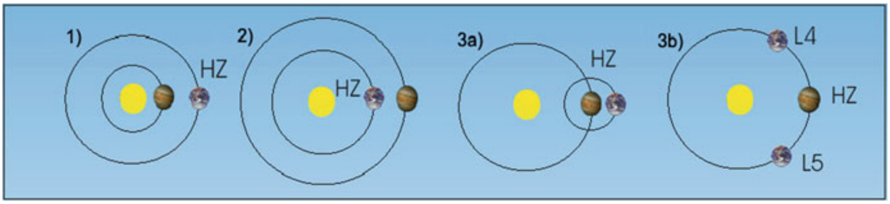

Figure 1. Different types of HZ—a classification from the dynamical point of view depending on the position of the giant planet's orbit (Courtesy of R. Schwarz).

More recent studies find a potentially larger HZ for a Sunlike star—see, e.g., Forget and Pierrehumbert (1997) or Mischna et al. (2000), both studies included CO_2 cloud effects.

From the given border of the Sun's HZ, one can see that the size of the HZ is limited to a small region, depending on the spectral type and the age of the host star; therefore, the planet's eccentricity has to be small enough if we require that the planet's orbit is completely in the HZ. In dynamical studies, we distinguish different types of HZ, depending on the position of the giant planet in the system (see Fig. 1):

1. The outer HZ (OHZ), where the HZ is outside the giant planet's orbit—like for systems hosting a hot Jupiter
2. The inner HZ (IHZ), where the HZ is between the host star and the detected giant planet—like in the solar system
3. The giant planet HZ (GP-HZ) where the detected giant planet moves in the HZ. In this case, we can only expect so-called habitable moons (see Fig. 1(3a)) or habitable Trojan planets (see Figs. 1(3b))—see Laughlin and Chambers (2002), Dvorak et al. (2004), and Érdi and Sándor (2005).

The study of habitability is an interdisciplinary venture including astrophysical, biological, geophysical, and chemical studies. From the astrophysical point of view, studies of the stellar luminosity and its influence on the distance of the HZ, as well as the planet's mass (to maintain an atmosphere) and planetary composition (assuming a terrestrial-like planet), are important to the science of habitability. Since the evolution of a biosphere takes a long time, the long-term orbital stability of the whole planetary system is a basic requirement for habitability. This emphasizes the importance of numerical stability studies, where two approaches to this problem have been used:

1. Studying stability and planetary formation in detected extrasolar planetary systems (see, e.g., Rivera and Lissauer, 2000, 2001; Jones and Sleep, 2002; Laughlin and Chambers, 2002; Menou and Tabachnik, 2003; Dvorak et al., 2003, 2010; Barnes and Raymond, 2004; Asghari et al., 2004; Érdi et al., 2004; Jones et al., 2005, 2006; Raymond et al., 2006; Barnes and Greenberg, 2006; Ji et al., 2005; Rivera and Haghighipour, 2007; Schwarz et al., 2007;

Beaugé et al., 2008; Murray and Correia, 2010; Fabrycky, 2010; Funk et al., 2010, 2011; Haghighipour et al., 2010; Michtchenko et al., 2010; Pilat-Lohinger and Funk, 2010; Pilat-Lohinger, 2010; Laskar and Correia, 2011; Rodríguez et al., 2011; and many others)

2. Performing general numerical studies that can be applied to many of the discovered systems (as described above; see, e.g., Sándor et al., 2007 and Pilat-Lohinger et al., 2011)

3. Dynamical Model and Computations

If one has to study a large set of orbital parameters, the computations will be very time-consuming; therefore, it is advisable to choose a model that allows gaining computation time. In dynamical astronomy, it is quite common to use the so-called elliptic restricted three-body problem (ERTBP), which describes the motion of a small body (of negligible mass) in the gravitational field of two primary bodies (that are either a star and a giant planet or two stars). The massless body has no influence on the motion of the two massive bodies, so that they move on Keplerian orbits around their center of mass. Numerous test computations have shown that this model provides good results for a system where the mass of one body is small compared to those of the others. For larger masses of the third body, the results of the ERTBP can be considered as a first approximation but should be examined additionally using the full n-body problem.

To determine whether a planet might be considered as "habitable planet" from the dynamical point of view, it is useful to calculate the *maximum eccentricity* (*max-e*) of its orbit over the whole integration time. Since the HZ is a quite narrow zone around a star, which does not allow high-eccentricity motion. The max-e maps indicate clearly regions of low eccentric motion in a system. This method has been successfully applied to studies of extrasolar planetary systems (e.g., Dvorak et al. (2003, 2004), Funk et al. (2009), Pilat-Lohinger and Funk (2010)).

To create fictitious multi-planet systems (of 2 and 3 giant planets) bearing resemblance to our solar system, we took the orbital parameters of Jupiter, Saturn, and Uranus (as given in Table 1) and varied the initial semimajor axis of Saturn from 8 to 11 AU in steps of 0.1 AU so that the mutual distance between the two giant planets Jupiter and Saturn—which dominate the dynamics in the solar system—was changed. Jupiter's orbital parameters were fixed to those given in Table 1. To consider systems with different mass ratios, we increased Saturn's

Table 1. Orbital elements of the giant planets.

Planet	*a*[AU]	*e*	inc.[deg]	T[deg]	Σ[deg]	M[deg]	Mass [m_{Sun}]
Jupiter	5.203	0.0483	1.305	275.201	100.471	183.898	0.95479e-3
Saturn	9.530	0.0533	2.486	339.520	113.669	238.293	0.28588e-3
Uranus	19.235	0.0473	0.773	99.866	74.033	111.688	0.43554e-4

mass (see Table 1) by a factor $6_S = 2$ to 40 (in steps of 1)—the maximum mass corresponds to about 13 Jupiter masses, which is the upper mass limit defining a planet, before stable H_2 burning sets in. From these variations, we obtained different configurations comparable with our solar system. We examined the interactions between Jupiter and Saturn and selected several values of 6_S for the stability study of test planets in the HZ.

For the test planets, we varied the semimajor axis between 0.6 and 1.6 AU[3] in steps of 0.02 AU and assumed Earth's orbital parameters for the eccentricity $e_{tp} = 0.0167$, the inclination $i_{tp} = 0.0008°$, the argument of perihelion $\omega_{tp} = 103.946°$, the node $\Omega_{tp} = 358.859°$, and the mean anomaly $M_{tp} = 206.900°$.

A stability map of the enlarged HZ consists of 1,581 orbits per mass ratio of the two gas giants—which is determined by 6_S. All orbits were computed over 10^7 years using the hybrid-symplectic integrator Mercury6 of Chambers (1999).

By calculating max-e over the whole computation time, we were able to distinguish between (i) orbits being in the HZ during the whole computation time, (ii) orbits being in the HZ for most of the time during its revolution, and (iii) highly eccentric orbits that probably provide problems for the habitability. In this context, we have to mention that Williams and Pollard (2002) claim that the Earth could also be habitable, according to the standard definition of habitability, if its orbital eccentricity would be 0.7. But this leads obviously to strong variations in the surface temperature. The max-e maps show regions of higher eccentricities resulting either from MMRs between the gas giants or from secular perturbations.[4] The latter were determined with the aid of the frequency analysis of Laskar (1990)—see also Robutel and Gabern (2006)—where the secular frequencies g and s are deduced by the following secular linear approximation (see, e.g., Murray and Dermott, 1999):

$$g = \frac{n}{4}\left(\frac{m_J}{M_{Sun}}\alpha_J^2 b_{3/2}^{(1)}(\alpha_J) + \frac{m_S}{M_{Sun}}\alpha_S^2 b_{3/2}^{(1)}(\alpha_S) + \frac{m_U}{M_{Sun}}\alpha_U^2 b_{3/2}^{(1)}(\alpha_U)\right)$$

$$g = -s$$

where $\alpha_J = a_{tp}/a_J$, $\alpha_S = a_{tp}/a_S$, and $\alpha_U = a_{tp}/a_U$; (a_J, a_S, and a_U are the semi-major axes of Jupiter, Saturn, and Uranus, respectively) and $b_{3/2}^{(1)}$ is a Laplace coefficient. The test-planets must have nearly zero initial eccentricities and inclinations. The term of Uranus is set in bracket, since it is not needed for all configurations.

The solution of this equation appears as black, bold line in the max-e maps (see Figs. 3, 4, and 5). In Pilat-Lohinger et al. (2008a, b), it is shown in detail that the application of this secular theory is in good agreement with the max-e maps.

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[3] We defined the "HZ" from 0.6 to 1.6 AU to include also the orbits of Venus and Mars.

[4] That is, an orbital perturbation which is cumulative and which causes a change (increase or decrease) in an orbital element. Gravitational forces between the celestial bodies in a system cause secular changes in the longitude of the ascending node and longitude of perihelion.

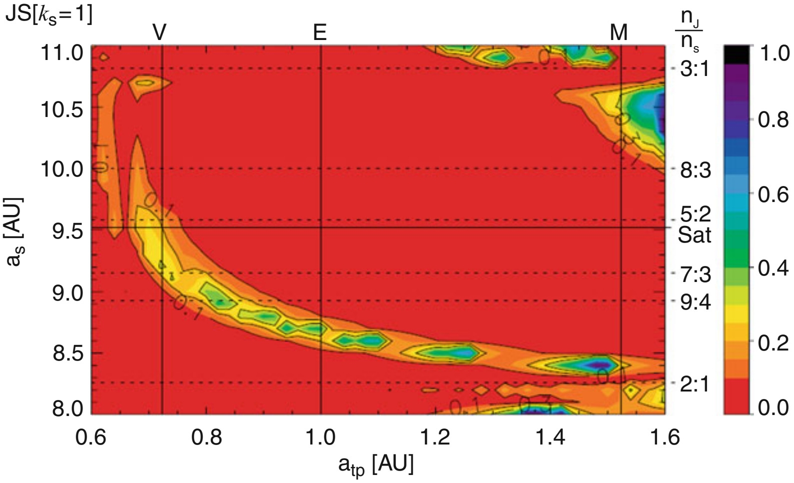

Figure 2. Max-e map for Earthlike planets under the influence of Jupiter and Saturn. The two axes show different initial semimajor axes either of the massless Earthlike planets (x-axis) or of Saturn (y-axis); the grid size is 0.02 AU in x, and 0.1 AU in y. The vertical, black, solid lines indicate the positions of Venus (V), Earth (E), and Mars (M), and the horizontal line represents Saturn (Sat). The dashed horizontal lines label the positions of different mean motion resonances. Different colors belong to different values of max-e—see the color scaling (This figure is taken from Pilat-Lohinger et al., 2008a).

4. The Modified Solar System: Jupiter–Saturn Configurations

First, we studied the influence of Jupiter and Saturn on the planetary motion in the HZ of a Sunlike star. We computed a cloud of test planets using the orbital parameters given in Sect. 3. The resulting stability map (see Fig. 2) representing the (a_{tp}; a_S) plane contains information on 1581 data pairs, when calculating the test planets for the different starting positions of Saturn. Figure 2 shows the perturbations of the Jupiter–Saturn pair via the max-e over the whole computation time; red shows the region of lowest e-max and blue the highest. The initial semimajor axes of Venus (V), Earth (E), and Mars (M) are marked by vertical solid lines and that of Saturn (Sat) by the horizontal solid line. The most important MMRs between Jupiter and Saturn (i.e., 2:1, 9:4, 7:3, 5:2, 8:3, and 3:1) are shown by horizontal dashed lines. Analyzing this result, we find that most of the HZ is not affected by Jupiter and Saturn (i.e., the red region). The stability map is dominated by an arched band that indicates higher eccentricities of the test planet due to a stronger influence of the giant planets in this region. The secular frequency analysis showed that the $g=g_J$ (J = Jupiter) frequency (i.e., the fundamental frequency of Jupiter's perihelion) is responsible for this perturbation. Within this band are several positions where the eccentricity is ~0.5 (green regions) or even higher (e.g., blue region near the position of Mars). Apart from this significant stripe, some smaller regions show an increase in eccentricity, especially in the outer part of the HZ ($a_{tp} > 1{:}2$ AU), e.g., when Saturn is nearly in 2:1 MMR with Jupiter. In that case, a test planet moving near the position of Mars would be influenced by the gas giants, causing an eccentricity of about 0.2 for the test planet. An even higher eccentricity can be observed when Saturn is placed between 10 and 10.7 AU, especially for the region outside the orbit of Mars. If Saturn orbits the Sun at 11 AU, perturbations in the area between 1.15 and 1.5 AU appear. Another interesting result is the heightened eccentricity for a test planet at the position of Venus, especially if Saturn is placed at its actual semimajor axis. The color scaling indicates an e-max 0.2; in contrast, the actual Venus is moving in a nearly circular orbit in our own solar system. In Pilat-Lohinger et al. (2008a), it was shown that such large fluctuations in eccentricity arrive regardless of the choice of initial orbital elements in this area. Only when we include the Earth in this system, a significant decrease in the eccentricity of Venus' orbit is observed.

5. Results for a Higher Saturn Mass

A similar study for a configuration where the two giant planets have nearly equal masses is shown in this section. The max-e of the orbital motion in the HZ for this system is shown in Fig. 3, where we have plotted the results of both studies (max-e and frequency analysis) together. Like for the real Jupiter–Saturn mass ratio, the arched band of higher max-e values, resulting from the g_J frequency, dominates this plot. Comparing this result with that of the solar system configuration (Fig. 3), one can recognize the following differences:

1. The secular resonance with Jupiter (g_J) is shifted slightly toward the outer border of the HZ so that the position of Venus (V) is no longer perturbed. But it is clearly visible that in this system, the eccentricity of the Earth was increased nearly up to 0.3, so that the Earth is no longer in the HZ during its whole revolution. Certainly, it is not clear yet whether a habitable planet has to be inside the HZ all the time. Williams and Pollard (2002) claim that eccentricities of the Earth up to 0.7 would not cancel out the habitability, but of course, there would be strong variations in the temperature. Maybe the eccentricity of 0.3, where the Earth leaves the HZ in its pericenter, would not affect the habitability of the planet.
2. Figure 3 shows that the outer region of the HZ is no longer influenced by the secular resonance with Saturn (g_S), when Saturn is placed between 10 and 10.7 AU. This frequency is shifted to a larger semimajor axis. The perturbation of the g_S resonance outside the 3:1 MMR is still visible (see the bold dashed line in the stripe of higher max-e at $(a_{tp}, a_S) = (1.4, 11)$).
3. If the gas giants are in 5:2 MMR, we now observe an influence on the HZ also in the max-e result. The frequency analysis of this system shows the perturbations

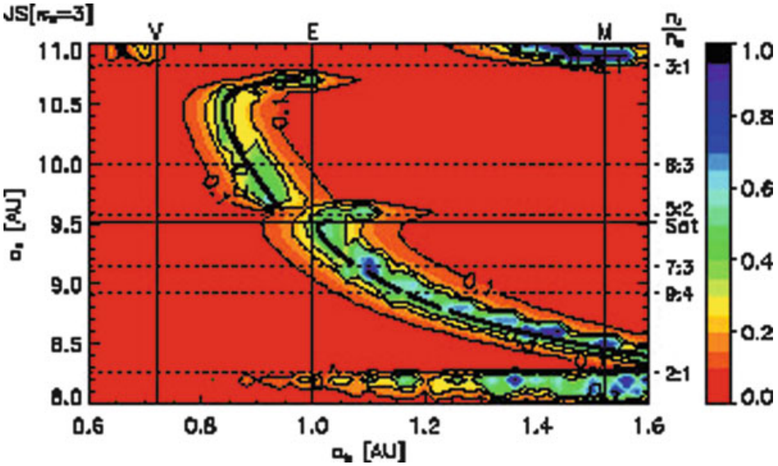

Figure 3. Max-e map for Earthlike planets in the HZ for an increased Saturn mass which is labeled by ($k_S = 3$). The color scale is the same as in Fig. 2.

of additional MMRs like the 7:3, 9:4, and 11:5 ones due to the gaps in the bold black line.

4. A stronger effect of the 2:1 resonance on the HZ can also be observed. Its influence reaches inside the Earth's orbit (see the horizontal stripe of higher eccentricity at $a_S \sim 8.1$ AU).

6. Systems with Three Giant Planets

The influence of Jupiter, Saturn, and Uranus on test planets moving in the region from $a_{tp} = 0.6$–1.6 AU for different semimajor axes of Saturn ($a_S = 8$–11 AU) is summarized in Fig. 4. A comparison of the results derived for the Jupiter–Saturn–Uranus (Fig. 4) and Jupiter–Saturn (Fig. 2) systems shows more or less the same dynamical behavior. So that no significant influence of Uranus on the HZ was detected within 10^7 years, when using the standard values of Uranus' mass (m_U) and semimajor axis (a_U). At the position of Venus (vertical full line labeled with "V"), we recognize again a maximum eccentricity of nearly 0.3 for a test planet placed in this region when Saturn moves in its actual orbit (see the horizontal full line marked with "Sat"). As shown in Pilat-Lohinger et al. (2008b), we have to include the Earth in our dynamical model to decrease the eccentricity in the area of Venus significantly.

In the max-e plot for $\kappa_S = 3$ (i.e., Jupiter and Saturn have nearly equal masses), we observe like in Fig. 3 a shift of the dominant secular structure toward the outer border in the HZ (see Fig. 5). This moves the position of Venus in the low-eccentricity region, while test planets around 1 AU enter in the area influenced by the g_J frequency, which induces higher eccentricities. This behavior near the Earth's orbit

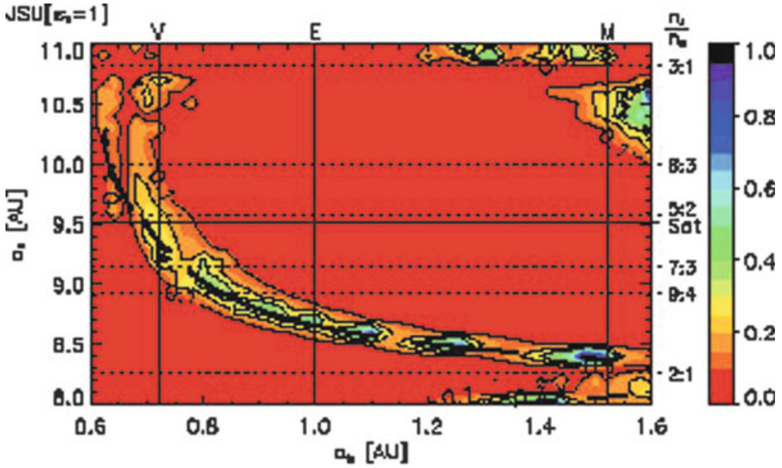

Figure 4. Max-e map for test planets in the HZ calculated in the Sun–Jupiter–Saturn–Uranus system. The panel shows the max-e result of the computations over 10^7 years. The color scale of the max-e is like in Fig. 2. The solution of the frequency analysis is given by black bold lines. Further details are given in Fig. 2.

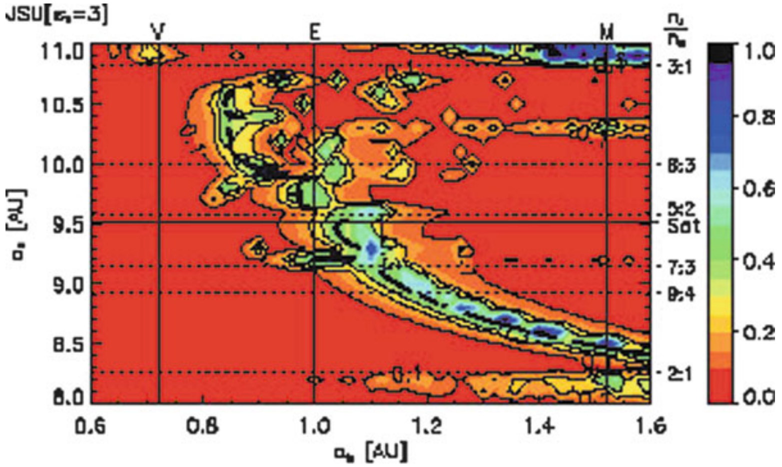

Figure 5. Same max-e map as Fig. 4 but with a higher Saturn mass—increased by a factor 3 (labeled by $[\kappa_S = 3]$).

(see the vertical line labeled by "E" in Fig. 5) can be observed for a_S from 9.15 to 10.3 AU. It follows that a more massive Saturn placed at its actual semimajor axis (labeled by the horizontal solid line at 9.53 AU) would cause a higher eccentricity for the Earth, as already found in the Jupiter–Saturn configuration when $\kappa_S = 3$ (see Fig. 3). A comparison of Figs. 3 and 5 illustrates the influence of Uranus that is visible due to the additional perturbations especially in the outer HZ (see Fig. 5). It seems that mainly MMRs between Saturn and Uranus, i.e., the 3:1 MMR near 9.2 AU, the 8:3 MMR near 10 AU, and the 5:2 MMR near 10.4 AU, are responsible for these perturbations. Following the bold black line, one can see that they appear also in the solution of the numerical frequency analysis, because of the gaps and fluctuations. To obtain more information about the influence of the third giant planet on the motion in the HZ, we increased the Uranus mass by factors of 3 and 5. Of course, then the perturbations are stronger. As a result, we have found additional perturbations due to the $g = g_U$ resonance at $a_{tp} = 0.8$ AU resulting from the increased Uranus mass, but no significant perturbations due to three-body resonances. For detailed information, see Pilat-Lohinger et al., 2008b.

7. Possible Influence on the Earth's Orbit

A summary of the max-e results for a test planet moving at 1 AU in the different Jupiter–Saturn–Uranus systems (for $\kappa_S = 1$–30) is given in Fig. 6. We see the influence of the giant planets depending on their mutual distances (due to the variation of a_S) and on the mass of Saturn. The max-e in the different 3-planet systems is defined according to the color scale, where yellow areas label the regions of lowest max-e and black labels that of highest max-e. The map shows:

1. Escapes of test planets for very high Saturn masses, when Jupiter and Saturn are in 2:1 MMR (i.e., a_S near 8 AU).
2. A quite large region (i.e., yellow area) with nearly circular motion of the test planets during the whole computation time of 10^7 years.
3. A large band of higher max-e between 9.6 and 10.8 AU, indicating that the test planets might leave the HZ periodically in certain configurations. Already for the double mass of Saturn and the standard a_S, the max-e is about 0.14 for the test planet placed at Earth' position. In this case, the perihelion of the orbit would be at 0.86 AU, and, therefore, no more in the HZ according to the definition of Kasting et al. (1993).
4. A quite high max-e value was found for the test planet when using the standard masses of Jupiter, Saturn, and Uranus, and Saturn's initial position is at 8.6 or 8.7 AU. This small displacement of Saturn would change the habitability of the Earth significantly. Orbital computations have shown that the motion of the Earth would be strongly perturbed if Saturn is located at 8.7 AU instead of ~9.5 AU, since secular perturbations acting at the position of the Earth lead to a periodic increase of the eccentricity up to a value of 0.6. With such a high eccentricity, the Earth's perihelion would be at 0.4 AU (i.e., close to Mercury) and its aphelion is at 1.6 AU (i.e., outside Mars' orbit) with a period of 20.5 million years. Moreover, for nearly 1/3 of this time, the eccentricity would be larger than 0.4. This high eccentric motion would not allow the existence of the other terrestrial planets.

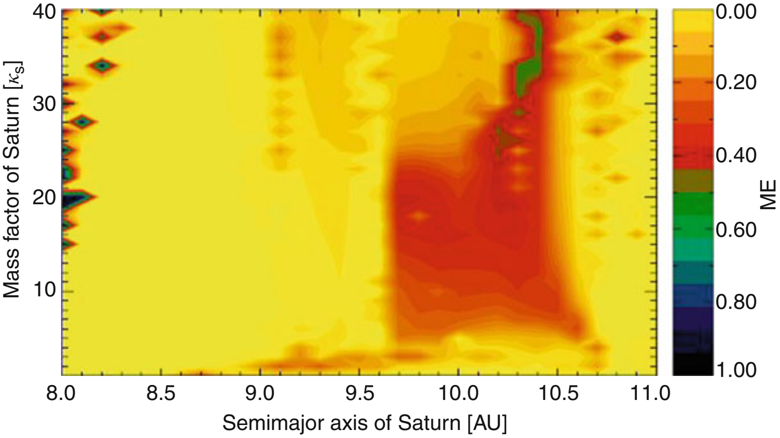

Figure 6. Max-e map for the Earth, where the x-axis labels different initial positions of Saturn and the y-axis shows different masses of Saturn. The colors (from yellow to blue) indicate the different max-e values: yellow for circular motion and blue for escapes. Along the x-axis ($y = 1$), one can see the results for the actual Jupiter–Saturn configuration, where we recognize an increase of the Earth's eccentricity if Saturn would be between 8.6 and 8.7 AU. Furthermore, the large red area shows that a more massive Saturn at positions between 9.6 and 10.5 AU would increase the Earth's eccentricity up to 0.4.

8. Conclusion

In this chapter we showed numerical investigations of solar system-like configurations, where we studied the dynamical habitability of terrestrial planets for the different systems. The fictitious system was generated by modifying Saturn's initial semimajor axis and mass. We increased the HZ and shifted the inner boundary from 0.93 to 0.6 AU and the outer boundary from 1.37 to 1.6 AU. This was done due to dynamical reasons, to be able to apply this study to the solar system. For the dynamical habitability, there are two main requirements:

1. Long-term stability of the planetary system
2. Low-eccentricity motion at least of the planet in the HZ

The numerical simulations have shown that not all system fulfill these requirements. An increase of Saturn's mass would lead to numerous configurations, where the Earth would move in a high eccentric orbit (with eccentricities of ~0.4, see the red area in Fig. 6). Very interesting is the high eccentric motion of the Earth in case of a small displacement of Saturn's orbit (from 9.5 to 8.7 AU), which leads to a maximum eccentricity of the Earth of 0.6. Then the Earth's orbit reaches from nearly Mercury's distance to outside Mars' distance and is less than 50% in the HZ. Imagine that this situation could be real if Saturn would have stopped at 8.7 AU when migrating outward during the late stage of formation. In that case, the Earth would undergo strong variations in the temperature (between 80° at perihelion and −40° at aphelion) as shown in the study by Williams and Pollard (2002). Fortunately, we are not faced with such a situation, and the Earth fulfills easily the conditions for habitability.

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Biodata of **Dirk Schulze-Makuch, Abel Méndez**, **Alberto G. Fairén, Philip von Paris, Carol Turse, Grayson Boyer, Alfonso F. Davila**, and **Marina Resendes de Sousa António** of “***A Dynamic Scheme to Assess Habitability of Exoplanets***.”

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A DYNAMIC SCHEME TO ASSESS HABITABILITY OF EXOPLANETS

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1. Introduction

As of this writing, more than 700 exoplanets have been detected, many in solar systems with multiple planets. Nearly all of the detected exoplanets are larger than Earth because large gas giants and planets close to their central star are easier to detect, but technologies currently operational or under development are hastening the discovery of smaller terrestrial planets. The Large Binocular Telescope Interferometer is currently under construction in Arizona. On Hawaii, the Keck Interferometer will combine light from the world's largest optical telescopes to enable the visualization of gas clouds, including large planets within them, around distant stars. The proportion of planets that may be smaller and more terrestrial-like is unknown, but should be answered, at least for neighboring systems in our own galaxy, within the coming decade. Missions such as the Kepler Mission and contemplated missions such as the Space Interferometry Mission and the Terrestrial Planet Finder will detect Earth-size planets and measure directly gases consistent with life, such as ozone and methane, of terrestrial-like atmospheres on planets around stars up to 50 light years away (Schulze-Makuch and Irwin, 2008). The possibility of Earth-type habitable planets in other solar systems has been modeled, and results indicate that this is a plausible scenario (e.g., planetary system of 47 UMa; Cuntz et al., 2003). Gliese 581d, a planet about 7–13 Earth masses and about twice Earth's radius, is currently the most promising candidate to be habitable (Schulze-Makuch and Guinan, 2010). The planet is at the outer edge of the habitable zone of the Gliese 581 system (Selsis et al., 2007; von Bloh et al., 2007; Wordsworth et al., 2010). If Mars would be that size in our solar system, Mars would likely still be a habitable planet in a traditional sense (stability of liquid water on its surface). The same is likely to be true for Gliese 581d. Regardless, with the new telescopes and missions on the way, we can expect the discovery of many more exoplanets, and with it the need arises for a classification scheme to assign research priorities on exoplanets based on their characteristics whether they may support life or not.

2. Observational Limits on Habitability

Given their increasingly large numbers and the diversity of possible environments, extrasolar planets are a likely candidate to provide the first evidence of life outside the Earth. Potential habitable candidates include terrestrial-size planets (Gaidos et al., 2005), super-Earths (Miller-Ricci and Seager, 2008), and exomoons (Kaltenegger, 2010). However, the assessment of the habitability of extrasolar planets is limited by our observational capabilities (Table 1). Currently, the easiest measurable parameters for extrasolar planets are their semi-mayor axis, eccentricity, inclination, period, and mass (Jones, 2008). This information alone will not be indicative of the presence or absence of life. Orbital parameters in combination with information from their parent star can be used for determining stellar habitable zone (SHZ) from orbital considerations (Kasting et al., 1993).

However, many more considerations are needed for reasonable habitability assessments at planetary scales (Table 2). For example, seasonal changes due to

Table 1. Basic planetary parameters that can be directly observed of terrestrial extrasolar planets by current or proposed space missions.

	Parameter	**Var.**	**Method**	**Missions**	**Refs.**
Orbital	Semi-major axis	a	DI, AM, RV, TP, GM[a]	SIM Lite, Kepler, Gaia	Jones (2008)
	Eccentricity	ε	DI, AM, RV	SIM Lite, Gaia	Jones (2008)
	Orbital inclination	I	DI, TP	SIM Lite, Kepler, Gaia	Jones (2008)
	Orbital period	T_{orb}	DI, AM, RV, TP	SIM Lite, Kepler, Gaia	Jones (2008)
Physical	Mass	m	DI, AM, RV[b], GM	JWST, Gaia	Jones (2008)
	Radius	r	DI, TP	Kepler, Gaia	Jones (2008)
	Density	ρ	DI, TP	Kepler, Gaia	Jones (2008)
	Mean surface temp.	T_{surf}	DI, TP[a]	TPF, JWST, Kepler	Jones (2008)
	Ocean areas	–	DI	TPF, JWST	Williams and Gaidos (2008)
	Atmos. composition	–	DI, TP[a]	TPF, JWST	Jones (2008)
	Vegetation red-edge	–	DI	TPF, JWST	Arnold and Brewer (2008)

[a] under some conditions, [b] lower limit.

Planets detection methods are direct imaging (*DI*), astrometry (*AM*), radial velocity (*RV*), transit photometry (*TP*), and gravitational microlensing (*GM*).

Table 2. Basic planetary parameters that can be used or derived from terrestrial extrasolar planets observations for habitability assessments.

	Parameter	Variable	Astrobiological relevance
Orbital	Semi-major axis	A	Surface temperature
	Eccentricity	E	Seasonal variations
	Orbital period	T_{orb}	Seasonal variations
Physical	Mass	M	Surface pressure
	Radius	R	Surface pressure and temperature
	Density	ρ	Composition
	Obliquity	ε	Seasonal variations
	Mean surface temperature	T_{surf}	Stability of liquid water
	Mean surface pressure	P_{surf}	Stability of liquid water
	Atmospheric composition	–	bioelements
	Land-ocean ratio	LOR	Habitat distribution/water cycle

obliquity can be complicated with high orbital eccentricities creating extreme variations in stellar flux received by the planet even beyond the SHZ (Williams and Pollard, 2002). In addition, high-ionizing or UV radiation levels from the parent star will have a long-term effect on the stability of a suitable environment for life unless the planet is protected by a magnetic field, a thick atmosphere (Smith et al., 2004), or UV absorber (Segura et al., 2003).

Space missions such as Terrestrial Planet Finder (TPF) will be able to sample the atmosphere of extrasolar terrestrial planets in great detail (Heap, 2010). The atmospheric composition of a planet can be used to assess their habitability. The combined presence of water, oxygen, methane, and carbon dioxide are suggestive of an environment appropriate for complex life. Particularly, the presence of oxygen, as indicated by ozone, and methane are detectable at low levels (Gardner et al., 2006). The sensitivity of these biomarkers is highly dependent on atmospheric temperature-pressure profiles as well as the geological evolutionary state of the planet (Kaltenegger and Jucks, 2005).

Another important limitation when assessing the habitability of extrasolar terrestrial planets is discerning surface features. They can be used to evaluate obliquity, rotation period, or even distinguish land-ocean features. Thermal variations in the annual IR light curve might be used to recognize seasonal variations due to obliquity (Gaidos and Williams, 2004). Light curves might differentiate between planets with no atmospheres and those with dense atmospheres (Mallama, 2009). Even the presence of oceans can be theoretically inferred from their glint under various orbital longitudes (Williams and Gaidos, 2008). Ocean versus land fraction, rotation period, seasonal and meteorological variability, and vegetation might be detectable with low-precision photometry (Ford et al., 2001). Spectral analysis of the characteristic vegetation spectra can also be used to identify vegetation signatures (Arnold and Brewer, 2008).

Besides instrumental limits (Sozzetti, 2005), many other factors can reduce our ability to interpret the light from extrasolar terrestrial planets. For example, the presence of dense clouds obscure surface characteristic and have an impact on the surface habitability (Kitzmann et al., 2010). In addition, a planet with satellites will show a complex light curve that will complicate the interpretation of its features (Moskovitz et al., 2009).

3. Habitability Criteria for Exoplanet Classification

It has become clear from the previous discussion on the observational limits of exoplanets with regard to habitability that any classification scheme can only be preliminary and is necessarily subject to updates based on new insights that are likely to be gained from advancing technology. Thus, any classification scheme to assess extrasolar planet habitability must be designed to be constantly updated as new insights about each exoplanet's or exomoon's parameters of planetary habitability are obtained.

However, at this point in time, we can propose a scheme to establish a comparative analysis of habitability, that is, a scheme that will allow us to quantify the habitability potential of one exoplanet or exomoon versus another by means of a habitability index. This scheme will have to be normalized to the only example of a habitable planet we know: Earth. And therefore, any other exoplanet or exomoon will have a habitability index equal or inferior to that of Earth. Because the scheme used to design the habitability index must be flexible and able to accommodate future observational capabilities, we are proposing a dynamic scheme.

As a first approximation, our dynamic scheme will be applicable to Earth-type planets; however, at least two alternative solar system models will also be considered, the Europa-type model and the Titan-type model. Though we do not know whether these moons contain life in our solar system, it is conceivable that they could or, at the very least, contain habitable conditions. The parameters of habitability for Earth-type planets include that (1) the planet must be terrestrial, the presence of (2) a significant atmosphere, (3) a magnetic field, (4) internal differentiation and plate tectonics, (5) detectable surface liquids (water if located within the habitable zone as traditionally defined), and (6) geoindicators and (7) bioindicators (including polymeric chemistry) of life.

4. Parameters of Planetary Habitability for Earth-Type Planets

While Earth-type planets are surely not the only type of planetary body on which we can envision life, it is the only type we are sure that life can exist and it is the example we are most familiar with. Thus, it is the case we will focus on our assessment:

4.1. THE PLANET OR MOON MUST BE TERRESTRIAL

As far as we know, the presence of a terrestrial planet is essential for life. Since we live on the surface of a planet with a substantial amount of subterranean biomass, we are biased toward the need for a planetary rocky surface. However, there are good reasons why we believe that these are essential requirements for life. Life needs a solid substrate and liquids to exchange nutrients with its environment (Schulze-Makuch and Irwin, 2008). A gas giant would not provide these requirements. A terrestrial planet with a rocky surface also implies an original process of planetary accretion that could have provided original compounds that are thought to be a prerequisite for the origin of life, such as volatiles and simple organic compounds. A rocky surface also provides a larger degree of stability, a higher density of chemical compounds, a substrate for chemical reactions, and protection from various types of radiation.

4.2. PRESENCE OF A SIGNIFICANT ATMOSPHERE

A significant atmosphere on the terrestrial planet or moon is required because any liquid or gaseous compounds would otherwise vaporize into the vacuum of space. In addition, an atmosphere also provides protection from various types of radiation. Additionally, atmospheres dampen the day-night and polar-equatorial temperature gradients on a planet, which is important for life to become a global phenomenon.

4.3. PRESENCE OF MAGNETIC FIELD

A magnetic field may not be essential for life, but it would provide significant protection from solar wind and other forms of radiation. It is particularly necessary for a thriving global biosphere as observed on Earth. Microbial life can be easily envisioned in an endolithic microbial form on the surface of a planet without a magnetic field or in the subsurface. Such may be the case on Mars. However, even Mars had a magnetic field early in its geological history, which probably made the planet more conducive to the origin of life and its persistence early in the history of the planet (whether life in fact originated on Mars or not). Magnetic fields also slow down or even completely stop atmospheric sputtering by the solar wind and, therefore, provide an extra degree of stability. Finally, a planetary magnetic field can also provide an adaptive advantage to microorganisms that live in specific chemical niches and require some sort of active or passive orientation mechanism to avoid dangerous or toxic environments, such as in the case of magnetotactic bacteria on Earth (Blakemore, 1975). However, the detection of a magnetic field of exoplanets will be challenging for the near future, and Energetic Neutral Atoms (ENA), very fast atoms produced by the stellar wind interaction with the exosphere of the planet, exosphere characterizations, and stellar wind pattern might reveal the first indications about the presence of a magnetic field (Ekenbäck et al., 2010).

4.4. PRESENCE OF INTERNAL DIFFERENTIATION AND PLATE TECTONICS

Life is easier to envision on any planetary body that is differentiated into a radioactive core, a mantel, and a crust. Internal differentiation is a sign of endogenic activity that is powered by radioactive decay. The likelihood of internal differentiation, in turn, is directly related to global mass, and that can be deduced by a planetary body's influence on orbiting or passing probes and by the gravitational attraction it exerts on other planetary bodies or light (Schulze-Makuch and Irwin, 2008). Especially useful for life is plate tectonics, which is driven by the internal heat of the planet. Plate tectonics or some other effective recycling mechanism for minerals and nutrients is important for the persistence of living systems, particularly a global biosphere. Nutrients and minerals would otherwise be quickly exhausted and evolving life, especially when still in its infant stage and not well established, would not be able to meet its nutrient demands within a relatively short time frame (on a planetary time scale). On Earth and probably early Mars, the recycling mechanism has been plate tectonics (Sleep, 1994; Connerney et al., 1999; Fairén and Dohm, 2004). Plate tectonics on Earth have also constantly produced greenhouse gases that have acted as a global thermostat providing stability for the evolution of life (Ward and Brownlee, 2000). Life can certainly be envisioned without plate tectonics, such as near hydrothermal vents or other conductive environments on Mars today, but in order to "go global", plate tectonics or some other effective recycling mechanism is needed. Plate tectonics and internal differential will be challenging to detect on exoplanets. Theoretical mass-radius relationship could be employed, but coreless planets are a theoretical possibility and model results are often inconclusive. Changes in orbital parameters could also provide some indication on the internal structure of the planet due to tidal interactions with other planets or its star. Plate tectonics is likely to be identified via direct imaging only, but modeling of the atmospheric composition consistent with plate tectonics may provide some first indications about its presence.

4.5. PRESENCE OF DETECTABLE SURFACE LIQUIDS

A liquid medium appears to be a necessary (but is not a sufficient) condition for living processes because macromolecules and nutrients can be concentrated within a bounded internal environment without immobilizing interacting constituents. This assumption is usually taken to mean liquid water as a medium, though organic compounds and water mixtures with ammonia and other miscible molecules can exist in liquid form at temperatures well below the freezing point of water (such as the hydrocarbon lakes on Titan). Certainly, by extrapolating from Earth and realizing how important oceans of water are for life, we would be exhilarated to discover oceans of liquid water on the surface of an exoplanet. Much of the notion of the habitability of Mars during the Noachian period is attributed to the (at least) temporarily persistence of oceans. If the surface liquid is water, then the exoplanet in question is located within the habitable zone as traditionally defined. However, even if the exoplanet or exomoon is located in that zone, it may not have any surface liquids present (e.g., Earth's Moon).

4.6. PRESENCE OF GEOINDICATORS

We define geoindicators here as alterations of the geological environment due to life processes, which can be global in nature (Schulze-Makuch and Irwin, 2008). Examples are atmospheric gas compositions, for example, O_2 and CH_4, resulting from biogenic processes or the rocks and sediments produced by life processes such as the banded-iron formation and stromatolite deposits of early Earth. Geoindicators would also include rate and type of erosion, if biologically magnified, and even roads and canals and local aggregates (such as termite mounds) constructed by more advanced life. However, even if these structures are large in spatial extent such as the Great Barrier Reef (which can be seen by the naked eye from the Moon), enormously high spatial resolution is required, which may not be available for some time. Caution is also advised for some of these geoindicators. For example, ozone is often cited as a geoindicator of life. However, ozone can also be produced without any involvement of biology. For example, Europa is covered with a thick layer of water ice in Jupiter's strong radiation field. If a large meteorite hits the Europan surface, much of the water ice will be ejected into the atmosphere, creating temporarily a surface water ocean with a water vapor atmosphere. Jupiter's radiation would split the water molecules into oxygen and hydrogen with hydrogen escaping to space and Europa gradually building up an O_2 and ozone-rich atmosphere without any involvement of biology.

4.7. PRESENCE OF BIOINDICATORS

Even though the size of an individual organism makes its detection on an exoplanet all but impossible, organisms in aggregate alter their environment and generate signatures of their functional processes. These direct consequences of biological activity we define as biosignatures (Schulze-Makuch and Irwin, 2008). Typical examples of biosignatures would be the presence of complex organic compounds including polymeric compounds of high molecular weight of 1000 or greater. This is based on the assumption that life regardless of origin would have a macromolecular machinery and would be complex compared to the inorganic background (Schulze-Makuch and Grinspoon, 2005). Another example would be the detection of the chlorophyll molecule. Spectra that can be linked to vegetation signatures and are not explainable with naturally occurring inorganic chemical processes are strong indicators for life (Arnold and Brewer, 2008).

5. A First Attempt of a Classification Scheme

A simplified classification scheme cannot account for all the variability that we expect to see in exoplanets (or exomoons). However, there is a need to employ some kind of filter in order to categorize the thousands of exoplanetary bodies that we will surely be detecting within the next decade or two. As a first screening tool, we suggest a habitability index score to be calculated by

$$\mathrm{HI} = \mathrm{T} * \mathrm{A} * \left(\mathrm{B} + \mathrm{ID} + \mathrm{PT} + \mathrm{SL} + \mathrm{GI} + \mathrm{PC} + \mathrm{BI}\right) \tag{1}$$

where:

HI = habitability index.

T = terrestrial planet. If the exoplanet or exomoon is terrestrial, it is assigned a value of 1. If it is not terrestrial, it is assigned a value of 0.25.

A = atmosphere. If the exoplanet or exomoon has a significant atmosphere, it is assigned a value of 1. If it lacks an atmosphere, it is assigned a value of 0.25. The exception are ice-covered planets or moons (which could have a subsurface ocean), which are assigned a value of 0.75

B = magnetic field. If the exoplanet has a measurable magnetic field, it is assigned a value of 0.1, otherwise 0.

ID = internal differentiation. If the exoplanet shows evidence of internal differentiation, it is assigned a value of 0.1, otherwise 0.

PT = plate tectonics. If the exoplanet shows evidence of plate tectonics, it is assigned a value of 0.1, otherwise 0.

SL = surface liquids. If surface water is detected on the exoplanet, it is assigned a value of 0.2. If any other type of liquid is detected, it is assigned a value of 0.1. If surface liquids can reasonably be assumed to be present on the exoplanet (e.g., within the habitable zone in a water-rich solar system) or reasonably be inferred to be present below the surface (Europa-type), or have been present in the past, a value of 0.05 is assigned.

GI = geoindicator. If a geoindicator can be detected on an exoplanet, it is assigned a value of 0.2.

PC = polymeric chemistry. If polymeric chemistry can be detected on the exoplanet, it is assigned a value of 0.1

BI = bioindicator. If a bioindicator can be detected on the exoplanet, it is assigned a value of 0.2.

The habitability index is set up that it falls between 0 and 1. It is designed that it can be updated as more data become available (an exoplanet for which we have no data has a HI score of 0 as default). The relative weight of each factor is established according to our current thinking of the driving mechanisms behind planetary habitability. This is based on the empirical observation that all life on Earth requires liquid water. The presence of liquid water or any other type of liquid alone does not necessarily imply life, but it would arguably place the exoplanet or exomoon high in the priority list, and therefore, it scores double than the rest of factors, excluding bio- and geoindicators. As more factors are introduced in the HI, it might be necessary to reevaluate the relative weight of each factor.

Also, the HI is clearly biased toward exoplanets which are closer and of which we have more information. As our technologies advance further, more exoplanets and exomoons will likely receive higher ranking and with it higher priorities to take a closer look toward habitability. However, this makes sense from a practical standpoint as we can only venture out to focus on a planet in more detail if we have indeed much available information to analyze.

As an example of how this system works, let us calculate the HI for some planetary bodies in our solar system:

$$\begin{aligned}
\mathrm{HI}_{\mathrm{Earth}} &= 1*1\big(0.1+0.1+0.1+0.2+0.2+0.1+0.2\big) = 1\\
\mathrm{HI}_{\mathrm{Europa}} &= 1*0.75\big(0.1+0.1+0.05(\text{unclear})+0.1+0+0+0\big) = 0.26\\
\mathrm{HI}_{\mathrm{Titan}} &= 1*1\big(0+0.1+0+0.1+0+0.1+0\big) = 0.3\\
\mathrm{HI}_{\mathrm{Jupiter}} &= 0.25*1\big(0.1+0.1+0+0+0+0.1+0\big) = 0.075
\end{aligned}$$

The situation for Mars, for example, depends whether the observations would be made today or 4 G.a. ago during the Noachian:

$$\begin{aligned}
\mathrm{HI}_{\text{Mars - today}} &= 1*1\big(0+0.1+0+0.05\,(\text{erosion features})+0+0+0\big) = 0.15\\
\mathrm{HI}_{\text{Mars - 4Ga ago}} &= 1*1\big(0.1+0.1+0.1+0.2+?+?+?\big) \square\ 0.5
\end{aligned}$$

The example for Mars is instructive. Since we can only obtain a short window of time in our exoplanet search, we lack information about the history of the planet. If we would not be aware of the history of Mars, the red planet would not be a high-priority target for astrobiology. This has to be kept in mind for assessing the HI for exoplanets and exomoons. Also, it is arguable whether Mars should be assigned a value of 1 for possessing a significant atmosphere, since the Martian atmosphere today is very thin and provides only a limited protection from radiation and desiccation. Perhaps, a value of 0.5 or 0.75 would be more sensible for an atmosphere that is thinner than Earth's atmosphere, but is still thick enough to provide some protection from space. The point has to be made again that the suggested habitability index (HI) here only provides a preliminary assessment and should be refined as more information of exoplanets becomes available.

6. Conclusions

The habitability index (HI), as proposed here, is a first attempt to prioritize the many exoplanets and exomoons that will be discovered in the near future in regard to their potential of harboring life. The HI is clearly biased toward exoplanetary bodies of which we have more information as the default value of each unknown exoplanet is 0. As technology improves, the scores of many exoplanets and exomoons will likely go up, and help us to focus our research efforts on worlds which likely host life.

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ASTROBIOLOGY OF TITAN

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1. Introduction

Mars, Europa, and Titan—these solar system bodies have a great exobiological significance. Here, we will focus on Titan, the largest satellite of Saturn, which has a dense atmosphere composed primarily of N_2 with about 5% CH_4 and a large number of minor constituents such as carbon monoxide, carbon dioxide, ethane, ethylene, acetylene, cyanoacetylene, hydrogen cyanide, benzene, and many others. An internal water ocean could exist below the surface crust of water ice. A 100-km-deep ocean considered in the recent model is buried below several tens of kilometers of ice (Lorenz et al., 2008). The temperature of such ocean corresponds to the temperature of water at its maximum density (4°C). The main requirements needed for exobiology are liquid water which exists within long geological period, complex organic and inorganic chemistry, and energy sources for support of biological processes. And all of these are on Titan.

2. Atmospheric Chemistry

Titan's atmosphere is a mystery to this day. The question is as follows: why does Titan have an atmosphere, while other large satellites (and planets for that matter, like more massive Mars) do not, and where does this atmosphere come from (Coustenis, 2005)? The second important question is the origin of nitrogen in Titan's atmosphere. Nitrogen is an essential element for life. Almost all biologically important molecules, such as amino acids, nucleic acids, and porphyrins, contain it in their structure. Nitrogen is among the most abundant elements in the cosmos. The question is the form of initial nitrogen in the composition of Titan, whether it was delivered in the form of N_2 or NH_3. The scientists led to the conclusion that the dominant carrier for nitrogen on Titan was rather NH_3 and other volatiles that can be trapped efficiently at higher temperatures (Owen, 2000). The ammonia was possibly incorporated in the form of ammonia hydrate in icy planetesimals accreted by Titan and in turn could have been derived from interstellar ices (Mousis et al., 2009). Subsequent photolysis of NH_3 on early Titan could easily produce the present amount of N_2 (Atreya et al., 1978). The other plausible scenario was offered by McKay et al. (1988). They suggest that the source of N_2 may be from shock formation due to impacts during the last stages of accretion.

Even though Titan receives only 1% of the solar ultraviolet flux that Earth does and is bombarded by charged particles from Saturn's magnetosphere, this energy is sufficient for photochemistry to proceed efficiently (Yung et al., 1984). Titan has no magnetic field, and it is directly exposed to the solar wind. The complex gas-phase organic chemistry induced by corona discharges (Ramirez et al., 2001) or lightning (Lammer et al., 2001) also is expected into dense nitrogen–methane atmosphere.

Reactions occurring in Titan's atmosphere produce a wide range of nitrogen-bearing organic molecules (Waite et al.*,* 2005; Vuitton et al.*,* 2007), which subsequently rain down onto the satellite's surface. The Cassini spacecraft has found evidence for organic molecules up to its mass limit of 100 at altitudes up to 1,200 km. Ammonia (NH_3) and hydrogen cyanide (HCN) have been identified as the main products *in situ* chemical analysis of Titan's aerosols by pyrolysis at 600°C (Israel et al., 2005). The potential presence of nitrile (−CN) and/or isonitrile (−NC) groups, amino ($-NH_2$, –NH– and –N<), and imino groups (−C=N–) in the refractory complex organics of the aerosol nucleus is inferred from the chemical nature of the compounds produced by pyrolysis. The presence of NH_3 in the pyrolysis products provides information about the general class of aerosols in Titan's atmosphere, including the presence of the NH_2 chemical group, which should be the major contributor to NH_3 released by pyrolysis. The presence of HCN is sign of polymeric species that include nitrile CN chemical groups or structural CN bonds. The presence of both NH_3 and HCN demonstrates that nitrogen can be incorporated into Titan's aerosol in different ways, and the general presence of nitrogen in the aerosol suggests that the aerosol acts as an important sink for atmospheric nitrogen.

The presence of ^{40}Ar in Titan's atmosphere suggests relatively late formation of at least part of Titan's atmosphere by cryovolcanic degassing of the ice-rock interior (Tobie et al., 2006). The interior was warm enough in the past for the water ocean to be enriched in potassium through interactions with the rocky core (Engel et al., 1994). Argon could then have ended up in the atmosphere if potassium-enriched water cryomagmas were directly deposited on the surface or, more likely, if it was outgassed simultaneously with methane.

Several observations suggest that the atmosphere, or at least part of it, is not primordial but has been outgassed during the last tens or hundreds of millions years. So there are some links between atmosphere and internal volume; as mentioned earlier, the presence of ^{40}Ar is produced by the decay of ^{40}K. The carbon isotopic ratio $^{13}C/^{12}C$ in hydrocarbons is close to the terrestrial value. This is consistent with the idea that hydrocarbons have been recently released from the interior (Tobie et al., 2006).

The main attention to Titan from astrobiological point of view was paid to its similarity with early Earth, especially its atmosphere, though at a much lower temperature. Titan provides an interesting model for some ideas about abiogenic synthesis of organic compounds on primitive planets. The N_2–CH_4 atmosphere appears to be one of the most interesting ones for prebiotic chemistry. It allows the formation of a wide variety of organics including compounds that are supposed to have played a key role in terrestrial prebiotic chemistry, such as hydrogen cyanide, cyanoacetylene, and cyanogen.

But tremendous importance for astrobiology has the presence of an internal water ocean on Titan, considered very likely from the Cassini radar observations.

3. Surface of the Satellite

3.1. LANDSCAPES

The surface of Titan has been described as "complex, fluid-processed, and geologically young." The Cassini–Huygens mission has revealed a remarkable diversity of Titan's icy landscapes, such as branching riverbeds, liquid methane and/or ethane pooled in polar lakes, and wind-blown equatorial dune fields of complex organic material settled from high altitude (Jaumann et al., 2009). The domes, flow-like features, and mountains present on Titan are the surface manifestation of internal processes active in the outer ice-I shell. We can see that the ice layer covered by a layer of unknown materials is likely to be a mixture of organics and ices. The organic compounds are shellacking over the water ice that makes up Titan's bedrock with a film of hydrocarbons at least a few millimeters to centimeters thick but possibly much deeper in some places. Mass spectra taken by the Huygen's probe after landing firmly identified the presence of methane and ethane on the surface and tentatively identified cyanogens (C_2N_2), benzene, and CO_2 (Niemann et al., 2005).

The surface of Titan is globally young in comparison with the surfaces of Rhea, Tethys, Dione, and other small Saturn's satellites which surfaces are scarred by impact craters. The Cassini radar images covered ~22% of the Titan's surface show only 50 craters which could be really impact craters (Wood et al., 2010). The images show that dunes embay, cross, and bury a number of craters, and another is detectable only because its rim rises above the lake that surrounds it. A number of craters are crossed by channels, probably cut by flowing liquids. The youthful age of Titan's surface overall, and the various erosional states of its likely impact craters, demonstrate that dynamic processes have destroyed most of the early history of the moon and that multiple processes continue to strongly modify its surface.

Near-infrared images of Titan obtained by the Cassini spacecraft show that a widespread methane ocean does not exist on the surface as was proposed earlier, but radar data strongly supported the possibility of smaller seas and lakes, mainly in the colder northern regions. Titan's lakes are probably a liquid ethane–methane mixture together with dissolved nitrogen. Also dissolved in them will be a variety of solutes, mainly organic compounds produced in the atmosphere and rained down in aerosol particles consisting of a nucleus of macromolecular materials coated with volatile hydrocarbons and nitriles (Israel et al., 2005). Such solutes will be much more concentrated in the lakes than in the atmosphere. Despite the low temperatures, the action of high-energy cosmic rays reaching the satellite's surface may produce additional organic compounds in this exotic chemical reactor.

There are some speculations on possible life in such environments (McKay and Smith, 2005; Schulze-Makuch and Grinspoon, 2005), especially in view of lacking of acetylene on the Titan surface (Clark et al., 2010). Scientists had expected the Sun's interactions with chemicals in the atmosphere to produce acetylene that falls down to coat the Titan surface. But Cassini detected no acetylene on the surface. The acetylene has been proposed by McKay and Smith (2005) as the best energy source for a methane-based life on Titan.

Cassini–Huygens observations have shown that Titan is a geologically active satellite. On average, Titan's surface is too cold for liquid water, but melting caused by meteoritic impacts and/or cryovolcanism may lead to its episodic availability.

3.2. CRYOVOLCANISM

Possible cryovolcanic features have been identified on several icy bodies of the solar system, including Europa, Ganymede, Enceladus, Triton, Miranda, and Ariel. Theoretical calculations suggest a heat flow at present about 8% that of the Earth, sufficient to mobilize water as liquid in the Titan's interior as the working fluid for cryovolcanism (Tobie et al., 2006). Observations of Titan by the Cassini spacecraft also suggest the presence of cryovolcanism on the surface. Where salty water reaches the surface and are possible locations for oxygen inserting chemistry along with impact craters. Such places as *Tortola Facula*, *Tui Regio*, and northern *Fensa* are discussed as candidate sites for cryovolcanic activity. There were found many bright circular features which could be interpreted as a cryovolcanic dome in an area dominated by extension (Sotin et al., 2005).

One of the first features imaged by Cassini, *Ganesa Macula*, resembles the geographic features called "pancake domes" found on Venus. Visual and infrared mapping spectrometers onboard of Cassini spacecraft show an unusual bright spot on Titan's surface. The feature is spectacularly bright at 5-μm wavelengths. The combination of VIMS and ISS images of this bright red patch shows a significant contrast between this oval area and the surrounding landscape. This 483-km-wide region may be a mixture of warm water ice and ammonia from the interior delivered by an ice volcano.

Two transient but unusually long-lived "bright spots" in Titan's atmosphere, which appear too persistent to be explained by mere weather patterns, have been discovered and attributed as the result of extended cryovolcanic episodes. Another circular feature thought to be an icy volcano is located in the dark area where the Huygens probe landed, not so far from the landing site. This is a dome, roughly 30 km wide, formed by upwelling icy plumes that release a mixture of methane, water ice, and ammonia onto the surface through lava flows, rapidly freezing. One can clearly observe a round shape with elongated wings extending westward. Those wings might be the sign of lava flows. The structure resembles volcanoes on Earth and Venus, with overlapping layers of material from a series of flows. In the center of the dome, a dark spot is clearly visible. It appears to be a caldera, a bowl-shaped structure formed above chambers of molten material.

The structures resembling lava flows were announced in a region of Titan called *Hotei Arcus*, which appears to fluctuate in brightness over several months (Nelson et al., 2009). Nelson et al. (2009), report that between October 2005 and January 2006, a region near the highland region of *Hotei Arcus* became much darker at infrared wavelengths. This change was still apparent after another pass in March 2006, suggesting that it was due to a permanent alteration of the surface rather than a transient effect, like low-lying clouds or fog. Though many phenomena were suggested to explain this fluctuation, the lava flows were found to rise 200 m above Titan's surface, consistent with it having been erupted from beneath the surface. There are changes in brightness and morphology that have occurred along time. At times when it appears highly reflective, it exhibits a spectral signature consistent with ammonia. The presence of NH_3 has been offered as evidence supporting cryovolcanic activity. Wall et al. (2009) have recently reported that Cassini radar images of *Hotei Arcus* where VIMS reports brightness changes are found in SAR images to exhibit lobate morphology consistent with cryovolcanic flows. The area seems to consist of a dark plain topped by brighter areas with lobate, or curved, boundaries with complex overlapping relationships. They also appear to cover channels running down from the highlands in the bottom right of the image. Based on the morphology and their apparent youth, Wall et al. (2009) propose that the bright units are recently erupted cryogenic lava flows.

The lack of obvious impact features in high-resolution Cassini imagery and inferred youth of the surface imply the possibility of active resurfacing and possible burial or subduction mechanisms that could supply subsurface liquid reservoirs with acetylene and other photochemical products. If volcanic processes are indeed emplacing new cryomagmatic material to the surface of Titan, such activity might be detected by recognizing their signatures, in particular, thermal anomalies on the surface and/or in the atmosphere, a unique morphology that is characteristic of the volcanic processes or has changed, or spectroscopic features that identify likely cryolava compositions.

Many people think that for the methane and other hydrocarbons in Titan's atmosphere to persist over long timescales, they must be replenished by eruptions of volatiles from Titan's mushy interior. The cryovolcanism provides a means of exposing Titan's organics to liquid water, transforming, for example, nitriles into more evolved and oxidized prebiotic species including amino acids.

3.3. LIQUID WATER POOLS ON THE SURFACE AFTER METEORITIC STRIKES

The strong meteoritic collisions with planetary surface could temporary melt a large amount of surrounding ice excavating a crater filled with a molten mass. These impacts could also serve a source of O-bearing species, such as H_2O (from molted ice) and CO_2 (from surface veneer, which could be, possibly, tens of meters thick). All molecules of biochemical interest (amino, nucleic and fatty acids, sugars) contain some oxygen. The sole way to insert oxygen into Titan's hydrocarbons, nitriles, and tholins is their hydrolysis. The hydrolysis of some types of Titan's tholins releases amino acids (Khare et al., 1986) with yields about 1%. Four amino acids and some nucleic acid bases were identified in a tholin sample that had been hydrolyzed in a 13 wt% ammonia-water solution at 253 and 293 K for 1 year (Neish et al., 2010). Nucleic acid bases also could be synthesized in such conditions (Levy et al., 2000). So, we can see that low temperatures did not prevent the hydrolysis; Alkynes are expected to be hydrolyzed to aldehydes and ketones, and alkenes to alcohols. The complex set of chemical reactions such as HCN addition, ammonolysis, nitrile oligomerization, and aldehyde–nitrile reactions are all thought to occur, plausibly also generating amino acids and nucleic bases (Thompson and Sagan, 1992).

The crater with diameter about 150 km will have 10 km depth of excavation and contain approximately 5–10% melt by volume. Thompson and Sagan (1992) estimated a lifetime of such liquid pools at about 10^4–10^6 years depending on crater's diameter and composition of water mixture. The conduction-limited cooling time of pool with volume about 1 km^3 is 10^5 years (Lunine et al., 1998). More precise estimations gave two orders of smaller lifetime of liquid environments in impact craters on Titan: 10^2–10^3 for 15-km craters and 10^3–10^4 for 150-km ones (O'Brien et al., 2005). And this time is still significant for chemical evolution, and the resulting aqueous organic phase may remain liquid for hundreds of years or longer, enough time for the synthesis of simple precursor molecules to the origin of life. Besides, we have to consider not only crater's melt but also a thin sheet of liquid water in the ejecta that has significantly larger surface coverage, even though lesser time of existing. O'Brien et al. (2005) have found that about 4% of the surface will have experienced the formation of a crater larger than 15 km in diameter over the last 3.5 Gyr. Craters larger than 15 km are formed about every 2 Myr, and craters larger than 150 km form about every 200 Myr.

These aqueous environments could serve as a connection between complex atmospheric chemistry and internal water one. All minor atmospheric constituents exceed their saturation vapor pressure condense and fall on the surface. The photochemical processes could supply about 0.25 m of solid aerosol on the surface every 20 Myr (Thompson and Sagan, 1992). The periods of existing for such large liquid bodies are long enough for different processes of chemical evolution. The melted water has to contain complex organic compounds derived from atmosphere, both unpolar (hydrocarbons) and polar (nitriles). Nitriles deposited as condensate on the surface can react with water to form exobiologically interesting compounds. For example, Ferris et al. (1978) show that moderately concentrated HCN solutions can hydrolyze to form oligomers that in turn yield amino acids and pyrimidines. These mixtures of unpolar, polar, and amphiphilic molecules could form more complex structures, such as micelles, inverted micelles, and other interphase surfaces. Monolayers formed at hydrocarbon–water interfaces could be a starting point on the route of the evolution of membrane structures which could result in the formation of double-layered membranes similar to biological ones. The origin of semipermeable membranes was an important step of chemical evolution. Such membranes could be elaborated from the more simple mono- and bilayered or inorganic ones. Amphiphilic molecules aggregate at hydrocarbon–water interphases and form the first simple membrane structures which have the liquid medium inside. The complex aqueous prebiotic chemistry could take place in such environments surely (Lorenz et al., 2001). An internal volume of micelles gives conditions of diminished water activity which may enhance the reactions of polymerization. An aqueous chemistry of a cometary material combined with mineral grains from a chondritic meteorite produces amino acids via the Strecker-type mechanism, through reaction of aldehydes with ammonium and HCN (Lerner et al., 1993).

During of the meteoritic impact into ice, a large amount of organic could be formed from the water ice, methane, and ammonia due to an electrostatic energy. Piezoelectric effect into ice could store energy as electrostatic charges over an extended area of ice after a meteoritic impact. Large currents flowing in the ice could form organics by similar to Miller synthesis (Borucki and Khare, 2001) and serve for the electrolysis of the ice with evaluating of oxygen and hydrogen mixture. Impact of cometary or meteoritic bodies should also alter the organic material through shock chemistry due to pressure and heat up to polymerization of amino acids, resulted in the formation of the peptide bond. The experimental investigation has shown the forming of dipeptides and diketopiperazines (cyclic dipeptides) under high-speed shocks from aqueous solution of amino acids (Blank et al., 2001). The diketopiperazines could be a starting point for the further synthesis of oligopeptides. After freezing, the complex structures could be intact into ice. The structures that do become embedded in the ice would have a good chance of being thawed and liberated later under the next melt events both meteoritic strike or internal liquid volcanism. So, there can be a cyclic evolution of complex prebiological structures in the upper ice layer, not only in the lower one.

Strong enough meteoritic impact could initiate a huge explosion of the clathrate hydrates containing electrolysis products, H_2 and O_2 inside icy shell forming internal liquid envelope (Drobyshevski, 2002). An electrostatic charge generated during a meteoritic impact into ice could serve as detonator. Such internal liquid “bubble” could have a huge mass, moderate temperature, and long lifetime.

Solid-state convection and endogenic resurfacing could supply organic molecules form organic frost surface to internal ocean for using by putative organisms after hydrolysis. Laboratory tholins react rapidly in 13 wt% ammonia water at low temperature, producing complex organic molecules containing both oxygen and altered nitrogen functional groups (Neish et al., 2009).

3.4. MACRO-, MINI-, AND MICROCAVES IN THE ICY LAYER

Subsurface cavities, both with external entrance and closed systems (plutonism), connected with different geological processes could provide a huge array of habitats. Lorenz and Lunine (1996) found that a karst landscape could be developed involving subterranean caverns and passages.

Caves could be formed through such processes as solid surface cracks, impact cratering, hydrocarbon liquid weathering, tidal forces, and volcanism. The gravity force could produce vertical bending fractures in the lower layer of the ice. Such cracks could have spacing of hundred of meters and form a closely packed fractured system.

The liquid hydrocarbons from the surface could soak into the caves connected with cryovolcanism and form complex interphase systems mixing with the cryolava. Here, we can propose a new kind of prebiological chemistry—inverted interphase catalysis—micelles existing in hydrocarbon fluids, which is expected to be very rich in organic solutes and having an aqueous solution of minerals and small organics inside. Amphiphilic molecules derived from atmospheric photochemistry aggregate at hydrocarbon–water interphases and layered membranes are easily formed. These membranes could form micelles and “microvesicles.” These structures both have an ability to concentrate molecules and have membrane-like components which are able to use membrane potential and/or ion gradients for the protometabolism. The size, form, and composition of these particles could be very close to single-celled organisms such as bacteria or nanobacteria, and similar vesicles could have been the precursors of the living systems on Titan. Large populations of these micelles would have provided an environment for the concentration of prebiotic molecules and for their chemical transformation after concentrating through exposure to the fluctuating fields of temperature, freezing–thawing cycle, and other internal factors.

Coagulation and division of the particles could have resulted in an increased diversity of molecular species and early mechanism for reproduction and replication of successful molecular populations. The main task of these systems is the sufficient concentrations of prebiotic molecules for future stages of the chemical evolution, particularly, polymerization. Caves, subsurface fissures, and microcracks all could provide homes for microbial life. The subsurface offers one of the best of all possible sites for exobiological activity when the environments could be the less hazard then on the surface. The caves could also preserve of the possible biosignatures owing to relatively stable environments over long geological periods. These caves also could provide a more easy access to the internal part of the upper ice crust.

3.5. CRACKS IN THE ICY LAYER

Cracks in the icy crust caused by tidal forces have been proposed for Europa (Greenberg et al., 2000). Thick ice shell can result in the development of large extensional stresses and fractures (Nimmo, 2004); in a cold, high-viscosity ice shell, fractures may penetrate to significant depths and form pathways for volatile migration. Such features could exist on Titan also and serve as the mechanism for transporting and mixing substances vertically and connection to the complex atmospheric chemistry. Acting as a pump, these cracks could deliver complex organic substances from Titan's atmosphere to its subsurface ocean on daily basis. There, it can be assumed the habitable niches in the ice crust periodically bathed in liquid water. The heat could be generated within the active cracks. As a result of tides, liquid water regularly bathed crustal cracks and surfaces with heat, transporting and mixing substances from the oceanic reservoir to surface and back. Within a given crack, any organisms would be expected to occupy a range of depths, with individuals either moving with the flow or attaching themselves to the crack wall for as the wall remained solid. Here, the organisms could exploit the range of conditions and available substances using chemical and energetic flows. The biological communities could be frozen into icy walls of the crack and be inactive through most of time depending on the orbital period and long-term evolution of Titan's orbit. Under action of upwelling flow, they thaw out for a brief period of activity and could produce microhabitats for other species. Open pools of liquid could be formed at time of opening of crack and served as driving force of aqueous organic alteration of the surface compounds which are products of the complex atmospheric chemistry. Regular basis of these events could serve as biological clocks for possible ecosystems delivering nutrients and warmth for them. Individual cracks may remain active for at least tens of thousands of years (Greenberg et al., 2000). The brine-filled cracks in Earth's sea ice (Bowman et al., 1997) or in the lithosphere (Bischoff and Rosenbauer, 1989) provide a suitable habitat for microorganisms, and these types of environments may offer close parallels with the cracks in the Titan's ice shell.

The heat produced in the interior of Titan by tidal and radiogenic decay can be transferred through the ice crust by conduction or by thermal convection. Under appropriate conditions, modest variations in the heat flux from the interior can force an ice-I shell to switch between a conductive and a convective state (Mitri and Showman, 2008). Thermally induced solid-state convection within ice shell could also circulate subsurface material between the ocean and the surface and provide a significant amount of biogenic compound to sustain an internal biosphere. Thermal fluctuations (heating from hydrothermal vents on bottom of the ocean maintained by tidal dissipation in the rocky interior) or differences in the ice composition (e.g., large amount of salts or organics) and water temperatures could raise an ice temperature on a fixed area and cause in diapirs. A diapir is a mass of low-density material rising buoyantly and distorting other surface as it pushes from underneath. Episodic oceanic plumes could rise to the base of the ice shell from magmatically heated regions of the ocean floor acting as a driving force for the diapir. The material in these masses can extrude onto the surface and partially melt it. The chaotic terrain on the surface of Europa is a good example of this process (Pappalardo et al., 1999). Such convection associated with diapirs might serve to mobilize and transport biological materials and organisms.

Several of the features on the images acquired by Cassini spacecraft show zones of extension which are thought to result from extension and resurfacing associated with upwelling. If the lineaments on Titan are related to upwelling "hot ice" and contain contaminants such as hydrocarbons that vaporize as they get closer to the surface, mechanisms similar to those operating for silicate volcanism may produce flows of differentiated ices on Titan that would not be H_2O-based. If tidal heating is focused in low-viscosity domains (hot ice in the upwelling plumes) as has been hypothesized for Europa, this energy may help ice melting and gaseous release in the atmosphere (Sotin et al., 2005).

4. Internal Water Ocean

Since water ice is one of the major constituents in the outer solar system, the most models of condensation and icy satellite formation predicted that incorporation of abundant water ice and silicate rock containing radionuclides could have led to ice melting and the formation of extended liquid water mantles beneath conductive outer ice shells. The presence of reservoir of salty liquid water beneath the satellite's outer icy shell is very likely for Titan. And such ocean may contain more water than the all terrestrial ones. Spohn and Schubert (2003) have shown that even radiogenic heating in a chondritic core may suffice to keep a water ocean inside large icy satellites.

On the surface of Europa and Ganymede, we can see non-icy, sulfur-bearing, and hydrated colored materials derived from internal oceans of these satellites. So we can say that the internal ocean is not a separate object, but it has a connection with the surface. Even Enceladus, a small Saturn's satellite, indicates past or present aqueous activity in the satellite's interior (Schmidt et al., 2008).

Since Titan might have been accreted from rocky materials similar to those present on asteroids, juvenile satellite could be consisted of metal, amorphous silicate phases (mainly Fe–Ni metal, FeS, Mg- and Ca-silicates, and Ca–Al oxides), hydrated and oxidized phases and salts (phyllosilicates, magnetite, Fe–Ni sulfides, carbonates, phosphates, chlorides, and sulfates), ices, and organic compounds. It could receive a lot of organic matter incorporated as a polymer in which polyaromatic groups are linked by O-, N-, and S-bearing aliphatic units. The comet material could delivered CO_2, CO, methanol, ethane, acetylene, ammonia, H_2S, formaldehyde, and a lot of organic species again.

At the first stages of oceanic origin, when there is a contact between mineral phases and water, a lot of phyllosilicates (serpentine, cronstedtite, saponite, etc.) and tochilinite (a hydrated Fe–Ni sulfide) formed through hydration, along with magnetite and forsterite.

4.1. PHYSICOCHEMICAL CONDITIONS

The present composition of the putative liquid layers of the ice satellites is probably very complex. Heavily hydrated salt minerals, such as magnesium and sodium sulfates, sodium carbonate, and their mixtures, have been detected on Jupiter's satellites by near-infrared mapping spectrometer (NIMS) on Galileo, along with organic material like tholins bearing different functional groups, including C–H, S–H, SO_2, CO_2, and C=N (McCord et al., 1999, 2001). Mass balance calculations that modeled an extraction of the elements into the aqueous phase from chondritic material show that Titan's extensive subsurface ocean likely also contains dissolved salts from endogenic materials resembling to carbonaceous chondrite rocks incorporated into the satellite during its formation and released at the time of planetary differentiation. The presence of solutes into oceanic water is probably unavoidable in the context of water–rock interaction either early in the history of the satellite or at present time. The low- and high-temperature alteration of primitive accreted material leads to form of a complex water solution of such cations as K, Na, Mg, Ca, Mn, and Fe, and anions as SO_4^{2-}, Cl^-, Br^-, CO_3^{2-}, HCO_3^-, and others along with nitrogen compounds. Phosphorus, sulfur, micro-, and macronutrients have to be abundant into Titan's ocean through aqueous weathering of the chondrites and basalts. Oxidation led to formation of ferrous (serpentine, saponite) and ferric (magnetite, cronstedtite) minerals, sulfides (tochilinite, pyrrhotite, pentlandite), Ni-rich alloys, chromite, and phosphates. Note that Fe^{2+} and Fe^{3+} solutes formed through aqueous oxidation of Fe–Ni metal and were also incorporated into secondary silicates. Hydrogen (H_2) could have formed through mineral oxidation by water. Separation and rapid release of H_2 to space through fractures in thin icy shells would have favored further oxidation reactions. The temperature of the internal water ocean into icy satellites could have been relatively warm (Melosh et al., 2004).

So we have a good place for origin of lithoautotrophic biochemical processes from the earlier stages of the satellite's evolution on base of biologically useful electron donor–acceptor pairs. There are multiple redox disequilibria which may have supported metabolism of chemotrophic organisms, if they were present, since the oxidized species (Fe^{3+}, carbonate and sulfate) could have coexisted metastably with reduced compounds (Fe^{2+}, sulfide and native sulfur, organic matter). As we can see earlier, convection in the upper icy shell may have delivered some H_2 and CH_4 toward the surfaces and atmospheres, and vice versa.

As the source of carbon, putative microorganisms could use such C-species as methanol, HCO_3^-, CO_3^{2-}, and formate ($HCOO^-$), which forms through hydrolysis of CO and HCN, and reactions among single C-species (Seewald et al., 2006).

Nitrate accumulated in the ocean at the first stage of atmosphere's evolution would have allowed the first protobiosystems to use it as the primary source of energy (Simakov, 2000). Recently, it was a common view that the first living organism was one with an anaerobic fermentation, the simplest form of energy conversation on a substrate-level phosphorylation. The energetic reactions can ever proceed in a homogenous solution and in the absence of any supramolecular elements of cells, such as membranes. These organisms had to grow on prebiotically formed organic compounds, but the sources of these compounds were probably limited on early Titan in comparison with the abundance of nitrogen and sulfur inorganic compounds. We would like to propose the idea that the first protoliving systems in Titan's ocean could had internal energy source, namely, the chemical potential of an inorganic reaction. The all-energetic metabolism should be constituted on the base of some kind of such inorganic redox reactions—"basic reaction" (BR).

There are some candidates on the role of the BR where the electron acceptors (NO_3^-, SO_4^{2-}, Fe^{3+}, Mn^{4+}, or CO_2) have to be coupled with the electron donors. Electron donors that may be important in such process include H_2, CO, CH_4, Fe^{2+}, Mn^{2+}, pyrite, sulfur compounds, and organic material. Four energetic full-operative biogeochemical cycles are possible inside Titan's ocean, namely, nitrogen (N-cycle), sulfur (S-cycle), iron (Fe-cycle), and carbon (C-cycle), and all of them could be connected each with other (Simakov, 2004). The BR of nitrate reduction to dinitrogen is a more thermodynamically favorable in the row of different inorganic substrates (Gaidos et al., 1999), and all gaseous nitrogen in the contemporary Titan's atmosphere can be the product of this reaction (Simakov, 2000).

Chemolithoautotrophic organisms will play an essential role in ecosystems by providing a continuous supply of organic carbon for heterotrophs.

Methanogenesis is a process on base of reduction of carbon dioxide by molecular hydrogen $4H_2 + CO_2 \ \ CH_4 + 2H_2O$, and carbon dioxide can be used as the sole carbon source. Instead, CO_2 methanogens could also use a few simple organic compounds, such as acetate, formate, methanol, and methylamines. A lot of Earth's methanogens are able to adapt to high salinity, and they are found in millions of years old permafrost sediments (Rivkina et al., 1998). Usually, hydrogen-consuming methanogenic microorganisms form the base of a food chain.

To survive in Titan's briny network, lithotrophic microbial communities require a sustainable energy source such as hydrogen. A volumetric electrolysis of dirty ice due to an action of electric currents excited by strong magnetic field of Saturn could supply the methanogens with a large quantity of H_2 (Drobyshevski, 2002). The electrolysis products H_2 and O_2 could form a solid solution (clathrate hydrates) with concentration up to ~10–15% and redistributed by solid-state thermal convection. Hydrogen will diffuse out from the icy matrix into brine drainage network, and it could be consumed by the microorganisms. At high contents of the H_2 and O_2 (more 15 wt%), ice becomes capable of detonation. Such global explosions inside icy satellites could serve as driving force of the chemical evolution on these objects and form a temporal habitats inside icy layer. Others sources of hydrogen could be (1) vented gas from hydrothermal vents. Berndt with coworkers (1996) found that both H_2 and CH_4 were generated by the reaction of CO_2-bearing, NaCl aqueous fluid with common igneous mineral olivine, $(Mg, Fe)_2SiO_4$ at 300°C and 500 bars. This mineral is abundant in the chondrites, the most common type of meteorites. Other electron donors (Fe^{2+}, Mn^{2+}, and S) are constituents of both volcanic rocks and meteorites also and could be released by weathering; (2) water reactions with fresh mineral surfaces and ferrous iron; (3) a process of H_2 production at time of rocks crushing (Freund et al., 2002); or (4) molecular hydrogen produced by the radiolysis of water and ice.

Very recently, it has been found that hydrogen is flowing down through Titan's atmosphere disappears at the surface, as it could be being breathed by alien bugs. Strobel found a disparity in the hydrogen densities that leads to a flow down to the surface at a rate of about 10,000 trillion hydrogen molecules per second. This is about the same rate at which the molecules escape out of the upper atmosphere (Strobel, 2010).

Some scientists consider methanogenic Archea as one of the initial organisms from the origin of life on Earth. The gas formed by methanogens could have been recycled, for example, via anaerobic oxidation of methane, leading to substrates (inorganic carbon and sulfide) for chemosynthesis of new biomass. Fortes et al. (2007) have suggested that conversion of NH_3 into NH_4^+ could have resulted in an $(NH_4)_2SO_4$-rich early ocean on Titan. These compounds are good candidate for energy metabolism of putative Titan's biosphere, especially sulfate as a good oxidizer for BR.

A rich chemosynthetic ecosystem could be associated with methane clathrate areas on the icy bed. Clathrates are cage structures formed by H_2O ice having 8 cavities for each 46 water molecules that can trap large "guest" molecules of nonpolar methane molecules. These clathrates could supply methane into Titan's atmosphere. A free or dissolved methane represents a major microbial source of energy. The cold methane vents induced by liquid methane could serve as a source for forming of the chemosynthetic ecosystems. The examples of such systems could be found around methane clathrate on the Earth's sea bed (Boetius et al., 2000). Some organisms are capable of disproportioning methanol, methylamines, or methyl sulfides to methane and carbon dioxide, oxidizing –CH3 groups to CO2 anaerobically. Microbial consortia based on anaerobic oxidation of methane coupled to sulfate reduction can support other microbial communities by generating substantial biomass accumulation derived from methane.

It is generally accepted that the main obstacle for life in such close ecosystems as internal ocean on Titan is the absence of permanent flow of metabolic component, especially soluble oxidants (Gaidos et al., 1999). All possible redox pairs are depleted by abiotic reactions and biological activity, and without an external energy source, chemical equilibration will ultimately extinguish all life. But it is very important to reckon with such main attribute of any living organisms as adaptation. At time of food deficit, microbial community easily gears to other possible energy source. For example, in process of anaerobic respiration *Shewanella oneidensis* could reduce O_2, V(V), Cr(VI), Fe(III), Mn(IV), Tc(VII), U(VI),

NO_2^-, NO_3^-, SO_3^{2-}, $S_2O_3^{2-}$, $S_4O_6^{2-}$, fumarate, glycine, and trimethylamine-*N*-oxide (Carpentier et al., 2005). We can see a great flexibility of living matter. Besides, microorganisms have a very useful ability to change a course of energetic reaction on backhanded one. On Earth, the anaerobic oxidation of methane might proceed as the reverse of archaeal methanogenesis from carbon dioxide. So-called hypothesis of "reverse methanogenesis," namely, the proposal that archaea anaerobically oxidize methane to CO_2 by using very similar enzymes and essentially the same catalytic steps as methanogens reducing CO_2 to methane but in the reverse direction (Scheller et al., 2010). It has been shown that the key Ni-enzyme in biological methane formation from a methanogen converts methane into methyl-coenzyme M at rates and apparent Km for methane consistent with the values estimated for the anaerobic oxidation of methane by archaea. This result lends support to the hypothesis of "reverse methanogenesis" in the anaerobic oxidation of methane with sulfate as the ultimate electron acceptor. Furthermore, this is the first experimental observation of activation of the strong C–H bond (439 kJ/mol) of methane by an enzyme without oxygen-derived reactive intermediates. So we can see that living systems could squeak through chemical equilibration deadly for unliving closed systems.

4.2. HYDROTHERMAL VENTS ON THE BOTTOM OF THE OCEAN

It has been suggested that the satellite may have a metallic core and silicate mantle, and this core can be assumed to provide internal heat through radioactive decay, subjecting the ocean floor to volcanic eruption. The first heat source that is likely present within Titan's interior today is radiogenic heating due to the decay of the long-lived radiogenic elements such as ^{40}K, ^{232}Th, ^{235}U, and ^{238}U. It is worth noting that tidal heating is an important heat source within Titan too, because of this satellite's large eccentricity.

Tidal dissipation and radiogenic heat could cause substantial heating in the rocky mantle, generating volcanism at the base of the water layer, similar to suboceanic one on Earth. On large and/or early formed satellites, continuous supply and transfer of heat within the silicate mantles could have induced hydrothermal circulation, dehydration of previously formed minerals, and further alteration of organic matter. If occurred, thermal dehydration of early formed minerals (phyllosilicates, tochilinite) would have caused upward migration of hydrothermal fluids, thereby affecting ocean mass and composition. Hydrothermal activity level could be estimated on calculations of internal heating. Up to 10^{10} l per year of hydrothermal fluid may be generated at Titan's hydrothermal vents (Jakosky and Shock, 1998). Hydrothermal alteration of silicates may have leached radiogenic elements from silicate core and concentrated them in the ocean. As a result, hot aqueous fluids with inorganic and organic species induced changes in ocean compositions.

The hydrothermal fluid is a major source of reductants such as H_2S, H_2, CH_4, and Fe^{2+}. Several abiotic processes could lead to synthesis of organic compounds and supply them to the putative ocean:

1. Fischer–Tropsch type (FTT) synthesis from cooling volcanic gases (Zolotov and Shock, 2000)
2. Aqueous synthesis in mixing zones of hydrothermal fluids and with oceanic water (Shock and Schulte, 1998)
3. Aqueous FTT synthesis during initial stages of chemical interaction of oceanic water with basic/ultrabasic igneous rocks (Berndt et al*.,* 1996)

Hydrothermal reactions between the ocean and underlying rock layer should have reprocessed a portion of the ocean, along with cometary and chondritic material, into more complex organic compounds. Hydrothermal vents provide a great source of disequilibrium and metabolic energy for chemolithotrophic microorganisms. A mixing of hydrothermal fluids with seawater gives a metabolic energy potentially available from chemosynthetic reactions involving S, C, Fe, N, and Mn compounds. Theoretically, hydrothermal vents present very diverse environments for microbial life, such as black smoker chimney walls and orifices, warm vents and seeps, surface-attached microbial mats, and floating particles in vent plumes. They could supply heat and biology useful substances for possible oceanic habitats. The hydrothermal systems could support a wide spectrum of chemolithoautotrophic processes, and they have been proposed as a possible site for the origin of the first life forms on any planet.

Molecular hydrogen along with other substances such as sulfide, sulfur, and ferrous serves as important electron donors for different types of anaerobic respiration (nitrate, sulfate, sulfur, and carbon dioxide respiration). Organic matter produced by chemolithoautotrophs serves as nutrients for heterotrophs. As examples, we can see an organism with the highest growth temperature on Earth—*Pyrolobus fumarii* exhibiting an upper temperature border above 113°C (Blochl et al., 1997), and it gains energy by chemolithoautotrophic nitrate reduction, forming ammonia (NO_3^- NH_4^+). The members of other genus *Pyrodictium* gain energy by reduction of S_0 (S_0 H_2S). The capacity to reduce of an elemental sulfur to H_2S is widespread among Bacteria and Archaea as an energy-conserving reaction. Other chemolithoautotrophs are able to grow by reduction of SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, with H_2 (Stetter, 2001).

Some microorganisms could gain energy by reduction of nitrate (NO_3^- NO_2^-) with Fe^{2+} (*Ferroglobus*), S_0 (*Aquifex*), and H_2S (*Ferroglobus*) as electron donors.

Carbon monoxide is regarded as the common component of volcanic gases in deep-sea environments, and this gas could serve as a sole source of carbon and energy to a variety of diverse bacteria on Earth (Sokolova et al., 2002). In anoxic environment, CO could be oxidized with production of acetate, methane, or CO_2, and H_2 and these vast products could be used as nutrients by the others microorganisms. For example, molecular hydrogen could be used in processes of methano- or acetogenesis, sulfate, sulfur, iron, and nitrate reduction.

Hydrothermal deep-sea vent fauna on Earth is naturally exposed to a peculiar environment enriched in potentially toxic species such as heavy metals and natural radionuclides. It is now well established that some of the organisms present in such an environment accumulate metals during their lifespan. Though only few radionuclide measurements are available, it seems likely that hydrothermal vent communities are exposed to high natural radiation doses. Vent organisms are characterized by high U and Po–Pb levels compared to what is generally encountered in organisms from outside hydrothermal vent ecosystems. Though the number of data is low, the results reveal various trends in relation to the site, the location within the mixing zone, and/or the organisms' trophic regime.

Some problems could arouse from the high-pressure ice layer on the silicate core. Thicknesses of the liquid and of the high-pressure ice layers at present would depend on the internal heat production rate and on the presence of antifreeze constituents in the liquid layer. From the calculation of the thicknesses of the outer ice Ih and the internal liquid layers, it can be inferred that Titan's high-pressure ice layer is of 300–600 km to 400–700 km for 5 and 0 wt.% primordial NH_3 in the H_2O mantle, respectively (Grasset and Pargamin, 2005). Depending on the models, the pressure at the ocean bottom is ranging between 400 and 700 MPa, with 800–1,200 MPa pressure at the bottom of the high-pressure ice layer. A basic scaling thus suggests that the ice V sublayer is up to ~150–200 km thick in a pure H_2O model, while it may be less than 50 km thick in the presence of 5 wt% NH_3. The ice VI sublayer would occupy the remaining portion of the high-pressure solid layer, that is, 200–400 km, depending on the pressure of the interface and on the abundance of NH_3 or another antifreeze constituent. So, such layers will defend the silicate core from action of oceanic water. However, the interior model is largely uncertain because the interpretation of the data is still debated at present time.

5. Conclusions

The environments mentioned above indicate that all conditions capable of supporting life are possible on Titan. All requirements needed for exobiology—liquid water which exists within long geological period, complex organic and inorganic chemistry, and energy sources for support of biological processes are on Saturnian moon. Subglacial life may be widespread among such planetary bodies as Jovanian satellites, Titan, and satellites of others giant planets, detected in our Galaxy at last decade. The existence of rich atmosphere is the main difference of Titan from the Jupiter's moons. This atmosphere could supply the large quantity of different organic compounds to putative ocean. There are some possible mechanisms for extensive, intimate interaction of a liquid water ocean with the surface of the ice crust. Titan provides also insights regarding the geological and biological evolution of early Earth during ice-covered phase. There is a huge deficiency of carbon in the contemporary environment, and this missing carbon could be contained as biomass and dissolved organic carbon in the putative ocean. Possible metabolic processes, such as nitrate/nitrite reduction, sulfate reduction, and methanogenesis, could be suggested for Titan. Nitrate and sulfate could be predominant forms of N and S in the ocean, and nitrate and/or sulfate reduction would have been potential sources of energy for primitive life forms. Given the possibility that organic compounds may be widespread in the ocean from synthesis within hydrothermal systems derived from atmospheric chemistry and delivered by comets and meteorites, these putative nitrate and sulfate reducers may have been either heterotrophic or autotrophic. Furthermore, at the presence of substantial amount of methane, the methanogenesis along with methanotrophs also have been energetically favorable. Excreted products of the primary chemoautotrophic organisms could serve as a source for other types of microorganisms (heterotrophs) as it has been proposed for Europa (Gaidos et al., 1999) and Mars (Boston et al., 1992).

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THE LIKELIHOOD OF HALOPHILIC LIFE IN THE UNIVERSE

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1. Introduction

The search for extraterrestrial life has been declared as a goal for the twenty-first century by several space agencies (Foing, 2002). Potential candidates are microorganisms on or in the surfaces of moons and planets. Extremely halophilic archaea (haloarchaea) are of astrobiological interest since viable strains have been isolated from million-year-old deposits of halite (McGenity et al., 2000; Stan-Lotter et al., 1999, 2002; Fendrihan et al., 2006), suggesting the possibility of long-term survival under desiccation. Extraterrestrial halite has been identified, for example, in Martian meteorites (Treiman et al., 2000), in chloride-containing surface pools on Mars (Osterloo et al., 2008), and in the presumed salty ocean beneath the ice cover of Jupiter's moon Europa (McCord et al., 1998). These discoveries make a consideration of the potential habitats fvor halophilic life in space intriguing. Recent data on the physical occurrence of liquid saline water on Mars (Smith et al., 2009; Renno et al., 2009) have added another novel aspect to this notion, since such "cryobrines" would provide liquid phases on the Martian surface, allowing perhaps metabolic activity of halophilic microorganisms.

This chapter presents available data for halophilic microorganisms in view of their potential occurrence and survival in extraterrestrial habitats, including currently known findings of halite in outer space and some information on their resistance to radiation, desiccation, and temperature extremes. Promising methods for their detection are also discussed.

2. Halophilic Microorganisms from Ancient Salt Sediments

During the Permian and Triassic ages (280 to 192 million years ago), large salt sediments were deposited on Earth, which originated from evaporation of vast hypersaline seas (Javor, 1989). Isolation of halophilic microorganisms from these ancient rock salt deposits had been reported occasionally since the early decades of the twentieth century (for references, see Grant et al., 1998; McGenity et al., 2000); however, thorough taxonomic characterizations of such isolates were attempted only fairly recently, starting in the 1990s.

Our group isolated from alpine Permian rock salt, which was obtained from the salt mine in Bad Ischl, Austria, a haloarchaeon named *Halococcus salifodinae* (Denner et al., 1994). This was the first isolate from ancient salt sediments, which was formally classified and deposited in several international culture collections. Two further strains, which had been isolated independently by different workers, Br3 (from solution-mined brine in Cheshire, England) and BG2/2 (from a bore core from the mine of Berchtesgaden, Germany) resembled *Hc. salifodinae* BIp in some phenotypic properties, including the characteristic morphology of tetrads arranged in large clusters (Fig. 1); in addition, rock salt samples were obtained 8 years later from the same site in Bad Ischl, and several halococci were recovered from these samples, which proved to be identical to strain BIp (Stan-Lotter et al., 1999). The data suggested that viable halophilic

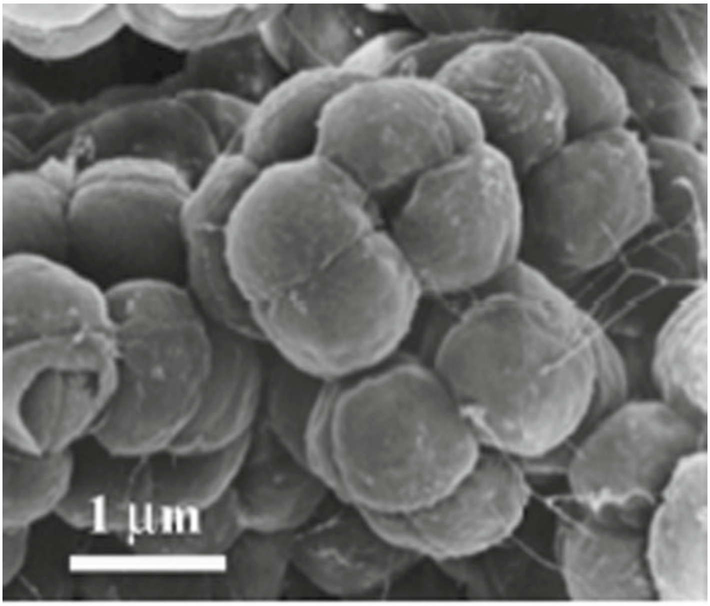

Figure 1. Scanning electron micrograph of *Halococcus salifodinae* Br3 DSM 13046 (Photograph taken by G. Wanner).

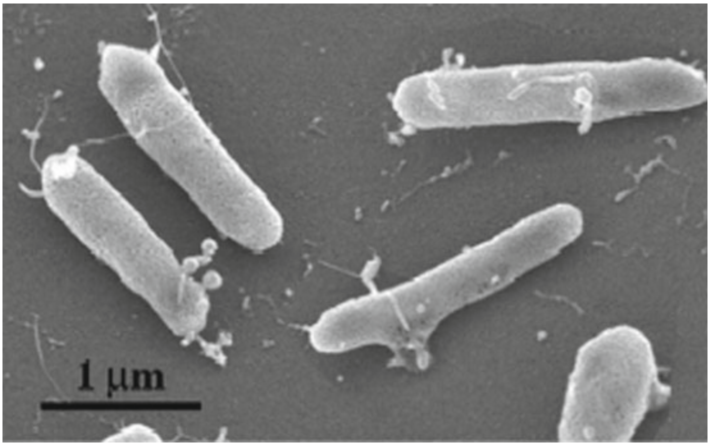

Figure 2. Scanning electron micrograph of *Halobacterium noricense* DSM15987^T (Photograph taken by C. Frethem).

archaea, which belong to the same species, occur in geographically separated evaporites of similar geological age.

A further halococcal isolate from the Bad Ischl salt formation, which differed from the previously described strains, was identified as a novel species in 2002 and named *Halococcus dombrowskii* (Stan-Lotter et al., 2002). Several non-coccoid strains were obtained from a freshly drilled bore core at the salt mine in Altaussee, Austria (about 40 km distance from Bad Ischl), which were similar in their 16S rRNA sequence to the well-described species *Halobacterium salinarum.* Taxonomic analysis, however, suggested placement into a novel species, *Halobacterium noricense* (Fig. 2; Gruber et al., 2004).

Halophilic isolates from ancient salt in other parts of the world include a single rod-shaped *Halobacterium* strain from 97,000-year-old rock salt in the USA, which was described by Mormile et al. (2003) and deemed to resemble *Hbt. salinarum* NRC-1. Vreeland et al. (2002) isolated a haloarchaeon termed *Halosimplex carlsbadense* from the Permian-aged Salado formation in New Mexico. Other isolates obtained by these workers include strains with similarities of their 16S rRNA genes to *Halobacterium* and *Natronobacterium* species (Vreeland et al., 2007); no further characterization of these strains is yet available. Recently, direct microscopic observations of fluid inclusions in a well-dated (0–100,000 years) 90-m-long salt core from the Death Valley, California, indicated the presence of prokaryotes, most likely haloarchaea and/or halobacteria (Schubert et al., 2009). Table 1 contains a list of the validly described haloarchaeal isolates from Permo-Triassic rock salt and a strain from a British salt mine.

Besides isolation of viable haloarchaeal strains, DNA was obtained from samples of ancient salt, and polymerase chain reaction (PCR) was applied. The first report about this approach was published by Radax et al. (2001), who

Table 1. Validly described haloarchaeal isolates from Permo-Triassic rock salt and salt mine brine. T denotes type strain.

Organism, strain	Type strain(^{T}); catalogue numbers	Origin	Reference
Halococcus salifodinae BIp	DSM 8989^T, ATCC 51437^T, JCM 9578^T	Rock salt (lumps), Bad Ischl, Austria	Denner et al. (1994)
Halococcus salifodinae BG2/2	DSM 13045	Salt drill core, Berchtesgaden, Germany	Stan-Lotter et al. (1999)
Halococcus salifodinae Br3	DSM 13046	Brine in salt mine, Chesire, England	Stan-Lotter et al. (1999)
Halococcus salifodinae N1	DSM 13070	Rock salt (lumps) Bad Ischl, Austria	Stan-Lotter et al. (1999)
Halococcus salifodinae H2	DSM 13071	Rock salt (lumps), Bad Ischl, Austria	Stan-Lotter et al. (1999)
Halococcus dombrowskii H4	DSM 14522^T, NCIMB13803^T, ATCC BAA-364^T	Rock salt (lumps), Bad Ischl, Austria	Stan-Lotter et al. (2002)
Halosimplex carlsbadense 2-9-1	ATCC BAA-75^T, JCM 11222^T	Permian salt near Carlsbad, NM, USA	Vreeland et al. (2002)
Halobacterium noricense A1	DSM 15987^T, NCIMB13967^T, ATCC BAA-852^T	Salt drill core, Altaussee, Austria	Gruber et al. (2004)

described the general procedure, which included dissolution of rock salt from Bad Ischl, Austria, under sterile conditions, filtering the solution through membranes of small pore size (0.22 µm) and subjecting the residue on the filter to DNA extraction. Nucleotide primers, which were specific for archaeal 16S rRNA genes, yielded a single PCR product of the expected size. The purified haloarchaeal PCR products of several independent extractions of DNA from rock salt were used for the construction of clone libraries (Radax et al., 2001). Phylogenetic analysis revealed several clusters, many of which had sequence similarity values of less than 95% when compared with known haloarchaeal species, with cultured haloarchaea from British, Polish, and Thai salt mines and with uncultured phylotypes (McGenity et al., 2000). The results suggested the presence of a halophilic community within the salt containing many representatives which have not yet been cultured (Radax et al., 2001). Fish et al. (2002) reported amplification of ancient bacterial and archaeal DNA from various halites 11–425 million years of age. Park et al. (2009) used salt samples from deposits of 23–419 million years of age and found that DNA similar to *Halobacterium* sp. was present in the older salt, but DNA with similarities to *Halorubrum* and *Haloarcula* sp. was present only in the younger salt (23 million years).

The microbial content of ancient rock salt is generally low—estimates range from 1–2 cells/kg of salt from a British mine (Norton et al., 1993) to 1.3×10^5 colony-forming units (CFUs) per kg of alpine rock salt (Stan-Lotter et al., 2000) and up to 10^4 CFUs per g of Permian salt of the Salado formation in the USA (Vreeland et al., 1998). Nevertheless, the reports cited here show that viable halophilic prokaryotes and/or amplifiable DNA were obtained reproducibly from ancient rock salt of different locations. The data support the hypothesis that the halophilic isolates from subterranean salt deposits may be the remnants of populations which once inhabited ancient hypersaline seas; in addition, they provide strong evidence against the notion that the strains could be the result of laboratory contamination. The strains can be regarded as resistant to long-term desiccation and nutrient depletion (see Grant et al., 1998), and it can be speculated that microbial life on other planets could have survived in subsurface deposits of salts for millions of years.

3. Extraterrestrial Halite

If halophilic prokaryotes on Earth can remain viable for very long periods of time, then it is reasonable to consider the possibility that viable microorganisms may exist in similar subterranean salt deposits on other planets or moons. This notion becomes all the more plausible in view of the detection of halite in extraterrestrial materials (Table 2).

Table 2. Halite in outer space.

Name/origin	Method of detection	Reference
Meteorites		
Nakhla + other SNCs (from Mars)	EM, infrared	Bridges and Grady (2000), Treiman et al. (2000)
Monahans (Asteroid)	Raman	Zolensky et al. (1999)
Murchison (+others)	EM, X-ray	Barber (1981)
Zag (Asteroid)	EM; RELAX[a]	Whitby et al. (2000)
Moons		
Europa (satellite of Jupiter)	Infrared	McCord et al. (1998)
Enceladus (satellite of Saturn)	cosmic dust analyzer	Postberg et al. (2009)
Planets		
Mars (rovers Spirit, Opportunity)	microscopy, X-ray	Rieder et al. (2004)
Mars (Southern highlands)	THEMIS[b]	Osterloo et al. (2008)

[a] Resonance ionization mass spectrometry.

[b] Thermal Emission Imaging System (infrared).

The SCN meteorites which fell in Shergotty (India), Nakhla (Egypt), and Chassigny (France) stem from Mars and were found to contain traces of halite **(Gooding, 1992; Bridges and Grady, 2000; Treiman et al., 2000), as do the Murchison and other carbonaceous meteorites (Barber, 1981). Even macroscopic crystals of** extraterrestrial halite, together with sylvite (KCl) and water inclusions, were found in the Monahans meteorite, which fell in Texas in 1998; the pieces of the meteorite were inspected days after being collected (Zolensky et al., 1999). The age of this meteorite was estimated by Sr/Rb dating to 4.7 ± 0.2 billion years. Analysis of the Zag meteorite, which fell in Morocco in 1998, revealed an ancient origin of the halite and water inclusions of this meteorite (4.57 billion years), which was interpreted to suggest the formation of evaporites as very early events in the solar system (Whitby et al., 2000). The Galileo spacecraft collected evidence that supports the existence of a liquid ocean on Europa. Galileo's onboard magnetometer, which measures magnetic fields, detected fluctuations that are consistent with the magnetic effects of currents flowing in a salty ocean (McCord et al., 1998). Postberg et al. (2009) focused on ice grains from the plumes of Enceladus, using the Cosmic Dust Analyzer instrument aboard Cassini. They found that, although all the grains were dominated by water ice, about 6% of them were quite salty, containing roughly 1.5% of a mixture of sodium chloride, sodium carbonate, and sodium bicarbonate. Sulfate and halite evaporite rocks have been discovered recently on Mars (Rieder et al., 2004). These facts suggest that brine pools might have been relatively common on the surface of Mars (Wierzchos et al., 2006). In addition, chloride-bearing materials have been detected on the Martian surface (Osterloo et al., 2008).

Recent data on the physical occurrence of liquid saline water on the surface of Mars (Smith et al., 2009; Renno et al., 2009) have added a novel aspect, since those brines would provide potential—if perhaps only temporary—liquid phases in the thin Martian atmosphere, allowing not only survival but metabolic activity of halophilic microorganisms.

4. Responses to Irradiation

Mars today is mostly a cold dry desert with an average surface temperature of minus 60°C and an atmospheric pressure, which is about 120 times lower than sea level pressure on Earth. Solar irradiation includes ultraviolet light at wavelengths down to 190 nm, which is not shielded, as on Earth, by an ozone layer, and fully hits the Martian surface (Rontó et al., 2003; Patel et al., 2004). It is believed that, before the accumulation of oxygen and ozone, the early Earth environment experienced an influx of short-wavelength UV of high intensity (Westall et al., 2006). Early life on Earth, therefore, was likely resistant to UV, and it can be concluded that potential microorganisms on Mars may have possessed resistance to this type of radiation as well or perhaps still do.

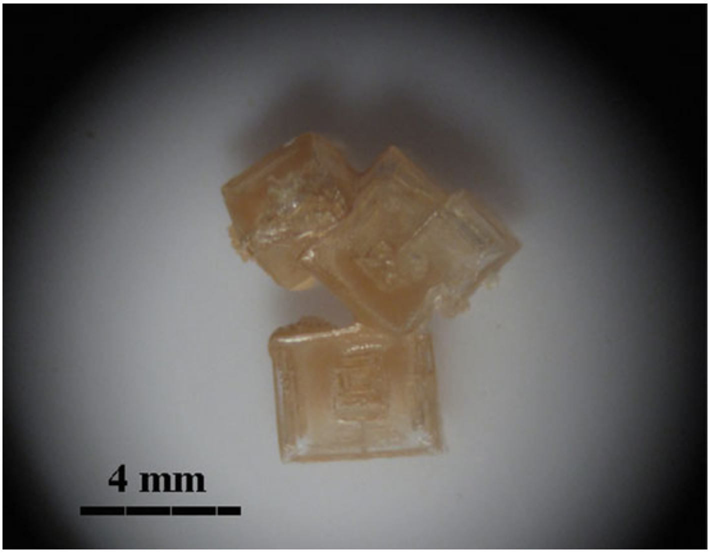

Figure 3. Halite crystals on a quartz disc with entrapped cells of *Halococcus dombrowskii* DSM14522^T.

Exposure of an extremely halophilic archaeon, *Haloarcula* sp. (which was recently renamed *Halorubrum chaoviator*; Mancinelli et al., 2009), to space conditions and an irradiation dose of about 10^4 kJ/m^2 had been performed during the "Biopan" experiments by Mancinelli et al. (1998), who demonstrated considerable survival of cells after 14 days in space. We studied the resistance of *Halococcus dombrowskii* DSM14522^T and other haloarchaea to simulated Martian UV flux (200–400 nm), using cells embedded in laboratory grown halite (Fig. 3). This procedure simulates the natural conditions of evaporating hypersaline waters containing microorganisms. The cells accumulated preferentially in fluid inclusions of halite, as could be demonstrated with prestained haloarchaea (Fig. 4), using the fluorescent dyes of the LIVE/DEAD BacLight™ kit (Fendrihan et al., 2006). The fluid inclusions were square or rectangular, as is common in the rectangular mineral halite, and the cells were rather densely packed within the fluid-filled spaces.

Halite-embedded cells showed no loss of viability after exposure to about 21 kJ/m^2, and they resumed growth in liquid medium with lag phases of 12 days or more after exposure up to 148 kJ/m^2 (Fendrihan et al., 2009a). The D_{37} (dose of 37% survival) for *Hcc. dombrowskii* was 400 kJ/m^2; by extrapolation, survival of a dose of ≥3,000 kJ/m^2 was estimated, which compared well to the dose received and survived by *Halorubrum chaoviator* mentioned above. It can be concluded that halite-embedded cells are protected to some extent against irradiation, since, compared to irradiation of liquid cultures, a marked increase in survival was noted (Fendrihan et al., 2009a).

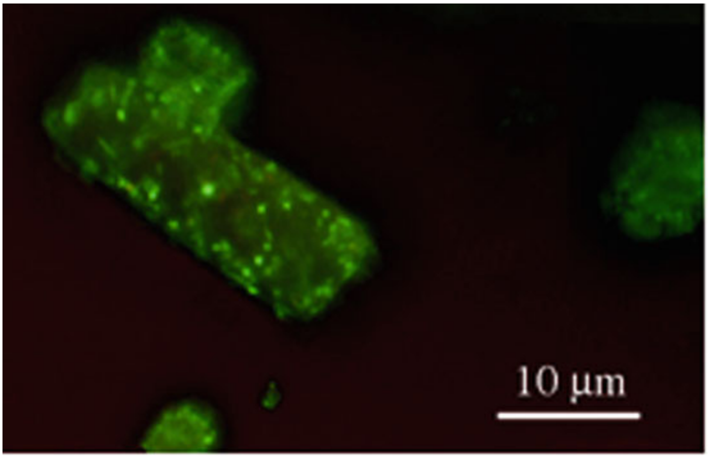

Figure 4. Localization of prestained *Halobacterium salinarum* NRC-1 cells in fluid inclusions of artificial halite. Cells were stained with the LIVE/DEAD BacLight™ kit prior to embedding and visualized by fluorescence microscopy.

Halite can cause attenuation of UV radiation due to the presence of color centers in halite's crystal structure that leads to a partial absorption of the light of shorter wavelength and reemission at longer wavelengths (Fendrihan et al., 2009a). In liquids, color centers do not appear; therefore, the absorption due to crystal vacancies (color centers) can explain increased survival of halite-embedded haloarchaeal cells. When natural brines are drying, formation of cubic crystals of different sizes will take place; if microorganisms are present, they will accumulate in various amounts within fluid inclusions. Dispersal of halite crystals can occur by wind (Wheeler, 1985), since cubes of halite from Sahara desert sands were found that had blown over Britain in the year 1984. The well-documented dust storms on Mars (e.g., Christensen et al., 1998) might provide conditions for the dispersal of crystals with potential microorganisms inside, regardless of whether they are indigenous or a result of forward contamination. Such crystals could afford sufficient shielding from UV radiation that might allow the microorganisms within them to remain viable on the inhospitable surface of Mars.

5. Response to Extremes of Temperature and pH

Most known archaeal halophiles are moderately to slightly thermophilic, showing optimum growth temperatures of 35 to 50°C (Grant et al., 2001), and their maximum growth temperature ranges between 50 and 56°C. Among bacterial halophiles, a few thermophiles are known, for example, *Halothermothrix orenii* and *Thermohalobacter berrensis*, which grow up to 68 and 70°C, respectively (Cayol et al., 1994, 2000), and several cyanobacterial species, which grow in hypersaline Solar Lake on the Sinai Peninsula, whose temperature can reach up to 61°C (Oren, 2002).

Warm environments are widespread on Earth, with temperatures of up to 50°C being due to solar radiation, while temperatures greater than 50–55°C are much rarer and are generally associated with geothermal activity (Brock, 1986). Halophiles have not been linked directly to volcanic activities on Earth or other planets and moons, as has been suggested for hyperthermophilic archaea (Stetter, 2002). These prokaryotes, however, have much higher temperature optima for growth (90–106°C) and often possess a sulfur-dependent metabolism. In our solar system, past and present volcanic activity is known; for example, extant active volcanoes are believed to be present on Jupiter's moon Io (http://www.solarviews.com/eng/iovolcano.htm). On Mars, several extinct volcanoes were found, some of which are vast shield volcanoes far bigger than any on Earth. In 2005, the European *Mars Express* spacecraft has found evidence that volcanic activity may have occurred on Mars in the rather recent past as well (http://www.esa.int/esaMI/Mars_Express/SEMLF6D3M5E_0.html). On Saturn's moon Enceladus, the presence of salty ice and probably warm subsurface water was reported (http://www.nasa.gov/mission_pages/cassini/media/cassini-20090624.html), which would make this satellite a possible habitat for halophilic microorganisms. Europa, the smallest of Jupiter's Galilean moons, also appears to have an active volcanic system, except that its volcanic activity is entirely in the form of water, which freezes into ice on the frigid surface, a process known as cryovolcanism (see Marion et al., 2003). Assuming internal heat flow and perhaps hydrothermal vents on the bottom of Europa's ocean, the possibility of microbial life below the icy surface of Europa was discussed extensively by Marion et al. (2003). These authors compared model simulations of hypothetical ion-containing brines below the ice on Europa with respect to water activity (a_w), pressure, and pH, concluding that halophilic microorganisms would be expected to survive a_ws of 0.6–0.72 but that the temperatures to be expected (−38 to −23°C) would be too low for biological activity. In addition, if SO_2 dissolution into the ocean, leading to H_2SO_4 and concomitantly low pH values, was considered in the model simulations, most halophilic microorganisms would not grow because generally they are not known to tolerate acidities below about pH 4.0 (Goh et al., 2006; Wang et al., 2007; Minegishi et al., 2008), although recently 16S rRNA analysis revealed the presence of bacterial genera close to *Proteobacteria*, *Bacteroidetes,* and *Actinobacteria* in an Australian acidic lake of high salinity (Mormile et al., 2009). Still, many points need yet to be clarified, before detailed plans for the biological exploration of Europa can be made, such as the thickness of the ice layer, the nature of the brownish streaks on the ice, the presence of thermal sources, and the chemical composition of the ocean (Marion et al., 2003).

Earth analogues of cold planetary environments, where halophilic microorganisms were found to live, are several lakes in the Vestfold Hills in Antarctica, with temperatures as low as −18°C (Oren, 2002). Bacterial isolates included *Halomonas* and *Psychroflexus* strains, an archaeal isolate was *Halorubrum lacusprofundi* (Oren, 2002). In addition, 16S rDNA analysis yielded numerous bacterial and archaeal representatives, most of them belonging to the *Proteobacteria* and *Cytophaga* (bacteria) and several genera of the *Halobacteriaceae* (Archaea), respectively (Oren, 2002). Cryopegs, which are defined as lenses of "cryosaline water" or "overcooled brine," can occur embedded in permanently frozen Holocene and Pleistocene strata and are the only hydrological systems on Earth with permanent subzero temperatures, high salinity (60–300 g/l), and isolation from external factors throughout their geologic history (Gilichinsky et al., 2005). In such cryopegs from an eastern Siberian permafrost location of 40–50 m below surface with temperatures ranging from −9 to −11°C, a wide diversity of fungi and bacteria was found, such as anaerobic and aerobic, spore-less and spore-forming, halotolerant and halophilic, psychrophilic and psychrotrophic bacteria, mycelial fungi, and yeasts, many of which were subsequently cultured at temperatures below 0°C (Gilichinsky et al., 2005).

6. Detection Methods

Detection methods for microorganisms and/or their constituents in extraterrestrial missions are under development. We discuss here two types of approaches, which have been considered by us and other workers and should be applicable to the search for potential halophilic microorganisms: Raman spectroscopy and the use of fluorescent dyes in microarrays or biomarker chips.

6.1. RAMAN SPECTROSCOPY

Raman spectroscopy has been suggested for the detection of both extinct and extant microbiota, since this technique is sensitive to organic and inorganic compounds as well as able to unambiguously identify key spectral markers in a mixture of biological and geological components (Ellery and Wynn-Williams, 2003; Jorge Villar and Edwards, 2006). The method also has the advantage of being a nonintrusive *in situ* analytical procedure, requiring very little effort in preparing the samples (e.g., no chemical extractions are necessary), and miniaturized instrumentation is being developed (Jorge Villar and Edwards, 2006). Halite itself does not give rise to any Raman peak (Downs, 2006). Nine different extremely halophilic archaeal strains which had been embedded in laboratory-made halite crystals (similar as in Fig. 3) were investigated with two methods: FT-Raman spectroscopy, using laser excitation at 1,064 nm and dispersive micro-Raman spectroscopy with laser excitation at 514.5 nm (Fendrihan et al., 2009b). The spectra showed prominent peaks at 1,507, 1,152, and 1,002 cm^{-1} (see Fig. 5), which were due to haloarchaeal C_{50} carotenoid compounds (mainly bacterioruberins). Other peaks were attributed to peptide bonds and nucleic acids (Fendrihan et al., 2009b). The results contributed to a growing database on Raman spectra of terrestrial microorganisms from hypersaline environments, whose establishment had been suggested by Marshall et al. (2006).

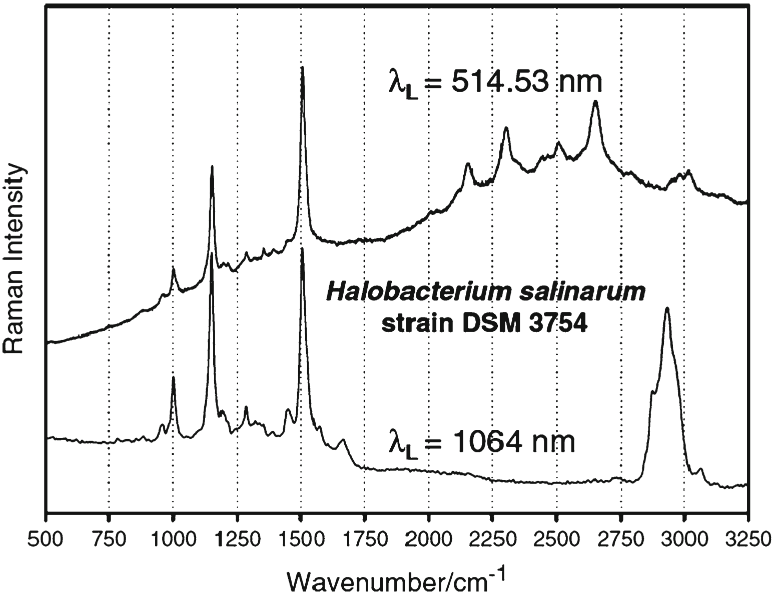

Figure 5. Resonance Raman spectrum with laser excitation at 514.53 nm (*upper* trace) and FT-Raman spectrum with laser excitation in the near infrared (*lower* trace) of *Halobacterium salinarum* strain DSM 3754, showing prominent peaks at 1,002, 1,152, and 1,507 cm^{-1}, which are due to carotenoids, for example, bacterioruberin (See Fendrihan et al., 2009b).

6.2. FLUORESCENT DYES IN MICROARRAYS AND BIOMARKER CHIPS

Fluorescent dyes such as SYTO 9 and propidium iodide from the LIVE/DEAD BacLight™ kit (see Fig. 4) provide strong signals, which are suitable for remote detection and are compatible with high ionic strength (Leuko et al., 2004; Stan-Lotter et al., 2006). Nadeau et al. (2008) examined several fluorescent stains for their applicability to direct microscopic analysis by robotic landers or for return samples. As pointed out by Parro et al. (2008a), most instruments in planetary missions are designed to detect organic volatiles, for example, the Urey instrument (Skelley et al., 2006), which is proposed for the upcoming ESA/NASA ExoMars mission (Vago, 2010) and will analyze fluorescently tagged volatiles by capillary electrophoresis. However, many organic compounds are nonvolatile under the foreseen experimental conditions. For such molecules, antibody microarrays are being developed, which permit the recognition of up to several thousand different antibody reactions on a single chip with a size of only a few square centimeters (Parro et al., 2008b; Sims et al., 2005). Antibodies are particularly useful receptors since they can detect simultaneously a broad range of biomarkers, from low molecular weight metabolites to whole cells and spores (Fernández-Calvo et al., 2006). Fluorescent reporter molecules which monitor the binding event are almost exclusively used now for obtaining signals in microarrays (Angenendt, 2005). Although the reactions in space missions will be carried out in heated compartments, stability and storage conditions of all reagents are of interest and are being investigated under simulated galactic particle radiation (Thompson et al., 2006). A field prototype of an antibody array-based life-detector instrument, Signs Of LIfe Detector (SOLID2), has been tested recently in a Mars drilling mission simulation, using automatic sample processing and analysis of ground core samples with protein microarrays that contained 157 different antibodies (Parro et al., 2008a). We submitted recently extracts from hypersaline environments (Permian salt from the Himalayas, from the shore of the Dead Sea, and from the Piranske salterns in Slovenia, respectively) to a 300 antibody-containing microarray system, similar as described by Rivas et al. (2008), at the Centro de Astrobiología (INTA-CSIC), Madrid, and obtained strong positive signals with antibodies which had been raised against exopolysaccharides from halophilic bacteria (Weigl et al., 2010), suggesting a very sensitive recognition of biomolecules in crude salty extracts.

7. Conclusions and Considerations for Astrobiology

The interest in potential extraterrestrial microbial life has increased considerably since the discovery of bacteria-like microfossils, together with polycyclic aromatic hydrocarbons and low-temperature carbonate globules, in the Martian meteorite ALH84001 (McKay et al., 1996). It was suggested that if these features constitute a proof for past or extant life on Mars, such life must have existed within the surface of Mars. The apparent longevity of haloarchaeal strains in dry salty environments is of interest for astrobiological studies in general and in particular, for the search for life on Mars. On Earth, microorganisms were the first life forms to emerge and were present perhaps as early as 3.8 billion years ago (Schidlowski, 1988, 2001). If Mars and Earth had a similar geological past, as has been suggested (Schidlowski, 2001; Nisbet and Sleep, 2001), then microbial life, or the remnants of it, could still be present on Mars. Interesting saline environments are stromatolites, whose earliest representatives date back to probably 3.5 billion years; since microorganisms are implied in the formation of stromatolites and since several haloarchaeal strains were isolated from modern stromatolites in Australia, Leuko et al. (2010) argued for plans to include the search for such formations on Mars. Other locations in outer space, where saline environments can be expected, are Jupiter's moon Europa, Saturn's moon Enceladus, and the asteroid belt.

The European Space Agency, together with NASA, is planning the revised ExoMars mission, which will include a mobile rover capable of drilling into the surface of Mars and of probing for traces of organics and biomolecules (Vago and Kminek, 2007; Vago, 2010). In addition, return samples from Mars or other celestial bodies might become available. Other samples may stem from meteorites, which could contain halite, as described above. In any case, the development of sensitive and specific methods for life detection in extraterrestrial samples will be crucial, since the requirement for authenticity—that is, proof, that any detected substances are not stemming from Earth—is severe, and these specifications will likely spawn applications for a variety of terrestrial samples.

Promising methods for the detection of halophilic microbial life in outer space are Raman spectroscopy and microarray-based methods, both of which are highly sensitive and suitable to miniaturization of the respective instruments.

8. Summary

Viable halophilic prokaryotes were found in ancient halite of probably more than 400 million years of age; stromatolites are another terrestrial formation which could contain potentially ancient halophiles. Therefore, intriguing locations for the search for halophilic microorganisms (or their remaining constituents) are Mars, which may have been a wetter and warmer place in the past, and recent data suggest the presence of halite on Mars, as well as the moons Europa and Enceladus in our solar system. Considering the harsh environments on other planets and moons, the chances for finding extraterrestrial life should be greater when drilling into the subsurface and searching with highly sensitive instrumentation for organic molecules and perhaps living fossils.

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Biodata for **Daria Tashyreva** and **Josef Elster**, the authors of "***Production of Dormant Stages and Stress Resistance of Polar Cyanobacteria***."

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PRODUCTION OF DORMANT STAGES AND STRESS RESISTANCE OF POLAR CYANOBACTERIA

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1. Introduction

Cyanobacteria are commonly considered as the dominant phototrophs in polar terrestrial and freshwater ecosystems. Although it is generally assumed that cyanobacteria prefer high temperatures for growth (>20°C), cyanobacteria are tolerant to cold conditions. They frequently represent the dominant autotrophic organisms with a high productivity in many types of habitats in the Arctic and Antarctic (Tang et al., 1997; Vincent, 2000). Their dominance can be explained by both a high tolerance for these extreme environments, the absence of grazing pressure, as well as the absence of competing species, which are excluded by the inhibiting effects of extremely low temperatures during much of the year (Nadeau and Castenholz, 2000). Cyanobacteria successfully colonize a remarkable number of terrestrial and aquatic polar habitats including rocks, glaciers, soils, streams, ponds, and lakes. Benthic communities of cyanobacteria in certain polar lakes form perhaps the thickest microbial biofilms known to occur in natural environments: the biomass accumulation can reach a thickness of several tens of centimeters (Vincent, 2000). In such extreme polar habitats as in desert soils and glacial streams, cyanobacteria are not only the dominant phototrophs but also constitute most of the microbial biomass (Vincent, 2000).

It has frequently been reported that filamentous cyanobacteria are the most abundant in the various polar habitats (Elster et al., 1997; Elster, 1999; Nadeau and Castenholz, 2000; Cavacini, 2001; Casamatta et al., 2005; Jungblut et al., 2005; Komárek et al., 2008). Motile species, such as Oscillatoriales, as well as nitrogen-fixing Nostocales, are very prominent in cyanobacterial communities. Filamentous cyanobacteria are extensively represented, specifically by *Phormidium* spp. and *Leptolyngbya* spp., which are present in the majority of polar habitats (Cavacini, 2001; Elster, 2002; Jungblut et al., 2005; Komárek et al., 2008). Coccoid cyanobacteria have a restricted distribution and never occur as dominant (Komárek et al., 2008). Bloom-forming cyanobacteria such as *Anabaena*, *Aphanizomenon*, and *Microcystis* are usually absent in the Arctic and Antarctic communities (Vézina and Vincent, 1997).

2. Classification of Polar Environments

Several habitat classifications are used to describe the various types of environments which occur in the polar regions. The following habitats and communities are usually distinguished: marine environments, snow and ice communities, rocks and soils, and aquatic habitats (which include rivers, streams, ponds, and lakes). However, the distinctions between these types of habitats are not clear, and overlap between them frequently occurs. Such classifications do not take into consideration seasonal and diurnal variations of the most important abiotic factors, which determine life strategies of polar living organisms.

Elster (2002) suggested a classification of polar habitats, with particular attention toward algal communities, according to the water availability and its state during summer and winter time. Accordingly, three environmental categories were proposed: lacustrine (unfrozen water is present throughout the whole year), hydro-terrestrial (liquid water is available during the summer but is frozen in winter), and terrestrial (water availability is temporary and even changes throughout diurnal oscillations). This type of classification is reasonable to use with respect to the study of survival strategies of cyanobacteria, including the production of dormant stages, because water is known to stabilize the temperature. Water has a high heat capacity and therefore moderates temperature fluctuations in natural environments.

The lacustrine (lake) environment is considered the most stable and favorable, due to the whole-year presence of liquid water, which consequently allows cyanobacteria to avoid both freezing and desiccation. Many lakes are covered by thick ice, but the water beneath remains unfrozen, despite winter temperatures which drop far below 0°C. Therefore, the richest cyanobacteria biomass accumulations in the polar regions occur in the benthic habitats of lakes and ponds. Filamentous cyanobacteria, such as *Phormidium* spp. and *Oscillatoria* spp. (Vézina and Vincent, 1997; Quesada et al., 1999) and *Nostoc* sp. (Vincent, 2000), are the most abundant species among polar benthic communities. Cyanobacteria reported to form planktonic communities include the genera *Phormidium*, *Chroococcus*, *Aphanocapsa,* and *Synechocystis* (Lizotte, 2008).

Hydro-terrestrial environments (wetlands) include temporary shallow freshwater or brackish, lotic, and lentic wetlands and saturated soils or meadows; shallow lakes and ponds; glacial and snow-fed streams and rivers, including their wet banks and periodically flooded surroundings; and irrigated rock faces or wet walls (Elster, 2002). Because of their high variability in habitat types, they are also disposed to high diversity in their cyanobacterial genera (Chroococcales—*Aphanocapsa, Aphanothece, Gloeocapsa, Gloeocapsopsis;* Oscillatoriales—*Leptolyngbya*, *Lyngbya, Schizothrix, Phormidium, Microcoleus, Oscillatoria;* Nostocales—*Scytonema, Tolypothrix, Calothrix, Dichothrix,* and *Nostoc*) (Elster, 2002; Mueller et al., 2005).

Polar terrestrial environments are represented by soils and rocks, which are periodically made wet. Water in the liquid form is only available for a short period or alternatively is available as air humidity; therefore, this unstable environment can be the principle source of resting stages, which are well adapted to harsh conditions (Broady, 1996; Elster, 1999). For example, Davey (1989) noticed that *Phormidium*, which occurs in fellfield Antarctic ecosystems, which are usually subjected to extensive periods of desiccation, displays a greater rate of recovery from dehydration and also a greater resistance to drying than aquatic cyanobacteria. Therefore, it is perfectly adapted to its habitat of unstable, regularly dehydrated soils. One of the most common species in polar soils is *Nostoc* sp., although non-akinete-forming *Microcoleus*, *Leptolyngbya*, *Oscillatoria,* and *Phormidium* also occur (Broady, 1996; Vincent, 1988; Elster, 2002; Smith et al., 2006).

3. Stressful Factors Acting in Polar Environments

Populations of cyanobacteria living in polar areas have to cope with harsh physical conditions and experience extremely high physiological stresses during much of the year. Polar habitats are highly selective, requiring organisms to be tolerant and to evolve specific adaptations to the unfavorable abiotic factors. Short summers and low inputs of thermal and solar energy are characteristic of polar ecosystems, and they have a major effect on photosynthetic organisms (Elster and Benson, 2004). Freezing and desiccation are the main forms of environmental stress in cold regions, although other stresses such as nutrient deficiency, osmotic stress, high rates of UV irradiation, and significant changes in irradiance during the year also affect polar organisms. Besides prolonged winter periods, when temperatures are constantly well below zero, cyanobacteria in polar environments may be exposed to frequent freeze-thaw cycles and water status oscillations during the short vegetative season (Davey, 1989; Thomas et al., 2008).

3.1. FREEZING INJURY

During freezing-melting, cells are exposed to a radically altered environment. Cryoinjuries are conventionally divided into two categories, occurring by different mechanisms, which are based on the rates at which the cells are cooled: (1) considerable cell dehydration and (2) mechanical disruption of membranes by the growth of ice crystals (Acker and McGann, 2003).

During slow cooling (0.5°C/min), intra- and extracellular solutes concentrate as a result of the formation of external ice and water outflow from the cells (Acker and McGann, 2003). There are two possible mechanisms for the cell water loss during extracellular freezing. As water freezes, salts are excluded from the growing ice crystals. These salts remain in the unfrozen extracellular fraction and act as an osmotic solution (Wharton and Ferns, 1995). However, in dilute water environments, dehydration is instead driven by the difference of vapor pressures between the extracellular ice and the supercooled water inside the cell, at the same temperature (Wharton et al., 2003). Freeze-induced dehydration has numerous effects resulting in cellular damage such as the denaturation of proteins and precipitation of various molecules. Additionally, the accumulation of extracellular ice can potentially result in the physical disruption of cells, caused by the formation of adhesions between ice crystals and the cell walls and membranes (Thomashow, 1998).

During rapid cooling, or in the absence of extracellular freezing, the cell is not able to lose water fast enough to maintain equilibrium. This results in the cytoplasm becoming increasingly supercooled, and the cell attains equilibrium by intracellular ice nucleation (Acker and McGann, 2003). Intracellular freezing has been found to be much more detrimental to cells than the dehydration effects of extracellular freezing. The formation of ice usually destroys the membranes, particularly if the crystals are formed intracellularly (Fuller, 2004).

Both dehydration and intracellular ice formation are associated with cellular injury to the plasma membrane and the cell wall. Membrane damage is considered more harmful to cells than is damage to the cell walls. An injury due to dehydration occurs because the membranes are not limitlessly elastic, and at a certain volume, membrane vesiculation develops (Steponkus and Lynch, 1989). Intra- and extracellular ice crystals are considered to rupture the membranes eventually resulting in the release of cellular components and consequent cell death (Mazur, 1984; Thomashow, 1998).

3.2. DRYING INJURY

An actively growing cell requires a high water content in order to maintain cell structure and enable enzymatic activity. Water allows the cell to create an environment for biochemical reactions and stabilizes the native structures of DNA, proteins, and lipids. The majority of organisms are unable to tolerate any extensive or complete removal of water from their cells (Alpert, 2006).

Cell dehydration can be provoked by osmotic stress, extracellular freezing, or the direct effects of dry air. However, the injuries caused by hypertonic solutes, as well as those caused by water deficit, are different. The water deficit due to direct air drying is far greater than can arise if a cell is exposed to osmotic stress, also being far greater than can arise in freeze-tolerant cells in the presence of extracellular ice (Potts, 1994).

The drying of cells commonly leads to substantial damage to both the cellular structures and molecules, which ultimately results in cell death. At the level of the cyanobacterial community, drying may lead to changes in the surface area of the community, its shrinkage, changes in texture, precipitation of salts, as well as color changes as pigments are oxidized (Potts et al., 2005). In the cells themselves, the main targets of desiccation damage are the nucleic acids, proteins, and membrane lipids. Upon drying, cell membranes undergo fusion and lipid phase transitions from the gel state to the liquid crystalline state and the inverse process upon rehydration (Crowe et al., 2002; Potts, 1999). Therefore, the structure of the membranes is destroyed resulting in leakage of the cells' contents. Damage to the nucleic acids includes chemical modifications (alkylation or oxidation), cross-linking, as well as base removals such as depurination (Potts, 1999). Maillard (browning) reactions in the presence of light also contribute to damages to nucleic acids and proteins by means of linking between reducing sugars and free amino groups of the dry proteins (Potts, 1994).

Cyanobacteria are organisms well known as being able to survive periods of drought (Davey, 1989; Gao et al., 1998; Potts, 1999). It is necessary to emphasize that drought is the objective low availability of water in the environment surrounding the organism, while desiccation is a considerable water loss of a cell or an organism (Alpert, 2005). Therefore, drought tolerance can be divided into two types of stratagems, which all drought-tolerant organisms embrace—preventing (or at least the reduction of water loss) and maintaining their viability in an extensively (or completely) dried-up state.

4. Avoidance Strategies

Polar cyanobacteria have evolved a diverse range of protective strategies, in order to avoid or tolerate various severe environmental factors which occur in the polar regions (Elster and Benson, 2004). These avoidance strategies include motility, which allows for an organism moving to a more favorable environment (Castenholz et al., 1991). Motile cyanobacteria can react to environmental stimuli such as temperature, pH, irradiance, etc. Since the hormogonia of many filamentous cyanobacteria have the capacity for both phototactic and aerotactic movements (Damerval et al., 1991; Meeks et al., 2002), this capacity can possibly facilitate their finding of more propitious conditions for wintering. Such an avoidance strategy is typical for cyanobacteria especially in such environments as lakes and wetland floors. These habitats may physically protect living organisms against stress factors caused by changes in temperature and water availability (Elster and Benson, 2004). For example, cyanobacteria in certain polar lakes (Vincent, 1988) can escape to the bottom, where the water never freezes. Therefore, such an active strategy allows the cyanobacteria to avoid direct freezing and desiccation. Motile soil cyanobacteria can escape from direct desiccation and excessive light through vertical migrations into the lower layers of the soil (Davey and Clarke, 1991; Ehling-Schulz and Scherer, 1999). The formation of dense multilayered mucilaginous mats can facilitate stream periphyton and soil communities to withstand fluctuations in temperature, partial drying, and freeze/thaw cycles during short summer periods, as well as the maintenance of metabolic activities over a wider range of environmental conditions (Hawes et al., 1992; de Los Ríos et al., 2004; Elster and Benson, 2004). For example, cells of the cyanobacterium *Nostoc commune* secrete significant amounts (more than 60% of the colonies' dry weight) of a complex extracellular polysaccharide, which retains water during periods of drought (Hill et al., 1997).

Another method to avoid the direct actions of a wide range of stressful factors is the formation of associations with other organisms (such as fungi in lichens). Furthermore, while this strategy provides a high degree of physical protection, it also brings metabolic advantages to the symbiotic organisms.

With respect to water management, lichen symbiosis is one of the most successful mutualistic life strategies in the polar regions (Elster and Benson, 2004).

5. Concept of Dormancy

One of the most widespread adaptations to survive environmental stress is dormancy. If we accept that cyanobacteria are a group of bacteria, we can accept the characterization of dormancy which has been confirmed for heterotrophic bacteria. Bacterial dormancy can be defined as “a reversible state of low metabolic activity, in which cells can persist for extended periods without division” (Kaprelyants et al., 1993). The word “dormant” is a synonym for inactive, and the most remarkable characteristic of the dormant stage is the significantly reduced (or even undetectable) rate of metabolism. And since dormancy is a survival strategy, dormant cells must possess a higher resistance to different stressful factors than do corresponding vegetative cells. Finally, dormant cells must retain vitality after the return of favorable conditions (Sudo and Dworkin, 1973; Mulyukin et al., 1997; Colwell and Grimes, 2000; Kell and Young, 2000; Oliver, 2005; El-Registan et al., 2006).

As summarized by Elster (1999), the strategy of dormancy, that is, changes in the rates of physiological processes, is combined with additional protective mechanisms against various stress factors:

1. Changes in the membrane and cell envelope structure/composition and consequent changes in their fluidity, etc. (in response to low or high temperatures, desiccation, and osmotic stress)
2. Production of special protective substances (pigments and amino acids against excessive light, ice-active substances against freezing, nonreducing sugars in response to desiccation, etc.)

A large variety of terms have been used to describe such less metabolically active and more resistant microbial cells, including “dormant,” “quiescent,” “ultramicrobacterial,” “minicell,” “cryptically growing,” and “resting” (Colwell and Grimes, 2000). We use the term “dormant” to describe any form of metabolically inactive stage, regardless of the factors required for its induction or termination. Therefore, dormancy can be subdivided into two types (diapause and quiescence), according to the cause arresting the metabolism. This classification is usually used to describe specific states of invertebrates (Alekseev et al., 2006; Rebecchi et al., 2007) and has not been applied to cyanobacteria before.

5.1. AKINETES AND DIAPAUSE

According to Rebecchi et al. (2007), diapause is under endogenous control and is connected with changes of environmental factors and those stressors which occur, but it is not directly induced by them. It represents a proactive mechanism, which combines environmental signals and an internal hormonal mechanism (Alekseev et al., 2006). Although its effect is usually to facilitate survival during unfavorable periods, it is not immediately reactive to the adverse environmental conditions. A classical example which represents such a category of dormant cells is the cyanobacterial akinete. It is considered either to be an overwintering or long-term survival structure (Sutherland et al., 1979; Livingstone and Jaworski, 1980), which develops when environmental factors are still able to maintain normal cell metabolism. They can persist even when favorable environmental conditions return (Livingstone and Jaworski, 1980; Nichols and Adams, 1982). However, akinetes are not completely dormant; they retain approximately 7 % of the oxygen evolution and consumption as the vegetative cells do (Thiel and Wolk, 1983). It has been reported (Sutherland et al., 1979) that environmental factors (decreasing or increasing of light intensity, low temperature, desiccation, and the absence of combined nitrogen) provoke akinete formation. However, numerous observations suggest that energy limitation is the primary signal for akinete differentiation. That is why akinetes are most often seen in the stationary phase of growth in cultures, which could be a consequence of the limitations of nutrient or/and light (Meeks et al., 2002).

Cyanobacterial akinetes are very distinctive compared to their vegetative cells. Akinetes are usually larger and have a thicker cell wall, with a condensed mucilaginous sheath and with a more granular cytoplasm than do vegetative cells (Sutherland et al., 1979; Nichols and Adams, 1982). The contents of lipids and fatty acids in akinetes have been shown to be less than in vegetative cells, with fatty acids being more saturated in akinetes (Yamamoto, 1972). The high contents of cyanophycin and glycogen in akinetes serve as sources of nutrients to maintain low metabolic activity when akinetes persist and to provide energy for growth upon germination (Sutherland et al., 1985). Akinetes are thought to be more resistant to desiccation, frost, and prolonged dark and cold periods than are vegetative cells (Sutherland et al., 1979).

The water content of akinetes appears to be lower than that of their corresponding vegetative cells. In *Anabaena variabilis*, the vegetative cells contain 77 % water, while akinetes contain 63 % (Potts, 1994). Thus, despite the fact that the formation of akinetes is associated with changes in their hydration level, the changes are small, and they contain too much water to be considered as desiccated cells. The formation of akinetes prevents water loss by modifying the structure and thickness of the cell envelope. Cyanobacterial akinetes possess in their cell wall additional layer of peptidoglycan and fibrillar layer (Nichols and Adams, 1982). These layers increase by more than by two times the total thickness of cell wall (Sutherland et al., 1979). As was shown in the study by Hori et al. (2003), akinetes of *Anabaena cylindrica* partially lose water during the drying process; however, they do not desiccate further in spite of prolonged drying, thus probably demonstrating that akinetes can keep water even in cases of the long-term effects of dry air. In this experiment, the remarkable recovery of the akinetes was demonstrated after drying, while the vegetative cells underwent shrinkage and lysis. Agrawal and Singh (1999) demonstrated the great number of germinated akinetes of *Anabaena iyengarii* and *Westiellopsis prolifica* after both short-term (1 hour) and prolonged (1 month) periods of desiccation, whereas vegetative filaments were highly susceptible to the drying process and were represented by almost total mortality. Very little is known about the molecular basis for the resistance of akinetes to environmental stresses (Agrawal, 2009).

Akinete formation is not a common feature of all cyanobacteria. It is only related to Nostocales and Stigonematales (Sukenik et al., 2007). The majority of cyanobacteria in the polar regions belong to Oscillatoriales, which are non-spore forming. However, even many species of Nostocales overwinter as vegetative filaments rather than akinetes and do not contribute to survival under adverse conditions (Agrawal, 2009). On the other hand, vegetative filaments usually are much more susceptible to desiccation (Agrawal and Singh, 1999) and freezing (Sutherland et al., 1979) than akinetes and often show 100 % mortality after drying or freezing/thawing. This suggests that other mechanisms than akinete formation, probably alternative dormant cells, contribute to survival of stressful factors.

5.2. STARVED, STATIONARY-PHASE CELLS, AND QUIESCENCE

It is known that various species of cyanobacteria can tolerate freezing and desiccation without the production of any morphologically distinct cells (Davey, 1989; Shaw et al., 2003; Agrawal, 2009) but do possess special biochemical adaptations. In those cases, the transformation to a resistant state involves adaptive mechanisms but not evident morphological differentiation such as the formation of akinetes.

The current state of information about quiescence and quiescent organisms was summarized by Rebecchi et al. (2007). The term quiescence is used for the decrease of metabolic activity under exogenous control. It is directly initiated and maintained by various environmental factors, for example, the absence of liquid water caused by drying or freezing, lack of oxygen, excessively low temperatures, and high salt concentrations. The quiescent state is immediately discontinued when the external stimuli are removed. Some quiescent organisms are capable of reducing their metabolic activities to an undetectable level (Rebecchi et al., 2007). To define this type of dormancy (a completely ametabolic state), Keilin (1959) suggested the term “cryptobiosis.” The cryptobiotic state implies metabolically inactive stages induced by freezing, lack of oxygen, high levels of solutes in the surrounding environment, and desiccation. These stages are termed as cryobiosis, anoxybiosis, osmobiosis, and anhydrobiosis. For example, cells in the anhydrobiotic state are restricted in their metabolism because of the absence or low content of water. At the absolute water content threshold of 10 %, there is no longer sufficient water to form a monolayer around the proteins and membranes; therefore, enzymatic reactions and metabolism stop (Alpert, 2006). Such dried or frozen cells can persist for years, even centuries, in an inactive state, and then resume their metabolic activity when melted and rehydrated (Gilichinsky et al., 1992; Crowe et al., 2002). It is recognized that *Nostoc*-dominant mats can quickly resume photosynthetic activity and respiration after complete desiccation, immediately upon rehydration (Hawes et al., 1992; Tamaru et al., 2005). However, it is not known whether all cells in natural populations of cyanobacteria can directly tolerate freezing, desiccation, and salinity stress or whether it is the ability of individual cells, with specific properties. We suggest that certain unfavorable factors such as starvation (Billi and Grilli Caiola, 1996; Oliver, 2005) can induce changes in the ultrastructure and biochemistry of stressed cells, which directly permit them to tolerate freezing and desiccation.

The properties of cells which enter the stationary phase, or the changes in starved cells, may explain why cyanobacteria are tolerant of various stressful factors without the production of any cells morphologically different from vegetative cells. It is known that eukaryotic alga (Morris, 1981), yeasts (Argüelles, 2000), and eubacteria (Siegele and Kolter, 1992) when entering the stationary phase possess a much higher resistance to various stressful factors and a lower metabolic activity, than in their logarithmic phase. Stationary-phase bacterial cells usually change the composition of the fatty acids in their membranes (Muñoz-Rojas et al., 2006), accumulate trehalose (Hengge-Aronis et al., 1991; Potts, 1994), accumulate storage products (El'-Registan et al., 2006), and thicken their cell walls (Suzina et al., 2004). In gram-negative bacteria, there is also a typical enlargement of the periplasmic space (Gorshkov et al., 2009) which is of particular interest because cyanobacteria belong to the gram-negative bacteria. Stationary-phase cells are thought to be more resistant to drying than logarithmic-phase cells, in part because trehalose accumulates during the stationary phase (Hengge-Aronis et al., 1991). On transition to the stationary phase, cells of *Escherichia coli*, *Lactobacillus bulgaricus,* and *Pseudomonas putida* decreased the content of unsaturated fatty acids and incorporated cyclopropane fatty acids into their membranes. These alterations of fatty acids composition have been proven to enhance the freezing and desiccation tolerances in bacteria (Cronan, 1968; Singh et al., 2002; Muñoz-Rojas et al., 2006; Li et al., 2009). Late-stationary-phase cells of *Micrococcus luteus* increased the total thickness of their cell walls by 1.5–3 times, compared to exponential or early-stationary-phase cells, and they formed a continuous thick microcapsule layer (Mukamolova et al., 1995; Mulyukin et al., 1996; Suzina et al., 2004). Thickening of the cell wall is a general adaptation of dormant prokaryotic and eukaryotic cells to various insults (Sudo and Dworkin, 1973). Also, the most frequent and common factors known to induce the production of more resistant stages are starvation and an inadequate energy supply (Roszak and Colwell, 1987; Siegele and Kolter, 1992; Votyakova et al., 1994); this for cyanobacteria means low light intensity and the lack of mineral nutrients. The changes in the starved cells' ultrastructure, metabolism, and biochemistry are similar to changes in stationary-phase cells because starvation can induce a stationary-phase response (Notley and Ferenci, 1996). Despite the fact that these cells are much more resistant to various stresses and have a lower metabolism, it is obvious that not all of them are completely metabolically inactive (Siegele and Kolter, 1992; Colwell and Grimes, 2000), decreasing metabolic activity under the direct action of drying or freezing.

It is commonly accepted that such stationary-phase cells or starvation-induced cells represent dormant stages of eubacteria, which do not produce endospores (Kaprelyants and Kell, 1993; Votyakova et al., 1994; Oliver, 2005; Colwell, 2009). These studies of heterotrophic bacteria have revealed that these alternative dormant cells can persist for a prolonged time (at least several months) without losing their viability in nutrient-depleted medium, and they are much more resistant to drying, freezing, osmotic stress, and moderate heating than are vegetative logarithmic-phase cells (Roszak and Colwell, 1987; Jenkins et al., 1990; Votyakova et al., 1994; Mukamolova et al., 1995). Similar studies on cyanobacteria are rare (Billi and Grilli Caiola, 1996; Grilli Caiola et al., 1996; Schwarz and Forchhammer, 2005); however, it is likely that cyanobacteria possess similar features to the other eubacteria. In the study by Billi and Grilli Caiola (1996), the coccal cyanobacterium *Chroococcidiopsis* sp. formed "spore-like" cells when cultivated on a nitrogen-free or nitrogen-limited medium. These cells shared some features with akinetes, that is, a thick multilayered envelope with polysaccharide and sporopollenin-like deposits between the plasmalemma and cell walls. These cells had very low metabolism, measured by their oxygen evolution and uptake, and remained viability for at least for 4 months. Similar cells were observed in aged (23 months), desiccated cultures of *Chroococcidiopsis* sp. (Grilli Caiola et al., 1996), and were considered as long-term survival forms appearing under stressful conditions.

Since cyanobacteria in natural populations, and even under laboratory conditions, are not synchronized (Nichols and Adams, 1982; Kell et al., 1991; Billi and Grilli Caiola, 1996) due to the heterogeneity of conditions in colonies and microhabitats, some proportion of more resistant "stationary-phase cells" may always be present. This could explain why cyanobacteria in natural habitats can tolerate quickly changing environmental conditions, for example, desiccation by the wind (Gao et al., 1998; Hawes et al., 1992; Agrawal, 2009) or sudden freezing (Davey, 1989, 1991).

The ability of cyanobacteria to withstand desiccation and freeze-drying differs among taxa, with *Nostoc*, *Calothrix*, *Microcoleus*, *Chroococcidiopsis*, and *Gloeocapsa* being among the most resistant (Potts, 1999).

6. Biochemical Mechanisms of Resistance to Freezing and Desiccation

The enormous resistance of some polar cyanobacteria to freezing and desiccation could be possible via biochemical mechanisms. Some of these have been demonstrated directly by cyanobacteria, while others have been described in different groups of organisms, but possibly can also be found also in cyanobacteria. It is not clear whether all cells in natural colonies and biofilms possess these protective substances or if it is a property of particular cells, nevertheless becoming a general property of a colony. Possibly, various taxa of cyanobacteria could possess different protective mechanisms. Strains or species with higher desiccation tolerance are usually more tolerant to freezing (Lin et al., 2004; Crowe et al., 2004), and their protective mechanisms to these types of stress frequently overlap. Therefore, mechanisms which are responsible for freezing and desiccation tolerance get examined concomitantly.

6.1. PRODUCTION OF SUCROSE AND TREHALOSE

One of the most widespread adaptations is the accumulation of nonreducing sugars, such as sucrose and trehalose. These compounds are known to either contribute to tolerance to desiccation (Potts, 1999; Crowe et al., 2002), freezing-drying (Tanghe et al., 2003), and osmotic tolerance (Reed et al., 1984). They can prevent intracellular freezing by decreasing the freezing point as well as through water replacement and stabilize membranes, proteins, and DNA during desiccation. Further, they serve as compatible solutes, thereby preventing from dehydration during osmotic stress (Hershkovitz et al., 1991; Leslie et al., 1995; Crowe et al., 2002, 2004).

In polar habitats, many cyanobacterial communities frequently appear as brittle, highly desiccated biofilms. Such biofilms contain low to undetectable amounts of water (Hawes et al., 1992; Tamaru et al., 2005). Consequently, these organisms can tolerate drought by desiccation to 10 % (and even less) of their initial water content without losing vitality. According to Alpert (2005), desiccation tolerance can be defined as "the ability of an organism to equilibrate its internal water potential with that of moderately dry air, and then resume normal function after rehydration." It is generally known that sucrose and trehalose bind to dried membranes, proteins, and nucleic acids as water substitutes, thereby preserving the native structures of the molecules (Billi and Potts, 2000; Crowe et al., 2002). Additionally, trehalose prevents the damage caused by Maillard reactions (Crowe et al., 2004). Besides their ability to directly interact with molecules and stabilize them, trehalose and sucrose form glasses in their dry state. Glasses provide resistance to further dehydration and reduce molecular diffusion, therefore stopping all chemical reactions and ensuring complete dormancy over prolonged periods (Billi and Potts, 2000). Sucrose and trehalose have been found to contribute to desiccation tolerance in cyanobacteria *Phormidium autumnale* and *Chroococcidiopsis* sp. (Hershkovitz et al., 1991) and trehalose alone in *Nostoc commune* (Sakamoto et al., 2009). These sugars are also thought to enhance freezing tolerance due to the stabilization of membranes and biomolecules (Strauss and Hauser, 1986; Tanghe et al., 2003), suppression of ice crystals growth (Crowe et al., 2004), and reduction the osmotic effects of freezing (MacKay et al., 1984).

6.2. SECRETION OF EXTRACELLULAR POLYSACCHARIDES

The secretion of extracellular polysaccharides (EPS) contributes to both increased freezing and desiccation tolerances. In their natural habitats, cyanobacteria often form macroscopic colonies and mats, in which filaments are embedded into EPS, or groups of cells are surrounded by EPS capsules (Helm et al., 2000; de Los Ríos et al., 2004; Tamaru et al., 2005). EPS are highly hydrophilic solutes, which are composed of various carbohydrates including glucose, xylose, galactose, and uronic acid (Helm et al., 2000). They conserve water content during desiccation and contribute to water absorption when rehydrated (Ophir and Gutnick, 1994; Shaw et al., 2003). Natural colonies of *Nostoc commune*, which contain EPS, show a much higher resistance to desiccation and freezing than did those in which EPS had been mechanically removed without destroying the cells (Tamaru et al., 2005). EPS is thought to provide desiccation tolerance due to its stabilization of membrane vesicles, as well as enhancement of the stabilizing effects of sucrose and trehalose (Hill et al., 1997). Vincent (1988) suggested that EPS enhanced freezing tolerance because they prevented from ice nucleation in immediate vicinity of cells, thereby avoiding the mechanical damage caused by ice crystals. In response to drying/rehydration cycles, cyanobacteria (*Phormidium*, *Nostoc*, and *Chroococcidiopsis*) can synthesize water-stress proteins; however, their exact functions are not known (Hill et al., 1994).

Other consequences of long-term desiccation is damage caused by UV and intensive photosynthetically active radiation, which produce cross-linking between reducing sugars and amino acids, creating damage within nucleic acids, photosystem II proteins, ribulose-1,5-bisphosphate carboxylase, nitrogenase, and phycobiliproteins (Ehling-Schulz and Scherer, 1999; Potts, 1999). Intensive light is especially harmful to inactive cells because the reparative enzymatic mechanisms cannot function due to the cells' arrested metabolic activity (Potts, 1999). In order to avoid light-induced damage, cyanobacteria produce protective pigments such as scytonemin (extracellular lipid-soluble sheath pigment) and mycosporine amino acids (intra- or extracellular water-soluble compounds) together providing an effective screen against harmful UV radiation (Dillon and Castenholz, 1999; Ehling-Schulz and Scherer, 1999). In addition, cyanobacterial cells which are exposed to high solar radiation can be damaged by the production of reactive oxygen species such as singlet oxygen, superoxide, and hydrogen peroxide (Vincent, 2007). Carotenoids (accumulated intracellularly) are well-known antioxidants, which can serve as photosynthetic accessory pigments and as cellular protection against reactive oxygen species (Mueller et al., 2005). Highly pigmented cyanobacterial mats are commonly present in both the Arctic and Antarctic (Mueller et al., 2005).

6.3. ANTIFREEZE PROTEINS AND ICE-NUCLEATING AGENTS

Another group of compounds which, as far as we know, have not yet been found in cyanobacteria, but may possibly be present, are ice-active substances such as antifreeze proteins (AFPs) and ice-nucleating agents. It is commonly believed that cells, including cyanobacterial, cannot tolerate intracellular freezing at natural cooling rates (Mazur, 1984). Therefore, cyanobacteria probably produce these compounds and thus survive freezing periods by preventing intracellular freezing and by modifying the structure of extracellular ice crystals. One possible mechanism by which cells are injured by freezing is the mechanical disruption of membranes and the cell envelopes. AFPs bind to ice crystals and modify their structure, preventing mechanical damage to the cells (Davies et al., 2002). It was demonstrated by Raymond and Fritsen (2000) that Antarctic cyanobacterial mats produce ice-active substances, possibly of proteinaceous nature, which incorporate themselves into ice crystals and modify their morphology in the same way as do antifreezes in fish.

It is known that cells can significantly supercool before freezing (Turner et al., 1990; Lundheim, 2002). In general, the more the cells supercooled before freezing, the more damage is sustained when the water freezes (Worland and Lukešová, 2001). The role of ice-nucleating agents is in the promotion freezing of the extracellular water at high subzero temperatures (Worland and Lukešová, 2001; Lundheim, 2002). This causes water to outflow from the cells and prevents from the supercooling of the cytoplasm, which is followed by intracellular freezing. Upon freezing, up to 90 % of cell's water can be removed, and residual 10 % cannot freeze at any temperature (Mazur, 1984). This mechanism is known as cryoprotective dehydration (Wharton et al., 2003). Although these agents have not been found in cyanobacteria themselves, ice nucleation at high subzero temperatures can be caused by both fungi and bacteria, which live in associations with the cyanobacteria. It has been demonstrated that field-collected *Prasiola crispa*, a eukaryotic green algae, had a mean freezing point 10° higher compared to axenic culture (Worland and Lukešová, 2001).

7. Conclusions

Despite the fact that the high stress resistance of polar cyanobacteria is a well-known phenomenon, as well as the mechanisms which contribute to their resistance having been broadly studied, little is yet known about the dormant stages of cyanobacteria. Many questions remain which need to be answered. Which physiological, ultrastructural, and biochemical properties do the dormant stages of polar cyanobacteria have? What seasonal changes occur within the cells? Which environmental factors or which combinations of them induce a dormant state in polar cyanobacteria? Is there any cross-resistance to the various environmental insults? What are the differences in resistance between taxa and between species originating from different areas (polar, temperate, and tropical regions)? And there are many, many other questions.

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CAN THE EVOLUTION OF MULTICELLULARITY BE ANTICIPATED IN THE EXPLORATION OF THE SOLAR SYSTEM?

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1. Introduction

Understanding the evolution of development in multicellular organisms is one of the most challenging problems in biology, along with the still-to-be understood origin of life on Earth (and in the universe), the central core of the modern science of astrobiology. One giant step in this direction has been taken by John Tyler Bonner (Bonner, 2001). He focused on a significant transition in the evolution of the Earth biota (Maynard Smith and Szathmáry, 1995, Ch. 12), when development was not complicated by the billion years that followed the late Proterozoic, at a time when the Earth had witnessed over two billion years of microorganism evolution. Some progress is possible with the development of a model for the origin of multicellular organisms based on the idea that it had a selective advantage to be multicellular; as in poor environments, microorganisms could use each other as nourishment to survive (Kerszberg and Wolpert, 1998).

The problem of the origin of metazoans is pivotal for astrobiological research. Besides the three possibilities in the quest for simplicity outlined by Bonner (cf., Sect. 2), in the long term, the exploration of the solar system seems to be another way to elucidate the key factors of evolutionary development. This may be attempted on the Galilean moons of Jupiter (cf., Sects. 3 and 4) or on Mars in spite of the difficulties faced by the fleet of past, present and future missions.

During the past decade, in the wake of the Mars Allan Hills meteorite controversy (McKay et al., 1996), the discovery of extrasolar planets (Perryman, 2000; Santos, 2008), evidence of life in extreme environments (Pikuta et al., 2007; Morozkina et al., 2010) and complex organic molecules in outer space (Kwok, 2009), as well as new research on the origins of life (Chyba and Hand, 2005; Schuster, 2010), provided the scientific foundation for studying the origin and distribution of life in the universe. By 1998, a detailed astrobiology road map was in place to answer three questions:

1. How does life begin and evolve?
2. Does life exist elsewhere in the universe?
3. What is the future of life on Earth and beyond?

NASA founded the Astrobiology Institute and funded academic and research institutions around the USA to undertake research to answer these questions (Dick and Strick, 2004).

In principle, it would be desirable to obtain preserved samples from putative biospheres, in a returning probe to Earth, in order to perform exhaustive analyses in search for biomarkers, most desirably putative forms of life showing high complexity. From the engineering point of view, there are many bioethical issues involved. Furthermore, the quest of information on the evolution of biotas from planets around other stars seems to be not feasible with present technology, particularly in two areas of research that employ direct but remote observation:

- Direct visualisation of living organisms (Schneider et al., 2010): The shape of an organism 10 m in length and width and a spatial resolution of 1 m would be required. Even on the putative closest exoplanet, Alpha Centauri, the required baseline B would be at 600 nm $B = 600{,}000$ km (almost the Sun's radius).

  In reflected light, the required collecting area to obtain 1 photon per year in reflected light is equivalent to a single aperture of $B = 100$ km. In addition, if this organism were moving with a speed of 1 cm/s, it would have to be detected in less than 1,000 s. All these numbers are unrealistic, unless laser-trapped mirrors, as proposed by Labeyrie (1996), are realised. But as pointed out by Schneider and collaborators, in their present conception, laser-trapped mirrors are fragile in the solar wind.

- SETI: The existence of so many exoplanets discovered up to the present (Fridlund et al., 2010) increases our expectations to find intelligent life forms evolving elsewhere in the universe. In spite of uncertainty over the origin of life on Earth, a landmark paper in 1959 spurred on the search for intelligence elsewhere. Its authors, physicists Giuseppe Cocconi and Philip Morrison, argued that artificial radio signals at a 'magic frequency' would be evidence of intelligent life. In the early 1980s, NASA began building a programme to detect such signals.

This ultimately produced a customised 'multichannel spectrum analyser', which became active in 1992 (Morrison et al., 1977). However, congressional politics wound up any SETI observations under the auspices of the US government only a year after they began. The remnants of the NASA SETI programme were transferred to the non-profit SETI Institute in California. With private funding, the SETI Institute has continued to search with the multichannel spectrum analyser and also secured funding for the Allen Telescope Array—an array of some 350 radio dishes that are each 6 m in diameter, which will operate as a dedicated SETI facility 24 h a day. Smaller SETI endeavours are carried out around the world by a variety of institutions. So although no longer government funded, the search for cognitive exobiology remains in place.

These examples, as well as further astrobiological research, rest under the implicit but simplifying assumption that exogenous life can achieve enough complexity to be detected. Altogether, this favours *in situ* analyses to be performed by probes in different places in the solar system, where conditions seem to be within the range to support life, at least the kind that is analogous to how we know it on Earth.

Even the simplest known unicellular organisms on Earth are capable of homeostatic adaptation and are genetically and metabolically complex. The capabilities for homeostasis are, in a sense, mandatory evolutionary precursors for the ability for differentiation. Even in unicellular organisms, homeostasis is a precursor for the further increase in complexity, involving dramatic changes in the signalling, cell cycle regulation, etc., that probably allowed the emergence of multicellular lineages (Hoenigsberg et al., 2008; Sebé-Pedrós et al., 2010). In this chapter, we have a twofold aim. First, we want to recount and evaluate how 'universal' are the conditions for the emergence of multicellularity. Arguably, multicellularity can be thought to be a prior step in the evolution of traits with high complexity, such as intelligence and language. From this remark, it does not follow that we can expect the latter to be an inevitable consequence of the former. We know that the selective pressures leading to human intelligence and language capabilities must be quite specific, and that the physiological possibilities of organisms are not enough to promote them (Maynard Smith and Szathmáry, 1995, Ch. 17; Fitch, 2010). Yet, multicellularity can be regarded as a prior step to achieve increased degrees of morphological complexity, range of sizes and novel traits that allow using empty niches. After all, it was not until nearly after 80% of the history of life on Earth when differentiated multicellularity appeared and which resulted on the early Ediacaran fauna and the great Cambrian explosion (Glaessner, 1983; Cloud, 1986). The latter ultimately led to traits unimaginable for an ecosystem composed of unicellular (or simple multicellular) organisms. We are aware that multicellularity might not be the only route towards complexity, but we consider that it is among the simplest strategies to achieve it.

A question that we would like to answer is, given the easiness with which multicellularity developed on Earth (about 25 times to the best of our knowledge; Buss, 1987; Bonner, 2001), can we expect that, if life in a unicellular form exists elsewhere in the solar system, multicellular forms will also be present? We will address this question in terms of the possible hereditary mechanisms and constrains that we could expect in any replicating entity composed by material building blocks (as opposed to information based). This would support the large body of astrobiological research that works under the 'complex life assumption' and which covers not only the theoretical background but also the practical aspects, that is, the search for biosignals in the solar system.

This will in turn lead us to our next goal: Can we devise a technique to identify and characterise simple multicellular organisms in the next generation of space missions, aimed specifically for the search of life in Mars and in the Jovian moons? We will try to base our proposals making the fewest biological assumptions, in order to avoid the inherent bias to search for life as we know it, and with the use of currently existing technology: spectroscopy, microscopy and cytometry.

2. How Can One Simplify the Mechanism of Development?

2.1. BASIC PRINCIPLES THAT UNDERLIE DEVELOPMENT

One might argue about the details of development that those details *are* development, but we will take a different approach. It seems that there are some basic principles upon which all the details may rest. The present chapter focuses on how to find those principles and what they might be. We consider three ways along which we could pursue this quest for simplicity:

- The straightforward, descriptive biological approach where the mechanisms are at least superficially exposed.
- The beginnings of multicellular development yield another clue. One might assume that at first, only the minimum steps necessary were present. What happens inside a cell is incredibly complex despite its small size, but with the evolutionary origin of multicellularity, some minimal signals between cells must have taken place, giving rise to the origin of multicellular development. Those extracellular beginnings were simple and were only subsequently followed by an increase in complexity.
- Mathematical modelling. One can ask what is the simplest way to achieve some developmental change in form. This is an approach to which we shall return periodically to see how it fits in with our more central message of what might have been the nature of the first cell signalling when multicellular development arose in early evolution.

These are not the only ways of achieving simplicity, for there are others as well. To give a well-known example, the foundations of molecular genetics were achieved by Max Delbruck and Salvador Luria using viruses (bacteriophages), and by the very simplicity of these naked, parasitic genes (and their rapid rate of reproduction), it was possible to gain an extraordinarily deep understanding into the fundamental nature of the effects of mutations. The use of viruses to solve important biological problems remains important today, as shown recently by Burch and Chao (1999), who have elucidated some basic questions of population genetics, again by taking advantage of the molecular simplicity of viruses.

2.2. COMPROMISING THE DEVELOPMENTAL STAGES: THE UBIQUITY OF TRADE-OFFS IN THE TRANSITION TO MULTICELLULARITY

Among the major evolutionary transitions, multicellularity (and perhaps sexual recombination) enjoys the privilege to have evolved many independent times, at different taxa and in different ways. This is a gift that allows us to understand the causes and benefits of this trait in some detail. The main steps in the evolution of multicellularity are succinctly summarised (Bonner, 2001; Grosberg and Strathmann, 2007): What we have, then, is the evolutionary origin of multicellular development. First, there was a selection for an increase in size by becoming multicellular, and once achieved, there was a selection for a better integration, a better coordination of the adhering cells to compete effectively for energy and for a way to reproduce successfully. Then, with each successive step of size increase, propelled always by the fact that the uppermost size niche is never filled, there has been a further selection pressure for integration and coordination, often by new and innovative devices to accommodate the newly created larger organism.

In dissecting the evolutionary stages of multicellularity, we must discriminate between *colonies of cells*: The individual cells might be related or not, but the colony as a whole works for a common good. The *undifferentiated multicellulars* are composed by cells that are all related with each other. These essentially reproduce clonally (at least a few times) and remain together after the division. In the *differentiated multicellular* organisms, the cells can adopt distinct specialised function, which usually affects their morphology, patterns of gene expression, etc., while still being composed by related cells developing from a single mother cell according to a specific developmental plan. Colonial forms are found in archaeans, bacteriae and eukaryotes, while true multicellular organisms are primarily in eukaryotes. Differentiated multicellularity is the one that involves many complex traits involving signalling and cell cycle regulation and which has allowed for highly complex organisms to develop.

The precise mechanisms that trigger and propel the passage to multicellular organisation seem to lie on the trade-offs between the uses of resources or traits from which the cells can benefit (Michod, 2007). Ultimately, individual cells gain or lose reproductive success by allocating varying amounts of resources to distinct vital functions or traits. For example, in becoming colonial, the individual cells give up their free motility (dispersal) but gain survival by the advantages of size (Bonner, 2001) or by minimising the interaction with non-cooperative individuals (Pfeiffer and Bonhoeffer, 2003). Also, cells might allocate their individual resources to either reproduction or specialised a function (e.g. photosynthesising), achieving in this way a differentiation between cell types (Michod et al., 2006). Even for simple organisms like cyanobacteria, the division of labour among cells is a stable solution for heterogeneous environments. However, each cell cannot individually perform the two ways of energetic harvesting: fermentation and photosynthesis

(Pfeiffer et al., 2006). In metazoans, a trade-off comes from the cytoskeleton because cells employ its elements to either form the mitotic spindle and divide or to assemble the cilia machinery and disperse (Buss, 1987; Solari et al., 2006). From different points of view, we confront the existence of trade-offs in the evolution of development. These trade-offs reflect the conflict that critically determine whether for a cell it results convenient to remain free or to include itself as part of a multicellular individual. Ultimately, a more efficient signalling system reflects the evolutionary maturity where conflicts are avoided and development proceeds efficiently (Hoenigsberg et al., 2008).

These examples illustrate that the existence of trade-offs between functions and traits is imposed as constraint to what individual cells can achieve. A mixed strategy, to which these individual cells can opt, is their aggregation and the subsequent division of labour. In this way, multicellularity confers the benefits of the different individual types or advantages at once, plus the conveniences of the emergent aggregated multicellular individual.

Besides the existence of a trade-off, a second but equally critical factor is that of genetic conflict. Within a colony, a mutant may appear that confers such individual a clonal advantage over the other cells. This mutant will first parasite the colony and eventually disrupts the multicellular development. Thus, mechanisms have to evolve in order to minimise the risk of appearance of these genotypes. As suggested above, cooperative cells might have already solved this problem (Pfeiffer and Bonhoeffer, 2003), for example, by some kind of cellular kin recognition, so when in passing to the multicellular stage, genetic conflict is a minimal disadvantage. Besides, because in multicellular organisms the cells are genetically related, genetic conflict is also kept to a minimum. But mechanisms to punish cheaters that only take advantage of the colony might be required, including cell death (Kerszberg and Wolpert, 1998).

Arguably, trade-offs can be considered a universal feature that is expected in virtually any type of reproducing entity evolving under ecological constraints. However, in the astrobiological context, assumptions on hereditary mechanisms are harder to argue. For instance, whether genetic conflicts can arise depends on such mechanisms. Still, in our hereditary system, genetic conflict is more an antagonistic factor, a problem to solve, than a requirement for the emergence of multicellulars. We will retake this part of the discussion in Sect. 2.3.

2.3. HANDICAP OF BIOLOGISTS IN RELATION TO DETECTION OF MULTICELLULARITY

We have been arguing that the potential discovery of exogenous life, for example, in Europa or Mars, is of immediate urgency. Under the assumption that life exists in any of these places, two possible scenarios unfold: The exogenous life forms are either unrelated to life on Earth or they are related (e.g. by panspermia). In either case, the search for multicellularity in the solar system becomes relevant because it might tell us about the ‘universality’ of the traits that Earth’s living creatures have developed.

We have no idea whether what we have learned about evolution is of universal validity. What has been called the *fundamental handicap of biologists*, ‘our knowledge in biology is restricted to only one example’ (Shklovskii and Sagan, 1966, Ch. 14), raises the question whether in attempting to do astrobiology we are instead just extrapolating biology to exoplanets, whose normal conditions are instead extreme on Earth. This line of research on extremophiles can rationalise such an extrapolation. This can be a trap, since we have been tempted to think that because life is able to exist on such exceptional habitats, then it could emerge in those environments. As a matter of fact, for long, we thought that the Archaean extremophiles lied at the root of the tree of life, but recently, this assumption has been challenged: These extremophiles may be instead subsequent adaptations of organisms that lived in moderate conditions (Gribaldo and Brochier-Armanet, 2006). This observation has not been fully taken into account in astrobiology.

In addressing the likelihood of the existence of exogenous multicellulars, are we confronted with this fundamental handicap? There is a very good body of mathematical modelling that has studied the origins and evolution of multicellularity. But how much of this can be extrapolated is evidently not clear. However, because many of the mathematical and computational models overlook many biological details (from signalling to inheritance), it suggests that some of the arguments are to some extent robust.

Yet, in practical terms, the search for complex forms of life is very much handicapped. Even in our own biota, it is easy to think of counterexamples to the simple stacked-cell archetype: (1) intertwined tissues (e.g. neural and gangliar) and (2) coenocytic organisms; they have pseudo continuous tissues where a compartmentalised yet continuous cytoplasm hosts multiple nuclei (e.g. the Bryopsidales algae, *Rhabdocalyptus*, the glass sponge) and (3) progressively contained cells, like Russian dolls, where one cell encloses other cells, which in turn enclose other cells, and so on (e.g. *Volvox*). We can imagine many alternative ways of complex multicellular-like organisations, but it would be most exciting to discover those arrangements that we did not conceive even theoretically and for which detection is even harder to anticipate.

2.4. DARWINIAN AND LAMARCKIAN EVOLUTION: THE ROLE OF HEREDITY FOR THE EMERGENCE OF MULTICELLULARITY

We suggested above that the transition to a multicellular stage is accompanied by trade-offs, and that in such transition, genetic conflict is a major problem to solve by the emerging species. We also advanced the hypothesis that genetic conflicts actually arise as a consequence of the limited and digital inheritance system that we know and have. (To be clear, our inheritance system, DNA, is practically unlimited due to the enormous amount of possible states. However, it is typically the case that because of the low mutation rates, there are simultaneously only a few numbers of mutants appearing in a population in a short interval of time, between the appearance of the first mutant and fixation of any of the genotypes. Thus, the available states over which selection can act are limited.) Other types of inheritance (Szathmáry, 1999) might not incur into genetic conflicts but may have other kind of hindrances of their own. However, under these distinct forms of inheritance, individual cells may find it detrimental or beneficial to become part of multicellular individuals. In any case, *some kind* of inheritance must exist, and trade-offs are expected. How much can we anticipate with these two elements? We have to consider the possible kinds of replicators, which roughly can be classified into four types. Replicators can have limited or unlimited heredity and can be genotypic or phenotypic.

If inheritance is *unlimited* (continuous traits with affected by polygenes approximate this situation), then there is a huge possibility to fine-tune the factors that stabilise multicellular aggregations. Specifically, signalling and homeostasis can be nurtured through selection to be sensitive to differential changes, rather than to a discrete presence/absence set of signals. In turn, this fine regulation may allow for refined mechanisms to avoid conflict (e.g. Michod, 2007). We will not deepen into this type of inheritance, since this class is realised by life as we know it on Earth, and most research in multicellularity comprises this class (see Grosberg and Strathmann, 2007 for a review).

Limited inheritance occurs when the number of states (e.g. mutants) is far much smaller than the number of individuals in the population. Only a few genes may allow enough degree of differentiation to support colonial aggregations. However, the potential for the evolution of novel complex traits might be strongly impaired by the few numbers of possible genotypes. Although this might be a reason to fix increases in the size in the genome in exotic life forms, there might well be constraints on the number of their analogous genetic units. Nevertheless, there is no reason to discard mechanisms that avoid genetic conflict and thus fix a stable multicellular level. As an obvious analogy, consider relatedness among the cells in a multicellular organism. Development from of a single cell might enforce relatedness, and this is not debauched by the limitedness of the inheritance system. The advantages of a multicellular might still apply over those of the unicellular alternative. As mentioned above, some traits with strict Mendelian inheritance could mimic this type of inheritance, and no wonder that many organisms have remained in evolutionary stasis, perhaps stuck in a local fitness optimum without apparent modifications for hundreds of millions of years.

Both types of genetic inheritance can support evolutionarily stable multicellular states, as long as mechanisms to avoid selfishness can exist. However, if life elsewhere emerges as a *phenotypic* replicator, there is little chance that differentiated multicellularity can arise. In this inheritance system, any phenotypic change is passed to the next generation in a Lamarckian fashion. Thus, a *phenetic conflict*—analogous to genetic conflict—is entangled with differentiation. Hence, a multicellular organisation would be much a more challenging evolutionary transition, compared to that of the genetic inheritance. Nevertheless, this does not exclude the possibility of cooperation and of strict regulation (essentially intra-colonial selection) of the potential defectors.

Differentiated multicellularity seems unlikely, for two reasons. First, because any phenotypic difference is heritable, the distribution of cell types (and their replication rates) within the organisms would be driven by, or at least correlated with, the environmental fluctuations. At best, this would be a way of senescence, but any strict control of cell types and proportions seems far-fetched. This immediately limits the possible degree of complexity (amount of differentiation into tissues, organs, etc.) that can evolve. The second reason is related to the effects of canalisation. In our genetic system, mutations and environmental factors are often ‘canalised’, meaning that their effect is buffered by other genetic modifiers and epigenetic factors, so that the phenotype shows minimal variation resulting from such mutations or environmental effects. This has played a critical role in the evolution of development. But by definition, canalisation is absent in phenotypic replicators.

In particular, if the phenotypic replicators have unlimited heredity, we face the worst-case scenario. This is allusive to the blending inheritance idea, where selection quickly consumes all variability and evolution halts. Similarly, differentiation would simply come to a standstill by an ‘optimal’ cell type that would propagate in the whole organism, leading to an evolutionary dead end. A possible way out would be a threshold mechanism in the replication, where each phenotype has metabolic basin of attraction that buffer slight phenotypic deviations, but if a threshold is crossed, the new phenotype progresses. In this way, simple versions of differentiation are possible but without a major increase in complexity. A clear-cut example of this type of systems is the lipid world’s replicating vesicles (Segre et al., 2001). This type of organisms (so far only a theoretical construction) consist of vesicles that have certain proportions of different lipids. These proportions convey information transduction and are heritable, at the same time. However, the number of possible states is limited, and the modification of the lipid proportions by environmental addition of certain chemicals is possible. Yet, these external modifications are inherited; thus, development is simply unstable. Although this model has been proposed as an alternative for life’s origin for its hereditary properties and physical-chemical plausibility (Shenhav et al., 2003), it is clear that it cannot account for increased levels of complexity.

Summarising, a colonial organisation and undifferentiated multicellularity are valid structures that could emerge in any kind of hereditary system (with different degrees of difficulty, though), which could confer the individuals with some additional advantage by size, cooperation, etc. Differentiated multicellularity would require hereditary mechanisms of a very specific kind: digital information transduction across generations. This narrows our expectations for detecting complex life form. However, we ultimately rely on evidence to proceed in our intellectual quest, but with these educated guesses, we can begin our journey towards the search for complexity.

3. Detection of Biomarkers in Solar System Exploration: Possible Single-Cell Evolution

The considerable evidence for the presence of a liquid ocean over a silicate core makes Europa a candidate for the emergence of a second evolutionary pathway of autochthonous life. A fundamental subject in astrobiology is the origin of habitable ecosystems—a question in geochemistry—rather than the alternative search for the origin of life itself—a question in chemical evolution (Chela-Flores, 2010). Since certain bodies may share a similar geophysical past with the Earth, a question suggests itself: *Can available instrumentation be the 'pioneer' in the discovery of habitable ecosystems in geophysical environments similar to the early Earth, where oceans were in contact with a silicate core?*

Evidence already exists for a positive answer. Although inconclusive, Dalton et al. (2003) compared the spectra of Europan surface elements with experiments where extremophile microorganisms were exposed to conditions that recreated the moon's surface radiation, pressure and temperature. The spectra showed good agreement with each other for certain bandwidths. Although encouraging, this is inconclusive. Spectrograms of small Europan ice regolith samples might give a more certain conclusion. In any case, we propose an alternative, based on a central piece of this dilemma: the element sulphur (S).

A reliable window on the nature of the early terrestrial habitable ecosystems is the Pilbara craton (Australia), a rich fossiliferous archive of the early steps of evolution, having preserved details of ancient hydrothermal vents. It contains a ~3.47 Ga barite deposit with microfossils of a complex set of sulphate-reducing bacteria (Shen and Buick, 2004). The large spread in the $d^{34}S$ values provides the earliest reliable biomarker from the early Earth. Europa may represent the only other case in the solar system in which liquid water has been in contact with a silicate core over geologic time in perfect analogy with the early Earth (Bland et al., 2009). It is therefore reasonable to make the following hypothesis:

> *On Europa, the presence of hydrothermal activity at the interface of the silicate core and the ocean can provide a variety of chemicals playing a role in sustaining microbial life at the ocean floor.*

This hypothesis is subject to a feasible experimental test: Europa's non-ice surficial elements were found to be widespread, patchy and, most likely, endogenous. We argue that penetrators should be inserted in orbital probes in the future exploration of Jupiter's system (Gowen et al., 2009). There are alternative views on the effect of space weather on the radiation-induced S-cycles produced on the surficial molecules, but S is common to both interpretations (Carlson et al., 1999; McCord et al., 1999). Fortunately, just because of this presence of sulphur on the icy surface of Europa, we are presented a unique opportunity to identify reliable biomarkers, since there are ways of identifying biomarkers by simply looking at the isotope anomalies when we suspect that microbes may have been present in the environment. The redistribution of the primordial isotopic mixtures can be followed up in terms of the appropriate parameter, namely, the ‘delta’ parameter (Chela-Flores, 2006):

$$\mathbf{d^{34}S = [(^{34}S/^{32}S)_{sa}/(^{34}S/^{32}S)_{st} - 1] \times 10^{3}0[^{0}/_{00}, CDM}$$

For simplicity, this function will be referred to as the delta-34 parameter, or simply as the delta parameter. Its value is close to zero when the sample coincides with the corresponding value of the Canyon Diablo meteorite (CDM), a triolite (FeS) that was found in a crater north of Phoenix, Arizona. This parameter allows a comparison of a sample (sa) with the standard (st) CDM. The relevant terms are the dominant sulphur isotope (^{32}S) and the next in abundance (^{34}S). In fact, $(^{34}S/^{32}S)_{st}$ coincides with the average terrestrial fraction of the two most abundant isotopes of sulphur. We obtain positive values of the delta parameter when by comparison we have a larger quantity of the less abundant isotope ^{34}S. Nevertheless, the advantage of having defined such a parameter is that negative values will indicate an abundance of the most abundant isotope ^{32}S. Besides, we remark that in non-terrestrial solar system materials (such as lunar dust or meteorites), the values of the delta parameter are close to the CDM average. This, in turn, signifies that biological processes will be more easily recognisable when sulphur, rather than the other biogenic elements (hydrogen, carbon or nitrogen), will be considered. There is an overwhelming amount of data supporting the view that metabolic pathways of sulphur bacteria have enzymes that preferentially select the isotope ^{32}S over ^{34}S. As pointed out above, this will be reflected in habitats that are depleted of ^{34}S. In other words, in lakes, seas or oceans, where the sulphur microbes are present, the value of the $d^{34}S$ parameter will have characteristic large negative values.

This suggests that focusing on sulphur might be more reliable means for estimating biological effects (if any) on Europa. In contrast, to the isotopes of hydrogen, carbon or nitrogen, sulphur shows fractionation with a relatively narrow distribution range in meteorites, as well as the Moon fines, breccias and fine-grained basalts retrieved by the Apollo missions. In the case of meteorites, these values are about 2‰ relative to the standard CDM average (Kaplan, 1975; Farquhar and Wing, 2005). The measurements of isotopic ratios of the biogenic elements were not considered during the Galileo mission. Fortunately, they are in principle measurable in future missions to Europa. A remarkable and most useful phenomenon is that the largest known S-fractionations are due to microbial reduction and not to thermochemical processes.

Besides, sulphate abiotic reductions are generally not as large as the biogenic ones (Kiyosu and Krouse, 1990). From experience with a natural population, this type of biota is able to fractionate efficiently S-isotopes up to $d^{34}S$ of −70‰ (Wortmann et al., 2001). Dissimilatory sulphate reducers are ubiquitous on Earth, producing the largest fractionations in the sulphur stable isotopes. These microbes are widely distributed in terrestrial anoxic environments. Consequently, they are the most evident candidates for the microorganisms populating a habitable Europan ecosystem. Microbial fractionation of stable S-isotopes argue in favour of penetrators for surveying the surface of not only Europa but also Ganymede, where surficial sulphur has been detected (McCord et al., 1997). The Europa Jupiter System Mission (EJSM) intends to explore in the 2020s both of these satellites.

According to our hypothesis, we predict that penetrators (supplied with mass spectrometry) should yield different results for fractionated sulphur. The icy patches on Europa should give substantial depletions of ^{34}S, while measurements on Ganymede should give significantly lower values for the depletion of ^{34}S. (As the largest of the Galilean satellites lacks an ocean-core interface, according to our hypothesis, it would not be able to support life.) These diverging results—large minus d^{34}S for Europa and small minus d^{34}S for Ganymede—would provide a clear test for the hypothesis that a habitable ecosystem has emerged on Europa. The test is within reach of available technology for planning the eventual penetrator payload.

4. Detection of Biomarkers in Solar System Exploration: Multicellular Evolution on the Europan Surface and the Martian Poles

4.1. INSTRUMENTATION SUGGESTED FOR A FUTURE SPACE MISSION

As discussed above, reduction states of sulphur in different forms may provide indirect but reliable evidence of life. Yet, this is far from any organismic characterisation. Perhaps, a following step would be to try to determine the range of traits of the putative organisms.

The most reasonable alternative is direct imagining. We are expecting to deal mainly with micro- and mesoscopic organisms, which require powerful optical or electron beam microscopy. Both technologies have already been implemented in the Phoenix Mars Lander, a mission that successfully reported the apparent presence of water including ice-rich regolith (in the soil) as well as snow (Smith et al., 2009; Whiteway et al., 2009), but unfortunately, there was no organic matter on the landing site. Not only organic matter was not detected by the wet experiments but also the micrographs showed no signs of organisms. Although the mission was not aimed at detecting biosignatures, the power of the microscope was enough to detect even small bacteria of 0.1 µm. This lack of evidence does not rule out the presence of life on Mars. A more probable place to detect life would be in the polar caps, where the dune spots have diffusion patterns that suggest biotic dispersion (Ganti et al., 2003; Horvath et al., 2009) and which most abiotic models fail to explain (Kereszturi et al., 2009).

Another phase, which is at the core of our interest in this chapter, is to characterise some organismic properties. With a rather simple technology, it may be possible to have a significant insight about the complexities that may be abundant in exogenous life. The favourite targets at the moments are Mars (in the poles) and Europa. In both worlds, life specimens might be trapped in the surface ice, which is in principle accessible to the penetrators. A piece of ice containing some organismic tissue can be minced in such a way that the biological sample, if multicellular, is disrupted into smaller constituents, tentatively cell analogues. It would be hard at this point to perform any biochemical or calorimetric analyses, particularly in the Jovian system.

Since we expect that the hypothetical organisms in Europa have metabolism based in sulphur, we require techniques that allow monitoring of distinct chemical states of this element. Although sulphur compounds are very elusive, particularly *in vivo*, spectroscopy in the X-ray wavelengths (near 2.47 eV) shows a characteristic peak (termed *K-edge*) that allows discerning among distinct radical types involving this element (Rompel et al., 1998). The advantage of spectroscopic observation of sulphur is that it is non-invasive and therefore may allow coupling with a flow cytometer.

Cytometry is a technique commonly used in diagnosis of diseases and experimental biology. This technique passes a suspension of cells through a narrow channel that is monitored by an optical device that characterises every single particle. Conventionally, cytometers employ optical emissions at clearly distinct wavelengths—usually those resulting from labels from specific antibodies (e.g. green fluorescent protein)—to categorise, accurately count and even sort the cells, organelles, chromosomes, etc., according to several physical, chemical, physiological and molecular properties that can be targeted using antibodies. However, as an astrobiological technique, immunologically based labelling seems to us a weak option, owing to the fact that the antibodies aim for specific macromolecules. With Europa in mind, we argue that X-ray absorption spectroscopy can be developed as an alternative to immunological labelling and to wavelengths which are less ambiguous than the infrared and optical spectra (Dalton et al., 2003), basing the categorisation of the putative cells on the spectrum of absorption near sulphur's *K*-edge.

4.2. DESCRIPTION OF THE *IN SITU* PROPOSED EXPERIMENT

An *in situ* experiment demands that the 'preparation' of the samples is simple, quick and energetically efficient. In order to create a suspension from an ice sample directly taken from the regolith, a simple procedure is needed. We assume that the ice contains frozen tissues, which can be mechanically disrupted. The most compelling way for automatisation and payload requirements in astrobiological research is tissue pressing (Singh, 1998) and a 'fine mincing' using the 'Medimachine®' (Novelli et al., 1996). In the first, a sample is pressed through a very fine mesh that disaggregates the tissue (this might require prior melting and filtering of the ice sample). The second is a small apparatus that scraps the sample into tiny particles on the order of microns, the typical size of a mammalian cell.

The disaggregated tissues can then be suspended into a solvent (water, alcohol) and circulated through the flow cytometer for precise scrutiny.

Thus, the instrumentation required for cell analyses and characterisations comply with the engineering constraints. The mass spectrometer has been already miniaturised to weigh a few grams. A flow cytometer can weight on the order of half a kilogram and occupy less than 100 cm^3. The tools for cell disaggregation occupy and weigh only a fraction of that. With astrobiological objectives in mind, it would be a worthwhile challenge to reduce these dimensions even more.

5. Discussion and Conclusions

We have discussed two main arguments in this chapter: Firstly, the likelihood that multicellular life can (or has) emerge(d) elsewhere (with views on Europa and Mars). The complexity level that we could expect would be, to be on a safe margin, colonial and undifferentiated multicellularity. These can be supported by any inheritance system that is based on chemical metabolisms. At the same time, ecological constraint might ease their existence.

Secondly, we have discussed ways to detect and analyse such life forms. We have in mind instruments that could be contained in small penetrators. These instruments could provide us with significant data on the physical and chemical properties of the icy surfaces of Europa or Mars. We aim to optimise our insights in two ways. We go beyond the now canonical goal of detecting life. This is certainly desirable. Our knowledge would advance upon characterisation of such life forms. The twofold gain comes first from identifying exogenous life, and second, from providing information about the evolutionary steps of our own biota.

Life on Europa might be found in the cracks, or we may find biosignatures of it on the icy surface. In either case, the penetrators are valid preliminary suggestions for adequate instrumentation. Direct observation of Europa's ocean, perhaps photographic, must await for the hydrobot/cryobot missions but not in the foreseeable future. Yet, the tectonics of Europa (Greenberg, 2005) stir and mix the ice, which is to our favour, because it can make these samples accessible at some point in the surface from which, in principle, ice samples can be extracted.

Initially, the sulphur isotopes may provide a first evidence for life. But samples can be analysed using a combination of microscopy, spectroscopy, tissue disruption and flow cytometry. All together, these techniques can provide us with an enormous amount of information about (a) the existence of multicellular organisms, (b) their degree of differentiation ('level of complexity') and (c) characteristic of individual cells. Even if we would not find multicellular organisms, the last point entirely justifies the use of the cytometer, since it would allow our first insight into the cell biology of exogenous unicellulars or even of molecular protobiotic replicators of high complexity.

We might find after all that life elsewhere in the solar system, if present, is not entirely unrelated to ours. If life exists on Mars, it is most likely struggling for survival under conditions that are at best extreme to our standards. On the other hand, if life exists on Europa, it is most likely close to its 'initial'. We may consider that the Earth, by comparison, to be at a most favourable stage between these other two, enjoying its golden age of a rich and vast biodiversity. Still, panspermic seeding, even if at a very low frequency raises the possibility that life-to-be-discovered may not belong to independent trees of life. Instead, our first contact with exolife may uncover new branches of the same phylogenetic tree, where the exo-organisms may successfully exploit niches unimaginable for us.

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ANTARCTICA AS MODEL FOR THE POSSIBLE EMERGENCE OF LIFE ON EUROPA

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1. Lake Vostok

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. Its surface is of approximately 14,000 km^2 and its volume is 1,800 km^3. 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 analogue provided by Lake Vostok for the Europa environment seems appropriate (Chela-Flores, 2001). At the time of writing, 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), diatoms, and most interestingly, 200,000 year old bacteria. Besides, it appears that 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 (Priscu et al., 1999). We shall discuss the possibility of the existence of Europan microbes within a liquid water environment sealed by a frozen surface, which is by now known in great detail with the help of the Galileo Mission (1995–2003).

Bacterial density is found to be two to sevenfold 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 (Dudeja et al., 2010). 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). With few exceptions, all other characterized species of the *deltaproteobacteria* are strict anaerobes that respire via the reduction of electron acceptors, such as sulfate, elemental sulfur, iron (III), and Mn (IV) (Lovley et al., 1995).

These bacterial communities are diverse and physically associated. Such ecosystems lead to fundamental questions regarding the physiology and metabolism of several microorganisms. Prominent among these microbes, we have *deltaproteobacteria* and *betaproteobacteria*. *Deltaproteobacteria* are phylogenetically linked with sulfate bacterial reduction, SBR (Shen and Buick, 2004). Detailed analysis on accreted ice has shown that bacterial cells are often associated with organic and inorganic particles (Priscu et al., 1999), implying that a portion of cells within the lake water are not free living. Similar results have been reported for the permanently ice-covered, lakes in the dry valleys (Lisle and Priscu, 2004).

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. Lake Vostok is the largest of more than 140 subglacial lakes (Siegert et al., 2005). The zone of ice layer up to 3,309 m (referred to as I), and the layer between 3,310 and 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. Despite 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, the insulating properties of the overlying icy sheet, and adiabatic lowering of the freezing point (Siegert 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 (Siegert 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 biogenic sulfur may be reaching the surface.

2. Perennially Ice-Covered Lakes in Antarctica and Microbial Mats

Microorganisms constitute the major part of all life on Earth, and they are mainly organized in microbial mats and into biofilms. For this reason, the description of different aspects of microbial mats are fundamental for our deeper understanding

Table 1. Statistics of the dry valley lakes in Antarctica.

Lake or pond	Maximum depth (meters)	Elevation (meters above sea level)	Lake type and morphology of mats
Lake Fryxell	18	17	Perennial ice cover; liquid water, MM, ICM, FM, CLM, APRM, ANPRM
Lake Hoare	34	73	Perennial ice cover; liquid water, MM, ICM, FM, CLM, APRM, ANPRM, PM
Lake Vanda	69	123	Perennial ice cover; liquid water, MM, ICM, FM, CLM, APRM, ANPRM
Don Juan Pond	0.1–2	116	Ice-free
Lake Chad	1	58	Perennial ice cover, liquid water, MM, ICM, FM, CLM, PRM
Lake Bonney	40	57	Perennial ice cover, liquid water, CLM

MM molecular mats, *ICM* intercellar mats, *FM* floating mats, *CLM* columnar mats, *PRM* prostate mats, *APRM* aerobic prostate mats, *PM* pinnacle mats, *ANPRM* anaerobic prostate mats.

of a major cross-section of microbiology in general, and especially for astrobiology. Among 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 our own solar system, such as on Europa, Mars, Enceladus, and Titan. Indeed, 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 thrive as exemplified by the startling colored microbial mats that live in Yellowstone National Park. Microbial mats are also present in volcanic vents on the ocean floor, called black smokers. Other environments suitable for microbial mats are deserts and, specifically, the dry valleys of Antarctica in the McMurdo region that is traversed by striking glaciers. The British explorer 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.

From the point of view of geology and microbiology, some of the best studied frozen lakes are in the Taylor Valley, namely, Lake Fryxell and Lake Hoare. Further north, in the Wright Valley, Lake Vanda is also remarkable for its biota. Among the microbial mats that are permanently thriving in the frozen lakes, there are examples of both prokaryotes and eukaryotes (cf., Table 2).

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* Fritsch, 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.

Table 2. Microorganisms living in the dry valley lakes, Antarctica.

Organism	Domain	Habitat
Cyanobacteria	Bacteria	Lakes Chad, Fryxell, and Vanda
Leptothrix	Bacteria	Lakes Fryxell and Hoare
Achronema	Bacteria	Lakes Fryxell and Hoare
Clostridium	Bacteria	Lakes Fryxell and Hoare
Chlamydomonas subcaudata (phylum Chlorophyta)	Eucarya	Lakes Bonney (east lobe) and Hoare
Diatoms (phylum Bacillariophyta)	Eucarya	Lakes Bonney, Chad, Fryxell, Hoare, and Vanda
Bryum cf. *algens* (a moss)	Eucarya	Lake Vanda

What is most significant is that single-celled eukaryotes are amply represented in this Antarctic biota. Among 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 not only the above-mentioned cyanobacteria, but also heterotrophic bacteria, eukaryotic algae (mainly diatoms), and fungi (Baublis et al., 1991). There are some dinoflagellates *Gymnodinium* and *Glenodinium* in Lake Fryxell, where in addition, protozoan taxa were associated with the algal mats (Cathey et al., 1981).

The existence of these permanently frozen lakes adds an extra bonus to our working model of the Europan Ocean. Modern organisms analogous to the early biota of the Earth are to be found in the dry valley lakes. Most importantly, single-celled eukaryotes are amply represented in this biota. In Table 3, we summarize some of the organisms known to inhabit in these lakes (Doran et al., 1994; Wharton et al., 1983; Parker et al., 1981, 1982; Ellis-Evans and Wynn-Williams, 1996).

The permanently ice-covered lakes of the McMurdo dry valleys present a legitimate earthly analogue to putative Europan ecosystems. The presence of dissolved organic carbon (derived from microbial mats, algae and bacteria), sulfate reduction (one of the most dominating process responsible for organic matter oxidation), and the adaptation of microorganisms (thriving on either oxygenic or anoxygenic photosynthesis) to low temperatures and low light conditions are the three most important analogies to look for while searching for extraterrestrial life or life-sustaining environments in Europa.

In addition, the influence of saline conditions on microbial life in dry valley lakes particularly Blood Falls (a subglacial iron-rich brine) and Don Juan Pond (one of the coldest, saltiest water bodies on earth) is also interesting since in such a system a microbial consortium facilitates a catalytic sulfur cycle which is a result

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

Organism	Domain	Habitat
Diatom shells	Eucarya (Bacillariophyta)	Lake Vostok (ice core, at depth of 2,375 m)
Caloneis ventricosa	Eucarya (Bacillariophyta)	Lakes Chad, Fryxell, Hoare, and Vanda
Hantzschia amphioxys	Eucarya (Bacillariophyta)	Lakes Fryxell, Hoare, and Vanda
Navicula cryptocephala	Eucarya (Bacillariophyta)	Lakes Bonney, Fryxell, Hoare, and Vanda
Chlamydomonas subcaudata	Eucarya (Chlorophyta)	Lakes Bonney and Hoare
Tetracystis sp.	Eucarya (Chlorophyta)	Lakes Fryxell, Hoare, and Vanda
Yeast	Eucarya (Ascomycota)	Lake Vostok (ice core)

of limited carbon supply because of the absence of contemporary photosynthesis (Mikucki et al., 2010). Such systems demonstrate the possibility of biogeochemical process capable of accumulating iron (Fe (II)) despite the presence of an active sulfur cycle. This alternative sulfur cycle could be important if the ocean beneath the surface of Europa contains iron (Fe(III)) (Mikucki, 2009).

3. Microbially Produced Patches in Antarctica: Can They Be Produced on Europa Too?

The extremophiles that are trapped in microbial mats may also be living under the Taylor Glacier in the Taylor Valley, a region that is bounded by the Ferrar Glacier and the Asgard Range. 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. An intriguing feature, named 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. This coloring is analogous to the colored microbial mats that live in the hot springs of the Yellowstone National Park.

Isotopic measurements of sulfate, water, carbonate, and ferrous iron and gene analyses imply that a microbial consortium facilitates a catalytic sulfur cycle that may be 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 sulfur patches were discovered by the Galileo Mission (1995–2003). These stains on the icy surface of the Jovian satellite are suggestive of chemosynthetic products of metabolism. In addition, underneath 4 km of ice in that continent, there is a subsurface lake that presumably contains living microbes. This hypothesis has been confirmed, since microorganisms have been found from accretion ice retrieved from ice 3,590 m below Vostok Station (Priscu et al., 1999). Extrapolation of the data to the lake itself, known as Lake Vostok, may support a microbial population, despite more than 10^6 years of isolation from the atmosphere. These examples are just the "tip of the iceberg," since by means of ice-penetrating radio-echo sounding surveys, we have discovered the existence of about 280 subglacial lakes, most of them being located under the East Antarctic ice sheet (Smith et al., 2009).

All the above facts encourage the search for biogenicity. Our main interest is the search for biosignatures on Europa on the sulfur patches discovered by the Galileo mission (Singer, 2003; Bhattacherjee and Chela-Flores, 2004; Chela-Flores and Kumar, 2008; Chela-Flores et al., 2009).

The Galileo mission discovered the sulfur patches of non-ice elements on Europa's icy surface. These patches have been inferred and confirmed over a 20-year period. They match the distribution of an ultraviolet absorber that was suggested all the way back by the Voyager data (McEwen, 1986). The New Horizons Mission on its way to Pluto has confirmed the patches of non-ice elements (Grundy et al., 2007). Based on combined spectral reflectance data from the solid-state imaging experiment (SSI), the near-infrared mass spectrometer (NIMS), and the ultraviolet spectrometer (UVS), it has been argued that the non-water ice materials are endogenous in three diverse, but significant terrains (Fanale et al., 1999). Effusive cryovolcanism is clearly one possible endogenous source of the non-water-ice constituents of the surface materials (Fagents, 2003). The most striking feature of the non-water surficial elements is certainly their distribution in patches. Implantation would be expected to produce a more uniform surface distribution if the source were ions from the Jovian plasma; it may be argued that if the plasma from the magnetosphere was responsible for the sulfur distribution, some geologic process has to be invoked to allow for a nonuniform distribution (Carlson et al., 1999).

Alternatively, the sulfurous material on the surface may be endogenous. In other words, the cryovolcanism on Europa would be from the bottom of the global ocean, more like the "black smokers" that are found on the Earth seafloor.

4. Discussion

On the strength of our Antarctic models sketched above, we may assume that on Europa, the presence of hydrothermal activity at the interface of the silicate core and the ocean can provide a variety of chemicals playing a role in sustaining microbial life at the ocean floor. To search for life, several options are in principle available. Astrobiological exploration should not be restricted to the remote observation of the surface for three main reasons (Gowen et al., 2010):

1. The likely habitable environments are in the subsurface, where liquid water may be present.
2. The surface has an intense radiation environment where many materials, including organic molecules, may not survive unaltered for a long time. If the

aqueous reservoirs are linked with the surface, by fractures for instance, materials indicative of the habitability may ascend from the interior. But exposed materials will be affected and may lose the signatures from the potential habitable environment. So, remote measurements will be from secondary materials, modified on the surface from the original conditions at the interior aqueous reservoirs.

3. The concentration of biosignatures at the surface may not be sufficient for remote detection.

We discuss some of the possible lines of research. Firstly, the Antarctic lakes are a starting point for the technology of submersibles: Mapping Antarctica's West Lake Bonney, is an ongoing project. This is a 2.5-mile-long, 1-mile-wide, 130-foot-deep lake located in McMurdo dry valleys. Bonney has a permanent ice cover 12–15 ft deep (cf., Table 1 for a comparison with other dry valley lakes). Earlier, we had envisaged submersible technology, under the name of cryobot-hydrobot-coupled probes (Horvath et al., 1997). In spite of the great challenge in proceeding in this direction, the more recent NASA project "ENDURANCE" is providing insights in completely independent light submersibles to map in 3D the geochemistry and biology of ice-covered lakes. (It is an acronym for Environmentally Non-Disturbing Under-ice Robotic Antarctic Explorer.) The nearly spherical submersible is about 2 m (6.5 ft) in diameter and in air weighs more than 1,300 kg (about 1.5 t). Clearly for the eventual use in the Jovian System (beyond the era of the EJSM), this concept has to go through several stages of miniaturization. The Endurance Project is led by Peter Doran with Bill Stone (caver) and John Priscu among the coinvestigators.

Secondly, closer to our time and the next mission being planned, the biogenic hypothesis can be subject to a more feasible experimental test: We may look for cellular life (a third, simpler, and possibly more feasible option will be discussed below). In this case, some penetration tool (beyond the simple effect of the gravitational force) has to be added to the penetrator. One option that has received attention in the literature is that of a mole-like thermal drill designed to cut through the icy surface. Such a device would use heat to melt through the ice and a rotating drill blades to clear away rocky material. The drill would be nestled inside a larger penetrator that would burrow itself into Europa's icy surface. To search for signs of life on Europa, the penetrator may have to go deeper into the surface ice, because space weather conditions erase any cellular traces in the top layer. The drill would be tethered to the penetrator by a communication cable, which would allow data to be sent to the instruments in the penetrator, to be later beamed up to an orbiter (Weiss et al., 2008, 2011).

Finally, biogenicity may be subject to a simpler test. If we do not focus on cellular life, but rather of its metabolic effects in the fractionation of sulfur, we can exploit the Galileo mission's remarkable discovery of Europa's non-ice surficial elements. They were found to be widespread, patchy, and most likely, endogenous. For probing the Europan patches, the most appropriate technology is the penetrator. This approach consists of small projectiles that can be delivered at high velocity to reach just beneath the surface of planets or their satellites for probing samples of surficial chemical elements, among other investigations. The recent interest in lunar science in the UK has led to the development of penetrators by the UK Penetrator Consortium (Gowen et al., 2009).

These instruments can have payloads that would allow a variety of measurements on the Jovian satellite surfaces for the search of biomarkers on Europa and Ganymede. While orbiting instruments can further investigate the nature of Europa's surface and interior, currently, only in situ instruments offer the realistic possibility to detect any evidence of life. The rough surfaces and extreme cold together with an extreme radiation environment mean that Europa is a challenging, but not impossible, object for in situ measurements. Penetrators of relatively low mass (~5–15 kg) are better suited to these conditions than a soft lander, since they are buried on impact in the near-surface regolith, thereby automatically accessing areas subjected to a significantly reduced radiation flux, while easing radiation shielding requirements (Gowen et al., 2010).

We argue that penetrators should be inserted in orbital probes in the future exploration of Jupiter's system. The Europa Jupiter System Mission (EJSM) intends to explore in the 2020s both of these satellites (Grasset et al., 2009). EJSM consists of two spacecrafts operating in the Jovian system: the NASA-led Jupiter Europa Orbiter (JEO) and the ESA-led Jupiter Ganymede Orbiter (JGO). JEO and JGO will execute a coordinated exploration of the Jupiter System before settling into orbit around Europa and Ganymede, respectively. JEO and JGO will carry several complementary instruments to map the Jovian magnetosphere and its interactions with the Galilean satellites, and characterize water oceans beneath the ice shells of Europa and Ganymede.

There are alternative views on the effect of space weather on the radiation-induced S-cycles produced on the surficial molecules; but S is common to both interpretations (Carlson et al., 1999; McCord et al., 1999). The largest known S-fractionations are due to microbial reduction and not to thermochemical processes. Besides, sulfate abiotic reductions are generally not as large as the biogenic ones (Kiyosu and Krouse, 1990). From experience with a natural population, this type of biota is able to fractionate efficiently S-isotopes up to d^{34}S of −70‰ (Wortmann et al., 2001). Dissimilatory sulfate reducers are ubiquitous on Earth, producing the largest fractionations in the sulfur stable isotopes. These microbes are widely distributed in terrestrial anoxic environments, especially in the Antarctic ecosystems described in the present chapter.

Europa's surface is an extremely hostile environment, as a result of space weather, namely, the constant exposure to Jupiter's intense radiation belts. The topmost ice layer is subject to harsh radiation, but changes other than chemical reactions (photolysis, radiolysis) are not to be taken into account since, for instance, there is no high-energy (several gigaelectron volt, GeV) deep-inelastic scattering that would change the nuclear identity of the atomic components of the surficial molecules participating in the above-mentioned S-cycles. Meteoroid gardening might lead to the contamination of the upper surface 1–2 m with regolith (Cooper et al., 2001). This would require penetrating deeper than this depth in order to reach *pristine material*, which would be challenging, even for penetrators. However, since sulfur processing by bacteria may lead to such radical and extreme depletions of ^{34}S compared to exogenous sulfur, even if some of the contaminated surface by the gardening process contains nonbiological sulfur, and penetrators do not reach the above-mentioned depths (1–2 m), robotic mass-spectrometry analysis of the non-water ice elements would identify biologically processed sulfur (e.g., dS^{34} reaching over –(50 to 60) ‰).

Consequently, they are the most evident candidates for the microorganisms populating a habitable Europan ecosystem. Microbial fractionation of stable S-isotopes argue in favor of penetrators for surveying the surface of not only Europa but also Ganymede, where surficial sulfur has been detected (McCord et al., 1997). According to our hypothesis, we predict that penetrators (supplied with mass spectrometry) should yield different results for fractionated sulfur. The icy patches on Europa should give substantial depletions of ^{34}S with penetrators supported by JEO, while measurements on Ganymede hopefully with penetrators supported by JGO, which should give significantly lower values for the depletion of ^{34}S. (As the largest of the Galilean satellites lacks an ocean-core interface, according to our hypothesis it would not be able to support life.)

These diverging results—large minus d^{34}S for Europa and small minus d^{34}S for Ganymede—would provide a clear test for the hypothesis that a habitable ecosystem has emerged on Europa. The test is within reach of available technology for planning the eventual penetrator payload. From what we have said in this chapter, the Antarctic dry valley lakes, as well as the many subglacial lakes, especially Vostok, and more recently Lake Concordia are excellent models for the possible ecosystems that we are unable to probe directly elsewhere in the Solar System. It seems reasonable from the point of view of instrumentation and costs that we should insist on the payloads for both JEO and JGO to include penetrators capable of doing mass spectrometry.

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Biodata of **Rob Hengeveld** author of "***The Likelihood of Extraterrestrial Intelligent Life.***"

Rob Hengeveld After my study at the University of Leiden in the Netherlands, I worked on various ecological problems, gradually focusing on spatial processes, such as the colonization of large inland polders by carabid beetles. After this, I first turned to biogeography and then to invasion research. I have written two books on these subjects, *The Dynamics of Biological Invasions* (1989), Chapman and Hall, and *Dynamic Biogeography* (1990), Cambridge University Press. Subsequently, I extended my interest to biogenesis on which I published several papers, part of them with Dr. M.A. Fedonkin of the Russian Academy of Sciences. A third book, *Wasted World* (2012), concerns long-term environmental trends causing problems for man's prolonged existence on Earth (University of Chicago Press). The Dutch translation of this book, *Verspeelde Wereld*, is due in 2013. A fourth book with Dr G.M. Walter of the University of Queensland, *Autecology*, on an alternative approach to ecology, is in press (Taylor and Francis).

In 2004, I became Extraordinary Professor at the Vrije Universiteit at Amsterdam, teaching Biogeography and Invasion Research. I am a founding member of the International Biogeographical Society. Working in the front line of research of various disciplines, my focus has been on theoretical and methodological aspects in biology. In this connection, I received the Distinguished Statistical Ecologist Award from the International Association for Ecology.

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THE LIKELIHOOD OF EXTRATERRESTRIAL INTELLIGENT LIFE

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1. Introduction

Ever since the discovery of planets, and even long before that time, people speculated on the possibility that we are not alone in the universe and that life can be found on those other planets as well, perhaps also developing there into intelligent beings, similar to us humans. These beings would even live in highly developed technological civilizations and be able to communicate with us. The question is how realistic such speculations are. I think they are rather unrealistic.

Usually, the proponents of ideas on intelligent extraterrestrial life are found among astronomers, geologists, or chemists, etc., that is among nonbiologists (see Simpson, 1947; Mayr, 1985). Occasionally, scientific arguments are openly religiously motivated (e.g., Conway Morris, 2003; see, however, Hengeveld, 2004). The astronomer Frank Drake came up with an equation containing parameters from astronomy, which define the astronomical side of the equation only. In contrast, biologists, along with some paleontologists, often question whether intelligent life is likely, certainly in a form that is able to communicate with us. Of course, as always in cases like these, distinctions between representatives of different disciplines are not sharp. Such distinctions suggest only points of gravity; there is often overlap.

The literature on the possibility of extraterrestrial intelligent life has recently exploded with the rapid growth of astrobiology during the last few decades, actually giving arguments for space exploration (e.g., Dick and Strick, 2004). Apart from stimulating astrobiology and space exploration, it also stimulated the study of life's origin, biogenesis. Recently, the study of extraterrestrial life has even received its own encyclopedia, according to its subtitle covering "*all* life in the universe" (Darling, 2000). Two books on the history of the idea of extraterrestrial life, each packed full of considerations and references, have been published during the 1980s, only covering the periods of Democritus to Kant (Dick, 1982), and that of 1750–1900 (Crowe, 1986). As indicated, much more has been published since then (e.g., Basalla, 2006). Obviously, all this literature cannot possibly be covered here within a single chapter. This chapter gives the main lines of argumentation, concentrating on the biological ones. We have to fill in the biological side of the equation with present-day arguments, and this in terms of probability similar to the astronomical side.

Both camps are phrasing their arguments in terms of probability, whereby the nonbiologists emphasize astronomical probability and the biologists the probability of the biological replicability of higher beings. This tune was already firmly set in 1904 by the codiscoverer of evolution by natural selection, Alfred Russel Wallace in his book, *Man's Place in the Universe*, a book which caused a shock wave through the scientific world of the time. In 1907, Wallace repeated his ideas in connection with the possibility of life on Mars in his response to Percival Lowell's book *Mars and Its Canals* of 1906. So far, most people had thought in terms of a plurality of worlds, all inhabitable by intelligent beings, and here was well known – even one of the foremost evolutionary biologists – suggesting that there was only one inhabitable world with such a life form, the planet Earth.

Over a century has passed since Wallace's days, which makes one wonder what the present arguments might be from both sides, although expressed here by me, a biologist. Reading Wallace's book of 1904 for this purpose, I realized I have to follow him by explaining life as one long process, ever interacting with the heterogeneous and continually varying and changing environment. Which interactions take place is uncertain, but at the same time, it is these interactions that determine whether life originates, maintaining itself and evolving or not, that is, which constitutes the biological parameter of Drake's equation. It may be that in this respect, nonbiologists are thinking too lightly about matters biological, particularly when they go so far as to speculate in detail about the similarity of ecosystems on foreign planets with those on Earth (Irwin and Schulze-Makuch, 2010). However, Wallace did even more than this: he suggested comparing the chances of the occurrence of habitable planets in the universe on the one hand with the development of life on the other. These chances have to match. He estimated the probability of the first as a million to one, and that of life developing on them as a hundred million to one. The probability of a creature similar to man developing would be one hundred trillion to one. Here too, I shall follow the same line of approach.

2. The Problem

The physicist Enrico Fermi once wondered why there are still no signs coming to us from the universe, if it would indeed be teeming with intelligent life (e.g., Aczel, 1998; Schulze-Makuch and Darling, 2010). His wonderment became known as the Fermi paradox: "If there are extraterrestrials, where are they?" (see Davies, 2010; Hart and Zuckerman, 1982; Webb, 2002). Later, the astrophysicist Frank Drake structured the debate by formulating a nonanalytical equation expressing the probable number of civilizations in our galaxy, N, with which we might be able to communicate:

$$\mathrm{N} = f_p.f_l.f_i.f_c.T_g.R^*.n_e.L \tag{1}$$

in which:

f_p = the fraction of stars in our galaxy with planets
f_1 = the fraction of those stars that develop life at some point
fi = the fraction of those stars that develop intelligent life
f_c = the fraction of civilizations with a technology releasing detectable signs
T_g = the age of the galaxy
R^* = the average rate of star formation in our galaxy per year
n_e = the average number of planets per star with planets potentially supporting life
L = the length of time civilizations of intelligent beings release detectable signals into space

The first four parameters are fractions and refer to the chance of N such planets are existing. Because they are multiplied with each other, the total chance of this happening is quite small. However, it increases by multiplying this product with the next set of four parameters.

3. The Astronomical Side of the Equation

Drake's equation mainly contains astronomical parameters defining the chance of receiving messages from civilizations in outer space. However, it still does not contain parameters defining our place within the galaxy, the distance of the planet to its central star, its size and influences by other planets or an accompanying moon, or geological parameters defining the elemental composition of the planet and its crust, and those of its geochemical development (see Ward and Brownlee, 2000; Gribbin, 2011). Also, the values of these parameters are difficult to estimate so that results vary widely. Frank Drake himself seems to have considered his equation, written up in 1961 in preparation for a small gathering of about a dozen people, as a way of "organizing our ignorance" of the subject. Drake himself seems never to have published his equation, not even in a book on this subject he published the next year (Drake, 1962).

Still, either explicitly or implicitly, his equation has become the basis of all further speculations and convictions that somewhere in the universe, a type of intelligent life must exist similar to that found on Earth (e.g., Shklovskii and Sagan, 1966; Aczel, 1998). Though there are still no signs from other planets, at some point, it might be detected. Others, such as Ward and Brownlee (2000) or Gribbin (2011) (see also Grinspoon, 2003; Comins, 1993), deny the possibility of extraterrestrial life. They point to the insufficiency of the set of astronomical parameters, which would lead to an overestimation of the chance of extraterrestrial life occurring. What happens is, in short, that students with similar ideas to Aczel (1998) estimated that the small probability defined by the first four parameters in Drake's equation is fully compensated for by the large value of the last four. Ideally, this could result in a value equal to or greater than one, meaning that the universe is teeming with life. In contrast, students who share the ideas like Ward and Brownlee (2000), feel that the product of these two sets remains too small for life to originate and evolve elsewhere.

4. The Biological Side of the Equation

These authors, therefore, keep using the original arguments from astronomy, still occupying themselves with the same one-sided approach, that is, with only one and the same astronomical side of the equation, P_a, rather than giving estimations on the other, biological side, P_b. As in any equation, it is the two sides that have to match. As it is now, Drake's equation contains only one biological parameter, *fi*, defining the chance of life originating and of developing in the same direction and to the same extent, resulting in a life form with the same kind and level of intelligence and having developed a civilization of a sufficiently high technological level to make itself known.

Instead, one could think of an equation which puts the probability of extraterrestrial life occurring according to astronomical parameters on one side, P_a, and that of biological ones, P_b, on the other, as

$$P_a = P_b \tag{2}$$

P_a is a probability largely defined by N in Drake's equation, and P_b is the probability of life originating, maintaining, and evolving over, presumably, billions of years and eventually resulting in some communicative intelligent being living in a technological civilization similar to ours. However, P_a is not identical to N because N contains a parameter that actually defines P_b: the fraction of stars that actually develop intelligent life, *fi*. This parameter, *fi*, holds that the probability that the direction and rate of change of the environment as well as of life adapting to these changes is the same for all suitable planets. Therefore, we should rather take *fi* out of the equation. Furthermore, it is far from certain that life would always originate and evolve in the same way, which is the point biologists are trying to make. It is debatable that the same, biological process could happen twice. Firstly, including this parameter introduces an element of circularity. Secondly, both the origination and all further processes of the maintenance and evolution of life would progress deterministically, not leaving any room for stochastic variation. Due to stochastic variation, life would typically follow a random walk process, and the outcome of evolution would be radically different from the expected, deterministic value. To biologists, the problem is whether or not the chance of a suitable planet occurring is the same as that of developing an identical kind of life. The equation should therefore be rewritten as

$$aP_a = bP_b \tag{3}$$

with a and b as parameters to balance the two sides. If, for example, Wallace (1907) was right in suggesting that $P_a = 10^{-6}$ and $P_b = 10^{-8}$, this would imply that

$a = 100$ and $b = 1$. In other words, if life has a chance of developing elsewhere, we would need a 100 universes like ours. If instead we want to calculate the chance of life occurring on, say, a 100 other planets in our universe, then $a = 1$ and $b = 100$. The number of planets in the universe seems to be in the order of 10^{20}, a very large number indeed. However, the number of planets on which life might occur is considerably smaller: Aczel (1998) proposed that the chance to find life on a planet anywhere in the universe to be one in a trillion, but the chance to find intelligent life is therefore even smaller than that. For biologists, the problem is therefore to estimate P_b.

5. Practical Impossibility

The physicist Walter Elsasser (1987, see also 1975) attempted to understand organisms in terms of probability: by how much do they deviate from random expectation? How large is a large deviation? At which probability value do we consider the repetition of a phenomenon as impossible? To give a feel for what he considers exceedingly large numerical values, he mentions the estimated total number of hydrogen atoms in our universe to be in the order of 10^{80}. Although this number is huge, the number of permutations among the atoms in a cell is infinitely – incalculably – larger. Conversely, the chance of finding a number of atoms in a particular combination within and between molecules furthermore assembled in a dynamic system like that of a cell is often exceedingly small. Elsasser conservatively calls any number with an exponent exceeding the value of 100 an immensely large number, and he considers the realization of any chance of an event of that size immensely small. Consequently, it is impossible to realize in practice. In comparison, in statistical analysis, a probability level of rejection of 5% is usually kept, meaning that a chance of making a mistake of 5×10^{-2} is considered unlikely to happen. Given the vastly larger number of atoms even within the very confined space of a single bacterial cell, communicating in a specific way with its broader chemical environment, the number of choices would readily become immense, incalculable, transcomputational.

To structure his ideas further, Elsasser (1987) classified numbers according to three size classes: ordinary numbers with sizes ranging from 1 to 10^{10}; very large numbers, ranging in size between 10^{10} and 10^{100}; and immensely large numbers, those greater than 10^{100}. Relative to the total number of hydrogen atoms in the universe, about 10^{80}, he calls probabilities of realization of the order of the first class ordinary, those of the second class very small, and those smaller than 10^{-100} immensely small. Accordingly, the latter can be considered impossible to be realized in practice. This would apply to many, if not to all, biological organisms. Here, it is not the number of components that counts in determining a probability, but the number of interactions between them, a number which increases exponentially relative to the number of components themselves.

Processes happening within a living system and in its chemical environment do not operate independently of each other but are dominated by a great number of interactions. Initially, all chemical reactions during and after biogenesis were interactions between atoms of various chemical elements or between the molecules they formed. This happened inside and outside a protocell, within their energy-generating membrane, and between protocells (see Hengeveld, 2012). These interactions, moreover, form hierarchies, the tiers of which also interact with each other. Thus, atoms interacting with each other form molecules, which themselves interact, changing their composition, such as typically happens in enzymes. After that initial chemical phase, the evolving bacteria interacted with each other and with also the nutrients and poisons in their environment, and so forth. This added a spatial component to their interactions and thereby a new tier of stochasticity, tier after tier after tier being added during evolution.

6. Combinatorics

When a more or less closed set of interacting components is relatively independent of external influences, we call it a system. A biological system can be considered in various ways, the living system itself, the system it forms with its immediate environment, a global biospheric system, or this biospheric system together with the Sun, a biological system of the largest scale. Ultimately, it is the energy from the Sun that keeps all systems going. In contrast, living systems do not interact significantly with processes in the Earth's core, or with planets such as Jupiter or Saturn, the influences of which are considered external. Living systems are either relatively simple, such as viruses and bacteria, or exceedingly complex, such as mammals and, hence, man in his biological and socioeconomic, technological environment. And within systems there are both linear, nonlinear, cyclic, and feedback processes, such as in biochemical pathways and cycles. These processes can best be studied when relatively simple, because the very rapid increase of the number of components results in an even faster increase in the number of many possible interactions between them. This number of interactions – the number of permutations between the system's components – increases exponentially. Thus, there is a one in six chance of a four turning up by throwing a six-sided dice. The chance of this happening is one in six because each of its six sides has an equal chance of turning up – of being realized. Over time, after many throws, four has turned up a good number of times, so that it becomes unlikely that this would not have happened at all, that is, $1/6 + 1/6 + \ldots = 1$, the reasoning Aczel (1998) followed to calculate his "probability 1." However, expecting to find a four twice in immediate succession, or at another, predicted throw is 1 in 36, because those two previously independent chances are now made dependent and therefore have to be multiplied: $1/6 \times 1/6 = 1/36$, or $(1/6)^2$, or 6^{-2}. That is 0.028 instead of $1/6 + 1/6 = 2/6 = 0.33$ when the chances would have been additive.

The historical process of the evolutionary development of life follows an additive process. For example, the by itself improbable word CAT ($p = 0.0000021$) results from a succession of letters with each a chance of realization of 1/26 if indeed they are all equal, first the C, then the A, and finally the T. This explains how very improbable end states of a process can still develop historically with a reasonable chance of realization ($p = 0.15$). It also explains that previous states increase the chance of the next stages developing: they have to fit the existing pattern. However, though the combined chance tends toward one, it never reaches this value because evolution is an open-ended process. As soon as one adaptation has occurred, conditions have changed, necessitating a following adaptation to develop. There is no final state.

7. Combined Chances

We can equate a dice with a particular environmental factor and throwing a four with the chance of observing a certain value. Throwing a four twice in immediate succession equates with observing this value for this factor twice. The same reasoning holds for expecting another specified number to turn up with the second throw, this second throw with one dice even representing another environmental factor when obtained with another dice. It is the chance of finding a specified combination of values at specific throws that matters. This can be generalized once more for the repetition of any combination of factors occurring over time, such as those of successive factors during the biogenetic, evolutionary, or ontogenetic development of organisms. Physically, these factors can represent anything, the availability of certain elements, the right temperature, the presence of an energy-generating mechanism, spatial interrelationships between organisms to allow reproduction to happen during evolutionary stages well after the early biogenetic ones, or the chance of being gobbled up by a predator, and so on. The number of factors in combination determines the chance of realization of the process of life's origination, and further development can thus be considerable. Moreover, all combinations contain a greater or smaller chance element of being realized. Apart from possible interdependencies, these factors can have different – and shifting – relative weights and frequencies of realization during the process, and affect each other. They can also occur in one order or the other, or be heterogeneously distributed in space. The chances of realization of these combined interactions often remain unknown.

Having one factor – one dice – with only six outcomes is not representative for the complexity of life processes happening, given the number of possible elements and compounds found in even a very simple cell. As a first step, we can take, instead of one, seven cubic dice and estimate the chance of one particular combination of values happening for those six dice together. This chance is 0.36×10^{-5}. Now, we can also take one coin and seven polyhedral dice with, respectively, 2, 4, 6, 8, 10, 12, 20, and 30 sides. The chances of one particular number turning up are now ½, ¼, 1/6, 1/8, 1/10, 1/12, 1/20, and 1/30, or 0.500, 0.250, 0.167, 0.125, 0.100, 0.083, 0.050, and 0.033. This means that the realization of certain factor values varies between the dice. When we equate each dice with a process component and each side with the value of each of these components, the chance of eight factors with a specific result – a specific number turning up – immediately becomes rather low, 0.32×10^{-7}. The chance is even lower when the same or another set of values for the same factors is required for the next step in the evolutionary process: 0.102×10^{-14}, 0.103^{-29}, and 0.107×10^{-59} after the next three steps, respectively. Thus, the increase in this exponent itself doubles at each step, making the total increase a double exponential one. If, furthermore, somewhere else in the universe the same four successive steps have to be taken in the same process with the same eight factor values and for an exact repetition on one other planet, the exponent becomes −118. This exponent exceeds Elsasser's minimum of −100, making the chance of realization immensely small, impossible to be realized in practice, therefore. Considering that an incredibly larger number of successive chance events has operated over the almost 4 billion years of astronomical, geological, geochemical, and biological evolution, a process identical or similar to the one followed here on Earth is unthinkable.

8. Reducing the Chance Exponent

According to this reasoning, the chance of life's origination and further evolution being repeated elsewhere and in a particular direction is immensely small; it is transcomputational. Some cautious biologists and paleontologists therefore suggested reducing the exponent. This would increase this chance, which would make it more likely to be realized. For example, Conway Morris (2003) pointed to the process of evolutionary convergence, the whole process over billions of years inevitably converging to man. The idea is that the various successive evolutionary steps are only partially independent or random: in some way, a previous step facilitates the next one to be taken. The basis of environmental and genetic variation remains random and unrelated to subsequent success, but this variation can be selectively constrained by biochemical, physiological, or morphological adaptations, or by environmental conditions. Accordingly, many species are perfectly camouflaged against the background of the pebbles or plants on which they live. Mimicry is the result of another form of evolutionary convergence, not within one but between two, unrelated species: an edible prey can fool predators by their striking similarity to another, inedible prey. Also, selection can direct the evolutionary process by allowing a predator to evolve with an appearance or with teeth similar to another predator, despite a dissimilar phylogenetic background. Thus, despite their very similar appearance and behavior, New World predatory birds are unrelated to the birds of prey from the Old World; they are phylogenetically related to the storks of the Old World.

I think, though, that we easily overestimate the relative importance of evolutionary convergence (Hengeveld, 2004). For example, the exuberance of colors of tropical fish of all different shapes and sizes indicates that camouflage or evolutionary convergence are not always the inevitable results of evolution.

Also, the argument that the shape of animals can converge for mechanical reasons, resulting in a torpedo shape of fast swimming fishes, applies to a relatively small number of animals. Moreover, the number of taxa with flying species, although often referred to in this context, is abysmally small relative to the total number of species. The swimming of elongated organisms goes back to the most ancient life forms and fanned out into millions of species. Given the hundreds of millions of very different forms of life from both the present and the past, to me evolutionary divergence seems more of the leading principle in evolution. It is the component of randomness that prevails.

The principle of backward compatibility from information science points to previously evolved structures having a greater constraining effect on those of the next phase. When building a next generation of computers, one is not entirely free in choosing a design: one has to keep to the basic design of previous machines and software. The very same principle constrains the direction in which living structures can evolve: previous forms dictate which forms will be feasible in the future and which ones will not. Also, the environmental conditions to which living systems adapt in order to survive do not vary independently either, but are constrained to relatively narrow bands of variation. Kauffman (1995) thought of a self-organizing process that would restrict the independence of biological variation and lead to higher forms of complexity. Thus, the components of the process would not be independent. Selection would thus lead to a gradual increase in intelligence among several interacting species. Gould (1996) pointed to yet another constraining historical factor, the size of individuals at the beginning of the evolutionary expansion of a taxon: if the individuals of the first taxon are small, those of most following taxa will remain small, whereas those changing in size necessarily become larger, causing the average size to increase.

Thus, one way or the other, evolution would broadly follow a path from unstructured and simple to structured and complex, although the exact path followed varies because of the randomness of continuous systems decay. On the point of self-organization, Kauffman (1995) differs from Wicken (1987) who sees the formation of structures and complexity as due entirely to entropic decay. Possibly, the two proposed processes of self-organization converge when we remember that over evolutionary time and with the dissipation of waste energy, it is common in the biochemistry of the cell for several molecules to build another molecule. This greatly – though not entirely – reduces the independence of processes and process components.

Genetic and biochemical variation on which evolution depends acts momentarily, remaining largely independent over time. Similarly, variation in space acts mostly locally in finding the right mate at the right time, or in being found by a predator or grazer. In general, biological variation is due to continuous entropic decay, and biological adaptations restrict the resulting stochasticity of the decay process. Stochasticity, though, is not restricted completely, with the result that the evolution of life follows a random walk process. This random walk process is unlikely to end up at the same point, a humanlike creature with a similar level of intelligence and living in a similar technological civilization. Moreover, as Gribbin (2011) argued, it is not only the intelligence of some being that is important, but so are the conditions under which it can develop a technological civilization as well. Such a development requires sufficient mineral and energy sources, and these have to develop over geological time as well, and tectonic processes and erosion have to bring them within reach, etc. Playing the evolutionary tape of life twice will unavoidably give another outcome (Gould, 1989; Gribbin, 2011) (see also Hengeveld, 2004).

The existence of constraints to independent variation means that simply multiplying chance values from one evolutionary stage to the next as if they were completely independent of each other overestimates the exponent of chance events based on the random combination of independent process components. The question is whether constraints to independent variation are sufficiently strong to give this exponent of the overall parameter P_b a value similar to or greater than the one obtained from astronomical considerations, P_a. To generate a convergent property like a color pattern on the abdomen of a fly similar to the pattern of another, unrelated species like that of a wasp requires a purposeful genetic adaptation for which often dissimilar genes operating in a different biochemical environment have to be activated or mutated. Because this is purposeful, the chance process is a multiplicative one. Any constraints to this process, though, are not multiplicative and have therefore a relatively small effect. In general – mathematically – effects of constraints to a multiplicative process are negligible (Elsasser, 1975).

9. Insufficient Knowledge

The operation of living systems depends on interactions within and between them, as well as on interactions with their abiotic environment. Their evolution is partly due to internal and external, random and nonrandom changes, but in the end, the changes have to match those of the independent variation in external environmental conditions. To evaluate the freedom living systems have to vary and adapt, we need to know how they are structured and work and what their limits of variation and adaptation are as a consequence. These limits determine the deviation from random variation they have achieved during evolution.

What is behind processes constraining genetic and phenotypic mutability remains largely misunderstood. This is partly because the information on them belongs to the far past and has been lost, and partly because of the exceedingly great intricacy of even the simplest life forms. The number of present and past interactions is too large and is therefore immeasurable. Without a detailed knowledge of those interactions, we would not be able to calculate the chance that – intelligent – life can also exist on other planets than the Earth. This chance, though slightly greater because of constraints to interactive processes, does remain incalculably small.

10. The Astronomical and Biological Arguments Are Unequal

In contrast to the biological parameters, the astronomical ones in Drake's equation are mutually independent. A mutually independent variation means that calculating the chance of some habitable planet somewhere else in the universe, although difficult and seemingly debatable at major points, remains relatively simple. Thus, the chance of the Sun occurring in the habitable zone of the galaxy can be taken to be independent of the chance of the Earth being hit by a planetesimal the size of Mars, which resulted in two moons which later collided forming our present Moon (Jutzi and Asphaug, 2011). Also, no single astronomical parameter concerns a developmental or evolutionary process (see Broecker, 1987), by nature an interactive process happening over time. For example, it is well known how the Moon originated, how it developed, how its distance to the Earth increased over time, what its biological impact may have been, etc. (see Comins, 1993), but these processes are irrelevant in Drake's equation.

Still, it does matter how such events as how the Moon originated occurred. The chance with which it came into existence was exceptionally small: it depended on an initial collision between an early Mars-sized planetesimal and Earth. A near miss due to a diversion of only 25,000 miles from its actual path would have meant that this collision could not have taken place. This would have meant, in turn, that the Earth's rotation round its axis would not have slowed down, which, again, would have resulted among other things, in lower tides, shorter days, and permanent high winds (e.g., Comins, 1993). Such environmental differences would have resulted in evolution taking quite another course than it in fact did. The high winds would put the plants under great mechanical stress and would have affected their evaporation rates. Who knows what type of plants would have evolved and what kinds of animals would have been associated with them, what shape and size they would have had, what type of physiology, and so on. Bird and insect life would have been very different, with all its consequences for pollination and for fruit bearing trees, etc. Moreover, without the Moon, the Earth's axis would not have been stable. Not only would this mean that the seasons varied considerably, the life cycles of plants and animals would in turn be affected. Moreover, a stable axis stabilizes the Earth's climate, which allowed life to maintain over 4 billion years. Despite the alternation of long and deep ice ages and very hot geological epochs, on a global scale, the temperature range has in fact kept between relatively narrow bounds so that all life never froze or boiled to death. With the larger variation of an unstable axis, life would have been different, if possible at all. In fact, a multitude of such direct and indirect factors, often due to seemingly insignificant events or processes, make up the habitability of a planet (Kasting, 2010). Still, on our habitable Earth, about 50% of the families of animals, 80% of the genera, and 90–96% of the species were lost at the end of the Permian, and during the Phanerozoic alone, each of the five other major extinction events eliminated more than 50 % of the biota. Each time, their strongly selective effect gave evolution another direction. How could this possibly be repeated elsewhere? At least, in all probability humanlike creatures would not have evolved.

11. The Origin and Early Evolution of Life

The search for extraterrestrial life is sandwiched between astronomy on the one hand and the study of biogenesis on the other (see, e.g., Sullivan and Baross, 2007). There are many theories on the way life may have originated (see, e.g., Fry, 1999; Lahav, 1999), based on a great number of definitions of life (e.g., Popa, 2004; Schulze-Makuch and Irwin, 2004). In fact, judgment of the suitability of a planet to develop life depends on several factors to which authors give different weights. The existence of water is the only one commonly mentioned and is usually considered as solvent for other molecules in a cell (e.g., Schulze-Makuch and Irwin, 2004). Carbon rather than hydrogen is considered an element pivotal to life, although hydrogen is more probably one of the initial elements in the start up of life, remaining pivotal as basic currency to the biochemistry of life ever since (e.g., Fedonkin, 2009; Hengeveld, 2012).

Instead, proposing that life started from individual molecules in some primeval organic soup, whether the molecules originally formed *in situ* or in outer space, a systems approach can better be followed, with the system having simple mechanisms both for generating energy and for processing it. Ultimately, it is this system of mutually interacting processes which dissipate energy that defines the chance of the evolution of life being exactly repeated elsewhere in the universe. Similar to the working of a present-day cell, the energy could have been generated in a membrane surrounding some cavity. The membrane would operate like a partition in a battery with a reducing environment outside and an oxidizing, biochemical one inside. The difference in the charge on the two sides could have generated a thermodynamic disequilibrium, creating an inward flow of electrons and protons from which energy can be tapped. The membrane may have consisted of an alloy of iron and nickel, and the cavity selenium as an alternating acceptor and donor of protons and electrons. Hydrogen, as electron donor, may have combined with selenium, after which the resulting compounds fell apart continually, making selenium a donor and hydrogen an acceptor, in turn. This initial cycle was only possible because both hydrogen and selenium are electron acceptors as well as donors with a relatively small and similar electronegativity. The electronegativity of other nonmetals in the upper rows of the periodic table of elements, especially carbon, nitrogen, and oxygen, was too strong. It gives the molecules they form their great stability, making them unsuitable for taking part in biochemical cycles without complex enzymes to break the bonds.

Thus, a primitive chemical cycle processed energy, initially without the help of enzymes. Metals, later still found in metalloproteins, were the ultimate electron suppliers (Fedonkin, 2009); iron, for example, was replenished in the iron cycle under the influence of ultraviolet radiation (de Duve, 1991). They could be included only later when other elements could form the catalysts with the help of an elaborate enzymatic apparatus (e.g., Hengeveld and Fedonkin, 2007; Hengeveld, 2012). Furthermore, the large amount of energy needed to form and break down the strong covalent bonds of carbon and nitrogen, and build up and maintain an elaborate and extremely complex chemical apparatus, could only be supplied by the input of energy from an external source, that of solar radiation. This radiation was itself converted into chemical energy by two highly complex photosystems in the membrane put in series, photosystems I and II. At some stages of complexification, the biochemical apparatuses of different cells were integrated by endosymbiosis (Kooijman and Hengeveld, 2005; Martin and Muller, 1998). From the very first stages of life onward, therefore, we are dealing with extremely complex evolutionary processes. For life to originate and evolve on some other planet as well, they need to be repeated.

This model for the beginning of life puts heavy constraints on the qualitative and quantitative chemical composition of the environment, on the direction and rate of its change, as well as on the thermodynamics behind the construction and maintenance of the energy-generating membrane surrounding a cavity. Right from the beginning, therefore, life could only have started under very specific physical and chemical conditions; that is, from a specific quantitative combination of many interacting factors. Later, as individual protocells had formed, stochastic spatial processes were added, such as the meeting between cells or with favorable conditions, and still later those between cells within multicellular organisms and between such complex organisms. Step by step, nutrient cycles evolved in the biosphere in which a distinction developed between plants as chemically constructive elements, and fungi and animals as destructive ones. Chemically, this meant a continuous recycling of carbon dioxide and water, with hydrogen as thermodynamic mediator. Water, therefore, is not only important as a solvent, but also as an ultimate supplier of hydrogen as the pivotal element for life. Thermodynamically, hydrogen as an energy carrier is central for all life processes, for that reason being continually exchanged in the biochemistry of all organisms.

12. Discussion

After half a century of listening with radio telescopes for which Cocconi and Morrison (1959) had earlier derived the bandwidth, there is still no sign of extraterrestrial life whatsoever, except for a meteorite with some highly enigmatic "fossil" from Mars (e.g., Schulze-Makuch and Darling, 2010; see, however, Walter, 1999). Webb (2002) gave 50 arguments why a possible "living" planet keeps silent, three important ones of which are the remoteness of a planet, the small size of the time window, and us not recognizing and understanding possible messages sent. Indeed, "If a lion could talk, would we be able to understand him?" Whatever we think about the possibility of intelligent life having developed elsewhere, we may never be able to test any idea.

The calculation of probabilities of combinations found within an organism and over ecological and evolutionary time using a number of unequal dice presumes the independence of the values obtained. As argued here, this is largely unrealistic. A specific – and therefore unlikely – combination of factors and factor values is more easily obtained under constraining conditions than under purely random conditions, although forming the optimum combination of constraining conditions remains subject to chance variation. A particular stage of evolution does not stand alone, independent of the immediately previous stage, but evolves directly from it. In that way, it uses adaptations present from all steps that were made previously. Also, the physical and chemical conditions within a cell can lead to the formation of a particular molecule, in a way comparable to what happens in a chemical reaction; the various cell components are not independent of each other, they often generate each other. In various ways, therefore, adaptation reduces the stochasticity of the life processes concerned, but as a process, adaptation itself is subject to chance.

Reducing chance does not imply that there is no space left for variation at all, so that processes within and between cells and organisms and over evolutionary time would be fully deterministic. Determinism of all processes would mean that, once the right condition for biogenesis occurred (n_e in Drake's equation), all next steps would follow automatically and with complete certainty. Indeed, should the outcomes – intelligent beings like us with highly developed technological civilizations – be similar for several planets, there would be hardly any place for variation, not even in the initial conditions, which would still lead to deterministic chaos. This is not realistic. It is true that to some extent, the variation in biological processes is reduced, and the evolution of new stages is constrained by previous ones, but this happens without all stochasticity being lost. The very great number of combinations, interactions, and evolutionary stages that need to be repeated exactly over the billions of years since life's origination on Earth, the value of the chance of reaching one particular outcome, a multicellular, eukaryotic being with the same intelligence and civilization as ours, will still be immensely small. Any repetition of the entire evolutionary process up to the same form of intelligent life is out of the question.

13. Conclusion

Factors leading to and maintaining living systems have always varied stochastically. The complex process of a great number of interactions between factors of a widely different nature and importance has been modeled in a very simple way by throwing dice. These represented factors, and their varying number of sides, factor values. We saw that, despite some constraints, even with a small number of both factors and the values they could reach, the chance of an exact recurrence of a certain combination soon becomes immensely small and therefore impossible to realize in practice. Even so, this immensely small biological chance, P_b, has to be matched by the number of planets in the universe with the right conditions for almost 4 billion years for this recurrence to happen, P_a, and, moreover, in the same direction and to the same extent. The number of habitable planets, necessarily being smaller than the number of hydrogen atoms in our universe, 10^{80}, is only very large in the sense of Elsasser (1987). According to this reasoning, the probability that the same life form exists elsewhere in the universe is too small to be considered realistic. As even the astronomical probability proves too difficult to estimate with any confidence, we are left with the task of balancing an equation comprising two large unknowns, a situation that should discourage speculation about the existence of extraterrestrial intelligent life.

14. Acknowledgments

My wife, Claire Hengeveld-Nicolls, corrected and improved both the English and the logic of this chapter.

15. Addendum

After having finished writing this article, I did find an article by Drake (1974) explaining his equation.

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THE ASTROBIOLOGICAL POTENTIAL OF POLAR DUNES ON MARS

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Abbreviations

CRISM	Compact Reconnaissance Imaging Spectrometer for Mars
DDS	Dark Dune Spots
HiRISE	High Resolution Imaging Science Experiment
HRSC	High Resolution Stereo Camera
MOC	Mars Orbiter Camera
MOLA	Mars Orbiter Laser Altimeter
TES	Thermal Emission Spectrometer
MSO	Hypothetical Mars Surface Organism

1. Introduction

At the polar regions of Mars H_2O ice accumulates on the surface annually, while in summertime elevated temperature prevails. In this work, we outline the possibility of life in this region, inside the seasonal surface features called dark dune spots (DDSs), which had been analyzed by the Mars Astrobiology Group at Collegium Budapest Institute for Advanced Study since 2003 (Szathmáry et al. 2002), and after the collapse of the institute was continued at the New Europe School for Theoretical Biology and Ecology.

Dark dune spots are transitional geomorphologic formations in the frost-covered polar regions of Mars that show a characteristic transitional sequence of forms during the springtime defrosting period. The subsequent stages of DDSs have been revealed as follows. Their spotlike gray initial form appears late winter. They form an umbra-penumbra-like pattern. When DDSs are formed on slopes, elongated streaks (seepages) emanate from them.

Sometimes new frosting forms a brighter collar around the spots. The majority of the DDSs appear in annually recurring pattern on the surface.

The shallow subsurface of Martian polar dark dunes as microhabitats was first suggested by our group in 2001 (Horváth et al., 2001, 2003; Bérczi et al., 2001; Szathmáry et al., 2001), the first detailed description was presented in Gánti et al. (2002, 2003) and the latest version in Szathmáry et al. (2007). Terrestrial cryptobiotic crust (CBC), dominated by cyanobacteria, was suggested as a partial analogue for possible Martian microbiological communities by Friedmann (1986) and Pócs et al. (2003, 2004). These polar terrains were suggested as favorable location for living organisms by several authors (Jakosky et al., 2003, Córdoba-Jabonero et al., 2005; Prieto-Ballesteros et al., 2006), where water ice is present seasonally, and thin interfacial water could be there based on theoretical arguments (Möhlmann, 2004, 2008).

2. Methods

In this work, we used remote observations by probes orbiting Mars: Mars Global Surveyor (MGS), Mars Express (MEX), and Mars Reconnaissance Orbiter (MRO). We also analyzed in situ cryptobiotic samples collected in hot and cold deserts of Earth. Both analyses were complemented with model computations and various results in the literature.

Based on data of the above-mentioned space probes, we analyzed morphological properties (with GIS softwares and methods), used topographic data from MOLA and HRSC digital terrain models (DTMs) based on stereo images, analyzed surface albedo (from HiRISE red channel with the formula I/F = (DN*SCALING_FACTOR) + OFFSET, and divided it with cos i, which gives an approach of Lambertian albedo), temperature data (from Thermal Emission Spectrometer (TES) (MGS) measurements (Christensen et al., 1992), with “vanilla” software for daytime between 12 and 14 local true solar time, showing repeated rough annual trends, with spatial resolution of around 3–8 km) and compositional data (from CRISM spectra with CAT-ENVI software) regarding the spatial and temporal changes of the observed features.

There are several craters on Mars that do not have specific name. To identify them more easily, we used the following unofficial names: Gánti for the crater at −68.1 1.3E (biochemist, founder of system biology, former member of the group who passed away in 2009) and Konkoly (astronomer, founder of the Konkoly Observatory, now the main observatory of the Hungarian Academy of Sciences) at −68.1 209.5E (Table 1).

3. Discussion

In the following sections, we first outline the basic observations on dark dune spots and related flow-like features, which focused our interest to the polar region.

Table 1. The analyzed locations of flow-like features on Mars.

Name/earlier id. no. in Kereszturi et al. (2009a)	Latitude (degree)	Longitude (degree, east)
1.	75.3	300.1
2.	77.5	300.1
3.	84.0	233
4.	83.7	235.9
5.	84.7	0.8
6. (Unofficially called “Gánti”)	−68.1	1.3
7. (Unofficially called “Konkoly”)	−68.1	209.5
Richardson	−72.0	179.4
Russell	−54.3	12.9
Jeans	−69.5	153.3

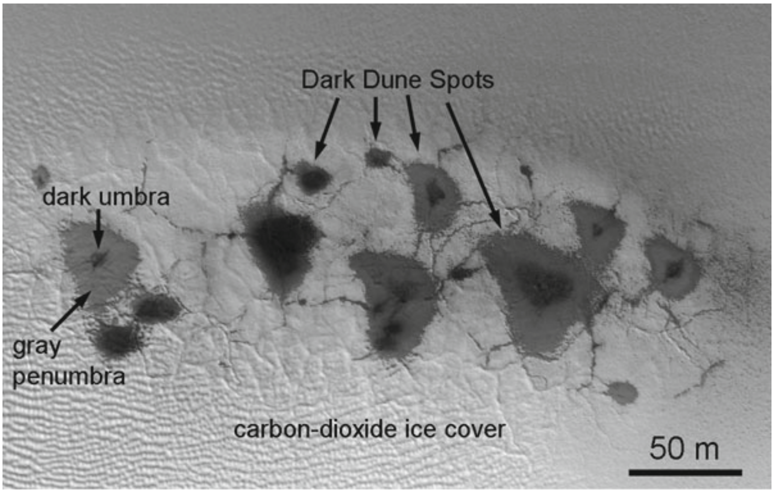

Figure 1. Example image showing *dark dune spots* in the Richardson crater on the HiRISE image PSP_003175_1080. Components of the spots: *dark umbra* (core) and *gray penumbra* (*ringlike* feature around the core) are visible.

Various environmental factors and stresses are analyzed in detail, in order to elucidate whether a hypothetic organism could withstand them, including the ranges of water (presence, availability, activity), temperature (values, possible heat-insulating methods), radiation shielding (solar UV and galactic cosmic rays), and chemical environment (oxides, peroxides). The observed analogues communities on Earth (with emphasis on salt tolerance) show suggestive survival strategies. In sum, we argue that DDSs may provide ephemeral habitable conditions on Mars.

3.1. THE DARK DUNE SPOTS

The features called dark dune spots (DDSs) are good candidates for the ephemeral presence of liquid water as their outer (penumbra-like) part holds water ice without carbon dioxide cover for an extended period in springtime (Fig. 1). These features

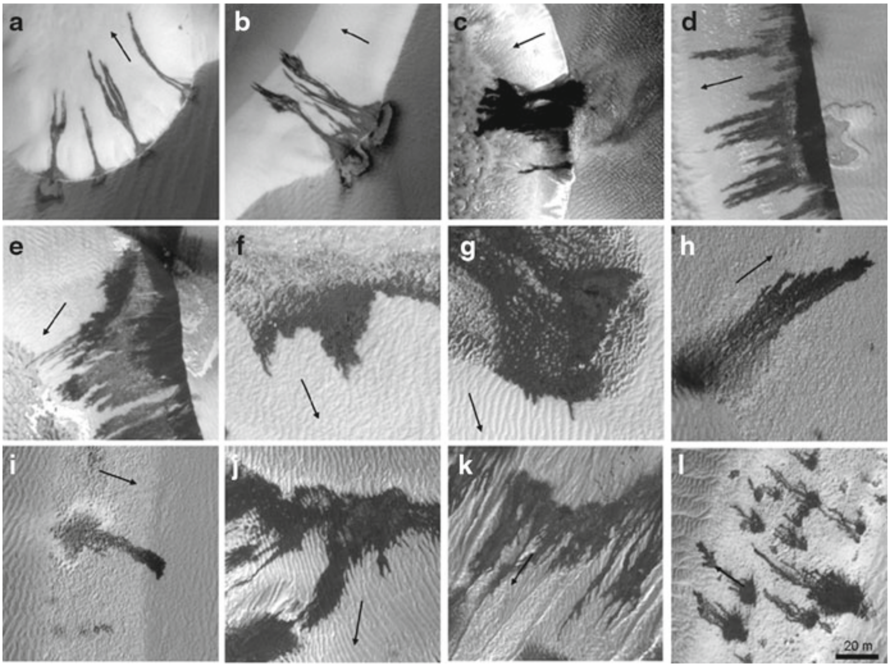

Figure 2. Examples for seepages on frost-covered polar dunes during local spring from the northern and southern hemispheres. *Arrows mark* the sloping direction on the 100 × 100 m sections, north is toward the top.

are present both in the southern and northern hemisphere, and in tilted terrains, they give rise to elongated slope streaks (Horváth et al., 2001, 2009; Gánti et al., 2003). Two groups of these streaks could be identified: diffuse ones, which may orient into various directions from the same spot and could be present on horizontal surfaces too in an earlier phase according to the seasons (Kereszturi et al., 2007), while the confined ones are present only on steeper slopes and move downward with a speed between 0.1 and 2 m/sol during a later seasonal period than the diffuse ones (Fig. 2). It is possible that this second type of slope features is produced by brines or brine-lubricated grains moving on the surface (Kereszturi et al., 2009b).

3.2. TEMPERATURES IN THE SPOTS

For any living activity, the temperature should be above a critical value for certain periods of time. Based on the general wisdom, the minimal temperature for metabolic activity is between −15 and −20°C (Junge et al., 2004), although there are suggestions for the possibility of metabolism at even lower temperature, for example, at −40°C (Price and Sowers, 2004). The following factors help raise the temperature inside the DDSs: 1. solar insolation, 2. dark dune material, 3. solid-state greenhouse effect of the ice cover, 4. heat-insulating effect of a possible water vapor layer formed between the dune grains and the ice cover by the volume decrease during the melting and vapor migration (see below).

The movement of the above-mentioned confined slope streaks happens when the daytime maximal temperatures were between 160 and 250 K—as measured on a 3-km spatial resolution (Horváth et al., 2009; Kereszturi et al., 2009b). Inside the dark dune spots (because of their low albedo), temperatures must be substantially higher than the above-mentioned value—as a result, it is possible that in springtime the temperature reaches the lower threshold limit around −20C. The situation could have been even better in the past. During the recent climate changes, the maximal surface temperature values, as well as the length of the periods above certain values, changed and occasionally could have been higher/longer. Based on the analysis of the historical habitability at the Phoenix landing site, better conditions could have prevailed in the past million years than today (Zent, 2006, 2008).

Based on the observation of Phoenix lander, falling ice crystals or snowflakes were observed in autumn at the northern polar region (Smith et al., 2009). The exact physical structure of the H_2O frost cover formed on the ground is not known, but it possibly has microscopic internal voids. We assume a porous H_2O ice layer below the later deposited topmost CO_2. When the sun heats up the dark dune grains at the bottom of the H_2O frost layer, individual H_2O molecules detached from the lowest part of the water ice layer may migrate inside the voids in vapor phase and freeze into solid form at the coldest upper locations, in physical contact with the CO_2 ice. If the transparency of the porous snow layer is low, small amount of radiation absorbed by the dark gains may produce a temperature gradient, since the CO_2 ice cover is very cold. Such migration of vapor may enlarge the voids at the bottom of the snow layer and seal the pore spaces from above (Fig. 3).

The lower, closed gaseous pore volume filled with water vapor keeps the gas-phased H_2O molecules there for an extended period and also serves as heat insulator. The thickness of this proposed H_2O layer is unknown; based on the atmospheric vapor content may be in the order of 10 μm. This model suggests that elevated temperatures may hold only for a short period.

3.3. WATER IN THE SPOTS

Inside the outer penumbra part of the spots, water ice is present and is in physical contact with dune grains (Kereszturi et al., 2011). Under such conditions, a thin interfacial liquid water layer may form (Möhlmann, 2004, 2008).

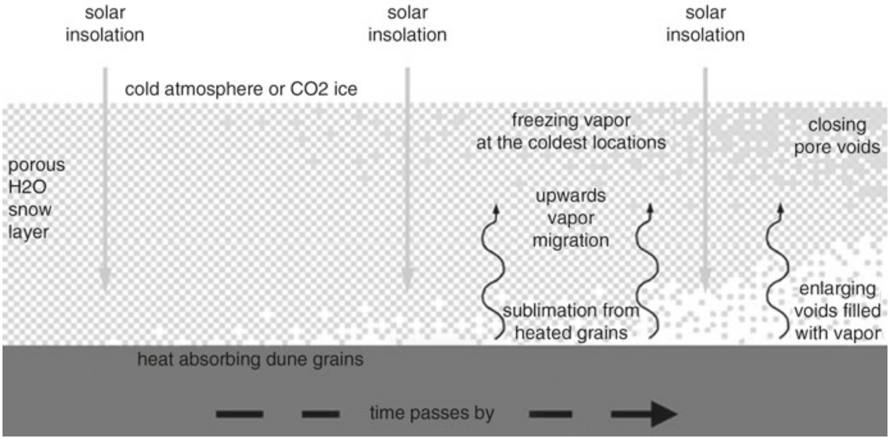

Figure 3. Hypothetical time sequence of the process inside the porous snow-like H_2O layer as time passes by, from left to right. Sunshine comes from the top through the water ice snow layer (*gray* color with *dotted pattern* showing porous structure) toward the *dark grains* at the *bottom*. Due to evaporation within the porous snow, lower pore spaces enlarge, and upper pores close.

Possible sources of the H_2O beyond the wintertime condensation are the nighttime freezing as observed by the Phoenix lander (Cull et al., 2009) and the migration of water from the interior of dunes (Tirsch et al., 2006; Feldman et al., 2008), which then sublimates away from the topmost layer of dunes in spring (Kuzmin et al., 2009a, b). Various salts, which are frequent on Mars, lower the melting point of water (Chevrier and Altheide, 2008) and may produce liquid brines at the polar regions (Chevrier et al., 2009), as it was probably observed by the Phoenix lander (Renno et al., 2009a, b).

At the elevated daytime temperature, various factors may decrease the H_2O loss rate:

- A porous layer cover of ice helps in H_2O trapping. Based on laboratory measurements (Chevrier et al., 2008), a 2-mm thin layer of unconsolidated clay grains decreases the ice loss to 0.2 mm/h at 260 K and below 1 mm/h under at 273 K (Bryson et al., 2008) under Martian conditions.
- For salty brines, water loss is even smaller (Knauth and Burt, 2002), which could be orders of magnitude lower than for pure water, and salts also expand the range of temperature values where solutions are stable. Based on laboratory experiments (Altheide et al., 2009), the hourly evaporation rate of 20–25 wt.% sulfate and chloride brines at 260–270 K is 0.1 mm or less.
- Strong adsorption by hygroscopic materials may keep H_2O molecules attached to grains for an extended period. Such hygroscopic materials, above all various salts, are present on the surface.

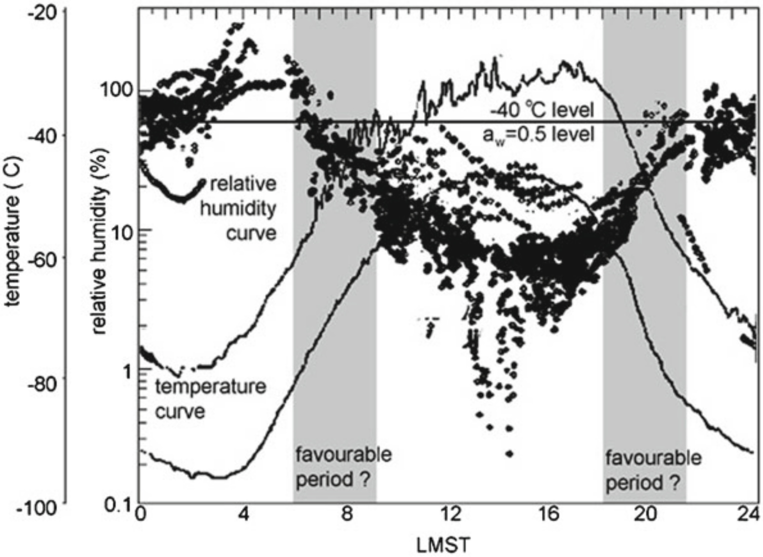

Figure 4. Overlapping daily temperature (*solid*) and water activity (*dotted*) curves at the Phoenix landing site measured at 1.2 m height (LMST is for local mean solar time). There are two favorable periods: in the morning 8–9 and afternoon 19–21 LMST, when both parameters are relatively close to the proposed threshold limit ($a_w = 0.5$ and −40°C). These values are below the tolerance limit of known extremophiles, but these parameters were measured not inside the near subsurface layer but above it. Both values could be higher in the top 2–4 mm of the regolith and may be barely tolerable (Hudson et al., 2009), as the temperature is higher at the sunlight absorbing dark dune grains, and the relative humidity may be elevated as the H_2O sublimates from the intergranular voids there.

Water activity (a_w) is to characterize the access to H_2O molecules. In the nighttime, frost a_w is around 1, and microorganisms may take up water in the cold nighttime and metabolize during the warm daytime if they are able to trap the H_2O. Aquaporin-like structures (Borgnia et al., 1999) are able to let the H_2O molecules into their cell. Phoenix measured a_w with the TECP (thermal and electrical conductivity probe). The nighttime a_w around 1 decreased to 0.1 till noon. There were two separate periods, when the parameters were close to the barely tolerable values (Fig. 4) around −40°C and a_w around 0.5. As these observations are valid for the open air, inside a surface porous layer water activity could be higher.

Beyond these arguments, it is also known from the Earth that there are organisms (especially desert lichens) which are able to use atmospheric water vapor for metabolism (Bertsch, 1966; Lange and Bertsch, 1965; Lange, 1969). If the same holds for hypothetical Martian organisms, water vapor sublimating from the subsurface ice may be used (when ice is no more present right at the shallow subsurface, as proposed by Wallis et al., 2009).

3.4. LEVEL OF RADIATION IN THE SPOTS

Shielding against the UV radiation is necessary on or close to the Martian surface (Schuerger et al., 2003; Cockell et al., 2005; Kminek and Bada, 2006). The following parameters help in the shielding:

- Shadow effects reduce the cumulative UV flux on the surface (Moores et al., 2007).
- Water ice snow with thickness of several cm could give enough radiation shielding, which is higher than expected at the seasonal cap. Solid ice needs to be on the meter-scale thickness to maintain enough shielding (Córdoba-Jabonero et al., 2005).
- Brines could also serve as UV filtering media (de la Vega et al., 2007) but probably do not provide enough shielding.
- The mm-thin mineral layer above the possible organisms that lets enough light to penetrate for photosynthesis (Cockell et al., 2005; Cockell and Stokes, 2006) can provide enough shielding.

Ionizing radiation is produced by heavy nuclei from solar particle events and galactic cosmic rays plus solar protons can reach and penetrate into the Martian subsurface to meter depth (Dartnell et al., 2006, 2007). This radiation does not necessarily inhibit the active life process instantaneously, but long-term accumulation without repair of damage should inactivate any cell in the order of 1,000 years. In the case of annual active periods when damages are repaired, hypothetical organisms in theory could survive.

3.5. CHEMICAL ENVIRONMENT IN THE SPOTS

Peroxides are often invoked to explain the lack of organic material at the Viking probes' landing sites (Oyama and Berdahl, 1977; Chevrier et al., 2004, 2006), which were toxic for known terrestrial living organisms. The exact composition and concentration of oxidants are unknown, although the presence of H_2O_2 and perchlorates has been observed (Catling et al., 2009). Three points are shown which may help for a hypothetic organism to overcome the problem of oxidants:

The dark color of the dunes suggests less red material there, for example, less oxidized regolith. Despite of the continuous falling of dust from the atmosphere, these basaltic dunes seem to be less oxidized than the average Martian surface.

Assuming that H_2O_2 is the main agent that produced the observed gas release during the Vikings' LR experiment, its concentration could be around 25–250 ppm (Mancinelli, 1989). Based on laboratory tests, certain organisms are able to tolerate H_2O_2 concentration up to 30,000 ppm (Mancinelli, 1989). There are arguments that the gas release was produced by living organism themselves (Levin and Straat, 1977, 1979) and also models suggesting that oxidants could be used by hypothetical organisms (Schulze-Makuch et al., 2007).

Table 2. Observed survival challenges (columns), survival strategies (rows), and examples (inside the cells).

Challenges/survival strategies	**Cold**	**Dryness**	**UV radiation**
UV protecting pigmentation			*Tolypothrix, Nostoc* (Tunisian Sahara, west of Matmata)
Rock coverage	*Gloeocapsa atrata, G. punctata*, (Devon Island, Arctic Canada) *Microcoleus chthonoplastes, Chroococcidiopsis* (Antarctica)	Hypolithic colonies (wind-shaded voids)	*Symplocastrum, Microcoleus* (Western Australia, Barlee Lake area), fiber optics strategy
Mucilaginous sheath		*Microcoleus chthonoplastes* (Tunisian Sahara)	
Dew-trapping “antennas”		*Microcoleus chthonoplastes* (Tunisian Sahara)	
Seasonal shifting into different layers		*Microcoleus paludosus* (Tunisian Sahara, El Hamma-Kabili road)	*Microcoleus paludosus* (Tunisian Sahara, El Hamma-Kabili road)

Water ice tends to decompose the strong oxidants during a short period when it is even only liquid form of interfacial layer, and this liquid form can produce an ideal circumpolar zone, where ephemeral liquid water is available.

3.6. POSSIBLE ANALOGUE COMMUNITIES ON THE EARTH

Based on the above-mentioned arguments, shallow subsurface locations with porous structure (Dor and Danin, 2001) could be important microhabitats on Mars in the circumpolar region. On Earth, the cryptobiotic crust is a possible analogous microhabitat with cyanobacteria inside them (Wynn-Williams, 2000; Friedmann et al., 1993). The cryptobiotic crust is a 0.05–5-mm thin layer on rock or soil surfaces, composed of weathered minerals and various organisms (bacteria, algae, small-sized fungi, lichens, and small bryophytes) plus mucilaginous sheath (envelope). We analyzed such samples from hot and cold deserts, collected by Tamás Pócs (2009) from 5 continents in the past 6 years. Cyanobacteria are good partial analogues for hypothetical Martian organisms, as they can photosynthesize, but most of the year are in dormant state, and they produce mycosporine-like components for UV screening and tolerate salt concentrations up to 200 g/l (Oren and Seckbach, 2001). They are adapted to wide range of temperatures (Price, 2000), and at many locations, the same taxon is present in hot and cold deserts (e.g., the *Microcoleus* species). Based on our observations of terrestrial extremophiles, important and Mars-relevant survival strategies are summarized in Table 2.

The following survival strategies have been observed by the authors and others:

- **UV-shielding** methods. *1. Rock coverage* maintains total UV screening, while it lets enough light through for photosynthesis. Organisms are present below the weathered top layer or inside the cracks of the rock (Devon Island, *Gloeocapsopsis pleurocapsoides*), but other taxa could be also present inside the unweathered surface layer of rocks (sandstone in Utah, *Chroococcidiopsis*). *2. Pigmentation*: the mucilaginous sheath contains UV-shielding scytonemin pigments (*Scytonema, Tolypothrix, Lyngbya*). Sometimes the crust is vertically stratified, and UV pigmentation is visible only in the upper layer (*Tolypothrix bissoidea, Microcoleus paludosus*, often in dried desert lakes) A special method we call optical fiber strategy was observed in *Microcoleus* and *Symplacastrum* bundles and their mucilaginous sheath, which conducted light below the surface (providing light for photosynthesis in the deeper layers), in salty lakebeds in Australia and Africa. Beyond the possible shielding methods, several organisms could maintain and adapt to extreme radiation level, above that on the surface of the Earth (de Vera et al., 2004, 2008).
- To have better **water balance**, the following methods were observed: 1. Bacteria used to make compact structures with small internal voids, cementing the mineral grains together. Such structures slow down the diffusion of water outside and provide larger internal surface for adsorption. 2. The mucilaginous sheath composed of hygroscopic polysaccharides also keeps the H_2O relatively strongly (e.g., *Nostoc* colonies can store much water in gelatinous substance, continuing photosynthetic activity in dry season). 3. Dew collection by small antennas, and seasonal shifting of Microcoleus (goes up for dew in certain periods) were observed in Tunisia, Sahara, first by Campbell (1979), and later in our samples from Sahara and Australia, too.
- **Temperature.** Our sample from Devon Island Houghton crater, with *Gloeocapsa atrata, G. punctata* tolerated temperatures down to around −50°C in wintertime for several months, and we identified *Microcoleus chthonoplastes and Chroococcidiopsis* from Antarctica, witnessed roughly to the same temperatures. The same species of Microcoleus is exposed in the Sahara and in the West Australian desert often to temperature above +50°C.
- **Long dormancy.** The organisms in the analyzed samples were inactive for a substantial period, somewhere most of the year (during polar winter or dry periods in deserts). There are examples from other researchers on extremely long dormancy: Cyanobacteria were revitalized from permafrost samples after up to 2–3 million years (Gilichinsky et al., 1995; Vishnivetskaja et al., 2001).
- **Fast recovery.** We found among other cyanobacteria taxa *Gloeocapsa, Gloeocapsopis,* and *Scytonema* in the observed crust samples showing quick water-absorbing ability and pretty fast change from dormant to active state within minutes, after long (3–6 years) dormant period. Even their vegetative reproduction (releasing hormogonia) started within 10–20 min (Peli et al., 2007). Garcia-Pichel and Belnap (1996) showed that both photosynthesis and respiration in cyanobacterial crust from Utah started within minutes of rewetting, just like similar process was observed by other authors (Potts and Friedmann, 1981).

4. Conclusion

Based on the observations and theoretical arguments plus model computations, we found that there are possible ideal microhabitats on Mars today in the circumpolar region, which maintain environmental parameters close to the threshold limit of extremophilic organisms on Earth. Below, we first summarize the favorable conditions at these locations, which are ephemerally present, then we outline a possible life cycle of the hypothetical organisms there, and last we give an outlook how this hypothesis could be useful.

In a global sense, the Martian circumpolar locations are favorable for living activity because of the following issues:

- The high latitude location is favorable for the presence of *water ice*, which could condense by winter and during the nights in spring there.
- Water ice thus accumulated might decompose aggressive *oxidants* if melted ephemerally.
- The *temperature* is still high enough for some possible metabolic activity in the "warmer" part of the day, despite the high latitude location. Although the water activity is probably very low in this period, several factors like densely packed grain structure, thin layer of porous dust cover, hygroscopic polysaccharides—as we observed in the case of Earthly habitats—may extend the duration of the "wet" period.

In a local sense, the dark-colored and porous-structured dunes are favorable because of the following issues:

- Based on observations, the surface and mm-shallow subsurface layer could be rich in *water ice* in springtime, and there could be a daily cycle of H_2O transport through this layer with downward transport in nighttime by condensation or accumulation of falling H_2O ice crystals and upward transport in daytime by the sublimation form the ice-cemented porous voids from below. The water budget could be improved by hygroscopic salts, which are known to be present on Mars, and could keep H_2O molecules from the wintertime or nighttime frost for an extended period. Observations of flow-like features also suggest the presence of flowing brines or movement of brine-lubricated grains in the polar region.
- Regarding the *radiatio*n environment, the solar UV could be reduced to tolerable level at 2–3 mm below the surface, where light is still enough for photosynthesis, and the cumulative annual radiation damage is lowered during the summertime dormant phase as any organism loses most of its internal water content during that period, and only less active radicals can form then.
- The *dark color* is important for two reasons: dark grains adsorb the sunlight and warm up relatively fast, and based on their color, these dunes are also less oxidized than the average Martian surface.
- A *weathered crust*-like layer on the top of dunes may produce favorable habitats using the water-trapping methods observed in microenvironments inside desert varnish on the Earth (Beaty et al., 2006; Kuhlman et al., 2008).

Based on our analogue studies and theoretical assumptions, we outlined strategies that could be used in the above-mentioned shallow microscopic locations and periods:

- Analysis of the cryptobiotic crusts on Earth from hot and cold deserts suggests various survival strategies, including seasonal shift of bacteria, light conduction below with optical fiber strategy, and rock coverage plus pigmentation. These may serve as examples as to what kind of survival strategies could be used by a hypothetical Martian biota.
- The short active-long dormant life cycle may work in theory, but the real Martian conditions are not well known to elucidate this issue. Observed examples on Earth show that bacteria are able to recover in minutes after a long dormant state. Observations (Smith et al., 2009) and models (Zent, 2008) indicate that the polar surface conditions have changed substantially on the scale of million years, and Martian polar terrains may have been more hospitable to life in the past than today. During these more favorable periods, hypothetical living organisms could have flourished, while the conditions today merely let them only survive.

The seasonal changes of dark dune spots and those of hypothetical Mars surface organisms (MSOs) could be connected via the following DDS-MSO model:

1. Early springtime sunshine causes sublimation and jets of CO_2 or geyser-like eruptions (Kieffer, 2000; Kieffer et al., 2006) on the ice-covered dunes.
2. Sunshine penetrates through the holes of CO_2 ice reaching the water ice layers below and heats up the light-absorbing dark basaltic dune grains inside this water ice (Horváth et al., 2002).
3. Thin interfacial water layer forms around the dune grains on a daily basis (Horváth et al., 2001), where sub-ice greenhouse effect (Gánti et al., 2002, 2003) and heat-insulating vapor layer may also contribute to the process.
4. Hypothetical MSOs start to metabolize, where wetting/drying and active/passive phases take place on a daily cycle (Horváth et al., 2001; Gánti et al., 2002, 2003).
5. The thickening of interfacial water layer causes seasonal seepage-like features (Fig. 2). These surface features produced here differ from those formed by geyser-like activity but resemble to seepages in the Antarctic Dry Valleys (Head et al., 2007).
6. At the end of spring, the surface gets dry, and the MSOs go into dormant state (Gánti et al., 2002, 2003).
7. A Martian year later, the DDSs and seepages reappear roughly at the same location and became active again (Gánti et al., 2003; Horváth et al., 2005).

This model is compatible with the observations of Mars, and the parameters in them are close to the tolerance limit of known extremophiles on Earth. Although important but problematic issues remain, especially concerning the conditions for water uptake and for metabolism, which are separately favorable in nighttime and daytime, respectively, it is also known that there are factors which decrease the H_2O loss at elevated temperature.

Although the present-day Martian surface environment is harsh and unpleasant for living organisms, there are reasons to believe in the possibility of life there today. If life appeared on Mars in the early warm and wet period, living organisms later had to face with strong environmental challenges that kept them from evolving to such a complex level than on Earth—but it still may be more complex than commonly assumed (Gibson et al., 2009). Any possible Martian biota had substantially longer period for adaptation than organisms on Earth, and such adaptation may have happened in substantially larger populations than in the smaller and localized extreme habitats on Earth. The selection pressures could be also more enduring on the red planet. Under such conditions, a larger number of rare mutations could have been selected for (Maynard Smith and Szathmáry, 1995), and the ability of multiple extremophilicity (against desiccation, salt content, cold, radiations together) may have been appeared in forms not present on the Earth. As a result, Martian organisms may better be adapted to the harsh environmental conditions.

Although we are far away from finding definitely habitable environments on Mars, the possible microhabitats and the possible life-cycle characterized in this chapter are suggestive. These suggestions are useful in the planning of Mars simulation chamber tests, in order to simulate the best possible and still realistic conditions on Mars and also to plan/realize abiotic tests on the analysis of the temperature and H_2O loss/retain issues alone. Our considerations may also inform the targeting of remote-sensed and in situ research, including the possible presence of hygroscopic materials, the ice cement inside the dunes, a weathered surface crust, or spectral characteristics related to the processes possibly going on inside these dunes.

Summarizing the above-mentioned considerations, we stress that in the upper crust of the polar dunes, small voids and hygroscopic materials may substantially decrease the loss of brines, formed from the nighttime condensed water ice. Such processes could produce a viable microenvironment there for metabolic activity around the lower end of the known temperature range of metabolic activity on Earth.

The requirements and elements of the DDS-MSO model are compatible with several observations (polar frost formation, estimated water activity, temperature, UV screening of minerals, etc.) of Mars, and the estimated environmental parameters on these dunes are close to the tolerance limit of known terrestrial extremophiles. As a result, we suggest hypothetical Martian organisms may find an ephemerally tolerable microenvironment at the polar dunes on Mars, and these dunes have high importance in astrobiology (Gánti et al., 2003; Szathmáry et al., 2007), as one of the best locations to search for the evidence of life on the Martian surface today.

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Biodata of **Diana E. Northup, Jennifer J. Marshall Hathaway, Jessica R. Snider, Monica Moya Balasch, Matthew G. Garcia, Maria L. N. Enes, Dapkevicius, and Cristina Riquelme Gabriel, Fred D. Stone, Michael N. Spilde.,** and **Penelope J. Boston,** authors of "***Life in Earth's Lava Caves: Implications for Life Detection on Other Planets***."

Dr. Diana E. Northup has been studying things that live in caves since 1984. She and her colleagues on the SLIME (Subsurface Life In Mineral Environments) Team are investigating how microbes help form the colorful ferromanganese deposits that coat the walls of Lechuguilla and Spider Cave in Carlsbad Caverns National Park; how these deposits compare to surface desert/rock varnish coatings; how microbes participate in the precipitation of calcium carbonate formations called pool fingers; and the microbial diversity located in the hydrogen sulfide cave, Cueva de las Sardinas in Tabasco, Mexico, using molecular, microbiological, and microscopy techniques. Dr. Northup has been honored by having her work featured on NOVA, the BBC, National Geographic, and the Discovery Channel. She is Professor Emerita in the University Libraries and a Visiting Associate Professor of Biology at the University of New Mexico, USA.

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Dr. Cristina Riquelme Gabriel is working in the identification of the diversity of microorganisms associated with the microbial mats in the Azorean caves using both culture-dependent and culture-independent techniques. She and her colleagues in the University of Azores are investigating what controls the diversity observed in this amazing environment. Their main aim is to elucidate the contribution of surface land use, precipitation, elevation, tourist activities, and soil composition in shaping these microbial communities. They are also evaluating the antimicrobial pattern of cave isolates against pathogen groups with previously reported resistance to antibiotics. She obtained her Ph.D. in Biology in the Autonomous University of Madrid and is working as a postdoctoral researcher with Prof. Maria de Lurdes Enes Dapkevicius at the Center for Agricultural Research and Technology of the Azores (CITA-A) in the University of Azores, Terceira, Portugal.

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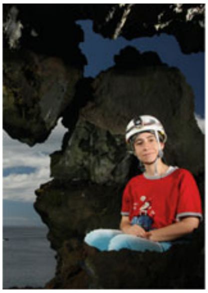

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Dr. Fred D. Stone has been studying lava cave invertebrates in Hawai‘i, USA, since the 1970s and in Australia since 1985. Since retiring as Professor at the University of Hawai‘i, he has continued studies of cave crickets in Hawai‘i and cave cockroaches in Australia, Southeast Asia, and Madagascar. He began working with Dr. Northup on the microbial mats in Hawaiian lava caves in 2008, studying the variables that might explain their distribution.

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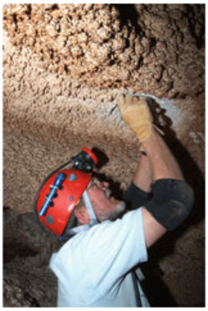

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Dr. Penelope J. Boston has been studying life in extreme Earth environments since 1979 and thinking about how to apply such information to the search for life beyond Earth. Boston's particular interests are in the precipitation of distinguishable biominerals, biotextures, and geochemical signals that can be used as biosignatures of life in Earth's ancient rock record and for future extraterrestrial life-detection missions. She is particularly concerned with the patterns that microbial communities may form in response to microbial, chemical, and physical processes. She is also engaged in trying to uncover the underlying ecological and energetic themes that can be seen in a wide range of geochemical microbial subsurface habitats. Penny does a great deal of public outreach and frequently appears in the print and broadcast press, for example, on NOVA, the BBC, National Geographic Channel, Discovery Channel, Learning Channel, History Channel, and many others.

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LIFE IN EARTH'S LAVA CAVES: IMPLICATIONS FOR LIFE DETECTION ON OTHER PLANETS

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1. Introduction

Caves of all types often present a barren, lifeless appearance to the casual observer. But, if you look carefully, caves contain a wealth of life. Lava caves, which occur in volcanic terrains, are especially rich in both macroscopic and microscopic life. Caves also are of astrobiological interest because they offer an excellent analog for detection of life on other planets, due to the protection they offer from surface conditions and their documented existence on extraterrestrial bodies.

Lava caves occur in many regions of the Earth, for example, Hawai'i, Washington, Oregon, New Mexico (USA), the Azorean (Portugal) and Canary Islands (Spain), and Iceland in the Atlantic Ocean, Japan, Korea, and Australia (Fig. 1). Invertebrates that inhabit these lava caves have been studied for many years in Hawai'i (e.g., Howarth, 1972, 1973, 1981a, b, 1983, 1993, 1996; Howarth and Stone, 1990; Stone et al., 2004), the Canary Islands (e.g., Ashmole and Ashmole, 1988; Ashmole et al., 1992), and the Azores (e.g., Borges and Oromí, 1991; Borges and Oromí, in press), as well as other locations (see citations in Northup and Welbourn, 1994). Microbial mats and organic oozes that occur in many lava caves were first described by Staley and Crawford (1975), who found the actinobacterial genus *Streptomyces* in lava caves in the state of Washington (USA).

Figure 1. Lava caves in Hawai‘i, New Mexico, Australia, and the Azores. Photos courtesy of Kenneth Ingham.

Stoner and Howarth (1981) were the first to describe the Hawaiian lava cave microbial mats. They found that the lava cave microbial mats were composed of bacteria and fungi and suggested that these organisms might participate in the cycling of nutrients such as nitrogen.

Microbial mats and organic oozes decorate the lava caves with a range of colorful deposits that can be yellow, white, gray, pink, butterscotch, and tan (Fig. 2) in color. White dominates among the microbial mats, with yellow a close second in locations studied to date. Most of the organic oozes observed are a butterscotch-gold or gray in color. These microbial mats and oozes have received remarkably little attention since their discovery. Our work (Northup et al., 2008, 2011; Garcia et al., 2009; Moya et al., 2009; Snider et al., 2009) has concentrated on lava caves in the Azores (Portugal), New Mexico, and Hawai‘i (USA), from which we will draw examples for the discussions below. Studies of life in lava caves have important implications for the search for life on other planets, where life might have taken refuge below the surface in lava caves.

2. Lava Cave Environments on Earth: Implications for Life

As with other types of caves, lava caves might be characterized as having up to five zones: entrance, twilight, transition, deep, and stagnant air zones, as characterized by Howarth (1993). The zones within a cave are delineated by physical characteristics

Figure 2. Lava microbial mats and organic ooze. Clockwise from the *top left*: overview of *yellow* microbial mat on wall; *white* and tan colonies; *white*, wrinkled colonies; *white*, worm-like, and tan colonies; *pink* colonies; *yellow* colony; *butterscotch-colored* organic ooze (Photos courtesy of Kenneth Ingham).

that include moisture content, amount of light, concentration of gases, airflow, and the degree to which evaporation can take place. These characteristics, and others, also act as selective pressures on organisms inhabiting them. Beyond the twilight zone of the cave, the combination of constant, absolute darkness, and low nutrient levels has led to morphological (e.g., loss of eyes and pigmentation; Moore and Sullivan, 1997) and physiological (e.g., fewer, larger eggs, and slower metabolic rates; Culver, 1982; Northup and Crawford, 1992; Northup et al., 1993) adaptations in cave invertebrates. Relatively constant temperatures and saturated relative humidity in deep zones of caves might lead to the loss of resistance to desiccation (Northup, unpublished results; Snider, 2010) and ability to survive in a saturated environment (Hadley et al., 1981). The low nutrient (i.e., oligotrophic) nature of many caves or areas within caves applies a strong selective force on organisms. The degree to which these selective pressures have led to adaptations in invertebrates is well studied, but little attention has been devoted to their effects in microorganisms. Caves also represent a protected environment due to the lack of ultraviolet radiation and strong weathering effects, such as hurricanes, extremes in temperature, or strong winds. Lava caves share many characteristics with other types of caves but differ in at least two important ways. First, because of their nature, they are shallower, with only a few meters of overlying rock (usually 10 m or less in thickness, Palmer, 2007). Secondly, they occur in basalt, rather than the limestone, granite, gypsum, or ice, in which other caves occur.

The degree to which the surface influences the area from which microbial mats are sampled might be an important determinant of the microbial composition. Because many lava caves have numerous collapses and skylights, true deep and stagnant air zones might not actually exist in many lava caves, although they are probably common in the smaller mesocavernous spaces interspersed throughout lava flows. Our studies of microbial mat composition include results from sampling in the twilight, transition, and deep zones.

3. Fueling Microbial Life in Lava Caves: What's to Eat?

Because of their shallow nature, numerous skylights, and numerous cracks and fissures, many lava caves might receive more nutrients than carbonate (limestone) and other caves. Organic matter from overlying vegetation and wind-blown detritus seeps and oozes into the cave through the many crevices and cracks in the overlying lava rock (Fig. 3). Also taking advantage of these cracks are plant roots from surface vegetation, seeking moisture and nutrients. Tropical plants have trouble maintaining their water balance (Howarth, 1993) and their roots often enter caves in search of moisture. Some plant families, such as Moraceae (*Ficus*), Bombacaceae (*Ceiba),* Sterculiaceae (*Brachychiton*), and Myrtaceae (*Eucalyptus, Metrosideros*) have species with special adaptations for growing in rocky substrates and sending their roots deep into spaces in the rock to reach water. Therefore, particularly in tropical areas, these roots are extensive and an important resource for invertebrates and possibly microorganisms in the caves (Howarth, 1993; Stone et al., 2004) (Fig. 3). Skylights bring in copious amounts of organic material and even animals that enter the cave or that survive the fall to the floor below. Because of the humid nature of many lava caves, even in the twilight zone, moss gardens flourish under these openings, and they become a refuge for life and a nutrient source for animals traveling to other parts of the cave, which might in turn bring nutrients to microbial mats (Fig. 3).

Our studies in lava caves in New Mexico have shown that organic carbon and nitrogen are present in rocks and soils in the caves. For organic carbon, these levels range from 43–68 % in roots, to 0.83–3.4 % in floor soils/rocks, to 0.18–1.87 % in wall rocks. Nitrogen varied from 0.1–1.1 % in roots, to 0.06–0.39 % in floor soils/rocks, to 0.01–0.03 % in wall rocks (Snider, 2010). These results demonstrate that essential nutrients such as nitrogen and organic carbon to fuel metabolism are available in the lava caves.

Another source of energy for life is the elemental makeup of the basaltic rock that can be used by chemolithotrophic microbes. Our recent elemental analysis of basalt substrates adjacent to microbial mats in Hawai‘i and the Azores shows

Figure 3. Sources of energy in lava caves include roots entering caves, infiltrating water, carrying nutrients, airflow from the surface, skylights that might have moss gardens below them, and plant detritus, such as this leaf that has been colonized by fungi (Photos courtesy of Kenneth Ingham).

5.7–9.3 wt.% of FeO[1] in Azorean samples and 5.2–14 wt.% of FeO in Hawaiian samples (Hathaway and Garcia, unpublished data). We infer that most of this iron is probably present in a reduced form in the basalt based on typical compositions of lavas in the region (HVGDS, 1995). Within the extensive GEOROC (Geochemistry of Rocks of the Oceans and Continents) database (http://georoc.mpch-mainz.gwdg.de/georoc/Entry.html), maintained by the Max Planck Institute for Chemistry in Mainz, Germany, FeO analyses, where Fe is in the reduced state of Fe(II), range on the order of 5–12 wt.% whereas Fe in the oxidized state (Fe(III)) is an order of magnitude less. Such reduced iron is a possible energy source for iron-oxidizing bacteria. Although present in much smaller concentrations, another element that might be useful for microorganisms is manganese, which ranges from 0.09 to 0.19 wt.% MnO in Azorean samples and 0.07 to 0.21 wt.% MnO in Hawaiian samples (Hathaway and Garcia, unpublished data). We infer that the oxidation state of these Mn compounds is likely to be reduced and thus also potentially available as an energy source. For the island of Hawai'i, values of MnO (Mn as Mn(II)) ranging from 0.1 to 0.3 wt.% have been reported (HVGDS, 1995). Small amounts of lead, copper, and cobalt are also present in the basaltic substrates and are typically toxic to organisms, but, if complexed with sulfides, could conceivably be a source of energy. Such potentially toxic metals might be challenging for some organisms, but tolerated by those

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[1] Original analyses were reported as elemental compositions but have been converted to oxide wt.% to conform to mineralogical standard reporting.

adapted to high metal environments as is known in many rock environments (Banfield and Nealson, 1998; Gadd, 2007). Sulfur is a common component of lava rock, especially in recent eruptions and in areas with gaseous effusions from magma bodies. Sulfur in the HVGDS database (1995) ranges from 0.01 to 0.31 wt.%, but its chemical state is not reported. Many sulfur compounds can be nutrient sources for organisms (Boston et al., 2006; Dahl and Friedrich, 2008).

4. Trends in Bacterial Phylogeny

4.1. DOMINANT BACTERIAL GROUPS

Culture-independent molecular analyses of microbial communities have revolutionized our characterization of “Who’s Home” in different environmental habitats and caves are no exception. Our studies of lava cave microbial mats have shown that 14 phyla within the domain *Bacteria* (see Fig. 4 to orient yourself to the placement of some of these phyla within the Tree of Life) are found in lava caves of all three of our study locations. The most ubiquitously found phyla include the *Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Nitrospirae,* and *Verrucomicrobia*. Found frequently, but not in both yellow and white microbial mats or in all locations, are the additional phyla of *Gemmatimonadetes, Planctomycetes, Bacteroidetes, Chlamydiae*, *OD1/OP11, Firmicutes, Ktedonobacteria,* and *TM7.* These results, at the phylum level, mirror those found in soils by Janssen (2006), who analyzed 32 different libraries of sequences from different soils. Thirty-two

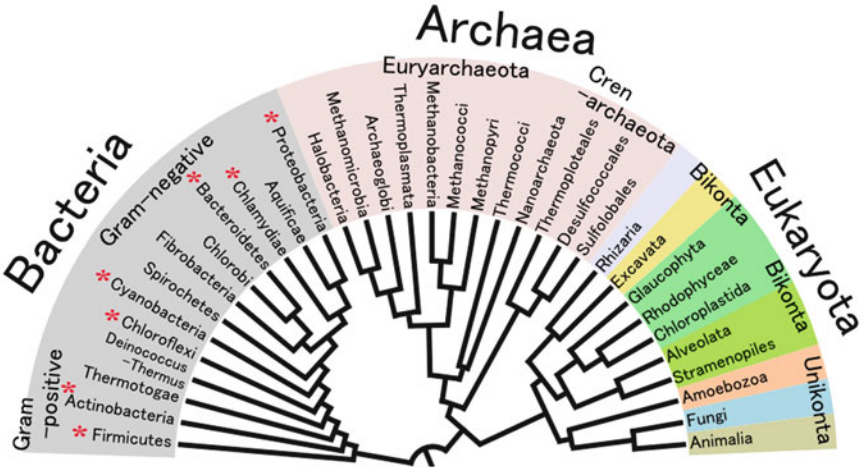

Figure 4. Phylogenetic Tree of Life from the Wiki Commons. Some of the phyla discussed in this chapter are in the domain *Bacteria* and have a *red asterisk* beside the phylum name. Illustration is in the public domain under the GNU Free Documentation License.

different phyla were found across the soils, with nine phyla dominating: *Proteobacteria, Acidobacteria, Actinobacteria*, *Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, Gemmatimonadetes*, and *Firmicutes* (Janssen, 2006). The *Proteobacteria* make up the largest percentage (39 % on average) of sequences found in the soils. Most of the sequences were novel, as is also true of the lava cave bacterial sequences, and the conclusions of Janssen's analysis differ markedly from the results of soil cultivation studies conducted over prior decades.

Our results also show some congruence with those of Gomez-Alvarez et al. (2007), who found that *Acidobacteria*, *Alpha*- and *Gammaproteobacteria*, *Actinobacteria*, and *Cyanobacteria* predominate in surface volcanic terrain bacterial communities on Hawai'i. One major exception to this is the occurrence of *Cyanobacteria*, which are rarely found in caves due to their usual requirement for sunlight to fuel photosynthesis. In our studies, we found substantial *Chloroflexi, Verrucomicrobia,* and *Nitrospirae*, which differs from the findings of Gomez-Alvarez et al. (2007).

One phyla of particular interest is the *Actinobacteria*, which are well known from caves (see, e.g., Groth et al., 1999) and soils. Many of our recovered clone sequences fall within this phylum. Within the *Actinobacteria,* many of our sequences are located within the order *Actinobacteridae*, a trend that is true of *Actinobacteria* in general. Within this order, our sequences fall predominantly in the subclasses *Actinobacteridae* and *Acidimicrobidae* (Zhi et al., 2009). These actinobacterial sequences are of particular interest because of the widespread ability of *Actinobacteria* to produce antibiotics, many of which have been developed for human use and the potential to identify new antibiotics from these genera (Lazzarini et al., 2000).

4.2. NOVEL DIVERSITY

Our lava cave studies routinely find novel genetic sequences across phyla and vary by cave studied. Several of the actinobacterial genetic sequences recovered from Hawaiian and Azorean microbial mats are quite novel, with several sequences being less than 90 % similar to any bacterial genetic sequence in NCBI's GenBank database. These results hold true in other phyla too, as illustrated by studies of the root communities in New Mexico lava caves, where only 5.9 % of wall and floor sequences and 8.5 % of root sequences grouped with cultured closest relatives. This suggests the presence of new families of *Actinobacteria* and other phyla in these lava cave microbial mats and roots (e.g., Fig. 5). Interestingly, several of the closest relatives of lava cave gene sequences are from studies of Oregon Caves National Monument (USA), Altamira Cave (Spain), Frasassi Cave (Italy), Mammoth Cave (USA), and Hawaiian volcanic deposits, as well as from studies of soils. Seven percent of the recovered Hawaiian and Azorean clone sequences were closely related to sequences retrieved from other volcanic terrain studies, which might suggest a community of organisms adapted to basaltic habitats.

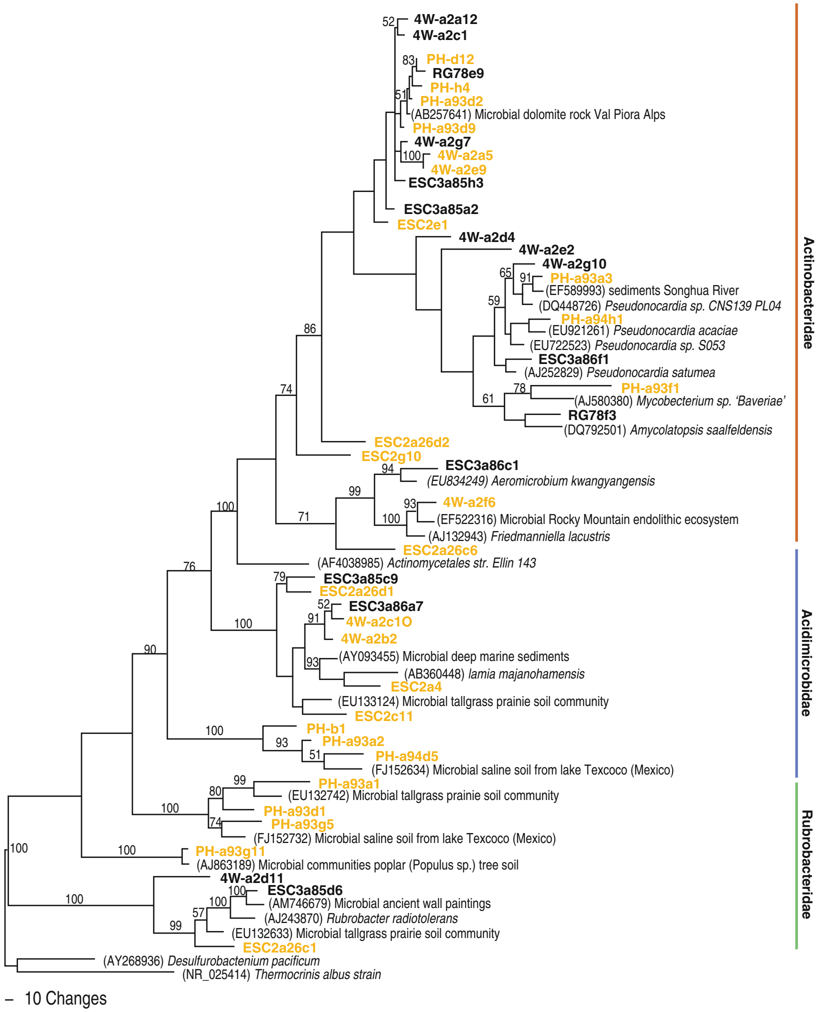

Figure 5. Phylogenetic tree of *Actinobacteria* clone sequences from Four Windows (4W), Pahoehoe (PH), and Escondido (ESC) Caves in El Malpais National Monument. Sequences that lack closest relatives illustrate the more novel organisms. Numbers above the horizontal branches represent confidence values (i.e., bootstrap values).

While the recovery of so many genetic sequences that are not closely related to any described, cultured organism is not unusual, it does suggest two avenues of future research. First, we propose to compare cave genetic sequences to those obtained from overlying soils. Secondly, our results suggest that cave microbial phenotypes might be identifiable and that subsurface indigenous communities might exist that can be characterized.

Table 1. Distribution of bacterial phyla found in white and yellow microbial mats in Hawaiian, New Mexican, and Azorean lava caves, based on 16S SSU genetic sequences from clone libraries.

Bacterial phylum	**White Hawai'i**	**White New Mexico**	**White Azores**	**Yellow Hawai'i**	**Yellow New Mexico**	**Yellow Azores**
Actinobacteria	*	*	*	*	*	*
Alphaproteobacteria	*	*	*	*	*	*
Betaproteobacteria	*	*	*	*	*	*
Gammaproteobacteria	*	*	*	*	*	*
Deltaproteobacteria	*	*	*	*	*	*
Acidobacteria	*	*	*	*	*	*
Chloroflexi	*	*	*	*	*	*
Nitrospirae	*	*	*	*	*	*
Verrucomicrobia	*	*	*	*	*	*
Gemmatimonadetes		*	*	*	*	*
Planctomyetes			*	*	*	*
Bacteroidetes	*	*	*		*	*
Chlamydiae	*		*	*		*
OD1/OP10	*		*	*		*
Firmicutes			*	*	*	*
TM7	*				*	*
Ktedonobacteria			*			

4.3. COMMUNITY COMPARISONS

Our studies suggest that the yellow microbial mats are slightly more diverse than white mats in terms of bacterial species present (Table 1). We also found that Hawaiian mats are the most species-rich, followed by the Azorean mats, and finally by New Mexican mats (Hathaway et al., unpublished data). The latter finding might be attributed to higher surface precipitation levels in the former two locations, but this is still being tested for all sites. Gomez-Alvarez et al. (2007) have shown that community makeup on surface volcanic terrains is controlled by the makeup and characteristics of the soil and trace gases. What actually controls the microbial diversity that we see in these lava caves is currently under investigation. Our preliminary results suggest that geography is the key factor in determining differences among community composition in Hawaiian and Azorean lava cave microbial mats (Hathaway et al., unpublished data). Other factors that might influence microbial mat community composition include surface precipitation, surface land use, surface vegetation, age of the lava flows that created the caves, elemental composition of the basalt substrate, and the microenvironment within lava caves.

Some anomalous occurrences of areas with no visible microbial mat on cave walls have led us to begin an investigation of whether passage shape, orientation, obstructions, water availability, and location within passage might influence the colonization and establishment of microbial mats. In Epperson's Cave, on the island of Hawai'i, we have observed a dead-end side passage with reduced airflow that occurs on the inner side of a large breakdown mound. Microbial mats are profuse before the mound but occur mainly on the higher ceiling in the deep cave zone after the mound. This suggests that nutrients carried on atmospheric currents or conditions created by the breakdown mound, such as constant high humidity due to trapped water vapor, might affect microbial mat initiation and growth. In New Mexico caves, our research has shown that floors are more nutrient-rich than walls/ceilings but supported a less diverse microbial community than that found on the walls/ceiling of the lava cave (Snider, 2010).

5. Roots as a Conduit for Nutrients and Immigration

Roots are a very obvious feature in some lava caves, especially in the Hawaiian Islands, New Mexico and Washington (USA), the Canary (Spain) and Azorean Islands (Portugal), Mexico, Japan, Australia, and Korea (Oromí and Martin, 1992), and have been studied for many years as habitats for subterranean invertebrates (Howarth, 1993; Jasinska et al., 1996; Stone et al., 2004; Howarth et al., 2007). What has received less attention is the role of roots as a conduit for microorganisms and nutrients that supply microbial communities (Snider et al., 2009; Snider, 2010). One can often observe water droplets and exudates on the roots that hang into caves, supporting the idea that they transport water and nutrients from both surface plant communities above the lava cave and from deeper, more water rich soils. Exudates produced by roots, dead root material (root cap cells that are shed or fine roots hairs), and young roots leaking sugars provide additional carbon and nitrogen inputs to the cave ecosystem (summarized in Snider, 2010). As the roots grow into the caves through the overlying basalt layer, they bring with them microorganisms from within the rhizosphere and thus act as a route for immigration of microorganisms into the caves. The studies by Snider et al. (2009; Snider, 2010) represent the first studies to investigate the influences of roots growing into caves on the microbial communities in caves. Her studies have revealed that the microbial community differs, especially at the species (or operational taxonomic unit defined at the 97% sequence similarity level), in the areas with roots as compared to those without roots and that levels of organic carbon and nitrogen are significantly higher in root samples (Fig. 6). The area directly beneath the roots receives increased organic carbon and nitrogen input also (Fig. 6).

One of the interesting findings from this study is the proportion of *Actinobacteria* clones recovered in the clone libraries. The highest number of actinobacterial clones was found in the clone libraries from the wet ceilings area and from the wet floor below the roots, suggesting that moisture might be essential for *Actinobacteria.* Approximately twice the number of actinobacterial clones was found in the wet areas of the caves than in the roots. *Actinobacteria* are known to inhabit caves (Moore and Sullivan, 1997) and soils, including the rhizospheres of numerous plants (Merzaevaa and Shirokikh, 2006; Norovsuren et al., 2007). The latter study found more *Actinobacteria* in the soil than in the rhizosphere, which mirrors the results from Snider's study (2010) and suggests that soils might provide some of the actinobacterial colonizers of caves.

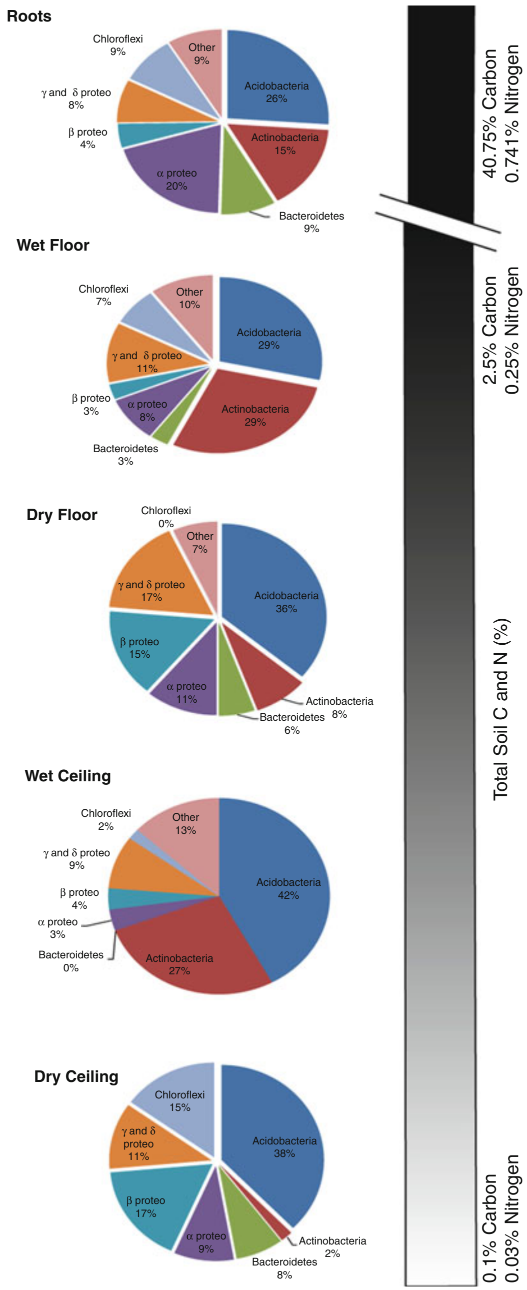

Figure 6. Phylogenetic analysis of bacterial phyla clone sequences from Roots Galore and Pahoehoe Caves in El Malpais National Monument. Habitats analyzed, from top to bottom, include communities on *roots*, the *wet floor* beneath the roots, the *dry floor* beyond the roots that receives no moisture dripping into the cave, *wet ceiling* in areas with no roots, and *dry ceiling* in areas with no roots and no dripping water seeping into the cave, arranged along a gradient of percent organic carbon and nitrogen found in samples from these areas.

Roots provide both an excellent habitat for microorganisms in caves, might be a conduit for microorganisms entering the cave habitat, provide nutrients, including organic carbon and nitrogen, to the cave environment and provide moisture to the surface plants.

6. Microbes That Masquerade as Minerals

In addition to the dazzling display of microbial mats in lava caves, we have discovered an array of deposits in these caves that on first glance, appears to be primarily mineral in nature (Fig. 7). Extracted DNA from samples and scanning electron microscopy (SEM) studies have shown that these deposits contain substantial microbial communities.

Clone libraries created from the DNA extracted from samples of several of these deposits show a diversity of bacterial phyla present with some important differences across samples (Table 2).

Figure 7. Mineral-like deposits in lava caves often contain extensive microbial communities. Clockwise from the *upper left*: iron oxide formations (Azores, Portugal); organic ooze icicle, colonized by microbial colonies (Azores); copper silicate stalactite (Hawai‘i, USA); *gold-orange* deposits (Hawai‘i); *pink* iron oxide hexagons (Azores); pointillistic deposits on top of organic ooze and basalt (Hawai‘i); and *gold-colored* veins on basalt walls (New Mexico, USA) (Photos courtesy of Kenneth Ingham).

Table 2. Distribution of bacterial phyla found in mineral deposits in Hawaiian, New Mexican, and Azorean lava caves, based on 16S SSU genetic sequences from clone libraries.

Bacterial phylum	Crusty gold-colored Hawai'i	Crusty gold-colored New Mexico	Pink hexagons Azores	Iron formations Azores	Organic ooze Azores	Blue-green Hawai'i	Yellow microbial mat Azores
Actinobacteria		*			*		*
Alphaproteobacteria	*	*	*	*	*	*	*
Betaproteobacteria	*	*	*	*	*	*	*
Gammaproteobacteria	*	*	*	*	*	*	*
Deltaproteobacteria	*	*	*	*	*	*	*
Acidobacteria	*	*	*	*	*		*
Chloroflexi	*		*	*			*
Nitrospirae	*	*	*	*	*	*	*
Verrucomicrobia			*				*
Gemmatimonadetes	*	*	*		*		*
Planctomycetes		*	*			*	*
Bacteroidetes		*	*		*		*
Chlamydiae					*		*
OD1/OP10					*		*
Firmicutes				*			*
TM7			*		*		*

Actinobacteria, while present in all microbial mats studied to date (Table 1), are not present in the gold-colored deposits in Hawai'i, the Azorean pink iron oxide hexagons, the Azorean iron formations, and the blue-green copper silicates from Hawai'i. *Firmicutes*, a phylum known to contain iron oxidizers, represented many of the clones retrieved from the iron oxide formations in the Azorean lava cave. The proteobacterial subdivisions were uniformly represented in both the mineral deposits and the microbial mats (Tables 1 and 2). This is not surprising since the *Proteobacteria* are one of the major components of the soil microbiome. The shallowness of lava caves dictates that soils, where present above the caves, will be a major source for lava cave microbial inhabitants. The other commonality is the uniform presence of *Nitrospirae*, a phylum that contains nitrite oxidizers, important players in the nitrogen cycle. The diversity across the mineral deposits is surprising and several of the closest relatives come from other cave studies.

Examining these mineral deposits with scanning electron microscopy (SEM, Fig. 8) reveals many key features about the microorganisms present. The gold-colored deposits, which resemble a crusty mineral deposit and vary from a light gold to an orange-gold in color, contain putative microbial morphologies that include coccoid-shaped forms and filamentous forms (Fig. 8, top left). The blue-green deposits, which XRD suggests are a poorly crystalline chrysocolla (copper silicate), contain a wealth of fuzzy filaments interlaced with many reticulated filaments, such as those seen in many other cave deposits (Melim et al., 2008). Our team has found these reticulated filaments in several carbonate and iron cave deposits, but this is the first occurrence in a copper mineral deposit. Their abundance in these deposits might provide an opportunity to cultivate them in order to scientifically describe these enigmas. The two images on the right in the middle row (Fig. 8) represent the pointillistic deposits shown in Fig. 7. These small white dots on the basaltic walls look like a cross between colonies and mineral deposits. The SEM reveals that an iron oxide deposit is present, through which the colonies erupt. A small piece of the carbon biofilm that covers the colony itself can be seen in the middle picture, on top the colony. Finally, the fascinating pink iron oxide hexagons (bottom row, Figs. 7 and 8) are shown to contain coccoid and filamentous morphologies on the hexagons and between the hexagons. The SEM images suggest that the microorganisms are degrading the hexagons.

An important take home message from this study is that mineral deposits should not be overlooked in studying the microbial life of lava caves in particular. The investigation of these deposits can help guide the interpretation of fossil and geochemical traces of ancient life in the rock record and potentially the detection of life on other planets, where only the mineral remnants of life might remain as biosignatures.

7. Implications for Life Detection on Other Planets

In the early 1990s, Boston et al. (1992) suggested that the subsurface of Mars would be a worthy target for the search for signs of extant or fossil life, because of the protection offered from harsh surface conditions by the enclosed cave environment.

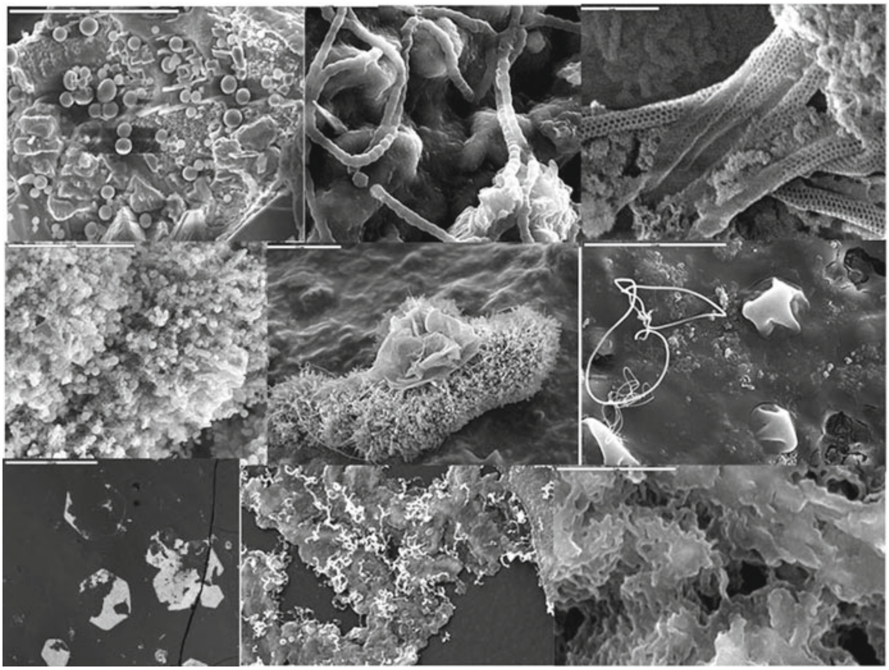

Figure 8. Scanning electron micrographs of mineral deposits in lava caves from the Azores, Hawai'i, and New Mexico. *Top row* from the *upper left*: coccoid-shaped putative bacteria present in *gold-colored* deposits in Thurston Lava Tube (Hawai'i, USA), scale bar = 50 μm; filamentous morphologies from *gold-colored* deposits in Four Windows Cave (New Mexico, USA), scale bar = 10 μm; reticulated filaments present in copper silicate stalactite (Hawai'i, USA), scale bar = 2 μm. *Middle row* from the *left*: typical microbial mat microbial morphologies for comparison (Azores, Portugal), scale bar = 20 μm; colony in the pointillistic deposits (Hawai'i), scale bar = 50 μm; colonies that have not yet erupted from an iron oxide biofilm in the pointillistic deposits (Hawai'i, USA), scale bar = 20 μm. *Bottom row*: *pink iron* oxide hexagons (Azores) overview, scale bar = 50 μm; closer view of an area colonized by filamentous bacteria with some coccoid shapes present; field of view is approximately 10 μm; close view of a portion of one hexagon showing putative coated filaments, scale bar = 20 μm (Photomicrographs by Michael Spilde, Diana Northup, and Penelope Boston).

Not only might life exist or thrive in this protected environment, but any remnants of former life would be preserved from destruction by solar radiation and high-energy galactic background radiation that reaches the surface of Mars through the extremely thin atmosphere (Westall et al., 2000; Villar et al., 2006; Izawa et al., 2010; Léveillé and Datta, 2010; Boston, 2010).

Lava caves in Earthly volcanic terrains make an especially attractive extraterrestrial analog environment due to the presence of large amounts of basaltic terrain on Mars and the identification of lava caves on the surface in a number of Martian localities and on other Solar System bodies (Boston, 2004; Cushing et al., 2007; Wynne et al., 2008; Haruyama et al., 2009). The potential for such cavities on Mars to harbor substantial amounts of ice over geologically significant periods of time has been suggested (Frederick et al., 2000) and strongly argued in a modeling study (Williams et al., 2010). It is also thought that Mars might undergo super annual climate cycles tied to obliquity variations on the order of 100,000-year duration (Laskar, 2004; Levrard et al., 2004). This means that an intermittent hydrological cycle might be restored on these types of timescales. Thus, water ice might have the opportunity to reenter Martian caves during the warm part of these so-called obliquity super seasons. If Williams and colleagues (2010) are correct, then such ices could last for time periods on the order of 30–100 ky before subliming away; thus, water could conceivably be available to potential Martian microbial inhabitants in caves (Williams et al., 2010).

As discussed above, the shallow nature of lava caves on Earth predisposes them to receive inherited organic carbon from the aboveground biosphere. But on Mars, no such surface biology exists today. Thus, alternative energy sources for microbial communities must be contemplated. There are two potential sources of energy available: (1) reduced gases coming from geologically deep sources and (2) oxidizable materials contained within the bedrock upon which organisms might be growing. The first case, reduced gases, has been suggested (Boston et al., 1992; Carr, 1996; Fisk and Giovannoni, 1999; McCollom, 1999; McKay, 2001), and examples of such energy sources supporting Earth subsurface microbial communities have been found in Columbia River basalt fractures (Chapelle et al., 2002), present in a uniquely self-reliant very deep subsurface organism (Chivian et al., 2008), and suggested to be produced by serpentinization (Parnell et al., 2010). Since hydrogen gas is the major reductant for CO_2-reducing methanogens in the deep subsurface (Boston et al., 1992; Chapelle et al., 2002; Ferry, 2010), any process that can produce H_2 is a potential energy source. Water-rock interactions in the Martian subsurface at latitudes where potential subsurface liquid water might underlie an icy overburden might facilitate such aqueous transformations.

Subsurface environments that are iron-rich, such as volcanic lava caves, are considered particularly appealing as astrobiological targets for several reasons. The most obvious is the potential for energy-producing reactions carried out on reduced iron compounds that are certainly present in Earth lavas as previously discussed. Secondly, palagonitization, a weathering process, which is known from both submarine and terrestrial lava surfaces on Earth, has been suggested for the Martian case (Bell et al., 2000) and has been identified as the best spectral analog for Mars surface sediments (Morris et al., 1993). On Earth, palagonitization has been shown to have a major microbial component (Thorseth et al., 1992, 1995; Stroncik and Schmincke, 2002). Such palagonitic lava rinds on the surface and interior of lava caves in Mars material could be evidence of biological processes, but a better understanding of all of these processes on Earth is necessary to help us better interpret data from future planetary missions.

8. Summary

Moving beyond studies of the invertebrate and vertebrate life to the microbial life that inhabits lava caves has allowed us to open up a whole new realm of research into life in Earth's extreme habitats. Our studies are laying the foundation to allow us to understand the lava cave ecosystem better, and in time, we may be able to shed light on what controls species diversity in these subterranean ecosystems. The variety of abiotic and biotic factors available in locations that range from Hawai'i in the Pacific Ocean, to New Mexico on a continental landmass, to the Azores in the Atlantic Ocean, provides an outstanding set of variables with which to tease apart these controls on species diversity. Additionally, we are discovering the range of the bacterial phyla present in these lava caves and discovering novel biodiversity. Finally, these studies allow us to better develop our understanding of biosignatures for use in life detection on other planets.

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LIFE IN THE SATURNIAN NEIGHBORHOOD

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1. Introduction and Context

Charles Darwin's vision, in 1859, of the origin and evolution of life on Earth, paved the way for future biological searches and studies on our planet and on other planetary bodies. The DNA decoding by James Watson and Francis Crick (1953) confirmed both the complexity and the symmetry of living particles. Following their strides, astrobiology, the study of evidence for life outside the Earth, is not only the research of the origin, distribution, and evolution of life in the whole universe but also that of structures and processes related to life and its destiny (Raulin, 2007). In general, astrobiology brings together different scientific disciplines such as astrophysics, geology, chemistry, geochemistry, biology, and more in order to shed light on the many aspects regarding the creation of our solar system as well as the initiation of life. Starting with the terrestrial paradigm, astrobiology focuses on extraterrestrial environments, posing the unanswered question on the origins of life on Earth and elsewhere, while investigating the more easily accessible organic compounds—and in particular the prebiotic chemistry—on other celestial bodies.

It is generally admitted today that life arose on Earth as the derivative of a long chemical evolution, implying three major raw ingredients: liquid water, carbonaceous matter, and energy, working together over time. Since life primitive structures should be able to emerge, evolve, and develop in suitable environments, the quest for possible habitats outside our planet is focused on places where these ingredients are or have been present.

With the discovery of planets beyond our solar system and the search for current living organisms or for favorable conditions for past and future life in exotic places such as Mars, Europa, Titan, and Enceladus, the notion of habitability takes a new dimension. In this chapter, we focus on habitability issues and possible living forms around Saturn. Saturn has 62 known natural satellites to this date, and the discoveries from the Cassini–Huygens mission, which started in 2004, have revolutionized our perception about whether these bodies could harbor life

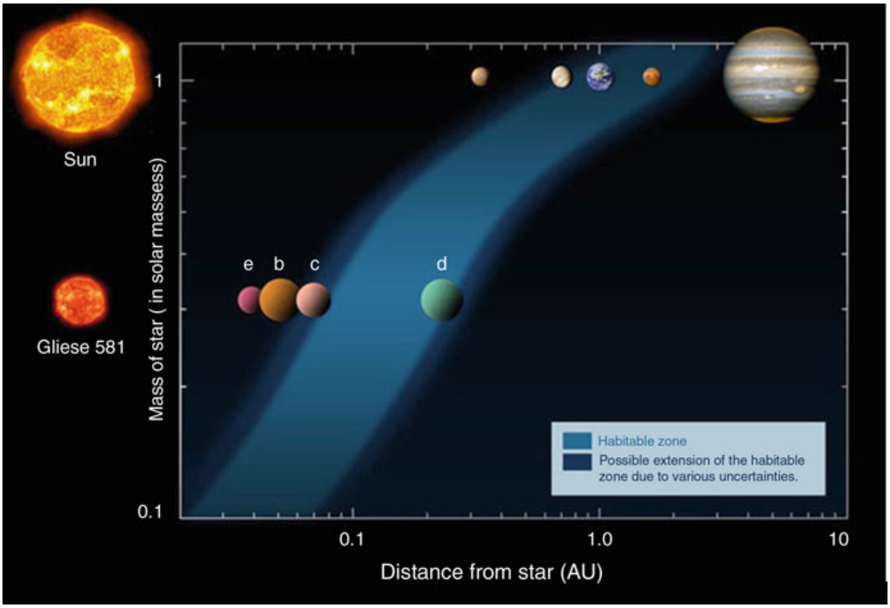


Figure 1. Map of the habitable zone's (*HZ*) limits. Earth is in the middle of the HZ for the solar system while Mars and Venus lie in its boundaries. The planetary bodies *e*, *b*, *c*, and *d* are exoplanets. Gliese 581 is *red dwarf* star 20.3 light years from Earth (NASA).

(now or in the future) or at least provide us with valuable information on the origin and evolution of life in the solar system. In the latter case, at least two of the Kronian satellites, Titan and Enceladus, can certainly offer a lot.

Discovered in 1655 by the Dutch astronomer Christiaan Huygens, Titan is the largest satellite of Saturn, bigger than planet Mercury, at 5,152 km in diameter. Titan rotates around the Sun within 29.5 years following Saturn on its trek. As a result, Titan experiences seasons, each of which lasts about 7.5 terrestrial years. Moreover, Titan orbits around Saturn within 16 Earth-days almost synchronously; thus, its solid surface rotates very slowly. Instead, due to strong zonal winds (Bird et al., 2005; Lorenz et al., 2008b), its atmosphere is in super-rotation. Due to Titan's distance from the Sun of about 9.5 astronomical units (AU), the satellite receives slightly more than 1% of the solar flux that the Earth registers at the top of its atmosphere at 1 AU (Fig. 1). In addition, Titan revolves far enough from the giant planet (about 20 Saturnian radii), to avoid any critical interactions with the rings or the magnetosphere. Although sometimes Titan moves close enough to Saturn to allow its atmosphere to interact with the energetic particles of the magnetosphere of Saturn. Together with the solar photons, these interactions play a key role in Titan's chemical evolution. Indeed, Titan possesses an extensive atmosphere made mostly of N_2 with a column density ten times that of Earth's (Fig. 2). Furthermore, Titan's astrobiological potential is enhanced by the presence

Figure 2. (*left*) Titan's thick, orange, and smoggy atmosphere (NASA); (*right*) Earth's atmosphere as seen from space (NASA); the cloudy nature of both atmospheric envelopes and also commonalities. Both Titan and Earth experience a greenhouse effect and complex photochemical reactions in their upper atmospheric layers.

of a rich organic chemistry which is produced in its atmosphere, thanks to the presence of its second most abundant gas, methane (about 1.4% in the stratosphere and 5% on the ground), and on the surface from the interactions among the various constituents.

Thus, Titan's unique and dense atmosphere harbors a whole host of organic trace gases: hydrocarbons and nitriles (e.g., Coustenis et al., 2007, 2010b). Its surface presents many morphological similarities with the Earth's, which surprisingly show similar structural diversity, but the raw materials are different from those on our own planet. With an environment very rich in organics, Titan, along with comets, is thus often considered as one of the best targets to search for prebiotic chemistry at a full planetary scale and a possible habitat for extraterrestrial life in all probability different from the terrestrial one. More importantly, our understanding of the origin of life on Earth could greatly benefit from studying Titan, where the low solar influx, the composition of the atmosphere, and the possible presence of an internal water ocean give us the opportunity to study the conditions prevailing on the primitive Earth.

After more than 8 years of close observations by remote sensing and *in situ* instruments on board the Cassini–Huygens mission, Titan is revealed as an evolving planet, geologically active, not only from erosional processes in the face of the lack of impact craters (Lopes et al., 2010) but also because of its possible cryovolcanism (Tobie et al., 2005; Barnes et al., 2006; Nelson et al., 2009a, b; Soderblom et al., 2009) and morphotectonism (Solomonidou et al., 2012), aeolian and fluvial erosion (Fulchignoni et al., 2005; Israel et al., 2005; Niemann et al., 2005; Owen, 2005; Tomasko et al., 2005; Zarnecki et al., 2005; Jaumann et al., 2008;

Lorenz and Mitton, 2008; Raulin, 2008; Lebreton et al., 2009), clouds and precipitations, and a methane cycle very similar to the water cycle on Earth (Bird et al., 2005; Atreya et al., 2006; Coustenis and Taylor, 2008; Brown et al., 2009a; Coustenis and Hirtzig, 2009). The organic chemistry products are found on Titan all the way from the upper atmosphere to the surface and possibly in the interior, indicating close exchanges between the different elements and planetary layers (interior-surface-atmosphere).

Enceladus, first observed by Sir Frederick William Herschel in 1789, is another intriguing moon of Saturn. Although it is quite small compared to Titan, with a mean radius of 252 km, the large plumes ejected from its south-polar region, as first discovered by the Cassini–Huygens mission magnetometer (Dougherty et al., 2006), make it very important for astrobiology. These geyser-like features mainly consist of water vapor and ice and include organic compounds (Dougherty et al., 2009; Waite et al., 2009). This strongly suggests the potential presence of a complex organic chemistry ongoing in the interior and the presence of liquid water, providing grounds for the search of a liquid ocean at short distances under the surface.

Enceladus is an unambiguous example of a cryovolcanically active icy satellite identified in the outer solar system and can be used to understand active processes that are thought to have once played and/or are still possibly playing (e.g., Titan's case) a role in shaping the surfaces of other icy moons. These processes include tidal heating, possible internal convection, cryovolcanism, and ice tectonics, which all can be studied as they currently happen on Enceladus. Moreover, the plume source region on Enceladus samples a warm, chemically rich, environment that may facilitate complex organic chemistry and biological processes.

What is the level of complexity reached by the organic chemistry in Titan and Enceladus? What is the correlation between the interior, the surface, and the atmosphere, especially regarding the biological aspects? What are the habitability potentialities in the Kronian environment, in particular for Titan? Such are the questions that further exploration of Titan and Enceladus should answer in the coming years (Brown et al., 2009b). After all, Darwin's evolutionary ideas could also be successfully applied *sensu lato* in the Saturnian neighborhood.

2. Habitability Issues for Outer Planetary Satellites

Liquid water is established as the necessary solvent in which life emerges and evolves. Water, as an abundant compound in our galaxy, can be found in various places, from cold dense molecular clouds to hot stellar atmospheres (e.g., Cernicharo and Crovisier, 2005; Hanslmeier, 2010). The thermodynamic behavior of water, which enables it to remain liquid in a large range of temperatures and pressures and to be a strong polar–nonpolar solvent, makes it essential for maintaining stable biomolecular and cellular structures (Des Marais et al., 2002).

A large number of organisms are capable of living in water. However, in a deposit of pure water, life will probably never spontaneously originate and evolve, since while there are many organisms living in water, none we know of is capable of living on water alone, because life requires other essential elements such as nitrogen and phosphorus in addition to hydrogen and oxygen. Moreover, no known organism is made entirely of water. "Just water" is therefore not an auspicious place for the emergence and evolution of life.

The concept of the habitable zone (Fig. 1) is, of course, based on our understanding of life on Earth and is related to the presence of liquid water on a body's surface. But the requirements for extraterrestrial life do not have to be the same, suggesting that life could exist outside the habitable zone (Cohen and Stewart, 2002), in particular if liquid water exists underneath the surface. Furthermore, the changes that occurred on Earth's primordial atmosphere under the influence of early primitive plant life (Wolstencroft and Raven, 2002) require that diachronic alternation be taken into account regarding the habitability opportunities of a planetary body. In addition, internal processes such as volcanic activity, hydrothermal movements, and radioactive decay that possibly occur within satellites located outside the habitable zone could affect the radiation and thermal level of the body and thus change significantly the environmental conditions favoring life (Horneck, 2008).

Of the large satellites of the gas giants, there are those that may house underground water deposits in direct contact with heat sources below their icy crust and those expected to have either liquid water layers encapsulated between two ice layers or liquids above ice. In the study for the emergence of life elements on such satellites, the timescale is of essence. If it is long enough, the liquid water underground ocean may host life. Thus, the icy satellites of the outer planets of the solar system, as well as the recently discovered exoplanets, host unique conditions which may inhibit the emergence of life precursors in isolating environments that can prevent the concentration of the ingredients necessary for life or the proper chemical inventory for the relevant biochemical reactions. Conversely, according to Trinks et al. (2005), a coupled sea/ice system could in theory provide the necessary conditions for life emergence in the primitive Earth. Additional laboratory experiments and *in situ* studies of deep subglacial isolated lakes in Antarctica (Kapitsa et al., 1996) would improve our understanding in this field, as the physical properties of deep subglacial lakes resemble those found on both Jupiter's moon Europa and Saturn's moon Enceladus (Bulat et al., 2009).

As a consequence, the satellites of the giant planets like Europa, Enceladus, Ganymede and Titan are possible habitable environments and valid targets in the research for life with space missions and/or telescopes. However, life structures that do not influence the atmosphere of their host planet on a global scale will not be remotely detectable. In the solar system's neighborhood, such potential habitats can only be investigated with appropriate designed space missions, as in the case of Europa, where the Europa Jupiter Space Mission (Clark et al., 2009) will attempt to look for the hypothesized internal liquid water ocean. In the case of the Saturnian satellites (see hereafter), proposed future missions shall also address this question.

3. Titan: Organic Factory and Habitat

Retracing the processes, which allowed for the emergence of life on Earth around 4 billion years ago, is a difficult challenge. Our planet has drastically evolved since that time, and most of the traces of what were the initial environmental conditions have been erased as a consequence of plate tectonics and erosion. It is thus crucial for astrobiologists to find extraterrestrial locales with similarities to our planet's early stages. This will provide a way to study in the present time some of the processes, which occurred on the primitive Earth, when prebiotic chemistry was in its young stages (Fig. 3). For instance, a subsurface ocean in the interior of the satellite of a gas giant (as on Europe, Enceladus, Titan, etc.) may be habitable for some kind of life form—even though not necessarily an Earth analogue-but also information on the terrestrial-like atmospheric and surface conditions on any planetary body can provide valuable information.

Titan is a good candidate in this instance as its atmosphere exhibits more similarities with the Earth's today—and even more so in the past than any other solar system body (e.g., Coustenis and Taylor, 2008). Recent Cassini–Huygens findings have revolutionized our understanding of Titan's system and its potential for harboring the "ingredients" necessary for life (Coustenis and Taylor, 2008;

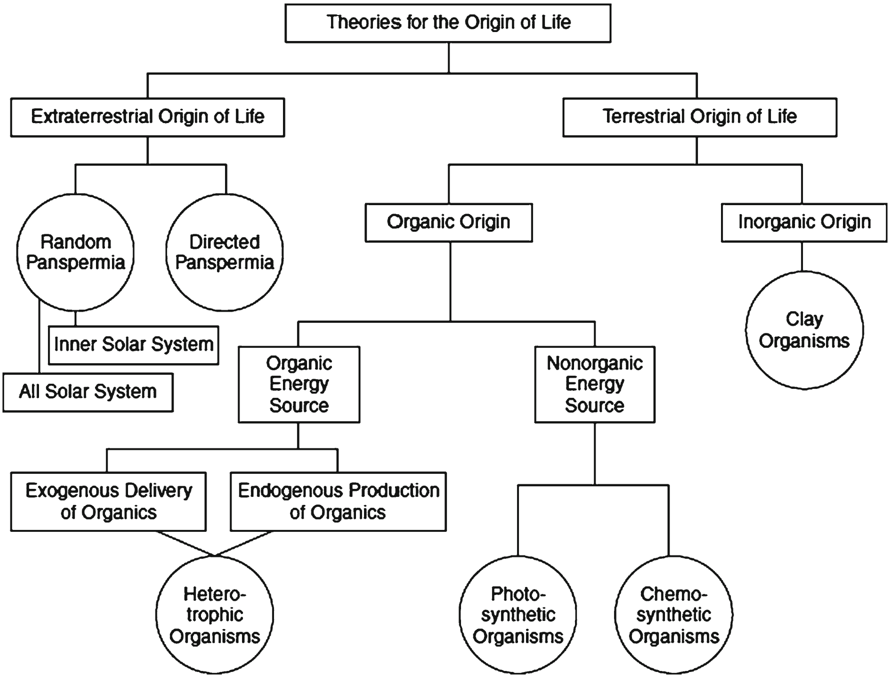


Figure 3. A scheme of possible theories on the origin of terrestrial and extraterrestrial life. The chemistry of the raw materials as well as energy sources plays key roles to each evolutionary path (From Davis and McKay (1996) and McKay et al. (2008)).

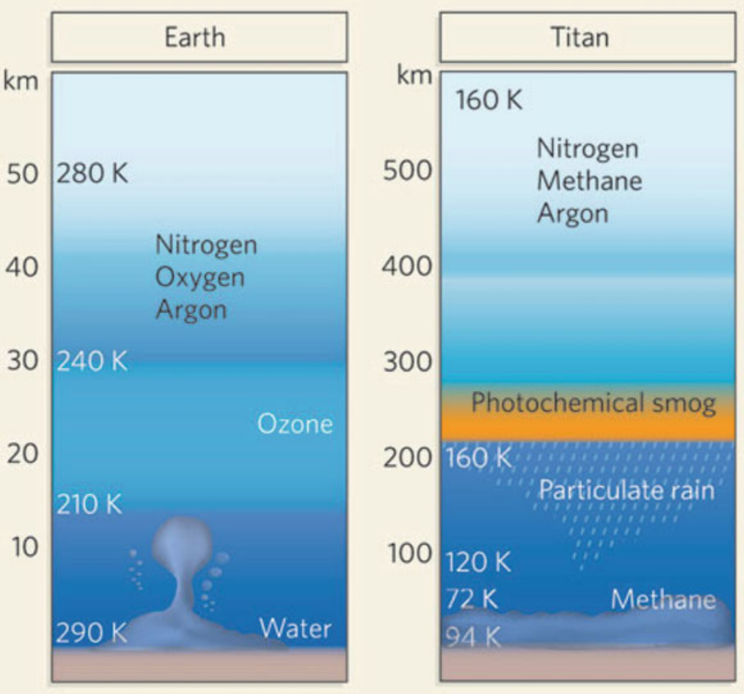


Figure 4. Atmospheric structure comparison of Earth and Titan. Both atmospheres are nitrogen-dominated, but on Titan, methane plays the role of water on Earth. Both atmospheres have an important haze content, and condensation processes are expected similarly on both bodies (Owen, 2005).

Lorenz and Mitton, 2008). Recent discoveries reveal that beyond its rich organic budget, and sufficient energy sources to drive chemical evolution, Titan also probably contains a vast subsurface ocean (Lorenz et al., 2008b).

Titan is indeed a very complex world much as our own planet. It is the only one other than Earth, that possesses a thick nitrogen-based atmosphere, four times denser than on our own atmosphere, with a rich organic chemistry (Fig. 4). It also has a geologically complex and active surface including lake-like features filled with organic liquid (e.g., Stofan et al., 2007). The physical processes within this world invite further close-up investigation that will provide a better understanding of the terrestrial processes as well.

Current investigations have shown that Titan fulfills many of life's prerequisites for a carbonaceous portfolio. Due to its nitrogen atmosphere, which is not in chemical equilibrium but like a chemical factory initiates the formation of complex positive and negative ions in the high thermosphere as a consequence of induced magnetospheric–ionospheric–atmospheric interactions involving EUV, UV radiation, energetic ions, and electrons (as recently demonstrated by the Cassini Ion and Neutral Mass Spectrometer, INMS). In this dynamic evolving environment, the second most abundant atmospheric constituent, methane, is dissociated irreversibly to produce a variety of trace gases such as hydrocarbons (e.g., ethane, acetylene, and propane) and in combination with the nitrogen, nitriles (e.g., hydrogen, cyanide, acetonitrile, cyanoacetylene), which are detected in the stratosphere (between 70 and 500 km in altitude) by the Composite Infrared Spectrometer (CIRS) onboard Cassini (e.g., Coustenis et al., 2007, 2010b). Literally, some of these gases would be considered as signs of life if they were on our planet (HCN is considered a prebiotic molecule, a precursor of life). Hence, finding how they form on Titan could give us clues on how life began on Earth.

The Cassini–Huygens mission has revealed the essential details of the organic and methane hydrologic cycles that we see today on Titan (Raulin et al., 2008; Brown et al., 2009b; Lebreton et al., 2009). Methane on Titan seems to play the role of water on Earth, with a similar complex cycle as shown in Fig. 5 (Atreya et al., 2006). On Titan, where methane is photodissociated and forms ethane and other organic products in the atmosphere, it should have disappeared after 10–100 million years, with around 34 Myrs as a nominal period (Atreya et al., 2006).

The intriguing question then is of how methane gets replenished in the atmosphere. On Earth today, it is life itself that refreshes the methane supply since methane is a by-product of the metabolism of many organisms. Hence, could this mean there is life on Titan? However, the Huygens Gas Chromatograph Mass Spectrometer (GCMS) data have shown that methane is not of biogenic origin, because the isotopic ratios are compatible with inorganic values (Niemann et al., 2005, 2010). Thus, the sinks of atmospheric methane on Titan are relatively well understood, but the major sources of replenishment are still very model dependent, as will be discussed later.

3.1. THE ATMOSPHERIC ORGANIC-RICH ENVIRONMENT OF TITAN

Although Titan's atmosphere is much colder than Earth's, it presents many direct similarities with our planet (Fig. 4), at different levels which have been pointed out since Voyager days. To begin with, both are made of the same main constituent, dinitrogen. A similar vertical structure from the troposphere to the ionosphere is also present, as well as a surface pressure of 40% larger than that of the Earth's (Fulchignoni et al., 2005). This is the only case of an extraterrestrial planetary atmospheric pressure so similar to that of Earth. Furthermore, a very exciting and complex organic chemistry takes place in Titan's atmosphere.

The direct analysis of the ionosphere by the Cassini Ion and Neutral Mass Spectrometer (INMS) instrument during the low-altitude Cassini flybys of Titan shows the presence of many organic species at detectable levels, at very high altitudes (1,100–1,300 km). Extrapolation of the INMS measurements (limited to mass up to 100 Da) and of Cassini Plasma Spectrometer (CAPS) data strongly suggests that high molecular weight species (up to several 1,000 Da) may be present in the ionosphere (Waite et al., 2007). These observations open a fully new vision of the organic processes occurring in Titan's atmosphere, with a strong implication of the ionospheric chemistry in the formation of complex organic

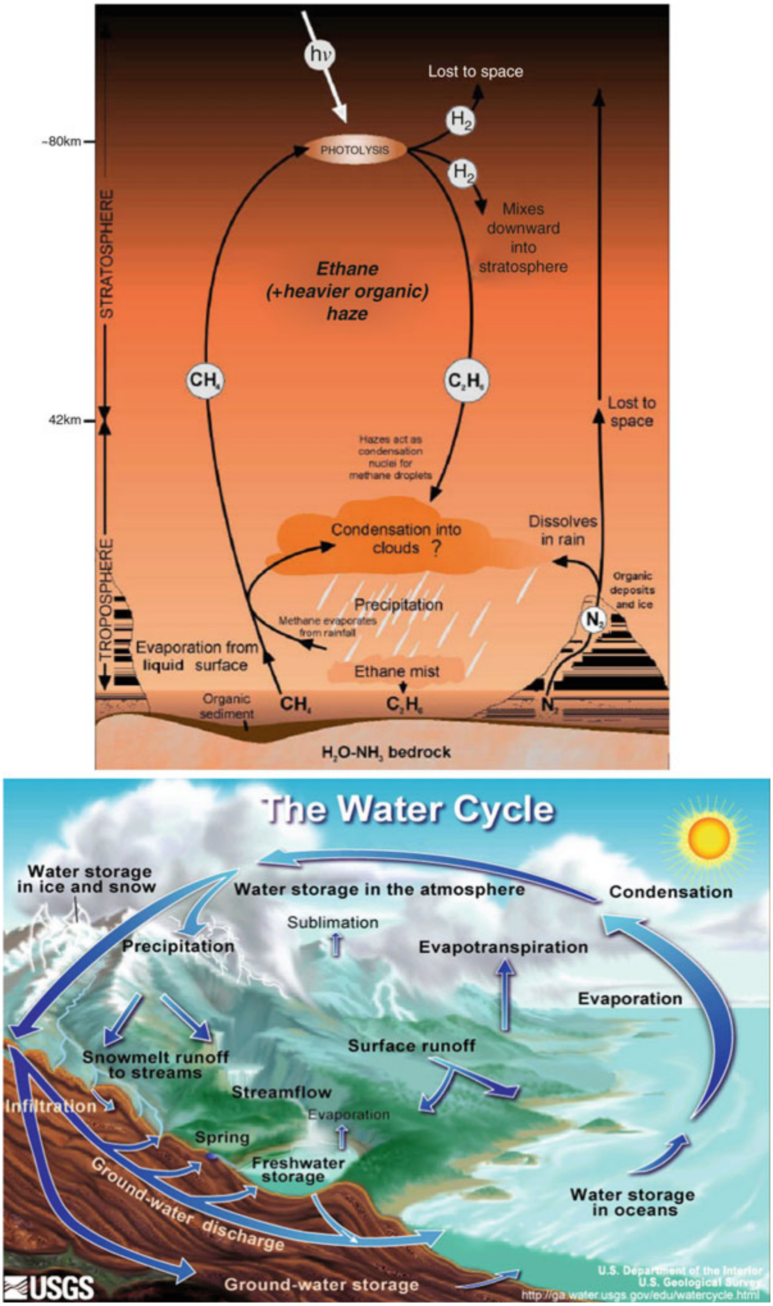


Figure 5. Titan's methane (*upper*) and Earth's water (*lower*) cycles in the atmosphere (USGS). Titan has a methane cycle resembling Earth's water cycle in its processes.

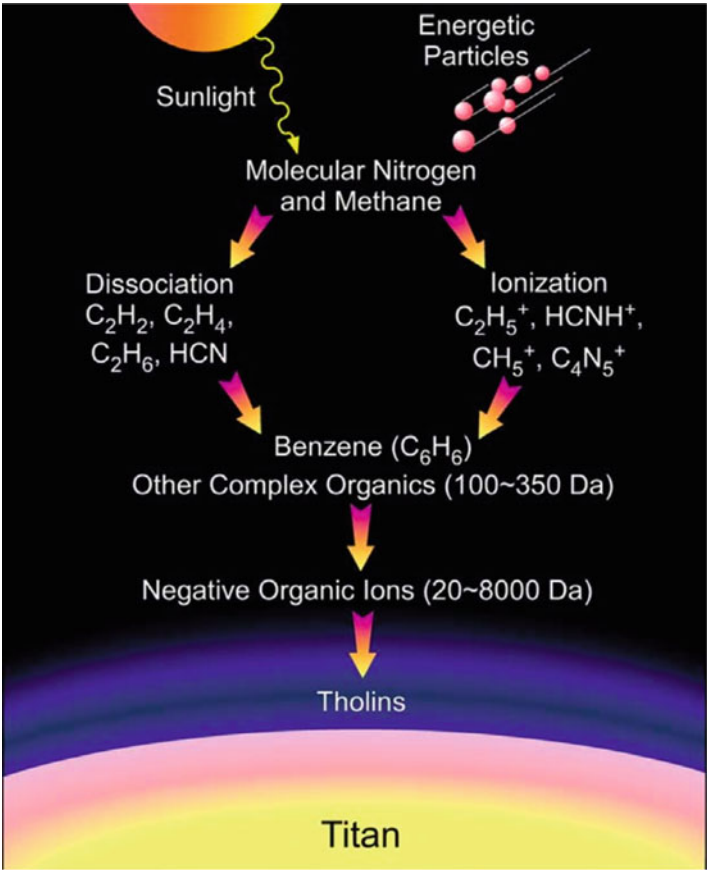


Figure 6. Organic chemistry in Titan's upper atmosphere. At the upper layers of the atmosphere, sunlight and energetic particles from Saturn's magnetosphere destroy CH_4 and N_2 and produce complex organic molecules that assemble to form negative ions from which tholins (hydrocarbon–nitrile aerosols) possibly form and diffuse in the lower parts of the atmosphere. Cassini/INMS measurements showed evidence for their formation around 1,000 km in altitude (Waite et al., 2007), and the Cassini/CIRS instrument detects the neutral chemistry in the stratosphere (Flasar et al., 2005).

compounds in Titan's environment (Fig. 6), which was not envisaged before. These compounds are detectable in solar and stellar UV occultations and initiate the process of haze formation starting at about 950 km (Waite et al., 2007) to finally condense out.

In the neutral atmosphere of Titan (between roughly 100 and 500 km), as we mentioned above, CH_4 chemistry is coupled with N_2 chemistry producing the formation of many organics in gas and particulate phase: hydrocarbons, nitriles, and complex refractory organics. Several photochemical models describing the chemical and physical pathways involved in the chemical evolution of the atmosphere of Titan have been published for the last 20 years (Yung et al., 1984;

Toublanc et al., 1995; Wilson and Atreya, 2004; Lavvas et al., 2008). These papers estimate the resulting vertical concentration profiles of the different molecules. Based on these models, the cycling of volatile chemicals starts with the dissociation of N_2 and CH_4 through electron, photon, and cosmic rays impacts in Titan's atmosphere. The primary processes allow for the formation of acetylene (C_2H_2) and hydrogen cyanide (HCN) in the high atmosphere. These molecules play a key role in the general chemical scheme: once they are formed, they diffuse down to the lower levels where they allow the formation of higher hydrocarbons and nitriles and perhaps aromatic compounds. Additionally, methane dissociation probably also occurs in the low stratosphere through photocatalytic processes involving acetylene and polyynes. The end products of the chemical evolution of methane in the atmosphere are complex refractory organic compounds and ethane.

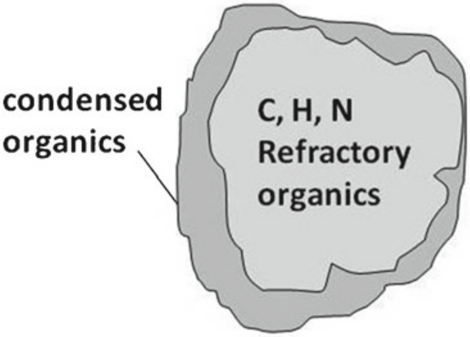
As these aerosols and haze particles fall through the atmosphere and grow, they become detectable with imaging systems such as the Cassini Imaging Science Subsystem (ISS) at about 500 km altitude and are ubiquitous throughout the stratosphere (Porco et al., 2005). They are strong absorbers of solar UV and visible radiation and play a fundamental role in heating Titan's stratosphere and driving wind systems in the middle atmosphere, much as ozone does in the Earth's middle atmosphere.

Experiments that simulate the reactions taking place in Titan's atmosphere (such as the Miller-Urey experiment) produce refractory organics, usually named tholins (Sagan and Khare, 1979) ("tholins" are solid products in the laboratory mimicking complex refractory organics: Nguyen et al. (2007); and references therein). Tholins represent laboratory analogues of Titan's aerosols and are useful to interpret many observational data which require information on the aerosols. As being experimental analogues of Titan's atmospheric particles, tholins also allow the study of the aerosols behavior in Titan's conditions with the tools available in the laboratory. Several organic compounds have already been detected in Titan's upper and lower atmosphere (Waite et al., 2007; Coustenis et al., 2010b). The list includes hydrocarbons (both with saturated and unsaturated chains) and nitrogen-containing organic compounds, exclusively nitriles, as expected from laboratory simulation experiments (Fig. 6). Moreover, since the Cassini arrival in the Saturnian system in 2004, the presence of water vapor and benzene has been unambiguously confirmed by the CIRS instrument.

3.2. TITAN'S PREBIOTIC RELEVANCE

Several of the organic processes that are occurring today on Titan form some of the organic compounds which are considered as key molecules in terrestrial prebiotic chemistry, such as hydrogen cyanide (HCN), cyanoacetylene (HC_3N), and cyanogen (C_2N_2). In fact, with several percent of methane in dinitrogen, the atmosphere of Titan is one of the most favorable atmospheres for prebiotic synthesis, although it almost lacks both oxygen and hydrogen. Concerning the hydrocarbon trace budget, photochemical models imply that light hydrocarbons are destroyed mostly

Figure 7. Composition of Titan's aerosols.



by reactions with OH^- and Cl^- radicals. Contrary to the very short lifetime of C_2H_2 on Earth (Rudolph et al., 1984), acetylene on Titan shows a seasonal variation during a Titan year (29.5 terrestrial years) and reaches almost the abundances recorded by Voyager 1 in 1980 (Coustenis et al., 2010a).

The Aerosol Collector Pyrolyser (ACP) experiment on Huygens provided the first direct *in situ* chemical analysis of Titan's aerosols. It collected haze particles from the stratosphere and the troposphere, heated them at different temperatures, and sent the produced gases for analysis to the GCMS instrument. The obtained results indicated that the aerosols are made of a refractory nucleus, composed of H, C, and N atoms (Fig. 7) and producing NH_3 and HCN after pyrolysis at 600°C (Israel et al., 2005). This strongly supports the tholin hypothesis. It also strongly suggests that Titan's aerosols may evolve once in contact with water ice on the surface and may produce a variety of organics of biological interest, such as amino acids (Neish et al., 2010; Ramirez et al., 2010).

Analogies are thus obvious between the organic chemistry activity currently occurring on Titan and the prebiotic chemistry which was once active on the primitive Earth, prior to the emergence of life (e.g., McKay and Smith, 2005). Indeed, in spite of the absence of permanent bodies of liquid water on Titan's surface, both chemistries are similar. As noted earlier, many resemblances can also be made between the role of methane on Titan and that of water on the Earth, with a complex cycle that has yet to be fully understood. Indeed, on Titan, methane can exist as a gas, liquid, and solid, since the mean surface temperature is almost 94 K (Fulchignoni et al., 2005), approaching the triple point of methane.

The atmosphere we enjoy today on Earth is probably radically different from the primitive one dating back to 4.6 billion years ago when our planet was nothing more than a molten ball of rock surrounded by an atmosphere of hydrogen and helium. In the absence of a magnetic field at those early stages, the intense solar wind from the young Sun blew this early atmosphere away. Then, as Earth cooled enough to form a solid crust, it was covered with active volcanoes, ejecting water vapor, carbon dioxide, and ammonia to form an early toxic atmosphere. Eventually, light from the Sun broke down the ammonia molecules exsolved by the volcanoes, releasing nitrogen into the atmosphere. Over billions of years, the quantity of nitrogen built up to the levels we see today. Although life formed just a few hundred million years later, it was not until the evolution of bacteria, 3.3 billion years ago, that the early Earth atmosphere changed into the one we know today. During the period from 2.7 to 2.2 billion years ago, these early bacteria—known as cyanobacteria—used energy from the Sun for photosynthesis and released oxygen as a by-product. They also trapped carbon dioxide in organic molecules. In just a few hundred million years, these bacteria completely changed the Earth's atmosphere composition, bringing us to the current mixture of 21% oxygen and 78% nitrogen. Schaefer and Fegley (2007) predict that Earth's early atmosphere contained CH_4, H_2, H_2O, N_2, and NH_3, similar to the components used in the Miller–Urey synthesis of organic compounds, often related to Titan's and Enceladus's atmospheric inventory. Furthermore, Trainer et al. (2006) looked at the processes that formed haze on Titan and on early Earth and found many similarities for what could have served as a primary source of organic material to the surface.

Before the rise of the atmospheric oxygen in the terrestrial atmosphere 2.5 Gy ago, it considered possible that the abundance of methane gas was 10–20 times higher than the today's value of 1.6×10^{-6} (Pavlov et al., 2003). Hence, if the atmospheric CO_2/CH_4 ratio had become equal to 10 at the mid-Achaean era, an organic haze could have formed on this early environment (Pavlov et al., 2000; DeWitt et al., 2009). This hydrocarbon haze produced the anti-greenhouse effect which reduced the temperature of the atmosphere (Kasting and Howard, 2006). Titan also hosts a thick methane-induced organic haze, similar to the one predicted for the early Earth and, consequently, experiences the same anti-greenhouse effect (McKay et al., 1999). The absence of vast amounts of CO_2 on Titan is one of the major differences between the two atmospheric envelopes. On the other hand, hydrogen cyanide and other prebiotic molecules are among the starting materials for biosynthesis. The existence of hydrocarbons, and in particular acetylene and benzene, has really enlarged the borders of photochemical organic products.

Especially, the presence of benzene (C_6H_6) seems extremely interesting, as it is the only polycyclic aromatic hydrocarbon (PAHs) discovered on Titan today. The presence of PAHs on Titan's atmosphere is very important as they could contribute to the synthesis of biological building blocks. Moreover, the combination of the liquid deposits on the surface of Titan and the low temperature could host the proper environment for this biosynthesis. Recent laboratory experiments showed that aromatic compounds could be plausibly produced on icy surfaces (Menor-Salván et al., 2008). Benzene was first detected at 674 cm^{-1} based on Infrared Space Observatory (ISO/SWS) data (Coustenis et al., 2003) with the mixing ratio of 4×10^{-10} and was then also detected in the thermosphere (950–1,150 km) from the analysis of Cassini/INMS data (Waite et al., 2007) and firmly in the stratosphere (100–200 km) at all latitudes by Cassini/CIRS measurements (Flasar et al., 2005; Coustenis et al., 2007, 2010b). Moreover, benzene has been tentatively identified on Titan's surface by Huygens/CGMS measurements (Niemann et al., 2005).

As Titan lacks oxygen and sufficiently high temperatures, as did primitive Earth, different evolutionary pathways from Earth must have been followed on Titan, and polyphenyls may possibly be created (Delitsky and McKay, 2010). The abundances of hydrocarbons are higher on Titan than those on Earth by a factor of about 10^2–10^4. Moreover, the temporal variations of the hydrocarbon traces on Titan experience a full cycle during the Titan year (Coustenis et al., 2010a) and are probably influenced by local or regional sources and sinks. Photochemical models are trying to reproduce these phenomena. Taking into account all the above-described characteristics, the prebiotic potential of Titan is enormous, and a huge effort in astrobiological studies is focused on its environment. Eventually, Titan still seems to be the ideal planet-size laboratory for increasing our knowledge of the evolution of the Earth's atmosphere.

3.3. ORGANIC CHEMISTRY AND MORPHOLOGY OF TITAN'S ACTIVE SURFACE

The Cassini–Huygens mission has significantly enhanced our understanding of Titan as the largest abiotic organic factory in the solar system. The abundance of methane and its organic products in the atmosphere, seas, and dunes exceeds the carbon inventory in the Earth's ocean, biosphere, and fossil fuel reservoirs by more than an order of magnitude (Lorenz et al., 2008a). As discovered by the Cassini/INMS, in the high atmosphere, heavy ions are formed (Waite et al., 2007).

Measurements throughout the atmosphere, both remote and *in situ*, have indicated the presence of numerous hydrocarbon and nitrile gases, as well as a complex layering of organic aerosols that persists all the way down to the surface of the moon (Tomasko et al., 2005; Coustenis et al., 2007; de Kok et al., 2007). Radar observations suggest that the ultimate fate of this aerosol "rain" is the generation of expansive organic dunes that produce an equatorial belt around the surface. Condensation of these species on aerosol particles is a probable explanation for these atmospheric characteristics. These particles, for which no direct data on their chemical composition were previously available, were analyzed by the Aerosol Collector and Pyrolyser instrument aboard Huygens probe. ACP results show that the aerosol particles are made of refractory organics which release HCN and NH_3 during pyrolysis.

This supports the tholin hypothesis (as described in section 3.1). From these new *in situ* measurements, it seems very likely that the aerosol particles are made of a refractory organic nucleus, covered with condensed volatile compounds (Israel et al., 2005). However, Huygens/GCMS did not detect a large variety of organic compounds in the low atmosphere (Niemann et al., 2005).

Figure 8. Linear dunes on Titan (Radebaugh et al., 2010). Titan's dunes are believed to be composed of ice and organics grains that possibly derive from a combination of the surface ice and the organic chemicals that fall through in Titan's atmosphere.

Moreover, the nature and abundances of the condensates have not been measured. Even more importantly for astrobiology, neither the elemental composition nor the molecular structure of the refractory part of the aerosols has been determined.

Eventually, these complex organic molecules are deposited on Titan's surface in large quantities, where data from Cassini's instruments hint at their existence. Hence, the upper thermosphere is linked intimately with the surface and the intervening atmosphere. In spite of the low surface temperature, the organics reaching the surface are probably evolving once in contact with water ice and may form organic molecules of biological interest.

Radar observations suggest that the ultimate fate of this aerosol "rain" is the generation of expansive organic dunes that produce an equatorial belt around the surface. Indeed, the surface of Titan shows also the presence of sedimentological and meteorological processes, as we see on Earth: There are many large dune areas (Lorenz et al., 2006; Radebaugh et al., 2008, 2010) (Fig. 8) where the terrestrial silica sand is probably replaced, once more, by water ice particles, mixed with the organic material of the aerosols.

Cassini's radar instrument finally unveiled what appears to be a land of lakes in Titan's northern polar regions (see Fig. 9) (Stofan et al., 2007). Cassini/ISS images also show a kidney-shaped dark feature about 200 km in length, named Ontario Lacus, that is outside the area of radar coverage and has recently been confirmed by the Cassini Visual and Infrared Mapping Spectrometer (VIMS)

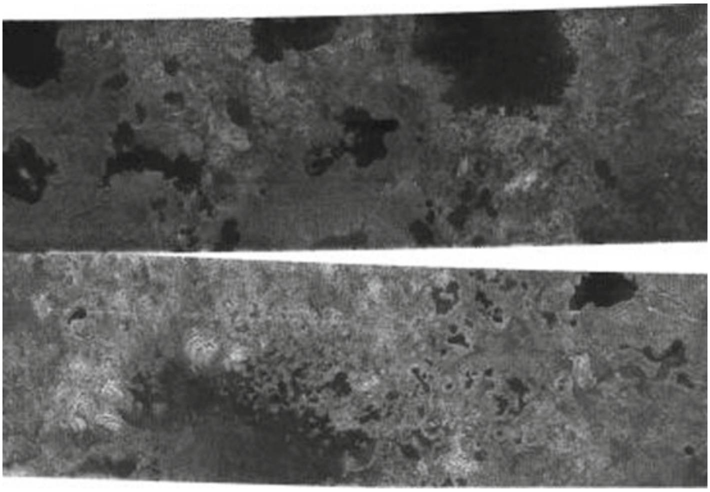


Figure 9. Lakes discovered in Titan's north-polar region by the Synthetic Aperture Radar (SAR) on board Cassini–Huygens mission (NASA/JPL). The dark patches are believed to be filled with hydrocarbon liquid.

not only to be a lake but also to be composed of ethane liquid (Brown et al., 2008). In the absence of a massive surface ocean but with analogues to all other terrestrial hydrological phenomena present, Titan's methane cycle is very exotic.

The liquid bodies are one of the main astrobiological aspects of Titan. Cassini's cameras (ISS) have allowed scientists to compile a nearly global surface map and to monitor the surface and atmosphere for activity. Intriguingly, repeated south-polar imaging by ISS revealed differences consistent with ponding of hydrocarbon liquids on the surface due to precipitation from a large storm. More recent ISS images of high northern latitudes illustrate the full extents (>500,000 km^2) of hydrocarbon seas, also observed by Cassini's radar. These observations demonstrate dynamic processes at work on Titan and indicate that the poles harbor liquid-hydrocarbon reservoirs, the extents of which differ from pole to pole and which may be coupled to seasonally varying circulation (Turtle et al., 2009).

The lakes and seas observed on Titan in the polar regions (Mitri et al., 2007; Stofan et al., 2007) make Titan the only body in the solar system having large liquid bodies on its surface. These very dark features at the high northern latitudes of Titan were finally shown to be liquid-filled (most probably with ethane-rich mixtures (Brown et al., 2008)) basins—classifying them as lakes.

The features range in size from less than 10 km^2 to at least 100,000 km^2. They are limited to the region poleward of 55°N. Currently, Cassini's instruments have identified and mapped almost 655 geological structures referred as lakes and/or basins (Fig. 10) (Hayes et al., 2008). Titan is thus the only planetary body, other than the Earth, with long-standing bodies of liquid on its surface (although direct observational evidence of the longevity of Titan's surface liquids remains

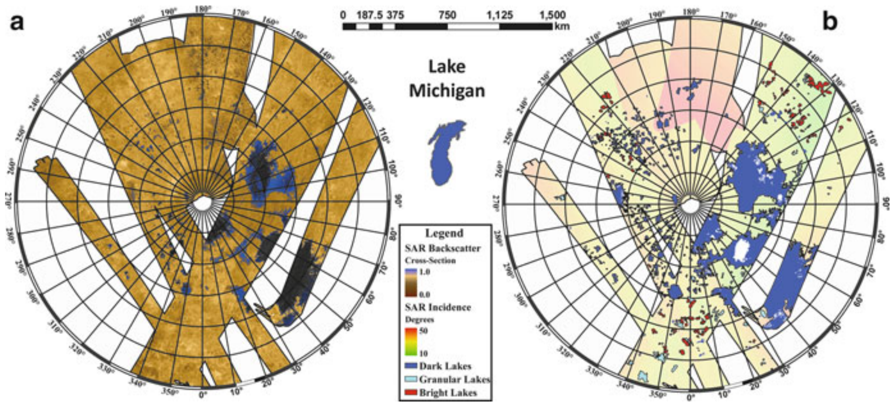


Figure 10. Map of almost 655 lakes and sea features by the Cassini radar system in azimuthal projection at the north pole of Titan. Map A (*left*) shows the radar swath mosaic up to May 2007 flybys. Map B (*right*) represents the spatial distribution of mapping units. Lake Michigan is illustrated for scale purposes (Hayes et al., 2008).

to be obtained). All of this suggests that Titan maybe even more similar to primitive Earth than we thought. However, the degree of complexity which can be reached from such an organic chemistry in absence of permanent liquid water bodies on Titan's surface is still unknown, although it could be quite high.

McKay and Smith (2005) noted the astrobiological importance of these geological features that are filled with liquid hydrocarbons, since there is a possibility for a different form of life to exist in such environments. It has been hypothesized that such a methanogenic life form consumes H_2 instead of O_2 that could be measured in the lower atmosphere. Two papers, by Strobel (2010) and Clark et al. (2010), based on data from the Cassini orbiter focus on the complex chemical activity on the surface of Titan. Strobel (2010) shows that hydrogen flows down through Titan's atmosphere and then somehow disappears on the surface. One of the most interesting phenomena occurring on Titan is that important quantities of atmospheric hydrogen precipitates and disappears when reach the surface. Such process resembles the oxygen consumption as occurring on Earth although in Titan's case, the element is hydrogen (Strobel, 2010).

Even though this is not supportive to the Titan's terrestrial-type life theory, it represents a hypothetical second form of life independent from water-based life we know on Earth. Strobel (2010) describes densities of hydrogen in different parts of the atmosphere and the surface. Previous models had predicted that hydrogen molecules, a by-product of ultraviolet sunlight breaking apart acetylene and methane molecules in the upper atmosphere, should be distributed fairly evenly throughout the atmospheric layers. The authors found a disparity in the hydrogen densities that lead to a flow down to the surface at a rate of about 10,000 trillion hydrogen molecules per second. This is about the same rate at which the molecules escape out of the upper atmosphere. Strobel (2010) states that it is not likely for hydrogen to be stored in a cave or underground space on Titan. Titan's surface is also so cold that a chemical process that involved a catalyst would be needed to convert hydrogen molecules and acetylene back to methane, even though overall there would be a net release of energy. The energy barrier could be overcome if there were an unknown mineral acting as the catalyst on Titan's surface.

Another possible indicator for life on Titan is the lack of acetylene on the surface since there is no clear evidence of this compound in the received data to date, while it is expected to have been deposited through the atmosphere. It has been suggested that this could be due to the fact that some form of life on the surface is using acetylene as an energy source (Clark et al., 2010). This theory is largely debated and controversial among the scientific community, especially due to suggestions of nonbiological origin of this phenomenon; however, it has the merit to propose new interesting astrobiological theories. In detail, Clark et al. (2010) map hydrocarbons on the surface from Cassini/VIMS data and find a lack of acetylene. McKay and Smith (2005) had identified acetylene as the best energy source for methane-based life on Titan. While nonbiological chemistry offers one possible explanation, these authors believe these chemical signatures bolster the argument for a primitive, exotic form of life or precursor to life on Titan's surface. According to one theory put forth by astrobiologists, the signatures fulfill two important conditions necessary for a hypothesized "methane-based life", which would consume not only methane but also hydrogen. However, one other possibility is that sunlight or cosmic rays are transforming the acetylene in icy aerosols in the atmosphere into more complex molecules that would fall to the ground with no acetylene signature.

To date, methane-based life forms are only hypothetical. Scientists have not yet detected this form of life anywhere, though there are liquid-water-based microbes on Earth that thrive on methane or produce it as a waste product. At Titan's low temperatures, a methane-based organism would have to use a substance that is liquid as its medium for living processes, but not water itself. Water is frozen solid on Titan's surface and much too cold to support life as we know it. The list of liquid candidates includes liquid methane and related molecules like ethane. While liquid water is widely regarded as necessary for life, there has been extensive speculation published in the scientific literature that this is not a strict requirement. The new hydrogen findings are consistent with conditions that could produce an exotic, methane-based life form, but do not prove its existence.

3.4. INTERIOR MODELS FOR TITAN AND ITS POSSIBLE SUBSURFACE OCEAN

Regarding Titan's interior structure, since the only *in situ* data from its surface are the Huygens probe recordings, only modeling assumptions can be presented. Structural models for planetary interiors suggest that Titan, like Europa, Ganymede,

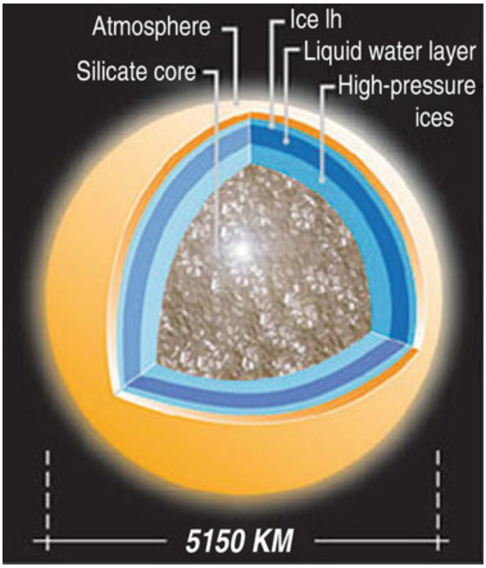


Figure 11. Illustration of Titan's internal structure with a liquid ocean between two subsurface ice layers (From Tobie et al., 2005). Cassini–Huygens recorded extremely low-frequency radio waves which supports the existence of this liquid subsurface layer (NASA/LPGN).

and Callisto, has maintained internal liquid water reservoirs, probably mixed with some ammonia and more speculatively sulfur and possibly entrained methane clathrates (Fig. 11).

Another possible location to look for life on Titan would be in an undersurface liquid water ocean. The presence of such an internal ocean is supported by Titan internal structure models (Tobie et al., 2005; Mitri et al., 2008), radar and gravity Cassini measurements (Lorenz et al., 2008b), and the Huygens Atmospheric Structure Instrument (HASI) experiment. Beghin et al. (2009) thus interpreted the extremely low-frequency electric signal recorded by HASI as a Schumann resonance between the ionosphere and a modestly conducting ocean (since the ice is not conductive) roughly 30–50 km below the surface. Thermal evolution models suggest that Titan may have an ice crust between 50 and 150 km thick, lying atop a liquid water ocean a couple of 100 km deep, with some amount (a few to 30%, most likely ~10%) of ammonia dissolved in it, acting as an antifreeze material (Lorenz et al., 2008b). This correspond to a pH around 11.5. The pressure reaches ~5 kbars at 200 km depth, and it could include hot spots reaching −20 °C. Such conditions are not incompatible with life as we know it on Earth (Fortes, 2000; Raulin, 2008; Raulin et al., 2009).

Tobie et al. (2005) suggested a layered interior structure of Titan, consisting of a rocky core overlaid by high-pressure ice, a liquid layer overlaid by low pressure ice, and finally a solid icy crust (Fig. 11). An earlier model by Fortes (2000) noted that underneath Titan's icy crust, at a depth of approximately 200 km, there lies an ammonia–water solution ocean in which life could survive. Another, more recent model by Mitri et al. (2008) suggests pockets of methane clathrates trapped within an ammonia–water ocean which could exsolute and produce overpressure and consequently the ammonia–water can erupt to the surface leading to cryovolcanic phenomena.

With regard to Titan's morphology and internal dynamic geology, it has been suggested that there may be active cryovolcanoes on Titan (Sotin et al., 2005; Lopes et al., 2007) since traces of former flows have been found across parts of the surface. A wide variety of Cassini data support the presence of cryovolcanism on Titan. Indeed, at least two regions have been observed to change reflectance on Titan's surface (Tui Regio 20°S, 130°W; Hotei Regio 26°S, 78°W) (Barnes et al., 2006; Nelson et al., 2009a, b), and one of them (Hotei Regio) in addition to a recent discovery (Sotra Facula 15°S, 42°W) in radar images exhibit lobate "flow" forms (Soderblom et al., 2009; Lopes et al., 2010), consistent with the morphology of volcanic terrain, supporting the hypothesis of cryovolcanic eruptions (Sotin et al., 2005).

However, on Titan, fluid water mobilized and made buoyant by ammonia and other materials could replace terrestrial melted silicates. Cryovolcanism suggests a dynamic process than involves the interior, the surface, and the atmosphere as well. It is a multi-complex activity that resembles terrestrial volcanic processes as it follows a similar pattern although in extremely altered conditions and different initial and depositional products. Cryovolcanism on Titan is believed to be a significant source of the methane in the atmosphere (Tobie et al., 2006). An underground liquid ocean, several hundred kilometers deep at the surface of Titan, is suggested to be the source of cryomagma, hence outgassing methane into the atmosphere and thus replenishing the destroyed amounts.

There are indeed studies suggesting the presence of an internal ammonia–water ocean (Grasset and Sotin, 1996; Grasset et al., 2000; Tobie et al., 2005; Mitri et al., 2007) while another study has modeled and suggested an ocean filled with methane clathrate pockets that lead to explosive cryovolcanism (Fortes et al., 2007).

The theory of trapped methane clathrates in the potential liquid ocean is of a major astrobiological interest. The presence of methane clathrates in an aqueous environment is attached to the "clathrate gun" hypothesis. This hypothesis suggests that potential movement and rise of the temperature in an underground liquid reservoir could trigger the sudden release of methane from methane clathrate compounds buried in permafrost or seabeds (Kennett et al., 2003) or an ocean like on Titan's case. The initiation of such process leads to further temperature rise and further methane clathrate destabilization that could easily cause and trigger cryovolcanic eruptions (Kennett et al., 2003). For Titan, a dynamic process like the one suggested by the clathrate gun hypothesis could result to increase of temperature values, creating an environment more favorable for life to exist.

With respect to the astrobiological interest, there is a controversy about the effectiveness of methane as a medium for life compared to water or ammonia. Water has higher solubility than methane, enabling easier transport of substances in a cell, while methane's lesser chemical reactivity allows for the easier formation of large structures corresponding to proteins (Benner et al., 2004). In addition, the cryovolcanic activity suggests higher temperatures within the ocean and the volcanic conduit where heat transfer between the interior and upper layers would be critical in sustaining any subsurface oceanic life (Grasset et al., 2000).

Thus, the possibility of life in this ocean cannot be excluded. Moreover, models also predict that during the first tens millions of years after Titan's formation, the ocean was in direct contact with the atmosphere on one side and with the bedrock on the side. This could have provided conditions very favorable for an efficient prebiotic chemistry toward the emergence of life, with the possible involvement of hydrothermal vents. Thus, the internal ocean of Titan not only is habitable but could be inhabited.

Hence, in spite of the low temperature, Titan is not a congealed Earth: The chemical system is not frozen. Titan is an evolving planetary body and so is its chemistry. Once deposited on Titan's surface, the aerosols and their complex organic content may follow a chemical evolution of astrobiological interest. Laboratory experiments show that, once in contact with liquid water, Titan tholins can release many compounds of biological interest, such as amino acids (Khare et al., 1986). Such processes could be particularly favorable in zones of Titan's surface where cryovolcanism may occur. The N_2–CH_4 by-products in Titan's atmosphere eventually end up as sediments on the surface, where they accumulate presently at a rate of roughly 0.5 km in 4.5 Gyr.

Long-term chemical evolution is impossible to study in the laboratory: *In situ* measurements of Titan's surface thus offer a unique opportunity to study by a ground-truth approach some of the many processes which could be involved in prebiotic chemistry, including isotopic and enantiomeric fractionation (Nguyen et al., 2007).

There are suggestions that Titan is presently geologically active on the surface (Nelson et al., 2006, 2009a, b) and in its interior. If Titan is currently active, then these results raise the following questions: What is the full extent of current geologic activity? What are the ongoing processes? Are Titan's chemical processes today supporting a prebiotic chemistry similar to that under which life evolved on Earth?

Although the chemical reactions that lead to life on Earth take place in liquid water, the reactions themselves are almost entirely between organics. The study of organic chemistry is therefore an important, and arguably richer, adjunct to the pursuit of liquid water in the solar system. Titan's organic inventory is impressive, and carbon-bearing compounds are widespread across the surface in the form of lakes, seas, dunes, and, probably, sedimentary deltas at the mouths of channels.

4. Enceladus: Liquid Water Far Away from the Sun

Discovered by William Herschel in 1789, Enceladus is arguably a place in the solar system where a demonstrably habitable environment already occurs and evaluating its astrobiological potential should be an overarching goal of Enceladus research. Although Enceladus is a relative small planetary body (500 km in diameter), significant chemical processes could produce primitive life structures. Obviously, oxidation/reduction reactions necessitate supporting redox-based life (Gaidos et al., 1999). Fe- and Ni-bearing dust particles can operate as reducing agents on Enceladus, which exist since planetary formation from the ancient solar nebula.

As concerns oxidant agents on Enceladus, Gaidos et al. (1999) also noted their production through E-ring particles, charged from the Saturnian magnetospheric environment. Then, some suitable geological process is needed to mix the reducing and oxidizing compounds. In a model by Cooper et al. (2009), the astrobiological parameters that support life on Enceladus are evaluated as higher than for Europa due to a less extreme state of oxidation and greater residual abundance of organics.

Indeed, Enceladus, Saturn's most active moon as observed from Cassini, presents a mystery in the studies of planetary science and, more specifically, in geophysics. The enigma evolves around how a small moon can possess sufficient dynamical energy to drive a geyser plume rising 600 miles in space out of the moon's south pole, eventually feeding its material to the outer E-ring of Saturn (Postberg et al., 2009). The heat source for Enceladus is still an open question, as is the possibility for life to exist on this small satellite if an underground liquid water ocean or liquid water subsurface pockets exist to explain the plumes (Kieffer and Jakosky, 2008).

The geyser plumes arise from the warm surface surrounding and including four parallel faults located at the moon's south pole, the "Tiger Stripes" fractures (Porco et al., 2006; Spencer et al., 2006), spewing a series of jets more than 600 km high (Fig. 12). The mass production rate of the plume gas has been estimated to be ~150 kg/s from occultation data (Tian et al., 2007). This value is surprisingly high, sufficient to remove a significant fraction (~20%) of Enceladus's mass over the age of the solar system (Kargel, 2006).

Data obtained by the VIMS instrument on board Cassini indicate CO_2 and organics as possible components (Brown et al., 2006). Data processing showed components that Cassini's INMS identified H_2O as the predominant component, CO_2 as the second most abundant, methane, and trace quantities of acetylene and propane (Waite et al., 2009). During the 9th of October 2008 flyby, Cassini dived into the south-polar plume, and INMS reported the presence of ammonia and other various organic compounds like deuterium and ^{40}Ar, as well as complex organics like benzene and other probable species such as methanol and formaldehyde (Waite et al., 2009). The chemical composition of the plume and surface material of Enceladus suggests the presence of a heat source in its interior, hot

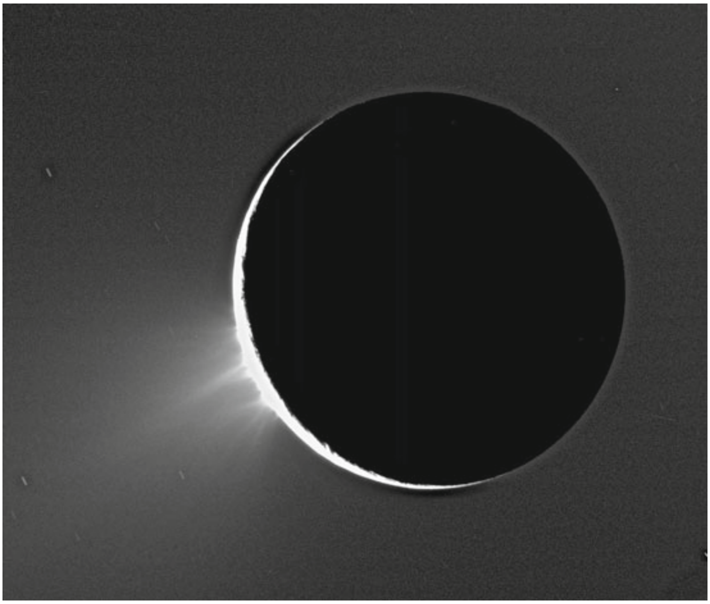


Figure 12. Enceladus's plumes ejected from the south-polar region captured by Cassini/ISS (Porco et al., 2006).

enough to decompose ammonia into N_2 and to drive reactions with hydrocarbons, implying internal temperatures on the order of 500–800 K (Matson et al., 2007).

There is a plethora of competing theories regarding the triggering and exsolution of geysers from the Tiger Stripes. The main controversy lies on as to whether the plumes are formed by a massive underground ocean (Tobie et al., 2008; Postberg et al., 2009) (Fig. 13a) or if the material generates from ice warmed, melted, or crushed by tectonic-like motions (Nimmo et al., 2007) (Fig. 13b).

Given the aforementioned observations and analysis, it is now known that Enceladus contains enough heat to drive complex and dynamic geologic activity. Interpretation of Cassini data with a view to explain the major internal reservoir that triggers and feeds this dynamic process points to a possible underground liquid ocean beneath the icy crust. Since the prerequisites for life to emerge are the simultaneous existence of energy, organic compounds, and liquid water that have been found on Enceladus, it seems that it possesses all the necessary components to support life.

In general, as described above, the south-polar region of Enceladus presents extremely high temperatures while the exsoluted vapor has also been shown to contain simple organic compounds. Most theories regarding the origin of this active region suggest that it is very likely for a liquid water environment to exist beneath the Tiger Stripes. This hypothesis enables the parallelism between potential biological ecosystems on Enceladus and the already existing on Earth (Fig. 14).

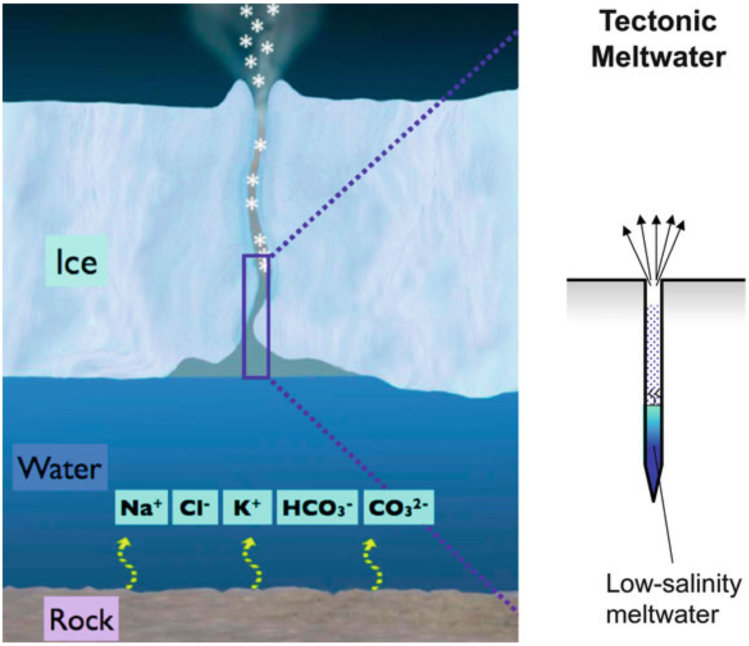


Figure 13. (*left*) Modeling of a possible internal ocean on Enceladus filled with water and chemical compounds. This model tries to explain the significant abundance of sodium salts of Saturn's E-ring, probably originating in Enceladus plumes (Postberg et al., 2006). (*right*) Internal model of Enceladus based on tectonic meltwater. Heating along fractures is caused by tidal flexing (Nimmo et al., 2007).

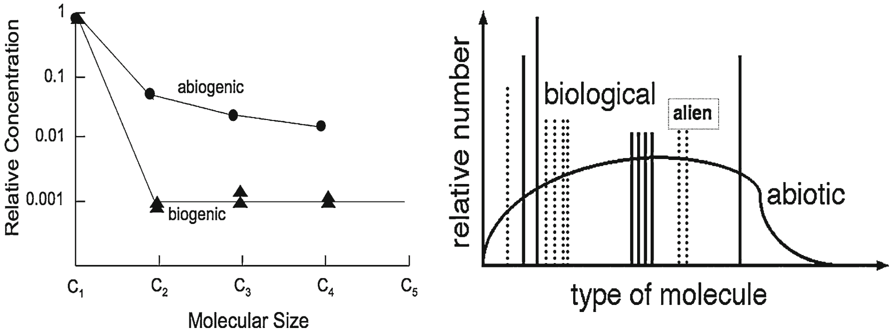


Figure 14. Indications for biological and nonbiological processes in relation to molecular parameter (*left*) terrestrial abiogenic production of hydrocarbons from McCollom and Simoneit (1999) and its biogenic pendant from Devai and Delaune (1996). (*right*) Organic distribution for abiotic, biological, and possible alien life on Enceladus (Both figures from McKay et al., 2008).

The standards for life that Enceladus' possible ocean does not fall into are the sunlight, the oxygen compounds, and the organics produced on a surficial-crust environment. Environments and ecosystems that do not meet the aforementioned prerequisites exist and evolve on Earth as well. Such an environment is located deep inside South Africa's surface, where sulfur-reducing bacteria consume hydrogen and sulfate, produced by radioactive decay (McKay et al., 2008; Muyzer and Stams, 2008). In addition, other analogous to Enceladus ecosystems are found within the magmatic volcanic rocks, which are produced through the activity of metasomatism. The metasomatosis of the volcanic rocks under the reaction of water produces methanogens of hydrogen on which the primary productivity is based on (McKay et al., 2008). Cassini's data showed that methane is present in the plumes. The terrestrial comparison regarding the ecosystems suggests that plume's methane may be biological in origin (McKay et al., 2008).

Specific questions relevant to the goal of understanding habitability on Enceladus include: Is liquid water present on Enceladus, either in a subsurface ocean, in the plume vent regions, or elsewhere? How extensive and long lived is the water, if present, and what is its chemistry? How does the liquid reservoir communicate with the surface? How thick is the ice crust and how uniform is that thickness? What energy sources are available for life? Is life present there now?

VIMS observations of several other satellites of Saturn, in the near-infrared region, show that their surface is covered by dark materials. This is particularly the case with Dione but also with Phoebe, Iapetus, Hyperion, Epimetheus and even with the F-ring (Clark et al., 2009). This dark material could be made of cyanide compounds and could be of cometary origin (Clark et al., 2009).

Spectral signatures of hydrocarbons have also been found from VIMS data on Iapetus and Phoebe suggesting the presence of organic compounds such as PAH's, kerogen-, or coal-like structure compounds (Cruishank et al., 2008).

Some of these satellites may also have internal liquid water pockets and thus may present the requisite essential for the emergence and development of life: liquid water and organic compounds. The solstice mission may be able to discover such properties and thus extend drastically the list of planetary bodies of important astrobiological interest in the Saturnian system.

5. Discussion: What Can Titan and Enceladus Tell Us About Life?

The complex mechanisms that have led to the emergence and development of life on Earth are still under investigation. Despite the great strides on biological sciences, the roots, the sources, and the initial conditions of life still remain unknown. Some answers can be found in extraterrestrial environments, hopefully in our solar system neighborhood or in exoplanets orbiting Sun-type stars. Without doubt, every planetary body, with a possible astrobiological potential, is a target for further investigations. Currently, after years of explorations, extended missions, and data analysis, it appears that among the main candidates for finding signs of past or current life within our solar system are Mars, Enceladus, Europa, and Titan (not in any particular order of priority or importance).

In this context, Titan and Enceladus, both orbiting Saturn, seem ideal locations for further investigation. As said before, their astrobiological importance is obvious, because they propose uniquely all the necessary ingredients for life emerging and evolution. Not surprisingly, scientists expect that their study will provide some important insights on the origin of life.

The surface of Titan appears, like the surface of Mars or Europa, as an unlikely location for extant life, at least for terrestrial-type life. Even though Titan presents terrestrial-type geology with complex structures formed mostly from dynamic processes, the absence of water on the surface makes it unlikely to support terrestrial-type life. Liquid water, if it exists, is not presently in contact with a silicate core, which is isolated from the subsurface ocean by a layer of a high-pressure ice phase (Tobie et al., 2005). However, Fortes (2000) noted that Titan's internal water ocean might support terrestrial-type life that had been introduced there previously or formed when liquid water was in contact with silicates early in Titan's history. According to McKay and Smith (2005), photochemically derived sources of free energy on Titan's surface could maintain an exotic type of life, using liquid hydrocarbons as solvents. Similarly, Stoker et al. (1990) stressed that terrestrial bacteria can in fact satisfy their energy and carbon needs by "eating" tholin. In this sense, a methane-rich atmosphere may act as a "poor planet's photosynthesis," providing a means to capture the free energy from ultraviolet light and make it available for metabolic reactions.

Consequently, it cannot be excluded that life may have emerged on or in Titan. In spite of the extreme conditions in this environment, life may have been able to adapt and to persist. Even though the possible current conditions (pH, temperature, pressure, salt concentrations) are not incompatible with life, as we know it on Earth (Fortes, 2000), the detection of a potential biological activity in the putative liquid mantle seems very challenging. Furthermore, as mentioned above, another possible location to look for life on Titan would be in a possible subsurface liquid water ocean, and thus, it seems astrobiologically essential to confirm its presence.

Marine geologists and Marine biologists are nowadays close to confirm after many years of research and analysis that in the lower part of Earth's ocean, below the thermocline, where the environmental conditions are extremely different than on the surface, life exists. In deep oceanic layers that suffer from low temperature and high pressure, there are two extreme environments where life is abundant. Such environments are the cold seeps (vents) (e.g., Ritt et al., 2010) and the hydrothermal vents. Cold seeps are areas resembling brine pools, from which methane and hydrogen sulfide and other hydrocarbon-rich fluid seepage are released into the ocean. In such conditions, types of life exist, feeding on single-cell Archaea and Eubacteria microbes that consume the methane and hydrogen sulfide from the seep. The environmental and living conditions described above resemble that of Titan's hypothesized internal liquid ocean. Similarly, the temperature is low, the pressure is high, and possible hydrovolcanic events exist, releasing methane and sulfides in the liquid ocean. Thus, there are many aspects of Titan that might argue for the presence of some sort of very basic life on Titan.

6. Future Exploration of the Kronian Satellites

Could Titan be providing us with hints as to the future of our own planet? Indeed, in addition to the past, Titan appears to be an analogue, albeit with different working materials, of the future state of the Earth when surface conditions preclude stable equatorial/midlatitude oceans. If we are to focus on the Earth and its climate, as well as on its organic chemistry, we need in the future to concentrate on another object in the solar system that sustains an active hydrologic cycle with surface liquids, meteorology, and climate change. The Cassini–Huygens mission has firmly established that Titan is such a body, in which the active working fluid of the hydrologic cycle is methane. The cycle is active but different from the Earth because Titan lacks a surface methane ocean. It possesses, however, methane lakes and seas, fluvial erosion, rounded pebbles, and liquid methane in the soil at the Huygens site.

With Titan, we are observing an active hydrologic cycle subjected to seasonal, annual, and longer term changes, as on the Earth. Moreover, the future increase in the solar luminosity make it almost inevitable that eventually water on the Earth will no longer be trapped in our ocean and troposphere but will escape rapidly in a process we see today for methane on Titan. The late stages of this evolution—an Earth with liquid water in the polar regions, in the crust, but no longer in an ocean—may be echoed by the configuration we see today in Titan's methane hydrologic cycle.

Our understanding of the future of living beings on Earth (and hence the habitability in many ways of our planet) may then have something to gain from a thorough exploration of Titan's current state.

Enceladus, on the other hand, may hold the key to understanding an important source of energy, plate tectonics, and volcanism. Indeed, many tectonic features on Enceladus may be analogous to features observed on other icy satellites such as Europa, Ganymede, and perhaps Titan. Thus, the study of the tectonics of Enceladus, which is currently active, can be used as a natural laboratory to investigate the response to stresses of the other surfaces of the outer solar system. Moreover, Enceladus possesses a warm, chemically rich, environment that may facilitate complex organic chemistry and biological processes.

The Cassini–Huygens mission has enormously advanced our knowledge of the Saturnian system and the satellites within. As far as Titan and Enceladus are concerned, the wealth of data retrieved by the Cassini–Huygens mission will definitely be the reference point for future planetary investigation. However, the key contribution to planetary science of Cassini may be the questions raised rather than those answered. Some drawbacks of the mission, such as insufficient

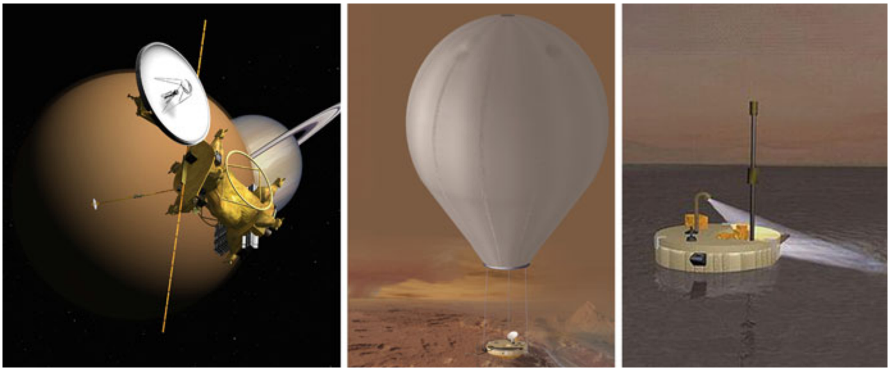


Figure 15. The TSSM basic concept. The orbiter (*left*), the balloon (*center*), and the lake lander (*right*) (Reh et al., 2008).

global coverage which has inhibited a full mapping of the atmospheric structure, composition, and temporal variations as well as the surface features of Titan and Enceladus, point to the need for further studies. Similarly, the part of the Titan's atmosphere between 400 and 950 km will remain unexplored (Coustenis et al., 2009). In addition, we will lack *in situ* measurements since the Cassini orbiter can only perform flybys of Titan and Enceladus, and the single vertical profile of the atmosphere taken by Huygens probe is limited to its landing site.

It is clear that Titan's organic chemistry and the possible subsurface ocean (among other) remain to be investigated. In particular, joint measurements of large-scale and mesoscale topography and gravitational field anomalies on Titan from an orbiter and from an aerial platform would impose important constraints on the thickness of the "lithosphere", the presence of mass anomalies at depth, and any lateral variation of the ice mantle thickness. As discussed above, it is astrobiologically crucial to confirm the presence of such an internal ocean, even though the water layer may not be in contact with the silicate core, like on Europa.

Lessons from Huygens will be used in the future to go back to Titan and explore in details its surface, in many locations. The intriguing discoveries of geological activity, excess warmth, and outgassing on Enceladus (due perhaps to the ejection of water and organics from subsurface pockets bathed in heat or by some other mechanism) mandate a follow-up investigation of that tiny Saturnian world that can only be achieved with high-resolution remote observations and detailed in situ investigations of the near-surface south-polar environment.

Among other options, a flagship (large) mission, TSSM (for Titan/Saturn System Mission), was proposed (Reh et al., 2008; Coustenis et al., 2009), jointly studied by NASA and ESA. TSSM could explore extensively one of the Titan's lakes and study for several months Titan's atmospheric and surface environment (Fig. 15).

This concept would also investigate the astrobiological perspective of Titan and Enceladus. In particular, since hydrocarbon lakes on Titan's surface may harbor evidence for present or past life, the proposed lake lander would play a crucial role. Similarly, the balloon platform would be responsible for understanding how volatile-rich worlds evolve and how organic chemistry and planetary evolution interact on large spatial and temporal scales.

The primary science goals of TSSM are to understand Titan's and Enceladus' atmospheres, surfaces, and interiors; to determine the pre- and proto-biotic chemistry that may be occurring on both objects; and to derive constraints on the satellites' origin and evolution, both individually and in the context of the complex Saturnian system as a whole. Many internal processes play crucial roles in the evolution of Titan and Enceladus. The formation and replenishing of Titan's atmosphere and the jet activity at Enceladus' south pole are intimately linked to the satellite's interior structure and dynamics. Open issues are listed below:

1. To determine their present-day structure and levels of activity
2. To determine whether the satellites underwent significant tidal deformation and whether they possess intrinsic or induced magnetic fields and significant seismicity
3. To identify heat sources, internal reservoirs of volatiles (in particular methane and ammonia), and eruptive processes
4. To detect plausible evidence for life by analysis of hydrocarbons in the plume during close encounters

A mission like TSSM would answer important astrobiological questions with precise measurements as follows:

1. What degree of complexity is reached by Titan's organic chemistry in the different parts of the geological system?
2. Is Titan a habitable world? Does it have an undersurface liquid water ocean or episodical liquid water bodies on the surface?
3. Is there currently, has there been or will there be biological activity on Titan?

To answer these questions and complement the Cassini–Huygens exploration of Titan, a dedicated orbiter and in situ elements would help by providing data and analyses directly in the atmosphere, on the surface, and the interior of Titan. The exchanges among the different media and the external processes that affect Titan on time lapses of days, years, or seasons beg for further investigation, even beyond the solstice Cassini mission which will be operating until 2017. Besides TSSM, other concepts for future missions to return to Titan have been proposed

Such as a simple orbiter to perform close-up investigations of the surface and the atmosphere of Titan (JET, C. Sotin, PI). Further more, several in situ elements have been proposed like:

The Aerial Vehicle for In-Situ and Airborne Titan Reconnaissance (AVIATR), an alternative proposal to a Titan balloon mission. Since Titan experiences low gravity and a dense atmosphere, such a nuclear-powered airplane could fly more than 20 times easier than on Earth. It could sample directly the atmosphere and cover huge swaths of Titan's landscape (Barnes et al., 2010; McKay et al., 2010).

Titan Mare Explorer (TiME) is a proposed probe focusing on exploring Titan's lakelike features. This lake lander could study the chemical composition and the geological characteristics of the hydrocarbon pools (Lorenz et al., 2009; Stofan et al., 2009).

Similarly, Titan lake probe is a lake lander which could be considered as part of the TSSM mission or as a stand-alone mission. The main objective of this proposal is to investigate the lake deposit and the physical properties of the liquids like the TiME concept (Waite et al., 2010).

Future exploration of the Saturnian neighborhood shall no doubt bring forth extremely important insights on our quest for the possibility of life and habitable sites elsewhere.

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