CELLULAR ORIGIN, LIFE IN EXTREME HABITATS AND ASTROBIOLOGY

From Fossils to Astrobiology

Records of Life on Earth and the Search for Extraterrestrial Biosignatures

Edited by
Joseph Seckbach and Maud Walsh

Springer

FROM FOSSILS TO ASTROBIOLOGY

Cellular Origin, Life in Extreme Habitats and Astrobiology

Volume 12

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The Hebrew University of Jerusalem, Israel

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Cover illustration:
Upper left: NGC 6188, a dust cloud in Ara OB1 association, which sprawls across the edge of an expanding bubble of gas that could be as much as 300 light years wide. Photo courtesy of Don Goldman.
http://www.astrodon.com/_img/image/gallery/2/NGC6188Web4.jpg

Upper right:: Solar system montage of Voyager images. Photo courtesy of NASA. Center: JPL. Image # PIA-02973.
http://grin.hq.nasa.gov/ABSTRACTS/GPN-2003-000006.html

Middle left: Hot spring in Yellowstone National Park Lower Geyser Basin White Creek area. Photograph by Maud Walsh

Middle right A spiny acritarch, *Meghystrichosphaeridium reticulatum*, preserved in 635-551 million year old phosphorite of the Doushantuo Formation at Weng'an in South China. Photograph by Shuahai Xiao.

Photo at the bottom: Intertidal stromatolites in Hamelin Pool, Shark Bay, Western Australia. Courtesy of B.P. Burns and B.A. Neilan (see their chapter in this volume).

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FOREWORD

From Fossils to Astrobiology: Records of Life on Earth and Search for Extraterrestrial Biosignatures

Astrobiology, the study of life in the universe, draws on many traditional areas of scientific study, including astronomy, chemistry and planetary science. This volume, number 12 in the **C**ellular **O**rigin**, L**ife in **E**xtreme Habitats and Astrobiology series (published by Springer) focuses on the study of the record of life on planet Earth, which is critical in guiding investigations in the rest of the cosmos, as well the evidence for and likelihood of extraterrestrial life. The 30 contributors to this volume are experts from 16 different countries: Australia; Austria; France; Germany; Hungary; India; Israel; Italy; Japan; Mexico; Norway; Romania; South Africa; Spain; Sweden, United Kingdom; the United States of America.

The editors thank the authors for their contributions and their cooperation during the compilation of this volume. We acknowledge the efforts of many individuals for their careful reviews of the chapters in this volume. Their names are listed in alphabetical order: Wlady Altermann, Peter Bahn, Stefan Bengston, Oliver Botta, Gary Byerly, Zachary Byerly, Jeffrey Chiaranzelli, Brent Christner, Alexandra Davatzes, Stephen Dornbos, J. Peter Gogartner, Jessica Goin, Richard Hugo, Hidrim Idriss, Carolina Keim, Joseph Lambert, Thomas Lindsay, Charles Lineweaver, Andrew Melott, Lori Marino, Harold Morowitz, Jared Morrow, Nora Noffke, Aharon Oren, Mary Parenteau, Russell Shapiro, Giovanni Strazzulla, Jan Toporski, Sergey Tsokolov, Peter Ward, and Frances Westall. We thank Shellie Miller and Maeghan Reese of Louisiana State University for their assistance with organization and proofreading of manuscripts. MMW acknowledges the support of the Louisiana Board of Regents/LaSPACE under the NASA Space Training Grant award NNG05GH22H.

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June 21, 2008

Biodata of **Joseph Seckbach**, editor of this volume and author of ***"Foreword"*** (both with M.M. Walsh) and the author with other coauthors of the ***"Summary and Outlook"***

Professor Joseph Seckbach is the founder and chief editor of ***Cellular Origins, Life in Extreme Habitats and Astrobiology*** **(COLE)** book series. See: www.springer.com/sereis/5775. He is the author of several chapters in this series. Dr. Seckbach earned his Ph.D. from the University of Chicago, Chicago, IL (1965) and spent his post-doctoral years in the Division of Biology at Caltech (Pasadena, CA). Then he headed at the University of California at Los Angeles (UCLA) a team for searching for extraterrestrial life. He has been appointed to the faculty of the Hebrew University (Jerusalem, Israel) performed algal research and taught biological courses until his retirement. He spent his sabbatical periods in Tübingen (Germany), UCLA and Harvard University, and served at Louisiana State University (LSU) (1997/1998) as the first selected occupant of the John P. Laborde endowed Chair for the Louisiana Sea Grant and Technology transfer, and as a visiting Professor in the Department of Life Sciences at LSU (Baton Rouge, LA). Recently (2006) he spent three months in Ludwig Maximilians University in Munich with a DAAD fellowship from the German service of exchange academicians, where several forward steps of this volume have been performed.

Among his publications are books, scientific articles concerning plant ferritin (phytoferritin), cellular evolution, acidothermophilic algae, and life in extreme environments. He also edited and translated several popular books. Dr. Seckbach is the co-author (with R. Ikan) of the *Chemistry Lexicon* (1991, 1999) and other volumes, such as co-editor for the Proceeding of Endocytobiology VII Conference (Freiburg, Germany, 1998) and the Proceedings of Algae and Extreme Environments meeting (Trebon, Czech Republic, 2000); see: http://www.schweizerbart.de/pubs/books/bo/novahedwig-051012300-desc.ht). His new volume entitled **Divine Action and Natural Selection: Science, Faith, and Evolution**, has been edited with Richard Gordon and published by World Scientific Publishing Company. His recent interest is in the field of enigmatic microorganisms and life in extreme environments.

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FROM FOSSILS TO ASTROBIOLOGY – A ROADMAP TO FATA MORGANA?

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1. Introduction

Joseph Seckbach, the editor of the present book series, invited me to write the introduction to "*From Fossils to Astrobiology*" during his 3 months visit to my Institute at the Ludwig-Maximilians-University under the fellowship of DAAD (German Academic Exchange Foundation) in 2006. Of course I felt honoured by his choice, but being sceptical towards the youngest and most multidisciplinary, flourishing and extremely fascinating of all science disciplines, astrobiology, I was also somewhat uncomfortable. Now, after extensive literature research and while writing, I realise how courageous it was of Joseph to entrust a notorious sceptic, like myself, with this work. I took up the challenge because I liked the idea of writing an introduction to a book on "Fossils and Astrobiology" as an essay, not necessarily complying with strict scientific rules and the dictatorship of a peer review process, but rather expressing a personal "qualified point of view" of a concerned scientist. I was eager to demonstrate the low chance for success of the endeavour to find extraterrestrial life. For me, a lot of fantasy and wishful, model driven thinking is associated with this new science discipline. The cases of reporting of Martian fossils and carbonates are good examples of how desire may influence scientific investigations.

It has always been a dream of mankind to find extraterrestrial life, intelligent life, of course, technically much more advanced than our civilisation, so we can learn from the aliens, discuss and explain our religious believes and perhaps demonstrate our religious and moral superiority. Next to these Hollywood-inspiring dreams, some researchers in astrobiology hunt for the evidence that terrestrial life has been introduced to the Earth from the infinite cosmos, riding on meteorites, comets and smaller impactors. The hypothetical proof of the theory of Panspermia, however, clearly will not explain any of the intriguing questions of the origin of life and its physical and chemical circumstances, but only

shift the problems to different unknown and less readily explicable environments. As an astrobiology-agnostic and scientist, I find the rise of expectations and promises suggested by astrobiology in the society alarming. They almost compel the researchers involved to turn up with positive, extraordinary results, in justification of the investments made in astrobiology. There can not be, however, compulsion for spectacular results when objectivity of interpretations is expected in science.

Astrobiology has been extremely successful in uncovering extraterrestrial environments and contributing to the knowledge of the history of the early Earth and life. This contribution over the last dozen of years is tremendous and fills almost uncountable pages of highly cited international astrobiology journals, volumes of conference abstracts and scientific and popular science books. Nevertheless, the young multidisciplinary science, especially when combined with paleobiology, Precambrian geology and geochemistry (the fossil aspect), requires humble and modest reports and should prescind from the temptation to impress with fast and spectacular shots and boulevard-type result reporting. Spectacular, extraordinary research interpretations need critical evaluation and extraordinary proof because of the difficulty in interpretation of indirect observations and measurements of events and environments extremely remote in time and distance. Fallacies, like P. Lowell's observation of a network of canals on Mars and their interpretation as evidence of advanced Martian civilisation more than a century ago are easily possible also with technically advanced, modern scientific equipment, allowing us to explore new, hitherto unreachable dimensions down to atomic scale and up to unimaginable distances in time and space.

2. What Is Astrobiology?

Astrobiology – a discipline fascinating and stimulating our thoughts but also being disparaged as "the science without an object" to investigate and thus illusive. If there is no life out there or it is too remote to be proven, astrobiology may be an expensive road to a fata morgana. However, astrobiology has undoubtedly boosted the research in Precambrian geology and paleobiology, biogeology, planetary geology, geochemistry, microbiology, and many other scientific disciplines, mediating between them and molecular biology, astrophysics, astronomy, oceanography and bringing together scientists that rarely spoke to each other before. These disciplines experienced a revitalising injection of funding that allowed them to combine forces and develop new research techniques, appoint many young and enthusiastic scientists, and turn up with intriguing results. These results and the pledge to find new worlds and perhaps extraterrestrial life brought astrobiology into the centre of public interest and called for ethical, philosophical and even religious assessment of this science.

Many respected scientific societies like the International Society of the Origin of Life (ISSOL) that has recently extended its name to "ISSOL – the International Astrobiology Society" or the SETI program (Search for

Extraterrestrial Intelligence) are very successful in mobilising researchers worldwide. Panels and commissions of scientists, politicians and social and religious activist have been set up to plan for the case of the discovery of extraterrestrial life or even possible contacts to extraterrestrial intelligence. Such panels not only prepare the necessary emergency plans against contamination of terrestrial and extraterrestrial environments, but also speculate on the possible impact on the society of such discoveries. All these activities are in spite of the hitherto absolute lack of any biological object or any, even equivocal, sign for life from outside of the physical boundaries of our planet Earth.

So, if astrobiology has no extraterrestrial biological objects to study, what is it exploring? According to the National Aeronautics and Space Administration (NASA) web page (2007) http://astrobiology.arc.nasa.gov/, "Astrobiology is the study of life in the universe. It investigates the origin, evolution, distribution, and future of life on Earth and the search for life beyond Earth. Astrobiology addresses three fundamental questions: How does life begin and evolve? Is there life beyond Earth and how can we detect it? What is the future of life on Earth and the universe?" – This is truly a universal subject of investigations, particularly when we consider the difficulties in the definition of life, despite of hundreds of years of research of life and living objects, and more than 60 years after Schrödinger's, 1944 book "What is Life?". Next to alleged extraterrestrial life and its fossilised remains hidden in the endless space, the above universal definition of astrobiology embraces all life sciences and all studies of terrestrial life. The major questions asked by this science, but especially the one on the future of life, require a truly prophetic capacity far beyond any responsibility and specialisation of natural sciences. They open ample space to unwholesome suppositions and religious disputes. In my atheistic opinion, however, astrobiology should be strictly restricted to natural sciences.

The dictum and the title of the present book "From Fossils to Astrobiology" demarcates a logical scientific philosophy for the search of extraterrestrial life. It follows the assumption that life in different worlds, on other planets, should obey the same chemical and physical rules as life on Earth. It must have evolved under conditions similar to those that prevailed on the early Earth and are witnessed by the oldest fossils and the environments recorded in rocks where these fossils are found. Like life on Earth, extraterrestrial life must be based on the reactivity and chemical properties of water, carbon and other crucial elements like nitrogen, phosphorus, iron, oxygen and few others. Thus, in order to set off through chemical reactions and thrive, extraterrestrial life also requires an environment of temperatures roughly between zero and few tens of degrees Celsius, although life on Earth can survive temperatures far beyond these boundaries. It also requires a rocky, silicate-oxide based environment, providing nutrients, a stable sources of energy, and shelter from extreme fluctuations of temperature and pressure and from damaging short wave radiation. From our knowledge of the life on Earth, we expect that within such conditions the emerging extraterrestrial life will undergo some kind of evolution, thus gradually changing its environment via its

metabolic products, just like it did on Earth. Actually, this expectation brings us to a new search strategy: Instead of directly searching for extant or fossilised organisms, extremely difficult to recognise from the distance, metabolic products of life and their environmental influence on the planets are sought.

Extraterrestrial life, therefore, is most likely to be found on planetary bodies with chemo-physical conditions similar to those prevailing on Earth during its long, 4.6 billion years of history. The second strategy of astrobiology appears thus logical: Life on Earth emerged under the conditions of the young Archean Earth, before 3.5 billion years ago, under presumably reducing atmosphere, perhaps elevated temperatures and oceans of different chemistry than today. Such conditions may prevail on other planets. It is therefore crucial to investigate the Precambrian Earth as a possible analogue to habitats of putative extraterrestrial life. A logical consequence from this strategy is that such planets could *vice versa* serve as analogues of the earliest conditions of the Hadean Earth, not preserved in the geologic record. The Earth is a dynamic planet driven by the internal forces as expressed through the plate tectonics, which are destructive to old rocks and preservation of organic matter. On planets devoid of plate tectonics, the chance of preservation of the very early steps of planetary evolution or perhaps even of the very first steps of genesis of life might be significantly higher than on Earth. But will such planets be akin enough to the Earth to offer conditions suitable for life, and will life have a chance to develop there?

3. Where To Search?

The above discussed conditions for the genesis and survival of life limit the seemingly endless number of candidates for a fertile environment in the Universe. Even if we take into account the myriads of stars in remote galaxies (which we will never be able to explore or to communicate with), planetary systems like our Sun and its satellites seem to be extremely rare. The chance to find a planet just like the Earth seems just a mathematical illusion, barren of any degree of certainty.

Some time ago, I was speculating together with Roger Buick, one of the Editors of the journal "Geobiology" and a supreme Precambrian geologist and geobiologist, about the "ten reasons why life probably evolved only on Earth". Equipped with the excellent Bavarian Augustiner beer brewed since AD 1294, sitting in a traditional nineteenth century pub in Munich, we were certain that life elsewhere must obey the same physical and chemical laws as on Earth and thus the factors listed above, such as solution chemistry (free availability and abundance of water, C, H; O; N; P; Fe; S …), temperature boundaries and bearable energy levels) were among our primary preconditions for the genesis of life. Further requirements in our discussion were gravity conditions similar to those on Earth, proportionally similar distance of the given planet to its star, cyclic changes of environment caused by orbital forcing and climatic fluctuation. To these necessities can be added an efficient shield from UV and cosmic radiation, sufficient atmospheric pressure, long

term equilibrium of planets internal and external energy budget (the lower luminosity of our Sun in the early Archean was buffered by Earth's higher energy flux and greenhouse gases), and last but not least, the necessary interplay of the right proportions of these preconditions, just as it was on the Archean Earth. Many of the above conditions are influenced by the internal dynamo and consequently by plate tectonic processes that regulate the energy budget of the Earth and allow geochemical cycles to operate. Plate tectonics significantly influenced the early atmospheric composition through degassing processes. Of course, the above requirements followed from life as we know it. We were wondering whether we would be able to recognise life if it is dramatically different.

Reaching out to other stars appears not very realistic considering the distances to overcome. Nevertheless, astronomers have discovered several stars orbited by planetary bodies and each of these discoveries (over 200 exoplanets since 1995) has been announced in the media as a possible site for life and as an "Earth-like" body. Exoplanets are discovered mainly by observation of a periodic shift in wavelength of a star caused by the gravitational pull of the invisible orbiting planet or by observing a periodical slight decrease in light intensity of a star when an orbiting planet passes in front of it. As far as researchers can say, all up to now discovered planets are gas giants composed mostly of hydrogen and helium, similar to Jupiter or Saturn. Only in April 2007, a newly discovered exoplanet was reported as "Earth-like" (Udry et al., 2007) and a possibly habitable "oasis in space". On a closer reading, however, it turns out that this possibly rocky planet of the mass of five times that of the Earth is orbiting the cold red dwarf, Gliese 581, in a distance of 10.7 million kilometres, which is just 0.07 Astronomical Units (AU = the distance between the Sun and the Earth). Every orbit is completed in 13 days (Schilling, 2007). Although modelling implies that giant rocky planets of five to ten times the mass of the Earth (often dubbed "Super-Earth") would inevitably have plate tectonics (Valencia et al., 2007), such planets can be called anything not "Earth-like". Nonetheless, it seems likely that with improved instruments and new spacecraft missions, eventually, somewhere an Earth-like planet will be discovered in the universe. Certainly, the speculations about its possible biology will be outermost.

Carbon and water are abundant in space. Carbonaceous matter in varying molecular forms, ranging from amorphous carbon, polycyclic aromatic hydrocarbons, to fulleranes is present in interstellar dust, meteorites and comets. H_2O, N, H and C-O compounds are ingredients of comets. Aminoacids and other prebiotic organic molecules have been detected in meteorites. Iron and sulphur are equally universally present, but all extraterrestrial bodies investigated up to date turned out to be sterile, although for some, like our neighbour planet Mars, big hopes for discovery of life still exist.

The above considerations make the genesis of life on other planets than Earth barely probable, but the universal adaptation ability of life to extreme conditions makes it possible that terrestrial life could survive on other planets. That allows for science-fiction scenarios of colonialized planetary bodies and thus,

literature on "Directed Panspermia", "Planetary Engineering" and the "Ethics of Terraforming" is profuse (e.g. Sagan, 1973; Friedmann et al., 1993; York, 2007, and many others). Experiments in which primitive life and its molecules are exposed to cosmic radiation on mineral shielded panels, carried on board of satellites, seemingly demonstrate that life could survive and travel in space. However, over long time cosmic radiation and other space conditions during such an interplanetary journey and the shock of an impact would certainly eradicate any living cell. "Lithopanspermia" experiments in which dry layers of biological test materials like bacterial endospores, endolithic cyanobacteria or lichens are sandwiched between gabbro discs and impact shock pressures are simulated, apparently determining the ability of the organisms to survive the harsh conditions (Horneck et al., 2008), indeed barely reflect the whole range of realistic circumstances and processes of an asteroid to planet collision and meteoritic interplanetary travel and impact. For this reason Panspermia both ways is not an option.

Despite of this unlikelihood, the most probable candidates for life (and colonialization) within our Solar system would be our neighbour planetary bodies Mars and although very improbable, the Jovian moon Europa (next to Io, Ganymede, and Callisto) and Saturn's largest moon, Titan. However, all these moons are tidally locked to their central planets and their energy balance depends rather on their distance to the Jupiter and Saturn than on their mass and distance to the Sun. Even if microbial life could survive the conditions of Mars, Europa or Titan, survival still is a big difference to the possibility of life's genesis.

The best of the above candidates for extraterrestrial life is the planet Mars. Our direct neighbour, the fourth planet from the Sun, more closely resembles the Earth than any other planet. The Martian day has a length of 24.6 hours and the Martian year a length of 669 days. The distance from the Sun ranges between 206 and 249 million kilometres. Mars has about half the diameter of the Earth's diameter and about 1/3rd of Earth gravity. Martian surface temperatures range between −125°C and + 25°C and the atmosphere contains about 95% of carbon dioxide next to 3% nitrogen, 1.5% argon and traces of water, with a surface atmospheric pressure of about 6 mb on average. It would be an interesting experiment to implement some bacterial strains on Mars and closely watch their fate after liberation.

Water on Mars must have been abundant during the early period of this planets history, the Noachian (c.4,500 to 3,500 million years ago). Some argue however, that liquid CO_2 has formed the suspicious drainage landscapes of Mars. Nevertheless, preference for liquid water is mounting and there are good hints for episodic, minor water flows expulsed from the subsurface throughout the post-Noachian (Hysperian and Amazonian) history of Mars (Fig. 1). It is controversial whether the Noachian occurrence of liquid water was conditional on the dense Martian atmosphere and the atmospheric greenhouse effect and lasted for millions of years or if it was only episodic, following enhanced volcanic activity or subsequent to giant meteorite impacts. Most interpretations postulate an ocean covering the Martian Northern Plains, about one third of the planets surface, during the Noachian. Remote sensing

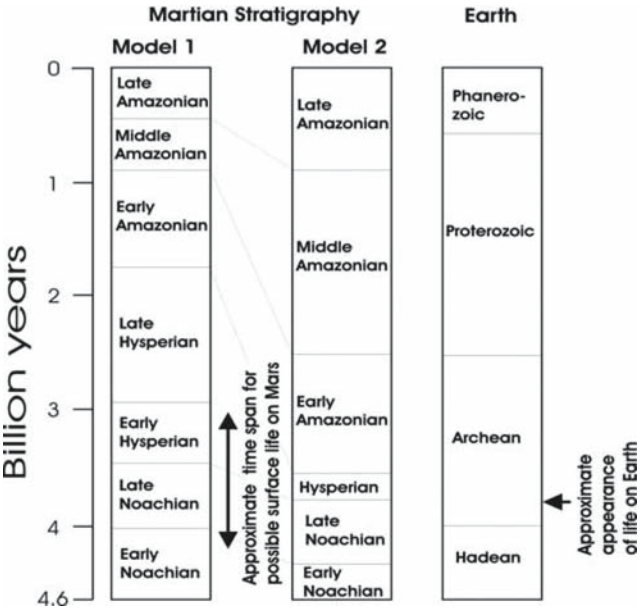

Figure 1. Two alternative models for Martian stratigraphic time table, compared to the Earth. Both models imply that the time span in which surface conditions for life on Mars were favourable, was relatively short.

images from the various Mars orbiters clearly show ancient coastlines. The Martian rovers have found mounting evidence for water-lain sedimentary rocks on Mars. However, the laterally continuous shorelines of the postulated Martian ocean show large topographic fluctuations, on an order of more than 2.5 km difference in elevation. This has been discussed as the result of deformation of the ancient coastlines after the loss of the oceans. Because of its small size and mass the internal dynamo of Mars was shut down early in the planet's history and led to the loss of the protective magnetic shield and to ionic erosion of Martian atmosphere by solar winds and made surface life on Mars impossible. The unweathered Noachian-Hysperian "olivine fields" (Hoefen et al., 2003) evidence thin and dry atmosphere since the Hysperian. However, if life existed on Mars during the Noachian, it might have migrated to the subsurface that still probably bears frozen water and to the ice-capped Martian polar regions.

4. What To Search For?

The search for life on Mars concentrates simultaneously on several strategies. The search for suitable habitats is mainly focused on the search for water and carbonate rocks. Carbonate sedimentary rocks on Earth that date from the early

Precambrian are biogenic precipitates and contain organo-sedimentary structures – stromatolites. Thus finding carbonates on the bottom of ancient Martian lakes and oceans or in Martian soil would be a good hint for ancient life. However, organic substances and metabolic products of life were directly searched for with negative results.

A pioneering direct approach to detection of biological activity was the 1975/76 Viking mission to Mars. The two Viking landers were equipped with a gas chromatograph mass spectrometer (GCMS) constructed to identify organic molecules in the Mars soil. However, no such molecules were found. Another experiment carried on board of the two landers was the Labelled Release (LR) life detection experiment designed to stir some Martian soil with a carried nutrient solution containing radioactive ^{14}C. It was envisaged that any living organism in the soil would digest the radioactively labelled carbon rich solution and release ^{14}C rich gas, as the organism metabolised the nutrients. In both Viking experiments, ^{14}C labelled gas was indeed detected but the results were discarded because no organic molecules were detected. It was assumed that the measured gas must thus have been released by an abiotic reaction with some unknown oxidant. The designers of this experiment, however, claim until today that their experiment has possibly found life on Mars, but the GCMS experiment missed the organic molecules. There is evidence for magnetic minerals in Martian soil, which could not last in the presence of strong oxidants because magnetic iron minerals would be turned into oxidised forms. Thus, they argue that in the absence of oxidants, the measured gas must have been produced by biological activity.

The fast loss of water on the surface of Mars also has important geochemical implications. It has been long assumed that the fast evaporation of Mars' oceans and lakes must have left a widespread crust of carbonate rocks on the planet surface. The CO_2-rich Martian atmosphere must have reacted with the rocks and liberated abundant Ca from Martian basalts. These calcium should have been left over on the surface when the oceans disappeared. From the 1980s on, a plethora of articles speculating on Martian carbonates was published. Eventually, Bandfield et al. (2003), in a paper in Science, identified carbonate minerals in the Martian dust by Thermal Emission Spectrometer (TES) orbiting the planet. These findings had tremendous implications, as on Earth carbonate rocks are clear evidence for biological activity and thus analogously, the images of possible Martian stromatolites and microbial deposits seemed an edge breaking discovery. However, some researcher did not "buy the story" for simple technical reasons.

Spectral analyses from the orbit do not detect minerals but measure radiance, which is then interpreted based on comparisons to terrestrial standards. The identification of 2–3 wt% carbonate minerals in the Martian dust, with calculated particle sizes of $<10\,\mu m$, was based on comparison of the Martian emissivity bands to laboratory spectra of labradorite standards. In general, the emissivity depends on the chemical composition of the dust or rock, mineral mixture, crystal orientation, grain size distribution and surface roughness, at that time all unknowns in the Martian soil. For the quantification of the spectral signatures of

minerals of up to 2–5% weight, the sensitivity of the sensor and the calculated particle size are essential; however, detection limits of TES for carbonate concentrations in weight percent were poorly specified. Earlier work by Bandfield et al. (2000) specified detection limits for the Martian TES spectra of 10–15 vol%. The comparison of TES and laboratory spectra is only possible at the same spectral and radiometric resolution for accurate definition of the position and spectral contrast of emissivity minima, not characterised in the report by Bandfield et al. (2003). Particle sizes and the concentration of mineral mixtures are important for the spectral response in the Thermal Infra Red (TIR). Particle sizes ≤63 µm were used for the calculations, although the high albedo surfaces on Mars were considered ≤10 µm particle size. The spectral bands (>1,350 cm^{-1}) thus did not necessarily indicate non-hydrous carbonates, as were believed to have been detected. Particle sizes <5 µm are responsible for spectral differences in the wavelength regions of <10 µm, due to the combination of surface and volume scattering effects, hence, the quantification of the presented spectra was ambiguous as well. All criticism, however, was in vein and our attempt of publication of this discussion was rejected (Altermann et al., 2004).

Thus far the extremely successful and long operating Mars Exploration Rover missions that landed in January 2004 has not encountered carbonate rocks. The Opportunity Rover that landed in the Gusev impact-crater, a former lake, discovered fields of small haematitic concretions dubbed "blueberries", probably formed in the presence of water and rocks composed of up to 40% evaporitic sulphate minerals instead of carbonates. The presence of sulphates indicates that the climate of the Noachian Mars was influenced by SO_2, where dilute sulphuric acids must have formed and prevented the precipitation of carbonates (even at traces of 0.1% of SO_2 in the atmosphere). A whole different story of the Martian atmosphere was revealed calling for caution with model driven thinking.

Carbonate minerals were also reported from the famous Martian meteorite ALH 84001. In tiny microscopic cracks in this meteorite, carbonate minerals were found and reported as "globules" and veinlets, filling fractures. However, the morphology of these carbonate "globules" is that of discs, as can be expected in cracks, and not globular. A Rb- Sr age of 3.9 ± 0.04 billion years (Ga) and an Pb-Pb age of 4.04 ± 0.1 Ga were measured on leachates from a 1.0 g chip containing c.5% carbonate (Borg et al., 1999) from these veinlets. On the other hand, a Rb-Sr 1.39 ± 0.10 Ga age on shock melted feldspathic glass and carbonate, thought to be in isotopic equilibrium, was measured by Wadhwa and Lugmair (1996). These carbonates are clearly not minerals within a "sedimentary system" and are not comparable with biogenic carbonates. They are formed from high temperature fluids during impact driven metasomatism or by impact melting of pre-existing carbonates rather than from low temperature fluids, as discussed by Borg et al. (1999). Elevated building temperatures of 80–200°C are suggested by $\delta^{13}C$ (&permile;) and by $\delta^{18}O$ (&permile;). The carbonates are zoned, with Ca- rich centres, Fe- rich mantles and Mg- rich margins. The zoning may be in isotopic equilibrium, but the isotopic composition has been differently interpreted and even claims of terrestrial led isotopy in these carbonates were raised. The zoning

might be the effect of high temperature crystallisation, but perhaps also of terrestrial alteration processes. Isotopic equilibrium of different zones would be, nevertheless, unlikely in the latter case. Which ever interpretation is correct, these carbonates can not be regarded as traces of the Martian Noachian ocean.

5. Martian Fossils

So how about looking directly for life or at least for fossilised life? Because life on Mars had little time to develop and flourish on the surface of the planet, possible fossil life will certainly be primitive, prokaryotic (Fig. 1). The earliest microfossils on Earth display already a very high degree of complexity. However, their authenticity and age and metabolic significance have been vigorously questioned in the recent years, showing how much uncertainty is connected to the business of morphological recognition of ancient and thermally altered microfossils. Finding and interpreting microfossils on Mars will be certainly, by orders of magnitude, more difficult.

Yet, the questioning of the authenticity of the Earth's oldest microfossils (Brasier et al., 2002, 2004) resulted in additional evidence that the Earth's oldest, 3.46–3.45 Ga, microfossils (Walsh, 1992; Schopf, 1993; Ueno et al., 2001) are indeed cellularly preserved remains of Archean microbial life (Altermann, 2005). They are closely associated with stromatolitic structures of the same ancient age and with various geochemical "biomarkers". Even Brasier et al. (2006) claim now to have found evidence of microbial (endolithic) life in sand-sized grains from the 3.4 Ga Strelley Pool Chert in Western Australia. The discussion on the authenticity of the Earth's oldest fossils led to the introduction and development of new investigation techniques like Raman spectroscopy, analysis of molecular biomarkers, atomic force microscopy (AFM) techniques and new, stable isotope methods into Archean paleontology. All these methods and the classical morphological studies on petrographic thin sections will have to be applied to putative candidates for Martian microfossils. Such complicated investigations require a sample return to Earth mission and will not be possible to perform on an automated, remotely controlled rover vehicle.

Considering that only few samples containing Archean microbial remains were found on Earth, despite intensive mapping and sampling campaigns, how big would be the chance to find Martian Noachian fossils in the few Martian meteorites collected on Earth? Such a claim was made by a group of NASA scientist for the same ALH 84001 meteorite, from which the above discussed carbonate minerals were detected (McKay et al., 1996). The meteorite found 1984 in the Allan Hills region of Antarctica appears to be essentially free of terrestrial weathering, after at least 13,000 years exposure to ice. The examination of surface textures of the cracked carbonate discs, at magnifications of 50,000 and greater, revealed the occurrence of ovoid to elongated structures. The straight forward interpretation was that the tiny elongated structures 20–100 nm in length, represent

fossilised Martian bacteria and the above discussed carbonate "globules" are of biogenic origin! From the occurrence of polycyclic aromatic hydrocarbons (PAHs) and the coexistence of carbonate minerals with Fe-sulphide phases and magnetite within partially dissolved carbonate, biomediated mineralisation in chemical disequilibrium was proposed, as is known to occur in some anaerobic bacteria. A resemblance of this magnetite to magnetite in magnetotactic bacteria was pointed out as additional evidence for Martian microbial activity. The nanoscopic structures shown in SEM microphotographs indeed strongly resemble single bacterial rods. A direct comparison to the size and shape of "nanobacteria in travertine and limestones" was made. Although the mere existence of nanobacteria is highly disputed because metabolic activity is impossible in such a tiny volume space, and no laboratory until today has succeeded in their cultivation, this report became a sensation.

This spectacular interpretation was diluted in an accompanying "News" article in the same issue of Science, by Richard Kerr (1996). It was clear that no single part of the above arguments would be acceptable evidence for life on Mars. PAHs and magnetite crystals, for instance, are found also in carbonaceous chondrites and in interplanetary dust particles or can be formed during hydrothermal decomposition of $FeCO_3$ (siderite), a common carbonate mineral. However, voices calling for caution like that of Bill Schopf, who, at the organised news conference, in the presence of the US president, suspected the findings as of low probability for truth, were not well received. The detailed arguments *pro* and *contra* the life on Mars interpretation are listed in his 1999 book "The Cradle of Life".

6. The Book "From Fossils to Astrobiology"

In my opinion, life is not a logical unavoidable consequence of the early Earth's conditions but rather a highly improbable coincidence of circumstances. The common assumption by astrobiologists that 'wherever there is water there should be life' is not even true on Earth, where life is indeed present almost everywhere. The most probable conditions for the origin of life and the most probable ingredients of life were experimentally brought together in countless attempts in the most sophisticated laboratories and still the coincidence was not reproduced. Excellent, equally educating and entertaining reading on such experiments can be found in R.M. Hazen's (2005) book "gen·e·sis".

The book in front of you, contrary to this introduction, is not a popular science book. It is addressed at scientists seeking precise information in various aspects of astrobiology. Perhaps the major paradox of life sciences and astrobiology is that after so many years of research, we still can not agree on an universal definition of life. The chapters of the book treat major problems in recognition of life on early Earth and discuss the habitats of life and problems in their comparison to extraterrestrial environments. New technologies as used for microfossil

recognition and the opening new perspectives for their application are considered, and general aspects of astrophysics and astrobiology are elucidated.

The young science of astrobiology remains controversial. Probably none of the authors of this book would agree unconditionally with the major synthesis of this introduction that life on Earth is matchless. On the other hand, I see little sense in discussing "non-protein based life," and I disagree with the criticism of the Earth's earliest fossils and their debate. I also see no signs for (astro)biological imprints on lunar regoliths. Although I trust that scientists, not long from now, will be able to reconstruct the first living cell *in vitro*, as they learn to better understand the circumstances of life's genesis, I radically doubt whether we will be ever able to reach far enough to have a chance to discover extraterrestrial life, if it ever exists or existed. But perhaps, and indeed hopefully, my scepticism towards astrobiology will soon be proven wrong. Life or fossil life will be found on Mars or another planet, and controversies will arise as to its origin on this planet or somewhere else in the Universe and as to its authenticity and criteria for recognition. And we will still be dreaming of finding intelligent life somewhere in the infinity of the Universe.

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to Fata Morgana? This painting of the life on the "Red Planet" Mars, by the 7 years old Hanna Altermann, elucidates the desire of finding extraterrestrial intelligent life. Science fiction and serious research programs result from this desire, but even microbial life will be an enormous exception in space. Life is not a logical and unavoidable consequence of 'suitable physicochemical' conditions but rather a highly improbable physico-chemical coincidence.

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NANOSIMS OPENS A NEW WINDOW FOR DECIPHERING ORGANIC MATTER IN TERRESTRIAL AND EXTRATERRESTRIAL SAMPLES*

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1. Introduction

Recognition of the earliest morphological or chemical evidence of terrestrial life has proved to be challenging, as organic matter in ancient rocks is commonly fragmentary and difficult to distinguish from abiotically-produced materials (Schopf, 1993; Van Zuilen et al., 2002; Altermann and Kazmierczak, 2003; Cady et al., 2003; Brasier et al., 2002, 2004, 2005; Hofmann, 2004; Skrzypczak et al., 2004, 2005). Yet, the ability to identify remnants of earliest life is critical to our understanding of the timing of life's origin on earth, the nature of earliest terrestrial life, and recognition of potential remnants of microbial life that might occur in extraterrestrial materials.

The search for earliest life on Earth now extends to early Archean organic remains; these tend to be very poorly preserved and considerably more difficult to interpret than the delicately permineralized microfossils known from many Proterozoic deposits. Thus, recent efforts have been directed toward finding biosignatures that can help distinguish fragmentary remnants of ancient microbes from either pseudofossils or abiotic organic materials that may have formed hydrothermally or in extraterrestrial processes (House et al., 2000; Boyce et al., 2001; Kudryavtsev et al., 2001; Schopf, 2002; Schopf et al., 2002, 2005a, b; Cady et al., 2003; García-Ruiz et al., 2003; Hofmann, 2004; Brasier et al., 2005; Rushdi and Simoneit, 2005; Skrzypczak et al., 2005).

An exciting area of biosignature research involves the developing technology of NanoSIMS. NanoSIMS is secondary ion mass spectrometry (SIMS) for

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* *Adapted from Oehler, D.Z., Robert, F., Mostefaoui, S., Meibom, A., Selo, M. and McKay, D.S. (2006), Chemical Mapping of Proterozoic Organic Matter at Submicron Spatial Resolution, Astrobiology 6(6): 838–850. Permission to reprint portions of that article has been granted by Mary Ann Liebert, Inc., publisher of Astrobiology.*

ultrafine feature, elemental and isotopic analysis. Its resolution approaches 0.05 μm for element mapping, which is 10–50 times finer than that attainable with conventional SIMS or electron microprobes. Consequently, NanoSIMS has the potential to reveal previously unknown, chemical and structural characteristics of organic matter preserved in geologic materials.

Robert et al. (2005) were the first to combine NanoSIMS element maps with optical microscopic imagery in an effort to develop a new method for assessing biogenicity. They showed that the ability to simultaneously map the distribution of "organic" elements [such as carbon (C), nitrogen (N), and sulfur (S)] and compare those element distributions with optically recognizable, cellularly preserved fossils could provide significant new insights into the origin of organic materials in ancient sediments.

This chapter details a recent NanoSIMS study which was designed to acquire new data relevant to establishing critical biosignatures (Oehler et al., 2006a–c). In this study, NanoSIMS was used to characterize element distributions of spheroidal and filamentous microfossils and associated organic laminae in chert from the ~0.85 billion year old (Ga) Bitter Springs Formation of Australia. Previous work established preservation of a diverse microbiota in the Bitter Springs Formation (Schopf, 1968; Schopf and Blacic, 1971), and there is no dispute within the scientific community regarding the biogenicity of any of the Bitter Springs structures evaluated in this new study. Thus, the NanoSIMS results described below provide new insight into – and can be used as a guide for assessing – the origin of less well understood organic materials that may occur in early Archean samples and in meteorites or other extraterrestrial samples.

2. Materials and Methods

Analysis was performed on structures within a polished thin section, ~30 μm thick, of chert, collected by D.Z. Oehler from the Ellery Creek locality of the Bitter Springs Formation. For the current study, spheroidal (*cf.* Myxococcoides) and filamentous (*cf.* Eomycetopsis) microfossils as well as organic laminae were located within the section using optical microscopy (Fig. 1). Specimens were selected for NanoSIMS based on the quality of preservation and occurrence at the top surface of the thin section. The specimens were photographed using an Olympus BX60 Research and Polarizing Optical Microscope, outfitted with a Nikon DXM 1200F Digital Camera; 4×, 10×, and 40× dry objectives were used, in both transmitted and reflected light, and sketch maps were constructed for use with the photographs for locating the structures of interest in the NanoSIMS instrument. Photomicrographic focal series were taken (using the same optical microscope) from the top of the thin section to the base of the structures of interest in transmitted light, using a 100× oil immersion lens and Cargill type DF immersion oil (Formula Code 1261). Focal planes imaged spanned about 20 μm of the thin

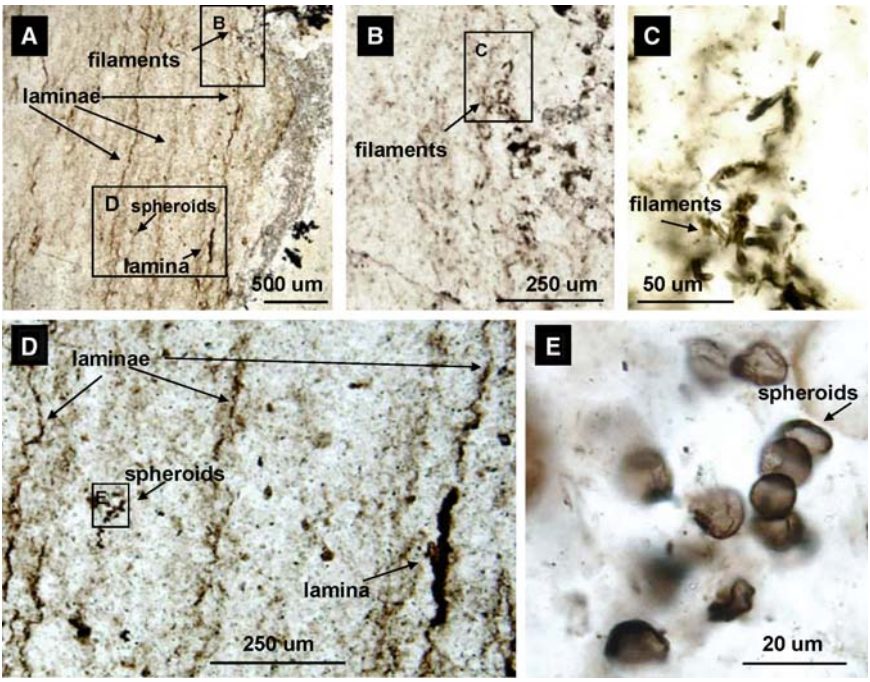

Figure 1. Optical photomicrographs in transmitted light of organic spheroids, filaments, and laminae in a polished thin section of chert from the ~0.85 Ga Bitter Springs Formation. (A–E) illustrate the spatial relationships among the different types of structures discussed in this paper. The small rectangles in (A) identify fields of view in (B) and (D), the small rectangle in (B) identifies the field of view in (C), and the small rectangle in (D) identifies the field of view in (E).

section, and 8 to 12 images typically were taken, so that step size between individual images of a focal series was 1–3 µm. The thin section was subsequently cleaned to remove all oil and any contamination from fingerprints by ultrasonication five times with reagent-grade ethanol for 2 min, each time. After the sample was cleaned with ethanol, it was dried in a 60°C oven for 1 h to drive off all solvents and finally coated with about 300 Å of gold.

It was assumed that the ultrasonication procedure was adequate to remove any traces of immersion oil or fingerprints on the slide. This assumption seems reasonable in view of the facts that (1) the NanoSIMS carbon maps are mirrored by both N and S maps (which is suggestive of sedimentary organic matter rather than the immersion oil, which is composed of hydrocarbons and chlorinated hydrocarbons), (2) there is a one-to-one correspondence of the C, S, and N maps with organic, kerogenous structures seen optically, and (3) the initial sputtering by NanoSIMS removes the most surficial layer, so any surface contamination is removed before data are collected. In addition, none of the kerogenous structures

imaged resides in any sort of a crack in the thin section, where traces of immersion oil conceivably could remain. Finally, it should be noted that the thin section studied was not embedded with epoxy during the thin sectioning process, as the sample was relatively dense, indurated and unfractured, so that none of the NanoSIMS data could be interpreted as originating from either epoxy or some combination of epoxy and immersion oil.

Chemical maps were produced with the Cameca NanoSIMS 50 of the Muséum National d'Histoire Naturelle in Paris, France. Using a focused primary beam of Cs^+, secondary ions were sputtered from the sample surface. $^{12}C^-$, $^{12}C^{14}N^-$, $^{32}S^-$, $^{28}Si^-$, and $^{16}O^-$ or $^{18}O^-$ were detected simultaneously (multicollection-mode) in electron-multipliers at a mass-resolving power of ~4,500 ($M\square M$). At this mass-resolving power, the measured secondary ions were resolved from potential interference by other ions or molecules that fall close in mass to the ions of interest. Because nitrogen is detected as CN^- in NanoSIMS instruments, it can only be detected in the presence of carbon. Images were obtained from a presputtered surface area by stepping the primary beam across the sample surface. Presputtering is done to remove the conductive coating and clean the surface of any contaminants before analysis. The primary beam was focused to a spot size of ~50–100 nm, and the step size was adjusted so that it was comparable to, but slightly smaller than, the size of the primary beam. An electron gun supplied electrons to the sputtered surface during analysis to compensate for positive charge deposition from the primary beam and to minimize specimen charging effects. Follow-up scanning electron microscopy was performed on the Jeol JSM-5910LV (at 15 kV, 10 mm W.D.) at Johnson Space Center, Houston, TX.

N/C atomic ratios were obtained from measured $^{12}C^{14}N^-$ and $^{12}C^-$ yields by normalization to a kerogen standard that we prepared from a sample of the Eocene Green River Shale. This kerogen, which was extracted from the shale by standard HF-HCl techniques (Beaumont and Robert, 1999), comprised ≥94% of the acid-insoluble residue; standard chemical techniques (Beaumont and Robert, 1999) were used to determine that the kerogen has an atomic N/C ratio of 0.025. The $^{12}C^{14}N^-/^{12}C^-$ ratio of the standard was then measured in the NanoSIMS using operating conditions identical to those used for analyzing the Bitter Springs fossils (e.g., same presputtering, spot size, e-gun, etc.).

Figure 1 illustrates the different types of organic structures analyzed in the thin section and their spatial relationships to one another. The spheroids studied are fairly abundant and occur in clusters of a few to ~25 cells, most commonly between dark brown organic laminae; the cells are typically less than 10 μm in diameter and have distinct reticulate walls, 0.3–0.5 μm thick. The filaments consist of sinuous hollow tubes, are also abundant in the thin section, and occur intertwined in mat-like layers that grade into the dark organic laminae; the filaments are 3–5 μm in diameter, up to hundreds of microns long, and have somewhat diffuse granular walls, 0.4–0.7 μm thick. The organic laminae are planar features composed of morphologically indistinct organic material, as seen in optical microscopy. In thin section, they

appear as strand-like fragments of organic matter that align to form parallel, wavy to crenulate surfaces. The material making up the laminae varies from morphologically diffuse and semitransparent to more distinct-bordered and dark brown in color. The laminae occur at intervals of a fraction of a millimeter to a few millimeters, and they have thicknesses from about 5 to 20 μm.

3. Results

NanoSIMS maps of C, N, S, Si, and O (measured as $^{12}C^-$, $^{12}C^{14}N^-$, $^{32}S^-$, $^{28}Si^-$, and $^{16}O^-$ or $^{18}O^-$) were acquired of the spheroidal and filamentous organic microfossils and the apparently amorphous organic laminae from a single thin section of the Bitter Springs Formation (Figs. 2–7). Results demonstrate an excellent correspondence between the optical images of the microfossils and the spatial (two-dimensional) distributions of C^-, CN^-, and S^- (Figs. 2–4). Intense sputtering into one sample allowed penetration by the NanoSIMS to a focal plane, 2–3 μm

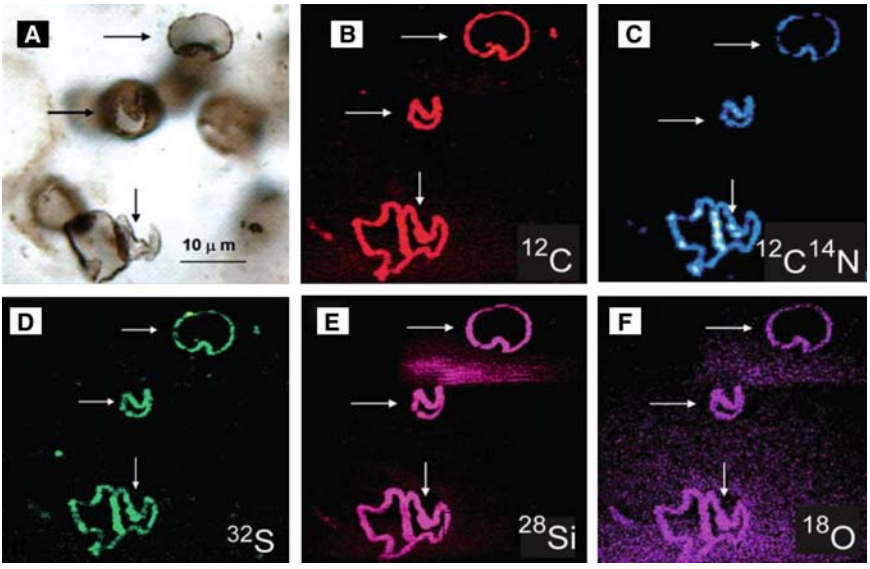

Figure 2. Spheroidal organic microfossils in a polished thin section of chert from the Bitter Springs Formation. (A) Optical photomicrograph in transmitted light; the cells of this panel are part of the cluster illustrated in Fig. 1A and E, but at a different focal plane and rotated so that the optical and NanoSIMS images can be directly compared. (B–F) NanoSIMS element maps of the same area as in (A). Arrows show corresponding cells in the different panels. Scale in (A) applies to all. ^{12}C, carbon; $^{12}C^{14}N$, nitrogen measured as CN^- ion; ^{32}S, sulfur; ^{28}Si, silicon; ^{18}O, oxygen. Color scheme is red for carbon, blue for nitrogen, green for sulfur, pink for silicon, and lavender for oxygen. The more intense the color, the stronger the response, with white being the strongest response in all cases.

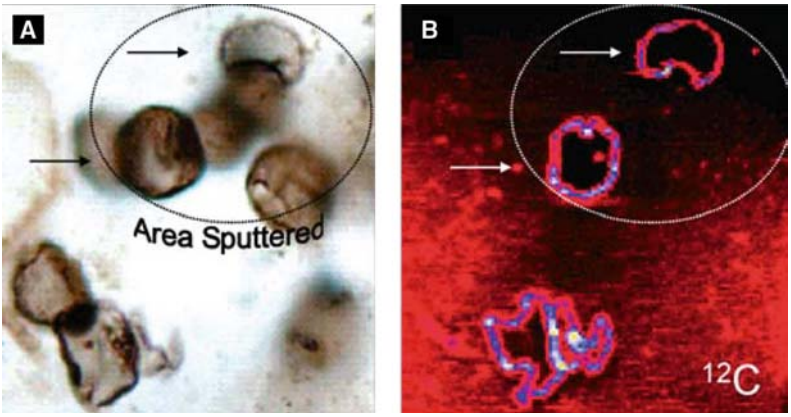

Figure 3. Spheroidal organic microfossils in a polished thin section of chert from the Bitter Springs Formation. (A) Optical photomicrograph in transmitted light, focused at a plane slightly below that of Fig. 2A. Dotted oval indicates area sputtered in the NanoSIMS. (B) NanoSIMS map, illustrating carbon image after sputtering into the thin section to a focal plane similar to that illustrated in (A). Nitrogen and sulfur maps (not shown) were similar to the carbon map. Arrows show corresponding cells in (A) and (B) and in Fig. 2. ^{12}C, carbon. Color scheme is red for carbon. The more intense the color, the stronger the response, with white being the strongest response in all cases.

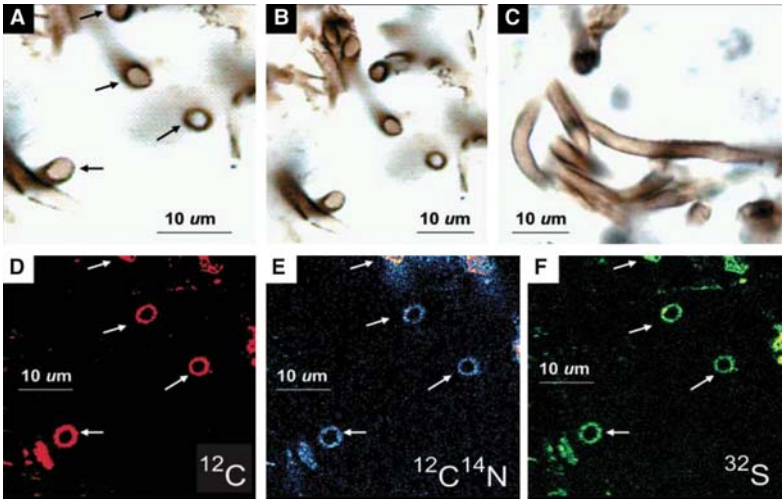

Figure 4. Filamentous microfossils in a polished thin section of chert from the Bitter Springs Formation. (A–C) Optical photomicrographs in transmitted light; (B) and (C) are at lower magnifications to illustrate the tube-like morphology and intertwined habit of these fossils. These filaments are part of the mass of filaments illustrated in Fig. 1A–C but at a different focal plane and rotated slightly so that the optical and NanoSIMS images can be compared. (C) is about 20 µm below the focal plane of (A) (but in the exact same locality of the thin section), and a focal series of 11 photomicrographs, each taken successively 1–3 µm lower in the section, demonstrates that these filaments form an entangled mass throughout the entire 20 µm that was imaged. (D–F) NanoSIMS element maps. Arrows show corresponding cells in the different panels. ^{12}C, carbon; $^{12}C^{14}N$, nitrogen measured as CN^- ion; ^{32}S, sulfur. Color scheme is red for carbon, blue for nitrogen, and green for sulfur. The more intense the color, the stronger the response, with white being the strongest response in all cases.

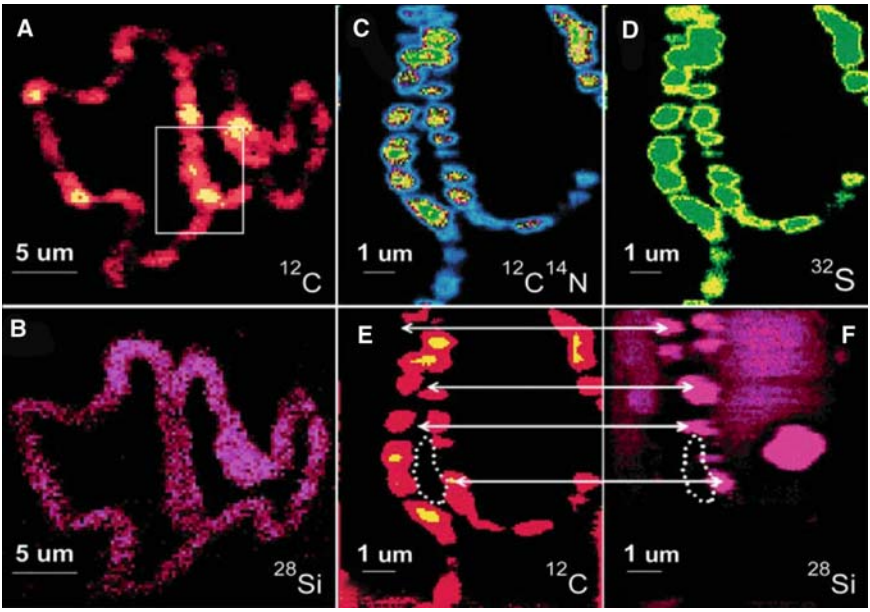

Figure 5. NanoSIMS images of a wall contact between two spheroidal microfossils in chert from the Bitter Springs Formation. (A and B) Relatively low magnification element maps. (C–F) High-resolution element maps. White rectangle in (A) shows area of high-resolution images in (C–F). Arrows in (E and F) tie locations of the silicon globules in (F) with corresponding locations on the carbon map in (E). In (F), the diffuse area of Si response in the central portions of the spheroids is likely due to silica in the host chert. Dotted white ovals in (E and F) are reference areas to tie the two images for comparison. ^{12}C, carbon; $^{12}C^{14}N$, nitrogen measured as CN^- ion; ^{32}S, sulfur; ^{28}Si, silicon. Color scheme is red for carbon, blue for nitrogen, green for sulfur, and pink for silicon. The more intense the color, the stronger the response, with white being the strongest response in all cases.

deeper in the section. A comparison of the optical image at a similar focal plane with the C^-, CN^-, and S^- maps at that lower plane in the NanoSIMS also demonstrates a three-dimensional correspondence between results of NanoSIMS and optical microscopy (Fig. 3). Importantly, the host chert matrix is essentially lacking in significant C^-, CN^-, and S^-, and these ions are present only in structures identified as microfossils using optical microscopy (Fig. 2).

Ultra-high-resolution images (obtained with the smallest possible primary beam spot, around 50 nm in diameter, which was rastered over a small area, typically $10 \times 10 \mu m^2$, in order to collect sufficient secondary ions from each pixel) show that the C^-, CN^-, and S^- distributions are identical to one another for both the spheroidal and the filamentous microfossils (Figs. 5 and 6). The spheroidal microfossils are defined by wall-like structures that consist of distinct globules enriched in C^-, CN^-, and S^- (Fig. 5). In contrast, the filamentous microfossils appear to consist of more diffuse, irregular, and "less packaged" material enriched in C^-, CN^-, and S^- (Fig. 6). These observations are likely to reflect differences in the structures of the biological precursors of the two types of microfossils: the spheroidal microfossils comprising

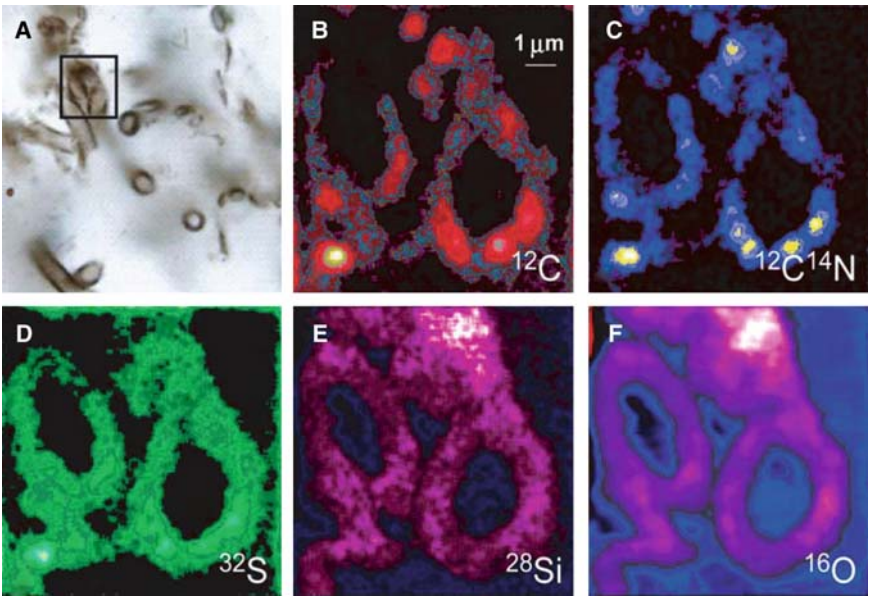

Figure 6. Filamentous microfossils in a polished thin section of chert from the Bitter Springs Formation. (A) Optical photomicrograph in transmitted light. (B–F) High-resolution NanoSIMS element maps. Black rectangle in (A) shows area of detail in (B–F). Scale in (B) applies to (B–F). ^{12}C, carbon; $^{12}C^{14}N$, nitrogen measured as CN^- ion; ^{32}S, sulfur; ^{28}Si, silicon; ^{16}O, oxygen. Color scheme is red for carbon, blue for nitrogen, green for sulfur, pink for silicon, and lavender for oxygen. The more intense the color, the stronger the response, with white being the strongest response in all cases.

remnants of actual cell walls and the filamentous forms probably representing remnants of extracellular mucilaginous sheaths common to filamentous cyanobacteria.

The Si^- and O^- maps also reflect the morphology of the microfossils (Fig. 2), even though some Si^- and O^- yields are detected from the host chert (SiO_2) as well (Fig. 5F). However, in the higher-resolution images of Figs. 5 and 6, the Si^- and O^- yields display differences in detail from the distributions of C^-, CN^-, and S^-. In the spheroids, the Si^- distribution shows a more open texture than is apparent in the C^- map (*cf*. Fig. 5A and B), and in the highest resolution (Fig. 5E and F), the globules of Si^- alternate spatially with globules of C^-. In the filaments, Si^- and O^- distributions appear to be thicker and more continuous than the simultaneously collected C^-, CN^-, or S^- ions (*cf*. Fig. 6B–D with Fig. 6E and F).

The NanoSIMS elemental maps of the organic laminae exhibit relationships among C^-, CN^-, S^-, Si^-, and O^- similar to those observed in the spheroids and filaments, and the images show densely packed structures reminiscent of the filamentous microfossils and collapsed spheroids (Fig. 7).

CN^-/C^- ratios of the spheroids, filaments, and laminae were measured in multiple localities on the NanoSIMS maps. Results show major differences in both absolute values and ranges (Table 1).

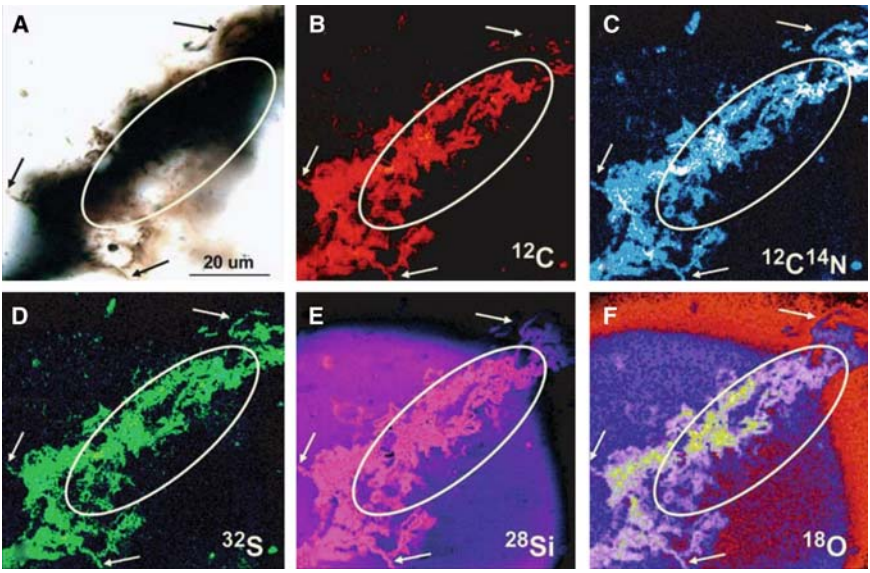

Figure 7. Organic lamina in a polished thin section of chert from the Bitter Springs Formation. (A) Optical photomicrograph in transmitted light; the location of this lamina and its spatial relationship to other structures discussed is illustrated in Fig. 1A and D. (B–F) NanoSIMS element maps of the area in (A). Arrows show reference points for comparison. The white ovals show the same region in (A–F). Scale in (A) applies to all. Sputtered areas are particularly evident in (E and F) as the large circular regions that extend well beyond the lamina. ^{12}C, carbon; $^{12}C^{14}N$, nitrogen measured as CN^- ion; ^{32}S, sulfur; ^{28}Si, silicon; ^{18}O, oxygen. Color scheme is red for carbon, blue for nitrogen, green for sulfur, pink for silicon, and lavender for oxygen. The more intense the color, the stronger the response, with white being the strongest response in all cases.

Table 1. Nitrogen to Carbon Ratios.

Sample	**Measured CN^-/C^-**		**N/C (atomic)**	
Kerogen standard	**0.414 ± 0.083**	**0.025**		
	Minimum	**Maximum**	**Minimum**	**Maximum**
Filaments	0.02 ± 0.008	0.04 ± 0.016	0.0012 ± 0.00048	0.0024 ± 0.00097
Spheroids	0.12 ± 0.047	0.22 ± 0.086	0.0073 ± 0.00284	0.0133 ± 0.00519
Laminae	0.12 ± 0.047	1.00 ± 0.039	0.0073 ± 0.00284	0.0604 ± 0.00236

4. Discussion

For spheroidal and filamentous microfossils, the NanoSIMS C^- and S^- distributions are virtually identical to each other and to the CN^- distributions, and a one-to-one correspondence exists with optical microscopic images (Figs. 2–6).

This suggests that all three elements (C, N, and S) are primarily remnants of biogenic organic matter. The size, shape, texture, and nature of the boundaries of the nanoscale remnants of C, N, and S of the filaments and spheroids constitute biosignatures for sedimentary remnants of these Proterozoic microorganisms.

Nitrogen is a good indicator of organic material because it is common in organic matter but rare in rock-forming minerals. Because nitrogen in rocks commonly derives from biological fixation processes, it can be considered an indicator of biological activity when associated with organic remains. While some chemical reactions might produce abiotic organics with nitrogen under certain hydrothermal or extraterrestrial conditions (Brearley, 2003; Ueno et al., 2004; Remusat et al., 2005), nitrogen associated with amorphous carbon in sedimentary rocks is most likely to be an indicator of biogenicity.

The S^- probably represents a mixture of cellular sulfur with sulfur incorporated during early diagenesis by the common process of sulfurization (Kohnen et al., 1989; Eglinton et al., 1993; Werne et al., 2000; Brocks and Summons, 2003). While not completely understood, the process of sulfurization is thought to incorporate sulfur derived from pre-existing microbial remains or biologically reduced sulfur and reactive, inorganic sulfur species [e.g., from sulfate-reducing bacteria (Werne, 2002)]. Given the low metamorphic grade of the Bitter Springs Formation (Schopf et al., 2005b), the added sulfur is unlikely to have been derived from thermochemically produced H_2S or volcanic sources. Therefore, the sulfur, though partially secondary, is nevertheless likely to be an indicator of microbial activity.

The general correspondence of relatively high Si^- and O^- yields with the microfossils was a surprise (Figs. 2E and F, 6E and F, and 7E and F). This is partly a reflection of the contrast that was used to illustrate those images. In Fig. 5F, for example, a background response for Si^- and O^- from chert can be seen within the cells imaged. The enhanced yields of Si^- and O^- associated mainly with the organic matter may be ascribed to (1) a matrix effect, in which secondary Si^- and O^- yields are greater in organic-rich regions compared with areas of "pure" chert lacking organic material and/or (2) the silicification process, whereby silica has nucleated on organic surfaces during permineralization (Oehler and Schopf, 1971; Oehler, 1976; Benning et al., 2002; Phoenix et al., 2000, 2002; Toporski et al., 2002; Yee et al., 2003), and as a result, the Si^- and O^- concentrations are actually greater in the region of organic matter compared with the concentrations imaged from the chert. Both processes may be at work, and follow-up studies will be aimed at investigating this further.

N/C ratios determined for the different structures range over two orders of magnitude, and such large variations are likely to be significant (Table 1), though we caution that the N/C results are preliminary. There are two types of instrumental fractionation (referred to as "matrix effects") that might affect measured CN^-/C^- and, therefore, the calibrated N/C ratios. These can occur because (1) the kerogen that makes up the microfossils and organic laminae is measured in its matrix of silica, while the standard was measured in purified acid extracts, and (2) the

standard is an Eocene type I kerogen from the Green River Shale (i.e., rich in aliphatic chains), whereas kerogen in the nearly 1 billion year old Bitter Springs chert is likely to be much more aromatic. However, such matrix effects typically only result in differences of a few percent, and so it is highly unlikely that such effects could account for the two-orders-of-magnitude variation among the measured CN^-/C^- ratios of the different Bitter Springs structures. Thus, the large disparity in CN^-/C^- ratios of the Bitter Springs spheroids, filaments, and laminae is likely to be real.

In addition, the 2σ error calculated for the CN^-/C^- of the kerogen standard includes the statistical ion counting and the reproducibility determined by measuring four different locations on the standard; that is $CN^-/C^- = 0.414 \pm 0.083$ (2σ). The 2σ errors reported for CN^-/C^- measured in multiple locations in each type of Bitter Springs structure would include similar effects (Table 1). In summary, while the statistical error on the standard can be used for precise comparisons with statistical errors determined for measured CN^-/C^- of Bitter Springs structures, the N/C atomic ratios determined for the structures should be regarded as semi-quantitative estimates.

The CN^-/C^- ratios of the filaments were found to be significantly lower (0.02–0.04) than the ratios of the spheroids (0.12–0.22). This difference is unlikely to be attributable to subtle diagenetic differences (since all structures are from the same thin section), and there is no evidence of hydrothermal activity or meteoritic contribution, which could account for abiotic formation of organic compounds. Thus, the large disparity in the CN^-/C^- ratios would seem likely to reflect original differences in the biological precursor materials. This interpretation is consistent with the preservation of an exopolysaccharide precursor for the sheath-like material, which would have surrounded the living filaments, and a peptidoglycan precursor (with much higher expected nitrogen content) for the material that would have been contained in the outer cell walls of the spheroids.

Similarly, a disparity in the original chemistry of the two types of microfossils may explain the apparently thicker and more continuous pattern of silicification in the filaments compared to that in the spheroids (*cf*. Figs. 6E and F and 5A, B, and F). If the filamentous forms are remnants of mucilaginous sheaths, then their originally exopolysaccharide chemistry may have promoted more extensive silicification (by a combination of permeation and encrustation) than occurred on the peptidoglycan of the walls of the spheroids. This possibility is suggested by artificial permineralization studies in which laboratory-fossilized microbial filaments with sheaths were both encrusted and permeated by silica (Oehler, 1976). It is supported further by recent studies showing that active permineralization favors exopolysaccharides of cyanobacterial sheaths (Phoenix et al., 2000, 2002; Yee et al., 2003).

Surprisingly, the organic laminae contain filamentous and apparently compressed spheroidal structures that are defined by strong enrichments in C^-, CN^-, and S^- (Fig. 7). These microfossil-like structures in the organic laminae are also characterized by sizes and thicknesses reminiscent of the well-preserved

microfossils in the mineral matrix of the rocks (*cf*. Figs. 2, 4, and 7). Thus, the laminae are interpreted as most likely representing remnants of densely packed microbial mats. This conclusion is consistent with the generally accepted view that such laminae are derived from biological precursors that are simply less well preserved than the optically recognizable filaments and spheroids. Since obvious microfossils were not apparent within the laminae, using optical microscopy or scanning electron microscopy (Figs. 1 and 8), this result also demonstrated the potential of NanoSIMS to reveal new structure in kerogenous organic materials that were presumed to be generally amorphous.

CN^-/C^- and N/C ratios for the laminae displayed higher absolute values and a much greater range than equivalent values from the individual spheroids and filaments (Table 1). The higher absolute values may reflect greater degradation in the laminae, which would result in increased CN^-/C^- values through oxidation of organic carbon and/or addition of nitrogen by microbial nitrification; such degradation also could account for the relatively poor state of preservation in the laminae, as noted above. The large range in CN^-/C^- values could also result from laminae that contain a mixture of microbial constituents; such a mixture might be composed of compressed filaments and spheroids that originally were similar in size and shape to the well-preserved microfossils and other microbial constituents of the ecosystem (e.g., Des Marais, 2003) or possible remnants of biofilms.

Modern bacteria have N/C ratios that range from 0.15 to 0.28 (Fagerbakke et al., 1996; Fukuda et al., 1998), values that are much higher than those from either the individual microfossils or the laminae (overall range of 0.0012–0.0604; Table 1). However, the N/C ratios from the laminae (0.0073–0.0604) overlap with

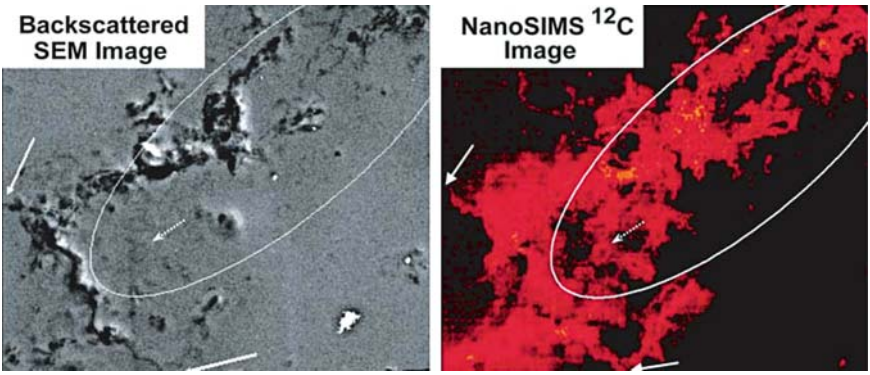

Figure 8. Scanning electron microscopy (SEM) (left) and NanoSIMS (right) comparison of organic lamina in a polished thin section of chert from the Bitter Springs Formation. The white ovals show the same area in each image and these ovals correspond to the ovals in Fig. 7; arrows show corresponding structures. The dashed arrows illustrate a structure suggestive of a cross section of a filament; in NanoSIMS, the structure is defined by C, CN, S, Si, and O enrichment (only C enrichment is illustrated here; see Fig. 7 for other element maps); in the backscattered SEM image, a faint hint of the same structure is seen.

the range of values reported in bulk kerogen samples from a variety of Precambrian cherts [0.0015–0.03 (Beaumont and Robert, 1999)]. The larger ranges of N/C ratios of the bulk kerogens and Bitter Springs laminae (compared to that reported for modern bacteria) likely represent a combination of (1) mixtures of precursor organisms, (2) early diagenetic changes that altered the original N/C ratios (for example, Gillaizeau et al., 1997; Bennett and Love, 2000), and (3) microbial degradation. Indeed, a large range in N/C ratios, such as we have observed in the organic laminae, may be a characteristic and, thus, a biosignature of a degraded biological community.

5. NanoSIMS of Organic Materials in Extraterrestrial Samples

The sub-micron scale resolution of NanoSIMS is ideally suited to studying elemental and isotopic composition of organic material that is found in some extraterrestrial materials, and the technique is now being applied to gain information about the formation and occurrence of organic compounds in the solar system. Particularly interesting recent studies include (1) that by Nakamura-Messenger et al. (2006) on the Tagish Lake carbonaceous chondrite meteorite which found organic globules that might represent types of prebiotic carbon compounds that might have been delivered to a young Earth; (2) those of Floss and Stadermann (2005) and Floss et al. (2004) on organics in interplanetary dust particles; (3) recently-published first results on particles collected from Comet Wild 2 in the Stardust Mission (Sandford et al., 2006); and (4) ongoing work on organics in the Nakhla Martian meteorite (Gibson et al., 2006; McKay et al., 2006). Eventually, NanoSIMS-derived chemical composition will be compared for extraterrestrial organic materials and ancient terrestrial organic residues; these comparisons should provide new insight into the sources of organic materials on earth, their relationship to the evolution of life on earth, and the potential for development of life elsewhere in the solar system.

6. Summary and Conclusions

The results of the NanoSIMS study described have demonstrated that *in situ* elemental composition of Proterozoic microfossils can be mapped and quantified with NanoSIMS at a spatial resolution of about 50 nm. The spatial correspondence of C, N, and S, along with the N/C ratios, provides new biosignatures for specific Proterozoic microorganisms and remnants of microbial communities. Moreover, N/C ratios as well as the distinctive patterns of silicification in the filaments and spheroids are suggestive of original differences in their chemical make-up (i.e., an exopolysaccharide precursor for the sheath-like filamentous forms vs. a peptidoglycan precursor for the walls of the spheroids). Finally, NanoSIMS

images of organic laminae previously thought to be amorphous reveal structures suggestive of densely packed remnants of microorganisms. These results are particularly notable, as the preponderance of organic matter in sedimentary rocks of any age occurs as similarly "amorphous," fragmentary remains, even in deposits with coexisting, *bona fide* microfossils (see Fig. 1, as an example). Therefore, it is possible that NanoSIMS will provide fresh insight into a large body of previously uninterpretable material.

Nevertheless, these first chemical maps of fossil cells are just a beginning. There are two additional types of chemical analyses possible with NanoSIMS that could provide significant new information about the nature of the organisms that produced these fossils: Namely, sub-micron scale analyses of (1) stable isotope ratios of H, C, N, and S and (2) elemental compositions of other potential indicators of biologic activity, such as Mg, Fe, and P. Both could provide insight into the metabolic pathways utilized by the ancient organisms that are fossilized in Precambrian-aged sediments.

Future work will aim at characterization of microfossils and organic fragments in Precambrian sedimentary rocks of varying ages, depositional environments, and lithologies. Key to selection of structures for this characterization will be their undisputed biogenicity, so that results can be used as a guide to interpreting less well-preserved, problematic materials. *In situ* stable isotope compositions from NanoSIMS will be performed and are expected to provide additional criteria for distinguishing biologically produced organic matter from that produced by abiotic mechanisms [e.g., $\delta^{15}N$ values of Precambrian kerogens generally are distinct from $\delta^{15}N$ of primitive organics in interplanetary dust particles and carbonaceous chondrites (Beaumont and Robert, 1999; Floss and Stadermann, 2005; Remusat et al., 2005)]. And finally, elemental analyses of the other potential metabolic indicators, such as Mg, Fe, and P, will be performed.

Thus, the new elemental and isotopic data obtainable with NanoSIMS will add significantly to the repository of criteria that can be used for assessing biogenicity and understanding the origin and significance of poorly preserved organic residues in some of the Earth's oldest rocks. In addition, establishment of nano-scale element and isotope ratios of early forms of life on earth and comparison with equivalent data from organic matter found in carbonaceous chondrites, Martian meteorites, cometary materials, and interplanetary dust particles, will provide new insight regarding the interplay of extraterrestrial organic compounds, the origin and early evolution of life on earth, and the potential for development of living systems on planetary bodies beyond earth.

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DISENTANGLING THE MICROBIAL FOSSIL RECORD IN THE BARBERTON GREENSTONE BELT: A CAUTIONARY TALE

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Abstract Morphological remains of microbes are one of several lines of evidence for the presence and nature of life on Earth and elsewhere. It is therefore critical to establish the timing of microbial influences on the rock record. This paper describes two examples of post-lithification colonization of rock surfaces by microbes that might confuse an authentic Archean biogenic signal in samples from the Barberton Greenstone Belt.

1. Introduction

The timing and environmental setting of early life on Earth is important for understanding past and present ecosystems on our planet as well as for exploring the possibility of life on other planets. The record of this early life, therefore, must hold up to the most rigorous scrutiny. One line of evidence for biological activity on the early Earth comes from microfossils preserved in sedimentary rocks from the eastern Pilbara block of Western Australia and the Barberton Greenstone Belt of South Africa that are up to 3,500 million years old (Altermann and Kazmierczak, 2003; Awramik et al., 1983; Rasmussen, 2000; Schopf, 1993; Schopf et al., 2002, 2007a, b; Walsh, 1992; Walsh and Lowe, 1985; Westall et al., 2001, 2006a, b). Recent studies have cast doubt on the genesis of some of the Australian fossils, however, with the contention that the structures formed abiologically in hydrothermal veins (Brasier et al., 2002) and other studies suggest that a variety of post-Archean microbial processes have affected the fossiliferous rocks. As new chemical techniques for detecting biomarkers emerge (Summons et al., 1999) it is essential that care be taken to discriminate between chemical and physical signals produced by syndepositional microbial activity and those resulting from later biological influences.

This paper describes two cases of post-Archean microbial growth on and within rocks from the Barberton Greenstone Belt. The first case reveals evidence of fungal colonization of internal rock surfaces and the second indications of

bacterial precipitation of post-greenstone belt (probably) modern-redeposition of Archean iron and manganese oxides.

2. Geologic Setting

The Barberton Greenstone Belt, located in the eastern part of the Kaapvaal Craton, South Africa contains rocks that are remarkably well preserved, often displaying only low greenschist facies metamorphism and little or no strain or recrystallization (Xie et al., 1997). It has been the focus of many studies of the early Earth because many primary fabrics and textures are preserved down to submicron scales. The Onverwacht Group is a predominantly volcanic succession, but contains important sedimentary deposits of both clastics and precipitates (Lowe and Byerly, 1999, 2007b). The two formations in the Onverwacht Group that have been the focus of this study are the Hooggenoeg and Kromberg Formations. The Hooggenoeg Formation is about 3,000 m thick and includes lavas that have been dated at 3,470 Ma (Byerly et al., 2002). The Kromberg Formation is a 1,700 m thick sequence of komatiites and basalts with minor interbedded sediments. The base of the unit is dated at 3,416 Ma and the top at 3,310 Ma (Byerly et al., 1996). The sediments of the Hooggenoeg and Kromberg Formations were deposited either in shallow-water and subaerial environments, where they tend to be associated with komatiitic or dacitic volcanic units, or under quiet, slightly deeper-water conditions associated with basaltic volcanism (Lowe and Byerly, 1999, 2007b). In their long history, the rocks of the Barberton Greenstone Belt may have been subject to subaerial exposure, weathering and microbial colonization in a variety of climates several times since their formation over 3 billion years ago (Beukes, 1999; Van Niekerk et al., 1999). The rocks are presently exposed in the temperate climate of southern Africa.

3. Preservation of Archean Microbial Signatures in the Barberton Greenstone Belt

Layers of cohesive fine laminations of carbonaceous matter, mainly found in black cherts and in banded black and white cherts represent the remains of layers of microbial mats interspersed with a chemical precipitate that is now very fine-grained silica (Walsh and Lowe, 1999) or silicified volcaniclastic sediments (Westall et al., 2008). Composite grains of clotted organic matter cemented with silica are commonly associated with the laminations. These pellets appear to have acted as loosely bound particles probably reflecting the influence of microbially-produced exopolymers. Within the laminated layers, including ones associated with evaporate deposits, are preserved rare fossil bacteria (Walsh, 1992; Walsh and Lowe, 1985; Westall et al., 2001, 2006a, b). Filamentous microfossils have

been reported by Walsh and Lowe (1985) and (Westall et al., 2006a, b). One occurrence is in an approximately 60-cm-thick black and white banded chert overlying silicified volcaniclastic sands in the upper Hooggenoeg Formation (Walsh and Lowe, 1985). The chert containing filamentous fossils is made up mainly of fine kerogenous laminations interlayered with thin accumulations of detrital kerogenous grains (Fig. 1). Pyrite grains 5–25 µm in size are scattered throughout the thin section. Solid threadlike filaments composed of kerogen and fine pyrite grains

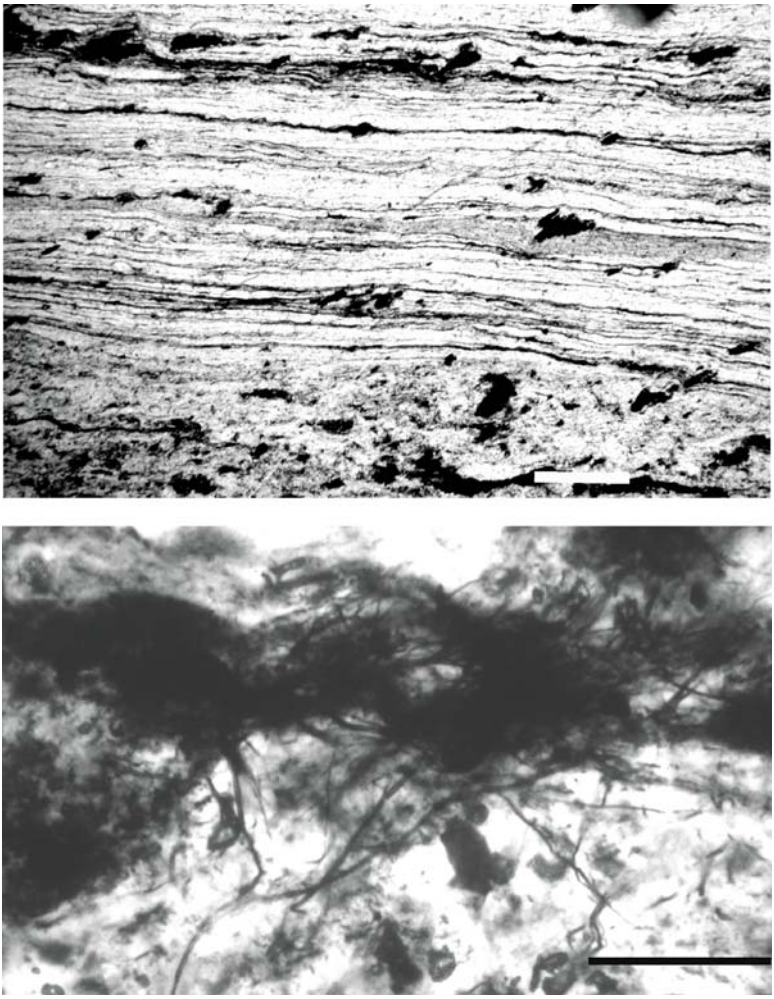

Figure 1. Microbial laminations preserved in carbonaceous chert. Top: Laminations in carbonaceous chert sample, Hooggenoeg Formation. Scale bar equals 200 µm. Bottom: Filamentous microfossils in carbonaceous chert, Kromberg Formation. Scale bar equals 20 µm.

occur mainly in clusters within both the fine laminae and the detrital layers. The filaments have cross-sectional diameters ranging from less than 0.2–2.5 μm and lengths up to 200 μm. A more complex community of filamentous microbes is preserved in a sample from the Kromberg Formation. The microfossils are preserved within layers of fine carbonaceous laminae that contain carbonaceous and lithic intraclasts. Hollow cylindrical filaments range from 1.4 to 1.2 μm in diameter and 10–150 μm in length and solid threadlike filaments range from less than 0.2 to 2.5 μm and lengths up to 200 μm. Most are non-septate, with a few exhibiting slight constrictions at intervals of approximately 1 μm, or breakage at intervals of several micrometers. The walls of the filaments are composed of carbonaceous matter and fine pyrite grains.

The filaments are commonly oriented subparallel to bedding, but in some cases extend downward between layers, or radiate from a tangle of filaments (Fig. 1). The filamentous microbes may represent only a portion of the original community, but seem to have had a role in the construction of the mats. Morphologically the preserved microbes are similar to both filamentous cyanobacteria and to filamentous sulfur bacteria, but their size range is closer to that of filamentous bacteria.

Yet another example of filamentous microorganisms occurs in a superbly preserved microbial mat from a biolaminite in the uppermost part of the Kromberg Formation (Westall et al., 2006a, b). In this case the microbial mat was formed in a littoral, evaporitic environment by microorganisms having diameters in the range of 0.25–0.3 ∝m and lengths up to tens of micrometers. The streamlined microorganisms are embedded in copious quantities of exopolymeric substances (EPS). Interlayered within the filamentous strata are very thin horizons of a suite of evaporite minerals that include aragonite, gypsum, Mg calcite and a halide (probably carrobite). Together with desiccation textures, the evaporite layers indicate periodic exposure of the mat. FIB-cut sections through the microbial mat show that it was coated by a thin layer (10–20 nm) of silica that perfectly preserved the filament and evaporite mineral morphologies on top of the mat. Beneath the surface, however, the mat is characterized by partly mineralized kerogen presenting an alveolar texture, similar to that found in modern photosynthetic microbial mats (Défarge et al., 1996) and representing the degraded remains of the microbial biofilm.

4. Fungal Colonization of Rock Surfaces

Scanning electron microscopy (SEM) was used to examine carbonaceous chert samples with filamentous fossils that had been detected by light microscopy. Rock sample chips were washed in distilled water and placed in an ultrasonic bath to eliminate superficial dust and rock particles. Some were etched in the fumes of HF acid for 1–3 h (for possible *in situ* microfossil investigation), then thoroughly rinsed with distilled water. When dry the etched and non-etched samples were coated with either Au or Pt. The chips were studied using a JEOL JSM-840A. One sample examined is the fossiliferous Hooggeneog sample described in the

preceding section. Some of the same filaments observed with light microscopy were detected by SEM. They appear to be kerogen-lined tubes completely encased by silica. Also observed on two contiguous fracture surfaces of this sample were abundant wide, septate filaments. Several protuberances at regularly spaced intervals along the filaments are similar to clamp connections in fungi of the Phylum Basidiomycota (Fig. 2). In addition, possible fungal spores with spiny exterior walls are present on the fracture surfaces. The fungal structures appear in some

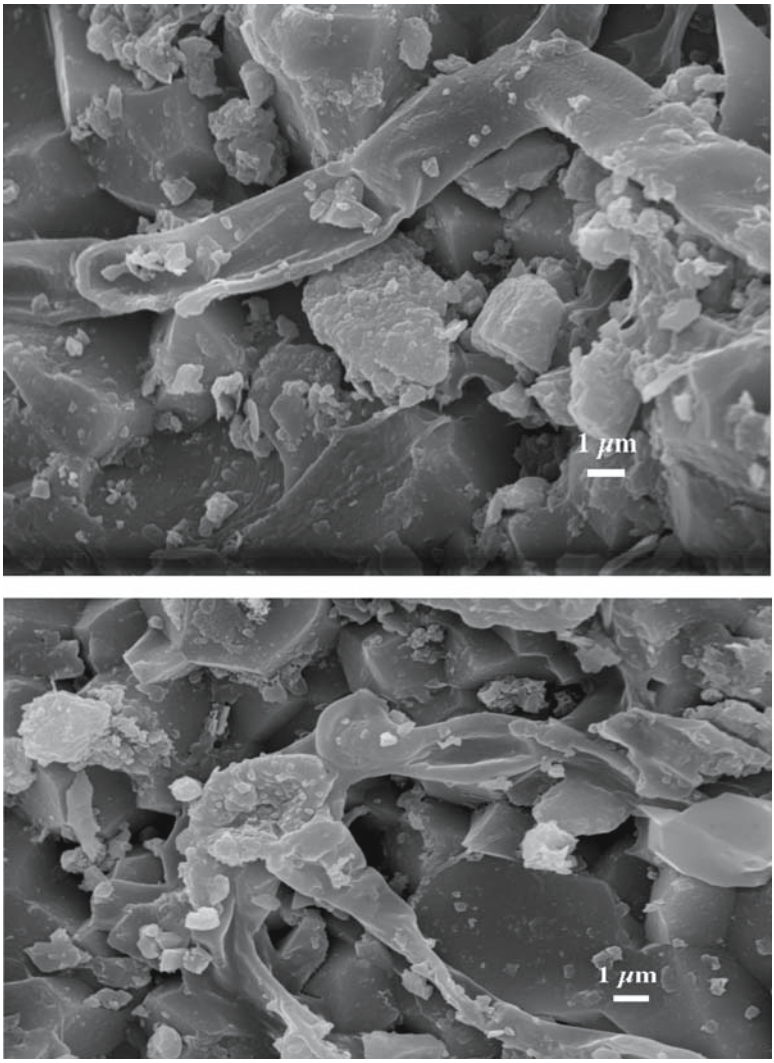

Figure 2. Fungus colonizing sample from the Barberton Greenstone Belt. Top: Collapsed segmented filament. Bottom: Clamp connections typical of the Phylum Basidiomycota. Scale bars equal 1 μm.

cases to be encased within the silica matrix, suggesting that they have been fossilized. It appears that two generations of filamentous structures are present in one sample: (1) Archean fossil bacteria and (2) younger fungi. Fungi are not present in the fossil record until the late Proterozoic, and clamp connections are not known before the Permian, approximately 290 Ma (Alexopoulos et al., 1996; Blackwell, 2000). The fungi clearly represent a younger episode of secondary contamination associated with the rocks containing primary Early Archean fossils. Since the Barberton Greenstone Belt has most likely experienced several episodes of subaerial exposure, including during the late Carboniferous to early Permian, the timing of the colonization of the fracture surfaces cannot be determined with any certainty. A similar situation in which younger silicified endolithic cyanobacteria and fungal hyphae and spores occur in fractures and crevasses around grain boundaries in very ancient sediments from Greenland (Isua, 3.8 Ga) was described by Westall and Folk (2003).

The presence of fungal contamination on interior surfaces of apparently unfractured samples reinforces the need for a combined use of light microscopy, valuable in determining the spatial relationships of fossil-like structures to sedimentary and petrologic features, and SEM, which allows examination of much finer structural features as well as chemical analysis, in the study of fossil bacteria. The SEM provides topographic information, but only surface features are visible. Transmitted light microscopy, which provides a view into the 40–60 μm depth of a thin section and gives a three-dimensional picture of the relationship of fossils or possible fossils with the surrounding rock matrix, yields valuable information about the context of the fossils and their mode of preservation. Veins and cracks that may be the venue of post-lithification colonization are usually readily apparent in transmitted light, particularly because of the difference in crystal size between matrix and veins.

5. Pleistocence Microbially-Mediated Mineral Precipitation on Barberton Greenstone Belt Rocks

Ironstone deposits that had been interpreted as remains of the world's oldest hydrothermal vents (de Ronde et al., 1994) have recently been reassessed and reinterpreted as much younger deposits that formed as subaerial spring deposits (Lowe and Byerly, 2003, 2007a; Roy et al., 2005). Evidence for Pleistocene ages for the deposits includes slope-parallel bedding and vertical dripstone (Fig. 3).

A microbial influence on the precipitation of the minerals is suggested by the presence in the goethite layers of a variety of structures that resemble filamentous bacteria and/or fungi. The manganese oxide layers do not appear to contain microbe-like structures, but the opacity of these minerals prevents ruling out a microbial presence. Delicate branches of goethite 10–20 μm wide and several hundred microns in length contain at their centers dark filamentous structures or linear arrangements of spherical structures that are 1–3 μm wide. The symmetrical

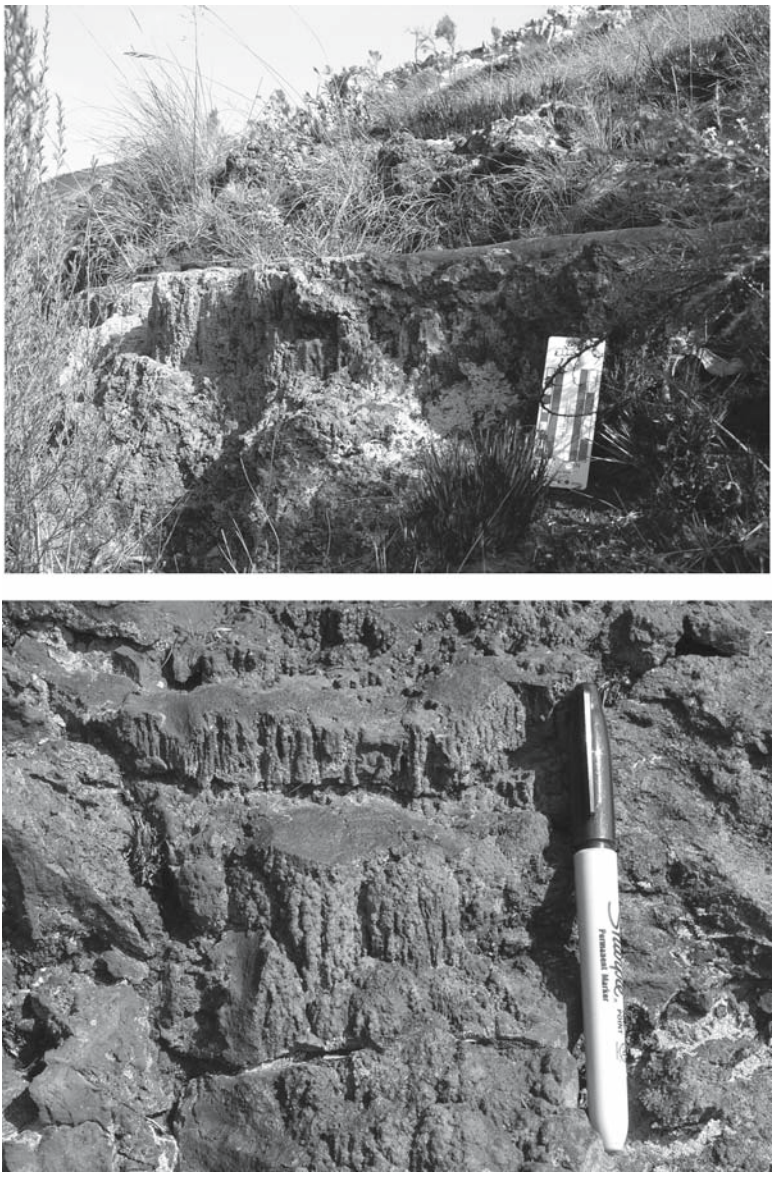

Figure 3. Pleistocene ironstone deposits associated with rocks of the Barberton Greenstone Belt. Top: outcrop showing horizontal terracing. Bottom: Close-up of vertical dripstone in ironstone deposit.

arrangement of goethite around these central structures suggests that iron oxide was precipitated on the cell walls of the microbes. Distinctly tubular structures with well-defined dark (carbonaceous?) boundaries are also found in some botryoidal goethite layers. These structures either form central cores to symmetrical

precipitate layers or cross-cut the laminations (Fig. 4). In some cases they are branching or appear to have originated from other structures (Fig. 4). The branching of these structures indicates that they are most likely the remains of filamentous fungi, and possibly associated bacteria. The tubes range in diameter from 2–10 μm, with lengths ranging from approximately 60–400 μm. Larger tubular structures are commonly associated with these filaments. The larger tubes, 10–25 μm in diameter and up to 300 μm in length, have indistinct, bumpy outlines. The size of the larger structures is greater than is commonly found amongst filamentous bacteria or fungi. The tubular structure and the wavy boundaries suggest that these represent filamentous microbial remains coated with mineral precipitates (primarily goethite, an iron oxyhydroxide, with minor gibbsite, an alumina oxyhydroxide, and todorokite, a manganese (+4 valence) oxyhydroxide). Similar goethite-encrusted filaments have been reported from stalactites in Lechuguilla Cave, New Mexico (Provencio and Polyak, 2001).

Modern weathering processes commonly produce coatings or varnishes on rock surfaces that contain iron and/or manganese (Adams et al., 1993; Perry et al., 2006). Because most geological samples are, of necessity, collected in the modern weathering zone, the possibility of modern mineral deposits always exists. The presence of iron and manganese, in particular, should be evaluated to eliminate the possibility of post-lithification mineral coatings being interpreted as syngenetic with the rocks being studied. It is essential to consider the outcrop context of a sample, including relationship to modern weathering surface, in establishing the timing of mineral formation.

6. Conclusions and Implications for Astrobiology

As new chemical techniques for detecting biomarkers emerge, extreme care must be taken to discriminate between chemical and physical signals produced by syndepositional microbial activity and those resulting from later biological influences. Both large-scale observations of outcrops and light microscopy of petrographic thin sections should be used in conjunction with electron microscope and chemical studies of terrestrial and, when possible, extraterrestrial samples to ensure that microbial remains are contemporaneous with parent rock formation.

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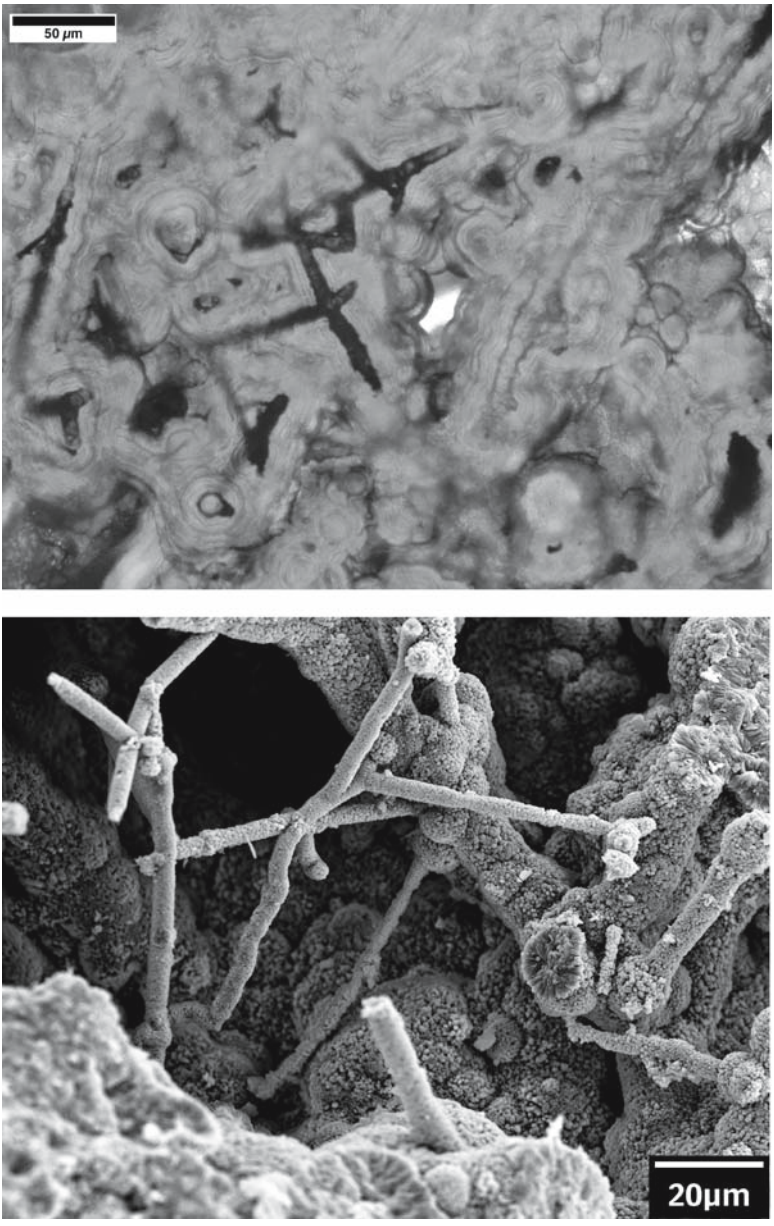

Figure 4. Branching filaments replaced and/or coated by goethite from ironstone deposit associated with rocks in the Barberton Greenstone Belt. Top: Petrographic light photomicrograph showing filaments cross-cutting botryoidal layers of goethite. Scale bar equals 50 µm. Bottom: Scanning electron microscope image of filaments in pore spaces. Scale bar equals 20 µm.

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LOOKING THROUGH WINDOWS ONTO THE EARLIEST HISTORY OF LIFE ON EARTH AND MARS

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Abstract We know that planet Earth is about 4.5 billion years old but what is less clear is when it first became home to life. Locating the first evidence for life on Earth is a question of considerable complexity and controversy. Biogeochemical signals or examples of cellular preservation from the early Archean (greater than ~3 billion years in age) are scarce and vigorously debated. Understanding the relationship between a specific signature in the terrestrial rock record and a specific organism and/or environment is a key issue that astrobiologists must address in order to succeed in any search for life, extinct or extant, on other planets. We here present an overview of putative biogenic signals described from some of Earth's oldest rocks and highlight the most promising areas for future research.

Keywords Abiogenic, Akilia, Archean, Astrobiology/Astrobiologist, Barberton, Biogenic, Carbon, Earth, Greenland, Isotope, Isua, Life, Mars, Microfossil, Microtube, Pilbara, Stromatolite, Sulfur

1. Introduction

The early fossil record here on planet Earth is essential to both designing the search strategy for seeking life elsewhere in our universe and to ratifying the evidence collected in this search. There are two fundamental challenges that face geologists and astrobiologists here. The first involves locating and recognizing preservational windows, that is to say, the types of rocks and fossil evidence needed to uncover the early history of life. The second involves the scientific community reaching a consensus on this evidence for the earliest appearance of life in the rock record. This terrestrial "ground truthing" process as reviewed below should proceed hand-in-hand with the exploration of other planetary bodies.

The search for the earliest life on Earth relies on finding ancient rocks where biosignals may still be preserved – these are our 'windows'. Unfortunately, suitable

minimally deformed rocks are relatively rare compared to the Phanerozoic rock record. Although several Archean greenstone belts have been studied worldwide, only two – the Pilbara of Western Australia and the Barberton Mountain Land in southern Africa contain intact stratigraphic piles of early Archean age. The rocks from these two regions are as old as ~3,500 Ma and neither region is highly metamorphosed. Older rock successions are known, some possibly as old as 3,850 Ma, like those of Greenland and Labrador. But these are of higher metamorphic grade, which makes identification of any putative biogenic signals even more difficult and controversial.

Efforts to find the earliest life in these rocks have been focused on meta-sedimentary rocks that are predominantly chemical in origin, for example cherts and banded-iron-formations and, more recently, on hydrothermal and even volcanic rocks. Detrital meta-sediments, such as sandstones and mudstones that are composed of fragments of older rocks, are less common in the Archean rock record, but may be some of the best preserved windows for investigation, where post-mortem processes which act to destroy biological information may have been minimized.

This chapter aims to provide an overview of putative biogenic structures and chemical traces from the three localities on Earth which preserve meta-sedimentary rocks in excess of ~3 billion years old: the Isua greenstone belt and Akilia Island, Greenland; the Pilbara craton of Western Australia; and Barberton mountain land, South Africa/Swaziland.

Two main questions need to be asked of any such reports. First, are the candidate structures or chemical traces truly ancient? In other words, can it be demonstrated beyond doubt that they are both indigenous and syngenetic to the rock in which they are found? Second, is the morphology of the candidate structures, along with associated chemical traces, indicative of biology? For example, do they show evidence of tiers of metabolic processing, and do they occur in a context that is conducive for life? At the same time, can abiogenic mechanisms be discounted for their formation?

Throughout the following discussion we use these and other criteria to evaluate putative biogenic structures or traces and to test whether they can provide *reliable* evidence for early life on Earth. We aim to present a balanced account of the currently available evidence for early Archean life and to also provide suitable notes of caution for interpreting the earliest candidate biosignatures. (Alternative recent reviews of this topic can be found in Schopf et al., 2007 and Van Kranendonk et al., 2007, the latter focuses more broadly on the early rock record.)

2. Greenland: The Earth's Oldest Supracrustal Rocks

The earliest rock record on Earth is somewhat fragmentary and although minerals have been dated from as far back as 3,910–4,270 Ma, these are single zircons contained within much younger c.3,100 Ma Jack Hills quartzites of Western Australia

(Wilde et al., 2001). We must therefore turn to the Isua Greenstone Belt and Akilia Island in southwest Greenland (Fig. 1) to find the oldest intact supracrustal, i.e. volcanic and sedimentary rocks on our planet. These have a minimum age of ~3,700 Ma in Isua (Moorbath et al., 1973; Nutman et al., 1997a) and could be as old as ~3,850 Ma on Akilia Island (Nutman et al., 1997b; Mojzsis and Harrison, 2000). Unfortunately these rocks have been subjected to intense metamorphism, so any fossilized morphological remains of Earth's earliest biosphere, if any ever existed at that time, have been destroyed by heat and pressure. Instead we must rely on chemical signatures within these rocks that may give clues to the former existence of life. The most widely used chemical signatures are isotopic ratios, in particular carbon isotope fractionations (reported as $\delta^{13}C$ PDB values) that are widely believed to record the signals of ancient metabolic activity and biological processing (see for example Schidlowski, 2001).

There has been much debate and controversy surrounding the value of the Greenland successions to provide the oldest possible window into earliest life (see for example a recent review by Whitehouse and Fedo, 2007). One such controversy centres on the tiny island of Akilia, just off the coast of southwestern Greenland (Figs. 1c–e) and is here used to highlight the importance of understanding the geological context of these ancient rocks. It begins with a report by Mojzsis et al. (1996) of evidence for life in a >3,850 Ma banded quartz pyroxene rock on Akilia. These authors analysed the carbon isotope signatures of graphite inclusions within ~10 μm^3 sized grains of apatite from a small outcrop that had previously been interpreted as a sedimentary banded-iron-formation (BIF) (Nutman et al., 1996). They found a strongly negative mean $\delta^{13}C$ value of −37 +/− 3‰ for this graphitic carbon within the apatite grains. Since organisms prefer to use the light isotope of carbon, Mojzsis et al. went on to state that this "*provides evidence for the emergence of life on Earth by at least 3,800 Myr before present*". The key to this statement relies upon two now widely scrutinized assumptions. First, is their interpretation of this rock unit as a primary sedimentary BIF deposit. Crucially, if the rock is indeed a BIF, then the carbonaceous material could preserve a primary biological signal that was shielded from later metamorphism by being trapped within the apatite grains. Second is their interpretation of negative $\delta^{13}C$ values as an *unambiguous* fingerprint for metabolic activity in the early biosphere.

Challenges to these assumptions are now legion. A second team of geologists (Fedo and Whitehouse, 2002) examined the Akilia site and determined that the outcrop, which is only a few tens of square meters in total area (Figs. 1d–e), is not a meta-sediment. A re-examination of the field relationships of the outcrop showed the banding to comprise discontinuous boudinage tails caused by multiple, intense deformation events. Furthermore, detailed geochemical data (major elements, trace elements and rare earth elements) from this team have pointed to an ultramafic igneous protolith. An igneous protolith for this rock means that the carbon would have little or no biological relevance; Fedo and Whitehouse (2002) therefore advanced two abiogenic scenarios that could also

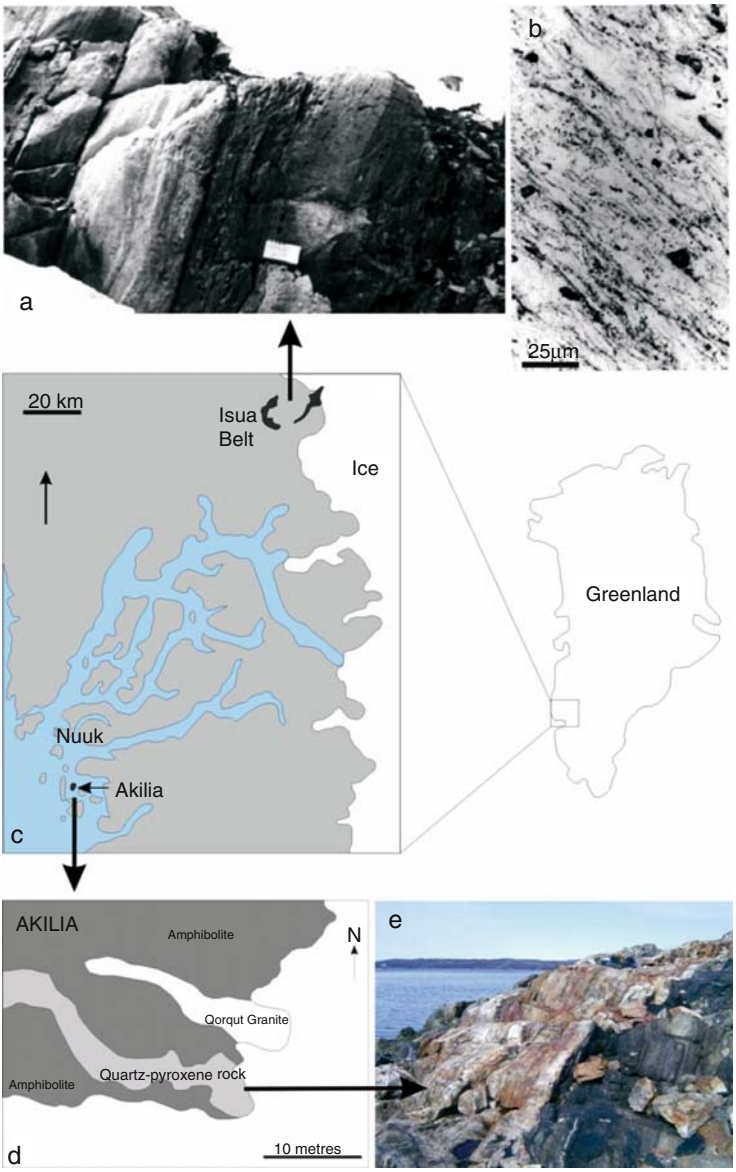

Figure 1. (a) Turbidite sedimentary rocks from the ~3,700 Ma ISB, West Greenland (Rosing, 1999); (b) photomicrograph of carbon grains within (a) (Rosing, 1999); (c) locality map for the Isua and Akilia localities in West Greenland discussed in the text; (d) sketch from aerial photograph of the location of the quartz pyroxene rock (purported BIF) on the southwest peninsula of Akilia Island; (e) field exposure of the purported ~3,850 Ma Akilia Island BIF in contact with surrounding ultramafic rocks. Figure 1b and c are reprinted with permission from Science, Volume 283. Rosing, M. T. ^{13}C Depleted carbon microparticles in >3,700-Ma sea-floor sedimentary rocks from West Greenland, pp. 674–676. Copyright (1999) AAAS.

explain the strongly negative $\delta^{13}C$ signal recorded in this outcrop. One involves the decarbonation of metasomatized ultramafic rock, and the other serpentinization of an olivine-bearing ultramafic protolith followed by metamorphic decarbonation.

The story took a further twist when Dauphas et al. (2004) came out in support of a sedimentary BIF origin for this deposit. They used a new approach involving iron isotopes and found that the Akilia rocks were enriched in the heavy iron isotope when compared to igneous rocks (see also a later paper that again uses Fe isotopes coupled to a greater array of additional geochemical traces, Dauphas et al., 2007). This, they argued, was best interpreted as evidence for the transport, oxidation and precipitation of ferrous iron from hydrothermal vents and thus consistent with these rocks indeed being the oldest water lain sedimentary deposit preserved on Earth. However, this finding was overtaken by yet another twist in this controversy when Lepland et al. (2005) re-examined 17 apatite samples from Akilia, including the sample originally used in the Mojzsis et al. study. These authors and another independent team (Nutman and Friend, 2006) failed to find graphite inclusions in any of the apatite grains raising the possibility that the original findings of Mojzsis et al. (1996) were an artefact.

The final convolution in this discussion to date came when some of the authors of the original studies replied to their critics in a pair of papers (Manning et al., 2006; McKeegan et al., 2007). In the first of these, new mapping, geochronology and geochemistry data are presented which support the originally reported >3,850 Ma age for these rocks, although ambiguity in the cross-cutting relationships due to the complex deformation history of the Akilia outcrops still leaves some doubts over these age constraints in some quarters (e.g. Eiler, 2007). The second paper uses laser Raman micro-spectroscopy to confirm the presence of graphitic inclusions in the apatite grains together with ion microprobe analysis to corroborate the original negative $\delta^{13}C$ measurement. It remains to be explained why two independent teams did not find these inclusions (Lepland et al., 2005; Nutman and Friend, 2006), and perhaps it is the case that these inclusions are much less abundant than was originally contended. Notwithstanding, these two recent studies have renewed hopes in some quarters that the Akilia rocks may after all hold evidence pertinent to the origins of life on Earth. However, considerable ambiguities do remain, especially regarding the exact nature of the protolith and the complex metamorphic and metasomatic history of the Akilia rocks, as explained in a recent review paper by Eiler (2007).

A more promising location to search for ancient biomarkers appears to be a low strain domain in the north east of the Isua greenstone belt where primary igneous and sedimentary fabrics are in part preserved. Researchers have for sometime now argued for a biogenic component to graphitic carbon in this area (e.g. Hayes et al., 1983). In addition to the Akilia Island sample, Mojzsis et al. (1996) also investigated apatite grains at a second locality, in an iron carbonate-rich rock from this low strain area. They again argued that these apatite grains contained carbonaceous inclusions whose isotopic values of $\delta^{13}C = -30 \pm 3‰$

are consistent with a biogenic origin. Once again, however, more recent work has cast doubt upon their claims. Lepland et al. (2002) investigated apatite grains from eight different meta-sedimentary samples and discovered that they were entirely free from graphite inclusions. They did, however, find abundant graphite inclusions in associated meta-carbonate rocks, formed by metasomatic processes; detailed petrography and rare earth element geochemistry led them to re-interpret the original Mojzsis et al. sample as metasomatic in origin. If the graphite in these metacarbonate rocks was formed via the reduction of carbonate ions during thermal decomposition of iron-carbonate as proposed by Van Zuilen et al. (2002, 2003), then it cannot be biogenic.

A third promising claim for life in these Greenland rocks comes from graphite globules in a meta-sedimentary unit within the low strain area of Isua (Figs. 1a–c; Rosing, 1999; Ueno et al., 2002). Trails of graphitic globules within some garnet and biotite metamorphic porphyroblasts suggest a primary origin for this graphite, while a $\delta^{13}C$ value of ~ −19‰ led Rosing (1999) to conclude that the carbon is biogenic in origin. As ever, extreme care must be taken to exclude the possibility of more recent contamination, especially given that modern endolithic coccoids have been found in cracks within BIFs of the Isua greenstone belt (Westall and Folk, 2003), and experiments have indicated post-metamorphic addition of organic matter to some Isua samples (Van Zuilen et al., 2002). The Rosing (1999) turbidite sample, however, was shown by Raman spectroscopy to contain well crystallized graphite that is markedly different from post-metamorphic contamination (Van Zuilen, 2005, Fig. 5b), and thus the original claim still stands.

The debate surrounding the ~3.8 billion year old rocks from Greenland is ongoing and, despite the great challenges associated with the Akilia and Isua areas, these rocks are unique in representing the period in Earth history where geological processes as we know them today may first be recognizable and where conditions for the emergence of life may have become tolerable. The fact that unambiguous signals for life have not been forthcoming, as yet, should not deter further detailed study into this earliest window into potential life on Earth.

3. East Pilbara Granite-Greenstone Terrane

The Pilbara craton of Western Australia (Fig. 2a) is composed of three ancient granite greenstone terranes; East Pilbara, West Pilbara and Kurrana. The oldest rocks are exposed in the East Pilbara where the lowermost group is the Warrawoona Group (Fig. 2b), deposited from 3,515–3,420 Ma. This consists mostly of mafic volcanic rocks of the Double Bar Formation, Table Top Formation, North Star Basalt, Mt Ada Basalt, and Apex Basalt.

These are interspersed with thin chert horizons and felsic volcanics of the 3,515–3,500 Ma Coucal Formation, the 3,472–3,465 Ma Duffer Formation, the ~3,460 Ma 'Apex chert' and the 3,458–3,427 Ma Panorama Formation (Van Kranendonk, 2006). The Kelly Group lies unconformably above these, consisting

of the >3,350 but <3,427 Ma Strelley Pool Chert, the 3,350–3,325 Ma Euro Basalt and the 3,325–3,315 Ma Wyman Formations. This pile, in turn, is unconformably overlain by the ~3,240 Ma Sulphur Springs Group.

These lower three groups of the Pilbara Supergroup house some of the Earth's oldest purported stromatolites, microfossils and microtubes (Fig. 2), as outlined below. (A recent alternative review of these rocks is available in Van Kranendonk, 2007.)

3.1. PILBARA STROMATOLOIDS

Stromatolites are laminated sedimentary structures that provide a controversial candidate signature for early life. In the following discussion, we therefore adopt the non-genetic term 'stromatoloid', thus making no assumptions about their biogenicity (cf. Buick et al., 1981). A stromatoloid can be defined non-genetically as "*…an attached, laminated, lithified sedimentary growth structure, accretionary away from a point or limited surface of initiation*" (Semikhatov et al., 1979), and in outcrop are macroscopically layered and wrinkled surfaces forming domes, cones and columns. Alternative biogenic interpretations which imply microbial mediation (e.g. Krumbein and Werner, 1983) are not favored by us, because they imply an origin which can be very difficult to establish in the early rock record.

In the Pilbara, nodular and wavy-laminated stromatoloids (Fig. 2g) have been described from a chert unit within the Dresser Formation at North Pole (Walter et al., 1980). Coniform stromatoloids have also been described from the Strelley Pool Chert (Lowe, 1980; Hofmann et al., 1999; Allwood et al., 2006, 2007). In both cases their interpretation as biogenic was initially based largely upon simple macro-morphological comparisons with modern day stromatolites, for example those found at Shark Bay in Western Australia.

Stromatoloids from this area have also been used to argue for the presence of oxidative photosynthesis by ~3,500 Ma (e.g., Awramik, 1992; Schopf, 1999) as well as phototrophic behaviour (Hofmann et al., 1999; Allwood et al., 2006). It is important to note, however, that neither convincing microfossils nor wrinkle mat fabrics have ever been found in these stromatoloids. This anomaly is usually excused by the low preservation potential of stromatolitic micro-facies and by the fact that microfossils are only found in an estimated ~1% of occurrences in Phanerozoic stromatolites. This line of argument is somewhat unsafe, however, given that the preservation potential of microbial remains in an Archean world lacking oxygen and supersaturated with respect to silica is expected to be much higher than in the Modern world with a highly oxygenated atmosphere, silica undersaturated seawater, and aggressive regenerative recycling of carbon. This lack of microfossil evidence should not therefore be dismissed lightly.

New nano-scale evidence for microbial processes in the form of aragonite nano-crystals intimately associated with organic carbon globules has recently

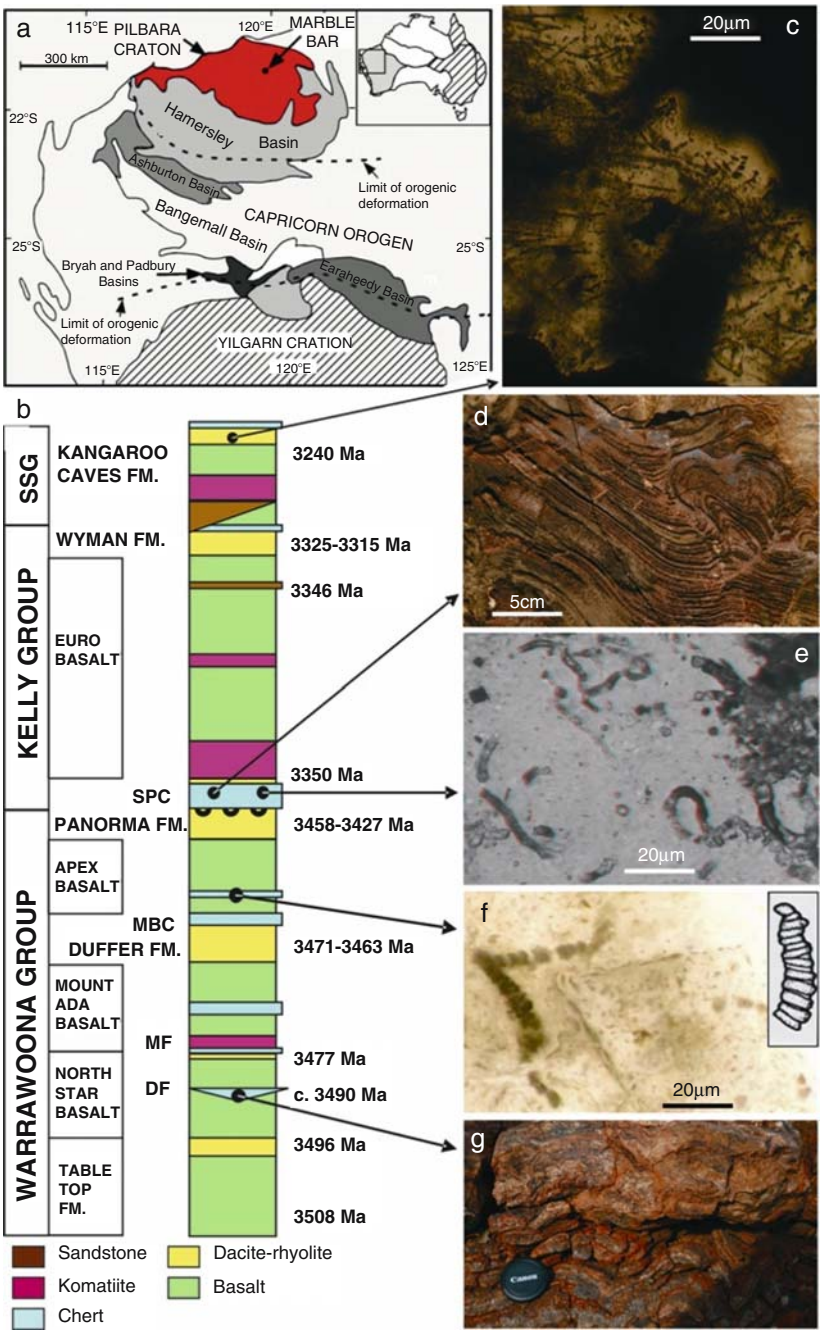

Figure 2. Selected putative biogenic structures from the Pilbara Craton, Western Australia. (a) Location of the Pilbara Craton (from Brasier et al., 2005); (b) stratigraphy of the ~3,500–3,200 Ga Warrawoona, Kelly and Sulphur Springs Groups. SPC = Strelley Pool Chert; MBC = Marble Bar

been found in younger 2.72 Ga stromatolites of the Tumbiana Formation in Western Australia; this has yet to be extensively sort in early Archean stromatoloids (cf. Lepot et al., 2008).

Several authors have re-examined the Dresser Formation stromatoloids; Buick et al. (1981) attempted to define universal stromatolite biogenicity criteria and concluded that these stromatoloids were only "*probable or possible*" biogenic stromatolites. Lowe (1994) re-interpreted the Dresser stromatoloids as produced by soft sediment deformation of originally flat layers. Lowe (1994) also directly questioned his original biogenic interpretation of the Strelley Pool stromatoloids, instead concluding that they formed through evaporitic precipitation. Van Kranendonk (2006) showed that the Dresser Formation stromatoloids occur in the vents of barite dykes and suggested that they may have been constructed by hyperthermophilic microbes. The biogenicity of these stromatoloids is questionable, however, because their macro-morphology appears to be largely controlled by the thickness of precipitated barite crusts and draping chert layers. Their distribution most likely reflects the supply of supersaturated solutions from which they precipitated. Robust micro-textural and isotopic evidence for the involvement of any kinds of microbes in the growth of these stromatoloids is still lacking.

The intriguing Trendall locality within the Strelley Pool Chert exhibits coniform stromatoloids that were first described by Hofmann et al. (1999). These stromatoloids are notable for their diverse range of coniform and rare columnar morphologies, their greater variation in size, plus one example of putative 'branching' (Fig. 2d). A biological origin for these structures has been advanced, based largely upon morphological arguments (Allwood et al., 2006, 2007; Van Kranendonk et al., 2003; Hofmann et al., 1999), but also rare earth element data suggestive of a shallow marine setting (Van Kranendonk et al., 2003). In their recent work Allwood et al. (2006, 2007) present a depositional model that is taken to support a shallow water phototrophic origin for the stromatoloids. Our own field work, undertaken across the whole of the outcrop belt, however, leads us to contest this interpretation. In the West Strelley belt, for example, small unbranched coniform stromatoloids are common and these do not show depth controlled changes in morphology or distribution (Wacey et al., 2008b). We also find a close interrelationship between coniform stromatoloids and crystal fan arrays, upon which they can be seen to nucleate. Further, they intergrade with

Figure 2. (continued) Chert; MF = McPhee Formation; DF = Dresser Formation; (c) automontage image of putative filamentous microfossils from the 3,235 Ma Sulphur Springs volcanogenic massive sulfide deposit; (d) stromatoloids preserved in carbonate from the 'Trendall Locality' within the ~3,400 Ma Strelley Pool Chert; (e) automontage image of microtubular structures from the ~3,400 Ma Strelley Pool Sandstone; (f) putative cyanobacterium-like structure *Archaeoscillatoriopsis disconformis* Holotype from the 3,460 Ma 'Apex chert' (after Schopf 1993) re-imaged by us to show growth as a self organizing structure around a rhombic crystal (inset is an interpretative sketch in the style of Schopf (1993) which omits the lower structure and side branch seen in the main image, adapted from Brasier et al., 2002); (g) stromatoloid preserved in ferruginous carbonate from the ~3,500 Ma Dresser Formation.

linguoid, lunate, sinuous and linear current ripples. We here argue that there was a strong chemical component in their accretion, with growth of carbonate crystals influenced by current velocities. In the absence of supporting micro-textural and biomarker evidence, the biogenicity of all of these stromatoloids remains to be demonstrated.

More generally, we caution that macroscopic self organising structures closely resembling stromatoloids are readily generated by abiogenic processes (see Brasier et al., 2006). These processes include diffusion limited aggregation of synthetic colloids in laboratory experiments (McLoughlin et al., 2008a), computer simulations using the Kardar Paris Zhang equation (Grotzinger and Rothman, 1996) and cellular automata (Wolfram, 2002). Given the absence of compelling microbial mat or microfossil remains in early Archean stromatoloids and the possibility that some of these deposits were formed from colloidal silica gel precursors (cf. McLoughlin et al., 2008a), questions remain as to whether, alone, Archean stromatoloids have anything to unambiguously tell us about microbes or early biology. We tend to agree with Schopf (2006), that unfortunately "*it is perhaps impossible, 'to prove beyond question' that the vast majority of reported stromatolites…are assuredly biogenic*".

3.2. PILBARA MICROFOSSILS

Perhaps the most well known report of microfossil evidence for earliest life comes from the 3,460 Ma 'Apex chert'. These Apex 'microfossils' (e.g., Fig. 2f) were described in detail in a series of high profile papers (Schopf and Packer, 1987; Schopf, 1992a, b, 1993; Schopf et al., 2002) and became the benchmark for *bona fide* early Archean 'microfossils', the only ones to gain wide acceptance by the scientific community during the 1980s and 1990s (e.g., Buick, 1990; Knoll and Walter, 1996; McClendon, 1999; Schopf, 1999). The eleven putative species of microfossils described in those papers are from 700 to 1,000 million years older than putative cyanobacterial biomarkers (Summons et al., 1999), genomic arguments for dating the appearance of cyanobacteria (Hedges et al., 2001) and an oxygenic atmosphere (Catling et al., 2001). Whilst the similarities between the Apex 'microfossils' and other more primitive bacteria were acknowledged, the morphology and size range of the supposed cells has been taken to suggest that oxygen-releasing cyanobacteria may have been present at least 3,460 Ma ago (Schopf, 1992a, b, 1993, 1999). For almost two decades, many of the key issues concerning the evolution of the early Earth hinged on this one discovery. For example, if these 'Apex chert' microfossils are accepted then they not only imply an early start for the contribution of photosynthetic oxygen to the atmosphere, they also imply that high levels of biological diversity were achieved at a very early stage in Earth history (Schopf, 1993), remarkably soon after the end of massive meteoritic bombardment of the inner solar system at ~3,800–3,900 Ma (cf. Kamber et al., 2001). A period of relative evolutionary stasis would then be

required, as there is little evidence for further diversification in the fossil record until the emergence of widespread eukaryotes nearly 2 billion years later (Knoll, 1994, 2003).

Such important claims clearly require watertight evidence. Unfortunately, the veracity of these reports has come into question (Brasier et al., 2002, 2005, 2006; García Ruiz et al., 2003). Brasier et al. (2002, 2005, 2006) re-examined both the geological context and morphology and geochemistry of 'Earth's oldest microfossils' and came to some surprising conclusions. The 'microfossils' are not in a seafloor chert as originally reported, but rather come from a chert breccia that lies some 100 m down a hydrothermal dyke system and well below the palaeo-sea floor. The microfossil-like structures are not confined to early formed clasts. They also occur in recrystallized, late stage hydrothermal fabrics. The 'microfossils' are chaotic and incoherent, not simple and unbranched as previously reported, and the 'stromatolite clasts' are in fact botryoidal cavity infills. In addition, Brasier et al. (2006) found no correlation between inferred 'cell shape', filament diameter and taxon-specific terminal cell morphology. Instead, they found that filament shape, septa and subdivisions could be best explained as self organizing structures resulting from silica re-crystallization of glass to spherulitic chalcedony that caused displacement of amorphous carbonaceous matter towards spherulitic margins. This created a morphological spectrum of arcuate to dendritic microstructures (see Brasier et al., 2006, Fig. 2) that includes microfossil-like artefacts and must therefore lead to the rejection of the biological nature of these putative fossils. Structures that resemble these supposed microfossils have also been produced abiogenically in the laboratory by García Ruiz et al. (2003).

With these cautionary findings in mind, we go on to discover that other claims of putative microfossils from the Pilbara area have also been re-examined, and similar concerns about their biogenicity and antiquity have been expressed. For example, minute spheroids were described from the chert barite unit of the Dresser Formation (Dunlop et al., 1978) whose size distribution and kerogenous composition were used to infer biogenicity. However, both the syngenicity and biogenicity of these structures were questioned and they were re-interpreted as either viscous bitumen droplets in secondary megaquartz and chalcedony laminae (Buick, 1990) or simple mineralic non-biogenic spheroids (Awramik et al., 1983). Tubular filaments were also described from the Dresser Formation (Awramik et al., 1983; Buick, 1984) and were initially interpreted as being biogenic based upon their morphology, carbonaceous composition and orientation within the rock, distinguishing them from more angular, crystalline micro-pseudo-fossils found in the same rock unit. On close examination they are remarkably similar to the 'Apex chert microfossils'. This means that an origin as self organizing abiogenic structures must also be considered for these structures. In addition, an origin as ambient inclusion trails (AIT's) has also been advanced (Buick, 1990). AIT's are formed by the propulsion of a mineral grain, often pyrite, through a partially or totally lithified chert substrate. They were once

thought to form purely abiogenically (Tyler and Barghoorn, 1963) but Knoll and Barghoorn (1974) highlighted the occurrence of organic matter in association with the AIT's and hypothesized that the propulsion of the mineral grains through the chert host is aided by decomposition of the biological matter, producing a gaseous driving force and acidic products that can etch the chert. Although the structures described by Awramik et al. (1983) and Buick (1984) are unlikely to be filamentous microfossils, it is possible there may be a biogenic component to their formation via this organically mediated AIT hypothesis. Further examples of filamentous structures found in the North Pole area by Ueno and co-workers (Ueno et al., 2001) have not been re-examined independently and still await a consensus on their biogenicity.

Perhaps more robust microfossil evidence for early life may come from the rather younger Sulphur Springs Group, a sequence of komatiites, basalts, dacites and rhyolites erupted at about 3,240 Ma (Van Kranendonk, 2006). Pyritic filaments (Fig. 2c) from a 3,235 Ma deep sea, volcanogenic, massive sulfide deposit were reported by Rasmussen (2000) and interpreted as the fossilized remains of thread like thermophilic, chemotrophic prokaryotes. The filaments are 0.5–2.0 μm in width and up to 300 μm long, can be straight, curved or sinuous and exhibit putative biological behaviour including preferred orientations, clustering and intertwining. They come from a subsurface drill core and only occur in the paragenetically early chert and coarse grained quartz that are cross cut by later fractures. This report appears to conform both to the criteria for syngenicity and many of the criteria for biogenicity. It awaits independent verification and supporting lines of geochemical evidence. Even so, it is an intriguing discovery that appears broadly consistent with the hypothesis of a thermophilic origin for life in the vicinity of sub-marine hydrothermal vents. It is, perhaps, the most convincing of the case histories that we have reviewed thus far.

3.3. PILBARA MICROTUBES

Remarkably preserved microtubular structures have recently been reported from a silicified sandstone unit at the base of the ~3,400 Ma Strelley Pool Chert (Wacey et al., 2008a; Brasier et al., 2006). Ongoing investigations of these structures show several lines of evidence consistent with a biogenic interpretation. The geological context of the sandstone unit appears conducive for life. This is evidenced by the presence of low angle cross bedding, channels and relatively high textural and compositional maturity, together indicating deposition in a shallow marine transgression, and providing the oldest such deposit in the rock record. That does not mean to say, of course, that any structures appearing in such a sandstone are necessarily biogenic. The microtubes are restricted to a small subset of early formed, well rounded clasts within the sandstone, and cross cutting relationships appear to exclude modern contamination, but as shown by Wacey et al. (2008a, Fig. 10), such contexts can be surprisingly complex and difficult to decode.

Pioneering work using nanoSIMS technology has detected biologically important elements such as carbon, nitrogen, phosphorus and sulfur, all occurring within or along the edges of the microtubes. Nano-scale *in situ* carbon isotope measurements from within the microtubes (average −26‰ PDB) also fall within the range consistent with biological processing (Wacey et al., 2008a). The occurrence of pyrite grains associated with the microtubes, together with their cross-sectional profiles, led Wacey et al. to conclude that these structures are likely biologically mediated ambient inclusion trails (AITs; cf. Knoll and Barghoorn, 1974). This discovery is not without its problems though; such trails can form at several stages through the diagenetic and metamorphic history of the rock, so great care must be taken to constrain their age. There is also no consensus, as yet, on the degree to which AITs are biologically-mediated; laboratory experiments are urgently needed to better understand this process.

Further mineralized microtubular structures have recently been reported from inter-pillow hyaloclastite within the 3,350 Ma Euro Basalt Formation and are argued to represent bioerosion traces created by euendolithic organisms within this volcanic glass (Banerjee et al., 2007). These microtubes are 1–9 μm in width (averages of 2.4 μm) and up to 200 μm in length (average ~50 μm).They are infilled with titanite (also known as sphene) which has been dated directly using U-Pb systematics to give an Archean age of 2.9 billion years old. This correlates with the youngest episode of regional metamorphism that affected the sample area and is interpreted to record metamorphic re-setting of the titanite which infills the microtubes and therefore a minimum age estimate for their formation. We await further results from these microtubular structures found in both meta-volcanic glass and silicified sediments with interest.

3.4. PILBARA SULFUR ISOTOPES

Sulfur isotopes are a promising additional tool in our armoury for investigating the early biosphere given that primitive bacteria which metabolize sulfur compounds are one of the most deeply rooted groups in the Tree of Life (e.g. Mojzsis, 2007, Figs. 7.5–12). Sulfur isotopes have also been utilized as a hotly debated tracer for the rise of atmospheric oxygen, an application that we will not discuss further here (see instead Kasting, 2006; Mojzsis, 2007). The analysis of sulfur isotopes preserved within ancient sulfides and sulfates can be used to recognize various processes in the sulfur cycle, in particular biological sulfate reduction and disproportionation of intermediate sulfur compounds (e.g. Shen and Buick, 2004). Evidence consistent with life at 3,490 Ma comes from the study of microscopic sulfides contained within barite crystals ($BaSO_4$) pseudomorphing gypsum ($CaSO_4$) in the Dresser Formation from North Pole, Western Australia (Shen et al., 2001). Fractionations of up to 21.1‰ (mean 11.6‰) between the sulfides and co-existing sulfates, together with the association of the sulfides with organic carbon are used to argue that sulfate reducing bacteria had evolved by ~3,490 Ma.

More recently, the $\delta^{34}S$ analyses of Shen et al. (2001) were repeated and corroborated using drill-core material from the North Pole by Philippot et al. (2007). More importantly, this study extended the analysis to include the minor sulfur isotope ^{33}S which yielded a positive anomaly. This implies the involvement of micro-organisms which disproportionate elemental sulfur in the formation of these sulfide grains. This is an exciting result in itself but in addition, the strict environmental requirements of currently known S-disproportionating bacteria (an anoxic environment and temperatures below 40°C, at near neutral pH and low H_2S concentrations) may help to settle discussions regarding the magnitude of hydrothermal inputs on the North Pole palaeo-environment. Caution is urged with respect to this inference, because this group of bacteria has received relatively little microbiological attention to date (Thamdrup, 2007).

4. Barberton Greenstone Belt

Ancient rocks of the Barberton Greenstone Belt (Fig. 3) include the Swaziland Supergroup, which comprises a lower mostly volcanic succession (Onverwacht Group) and an upper mainly clastic succession (Fig Tree Group and Moodies Group) (Anhaeusser, 1973; Lowe and Byerly, 1999). Here we focus on the oldest of these, the Onverwacht Group which spans the time interval ~3,500–3,200 Ma (Armstrong et al., 1990). In detail (Fig. 3b), it is composed of komatiitic and tholeiitic basaltic rocks interbedded with thin sedimentary units of silicified ash and black chert, together with rare felsic volcaniclastic and intrusive rock. Here we review the literature from the last twenty years pertaining to the description of several putative biogenic structures from the Onverwacht Group. Some putative biological structures were described prior to this time (e.g. Schopf and Barghoorn, 1967; Pflug, 1967; Nagy and Nagy, 1969; Engel et al., 1968; Muir and Grant, 1976; Knoll and Barghoorn, 1977), but a comprehensive review of these has already been given by Schopf and Walter (1983) who concluded that none of these discoveries gave compelling evidence for ancient life.

4.1. BARBERTON MICROTUBES

The most ancient structures described from the Onverwacht Group are mineralized microtubes from the formerly glassy margins of pillow basalts and inter-pillow

Figure 3. (continued) Research, Volume 106. Westall, F. de Witt, M.J., Dann, J., van der Gaast, S., de Ronde, and Gerneke, D. Early Archean fossil bacteria and biofilms in hydrothermally-influenced sediments from the Barberton greenstone belt, South Africa, pp. 93–116. Copyright (2001), with permission from Elsevier. Figure 3d and f are reprinted from Precambrian Research, Volume 54. Walsh, M.M. Microfossils and possible microfossils from the early Archean Onverwacht Group, Barberton Mountain Land, South Africa, pp. 271–293. Copyright (1992), with permission from Elsevier.

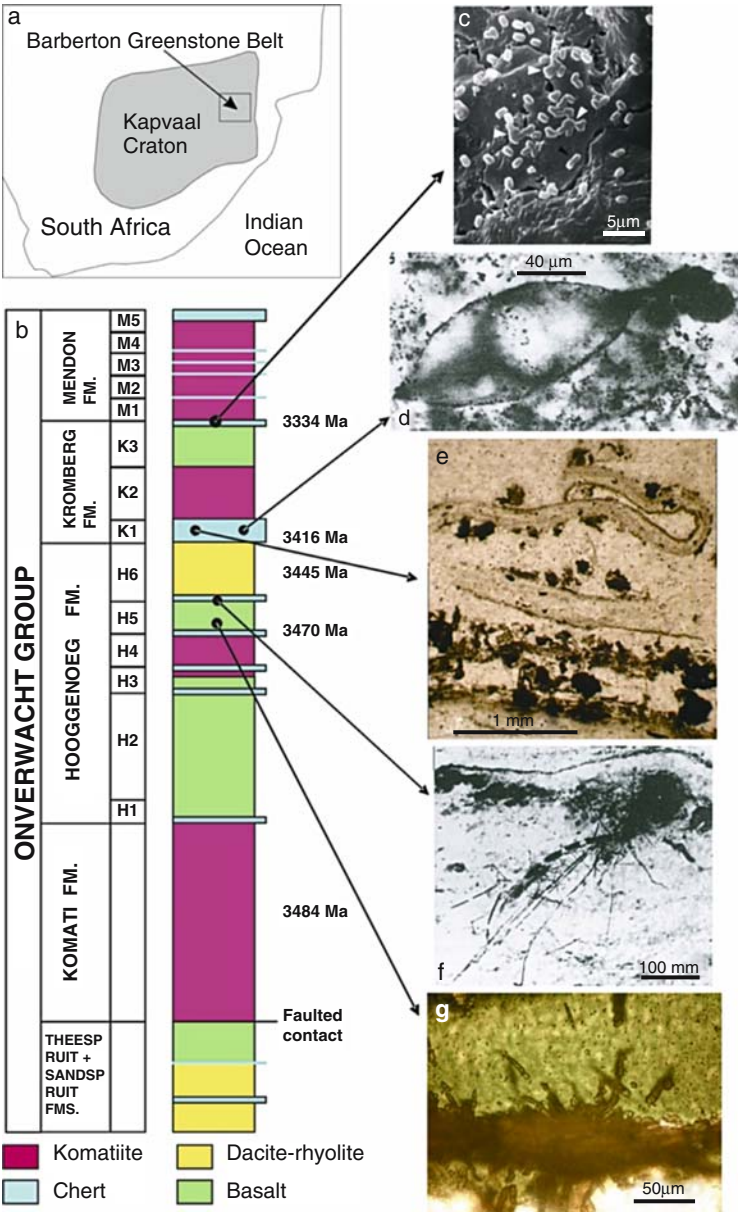

Figure 3. Putative biogenic structures from the Barberton greenstone belt (BGB), South Africa. (a) Location of the BGB on the South Africa–Swaziland border; (b) stratigraphy of the ~3,500–3,200 Ma Onverwacht Group; (c) etched fracture surface of chert from the ~3,400 Ma Kromberg Formation showing short rod-shaped bateriomorph structures embedded in quartz (Westall et al., 2001); (d) petrographic thin section of spindle structure in chert from the base of the Kromberg Formation (Walsh, 1992); (e) petrographic thin section of putative microbial mat layer from the 3,416 Ma Buck Reef Chert; (f) petrographic thin section of solid filaments in chert from the ~3,450 Ma upper Hooggenoeg Formation (Walsh, 1992); (g) petrographic thin section of mineralized microtubular structures within the formerly glassy margin of pillow basalt from the ~3,450 Ma upper Hooggenoeg Formation. Figure 3c is reprinted from Precambrian

hyaloclastites (Fig. 3g). These have been described from within the Komati, Hooggenoeg and Kromberg Formations, with the best developed structures coming from the 3,472–3,456 Ma upper Hooggenoeg Formation (Furnes et al., 2004; Banerjee et al., 2006). The mineralized tubular structures are 1–10 μm in width and up to 200 μm in length and extend away from "root zones" of fine grained titanite associated with re-healed fractures. The tubular structures are segmented in some areas (Fig. 3g) by cross-cutting chlorite formed during ocean floor alteration very soon after initial eruption of the basalts (Furnes et al., 2004). The replacement of titanite by low grade metamorphic chlorite within the tubes suggests the tubes were formed prior to this alteration and are indeed ancient structures. X-ray element mapping reveals the presence of linings enriched in carbon along the margins of the tubes, and this carbon is argued to represent decayed organic material from the trace maker (Banerjee et al., 2006). Moreover, carbon isotope analysis of disseminated carbonates in the pillow rims gives $\delta^{13}C$ values of +3.9‰ to −16.4‰ compared to +0.7‰ to −6.9‰ for the crystalline pillow interiors, and these lower values from the pillow rims are consistent with microbial fractionation (Furnes et al., 2004). Higher resolution in situ chemical and isotopic analyses are now needed to substantiate this claim. These findings highlight the preservation potential of volcanic rocks that have until recently been somewhat neglected in studies of Archean life. (Extensive reviews of microbial ichnofossils found in volcanic glass from the modern seafloor, Phanerozoic ophiolites and Precambrian greenstone belts are given in McLoughlin et al., 2008b and Furnes et al., 2007.)

4.2. BARBERTON MICROBIAL MATS AND MICROFOSSILS

Climbing the stratigraphy slightly, there are reports from the Buck Reef Chert (K1 in Fig. 3b) of putative microbial mat remains in a sedimentary setting (Tice and Lowe, 2004, 2006). This contrasts with many recent studies of Archean cherts that have been interpreted as formed from either hydrothermal exhalites or injections, or as hydrothermally modified sediments (e.g. Hofmann and Bolhar, 2007; Van Kranendonk, 2006; Brasier et al., 2005). In the case of the Buck Reef Chert, however, Tice and Lowe argue that the thickness, lateral extent, trace metal profiles and development across a range of depositional environments represents deposition under "normal" marine conditions followed by silicification by supersaturated marine waters. If this is indeed the case, then the associated microbial mat-like laminations, carbonaceous grains and 'rip up' clasts (Fig. 3e) of this 3,416 Ma unit may represent one of the first environments truly analogous to a modern day shallow marine setting where it may have been possible for life to gain a foothold (cf. Westall, 2005).

Combined with measured carbon isotope fractionations of $\delta^{13}C = -20‰$ to $-35‰$ and apparent ecological control on the distribution of the laminated mat-like structures and carbonaceous grains, this is at least consistent with a biogenic component to these structures (Tice and Lowe, 2006).

Similar reports of fossilized microbial mats with more numerous filamentous and coccoid microfossils come from carbonaceous cherts lower in the Barberton succession from within the upper Hooggenoeg and lower Kromberg Formations (Walsh and Lowe, 1985, 1999; Walsh, 1992). These include examples of plastically deformed carbonaceous fragments interpreted as microbial mat rip-up clasts and two examples of purported filamentous microfossils. Both threadlike (0.1–0.6 µm in diameter) and tubular (1.4–2.2 µm in diameter) filaments are present (Fig. 3f), and are composed of carbonaceous matter with some exhibiting slight constrictions at 1 µm intervals (Walsh and Lowe, 1985). These putative wrinkle-mat textures and microfossil morphologies are suggestive of microbial processing, but their morphological similarity to laminar and wispy textures in the hydrothermal cherts of the 3,460 Ma 'Apex chert' (cf. Brasier et al., 2005) raises the possibility of an abiotic origin for this carbonaceous material which needs to be more thoroughly tested. The carbon isotopic analyses are from 'bulk rock' analyses, so younger contamination cannot be completely eliminated. In addition, only weak evidence (the colour of the carbonaceous matter) is presented for syngenicity of the 'microfossils' with their host rock. A further structure of note within these cherts is the discovery of ~40 µm diameter 'spindles' (Fig. 3d). These intriguing morphologies have been variously interpreted as being biogenic and the outer sheaths of colonies of bacterial cells or, alternatively, as abiogenic and the carbonaceous coatings of ghosted gypsum crystals. These scenarios may merit further investigation, especially in light of the recent discovery of similar structures in the 3,400 Ma Strelley Pool Chert in Western Australia (Sugitani et al., 2007).

Westall et al. (2001) also describe spherules around 1 µm in size (Fig. 3c) commonly occurring as pairs, 'zig-zags' or 'strings' which exhibit a uniform size range and are associated with very fine, dark, discontinuous, wavy laminae and lenticular clots. Sausage shaped, rod shaped, oval mould structures and films are also described and are interpreted to represent fossil bacteria or phenomena associated with bacteria by direct analogy to modern equivalents. Here, detailed geological and petrographic context like that presented for the Buck Reef Chert is lacking. (It is not clear, for instance, from which chert units the samples precisely originate, nor whether they are from primary fabrics. Neither is detailed geological mapping presented to support the postulated depositional environment of a shallow water, periodically sub-aerial depositional setting.) Carbon isotope data are provided to show $\delta^{13}C$ values as low as $-27‰$, but caution must be used with this interpretation because such values can be derived abiogenically (cf. Horita and Berndt, 1999; McCollum and Seewald, 2006). Indeed, Westall et al. (2006a) acknowledge this problem in a more recent discussion of these structures. On the other hand, a recent study of the isotopic, chemical and structural characteristics of selected carbonaceous material from the Hooggenoeg

and Kromberg cherts concluded that this is indeed indigenous to the rock and has all the characteristics of metamorphosed biological material (Van Zuilen et al., 2007). However, this study also showed that small scale migration and re-deposition of organics had occurred and so morphological interpretations of putative microfossils and micro-laminae should be made with caution.

There are several reports of wrinkle structures in Archean clastic sediments. Amongst the best-preserved are those from the 2,900 Ma Mozaan Group (Noffke et al., 2003) and the 3,200 Ma Moodies Group (Noffke et al., 2006) of South Africa. These horizons contain organic carbon remains with $\delta^{13}C$ values consistent with biological processing and sufficiently good micro-textural preservation to suggest the presence of filamentous microbial mats that trapped and bound sediment grains in shallow marine to tidal flat environments (Noffke et al., 2003, 2006). Older cherts from South Africa and Australia also contain purported wrinkle mat-remains (e.g. Westall et al., 2001, 2006a, b), but the degree of preservation is typically less good in these early Archean cherts. The paucity of detrital sedimentary fabrics and widespread graphitization of carbonaceous remains in these early Archean cherts makes testing the biogenicity of wrinkle structures more challenging. Furthermore, the experimental work of McLoughlin et al. (2008a) has synthesized *abiotic* wrinkle structures from colloidal media that may be comparable to the silica gel precursors of some Archean cherts. This urges caution when inferring the biogenicity of wrinkle structures.

4.3. BARBERTON SULFUR ISOTOPES

A similar sulfur isotope story to that in the Pilbara emerges in the Barberton area from the work of Ohmoto et al. (1993). These authors sampled a shale and three black cherts from the uppermost Onverwacht Group (~3,300 Ma Mendon Formation) in the Barberton and used high resolution laser ablation mass spectroscopy to analyse individual pyrite grains. The range of $\delta^{34}S$ values (up to 12‰ variation) from these pyrites was argued to be greater than that expected if they had formed from purely magmatic or hydrothermal H_2S, and an origin from bacterial sulfate reduction was again invoked. This evidence of sulfate reduction from the Pilbara and Barberton predates previous evidence (Goodwin et al., 1976) by some 600–750 Ma and potentially allows calibration of an important deep branching node on the Tree of Life.

We remind the reader that the use of only one line of evidence, in this case isotopic evidence to constrain a key event in the evolution of life needs always to be regarded with care. Additional supporting lines of evidence from geochemistry, morphological remains and geological context are needed to strengthen the conclusions. Towards this end, multiple isotope systems are being developed and integrated with palaeontological observations from the Archean. For example, in younger c.2.7 Ga stromatolitic and non-stromatolitic sediments from the Belingwe Greenstone Belt of Zimbabwe, sulfur and carbon isotopes in conjunction with

rare earth element patterns have been used to argue for the presence of bacterial and archaeal consortia which are water-depth dependant and capable of anoxygenic photosynthesis, methanogenesis and methanotrophy (e.g. Grassineau et al., 2001). The sulfur and carbon isotopic systems discussed during this review have provided most of our isotopic data thus far for early Archean biological studies. Further isotopic systems are currently being developed which will hopefully further constrain Archean environmental and biological processes. A description of these is beyond the scope of this review and we refer the reader to the following: Nitrogen isotopes (Beaumont and Robert, 1999; Van Zuilen et al., 2005); Iron isotopes (Anbar, 2004; Dauphas et al., 2004, 2007; Johnson and Beard, 2006); Oxygen isotopes (Knauth and Lowe, 2003).

5. From Earth to Mars and Beyond

The potential existence of life on other planets is an exciting prospect. On Mars in particular which had a "warm wet" climate and similar environmental condition to the early Earth 4.5–3.5 million years ago, the possibility of the emergence of life is very real (e.g. McKay and Stoker, 1989). Given, however, the numerous problems and controversies we have highlighted when looking for earliest life here on Earth, what hope do we have for confidently identifying life elsewhere in the universe? We attempt to address this question here by looking in turn at three lines of evidence for life on other planets and the challenges that these face: first putative microfossils found in Martian meteorites; second microbial trace fossils created by endolithic bacteria on other planetary surfaces; and thirdly the insights stable isotope ratios measured on extra-terrestrial samples may yield. In each case we emphasize how critical analysis of this evidence goes hand-in-hand with re-examination of the early terrestrial rock record to provide the best strategy for seeking life beyond Earth.

To date, claims for extraterrestrial life have, unsurprisingly, been debated as vigorously, if not more so, than claims for the earliest evidence of life on Earth. The most famous of these is the claim for life in the ~4,500 Ma ALH84001 Martian meteorite found in Antarctica in 1984 (McKay et al., 1996). Here we summarize the four lines of evidence that were originally presented for fossil life in this meteorite and why each has been subsequently dismissed or re-interpreted. (1) Carbonate globules: the density and composition of the carbonate being comparable to phases associated with terrestrial bacteria. Carbonate by itself, however, is abundant in non-living materials. (2) Organic compounds known as polycyclic aromatic hydrocarbons that are created by bacteria were present in the meteorite. It is now thought that these were contaminants from the Antarctic environment (e.g. Jull et al., 1998). (3) Magnetite globules with morphologies only known to be produced by magnetotactic bacteria (Thomas-Keprta et al., 2001) were reported. Six criteria were subsequently outlined to test the biogenicity of magnetosomes; only a small proportion of this population satisfy these criteria

and very few of the magnetosomes are aligned in chains (Weiss et al., 2004). Nevertheless, this remains probably the strongest line of evidence for biogenicity with Thomas-Keprta et al. (2000) reporting that 27% of the magnetite grains do indeed satisfy the criteria for biogenicity. (4) "Nanofossil"-like structures were described. These, however, were smaller than the accepted minimum size range for even the smallest cells capable of independent growth and are likely just mineral artefacts. ALH84001 continues to be studied but the consensus at present is that unambiguous biosignatures have yet to be found. There are also a number of other reports of candidate microfossils found in other carbonaceous meteorites (e.g. Hoover et al., 2004) although these have been rather less celebrated and debated by the Astrobiology community in comparison to ALH84001.

This debate has been re-awakened by the recent discovery in the Martian Nakhla meteorite of carbonaceous, tubular and bleb shaped microstructures that show some similarities to endolithic microtubes in volcanic glass (McKay et al., 2006; Gibson et al., 2006). New technology has allowed identification of distinct CN^- signatures and an in situ carbon isotopic composition of −18‰ to −20‰ associated with these structures (Gibson et al., 2006). This carbonaceous phase appears to be distinct from a contaminating terrestrial phase which decomposes at much lower temperatures. It has therefore been suggested that the carbonaceous phase is either: (i) abiogenic, derived from an impact on Mars that also produced the fractures and veins in the Nakhla meteorite; or (ii) biogenic, the products of Martian euendoliths that may be similar to terrestrial, volcanic microborings. The latter is a plausible scenario as it is envisaged that the intense UV flux, absence of liquid water and freezing temperatures on the outer surface of Mars may have encouraged an endolithic mode of life (e.g. Friedmann and Koriem, 1989). Microtubular tunnels and galleries in olivine and pyroxene crystals from the Nakhla meteorites that are remarkably similar to bioerosion textures found in terrestrial iron-magnesium silicates have also been recently described by Fisk et al. (2006). These authors made cautious conclusions about their microtubular weathering textures: "*though the tunnels found in Nakhla are similar to the biosignatures found in terrestrial minerals, their presence cannot be used to prove that the Martian alteration features had a biogenic origin*." This conclusion underscores the need for further investigation into biotic and abiotic mechanisms of microtube generation and the refinement of criteria for establishing their biogenicity (cf. McLoughlin et al., 2007).

An alternative strategy for seeking life on other planets is the use of stable isotope ratios as a biomarker. Above we have reviewed the use of C and S isotopes in seeking evidence for biological processes on the early Earth. These isotopic investigations, both on Earth and other planetary surfaces, rely on several key assumptions, which we now remind the reader of before discussing whether these assumptions hold on other planetary surfaces: (1) biological processes generate an isotopic signature which is distinguishable from that resulting from abiological processes (but with regards to C isotopes, the growing appreciation of Fischer-Tropsch type reactions have questioned this assumption); (2) the isotopic

signatures of the various reservoirs i.e. geo- hydro- atmos- and bio- spheres on the planet concerned have been characterized and have remained constant over geological time; (3) secondary geological processes have not modified the isotopic signature measured; and (4) the isotopic signature is syngenetic to the rock sample on which it is measured (see also Van Zuilen, 2007). On Mars, the study of C isotopes is hampered by our relatively poor understanding of how the different C reservoirs and the fluxes between them have changed over time (e.g., changes in volcanic out-gassing, loss of CO_2 from the atmosphere) and also by the absence of a well characterized, inorganic carbonate reservoir to act as an isotopic standard (for further discussion see Van Zuilen, 2007; Grady and Wright, 2006). Alternatively, S isotopes could be employed to understand bacterial sulfur cycling given that there are evaporitic sulfates in sedimentary rocks on Mars (Squyres et al., 2004) which could provide an abiogenic standard – but here again relatively little is known about the evolution of the S cycle on Mars and so great care would need to be taken with the interpretation of any potential S isotopic measurements. These should be considered in concert with additional lines of morphological and chemical evidence.

6. Summary

In summary, extinct or extant life may await discovery on Mars and indeed on other planetary bodies such as Jupiter's moon, Europa, perhaps near subsurface hydrothermal vents (e.g. Fisk and Giovannoni, 1999). The review of the early terrestrial rock record presented here highlights the importance of volcanic and hydrothermal rocks between 3.5 and 3.0 Ga to the early history of life on our planet and is consistent with hypotheses which advocate a hydrothermal cradle for life (e.g. Russell and Arndt, 2005; Nisbet and Sleep, 2001). It also highlights the importance of siliciclastic rocks which may contain some of the best preserved fragments of the earliest rock record. However, studies of the 'Apex chert' and other early Archean hydrothermal systems caution that the unambiguous identification of fossil microbes may be hampered by abiotic carbon synthesis (cf. Brasier et al., 2005 and references therein). One thing is for certain. Improving our understanding of the earliest fossil record here on Earth is critical to our chances of confidently interpreting putative biological structures and signals found in the future on other planets.

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MODELS FOR SILICATE FOSSILS OF ORGANIC MATERIALS IN THE ASTROBIOLOGICAL CONTEXT

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Abstract Silicates are abundant on both Earth and Mars, and hold great potential for harboring biosignatures. Biosignatures are signs of past or present life and may be either organic or inorganic in nature. Our most recent work, which we review here, is a survey of how different classes of organic compounds interact with highly basic sodium silicate solution to model the formation of biosignatures in nature. Our work focuses on using IR (infra-red) spectroscopy as a way to determine the mechanisms by which organics are preserved within silicates. Throughout the chapter, we cite relevant studies by others, while still maintaining the focus on the review of our own work. We ultimately summarize how various classes of organics interact with sodium silicate in terms of both physical and spectral properties and describe their astrobiological significance.

1. Introduction and Objectives

One of the goals listed in NASA's Astrobiology Roadmap is to determine how to recognize biosignatures, which are signatures of life on early Earth and potentially on other worlds. A central requirement for this goal is that biosignatures must be defined in terms that can be measured and quantified (Morison, 2001; NASA, 2007a). A recent report on the astrobiology strategy for the exploration of Mars discusses various aspects of biosignatures (National Academies Report, 2007). It lists various recommendations and goals, such as to develop a catalog of biosignatures that reflect fundamental and universal characteristics of life that are not limited to an Earth-centric perspective, to determine which characteristics of Martian materials result from non-biological processes and which result from biological processes, and to follow the carbon to get to the biosignatures, among others. The report also points out that biosignatures may be mineralogical and inorganic in their nature. An example is the formation of biosignatures by the rapid mineralization that can protect microorganisms and organic molecules against degradation. This process is known as entombment. The database of terrestrial biosignatures needs to be expanded to facilitate determination of the mineralogical biosignatures of the Martian minerals (National Academies Report, 2007).

Various aspects of silicon biomineralization have recently been reviewed (Mann, 2001; Perry et al., 2003; Vrieling et al., 2003). While building on the background of others, we address several new questions that are associated with the silicification of the organic materials. The first question is if the silicified organic material can be identified *in situ* by IR (infra-red) spectroscopy. Such an approach is of interest to astrobiology, since the IR instrument has been miniaturized and made robotic, and it has been already used on extraterrestrial missions. The second question addresses silicification of the metal complexes of some bio-organic materials and their *in situ* identification by the IR. The *in situ* identification of the organic materials within their silicate and silicate-metal complexes is important for the upcoming missions to Mars, which are not of the return-sample type. Our ultimate goal is to identify the silicified organic materials by their characteristic IR bands. The final question addresses the changes that the organic material causes in the structure and appearance of the silica gel that it helps form. We do address this question and bring up a possibility that such changes in the silica gel need to be considered as inorganic signatures of various groups of organic compounds. We cite the relevant studies by others, while still remaining the focus on the review of our own work.

We are investigating the role of organic compounds in silicification. Silicate fossils may contain organic material preserved within the silica matrix. Alternatively, the organic material may induce morphological changes of the silica, but may no longer be present in the silica fossil. We have created model systems in the laboratory that provide insight into the nature of silicate fossils. These models have also been evaluated for the presence of organic materials via IR (infra-red) spectroscopy, and have been assessed for astrobiological relevance (Kolb et al., 2004; Perry et al., 2005; Kolb and Liesch, 2006, 2007; Liesch and Kolb, 2007a, b).

We are particularly interested in exploring various roles of silicate fossils in astrobiology, such as in the preservation of organic material on meteorites, during interplanetary transport, and possibly on Mars, and also as indicators of past life. Our model is comprised of a basic aqueous solution of sodium silicate, which we have treated with various classes of organic molecules, primarily those that are present on meteorites. Examples include amino acids, sugar-like compounds, Maillard products (which are formed from the reaction between the amino acids and sugars), alcohols and amino alcohols (Kolb and Liesch, 2006; Liesch and Kolb, 2007a). We have also initiated study of some phosphorylated biomolecules that are associated with the life on Earth, such as ATP, AMP, and DNA (Kolb and Liesch, 2007).

2. The Mechanisms of Silicification

The key part of any silicification process is the formation of silica gel from silicic acid or its salts, such as sodium silicate (Iler, 1979). Figure 1 shows the polymerization

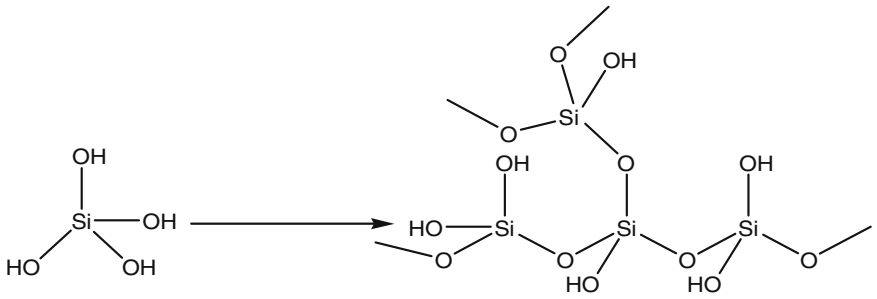

Figure 1. Polymerization of silicic acid.

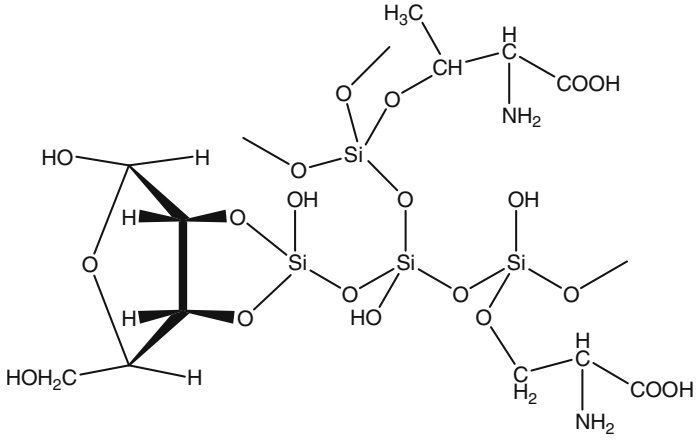

Figure 2. An example of organo-silicate: ribose (bottom left), serine (bottom right), and threonine (top right), make Si-O-C bonds with silicic acid within the silica gel.

of silicic acid to silica gel. Silica gel is characterized by the presence of the Si-O-Si bonds and Si-OH groups. Organic materials can interact with silicic acid or silica gel by forming covalent Si-O-C bonds, to give an organo-silicate. Several authors have proposed that hydroxyl-containing bio-organic materials can react to form organo-silicates, which will help stabilize and preserve those organics (Sullivan, 1986; Williams, 1986; Zubay, 2000; Perry and Kolb, 2004). This is shown in Fig. 2, using the examples of threonine, serine and ribose.

The preservation of organic material within silicates can come about by two different mechanisms (Perry and Kolb, 2004). The first mechanism is entombment, in which organic material is entrapped in a silica gel, during the polymerization. In the second mechanism, organic compounds covalently bond with the OH groups of the silicates, to form Si-O-C bonds. This mode could be feasible for the

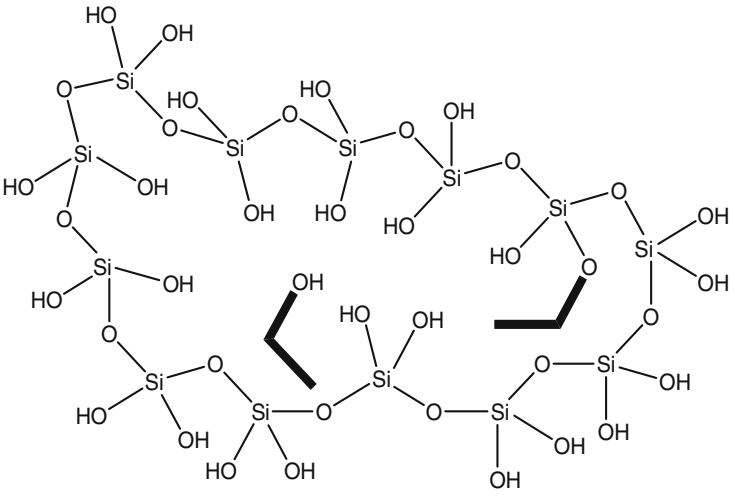

Figure 3. Models for biosilicification. Ethanol entombed in silica gel (inside, left), or reacted to make an organo-silicate (inside, right).

preservation of sugars, alcohols, and other bio-organic materials that have an OH group. These two mechanisms are shown in Fig. 3, using ethanol as an example.

The experimental support for entombment comes from the work by Coradin and co-workers (Coradin and Livage, 2001; Coradin et al., 2002). These authors studied the interaction of amino acids and peptides with sodium silicate. They have found that various amino acids and especially peptides promote polymerization of silicic acid to give silica gels. The experiments were performed with a dilute solution of sodium silicate at various pH values in the presence of appropriate buffers. The surface of these gels was studied via SEM (scanning electron microscopy), and the bulk of the gels was investigated by the IR spectroscopy. The IR bands in the regions for the amide, carboxylate and alkyl groups were observed, but the Si-O-C bond was not identified. These results are compatible with the entombment mechanism.

The most recent support for the second mechanism, which is relevant to astrobiology, came from studies of Lambert and co-workers (Lambert et al., 2004) and Kastele and co-workers (Kastele et al., 2005). These studies show in an unambiguous manner that covalent Si-O-C bonds form between sugars, sugar alcohols, or nucleosides and silicates. Si-O-C bond formation was proposed to stabilize sugars and to preserve them during transport in space and on meteorites (Lambert et al., 2004). The experimental demonstration of the Si-O-C bonds was made via Si-29 NMR (nuclear magnetic resonance) spectroscopy (Lambert et al., 2004; Kastele et al., 2005) and X-ray crystallographic studies (Kastele et al., 2005). Unfortunately, Si-29 NMR and X-ray diffraction instruments have not yet been

miniaturized and made robotic, and are thus not feasible for an *in situ* search for Si-O-C bonds on Mars.

3. Our Experimental Models

Our experimental models include the interaction between sodium silicate and the following groups of organic compounds: Amino acids, biological and meteoritic; Maillard products (Kolb and Liesch, 2006); Metal-Maillard complexes (Liesch and Kolb, 2007a); sugars, alcohols and amino alcohols (Liesch and Kolb, 2007a); acid halides; and biomolecules, such as urea, AMP, ATP, and DNA (Kolb and Liesch, 2007a). With the exception of acid halides, which we have studied as a model for very reactive molecules, all other compounds are of biological and astrobiological importance.

We became interested in developing a variation of the model of Coradin and co-workers (Coradin and Livage, 2001; Coradin et al., 2002). They used dilute sodium silicate and buffers, in order to mimic biogenic media of diatoms. In our model we use concentrated sodium silicate solution without buffers. Our model would be more applicable to basic niches on Earth, such as soda lakes. Upon the addition of the various organic compounds mentioned above, the sodium silicate solution polymerized into a viscous gel in nearly every case. In some cases, such as with the amino acids, polymerization similar to that described by Coradin and co-workers (Coradin and Livage, 2001; Coradin et al., 2002) had been observed. In other cases, the interaction with silicates had been hinted at (Azrak and Angell, 1973; Sweryda-Krawiec et al., 1999) but our studies led to the discovery of novel physical and spectral properties of these polymerized gels (Liesch and Kolb, 2007a). The various organic compounds we have investigated interact with sodium silicate solution in a variety of different ways and possess unique characteristics in terms of physical appearance, sol-gel-sol transformations, and the properties of their IR spectra. We also set as a goal to identify the Si-O-C band in the IR spectra, and had some literature guidance on this subject (Hino and Sato, 1971; Bellamy, 1975; Benning et al., 2004).

4. Results and Discussion

4.1. IR RESULTS

As mentioned in the previous section, a key goal of our work had been to elucidate the mechanisms of silicification. The key to distinguishing between the two main possibilities of entombment and covalent bond formation required the determination of the exact ranges of Si-O-Si and Si-O-C bands in the IR spectra. This in itself was a challenge that took nearly two years to finally sort out, because the Si-O-Si

and Si-O-C IR bands are both found in the 1,000–1,100 cm^{-1} region and overlap significantly according to the literature (Hino and Sato, 1971; Bellamy, 1975; Benning et al., 2004). In an attempt to resolve the issue, we conducted a series of deuteration experiments inspired by the research of Hino and Sato (Hino and Sato, 1971). Our deuteration experiments did result in some shifts in the IR bands, but only confounded the matter in the long run (Kolb and Liesch, 2006).

Clarification of the matter ultimately came about from a set of experiments involving the gradual hydrolysis of known organo-silicates, such as poly diethoxysiloxane and poly dimethoxysiloxane, and an IR study of the resulting partially hydrolyzed products. As hydrolysis proceeded, the ethoxy or methoxy groups were replaced by hydroxyl groups, with the concomitant decrease of the Si-O-C band in the IR spectra (Liesch and Kolb, 2007a). These experiments allowed us to determine the ranges of the Si-O-Si (1,125–1,000 cm^{-1}) and Si-O-C bands (1,225–1,150 cm^{-1}) (Kolb and Liesch, 2007a). This information helped clarify the ambiguity of the given literature values (Hino and Sato, 1971; Bellamy, 1975; Benning et al., 2004), and ultimately allowed us to differentiate between the mechanisms of entombment and covalent Si-O-C bond formation. Our research focused on gathering and interpreting the spectral data. The key features gained from the study of the IR spectra are the location of the Si-O-Si band (as dramatic shifts occurred in some cases) as well as the presence of organic peaks and/or the Si-O-C IR band. These trends and features are depicted in Table 1. This table also shows silicates demonstrating the different mechanisms of silicification.

Table 1. Summary of main trends of IR spectral analysis.

Compound	Si-O-Si band (cm^{-1})	Si-O-C band (cm^{-1})	Hydrocarbons in 2,800–3,200 cm^{-1} range	Mechanism of silicification
Polydimethoxysiloxane (known organo-silicate)	1,091	1,197	2,950, 2,847 (alkyl)	NA
Acidified Na-silicate	1,088	–	–	Catalysis
Amino acid (Serine)	1,077	–	–	Catalysis
(Proline)	1,060	–	2,952, 2,925, 2,859 (alkyl)	Catalysis/ entombment
Maillard mixture (Serine)	1,095	–	2,952, 2,926, 2,855 (alkyl) –	Catalysis/entomb-ment catalysis
(Glycine)	1,093	–		
Maillard complex (Iron (III) sucrose complex)	1,039	–	–	Catalysis, entomb-ment of metal ions
Sugar & (ribose)	1,086	–	–	Catalysis
Sugar alcohol (sorbitol)	1,076	–	–	Catalysis
Alcohol (n-butanol)	1,009	–	–	Catalysis, covalent bonding
Amino alcohol (ethanolamine)	1,023	1,158	2,963, 2,890 (alkyl)	Covalent bonding
Acid halide	1,087	1,178	3,107 (aryl)	Covalent bonding

The physical properties and unique characteristics of each gel will be discussed over the next few pages.

4.2. AMINO ACIDS

Amino acids play a unique and vital role in biological systems. They are the monomeric building blocks of polypeptides and proteins which can serve multitudes of different functions in the cell, ranging from catalysis to transport (Lodish et al., 2004). On Earth, 20 key "biological" amino acids (as well as a few lesser-known modified versions) are used to build the larger polypeptides and proteins (Lehninger et al., 2004). Various amino acids have been found on meteorites, such as the famous Murchison and Murray meteorites (Cronin and Chang, 1993; Cronin et al., 1995; Cronin, 1998).

Chemically, amino acids have fascinating, if not contradictory structures. The very name "amino acid" refers to the presence of both a basic "amino" group and an acidic "carboxyl" group. These compounds are often referred to as "zwitterions" (Lehninger et al., 2004). The etymology of the word strongly reflects the nature of these compounds, with the root word, "zwitter" meaning "hermaphrodite" in German (Zwitter definition, 2007). The presence of functional groups on the side chains of the amino acids, such as hydroxyl or amino groups, further increases the possibility of interaction with silicates.

Work by Coradin and co-workers (Coradin and Livage, 2001; Coradin et al., 2002) showed that polymerization of sodium silicate occurs upon the addition of amino acids and peptides. We have also observed and described similar polymerization with the amino acids in our model system (Kolb and Liesch, 2006). Upon the addition of the "biological" amino acids, the sodium silicate solution instantly polymerizes into a hard or rubbery whitish gel. After gel formation and a standing period, our gels were isolated, washed, pulverized, and dried as described in our recent paper (Kolb and Liesch, 2006). We ultimately obtained a whitish material consisting of small chunks and powder from these dried gels.

Upon standing (and before processing the samples), some dissolution of the gels was observed. We refer to this transition from soluble (initial sodium silicate) to solid gel and back to a soluble portion as the sol-gel-sol transformation. The sol-gel-sol transformation could feasibly play a key role in the transport and protection of organics in the astrobiological context – particularly Mars. Recent discoveries have indicated the presence of both water and silicates on Mars (NASA, 2007b). If organics such as amino acids had been in aqueous solution (sol) on Mars at some point, they could have been preserved within silicates (gel) and then released again when water became abundant once again (sol).

We have also studied meteoritic amino acids (those found on meteorites, but not in known proteins). Some of these acids, for example sarcosine, behaved very much like the biological amino acids, while some other, such as 5-aminovaleric acid, behaved entirely differently. The case of 5-aminovaleric acid was unique

among the amino acids because this was the only one we tested that formed a water-soluble gel. It was not until we worked with some of the sugars and alcohols that water-soluble gels were seen again in our laboratory.

Our IR analysis of the amino acid gels indicated the general absence of organics except in a few cases (Kolb and Liesch, 2006). Overall, it appears that amino acids merely catalyze the polymerization of sodium silicate. The trace organics indicate that entombment may be occurring to some minimal extent. In addition, it is possible that the washing and pulverization of the samples may remove some entombed, water-soluble organic materials.

4.3. MAILLARD MIXTURES

Maillard mixtures are a complex set of chemical compounds resulting from reactions between amino acids and sugars in either aqueous or solid states. These products have complicated structures, and possess numerous functional groups (Kolb et al., 2005). The astrobiological significance of these mixtures has been revealed by the similarities between the solid-state C-13 NMR spectra of the solid Maillard mixtures and the insoluble organic material from Murchison meteorite (Kolb et al., 2005, 2006).

Like the amino acids, Maillard mixtures also rapidly cause polymerization of sodium silicate (Kolb and Liesch, 2006). However, unlike the amino acids, which produce whitish gels and colorless solutions, the Maillard mixtures ultimately result in brownish or black gels and solutions. These colored gels have a rubbery consistency, but like the amino acids gels, yield small white chunks and powder after being processed. Also, like the case of amino acids, sol-gel-sol transformations were observed in the gels from the Maillard mixtures. This strengthens the astrobiological relevance of the Maillard mixtures as the sol-gel-sol transformations could lead to the preservation and subsequent transport of these compounds.

Analysis of these processed and dried gels also revealed an overall lack of organics (Kolb and Liesch, 2006). It appears that the Maillard mixtures catalyze the polymerization of the sodium silicate solution. Some entombment may be occurring, but it appears that much of the entombed organic material is removed during the processing of these gels.

4.4. MAILLARD-METAL COMPLEXES

The Maillard-metal complexes share some similarities with the Maillard mixtures described above, but a few key differences exist. The Maillard mixtures generally lead to the production of dark, insoluble materials which chemically resemble the black, insoluble materials found on the Murchison meteorite (Kolb et al., 2005, 2006). On the other hand, the Maillard-metal complexes are generally water soluble

(Trusovs, 2006). The Maillard-metal complexes are formed via a multi-step process in which chelation of metal ions, such as Fe^{+2}, Fe^{+3}, Ca^{+2}, Mg^{+2}, Zn^{+2} and Cu^{+2}, by the Maillard organic components occurs (Trusovs, 2006; Liesch and Kolb, 2007a).

The Maillard-metal complexes consist of fine brown or orange powders resembling finely ground cinnamon. Upon addition to sodium silicate, these complexes result in polymerization, which, in some cases, can be very rapid or which can take 20–30 minutes in others. The resulting colored gels were not rubbery like the amino acid gels, but instead were soft like the sugar and sugar alcohol gels, which will be discussed later in the chapter. These gels resembled those of the Maillard-mixtures, but were somewhat lighter in color.

As mentioned earlier, the gels resulting from the Maillard-mixtures consisted of small white chunks and powder after being processed and dried. In cases where the metal complexes contained chelated Ca^{2+}, Mg^{2+}, or Zn^{2+}, the dried gels also consisted of small whitish chunks and powder. An interesting trend was observed while processing the gels containing chelated iron. These gels originally possessed a dark brown or blackish color. Upon pulverizing and washing these gels, the color changed to olive and finally a blue-gray color. Drying these gels ultimately yielded small gray chunks. A notable observation was that this trend was observed for complexes that had been formed from both Fe^{+2} and Fe^{+3} ions. The Fe^{+2} and Fe^{+3} ions are typically associated with reddish or orange colors. Thus it is interesting that both ions result in the same gray color.

Nonetheless, the isolation of these colored gels was a significant event in our research. Of primary importance is the fact that these were the only gels produced in our laboratory that possessed a distinct color after being processed and dried – readily indicating the incorporation of some type of material into the silica gel. The IR analysis did not indicate the presence of organics, which suggests that the color is due to the presence of metal ions in the gel. Therefore, this discovery is important because it suggests a method for the introduction of metal ions into silicates. This brings new light to the debate on the formation of rock coatings and desert varnish (Perry and Kolb, 2004; Perry et al., 2005), which are of astrobiological significance since their presence is suspected on Mars.

Like the gels from the amino acids and the Maillard mixtures, noticeable sol-gel-sol transformations were observed. Often these transformations were accompanied by gels that became very compact, as well as an increase in the amount of liquid present in the experimental vials. Overall, the Maillard-metal complexes appear to catalyze the polymerization of sodium silicate solution. While it appears that the entombment of metal-ions is possible, it does not appear that the organics follow this mechanism.

4.5. SUGARS AND SUGAR ALCOHOLS

Like amino acids, sugars play an important role in life as we know it. As any student of introductory biology or biochemistry can explain, sugars are an excellent

energy source, make up part of our genetic material, and play key roles in a plethora of metabolic pathways (Lehninger et al., 2004). Sugars also play key roles in cellular recognition and provide structural support (Lodish et al., 2004). While structurally similar to sugars, sugar alcohols differ due to the reduction of their carbonyl groups to hydroxyl groups. In addition to their biological relevance, sugar-like compounds have been identified on meteorites (Cooper et al., 2001), thus establishing their astrobiological relevance. Some aspects of sugar-silicate interactions have already been studied (Kubicki and Heaney, 2002; Lambert et al., 2004).

Unlike the other classes of organic compounds already discussed, the sugars and sugar alcohols interact with sodium silicate gradually to form gels over a matter of days. These gels are typically soft, and some sol-gel-sol transformations have been observed. In the cases involving sugar alcohols, the sol-gel-sol transformations could be quite dramatic as these gels were water soluble. Often, a darkening can be observed in the solutions and gels resulting from sugars or sugar alcohols. Any traces of color seemed to be eliminated during the washing process, as the dried gels consist of small whitish chunks.

Taken as a whole, it appears that sugars and sugar alcohols merely catalyze the polymerization of sodium silicate solution. Entombment of organics seems unlikely as no organics have been observed in our IR spectra to date. While we observed no organics in the IR spectra, previous studies actually suggest a mechanism of covalent bond formation—not in the solid state, but as aqueous hypercoordinate chemical species (Kubicki and Heaney, 2002; Lambert et al., 2004). Thus, while the focus of our research has been on the mechanisms of silicification in the solid-state gels, it is possible that in addition to catalysis or entombment the mechanism of covalent bond formation is also occurring to produce water-soluble organo-silicates.

4.6. ALCOHOLS

The low molecular weight alcohols, such as methanol and ethanol, have been identified in interstellar clouds (Cronin and Chang, 1993). Alcohols through C-4 were found in aqueous extracts from meteorites such as Murchison and Murray meteorites (Cronin and Chang, 1993; Cronin, 1998). In addition, branched-chain isomers have been found to be more abundant than the straight-chain isomers. In general, on meteorites, there is a predominance of branched carbon chains over the straight ones, suggesting a process in which radical or ion stability of the intermediates may have been influential in the synthetic processes (Cronin, 1998). We were thus very interested in studying alcohols.

We have found that of all the compounds investigated in our lab, the alcohols were perhaps the most interesting and puzzling. The interaction with silicates had been hinted at in the literature (Azrak and Angell, 1973; Sweryda-Krawiec et al., 1999) but research investigating the interactions of alcohols with sodium silicate is rare in the literature. In cases where the interactions between alcohols and sodium silicate had been studied, work had been done entirely in the aqueous state, and no gels had been isolated (Samadi-Maybodi et al., 2001). We have

investigated nearly a dozen different alcohols of varying structures, and have found that smaller alcohols with more branched or compact structures are more effective in causing silica polymerization. This may have been a factor in the possible alcohol-silicate processes on meteorites. The alcohol silicates revealed unique physical and spectral properties (Liesch and Kolb, 2007a).

Upon the addition of a small amount of an alcohol to the sodium silicate solution, the two substances appear to be fully miscible. However, with a greater volume of alcohol, the mixture begins to separate into two distinct layers. The lower whitish layer consists of partially polymerized silicate, which is fluid in nature and has a low viscosity. By adding additional alcohol, the lower layer eventually solidifies into a white, soft, rubbery gel. We have described this volume-dependent trend in further detail in the literature (Liesch and Kolb, 2007a). Curiously, the alcohol gels were the only ones from our laboratory where we could feasibly control the viscosity and extent of polymerization by changing the volume of the alcohols added.

Processing and drying these gels presented quite a challenge. The water-soluble nature of the gels and the dramatic gel-sol transformations observed while processing these gels suggests that sol-gel-sol transformations could play a major role with the preservation and transport of alcohols.

Interestingly, even the dried gels behaved unlike any other gels studied in our lab. These dried gels were opaque and rubbery, yet were not entirely solid. Within days, chunks of gel would go from having crisp, distinct edges to having rounded edges. Ultimately, this gradual loss of integrity led to the fusion of chunks into a single large mass (Liesch and Kolb, 2007a). Analysis of these unique gels indicated no organics, within the detection limit of our IR instrument. It appears that alcohols do catalyze the polymerization of sodium silicate. It may be possible that water-soluble organo-silicates are formed in the process (Samadi-Maybodi et al., 2001; Lambert et al., 2004), but are largely removed by washing gels with water. This possibility has yet to be fully investigated.

In addition to the volume-dependent trends and water-solubility, the gels from alcohols were unique in terms of their IR spectra. As summarized later in Table 2, the Si-O-Si band can be typically found just under 1,100 cm^{-1}. In gels formed from the alcohols, this band had been dramatically shifted by over 50 cm^{-1}, in some cases towards lower wavenumbers (Liesch and Kolb, 2007a). Dramatic shifts in the location of the Si-O-Si IR band were seen only in the gels of alcohols and, to a lesser extent, in the gels of the amino alcohols and the Maillard-metal complexes. The nature of these shifts has not been elucidated, but the shifts can be used as an empirical tool for the cataloguing and characterizing these gels.

4.7. AMINO ALCOHOLS

After reviewing the work of Azrak and Angell (Azrak and Angell, 1973), which suggested that amino alcohols may covalently bond more readily with silicates than regular alcohols, it was only logical to investigate amino alcohols in addition

Table 2. Summary of the interactions of various organic compounds with sodium silicate.

Class of organic molecule	Notes
Amino acids (AA)	**Mechanism**: Catalysis. Entombment possible in trace amounts; low levels of organics detected in some cases.
Biological AA	Gel formation and appearance: Immediate formation of rubbery or hard whitis
Meteoritic AA (Sarcosine a nd 5-aminovaleric acid)	h opaque gels. Gels are not water-soluble. Dried gels consist of small white chunks and powder. **Sol-gel-sol transformation**: Some transformations were seen. **Gel formation and appearance**: Sarcosine behaved similarly to the biological A.A . 5-Aminovaleric acid did not result in immediate gel formation. Gel isolated from 5-aminovaleric acid was water-soluble. **Sol-gel-sol transformation**: Some transformations were seen with sarcosine.
Maillard mixtures	**Mechanism**: Catalysis. Entombment possible in trace amounts; low levels of organics detected in some cases. **Gel formation and appearance**: Rubbery gels quickly form, often within a minute or less. Gels are originally whitish but darken to orange and brown within days. Dried gels consist of white chunks and powder. **Sol-gel-sol transformation**: Major transformations were seen.
Maillard-metal complexes	**Mechanism**: Catalysis. No organics detected in the IR spectra. **Gel formation and appearance**: Soft gels quickly form, often within seconds, but may take up to 30 minutes to form. Gels are brownish. Dried gels from Fe-containing complexes were gray in color. All other metals resulted in whitish dried gels. **Sol-gel-sol transformation**: Major transformations were seen. **Other**: The gels from Fe-containing complexes were the only ones in our laboratory that kept a distinct color after being dried.
Sugars and sugar alcohols	**Mechanism**: Catalysis. No organics detected in the IR spectra to suggest otherwise. C-13 and Si-29 NMR studies suggest covalent bond formation occurs leading to the formation of water-soluble organo-silicates. **Gel formation and appearance**: Soft, transparent gels form slowly and darken over hours and days. Gels partially water-soluble. Dried gels consist of small white chunks. **Sol-gel-sol transformation**: Some transformations were seen.
Alcohols	**Mechanism**: Catalysis. No organics were detected in the IR spectra. **Gel formation and appearance**: Gel appearance depended upon the volume of alcohol added. Initially a low-viscosity gel formed. Firm, rubbery gels could be formed upon adding an additional amount of alcohol. Gels are partially water-soluble. Dried gels were opaque and rubbery. These gels lost their integrity upon standing and several chunks would fuse together to form a single mass. **Sol-gel-sol transformation**: Unique transformations were seen related to viscosity trends and water-solubility. **Other**: The volume-dependency and appearance of these gels was unique. Small alcohols with more compact structures are more efficient in the silica polymerization.

(continued)

Table 2. (continued)

Class of organic molecule	Notes
Amino alcohols	**Mechanism**: Entombment or covalent bonding are possible. Organics were observed in IR spectra. Si-O-C IR bands were observed in some cases. **Gel formation and appearance**: Immediately formed hard, white gels. Needed a very small volume to form gel compared to an alcohol of similar molecular weights. Gels were partially water-soluble. Dried gels consist of hard, rubbery, opaque chunks. **Sol-gel-sol transformation**: Some transformations were seen related to water-solubility. **Other**: One of the rare cases (in addition to acid halides) where Si-O-C bond formation was observed in the IR.
Acid halides	**Mechanism**: Varies. Catalysis was observed in some cases, while the strong presence of organics and the Si-O-C IR band indicates covalent bond formation in others. **Gel formation and appearance**: The length of time required to form a gel varies from seconds to days depending on the compounds tested. Gels are yellowish, and partially water-soluble in some cases, depending on the nature of the acid halide. Dried gels consisted of small white chunks. **Sol-gel-sol transformation**: Some transformations were seen. **Other**: These and amino alcohols are the only compounds that gave the IR evidence for the formation of Si-O-C covalent bonds.
Other key biomolecules	**Mechanisms**: Not yet determined. For ATP and DNA soluble phospho-silicates are possibly formed.
Urea	**Gel formation and appearance**: No gel formation.
AMP	**Gel formation and appearance**: Soft, whitish gels form quickly within seconds. Gels highly water-soluble. In some cases, gel entirely dissolved within a 24–48 hours of forming.
ATP	Sol-gel-sol transformation: Dramatic transformations were seen related to water-solubility, preventing analysis at this point in time
DNA	**Gel formation and appearance**: No gel formation. **Gel formation and appearance**: No gel formation.

to the other alcohols we had already studied. Some notable amino alcohols, such as ethanolamine, have long been known to be important metabolic precursors (Weissbach and Sprinson, 1952).

Additionally, polyamines assist condensation of silica in diatoms (Pohnert, 2002), which make great biomarkers. Amines alone effect condensation of silica in model systems in which the naturally occurring silica was replaced by tetraethoxysilane under circum-neutral pH values (Delak and Sahai, 2005, 2006). The latter studies suggest the nucleophile-driven reaction mechanism, of an S_N2 type.

We have found that the amino alcohols behaved strikingly different from the alcohols we studied (Liesch and Kolb, 2007a). In some ways, the amino alcohols reacted more like amino acids than alcohols. For example, the sodium silicate polymerized nearly instantly upon addition to form hard white gels. These gels were unexpectedly the hardest produced in our laboratory to date. In fact, on at least one

occasion, an experiment needed repetition because the vial had broken while trying to scrape the gel from the inner surface. Furthermore, the amino alcohols did not demonstrate the volume-dependent trend seen in the alcohols. Interestingly, alcohols required the addition of up to ten times the volume of an amino alcohol of comparable molecular weight to form a firm gel (Liesch and Kolb, 2007a).

As in the case of alcohols, the gels from amino alcohols were somewhat water-soluble and dissolved partially while being processed. After processing and drying these samples, the resulting gels consisted of hard, rubbery, opaque chunks. These dried gels were quite similar to the alcohol gels in appearance. However, a key difference from the alcohol gels was the confirmed presence of organics in the IR spectra. Not only was this one of the few cases where organics were observed in the IR spectra, but Si-O-C bond formation was also observed in the case of ethanolamine. Thus, for the amino alcohols, entombment and covalent bond forming mechanisms may be at work in addition to merely catalyzing the polymerization of the sodium silicate (Liesch and Kolb, 2007a).

4.8. ACID HALIDES

Acid halides are reactive organic compounds, which we have used as model compounds in order to effect a rapid Si-O-C bond formation. This model was successful, at least in some cases, as we were able to observe high levels of organics in the gels, as well as the formation of Si-O-C bonds (Kolb and Liesch, 2007a).

The gels resulting from different acid halides vary greatly in the length of time required for polymerization to occur. In some cases polymerization occurred nearly instantly, in others it took days (Kolb and Liesch, 2007a). The gels resulting from acid halides were hard, somewhat brittle, and pale yellow in appearance. Like the alcohols, some of these gels were found to have water-soluble properties, and sol-gel-sol transformations were observed.

Like the highly variable temporal aspects of gel formation, the mechanisms responsible for interaction with sodium silicate seem equally as variable. In the case of 2,4-dinitrobenzoyl chloride, organics were clearly seen in the IR spectra as well as Si-O-C bonds – suggesting a mechanism of covalent bond formation. In some other cases, no organics were seen in the IR spectra, and it appears that acid catalysis may cause polymerization of the highly basic sodium silicate solution in some cases, as the formation of the corresponding organic acids was observed in some cases (Kolb and Liesch, 2007a). At this point, we believe that the miscibility of the individual acid halides may play the greatest role upon determining which mechanism is involved in gel formation.

4.9. BIOMOLECULES: UREA, AMP, ATP, AND DNA

This last section briefly discusses some of the most peculiar and least understood cases examined in our laboratory. These biomolecules serve key roles in metabolic

pathways, and the storage of genetic information (Lehninger et al., 2004). While some of these experiments may be considered "failures" on the grounds that polymerization did not occur under our experimental conditions, vital information can still be gained from the study of these compounds. Take DNA for example: using <50 bp oligonucleotides extracted from Herring sperm, we found that polymerization and subsequent gel formation did not occur (Kolb and Liesch, 2007a). The solubility of DNA–silicate complex remains a possibility. An intriguing possibility is a formation of the soluble DNA-phospho-silicate complex, such as the proposed phospho-silicate gel of the phytic acid, a compound that is derived from myo-inositol (an isomer of the hexa-hydroxy cyclohexane in which all six hydroxyl groups are phosphorylated) (Samba-Fouala et al., 2000). The solubility of the DNA complex may be a plus, since the precipitated or bound polymer could no longer function as an effective template for replication (Liesch and Kolb, 2007c).

As in the case of DNA oligonucleotides, we did not observe any gel formation with either urea or ATP. These substances resulted only in clear, colorless solutions. Urea may make soluble silicates, and the ATP may form soluble phospho-silicates. Unlike the DNA, ATP, and urea, AMP did result in the polymerization of the sodium silicate solution and led to the formation of soft, whitish gels. Dramatic sol-gel-sol transformations were also observed in the AMP gels. In some cases, transformations were so dramatic that the gels completely transformed back to the sol phase and processing could not be conducted. When we finally managed to extract a gel from AMP, we found that these gels were highly water-soluble (Kolb and Liesch, 2007a). In fact, these gels completely dissolved during processing, which prevented the final isolation and drying of the gel, and ultimately prevented the analysis of the gel via infra-red spectroscopy. The inability to study the solid gels therefore leaves the mechanisms of interaction as an open question at this point in time.

In conclusion of this section, our initial results show that the silicification of the biologically relevant molecules that are associated with the genetic material (ATP, AMP, and DNA) do not yield solid gels, and are thus not expected to be preserved via the entombment mechanism, at least not in our experimental model. The same is true for urea, which is also found in biological systems.

5. Conclusions and Summary

Our lab has made great advances in the study of the silicification process over the past few years. The determination of the exact range of the Si-O-C IR band, and the ability to determine and distinguish between the mechanisms of silicification may provide crucial insight in helping achieve the goals set forth by NASA's astrobiology strategy (Morison, 2001; NASA, 2007a). We have gained a great deal of knowledge on the aspects of cataloging biosignatures, determining what characteristics of the Martian landscape may be due to non-biological processes, and showing that certain biosignatures may actually be inorganic in nature (such as

the highly dramatic Si-O-Si band shift seen with alcohols). Perhaps our greatest achievement has been to survey a broad spectrum of organic compounds, identifying key characteristics of each, and summarizing our findings for the advancement of the study of biosignatures. Our key findings are summarized in Table 2.

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MICROFOSSIL PHOSPHATIZATION AND ITS ASTROBIOLOGICAL IMPLICATIONS

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1. Introduction

One of the major tasks of astrobiology is to critically examine evidence of past or present ecosystems beyond our planet. As Earth is the only planet that is known to have hosted life, perhaps as early as 3.8–3.5 billion years ago (or Ga, *Giga anna*) as illustrated by biologically-meaningful carbon isotopic signatures and prokaryotic microfossils (Mojzsis et al., 1996; Schopf, 2006; but see van Zuilen et al., 2002; Brasier et al., 2006; Fedo et al., 2006), it provides the only model for us to learn how traces of life can be preserved and recognized. In this contribution, we focus on fossil preservation through phosphate mineralization and discuss its implications for the identification of possible life (particularly ancient life if it did exist) on other planets.

If cellular organisms had once existed on other planets, by either independent origin or shared descent with life on Earth, they were most likely to be microscopic, soft-bodied life forms akin to microorganisms (microbial life: prokaryotes, protists, and microalgae) that have populated various environments on Earth. This inference is based on two arguments. First, the striking ecological and metabolic diversity of microbial life on Earth (Nisbet and Sleep, 2001) indicates that it has a better chance populating extreme environments on other planets. Second, consideration of phylogeny and morphological complexity requires that the earliest life be represented by simple forms that are microscopic, unicellular (single-celled), and lack skeletons. Indeed, the evolutionary history on Earth shows that non-skeletal microbial life preceded macroscopic life by slightly more than 1.5 billion years, so that the Archean–Proterozoic biosphere (3.5–0.54 Ga) was entirely dominated by microorganisms. Macro-organisms visible to naked eye (e.g., *Grypania*) did not appear until about 1.9 billion years ago (Han and Runnegar, 1992; Fralick et al., 2002; Schneider et al., 2002) and they did not become ecologically dominant until the Proterozoic–Cambrian transition at about a half billion years ago. Similarly, the Archean-Proterozoic biosphere consisted almost exclusively of non-biomineralizing, soft-bodied organisms; biologically controlled mineralization evolved in only a few Neoproterozoic (1.0–0.54 billion years ago) taxa (Allison and Hilgert, 1986; Grant, 1990; Grotzinger et al., 2000; Porter and Knoll, 2000; Wood et al., 2002; Hua et al., 2005) and did

not become widespread until after the Cambrian Explosion (Bengtson, 1994; Knoll, 2003). Thus, the Precambrian world serves as a plausible model in the search for ancient ecosystems on other planets.

In this contribution, we ask the question how morphological evidence (as opposed to geochemical evidence) of microbial life – if it did exist – would be best preserved in extraterrestrial environments. We approach this question by briefly reviewing the taphonomic pathways in the Proterozoic (2.5–0.54 billion years ago) fossil record. This is followed by a more detailed analysis of three-dimensional phosphatization of non-biomineralizing microorganisms in the Neoproterozoic Doushantuo Formation. We focus on the phosphatization window of the Doushantuo Formation because it represents one of the most powerful taphonomic pathways through which soft-bodied microorganisms can be preserved. We then close our chapter by discussing the astrobiological relevance of phosphatization.

2. Preservation of Proterozoic Fossils

In contrast to the Phanerozoic fossil record that is characterized by macroscopic skeletal fossils, the Precambrian fossil record is dominated by microscopic, soft-bodied organisms. Thus, from a Phanerozoic point of view, the fossilization of such organisms is regarded as exceptional preservation. Butterfield identified six different taphonomic styles of exceptional preservation of Proterozoic–Cambrian non-biomineralizing organisms (briefly reviewed below, Fig. 1), which are named after well known biotas that exemplify each of these taphonomic styles (Butterfield, 2003).

Bitter Spring-type preservation is characterized by silicification of microorganisms in peritidal cherts (Schopf, 1968; Knoll, 1985; Zhang et al., 1998). Classical silicified biotas, for instance the Bitter Spring assemblage, typically show evidence of cellular preservation of microbial communities dominated by prokaryotic forms such as cyanobacteria, although unicellular and multicellular eukaryotes are often preserved as well (Xiao, 2004). In marine environments, this taphonomic window was open in its fullest in the Precambrian, particularly in the Proterozoic, most likely because of the higher availability of dissolved silica in marine waters before the rise of silica biomineralizers such as hexactinellid sponges, demosponges, and diatoms (Maliva et al., 1989, 2005).

Doushantuo-type preservation is known for the exquisite phosphatization of mostly eukaryotic microorganisms that thrived in shallow subtidal environments (Xiao and Knoll, 1999; Dornbos et al., 2006; Hagadorn et al., 2006). Often, labile cellular and subcellular structures are preserved in Doushantuo-type phosphatization. Similar to Doushantuo-type preservation is Orsten-type preservation, which represents a taphonomic pathway in which more recalcitrant tissues, such as ecdysozoan cuticles, are preserved through phosphatization (Müller, 1985; Walossek, 2003). The Orsten biota, however, is distinct from the Doushantuo biota in its carbonate (as opposed to phosphorite) depositional setting and the

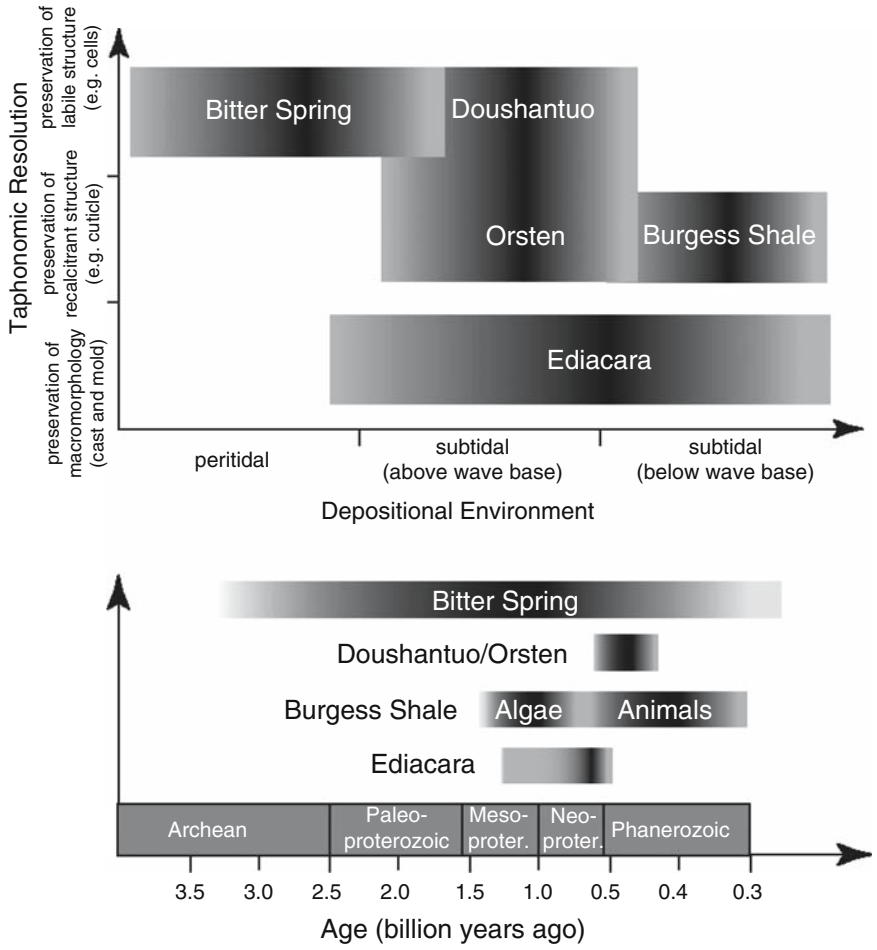

Figure 1. Schematic summary of preservation resolution, temporal distribution, and environmental distribution of various taphonomic pathways (Butterfield, 2003).

lack of phosphatization of more labile substrates such as cellular and subcellular structures. Some phosphatized biotas in Cambrian successions (Zhang and Pratt, 1994; Bengtson and Yue, 1997; Dong et al., 2004) may be considered as transitional taphonomic windows that bridge the end members of the Doushantuo and Orsten biotas, because they show evidence of both cellular and cuticular phosphatization. When considered together, the two phosphatization windows seem to be open only in the Ediacaran and early Paleozoic (Donoghue et al., 2006).

Beecher's trilobite-type preservation represents pyritization of relatively recalcitrant tissues, for example chitinous arthropod cuticles (Briggs et al., 1991) and cellulose-based cell walls (Yuan et al., 2001; Grimes et al., 2002). The preservational

resolution of this taphonomic window is limited by crystal size of authigenic pyrite and controlled by the balance between organic degradation through bacterial sulfate reduction (as a source of sulfide) and authigenic precipitation of pyrite. The temporal and environmental occurrences of this taphonomic window are not well characterized, but are potentially widespread given that pyrite formation is sensitive to local geochemical conditions rather than global secular trends (Rickard et al., 2007). Possible Proterozoic examples of this type of preservation include pyritized chuarid vesicles (Yuan et al., 2001) and pyritized tubes (Cai and Hua, 2007) in Ediacaran successions of South China.

The four preservational pathways discussed above are collectively known as permineralization – the preservation of soft tissues with three-dimensional detail by authigenic minerals (Briggs, 2003). In contrast, Burgess Shale-type preservation is characterized by two-dimensional compression and preservation of more recalcitrant tissues as carbonaceous films (Butterfield, 1995; Gaines et al., 2005), typically on bedding planes of fine-grained sediments deposited in deep-water environments below fair weather wave bases. Several mechanisms have been proposed to explain Burgess Shale-type preservation, although these need not to be mutually exclusive. Some argue that authigenic aluminosilicate minerals may have played a role in delaying organic degradation, therefore promoting organic preservation (Butterfield, 1995; Orr et al., 1998). But recent investigation of the Chengjiang biota – an early Cambrian example of Burgess Shale-type preservation – seems to indicate that pyrite mineralization was at least partly responsible for the exceptional preservation of soft tissues (Gabbott et al., 2004; Zhu et al., 2005). These authors argue that the degradation of more labile tissues by sulfate reduction bacteria provides a source of hydrogen sulfide (H_2S), which in the presence of reactive iron (Fe) would promote pyrite mineralization and the preservation of more recalcitrant soft tissues. Burgess Shale-type preservation is most common in the Cambrian, but also occurs in the Proterozoic (Xiao et al., 2002) and post-Cambrian Paleozoic (Butterfield, 1995). The secular trend of Burgess Shale-type preservation may be controlled by several factors, two of primary importance may be clay mineral geochemistry (Butterfield, 1995; Orr et al., 1998) and bioturbation (Allison and Briggs, 1993; Orr et al., 2003).

Finally, the Ediacara-type preservation is a non-actualistic taphonomic window, characterized by the casting and molding of macroscopic organisms in siliciclastic rocks, including sandstones. Microbial mats, through the formation of a "death mask" on degrading Ediacara organisms and the promotion of mineralization beneath microbial mats, may be responsible for the casting and molding of Ediacara fossils in South Australia (Gehling, 1999). Other Ediacara fossils were probably "masked" by volcanic ashes (Narbonne, 2005) or event deposits of fine-grained silts (Xiao et al., 2005). Ediacara-type preservation mostly occurs in Ediacaran rocks, although rare occurrences in Phanerozoic and Mesoproterozoic successions have been reported (Jensen et al., 1998; Hagadorn et al., 2000; Zhang and Babcock, 2001; Fedonkin and Yochelson, 2002).

From an astrobiological perspective, the Bitter Spring-, Doushantuo-, and Orsten-type preservation pathways are most important because of their likelihood of capturing morphological information about microscopic organisms consisting of more labile organic structures. In the following section, we will take a closer look at the Doushantuo-type preservation at its type locality.

3. Phosphatization in the Neoproterozoic Doushantuo Formation

The late Neoproterozoic phosphorite of the Doushantuo Formation at Weng'an, South China, hosts some of the best preserved microfossils, providing both cellular and subcellular insights into a variety of eukaryote organisms, including animals and algae (Xiao et al., 2004; Hagadorn et al., 2006). Because the Doushantuo Formation at Weng'an hosts the earliest record of animal life in the form of fossilized metazoan embryos (however, see Bailey et al., 2007 for an alternative interpretation and Xiao et al., 2007 for a rebuttal), past investigations have focused primarily on their evolutionary significance (Xiao et al., 1998). Only a few studies were designed specifically to understand the taphonomy of the Weng'an biota (Xiao and Knoll, 1999; Dornbos et al., 2005, 2006). The lack of in-depth understanding of Doushantuo taphonomy not only makes the biological interpretation of certain Doushantuo fossils controversial (Chen et al., 2000, 2004; Xiao et al., 2000; Bengtson and Budd, 2004), but also weakens the role of the Doushantuo biota as a model to guide further exploration of Doushantuo-type preservation in other ages and on other planets.

Previous examination (Xiao and Knoll, 1999) using scanning electron microscopy provided evidence that the preservation of Doushantuo microfossils critically depends on two mineralization processes: (1) encrustation through mineral nucleation and precipitation on organic substrates, such as cell membranes, cell walls, and mucus strands; and (2) impregnation of phosphatic minerals within organic substrates. Recent analysis provides further evidence to substantiate these processes.

In our investigation, we extracted Doushantuo microfossils using the standard acid digestion method (dissolution in 10% acetic acid for 3–6 days). Extracted specimens were gold-palladium (Au-Pd) sputter-coated to ~20 nm in thickness and then observed using scanning electron microscopy. We recognize four different styles of phosphate mineralization, each characterized by distinct crystal size, orientation, and organization. These are interpreted as different phosphatization processes related to availability of nucleation sites and degradation of organic substrate. The following sections explore these four styles of phosphate mineralization (Figs. 2–11), including (1) perpendicularly oriented, prismatic apatite crystals; (2) tangentially oriented, bladed apatite crystals; (3) randomly oriented, equant apatite crystals; and (4) phosphatic filaments, rods, and granules.

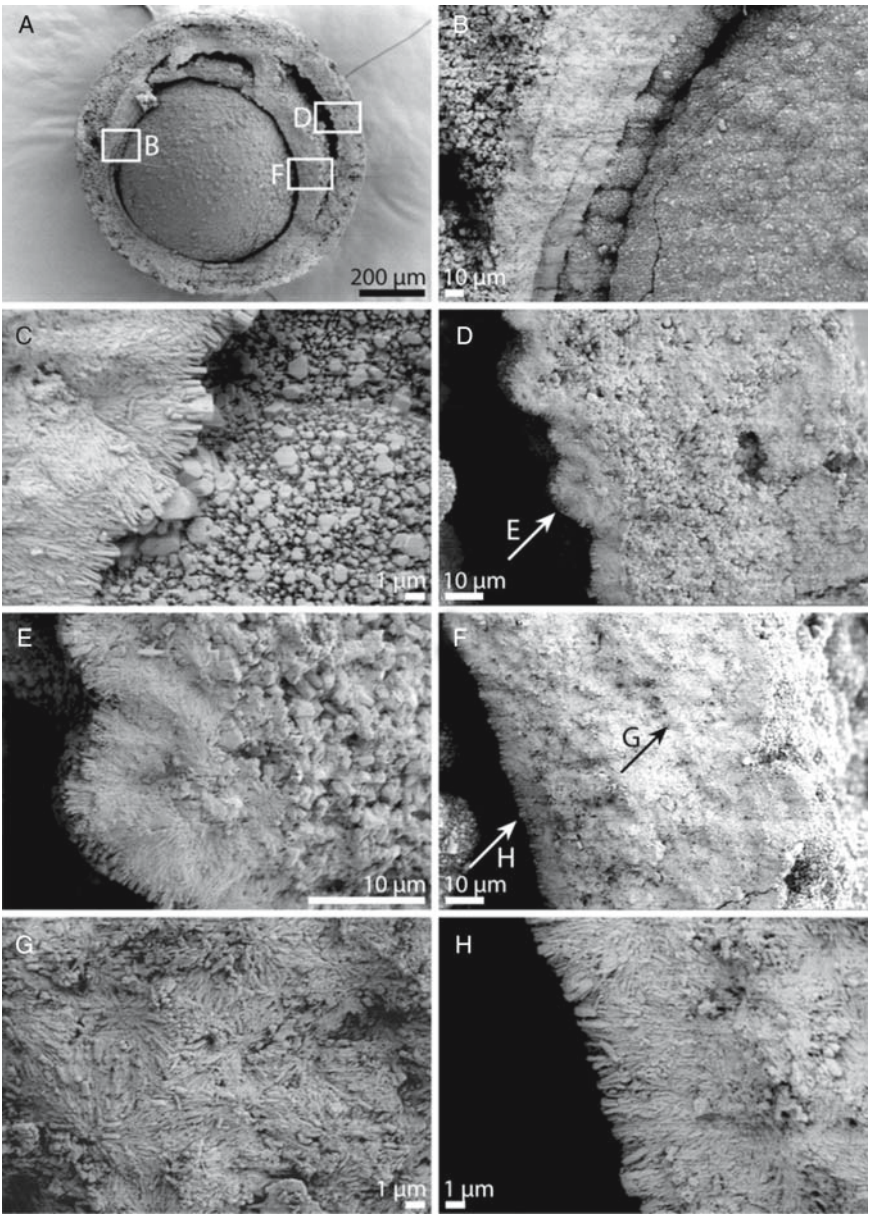

Figure 2. Phosphatic encrustation on *Megasphaera inornata*. (**A**) Eccentric egg cell and phosphatic envelope with uneven thickness. Boxed areas magnified in (**B, D, and F**). (**B**) Botryoidal cements on cell surface and in space between cell and envelope. (**C**) Magnification of (**B**) showing crystal terminations (lower right) and inward growing crystals (upper left). (**D–E**) Botryoids with inward growing crystals. Arrowed area in (**D**) magnified in (**E**). (**F–H**) Isopachs with inward growing crystals (**H**) and randomly oriented crystals (**G**). Arrowed areas in (**F**) magnified in (**G**) and (**H**).

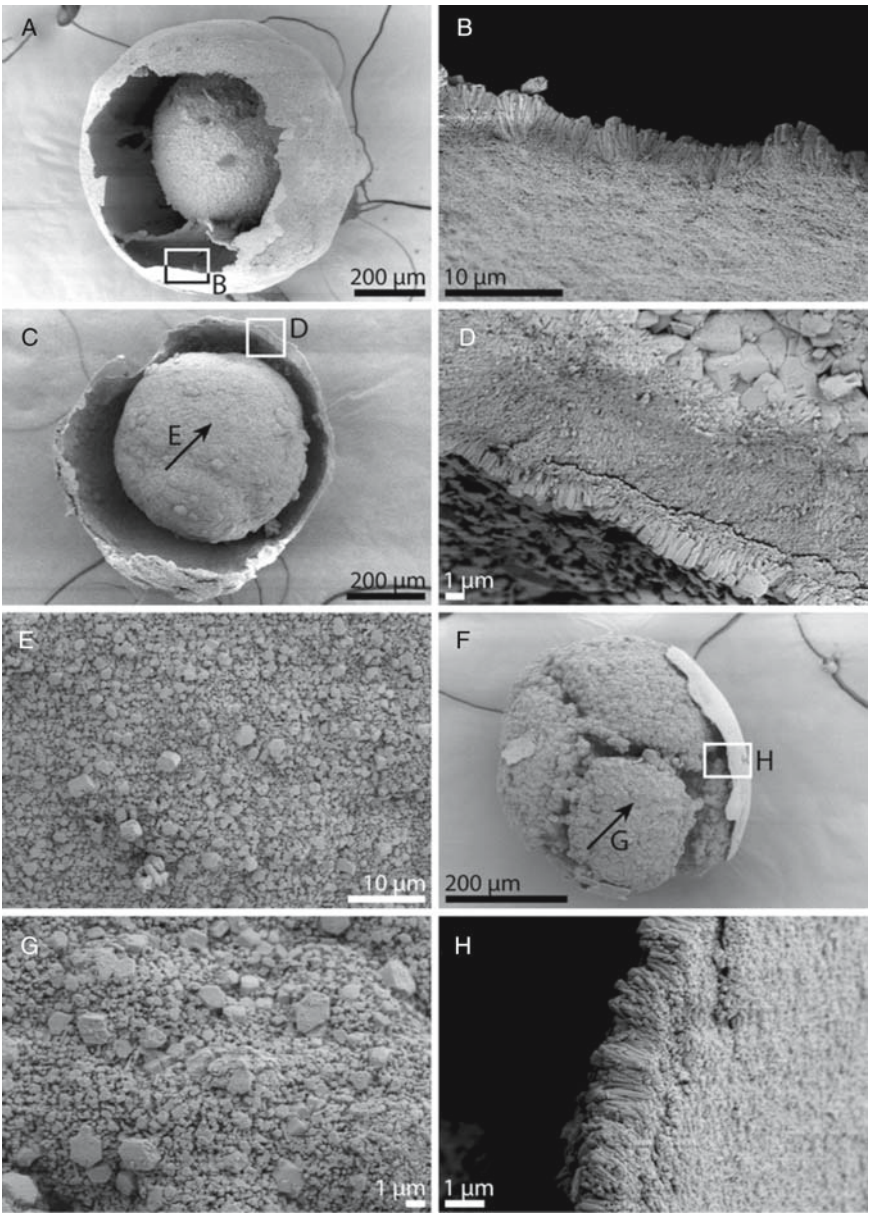

Figure 3. Phosphatic encrustation on *Megasphaera inornata* (**A–E**) and *Parapandorina rhaphospissa* (**F–H**). (**B, D, and H**) are magnified views of boxed areas in (**A, C, and F**), showing isopachous cements with inward growing crystals on inner surface of envelope. (**E and G**) are magnified views of cell surface in (**C and F**), showing distal views of hexagonal crystal terminations.

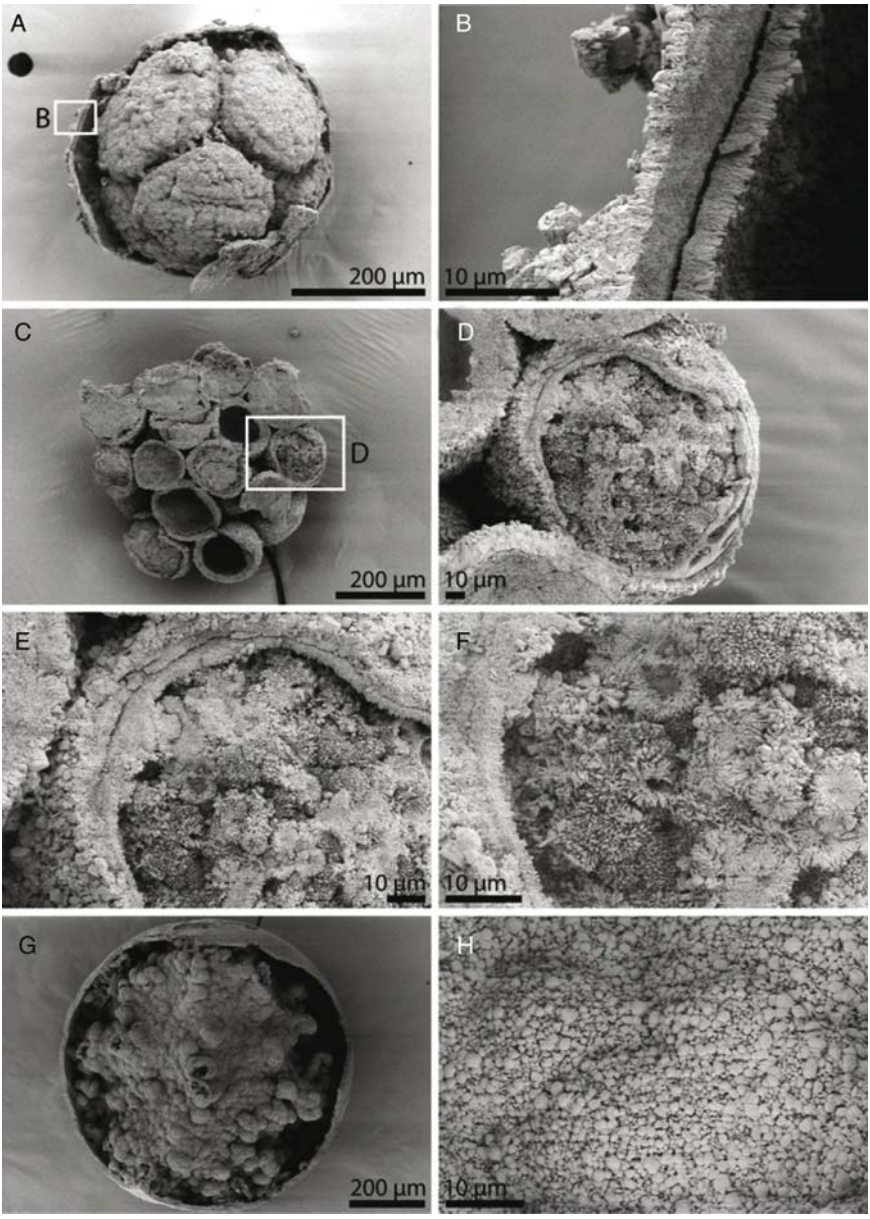

Figure 4. Phosphatic encrustation on *Parapandorina rhaphospissa* (**A–B**), *Megaclonophycus onustus* (**C–F**), and a strongly degraded spheroidal fossil (**G–H**). (**B and D**) are magnified views of boxed areas in (**A and C**), showing isopachous cements on both inner and outer surfaces of envelope/membrane, with crystals growing centrifugally from the original organic envelope that may be represented only by the gap between centrifugally growing cements. (**D**) is further magnified in (**E and F**), showing botryoidal cements within cells as well as isopachous cements on membrane. (**H**) is magnified view of (**G**), showing crystal terminations and botryoidal cements on strongly degraded cellular content.

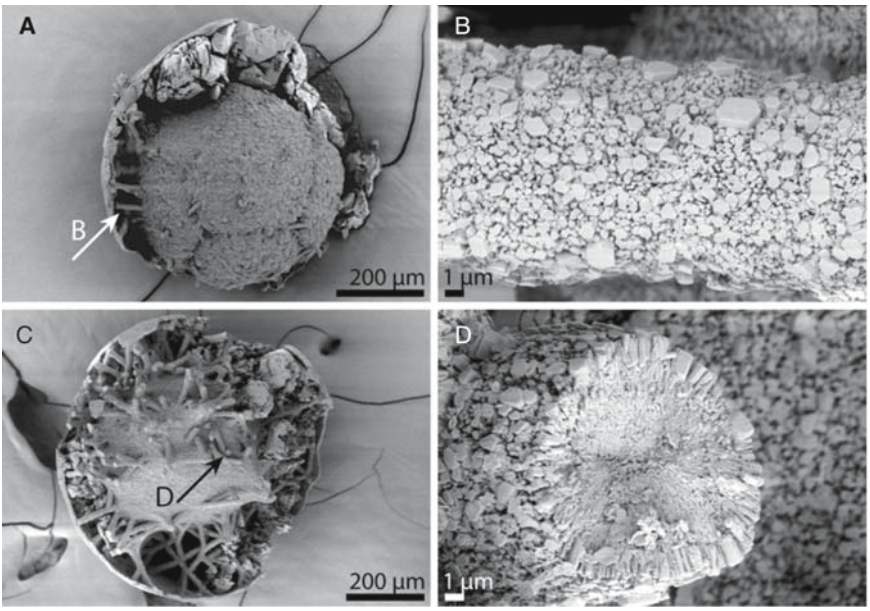

Figure 5. Phosphatic encrustation on organic filaments (mucous strands, bacterial filaments, or fungal hyphae). (**B and D**) are magnified views of (**A and B**), showing external and cross section views of phosphatic filaments. Crystals are oriented radially and coarsen centrifugally.

3.1. PERPENDICULARLY ORIENTED, MICROMETRIC, PRISMATIC APATITE CRYSTALS: PHOSPHATIC ENCRUSTATION ON ORGANIC AND INORGANIC SUBSTRATES

3.1.1. Description

We begin by examining one Doushantuo specimen of *Megasphaera inornata* (Fig. 2A), interpreted as a phosphatized animal egg cell encased within an egg envelope. At a closer look, there is abundant evidence for encrusting apatite crystals of micrometric size (Figs. 2C, E, H). These crystals are often oriented perpendicular to encrusted surfaces, which can be the egg cell surface (Fig. 2B, lower right) or the outer envelope (Fig. 2E). The egg cell is eccentrically located within the envelope and entirely covered with apatite botryoids approximately 10–20 μm in size (Fig. 2B, lower right). There is evidence that the botryoids overgrow on each other and sometimes aggregate to form cauliflower-like structures (Fig. 2B). The botryoids are made of prismatic apatite crystals that are radially oriented. Thus, they appear as hexagonal terminations when viewed distally on botryoid surface (Fig. 2C, lower right). The crystals are prismatic euhedra, typically ~0.09–0.85 μm in width and ~0.40–2.25 μm in length (Fig. 12).

Similarly, the envelope is covered with both botryoidal (Figs. 2D–E) and isopachous apatite cements (Fig. 2H). Thus, the apparent thickness of the phosphatic

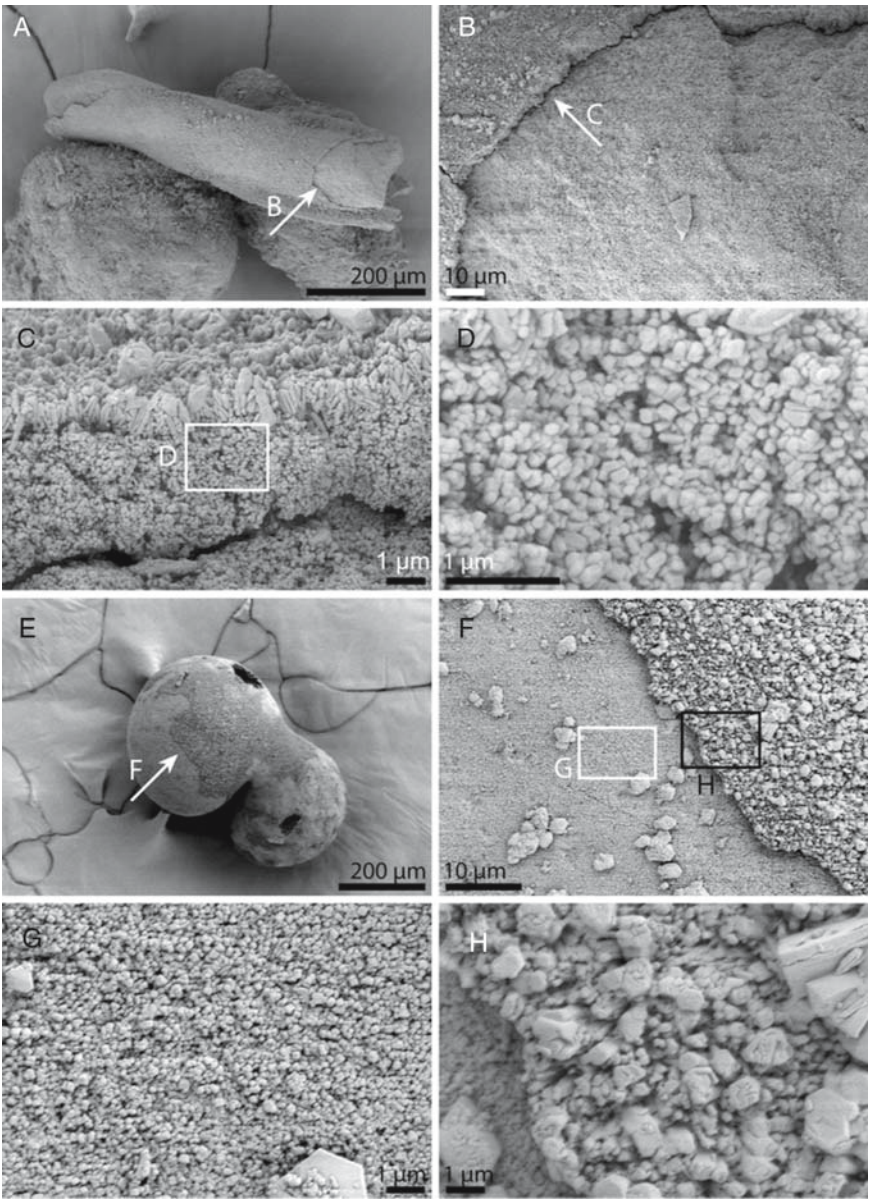

Figure 6. Phosphatic encrustation on a tubular microfossil (possibly *Sinocyclocyclicus guizhouensis*; **A–D**) and a problematic microfossil (**E–H**). (**B–D**) are successively magnified views of (**A**), showing phosphatic encrustation (with perpendicularly oriented crystals) overlying small randomly oriented crystals (**D**), interpreted as phosphatic impregnation of organic tube walls. (**F–H**) are successively magnified views of (**E**), showing phosphatic encrustation (**H**, distal view) mantling small randomly oriented crystals (**G**).

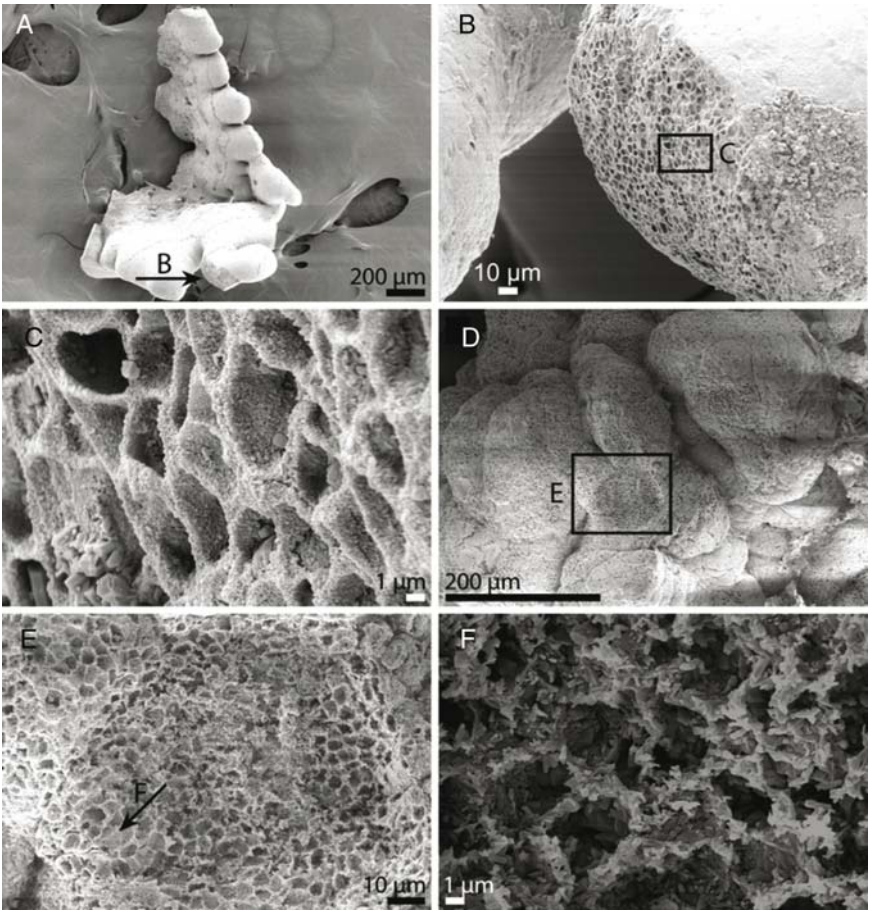

Figure 7. Phosphatization of algal cell walls. (**B and C**) are successively magnified views of (**A**), showing fractured algal thallus with cellular preservation. Small apatite crystals nucleated on cell walls. (**E and F**) are successively magnified views of (**D**), showing poorly organized crystals on cell walls.

envelope depends on the degree of encrustation and may be significantly different from the thickness of the original organic envelope. In the specimen illustrated in Fig. 2, because of the eccentric location of the egg cell and asymmetrical encrustation, its phosphatic envelope is strongly uneven in thickness. Indeed, the phosphatic envelope splits into two parts, creating a cavity between what appear to be layers of phosphatic encrustation (Fig. 2A, upper right). Phosphatic encrustation occurs on all surfaces – the inner and outer envelope surfaces, as well as the walls defining the cavity. These apatite crystals are also approximately 0.09–0.85 µm in width (Fig. 12). They terminate and increase in size toward the cavity (Figs. 2D–E),

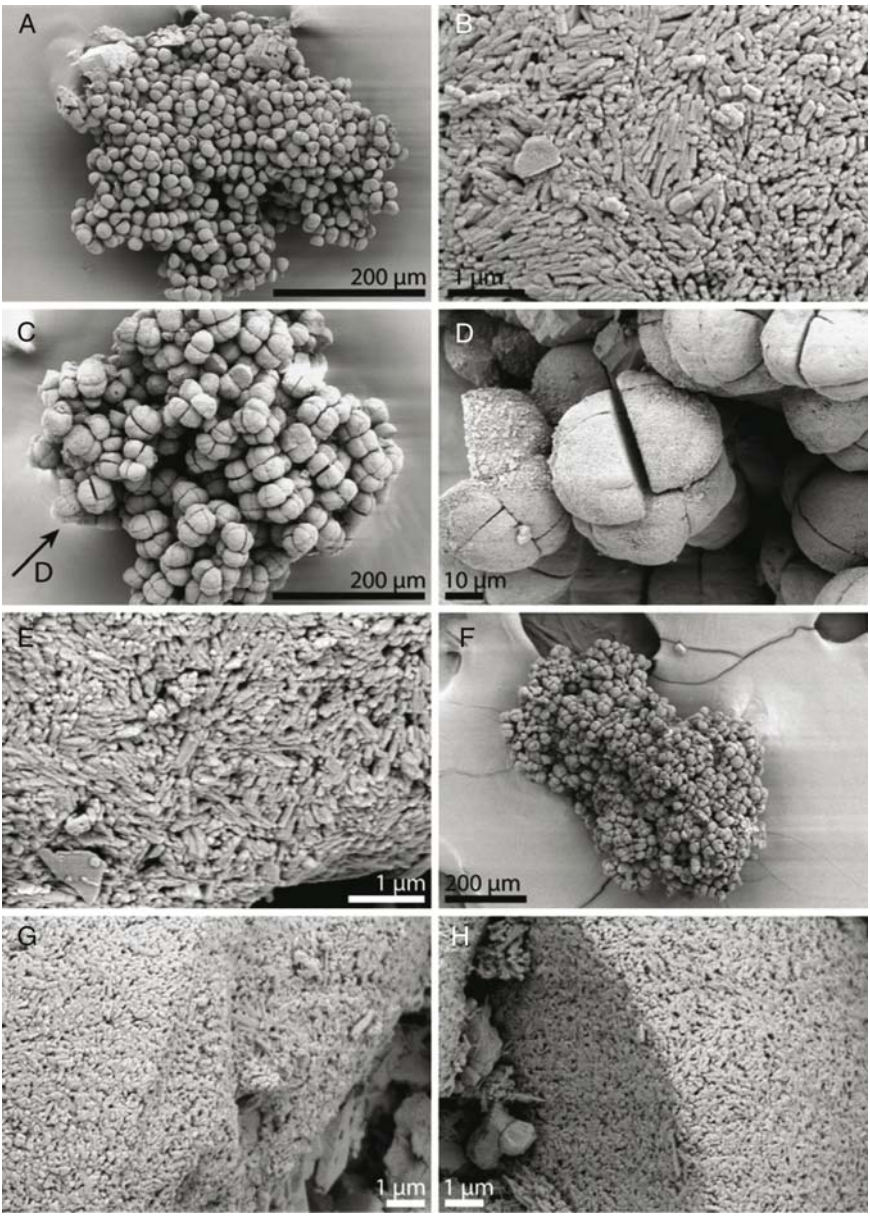

Figure 8. Phosphatic infilling of *Archaeophycus venustus* cells. (**B**) is magnified view of (**A**), (**D and E**) are successive magnifications of (**C**), and (**G and H**) are magnified views of (**F**), showing tangentially oriented crystals.

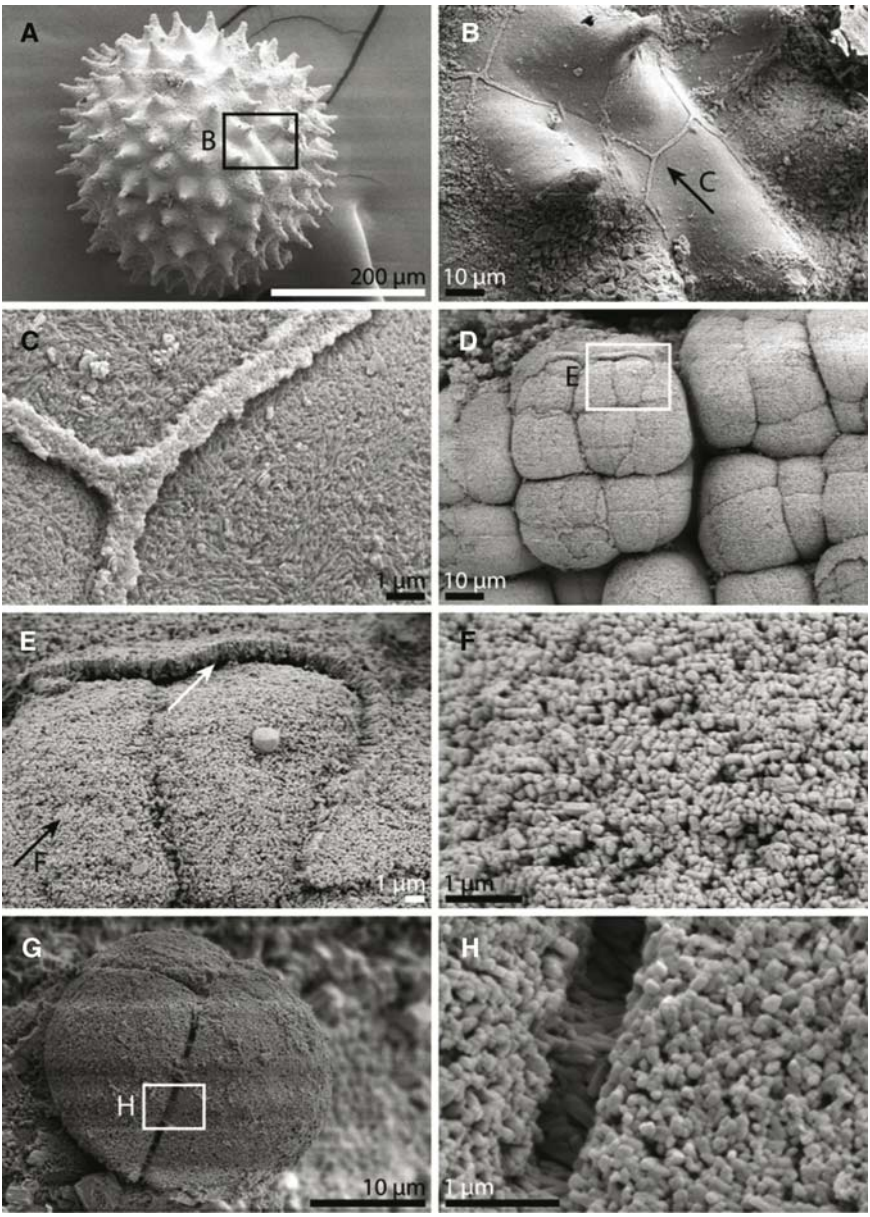

Figure 9. Phosphatic infilling in *Meghystrichosphaeridium reticulatum* vesicle (**A–C**), and phosphatic encrustation (**E**, white arrow) and impregnation (**F and H**) of *Archaeophycus venustus* (**D–H**). (**B and C**) are successive magnifications of (**A**), showing tangentially oriented crystals. (**E and F**) are successive magnifications of (**D**), and (**H**) is magnified view of (**G**), showing randomly oriented sub-micrometric crystals.

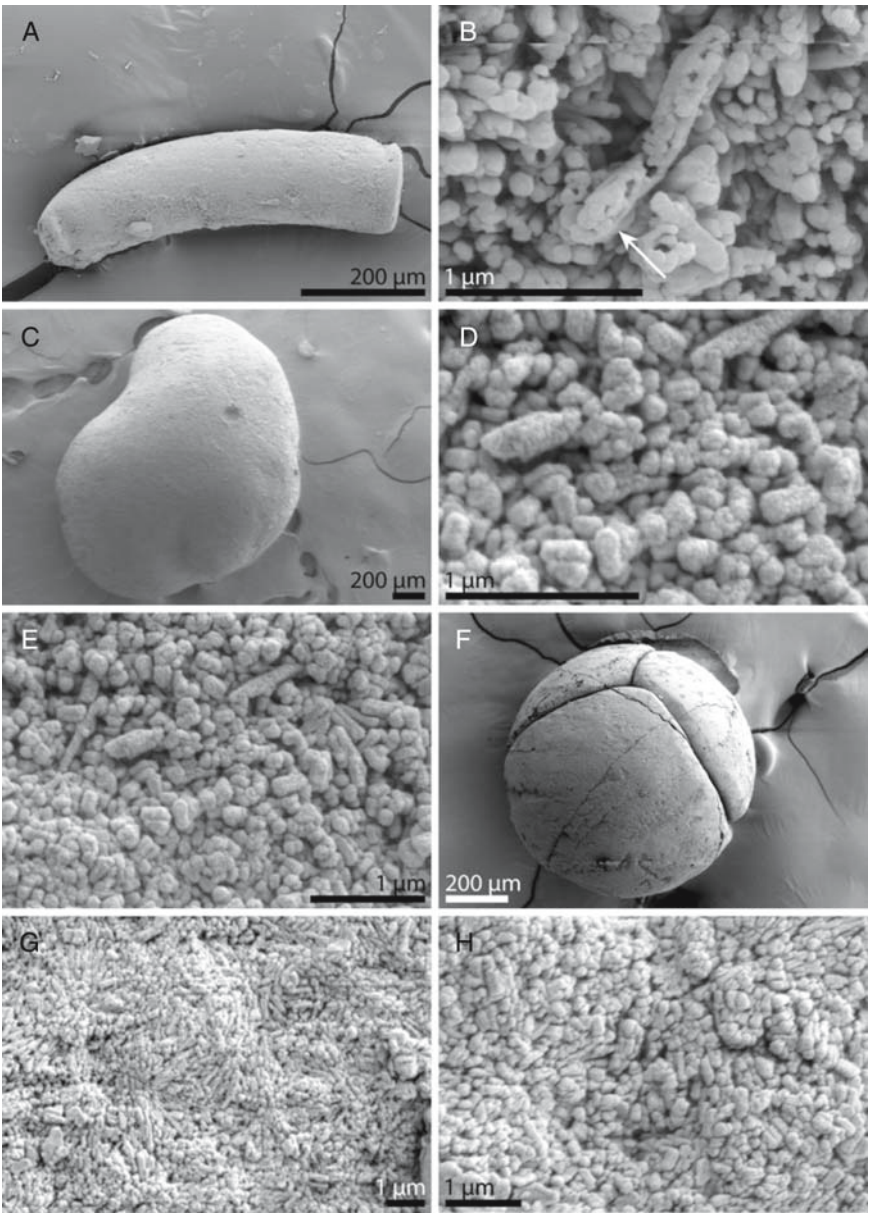

Figure 10. Phosphatic rods (**B**, arrow) and granules (**D–E and G–H**) on a tubular microfossil (possibly *Sinocyclocyclicus guizhouensis*, **A–B**), an unidentified algal fossil (**C–E**), and animal embryo *Parapandorina rhaphospissa* (**F–H**). (**B**) is magnified view of (**A**), (**D and E**) magnified views of (**C**), and (**G and H**) magnified views of (**F**). Note granular texture in (**D–E and G–H**).

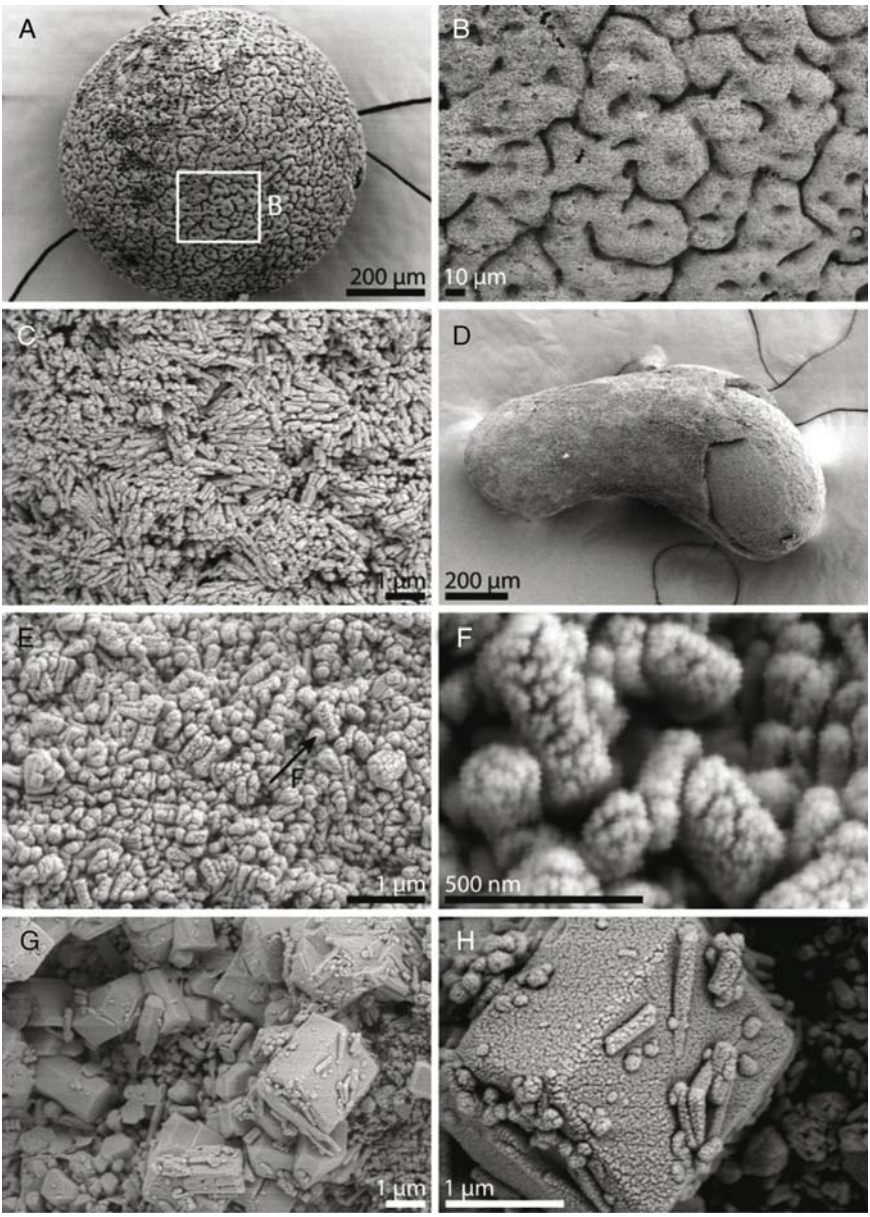

Figure 11. Granules on animal egg/embryo fossil *Megasphaera ornata* (**A–C**) and an algal fossil (possibly *Paramecia incognata*, **D–H**). (**B and C**) are successive magnifications of (**A**). (**E and G**) are magnifications of (**D**), and are further magnified in (**F and H**), respectively. Granular texture best seen in (**F and H**).

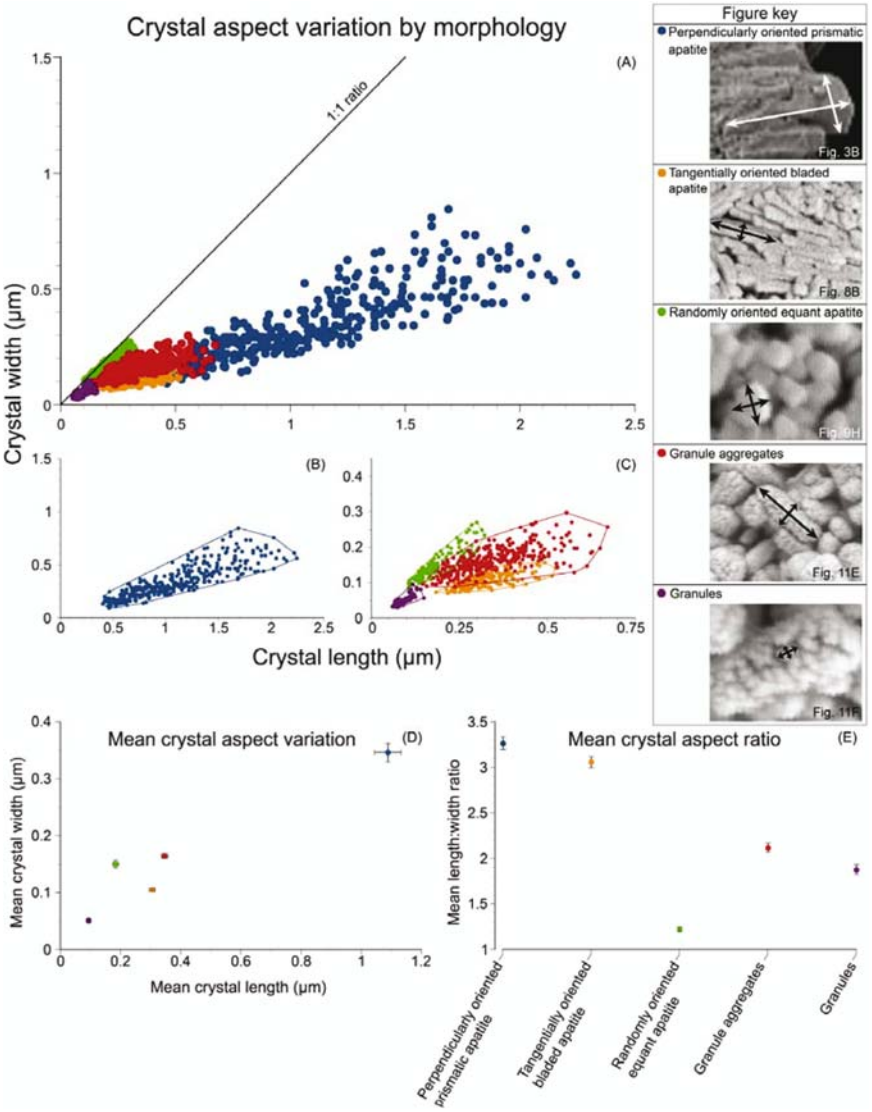

Figure 12. Crystal aspect variation by morphology. (**A**) Length–width plot of four crystal morphologies. Examples of crystal morphologies with marked dimensions and color coding are shown to the right in figure key. (**B**) Length–width plot of perpendicular prismatic apatite crystals. (**C**) Length–width plot of comparatively smaller crystal morphologies (tangential blades, random equant crystals, granule aggregates, and granules). (**D**) Bootstrapped mean crystal length versus mean crystal width, with 95% confidence intervals. (**E**) Bootstrapped length:width ratio with 95% confidence intervals.

toward the egg cell (Figs. 2F, H), and away from the envelope, indicating that they grew as cavity infillings and surface encrustations.

Similar isopachous and botryoidal encrustation occurs in virtually all Doushantuo animal eggs/embryos (Figs. 3–5), tubular fossils (Figs. 6A–D), and multicellular algae (Fig. 7). Apatite crystals are perpendicularly oriented on egg envelope surfaces (Figs. 3B, D, H, 4B), egg/embryo cell surfaces (Figs. 3E, G, 4E), filaments (possibly mucus strands, bacterial filaments, or fungal hyphae; Figs. 5B, D), the surface of a problematic fossil (Fig. 6H), and algal cell walls (Fig. 7C).

It appears that botryoidal and isopachous cementation is selective with respect to encrusted substrate. Botryoidal cements tend to occur on egg/embryo cell surfaces or cell interiors. In some *Megasphaera* specimens, the egg cell was significantly reduced (Figs. 3A, C) or strongly degraded beyond recognition (Fig. 4G). However, their cell surface, regardless of the degree of degradation, is often completely covered with botryoidal apatite cements. Similar botryoidal cements also occur on the cell surfaces of *Parapandorina rhaphospissa* (Figs. 4A, 5A), interpreted as blastula-stage embryos (Xiao and Knoll, 2000). These botryoidal cements are sometimes continuous with phosphatic filaments (Fig. 5). The cells of *Megaclonophycus onustus*, interpreted as possible blastula-stage embryos (Xiao and Knoll, 2000), are also covered with cements (Figs. 4C–E), although these are more isopachous than botryoidal. However, in the same specimen, abundant botryoidal cements occur within cells, as revealed by natural fractures of the cells (Fig. 4F). Additionally, some botryoidal cements also occur on the inner surfaces of egg envelopes (Figs. 2C–E).

In contrast, isopachous cements preferentially occur on egg envelopes, both on the inner and outer surfaces, with perpendicularly oriented crystals growing inward (Figs. 3B, D, H) or outward (Fig. 4B), respectively. Occasionally a thin veneer of isopachous cement overlies phosphatic substrate consisting of smaller, randomly oriented crystals (Figs. 6C, F, 9E). Finally, some algal cell walls appear to be phosphatized by poorly oriented, relatively small (sub-micrometric) apatite crystals (Fig. 7).

3.1.2. Interpretation

Phosphatic encrustation is interpreted as a relatively late diagenetic process. This is supported by the following observations: (1) it consists of relatively larger (micrometric) crystals and (2) it mantles early diagenetic phosphate that consists of smaller (sub-micrometric) and randomly oriented crystals (Figs. 6C, F, 9E). It is interesting to note that, with some exceptions, botryoidal cements tend to occur on animal egg/embryo cell surfaces and inner surfaces of egg envelopes, whereas isopachous cements tend to occur on envelopes or mantle early diagenetic phosphate. It is likely these two types of cements represent two generations of cementation that were controlled by nucleation processes (e.g., nucleation on small isolated particles vs. surfaces). We hypothesize that more recalcitrant substrates (e.g., envelope) can maintain their integrity and provide coherent surfaces on which late diagenetic, isopachous cements nucleate and grow. In contrast, more labile substrates

(e.g., cytoplasm, cell membranes) were easily degraded into organic particles or macromolecules that served as isolated nucleation sites for the growth of early diagenetic botryoidal cements.

3.2. TANGENTIALLY ORIENTED, SUB-MICROMETRIC, BLADED APATITE CRYSTALS: PHOSPHATIC INFILLING OF INTRACELLULAR SPACE

3.2.1. Description

Tangentially oriented, sub-micrometer-sized apatite crystals occur in a number of *Archaeophycus venustus* (=*Paratetraphycus giganteus*) specimens, interpreted as algal fossils (Zhang et al., 1998). *Archaeophycus venustus* cells are polyhedral in shape and approximately 10–30 μm in size (Figs. 8, 9D–H). They are often packed into sarcinoidal clusters. The cells are phosphatized by tangentially oriented, sub-micrometric (<0.20 μm in width, ~0.20–0.55 μm in length; see Fig. 12), apatite crystals – many of which with long axes parallel to the cell surface (Figs. 8B, E, H). One polyhedral cell has two of its facets exposed (Fig. 8H), and it can be observed from this cell that exposed crystals on both facets are tangentially oriented. Tangentially oriented crystals also occur in the acritarch *Meghystrichosphaeridium reticulatum* (Xiao and Knoll, 1999), whose vesicle surface is defined by sub-micrometric crystals that lie parallel to its vesicle surface (Figs. 9A–C). With a mean width of ~0.10 μm (ranging from 0.07–0.15 μm), tangentially oriented crystals are more slender than the perpendicular crystals in botryoidal and isopachous cements (Fig. 12). Moreover, they are often bladed and less euhedral than the prismatic crystals in botryoidal and isopachous cements.

3.2.2. Interpretation

Tangentially oriented apatite blades exclusively occur in algal and acritarch fossils, but not in phosphatized animal embryo cells or embryonic envelopes, which are typically characterized by botryoidal and isopachous cements, respectively. The tangential orientation of the crystals indicates that they did not nucleate on cell walls; instead, their orientation seems to be constrained by cell walls. We hypothesize that these tangential crystals grew on floating nuclei within algal cells or acritarch vesicles and were pushed against the cell/vesicle walls, essentially making an internal mold of the cells or vesicles.

It is uncertain why tangentially oriented crystals are not present in animal embryo cells or envelopes. It is possible that nucleation sites within algal cells and acritarch vesicles were abundant, so that randomly nucleated crystals tend to be smaller and their tangential orientation was constrained by the relatively recalcitrant algal cell walls and acritarch vesicle walls. In contrast, the space between animal egg/embryo cells and egg/embryonic envelopes is usually significant (partly because of shrinkage of egg/embryo cells), and nucleation sites may have been relatively rare in this space, thus nucleation was focused on egg/embryo cell

surfaces and egg/embryonic envelopes. The ample space between egg/embryo cells and encasing envelopes allowed apatite crystals to grow larger than tangentially oriented crystals.

3.3. RANDOMLY ORIENTED, SUB-MICROMETRIC, EQUANT APATITE CRYSTALS: PHOSPHATIC IMPREGNATION

3.3.1. Description

Some Doushantuo fossils were preserved through the precipitation of randomly oriented, sub-micrometric, equant apatite crystals. Such sub-micrometric crystals occur on the tube walls of *Sinocyclocyclicus guizhouensis* (Fig. 6D), the phosphatic wall of a spherical fossil (Fig. 6G), and cell surfaces of *Archaeophycus venustus* (Figs. 9F, H). In all cases, the randomly oriented crystals are subsequently mantled by isopachous cements of larger and perpendicularly oriented apatite crystals. Like the tangentially oriented crystals described above, the randomly oriented crystals are less euhedral and smaller (~0.08–0.30 μm in width, ~0.10–0.35 μm in length; see Fig. 12) than the perpendicularly oriented crystals, but they are equant rather than bladed.

3.3.2. Interpretation

Clearly, precipitation of the randomly oriented crystals predates the perpendicularly oriented crystals. Thus, randomly oriented crystals have the greatest potential to replicate the most labile organic structures. We hypothesize that the tube walls and cell walls were impregnated with microcrystals after only minimal degradation. In the impregnation process, crystal orientation and morphology are constrained by available "interstitial" space within the organic structures being impregnated. Of course, subsequent crystal overgrowth after complete degradation of organic structure is likely, so that the "interstitial" space is unlikely to have been faithfully replicated by the sub-micrometric crystals.

3.4. PHOSPHATIC FILAMENTS, RODS, AND GRANULES: EVIDENCE FOR MICROBIAL ACTIVITIES?

3.4.1. Description

Phosphatic filaments, described above (Fig. 5), are typically 10–20 μm in diameter and up to 100 μm in length, and often form pillars or networks in the space between shrunken eggs/embryos and envelopes. At closer look, they consist of radially oriented crystals that form botryoidal or isopachous cements. Crystals in the axial region (~0.1 μm in width) are much smaller than in the peripheral region (0.3–2.0 μm in width). Similar phosphatic filaments have been found in Phanerozoic phosphatized biotas (Bengtson, 1976; Conway Morris and Chen, 1992; Ding et al., 1992; Müller and Hinz-Schallreuter, 1993; Martill and Wilby, 1994; Duncan

and Briggs, 1996; Duncan et al., 1998; Yue and Bengtson, 1999). Some of these (e.g., Fig. 9D of Yue and Bengtson, 1999) have an axial lumen surrounded by isopachs or botryoids of radially oriented and outward growing crystals. The Doushantuo filaments do not have an axial lumen (Fig. 5D); instead, the axial region is characterized by much smaller crystals.

One specimen of the tubular microfossil *Sinocyclocyclicus guizhouensis* (Liu et al., 2008) bears rare phosphatic rods (Fig. 10A–B). The rods are slightly curved, about 0.2 μm in diameter and up to approximately 1.3 μm in length. Furthermore, these phosphatic rods consist of small granular sub-structures that are approximately 0.05–0.08 μm in size. It is uncertain whether they have a central lumen but they appear to have distally closed ends.

Some (but not all) Doushantuo fossils are covered with a granular texture (Figs. 10C–H, 11). Such granular texture occurs on the surface of multicellular algae (Figs. 10C–D), animal embryo cells (Fig. 10F), and egg envelopes (Fig. 11A). The texture consists of granules of relatively uniform size, on average about 0.05 μm in width and 0.09 μm in length (range of ~0.03–0.09 μm and ~0.06–0.15 μm, respectively). The granules forms aggregates about 0.30–0.70 μm in length and 0.08–0.15 μm in width (Figs. 11E–F, 12) or cover the surface of individual crystals (Figs. 11G–H). The occurrence of such granules on larger euhedral crystals (Fig. 11H) indicates that they postdate such euhedral crystals and are likely late diagenetic in origin. But it is uncertain whether the granular aggregates (Figs. 10D, 11F) and the granules on extremely small crystals (Fig. 11C) were also of late diagenetic origin, because they may or may not share the same origin with the granules on large euhedral crystals.

3.4.2. Interpretation

We interpret the phosphatic filaments, rods, and granules – in order of decreasing confidence – as possible evidence of microbial activities. The phosphatic filaments are almost identical in morphology to silicified microbial filaments in modern hot-spring environments (Jones et al., 1997, 2004; Renaut et al., 1998). Microbial filaments in modern hot-spring environments are rapidly encased by opal-A microspheres, often leaving an axial lumen (Fig. 10M of Jones et al., 1997) similar to those described by Yue and Bengtson (1999, their Fig. 9D). Jones et al. (2004) also showed that extremely thin mucus strands can serve as nucleation substrates for the nucleation of opal-A microspheres, which upon growth can be coalesced to form siliceous pseudofilaments. These siliceous filaments are plausible modern analogs for the phosphatic filaments from the Doushantuo Formation. Although Doushantuo filaments do not have a well defined axial lumen, the extremely small apatite crystals in the axial region (as compared with crystals in the peripheral regions) suggest the former presence of an organic or microbial filament, which either escaped from entombment or degraded after being entombed, and the former axial lumen was then filled with diagenetic phosphate. The phylogenetic affinity of the microbial filaments, however, is more difficult to determine. They can be bacterial filaments, fungal hyphae, or simply mucous strands produced by any microbes (Xiao and Knoll, 1999). Bailey et al. (2007) suggested that the

filaments may represent symbiotic epibionts, but they can also be interpreted as saprophytic bacteria given that such filaments typically occur on shrunken and degraded cells (Fig. 5; Xiao and Knoll, 1999).

Doushantuo phosphatic rods (Fig. 10B) can also be interpreted as phosphatized bacteria. Indeed, their morphology and granular texture is very similar to silicified bacterial rods reported in Jones et al. (1997, their Fig. 5G), except they are about five times smaller. Their smaller size (about 0.2 μm in diameter and 1.3 μm in length), however, does not preclude a bacterial interpretation (Southam and Donald, 1999).

Doushantuo phosphatic granules (0.09 μm in length and 0.05 μm in width; Figs. 11E–H), on the other hand, may approach the size limit of cellular life (Nealson, 1997; Southam and Donald, 1999). Structures of similar size and shape have been interpreted as nanobacterial fossils (Folk, 1999; Folk and Rasbury, 2002), but this interpretation has been met with skepticism because their extremely small size may not be sufficient to house necessary metabolic machineries (Nealson, 1997; Southam and Donald, 1999). A recent report describes granular-textured sheets from Triassic stromatolites (Perri and Tucker, 2007). These granular-textured surfaces in Triassic stromatolites are strikingly similar to those in the Doushantuo Formation (compare Figs. 11G–H with Figs. 2C, 3B, and 4C in Perri and Tucker, 2007), and a similar origin for both occurrences is plausible. Perri and Tucker (2007) interpreted the Triassic granular-textured sheets as mineralized extracellular polymeric substances (EPS), on the basis of their similarity to the sub-polygonal honeycomb structure of modern EPS. This interpretation may also be applicable to the Doushantuo Formation, but more research is needed to test the possibility that the granular texture in the Doushantuo Formation may be abiotic precipitation formed during acetic acid treatment in the laboratory or, less likely due to their irregular nature, artifacts resulting from excessive Au-Pd coating during sputtering.

3.5. QUANTITATIVE EVALUATION

To quantify the crystal aspect variability, we conducted detailed imaging and statistical analyses on high resolution scanning electron micrographs of the four crystal morphologies described above. For each crystal type, the dimensions of length and width were measured. The raw results are presented in Fig. 12A. The figure also shows the length and width of granule aggregates (red color). A total of 1,075 randomly chosen crystals (specifically 350 perpendicularly oriented prismatic apatite crystals; 200 tangentially oriented bladed apatite crystals; 125 randomly oriented equant apatite crystals; 325 phosphatic granule aggregates; and 75 granules) were measured on scanning electron micrographs using Adobe Photoshop CS2. The data were analyzed using SAS 9.1 and plotted using DeltaGraph 5.0. Figure 12A illustrates the length–width plots of all four different types of crystal morphology. Figures 12B–C shows less obscured view of the four crystal morphologies – including measurements of granule aggregates.

To estimate the confidence intervals, the data were subject to a naïve bootstrapping analysis (Efron, 1981; Kowalewski et al., 1998), with 1,000 iterations (SAS code written by M. Kowalewski and modified by R. Krause). Means and corresponding 95% confidence intervals of the lengths, widths, and aspect ratios for each of the above-described crystal morphologies are presented in Figs. 12D–E. The quantitative analysis shows that perpendicularly oriented, prismatic crystals are significantly larger than tangentially oriented, bladed crystals (Fig. 12D), although they have similar length:width ratios (Fig. 12E). The randomly oriented equant crystals and phosphatic granules are even smaller, and as expected have smaller aspect ratios. Thus, these four crystal morphologies are distinct in both shape and size. And the distinction of these crystal morphologies may aid in understanding factors affecting nucleation and growth of crystals in varying regions of multiple fossil taxa during the phosphatization process.

3.6. SUMMARY

To summarize, we recognize several different phosphatic textures, probably indicating different phosphatization processes responsible for the preservation of Doushantuo microfossils. Phosphatic encrustation by micrometric, perpendicularly oriented crystals is most pervasive in the Doushantuo Formation. It occurs as botryoidal and isopachous cements on a variety of substrates, including animal egg/embryo cell surfaces, egg/embryonic envelopes, algal cell walls, organic filaments, as well as secondary coatings on pre-existing phosphatic surfaces. Phosphatic infilling of intracellular space by sub-micrometric, tangentially oriented crystals was probably driven by abundant random nucleation within algal cells or acritarch vesicles. These random crystals probably became tangentially oriented when pushed against the relatively recalcitrant algal cell walls and acritarch vesicle walls. In essence, the cells and acritarchs were molded by crystals growing inside. Tangentially aligned crystals do not occur in animal cells perhaps because animal cell membranes were more labile and would be deflated and degraded before cell lumens were completely phosphatized. Phosphatic impregnation by sub-micrometric, randomly oriented crystals occurred when nucleation was initiated within organic substrates (such as more recalcitrant tube walls and cell walls) after only minimal degradation. Finally, there is tentative evidence for bacterial (or nanobacterial) activities preserved in the Doushantuo microfossils. At the present, the exact taphonomic roles of these bacterial activities are still uncertain.

Our analysis shows that the preservational quality and taphonomic resolution of phosphatic impregnation and intracellular infilling is much better than phosphatic encrustation. This is because the former processes tend to preserve more recalcitrant structures, during earlier diagenesis, after less degradation, by smaller crystals. Thus, analysis of Doushantuo microfossils suggests that the recalcitrance, degree of degradation, and crystal size all play a significant, controlling role in phosphatization of soft-bodied microorganisms (Briggs, 2003).

4. Relevance to Astrobiology

As discussed in the opening section, any extraterrestrial ecosystems likely started with a biosphere dominated by soft-bodied microorganisms. Additionally, from a practical point of view, the amount of extraterrestrial samples available for astropaleobiological investigation is likely small (even if a Mars sample return mission is conceivable in the near future). Thus, the astropaleobiological focus has been and must continue to be on microscopic fossils, and the Precambrian biosphere is the most suitable analog for such investigation. Among all of the taphonomic pathways discussed in the introduction section, Bitter Spring-type (silicification) and Doushantuo/Orsten-type preservation (phosphatization) hold the greatest potential in astropaleobiological investigation. Beecher's trilobite-type preservation (pyritization) has poor resolution because of the large size of pyrite crystals and its intrinsic dependence on organic degradation and destruction as a source of sulfide. Burgess Shale-type preservation and Ediacara-type preservation tend to preserve macroscopic organisms. In addition, the two-dimensional compression in Burgess Shale-type preservation also decreases its taphonomic fidelity, and instability of organic carbon in many strongly oxidative extraterrestrial environments also makes Burgess Shale-type preservation less relevant. Thus, in the search for ancient extraterrestrial ecosystems, we need to follow the silica and phosphate. Detailed investigation of silicified and phosphatized biotas preserved in ancient rocks on the Earth, together with an experimental approach to better understand of the molecular and geochemical processes of silicification and phosphatization (Martin et al., 2003; Raff et al., 2006), will certainly help us to more effectively choose astrobiological landing/sampling sites.

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PROTEROZOIC UNICELLULAR AND MULTICELLULAR FOSSILS FROM INDIA AND THEIR IMPLICATIONS

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1. Introduction

A comparative study of possible genesis of life on planet Earth and Mars reveals that similar conditions would have been present on Mars supporting a probable occurrence of unicellular Martian microfossils and stromatolites. The genetic aspect of eukaryotes or eukaryogenesis, eukaryotic evolution and probable geological and recent astrobiological possibilities from Mars and Europa has been discussed by many astrobiologists in Europe and U.S.A. (Chela Flores, 1998, 2007; Seckbach,1994; and the references therein). Westall and Southam (2006) and Westall and Walsh (2003) hypothesise that primitive life (prokaryotes) could have existed on Mars. Recently Schopf et al. (2008) have suggested that search for fossilized evidence of ancient life in rocks from other planets is likely to be constrained severely by the amount of rock available for study. Precambrian fossil record on the planet earth may be used as a model for the search for evidence of ancient life on Mars and other earth like planet.

The molecular evidences indicate that unicellular organisms (prokaryotes) lack nuclear membrane, mitochondrion and the chloroplast and its DNA is normally a single ring shaped chromosome which is not grouped with protein. The multicellular organisms (eukaryotes) have their DNA linked in chromatin, the main organelles are normally in its cytoplasm (Chela Flores, 1998). Seckbach (1994b) and Tewari (1993, 1999) studied the multicellular red algae (rhodophytes) *Cyanidium caldarium* and red brown algaeVendotaenid *Krolotaenia gniloskayi* Tewari from the Ediacaran Krol Formation of the Lesser Himalaya, India for its genetic aspects. The Ediacaran multicellular fossils of the Lesser Himalaya India are found in the Krol basin (Fig. 1) in the northwestern part and recently discovered diversified unicellular–multicellular microfossils including sponges come from the Buxa Dolomite, northeastern Lesser Himalaya (Figs. 1 and 2). Ediacaran fossils occur above the highest Marinoan glaciation (=Blaini diamictites) and below the lowest Cambrian deposits (=Tal Formation) globally (Tewari, 2007; Tewari and Sial, 2007). The present chapter deals with the important aspect of the Proterozoic unicellular and multicellular microfossils from the Buxa Dolomite of the NE Lesser Himalaya, India and its global and astrobiological implications.

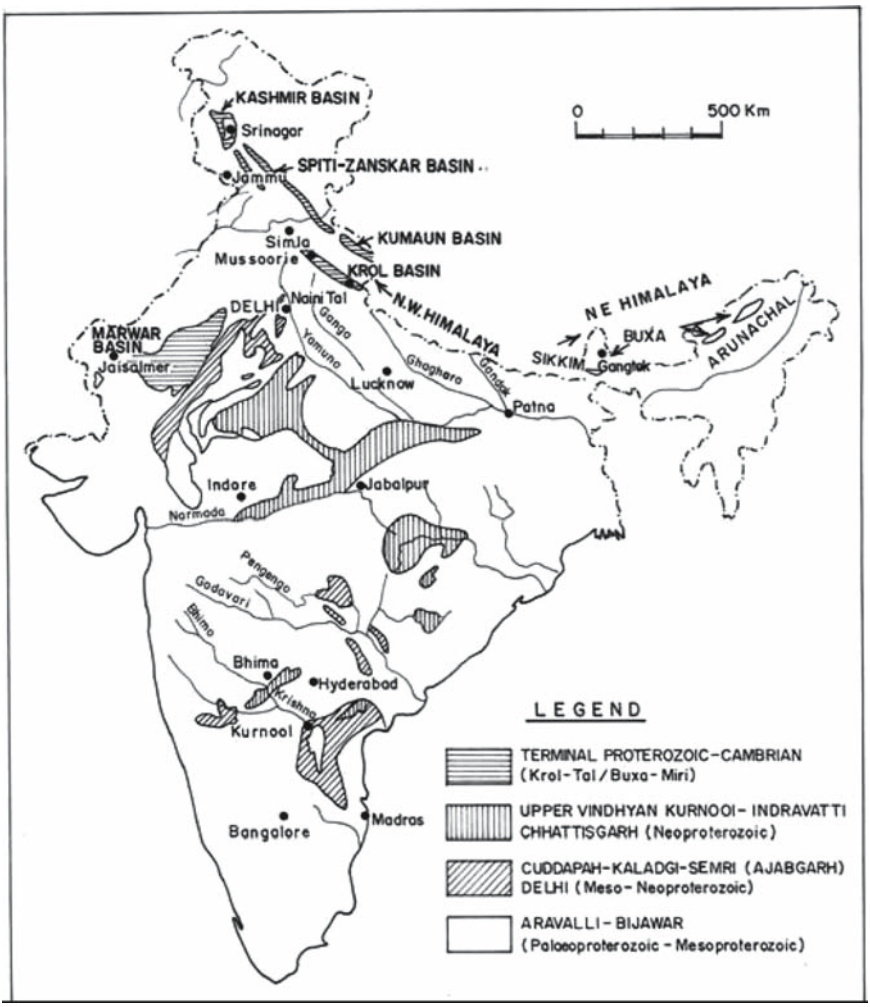

Figure 1. Geological map of India showing occurrences of the fossiliferous formations.

2. Diversified Unicellular and Multicellular Microfossils from Buxa Dolomite

A diversified assemblage of organic-walled microfossils comprising 27 taxa of the benthic and planktonic forms (cyanobactera, acritarchs and Vase shaped microfossils) has been recovered in petrographic thin sections from the lenses and bedded chert belonging to Buxa Dolomite exposed near Igo Bridge, Daring – Basar road in West Siang District of Arunachal Pradesh (Figs. 2, 5 and 6). In

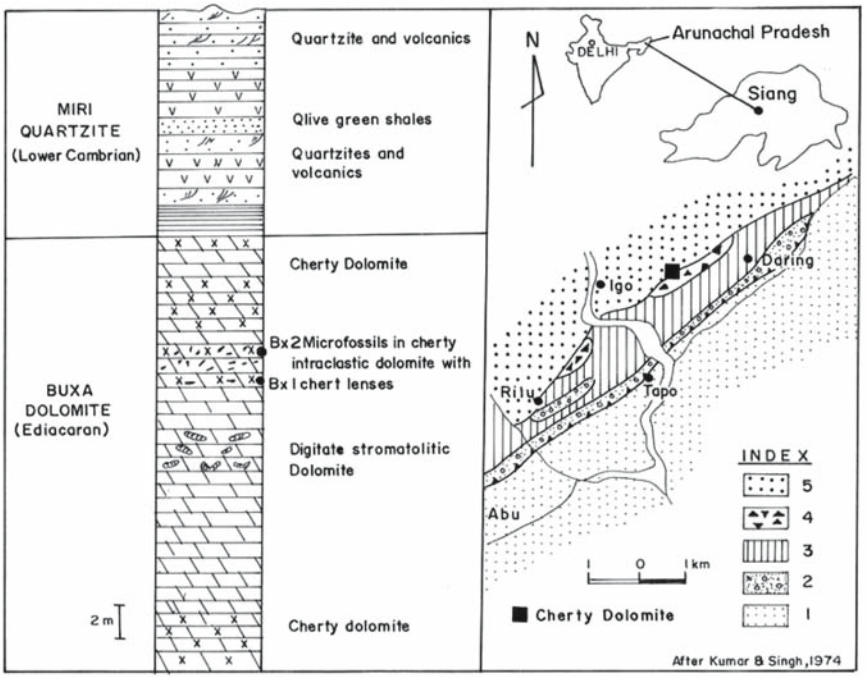

Figure 2. Geological and location map of the Buxa Dolomite in the NE Lesser Himalaya.

this assemblage 13 cyanobacterial remains belonging to Chroococcaceae, Nostocaceae and Oscillatoriaceae, 13 taxa of Acritarchs belonging to Sphaeromorphida, Scaphomorphida and Sphaerohystricho-morphida subgroups of acritarchs and one VSM (Vase shaped microfossils) have been reported in this paper. The cyanobacterial remains are *Huroniospora psilata*; *Eosynechococcus moorei*; *Paratetraphycus giganteus*; *Glenobotrydion aenigmatis*; *Myxococcoides minor*; *Palaeoanacystis suketensis*; *Oscillatoriopsis breviconvexa*, *O. robusta*, *O. rhomboidalis*; *Palaeolyngbya contenada*; *Siphonophycus typicum*, *S. rugosum*; *Polythrycoides lineatus*; *Obruchevella parva*; *Volyniella valdaica*; *Vetronostocale amoenum* and *Vetronostocale equale* sp. nov. The 13 taxa of acritarchs are *Margominuscula rugosa*, *M. simplex*; *Leiosphaeridia visingsa*; *Granomarginata vetula*; *Lophosphaeridium rarurm*, *L. jansoniusii*; *Trachysphaeridium robustum*; *Micrhystridium lanatum*, *M. ampliatum*; *Baltisphaeridium cerinum*; *Archaeohystrichosphaeridium semireticulatum*; *A. cellulare*; *Vandalosphaeridium reticulatum*; *Trachyhystricho-sphaera aimica*; *Gorgonisphaeridium pindyium*; *Meghhystrichosphaeriium perfectum* (Kolosova) comb nov.; *Navifusa segmentatus* and *N. bacillaris*. The single genus of VSM viz. *Melanocyrillium hexodiadema* has been recorded.

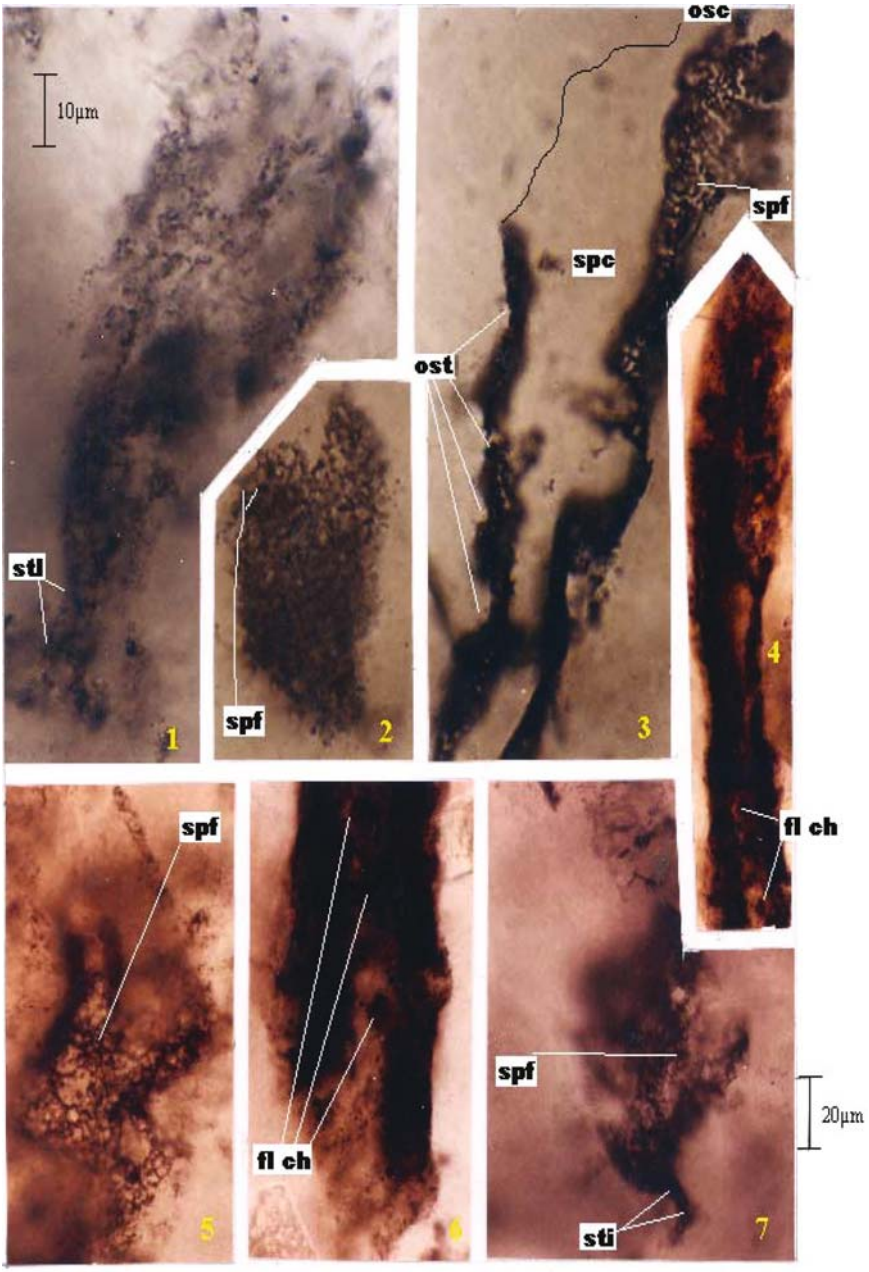

Figure 3. (Plate 1). Fossil sponges from the Buxa Dolomite, NE Lesser Himalaya, India.

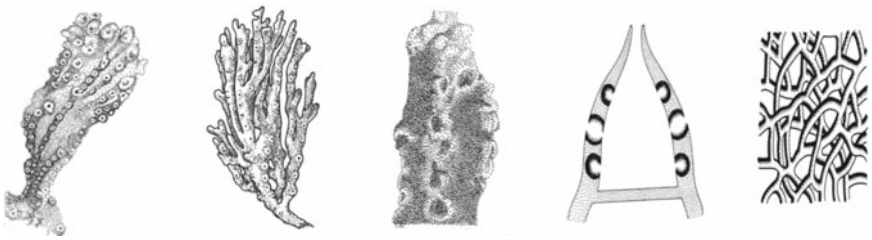

Figure 4. A variety of Demosponges.
1. Text figure of Plate. 1, Fig. 1 cf. *Chalina*.
2. Text figure of extant *Chalina*.
3. Text figure of Plate. 1, Fig. 6 showing canal system cf. *Chalina*.
4. Text figure showing Ascon type of water canal system as found in *Chalina*.
5. Text figure showing spongin fibers. Note the comparison with sponging fibers in Figs. 2, 5 and 7.

A. Stalk
B. Flagellated chamber
C. Finger like projections
D. Spongocoel
E. Spongin fiber net
F. Osculum
G. Ostium

The present assemblage of microbiota compares well with the known assemblage from the Latest Proterozoic/Vendian sediments of Northwest and Central Lesser Himalaya, India and its equivalent sediments in other parts of the world (Table 1) The Buxa dolomite is well exposed in Arunachal Pradesh as windows or linear belts from Kameng District in the west to Siang district in the east. Cherty bands, oolites, stromatolites and intraclasts characterize this dolomite. The stromatolites and microbiota are known from Kameng area and the Menga window in the Upper Subansiri District. Recently an assemblage of diversified organic-walled microfossils has been recovered in petrographic thin sections of lenses and bedded chert belonging to the Buxa Dolomite exposed near Igo Bridge, Daring – Basar road in West Siang District of Arunachal Pradesh. This assemblage which shows dominance of the spinated forms and coiled cyanobacterial remains viz. *Obruchevella* indicates Vendian age for these beds. The non-mineralized sponges are being reported for the first time from the same location. The assemblage of these fossil specimens represents three types of extinct sponge forms.

3. Sponges from the Buxa Dolomite

The present forms are simplest metazoan having numerous small pores on their body surface and hence justify their placement in Phyllum Porifera (Pore bearers). They show cellular grade constructions and loose aggregation of cells bound into soft tissues. Sponges are the most primitive of multicellular animals with a low

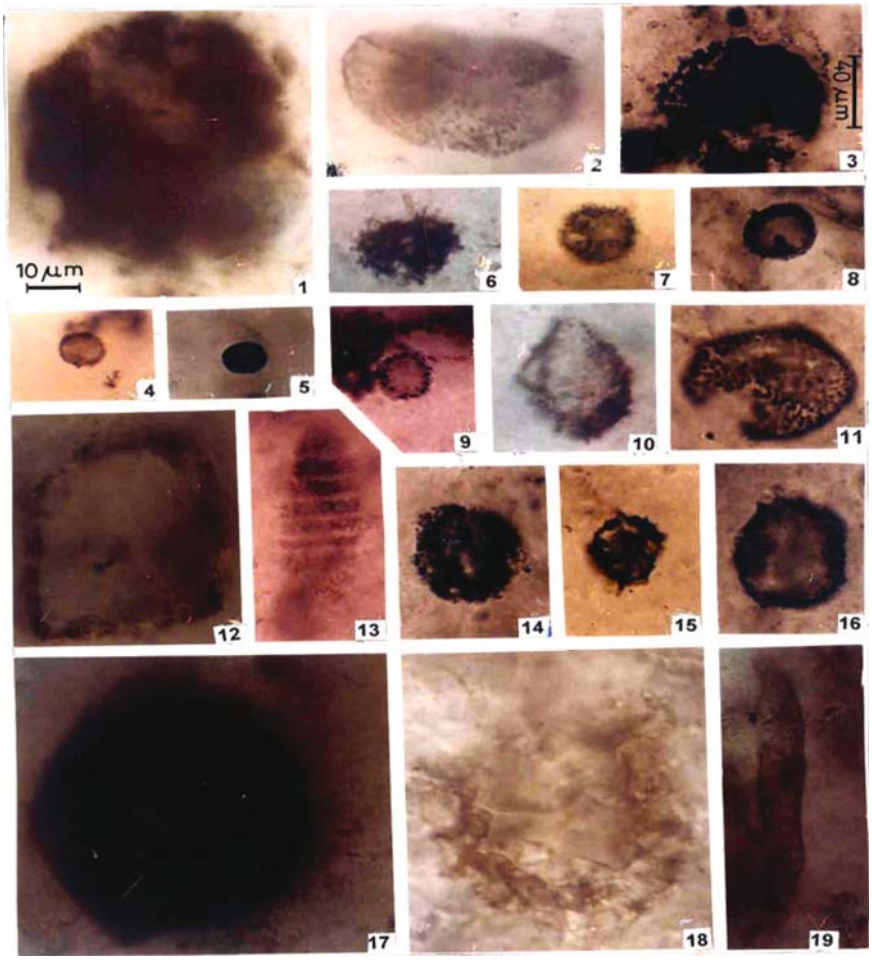

Figure 5. Coccoid assemblage of the Buxa Dolomite, NE Lesser Himalaya, India.

grade of organization. They are incapable of movement being attached to the substratum as plant. In other words sponges are fixed to some submerged object in water. All sponges have skeleton which provides them strength and rigidity. These skeletons are of three type – (i) consisting of soft colloid, varying from a mucous like sol to rather stiff gel; (ii) mineralized skeletons, composed of spicules made of opaline silica or calcium carbonate, and (iii) skeletons comprising of stiff and tough organic material called spongin. The first type of skeleton comprising of mucous like sol or stiff gel is often transient. In some forms the mineral matter in concentric layers covers the original axial thread, which is formed by longitudinal fission of pre-existing thread inside the living sponge cell. We believe that the ancient sponges may not be having the mineralized skeleton made by spicules

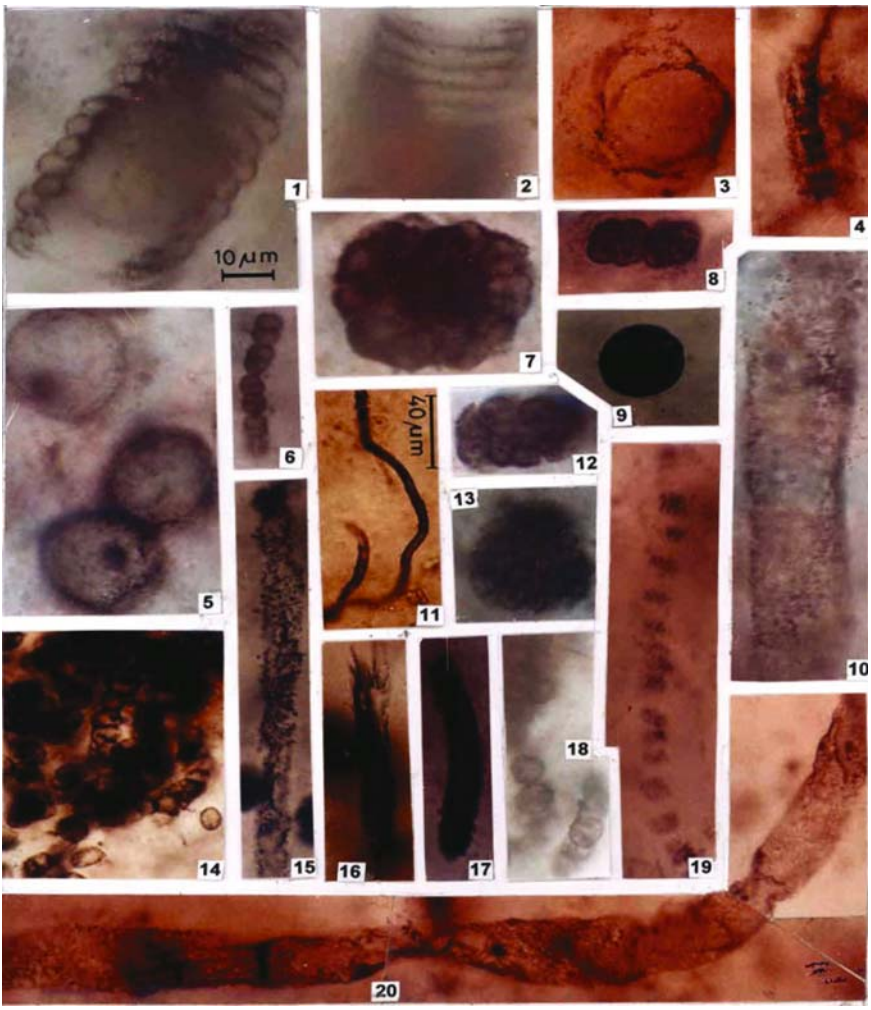

Figure 6. Filamentous assemblage of the Buxa Dolomite, NE Lesser Himalaya, India.

and forms with the organic skeleton and morphology similar to existing forms may be found in ancient sediments.

4. Systematic Description of Sponges

4.1. TYPE A

The present specimens are colonial forms having several fingers like structures hence may be finger sponge of the class Demonspongia (Fig. 3, Plate 1, Figs. 1, 4 and 6).

Table 1. Comparative Chart of O.W.M. (cyanobacterial remains and acritarchs and VSM) in present assemblage belonging to Neoproterozoic/Vendian sediments of the world.

				Cyanobacteria						Acritarch			vsm
Country	Authors	Lithology	Formation/group	□	✹	∞	&	Y	§	●	✷	◆	Ω
India	Shukla et al., 2006	Sh + C	Buxa Dolomite	+	−	+	+	+	+	+	+	+	+
	Tewari, 2004	C + Lst.	Deoban Limestone	+	+	+	+	+	+	+	+	+	+
	Kumar et al., 1984	Sh	Lolab, Karihul & Kunzam La	+	_	+	+	_	_	+	+	+	_
	Shukla et al., 1986	Nl + C	Deoban Lst.	+	_	+	+	_	_	+	_	_	_
	Venkatachala et al., 1990	Nl + C + Sh	Infra-Krol	+	_	+	+	_	+	_	_	_	+
	Tiwari and Pant, 2004	Nl + C	Infra Krol	+	+	_	+	_	+	+	+	_	+
	Maithy and Babu, 1997	Sh + C	Bhander Gr.	+	+	_	+	+	+	+	+	+	+
Canada	Shukla et al., 2005	Sh + C	Infra Krol	+	_	_	_		+	+	+	_	+
	Butterfield and Rainbird, 1998	Sh	Wynnaitt	_	_	_	+	_	+	+	+	_	_
China	Wang et al., 1983	Ph + C	Dengying & Meishucun	+	_	+	+	_	+	+	+	+	_
	Zhongyang, 1984	C	Jiudingshan	+	_		+	+	+	+	_	_	_
	Zang and Walter, 1992	Sh + Slt.	Huanian Gr	+	_	+	+	_	_	+	+	+	_
	Yin and Gao, 1993	Sh + Slt + C	Shuijigtuo	+	+	+	+	+	+	+	+	_	_
	Yin and Gao, 1995	C	Dengying	+	_	_	+	+	_	+	+	_	_
	Xiao and Knoll, 1999	P + D + C	Doushantuo	_	_	_	_	_	_	+	+	_	_
	Yun et al., 1998	Nl + C + Ph	Doushantuo	+	+	+	+	+	+	+	+	+	_
Green Land	Yin et al., 2003	Slt + C	Doushantuo	+	_	+	_	_	+	+	+	_	_
	Knoll et al., 1987	Sh + Sst	Polarisbreen Gr	_	+	_	_	_	_	+	+	_	_
	Strother et al., 1983	Slt + Sh	Narssarssuk	+	+	+	+	_	_	+	+	_	_

Spitsbergen		Knoll, 1982	Siliciclastic	Draken Conglte	+	+	_	_	+	_	+	+	+	_
		Knoll and Swett, 1985	Sh + Slt	Veteranen Gr.	+	+		+	_	+	+	+	_	_
		Butterfield et al., 1988	Sh	Svanbergellet	_	_	_	_	+	_	+	+	_	_
		Knoll et al., 1991	SiliciCarbonate	Conglomerate	+	_	_	+	_	_	+	+	−	+
		Knoll, 1992	C	Backlundtoppen	+	_	_	+	_	_	+	+	_	_
		Butterfield et al., 1994	Sh + C	Svanbergfellet	+	+	+	+		+	+	+	+	_
SW Sval-bard		Knoll et al., 1981	Sh + slt	Mineral fork, Utah	+	_	_	+	_	_	+	+	_	+
		Knoll, 1982	Slt	Kwagunt	+	_	_	+	_	+	+	+	_	_
		Knoll and Ohta, 1988	Sh + D	P. K. F.	_	_	_	+	_	+	+	+	_	_
Finland		Vidal and Nystuen, 1990b	Sh + Sst + Conglomerate	Hedmark Gr	+	_	_	+	_	_	+	+	+	_
		Tynni and Donner, 1980	Slt + Sh	Muhos	_	_	+	+	_	_	+	+	_	_
Africa		Maithy, 1975	Sh	Kanshi, Zaire	+	_	+	+	_	_	+	+	_	_
Czecho slovakia		Konzalova, 1974	Sh + Slt	Bohemia Massif	_	_	_	_	_	_	+	+	_	+
Russia		Konzalova, 1981	Graphite	Spilitic Gr	_	_	_	_	_	_	+	+	_	_
		Pychova, 1973	Sh	S Urals Siberia	_	_	_	+	_	_	+	+	_	_
		Timofev, 1973	Sh	Siberia	_	+	_	+	_	+	+	+	+	_
		Lo, 1980	C + Lst	Lr. Yudoma Suite	+	+	_	+	+	_	+	+	_	_
		Sergeev, 1989	Silis + C + D	Maly and Karatau Ridge, Kazakhstan	+	+	+	+	_	+	+	+	+	
		Weiss, 1989	C	Judma Majurgian	+	+	+	+	_	_	+	+	_	_

(continued)

Table 1. (continued)

Country	Authors	Lithology	Formation/group	Cyanobacteria						Acritarch			vsm
				□	✹	∞	&	Y	§	●	✷	◆	Ω
	Jankauskas, 1990	Sh + Slt + C	South and CIS Urals	_	_	_	+	_	_	+	+	_	_
	Pyatiletov and Rudavskaya, 1990	Sh + Slt + C	Siberian platform	_	_	_	+	_	_	+	+	_	_
	Ragozina and Sivertseva, 1990	Sh + Slt + C	Valdai Series	_	_	_	+	_	_	+	+	_	_
	Yakshin, 1989	Sh + C	East European	+	+	+	+		+	+	+	+	_
	Yakshin, 1990	Sh + Slt + C	Tinnovka	_	_	_	+		+	+	+	_	_
	Volkova, 1990	Sh + Slt + C	Regions of E. Europe	_	_	_	+	_	_	+	+	_	_
Australia	Damassa and Knoll, 1986	Sh	Arcoona Quartzite	_	_	_	+	_	_	+	_	_	_
	Zang and Walter, 1989	Sh +	Pertataka Fm	_	_	_	_	_	_	+	+	_	_
	Zang and Walter, 1992	Sh + C	Pertataka Fm	+	+	+	+	+	+	+	+	_	_
	Zang, 1995	C + Sh + Slt	Alinya Fm	+	_	+	+	+		+	+	+	_

D = Dolomite, **Ph** = Phosphate, **Nl** = Nodular, **C** = Chert, **Sh** = Shale, **Slt** = Siltstone, **Sst** = Sandstone, **Lst.** = Limestone, □ = Chroococcaceae, ✹ = Pleurocapsaceae, ∞ = Nostocaceae, **&** = Oscillatoriaceae, **Y** = Branched filaments, **§** = Coiled sheath, ●; = Sphaeromorphida, ✷ = Sphaerohystrichomorphida, **Ω** = Scaphomorphida, ♦ = VSM, + = Present, − = Absent

This sponge may be called as mermaid globe or dead man fingers as it is palm shaped with several fingers perforated with oscula. The skeleton of the present specimens apparently made of spongin fibers. Siliceous spicules are not visible. These specimens also show radial symmetry and Leuconoid type canal system as its body is massive and shows numerous flagellated chambers.

The present form is morphologically comparable with the known extant genera, *Chalina*. Though, the general morphology of the dead man finger is very clearly visible in the present specimens, it differs in not having siliceous skeleton. It is possible that this taxon may be representing ancestral lineage of *Chalina*. The skeleton in this case is made up of organic material, apparently similar to mucus lie sol or stiff gel, which is a transitional stage in the development of mineralized skeleton in *Chalina*.

4.2. TYPE B

This body is somewhat rounded leaf like structures with a leathery surface. The specimens show radial symmetry and a skeleton of spongin fibers. Spicules are absent (Fig. 3, Plate 1, Figs. 2, 5 and 7). The canal system may be of Leuconoid type.

The present specimens resemble with leaf sponge, *Phylospongia* of subclass Karatosa that are known as horny sponge. Spongin fibers are protein secretions of amoeboid cells, which form anatomizing network on the body wall. They are resistant to digenesis and decay and resemble hair and silk in chemical composition.

The Buxa dolomite is well exposed in Arunachal Pradesh as windows or linear belts from Kameng District in the west to Siang district in the east. Cherty bands, oolites, stromatolites and interaclasts characterize this dolomite. The stromatolites and microbiota are known from Kameng area and the Menga window in the Upper Subansiri District. Recently an assemblage of diversified organic-walled microfossils has been recovered in petrographic thin sections of lenses and bedded chert belonging to the Buxa Dolomite exposed near Igo Bridge, Daring – Basar road in West Siang District of Arunachal Pradesh. This assemblage which shows dominance of the spinated forms and coiled cyanobacterial remains viz. *Obruchevella* indicates Vendian age for these beds. The non-mineralized sponges are being reported for the first time from the same location. The assemblage of these fossil specimens represents three types of extinct sponge forms.

4.3. TYPE C

This specimen of sponge is in the form of a cylinder with a large opening on the top of its body interpreted here as osculum (Fig. 3, Plate 1, Fig. 3). There are numerous small pores or ostia in the wall of the cylinder and inside the cylinder is a body cavity (large central cavity) or spongocoel (Paragastrc cavity). Ostia open in this spongocoel and permit entry of water in this cavity, which exits

through osculum. The present specimens show radial symmetry and simplest type of (Ascon types) canal system.

Almost all sponges are provided with a skeleton embedded in mesenchymes. The mineralized skeleton is absent. Instead a reticulation made by interlacing of spongin fibers or hard mucus like sol to rather stiff gel is present.

5. Early Evolution of Life and Its Evidences from the Lesser Himalaya

The oldest known record of life on Earth is bacteria described by Walsh (1992), Westall (1999, 2006) and Westall et al. (2001, 2006) from Early Archean Onverwacht Group (3.3–3.5 Ga), South Africa. The oldest well preserved stromatolites have been recorded in 3.5 Ga old Warrawoona Group sedimentary rocks in Western Australia. Schopf (1968, 1983, 1993) and Schopf and Klein (1992) have discussed the global occurrence of Archaean and Proterozoic microfossils and their significance. The Laser Raman Spectroscpic study and 3D imagery of the oldest Archaean and the Proterozoic microfossils confirm the presence of kerogen in high concentration (Schopf et al., 2008).

The Meso-Neoproterozoic microbial diversification has been recorded from all continents and the microfossils are preserved in early diagenetic silicified carbonates Table 1). The Deoban Limestone is a well developed carbonate buildup in the Lesser Himalaya of northern India near Mussoorie (Fig. 1). It has been assigned Meso-Neoproterozoic age on the basis of Riphean stromatolite assemblage *Conophyton garganicum*, *Kussiella kussiensis*, *Colonnella columnaris* and *Baicalia nova* (Tewari, 1989, 1993a, 2002, 2007). Deoban and Buxa cherts show highly diversified microbial assemblage in the Meso-Neoproterozoic of the Lesser Himalaya in India and its global distribution is shown in Table 1. Twenty species belonging to eleven genera of coccoid forms are *Myxococcoides minor*, *M. inornata*, *Huronispora psilata*, *H. microreticulata*, *Eoentophysalis belcherensis*, *E. magna*, *E. cumulus*, *Glenobotrydion aenigmatis*, *G. majorinum*, *Tetraphycus major*, *T. conjunctum*, *Melasmatosphaera media*, *M. parva*, *Gloeodiniopsis lamellosa*, *G. gregaria*, *G.* sp., *Globophycus rugosum*, *Sphaerophycus parvum*, *Eosynechococcus isolatus* and *Caryosphaeroides pristina* (Shukla et al., 1987; Tewari, 1989, 2001b, 2002, 2004; Kumar and Srivastava, 1992). Five genera and eight species of filamentous forms have been recorded by Shukla et al., 1987; Tewari, 1989, 2001b, 2002 and Kumar and Srivastava (1992). These are *Eomycetopsis robusta*, *E. filiformis*, *E. siberiensis*, *Gunflintia minuta*, *G. grandis*, *Biocatenoides* sp., *Oscillatoriopsis* sp. and *Siphonophycus kestron*. The above named twenty eight species are recorded in the Deoban assemblage, whereas the maximum number of common species is known from Bitter Springs Formation of Australia (900 Ma, Table 1). According to Hofmann (1976) *Glenobotrydion*, *Myxococcoides*, *Globophycus*, *Caryospheroides*, and *Melasmatosphaera* possibly represent the degradational variants. Tiwari et al. (2000) and Tewari (2002) have reported isolated hexactinellid and monaxon sponge spicule and calcified algae *Epiphyton* sp. and *Renalcis* sp. from the

Gangolihat Dolomite (eastern extension of Deoban Limestone in the Uttaranchal Lesser Himalaya) which suggest Neoproterozoic age for the Gangolihat Dolomite.

Vendotaenid algae from the Lower Krol Formation of the Lesser Himalaya (a new genus *Krolotaenia gnilovskayi*) was established and subsequently the genus *Tyrasotaenia* and *Vendotaenia* have been recorded from the Nainital Syncline (Tewari, 1993b, 1999b). Hofmann (1992) regarded some carbonaceous megascopic ribbon shaped remains as algae (metaphyte) affinities of Neoproterozoic age. Schopf et al. (1973) described *Vendotaenia* as a multicellular macroscopic benthic metaphyte. The Vendotaenids are also recorded from the Sinian System (Late Proterozoic) of China and this eukaryotic algae has a global distribution. Ediacaran life diversified in the Terminal Proterozoic and includes soft bodied metazoans (coelenterates-medusoid, frondoid forms) and trace fossils. These evolved after the Varangian glaciation and are well known from the Ediacara type locality in Australia. These have been recorded from China, Eurasia, India and Wernecke and Mackenzie mountains of Canada (Glaessener, 1984; Narbonne and Hofmann, 1987; Hofmann, 1992; Walter, 1989; Mathur and Shankar, 1989; Shankar and Mathur, 1992; Shankar et al., 1997; Tewari, 1992, 1996, 2001b, 2004, 2007). The oldest pre Ediacaran fauna has been recorded from the intertillite beds of Windermere Supergroup, Mackenzie Mountains, Canada (Hofmann et al., 1990).

In India, the Ediacaran assemblage has been recorded from the Upper Krol Formation of the Lesser Himalaya (Fig. 1). The assemblage includes the soft bodied metazoans *Cyclomedusa davidi*, *Charniodiscus* sp. fronds and disc, *Kimberella* cf. q*uadrata*, *Zolotytsia biserialis* Fedonkin and *Conomedusites lobatus* Glaessener and Wade (Shanker and Mathur, 1992; Tewari, 1992, 1996, 2004, 2007).

6. Discussion and Conclusions

The recovered organic-walled microfossils comprise of 36 taxa of cyanobacterial, acritarchs and VSM. In which 17 taxa of cyanobacteria belong to Chroococcaceae, Nostocaceae and Oscillatoriaceae, 18 taxa of Acritarchs belong to Sphaeromorphida, Scaphomorphida and Sphaerohystrichomorphida subgroups and one VSM (Vase shaped microfossils now considered testate amoebae) are present. The recovered assemblage compares well with the known Late Neoproterozoic assemblages from other parts of the world (see Table 1).

The cyanobacteria is the most tolerant and primitive group and has remained morphologically unchanged since Archaean. The filamentous and coccoidal forms recorded here are known from the shales, bedded chert and chertified stromatolites of Archaean to recent sediments exposed in different part of the world, viz. Africa, Australia, Canada, China, India, Spitsbergen, Svalbard and thus, these forms do not have any stratigraphic significance. However, the helically coiled morphology, as shown by *Obruchevella* has wide spread occurrence in Upper Riphean – Early Cambrian sediments (Yakschin, 1989; Sergeev, 1989;

Knoll, 1992; Wang et al., 1983; Yin and Gao, 1993; Yin et al., 2003; Srivastava and Kumar, 2003). Though, there are records of *Obruchevella* even from older sediments viz from Archaean and late Palaeoproterozoic to Mesoproterozoic, this form is generally considered marker for Vendian. Morhologically close form *Volyniella valdaica* with coiled morphology reported here is known from the sediments belonging to Vendian age of Russian platform.

The acritarchs show morphological changes through time and hence have been used as stratigraphic markers (Timofeev, 1966; Downie, 1984; Timofeev, 1973; Jankauskas, 1989; Vidal and Ford, 1985; Knoll, 1992b; Zang and Walter, 1992; Jenkins et al., 1992; Butterfield and Rainbird, 1998; Yin, 1985; Spjeldnaes, 1963; Germs, 1995; Xio et al., 1997; Tewari and Knoll, 1994; Maithy and Babu, 1997; Weiss, 1989; Knoll, 1996). The large size acanthomorph acritarchs along with leiopshaerids are present in the early Vendian and these large forms disappear near the advent of the Ediacara fauna (Knoll, 1992; Zang and Walter, 1989). The size of the acanthomorphs reduces in younger sediments till we get dominence of small forms in the late Vendian (Volkova, 1968; Jankauskas, 1989). The acanthomorphs in the present assemblage, which include *Trachysphaeridium*; *Micrhystridium*; *Baltisphaeridium*; *Archaeohystrichosphaeridium*; *Vandalosphaeridium*; *Trachystrichosphaera*; *Gorgonisphaeridium*; *Meghystrichosphae-rium*; *Navifusa* are generally of small size indicating late Vendian affinity. The comparision of the present assembalages with other known assembalages of the world shows close comparison with the Terminal Proterozoic – Cambrian assembalages described from Canada, China, Australia, Greenland, Spitsbergen Russia and Svalbard (Table 1) Thus, the overall analysis of the recovered assemblage indicates late Vendian age for Buxa Dolomites.

The carbon and oxygen isotopic ratios of the Buxa Dolomite from Subansiri, Chillipam and Dedza areas of the Arunachal Lesser Himalaya suggest that these signatures are of Neoproterozoic (Vendian) age and represent pristine marine environment (Tewari, 2002, 2003; Tewari and Sial, 2007). The carbon isotope ratios are significantly positive and quite consistent with δ^{13} C (carbonate carbon) values ranging from +3.7‰ to +5.4‰ (PDB). The Oxygen-isotope data also shows remarkable consistency with the δ^{18}O value fluctuating within a narrow range between –8.9‰ and –7.2‰ (PDB). The Buxa Dolomite of the Arunachal Lesser Himalaya can be correlated with the Vendian Krol Formation of the Uttaranchal Lesser Himalaya on the basis of the very high positive carbon isotopic ratios and the present palaeobiological assemblage. The Doushantuo carbonates (Terminal Neoproterozoic) of the Yangtze Platform, South China also display very high carbon isotopic ratios identical to Krol- Buxa signatures of the Himalaya and were deposited after the global Neoproterozoic low latitude glacial event (Tewari, 2003, 2004, 2007).

A major event in the diversity of fossil algae and unicellular eukarya is recorded in 1.2–1.0 Ga old rocks (Knoll, 1985). The Meso-Neoproterozoic and Terminal Proterozoic succession of the Lesser Himalaya in the northern India shows excellent preservation of the highly diversified microbial assemblages.

The microbiota of the Deoban cherts and the Bitter Springs Formation of Central Australia (Schopf, 1968) are remarkably similar.

The Terminal Proterozoic diversification of life that led to the radiation of animals and plants occurred between 0.59 and 0.53 billion years ago on Earth. The prokaryotic to eukaryotic evolution and diversification of life on Earth, palaeoclimatic events of Neoproterozoic snowball Earth and the extinction and further emergence of highly organized life after Varanger (*Blainian*) glaciation can also be used as a possible model for the search of extraterrestrial life (astrobiological research). The stromatolites can also be used in the search for past life on Mars and elsewhere in the universe (Tewari, 1998, 2001b). In summary it is possible that the early life (4–3.8 Ga) must have been preserved on Mars as compared to Earth. Planet Earth has undergone Archaean Proterozoic plate tectonics and earliest life forms must have been destroyed or not preserved due to very high grade metamorphism (Tewari, 1999). Westall and Southam (2006) have also interpreted that the first life forms may exist on a planet (most probably Mars) where plate tectonics has not destroyed the early primitive evidence of life. The southern Highlands of Mars is the potential area for astrobiological research.

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Top: Brendan Burns, Middle: Brett A. Neilan, Bottom: Malcolm R. Walter

MICROBIAL COMMUNITIES OF STROMATOLITES

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1. Introduction

One of the major challenges in science is to identify modern living systems that present unique opportunities to address fundamental questions in fields ranging from microbial ecology, evolution, chemical biology, functional genomics, and biotechnology. Stromatolites represent such a system. One of the earliest pieces of evidence of planetary life is in fact contained in the microfossils of stromatolites. These extant analogues provide an insight into the nature of ancient microbial systems that dominated early life on Earth (McNamara and Awramik, 1992), and may also provide clues as to their resilience over such immense periods of geological time. This review will focus on microfossil evidence from ancient stromatolites, the significant microbial diversity shown in these living systems, and recent results on lipid profiling that link stable chemical signatures with the biotic components in modern stromatolites. We will also discuss throughout how these early life analogues fit into the emerging and exciting field of astrobiology, a multi-disciplinary field of science that allows us to address fundamental questions on our own origins and existence.

2. Microfossils in Stromatolites

As might be expected, fossil microbial mats, when they are preserved as stromatolites, are known throughout the geological record from the time of the oldest well-preserved sedimentary rocks, which are 3.4–3.5 billion years old (references in Walter et al., 1976; Allwood et al., 2006; Lowe and Tice, 2007; Van Kranendonk, 2006). In the broadest perspective, from 3.5 Ga to about 0.6 Ga stromatolites are by far the most abundant macrofossils of life on Earth. What happened then or

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somewhat before to lead to a great decline in abundance and diversity is controversial: the chemistry of seawater changed, microbial mats were out-competed by macroscopic algae, or that mats were grazed and burrowed by newly emergent metazoa are the main hypotheses. All are likely to be correct. From 0.6 Ga until now the pattern is uniform: stromatolites are abundant in "extreme" environments such as places that are hypersaline, hot or otherwise exotic. What such environments have in common is a paucity of grazing and burrowing metazoans, lending credence to the hypothesis that the dominant control on the distribution of benthic microbial mats is ecological pressure from metazoans. As detailed later in this review, this is further supported through microbial population analyses on living mats and stromatolites that reveal a scarcity of eukaryotes. Intriguingly, there are resurgences of stromatolites at times of extensive metazoan extinction, such as at the Permo-Triassic boundary, at about 253 Ga.

The dominance of stromatolites in the early rock record indicates that these microbial communities are a highly persistent mode of life and a significant stage in Earth's evolution. Indeed the oxygenation of the Earth's atmosphere is attributed to the oxygenic photosynthesis and other gas production performed by Archean stromatolite communities (Hoehler et al., 2001). This major step in the evolution of the geosphere contributed to the further evolution of the biosphere, such as the diversification of eukaryotic organisms observed in the Cambrian explosion. It has even been suggested that some eukaryotes may have originated from a fusion of symbiotic partners in microbial mat and stromatolite ecosystems (Nisbet and Fowler, 1999).

The oldest record of stromatolites is controversial (Brasier et al., 2002; Schopf et al., 2002). That is because it can be difficult to demonstrate the biogenicity of what are partly sedimentary structures. The original microbial mats are rarely preserved in any stromatolites, so interpretation usually relies on other features. The stromatolites of the 3.43 Ga Strelley Pool Chert of the Pilbara region of Western Australia are a good example (Fig. 1). No fossil microbes are known from those stromatolites. However, detailed mapping reveals that the form of the stromatolites varies systematically with the past environments in which they formed. A microbial ecosystem can be reconstructed in which benthic microbial mats extended from a rocky shoreline across a subtidal platform in to relatively deep water (Allwood et al., 2006). While no microfossils are known from these rocks there is degraded organic matter, kerogen, which has a carbon isotopic composition consistent with biogenicity. In other rocks of the same age and older, closely associated microfossils are abundant (Schopf et al., 2007), however their affinities are obscure.

It is not until the late Archean, at 2.5–2.8 Ga, that stromatolites became abundant, and their biogenicity is widely accepted. The most thoroughly studied examples are in the Fortescue Group in Western Australia (Walter, 1983; Figs. 2 and 3). Microfossils in a strict sense are still unknown from these stromatolites but there are abundant faint remnants of filamentous structures. In addition, there is a rich biological record preserved as carbon and sulfur isotope patterns and hydrocarbon "biomarkers", even in the absence of preserved microfossils. These have been used to indicate the presence of various microbial metabolisms in ancient samples (Kakegawa and Nanri, 2006). Further, minerals within fossil

Figure 1. 3.43 billion year old stromatolites in the "North Pole" area of Western Australia, seen in a natural vertical section. Width of the field of view about 1 m.

Figure 2. Fortescue stromatolites in outcrop near Redmont, Western Australia. (Photograph by S. A. Sweetapple.)

stromatolites can provide valuable information regarding ancient seawater chemistries, climates and other environmental parameters that influenced their deposition (Grotzinger and Knoll, 1999; van Kranendonk, 2006).

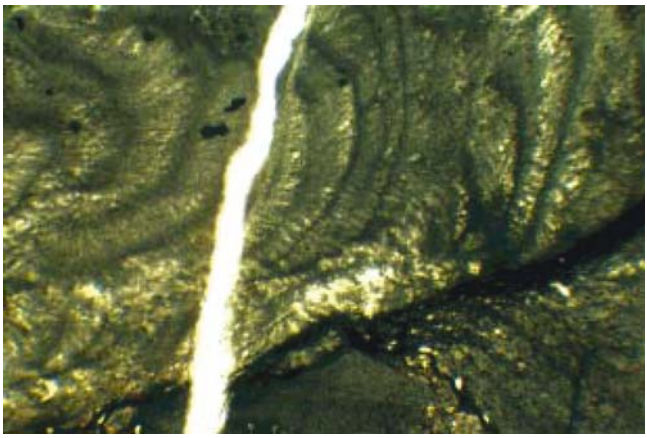

Figure 3. Thin section of Fortescue stromatolite preserved in calcite, from near Redmont, Western Australia. This is from the side of a large bulbous stromatolite; top is up. Biological filament traces are prominent. Dark laminae have prostrate filament traces (not visible here). Width of field of view is 5 mm.

By the early Proterozoic, 2.5 billion years, microbial mats dominated the benthic aqueous ecosystems of the Earth. They constructed reefs as extensive as any coral/algal reefs currently extant (Grotzinger, 1989). It is still difficult to elucidate the biological affinities of the constructing microbes, but most likely the dominant organisms were cyanobacteria. However, as outlined in the following sections on the microbial diversity and lipid biomarker analyses of modern stromatolites, it is now evident that these formations are home to an incredibly diverse microbial community.

3. Microbial Diversity of Living Stromatolites

In contrast to the abundance of fossilised stromatolites, extant stromatolites are rare. The best-studied modern stromatolites are those forming in open marine waters in Exuma Sound, Bahamas (Macintyre et al., 2000), as well as those from a hypersaline marine environment, that of Shark Bay on the western coast of Australia (Logan, 1961). Extant stromatolites have also been discovered in several locations, including in a Tongan caldera (Kazmierczak and Kempe, 2006), in Green Lake, New York (Eggleston and Dean, 1976) and in Lake Clifton, Australia (Moore, 1987). We will concentrate our detailed review, however, primarily on the microbiology of the living analogues in the Bahamas and, in particular, Shark Bay.

3.1. BAHAMAN STROMATOLITES

From studies on the Bahaman stromatolites and associated microbial mats a great deal of information on microbial mat lithification and stromatolite formation has been elucidated.

Carbon, nitrogen, oxygen and sulfur cycles have been studied (Pinckney et al., 1995). The cyanobacteria, aerobic heterotrophs, anoxygenic phototrophs, sulfate-reducers, sulfide-oxidizers and other anaerobic fermenters have been identified as the key metabolic groups within the stromatolite community (Dupraz and Visscher, 2005). Certain species of sulfate-reducing bacteria have been identified by 16S rDNA sequencing, and their location within the community pinpointed using fluorescent in situ hybridisation (Baumgartner et al., 2006).

The surface population of the stromatolites was found to cycle between several community types (Reid et al., 2000). Initially a pioneer community of the filamentous cyanobacterium *Schizothrix* sp. formed, which bound and trapped carbonate sand grains. This was followed by a heterotrophic bacterial biofilm containing abundant extracellular polymeric substances (EPS) developed, during which time cyanobacterial EPS production and bacterial sulfate reduction regulated calcium carbonate precipitation (Kawaguchi and Decho, 2002; Decho et al., 2005; Visscher et al., 1998). Finally a climax community developed, dominated by the endolithic cyanobacterium *Solentia* sp. which strengthened the lithified layer by fusing adjacent carbonate grains via microboring (Macintyre et al., 2000). From this current information regarding mat lithification and stromatolite formation processes in the Bahamas, it is possible to infer analogous processes that also may have occurred within Precambrian stromatolites. In addition to the study of stromatolite communities, the important question of why some mats lithify while others do not, has also been addressed. Possible answers include the need for an uncoupling of the metabolism of the key functional groups by spatial and/or temporal separation, and the influence of physicochemical properties such as calcium carbonate saturation indices and iron availability (Visscher et al., 1998; Dupraz and Visscher, 2005).

3.2. SHARK BAY STROMATOLITES

The stromatolites of Hamelin Pool at Shark Bay (Australia) are well recognised as the best examples of actively lithifying marine stromatolites (Fig. 4). Hamelin Pool is the innermost basin of Shark Bay, a shallow hypersaline bay on the western coast of Australia. Across the mouth of Hamelin Pool is a sea-grass covered sandbank that restricts water flow into Hamelin Pool. Combined with the high evaporation rates, low rainfall, and the lack of freshwater input from the extremely arid land surrounding the bay, this has resulted in salinity that reaches at least twice that of normal seawater (Arp et al., 2001). As alluded to earlier, this increase in salinity may result in a reduction in the level of grazing by higher eukaryotes.

Extant stromatolites of Hamelin Pool are relatively young; the oldest were radiocarbon dated to be 1,000–1,250 years old, with very slow growth rates of around 0.4 mm/year (Chivas et al., 1990). Early studies often reported only taxonomic and physiological properties of the dominant type of cyanobacteria found in Shark Bay stromatolites (Logan et al., 1974), however, recent reports have

Figure 4. Intertidal stromatolites in Hamelin Pool, Shark Bay, Western Australia.

begun to reveal an incredible microbial diversity of these formations, allowing researchers to make more specific and informed inferences about stromatolite functional complexity. One of these studies was the first polyphasic examination of the microbial communities of Shark Bay stromatolites, combining culture-dependent and culture-independent nucleic acid-based methods (Burns et al., 2004). This study showed that the stromatolite community was characterised by microorganisms of the cyanobacterial genera *Synechococcus*, *Xenococcus*, *Microcoleus*, *Leptolyngbya*, *Plectonema*, *Symploca*, *Cyanothece*, *Pleurocapsa, Prochloron* and *Nostoc*. Several of these cyanobacteria isolated from the extant analogues in Shark Bay are filamentous, a characteristic known to aid sediment trapping in stromatolites (Reid et al., 2000). Extracellular polymeric substances, known to be produced by several of the cyanobacteria identified also contribute to stromatolite structure by providing an adhesive matrix to physically bind sediment, as well as providing nucleation sites that promote carbonate precipitation (Arp et al., 2001). Furthermore, phylotypes related to *Synechococcus* were observed in the stromatolite 16S rDNA libraries (Burns et al., 2004), and the outermost cell surface of members of this genus has been shown to have a role in fine-grain mineral formation (Schultze-Lam et al., 1992). Interestingly, this formation occurs with both live and dead cells, so such a process could be important in stromatolite lithification even after cell death. A Precambrian counterpart to *Synechococcus*, *Eosynechococcus*, has been described in ancient stromatolites (Hofmann, 1976), and it has been suggested that characteristics of Shark Bay microbial mats may allow the preservation of cyanobacterial cells as microfossils (López-Cortés, 1999).

In addition to the considerable salinity and desiccation stress stromatolite microbial communities must tolerate, high temperatures and the relatively thin atmospheric ozone layer contribute to a high ambient UV irradiance at Shark Bay (Palmisano et al., 1989). This low ozone also makes this location a better analogue for Early Earth conditions. The sheath pigments known to be present in many of the surface-dwelling cyanobacteria identified (Burns et al., 2004) are likely to play a photoprotective role in screening deeper members of the community from physiological damage. Of further interest was the finding that a number of 16S rDNA clones clustered with the genus *Prochloron* (Burns et al., 2004). *Prochloron* is usually symbiotic with didemnid ascidians and to date there is no report of their existence as a free-living organism (Kühl and Larkum, 2002). There are also no reports of ascidians in Shark Bay, and thus the discovery of potentially free-living *Prochloron* associated with stromatolites was unexpected.

As described earlier, non-cyanobacteria microorganisms are also prominent in these systems, and another recent study on the Shark Bay stromatolites revealed that the most dominant sequences were in fact novel proteobacteria (Papineau et al., 2005). An example of the significant microbial diversity identified is shown in Fig. 5 and Table 1. It is quite interesting that in contrast to earlier notions, cyanobacteria do not appear to dominate in these stromatolites, though it is quite likely they still have major roles in primary production (Papineau et al., 2005). Both of the recent studies using molecular methods concluded that many of the stromatolite microorganisms were unique with no close relatives in the database (Burns et al., 2004; Papineau et al., 2005), and these microorganisms may also possess novel physiologies vital to the survival, integrity, and persistence of stromatolites. Examples of this are several novel Archaea related to *Halococcus* sp., that were recently isolated and characterized from the Shark Bay stromatolites (Goh et al., 2006; Allen et al., 2008). These organisms possess numerous novel physiologies, including an oxidase-negative phenotype, as opposed to all other *Halococcus* species. Characterisation of other novel microorganisms identified from extant stromatolites may reveal further unique metabolisms. Furthermore a diversity of other unculturable Archaea have been identified from living stromatolites for the first time, and although their exact roles in stromatolite biology are unknown, they are likely to be important community members involved in nutrient cycling. Some Halobacterial species have been shown to be capable of fixing CO_2 (Javor, 1988), and it would be intriguing to ascertain whether halobacterial species in stromatolites are involved in the calcification process, in addition to the accepted role that cyanobacteria play in formation of these biogeological formations.

An immense diversity of prokaryotic life associated with modern stromatolites has been revealed, and combined with our knowledge on the prevailing environmental conditions reveals an intimate association between biotic and abiotic factors in stromatolite formation. Most studies on both the Shark Bay and Bahaman stromatolites also revealed that eukaroytes were scarce in these extant formations (Reid et al., 2000; Burns et al., 2004; Papineau et al., 2005), although one study has documented various flagellates in Shark Bay (Al-Qassab et al., 2002). This supports the

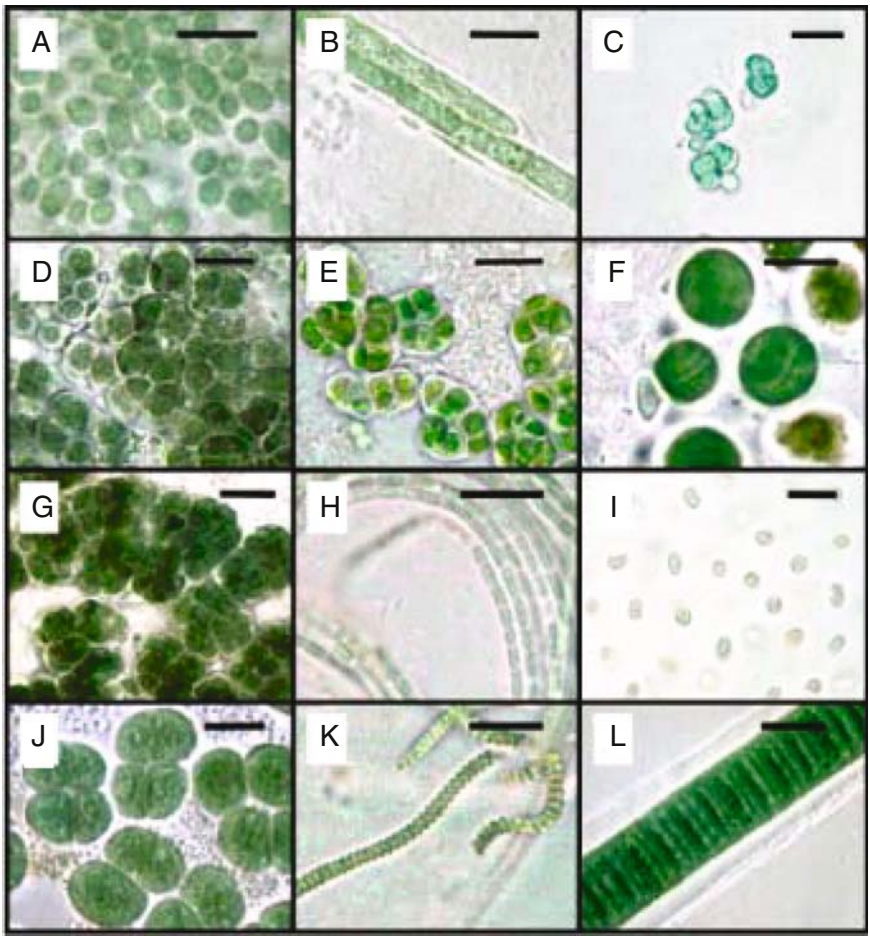

Figure 5. Light microscopy of cyanobacterial isolates from Shark Bay stromatolites. (A) *Euhalothece* (B) *Microcoleus*, (C) *Pleurocapsa*, (D) *Pleurocapsa*, (E) *Stanieria*, (F) *Chroococcidiopsis*, (G) *Xenoccocus*, (H) *Halomicronema*, (I) *Halothece*, (J) *Chroococcus*, (K) *Spirulina*, (L) *Lyngbya*. Scale bar is 10 μm in each image. (Photo courtesy of Michelle Allen.)

original theories that modern stromatolites appear to thrive in environments that exclude most higher organisms. In addition, the differences observed between the microbial community composition of extant stromatolites in different locations (Reid et al., 2000; Burns et al., 2004; Papineau et al., 2005), suggests that different stromatolite morphotypes will depend on the community present and therefore will be determined by it. For example, specific microorganisms trap sediments differently (Papineau et al., 2005), resulting in different stromatolite morphologies. The numerous novel or unknown microbes yet to be identified may also play pivotal roles in stromatolite systems that we do not yet understand.

Table 1. Summary of microorganisms identified from stromatolites in Shark Bay (Burns et al., 2004).

		Sequence analysis of 16S rRNA gene		
Prokaryotic group	**Isolate ID**	**Nearest relative in GenBank database**	**% Similarity**	**Accession no.**
Bacteria	HPB25	*Bacillus* sp. SD-18	98	AF326372
	HPB26	*Bacillus* sp. BA-54	95	AY557616
	HPB28	*Bacillus hwajinpoensis*	99	AF541966
	HPB29	*Bacillus marismortui* strain 123	99	AJ009793
	HPB9	*Halomonadeaceae* LA44	99	AF513453
	HPB55	Bacterium K2-13	99	AY345436
	HPB30	*Bacillus* sp. PL30	98	AF326370
	HPB31	*Halobacillus* sp. D-8	95	AY351395
	HPB32	*Halobacillus trueperi* GSP38	99	AY505522
	HPB13	*Marinobacter* sp. MED104	98	AY136120
	HPB14	*Salinivibrio costicola* GSP14	97	AY553070
	HPB33	*Bacillus* sp. SG-1	98	AF326373
	HPB34	Bacillus litoralis	99	AY608605
	HPB8	*Halomonas alimentaria* GSP27	98	AY553077
	HPB10	*Idiomarina* sp. NT N118	99	AB167034
	HPB35	*Bacillus megaterium* SAFB-011	99	AY167865
	HPB36	*Bacillus* sp. KMM 3737	97	AY228462
	HPB6	*Porphyrobacter tepidarius* DSM10	97	AF465839
	HPB37	Bacillus firmus	97	AJ509007
	HPB56	Bacterium K34	97	AY345475
	HSC29	*Euhalothece* strain MPI95AH13	96	AJ000710
	HSC25	*Cyanothece* sp. ATCC 51142	92	AF132771
	HSC31	*Gloeocapsa* sp. PCC 73106	94	AF132784
	HSC36	LPP-group MBIC10087	97	AB058225
	HSC37	*Geitlerinema* sp. PCC 7105	92	AF132771
	HSC22	*Xenococcus* PCC 7305	92	AF132783
	HSC20	*Xenococcus* sp. Cyano35	96	DQ058859
	HSC17	Lyngbya hieronymusii	94	AF337650
	HSC19	*Chroococcidiopsis* sp. PCC 6712	94	AJ344557
	HSC3	Dermocarpella incrassata	98	AJ344559
	HSC34	*Gloeothece* sp KO11DG	92	AB067577
	HSC24	*Uncultured* Chroococcus sp.	94	DQ058856
Archaea	HSA8	Uncultured archeon, clone HW25	92	AJ344315
	HSA16	*Halococcus* sp.	92	Z28387
	HSA21	*Halobacterium* NCIMB 734	94	AB074302
	HSA22	*Haloarchaeon* 14AHG	95	AY292398
	HSA23	Uncultured archeon clone HW54	93	AJ344317
	HSA24	Uncultured archeon IMT315	95	AF015964
	HSA25	Uncultured archeon clone HW11	92	AJ344308

4. Lipid Biomarker Profiling in Modern Stromatolites

In addition to morphological and molecular investigations into modern stromatolites, recent studies examining lipid profiles in these ecosystems have revealed both complementary and novel information. Lipid biomarkers, specifically fatty acids (FAME), hydrocarbons, ether-bound hydrocarbons, wax esters, sterols, and hopanoids, have been investigated in Shark Bay microbial mats and stromatolites (Allen, 2006). Input from cyanobacterial oxygenic photosynthesis was detected in each sample by FAME, hydrocarbon and hopanoid markers, while anoxygenic phototrophs were detected by FAME and wax ester markers. Lipids indicative of heterotrophic metabolism from the FAME, hydrocarbon, wax ester and sterol fractions were observed. Sulphur-cycling microorganisms were tentatively identified by FAME markers, and by a diverse array of branched quaternary carbon alkanes (BAQC) attributed to the sulphur-oxidising bacteria (Kenig et al., 2003). Actinobacteria, some species of which are active in nitrogen-cycling, were tentatively identified by FAME. Contributions from Archaea were indicated by ether-bound phytane, however, markers specific for methanogenic Archaea were not detected in these stromatolites (Allen, 2006). Sterols indicated the presence of bivalves and their dinoflagellate symbionts, and higher plant input, likely due to aerosols, was also identified in the FAME and hydrocarbon fractions. Of particular significance, was the fact that in general the functional groups of organisms detected by signature lipid markers correlated well with the metabolisms inferred for microbial mat and stromatolites from 16 S rDNA gene sequences (Allen, 2006; Burns et al., 2004; Papineau et al., 2005). The lipid biomarkers produced by the microbial mat communities were very similar to those observed from stromatolites in the same environment (Allen, 2006), suggesting that the microbial mats in Shark Bay are excellent analogues for the extant stromatolites in this location. Further, as the lipid profiles of many modern hypersaline or hot spring microbial mats do not contain BAQC such as those detected in Shark Bay (Allen, 2006), the microbial mats are likely to represent better analogues for the Shark Bay stromatolites than many other mat systems.

Not all lipid classes detected in stromatolites can be compared with ancient sediments since fatty acids are relatively quickly degraded, and wax esters may only survive up to 50,000 years (Cranwell, 1986). In addition, many studies do not investigate the presence of ether-bound lipids, indicative of archaea, although they have been detected in 10,400 year old sediments from Ace Lake, Antarctica (Coolen et al., 2004). Hydrocarbons, however, including the multiple-ring backbones of hopanoids and sterols, are very stable, and a number of these lipid biomarkers have been detected in Archean sediments. Steranes and 2-methyl hopanoids have been detected in 2.7 Ga shales from the Pilbara craton, Western Australia (Brocks et al., 1999; Summons et al., 1999; Brocks et al., 2003) suggesting that both eukarya and cyanobacteria were present at this early stage in Earth's history, while BAQCs, tentatively assigned to sulfur-oxidising bacteria, have been detected in 2.2 Ga sediments (Kenig et al., 2003). These same compounds have

also been detected in the extant stromatolites of Shark Bay (Allen, 2006), suggesting they are good analogues of ancient sediments. Unfortunately, lipid profiles from Archaean stromatolites are yet to be investigated, so direct comparison with the extant stromatolites of Shark Bay is not possible. On the basis of currently available information, however, Precambrian stromatolite microbial communities appear to have been similar to the diverse microbiological assemblages observed in Shark Bay today.

Further indications that the Shark Bay microbial communities represent reliable analogues of ancient stromatolites come from varied approaches: carbon and sulphur isotope data indicating the presence of oxygenic phototrophy and sulphate-reducing metabolism in 2.7 Ga stromatolites (Kakegawa and Nanri, 2006), correlates with the detection of these processes in Shark Bay sediments (Bauld et al., 1979). The detection of cyanobacterial microfossils as outlined earlier, and the similarity of Shark Bay stromatolite microfabrics with ancient stromatolites also supports this conclusion (Reid et al., 2003). The microbial mats and stromatolites of Shark Bay are therefore highly significant resources for understanding life on early Earth.

5. Conclusions

Stromatolites are excellent natural laboratories for the study of microbial ecosystems that may have shaped the biology of early Earth (Des Marais, 2003). Stromatolites provide us with a glimpse of what life may have been like on the early Earth, the kinds of complex microbial interactions that occur, and what kind of metabolisms/physiologies may have been important in early microbial communities. Indeed evidence suggests Archean life forms may have been relatively advanced (Altermann et al., 2006), and thus metabolic pathways observed in present stromatolites may have already been utilised by their ancient counterparts. Although culture-independent molecular analyses alone do not allow us to absolutely determine whether sequences represent active stromatolite organisms, the studies discussed in this review on extant stromatolites show we can take advantage of phylogenetic affinity with well-studied species to make predictions about the metabolic contributions of the organisms identified. Researchers can now build constructively on this platform of microbial community analyses by targeting specific functional genes and enzyme activities, as well as conducting large-scale functional genomics studies, thereby furthering our knowledge on how individual and combined physiologies contribute to stromatolite systems. The close physical association of microorganisms in this setting may also facilitate horizontal gene transfer of adaptive and evolutionally significant traits such as antibiotic resistance, which has implications both for organism evolution and the field of biotechnology.

Finally, the identification of stable biomarkers in stromatolites that are uniquely produced by microorganisms is an area of increasing interest. Characterising

the breadth of biomarkers, including lipids and characteristic pigments, in ancient microbial systems such as stromatolites, has important implications in the field of astrobiology, with the exciting potential for using the knowledge gained in the rational search and detection of possible biosignatures of life outside Earth. Furthermore, communities of microbes in stromatolites are responsible for the production of important trace gases, including photosynthetic oxygen, and the use of remote sensing to interpret infrared spectra may help us identify biological signatures arising from life on distant planetary atmospheres (Des Marais et al., 2002). The quest to understand early life on Earth and the prospects for life elsewhere addresses some of the most profound questions of humankind, and one of the extant analogues of early life, stromatolites, may be key in providing these answers.

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BIOSEDIMENTOLOGICAL PROCESSES THAT PRODUCE HOT SPRING SINTER BIOFABRICS: EXAMPLES FROM THE UZON CALDERA, KAMCHATKA RUSSIA

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Abstract Though the majority of microorganisms in hot spring ecosystems fail to be preserved as bona fide (carbonaceous) microfossils, the presence of microbial biofilms on accretionary surfaces of hot spring sinters can influence the development of sinter fabrics. The extent of biological influence on a primary sinter fabric depends upon the behavior of the microbial community at the time the sinter accretes as well as on the input of sedimentary processes – chemical and physical – that occur during sinter growth. Our ability to recognize the influence of benthic microbial communities on the fabric of a hydrothermal deposit, whether on Earth or, potentially, another rocky planet like Mars, requires an understanding of the interactions between microbial communities, authigenic mineral deposition, and detrital grain accumulation during sinter formation. Examples of hot springs in Uzon Caldera, Kamchatka, Russia, are discussed to illustrate how changes in the relative input of biological, chemical, and physical processes contribute to sinter biofabric formation and preservation. The conclusions drawn from this comparison are relevant to the search for evidence of life in any type of hydrothermal deposit found on a rocky planet.

1. Biosignatures in Hydrothermal Deposits

Understanding the early history of life on Earth is important not only for the advancement of origin of life hypotheses, but also for the quest to determine whether life may occur on other planetary bodies. Astrobiologists face the same challenge as paleontologists who search for and study life's earliest history, namely, how to recognize definitive evidence for life once a potential paleobiological repository is discovered. The types of biosignatures that could be found in hydrothermal deposits on a rocky planet include bona fide microfossils and the carbonaceous remnants of microbial communities, a variety of organic and inorganic chemical fossils, and microbially influenced sedimentary deposits.

Bona fide microfossils, which retain enough morphological fidelity to be recognizable and, by definition (Schopf, 1975), are carbonaceous (composed of

complex organic biopolymers), provide definitive evidence for life (Schopf, 1999). To demonstrate that a microfossil-like object is indeed a bona fide microfossil requires confirmation that it contains carbonaceous cell structures, has a recognizable three-dimensional cellular morphology, and is syngenetic with the deposit in which it was found (Schopf and Walter, 1983; Buick, 1990). The latter criteria is required to date microfossils and avoid mistaking younger contaminants as being the same age as the rock in which they were found. An important constraint on syngenicity is the geochemical maturity of the fossilized carbonaceous matter, whether it is from cellular or extracellular remains, which must be consistent with the degree of alteration experienced by its host rock. Though bona fide microbial fossils and evidence of fossilized extracellular substances become increasingly rare in rocks of progressively older age, life can leave a variety of traces in the rock record (Cady et al., 2003; Westall and Southam, 2006).

Chemical fossils include, for example, organic remains and biominerals characterized by metabolically fractionated stable isotopic signatures; biominerals characterized by a degree of chemical purity or structural order unattainable without biomolecular templates and controlled growth; and lipid biomarkers indicative of cellular remains and relict biomolecules from cells and their extracellular matrix. Chemical fossils have been particularly important in the search for ancient life on Earth (Schidlowski et al., 1979; Rosing, 1999).

Microbial communities also leave fossil evidence of their presence, behavior, and metabolic activities by generating or influencing the development of biofabrics and sedimentary structures such as microbialites and biogenic stromatolites. Microbially influenced sedimentary structures are commonly generated by the interaction of benthic microbial communities and the accumulation of minerals or mineraloids, either through trapping and binding of detrital grains or via authigenic mineral precipitation. Microbialites can occur as thrombolites (characterized by a clotted internal structure) or stromatolites (characterized by a laminated internal structure) (Burne and Moore, 1987). Biogenic stromatolites retain information that has been essential in understanding the paleoecology of early life and its environmental setting (Grotzinger and Knoll, 1999). The various contributions of biological, chemical, and detrital processes involved in the formation of biogenic stomatolites are reflected in the characteristics of their biofabrics (Hofman, 1973). The emphasis here is how these different biosedimentological processes contribute to the development and preservation of sinter fabrics that form in surficial hot spring environments.

2. Implications for Astrobiology

In March of 2007, the Mars Exploration Rover Spirit traversed a deposit that consists primarily of non-crystalline silica and is hypothesized to be the remains of a hydrothermal deposit (Fig. 1) (NASA/JPL News Release). Hydrothermal

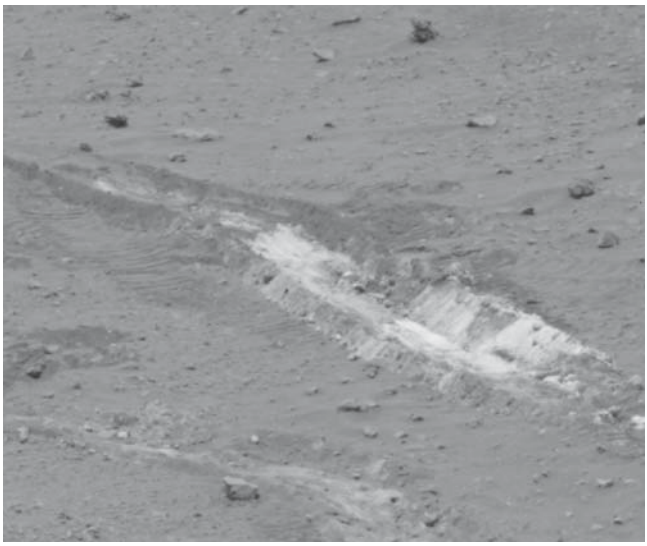

Figure 1. Deposit of amorphous silica uncovered by the Mars Exploration Rover Spirit (Image courtesy of NASA/JPL/Cornell, http://marsrover.nasa.gov/gallery/press/spirit/20070521a.html). The tire track marks are approximately 20 cm wide.

deposits can form on any rocky planet where subsurface water is present (e.g., Farmer and Des Marais, 1999). The potential for crater impacts to have initiated hydrothermal activity on Mars, as discussed by Newsom et al. (2001), suggests that any evidence observed on Mars that is consistent with hydrothermal activity should be explored for biosignatures. Recent models also indicate that the presence of subsurface water (liquid or solid) during impact events on Mars would lead to hydrothermal activity (Rathbun and Squyres, 2002). These impact hydrothermal systems could be relatively long-lived; an impact with a crater diameter of 30 km could generate a system that would endure for up to 67,000 years (Abramov and Kring, 2005). As discussed by Hode and colleagues (2008, this volume), mineral assemblages associated with an impact crater in Sweden were precipitated from fluids with temperatures that may have supported thermophilic life (Hode et al., 2003).

Though it is not yet known whether life ever occurred or thrived on Mars in the form of microbial mats and biofilms in hydrothermal systems, any evidence of sinter-like fabrics in highly siliceous Mars rocks will lead to extensive interrogation of the deposit over the full range of spatial scales for possible biosignatures.

3. Stromatolites

Though stromatolites have been studied for over 150 years, the meaning of the term "stromatolite" is still debated. Early stromatologists applied *Linnean* names to stromatolites based upon their morphology. It has since become apparent that stromatolite morphology is strongly dependent upon environmental factors (e.g., Hofmann, 1969; Grotzinger and Knoll, 1999). Historically, there have been two competing definitions of a stromatolite: (1) one descriptive of the structure – a laminated, lithified sedimentary structure – and (2) the other focused on the mechanism of formation – a laminated structure generated through authigenic mineral precipitation onto benthic microbial communities or detrital mineral grain trapping by benthic microbial communities (e.g., Hofmann, 1969, 1973; Walter, 1976; Burne and Moore, 1987). Of course, a biologically influenced stromatolite could be described by either definition, but because of the difficulty in determining the biogenicity of ancient stromatolites (Lowe, 1994; Grotzinger and Rothman, 1996), it should be clear when using this term which definition is implied. Here, we use the descriptive definition of the term stromatolite and preface it with the modifier biogenic when we can be certain that biology influenced the way it formed. Riding (1999) suggested that the term stromatolite be defined as "a laminated, benthic microbial deposit" and the modifiers "possible" or "probable" be used to indicate when biogenicity is uncertain. Regardless of the formation mechanism, the key characteristic of any stromatolite is its internal lamination (Hofmann, 1969).

Stromatolitic deposits are widespread in the Proterozoic rock record, with maximum diversity and abundance from 2.8 to 1 Ga (Riding, 2000). There are fewer exposures of stromatolites that formed during the Archean Eon, yet stromatolitic structures as old as 3.5 Ga have been described (Schopf, 1994; Allwood et al., 2006). The decline of stromatolite abundance after 1 Ga coincides with the rise of metazoans, and grazing activity is proposed as a major cause of the decline of stromatolite diversity toward the end of the Proterozoic Eon (Awramik, 1971).

Of relevance to the search for ancient and extraterrestrial life are the different types of stromatolites that form in a wide range of modern environments, such as in Shark Bay, Australia (e.g., Awramik and Riding, 1988); Exuma Sound in the Bahamas (e.g., Reid et al., 2000); and in hot spring ecosystems (Walter et al., 1972). A question commonly asked in ancient stromatolite studies is whether modern stromatolites formed in an analogous way to ancient structures that display similar morphologies? For example, the Shark Bay stromatolites are marine carbonate structures that formed in a sedimentary setting and display shapes similar to the majority of ancient stromatolites. Yet the participation of eukaryotic algae, which entrains much larger detrital grains in the modern structures (Awramik and Riding, 1988), along with dramatic secular changes in the composition of the Earth's atmosphere and oceans, limit the use of this modern locale as a biological and biosedimentological homologue for ancient biogenic-stromatolite

forming environments. Regardless, modern biogenic stromatolites provide exceptional opportunities to document how the interaction of biosedimentological processes contribute to the formation of such structures.

4. Sinter Biofabrics

The formation of a stromatolite requires (1) a substratum for the attachment of a microbial community and growth of the stromatolite, (2) a fluid medium, generally fresh or marine water, (3) a benthic microbial community, (4) the deposition of small detrital grains or authigenic mineral precipitates, and (5) a rhythm in the deposition of sediment or growth of the community (Hofmann, 1973). During the growth of a stromatolite, the type of fabric that forms in association with the structure depends on the contributions of four main factors: (1) the accumulation of skeletal remains of organisms (e.g., corals), (2) the input of clastic material (e.g., varves), (3) chemical precipitation (e.g., stalactites), and (4) the input of non-skeletal biological remains (e.g., algal mats) (Hofmann, 1973). As discussed below, the main factors that contribute to hot spring sinter fabrics are chemical, microbial, and detrital processes.

The description of *Conophyton*-like stromatolitic structures in hot spring sinters in Yellowstone National Park led to the recognition of one of the first modern analogues for ancient siliceous stromatolites (Walter et al., 1972). The study of modern sinter deposition has increased our understanding of how microfossils form, sinter fabrics develop, and chemical biosignatures become preserved in sinters (e.g., Walter and Des Marais, 1993; Cady and Farmer, 1996; Jones et al., 2001; Konhauser et al., 2001; Konhauser et al., 2003; Lalonde et al., 2005). The environment in which most mid-to-high temperature sinter stromatolites form excludes metazoan grazers and allows for the encrustation and entombment of components of microbial mats and biofilms.

The relative importance of biotic and abiotic factors in sinter stromatolite formation has not been firmly established. Modern sinters show evidence for microbial involvement in sinter fabric formation across a broad range of temperatures (Cady and Farmer, 1996). Yet arguments have been made for either abiotic or biotic factors playing a dominant role in sinter stromatolite morphogenesis. For example, a study of high-temperature siliceous sinter at Yellowstone National Park led Lowe and Braunstein (2003) to conclude that, though organisms were present, they did not control the development of stromatolite-like features in the sinter; hence, they consider the sinters abiotic in origin. Detailed study of Icelandic hot springs led Konhauser and colleagues (Konhauser et al., 2001) to propose that laminated moderate temperature sinter fabrics result from the annual growth and die-back of cyanobacteria, a process that favors a biogenic origin such stromatolites.

The examples discussed below illustrate how, in several hot springs in the Uzon Caldera, biosedimentological processes contribute to the formation of

sinter fabrics. The Uzon Caldera, in fact, is an ideal location for this comparative biosedimentological study of sinter fabrics, given the presence of multiple thermal basins with a wide variety – geochemically and sedimentologically – of hot springs, the remote location and international preserve status of the area (which limits the impact of human visitors on the site), the presence of natural and a few man-made hydrothermal features (the latter of which constrains their date of origin), and the distribution of numerous and diverse hot springs within a small geographic area. It is worth noting that the conclusions drawn from this comparison are relevant to the search for evidence of life in any type of hydrothermal deposit found on a rocky planet.

5. The Uzon Caldera

The Kamchatka peninsula of far eastern Russia is an active volcanic region characterized by numerous hydrothermal basins. The Uzon Caldera, in the Kronotsky National Biosphere Reserve, is located approximately 200 km north of Petropavlovsk, the only major city in Kamchatka (Fig. 2A). The Uzon Caldera formed during the collapse of Mount Uzon ~40,000 years ago, an age based on carbon-14 dating of the ignimbrites produced during the eruption (Florenskii, 1988). The caldera, a 7 by 10 km oval depression, is associated physically and geochemically with the Valley of the Geysers (Fig. 2B). The large

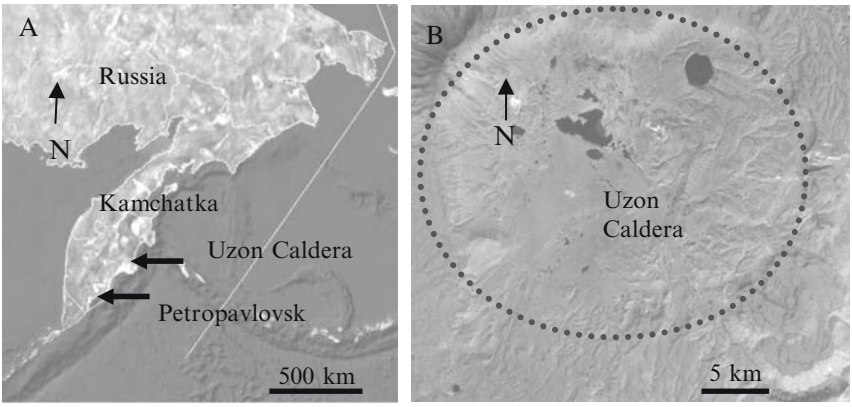

Figure 2. A: The Kamchatka peninsula of far eastern Russia (Image Google Earth). Petropavlovsk is the largest city in Kamchatka. B: Satellite image of the Uzon Caldera. The dotted line represents the approximate location of the caldera rim. Note that Valley of the Geysers is located in the bottom right-hand corner of the satellite image, beneath the bar scale. NASA Landsat 7 ETM + Satellite image at 15 m resolution.

Lake Dal'nee Maar was formed through a phreatomagmatic eruption 7,600 to 7,700 years ago (Ponomareva and Braitseva, 1991). The Uzon Caldera is underlain primarily by basalt and dacite flows and tuffs (Belousov et al., 1984; Zolotarev et al., 1999). A large, deep heat source and a deep water-bearing layer feed both the Uzon Caldera and Valley of the Geysers systems, within which occur several hydrothermal areas with many distinct geochemical features (Belousov et al., 1984).

6. Biosedimentological Comparison of Sinters

The main factors that contribute to modern sinter biofabric formation include (1) chemical input via authigenic mineral precipitation, (2) biological input via growth/death of benthic microbial communities, and (3) detrital input via the deposition of clastic sediment grains. Nearly every hydrothermal feature in the world supports some degree of microbial activity. Microbial biofilms and mats thrive in thermal fluids even though the ecosystem may not be receiving authigenic minerals or detrital input. On the other hand, when the rate of microbial replication is slow compared to the rates of detrital accumulation or mineral deposition, the influence of biology on the fabric of a sinter may be difficult, if not impossible, to recognize. Field- to microscopic-scale observations of the biosedimentological regimes studied are used here to assess the likelihood that such features would be preserved in the geological record.

Observations were made over a period of 3 years of summer field excursions to the Uzon Caldera. The hydrothermal features described here include Thermophile Spring, Burliashiy Pool, Zavarzin Pool, Ochki Pool, and the outflow channel associated with the capped but leaky well head of the K4 Well. In 2003–2006, these hydrothermal features were characterized by relatively similar, undersaturated dissolved silica concentrations (~100 ppm) and circumneutral pH, though some vents at Ochki Pool were found to be more acidic (bulk geochemical analysis was provided by the University of Georgia, Athens Geology Department, personal communication per Doug Crowe).

7. Thermophile Spring

Thermophile Spring is a moderately sized hydrothermal feature located near the rim of the Eastern Thermal Field in Uzon Caldera. The ~74°C vent pool (~1 m in diameter) drains into an outflow channel ~7 m long. The pH of the fluid at the vent increases from 6 to a maximum of just over 7 at the distal portion of the outflow channel. The main effluent of the pool is characterized by small islands composed of white silica sinter. Beyond the sinter islands, the channel floor is coated by black sinter and sediments. The white and black sinters are covered by

white microbial streamers. Thick, laminated phototrophic (green-orange pigmented) microbial mats form in the distal portion of the outflow channel (Fig. 3).

It is interesting to note that the identity and distribution of the microbial communities that presently occupy this system are the same as that described by Gorlenko and colleagues (1987) on the basis of their observations made nearly 20 years ago. Permanently submerged white microbial streamers – free floating string-like mats – occupy a considerable portion of the outflow channel. Near the vent effluent they colonize the outer rims of the high-temperature sinter islands.

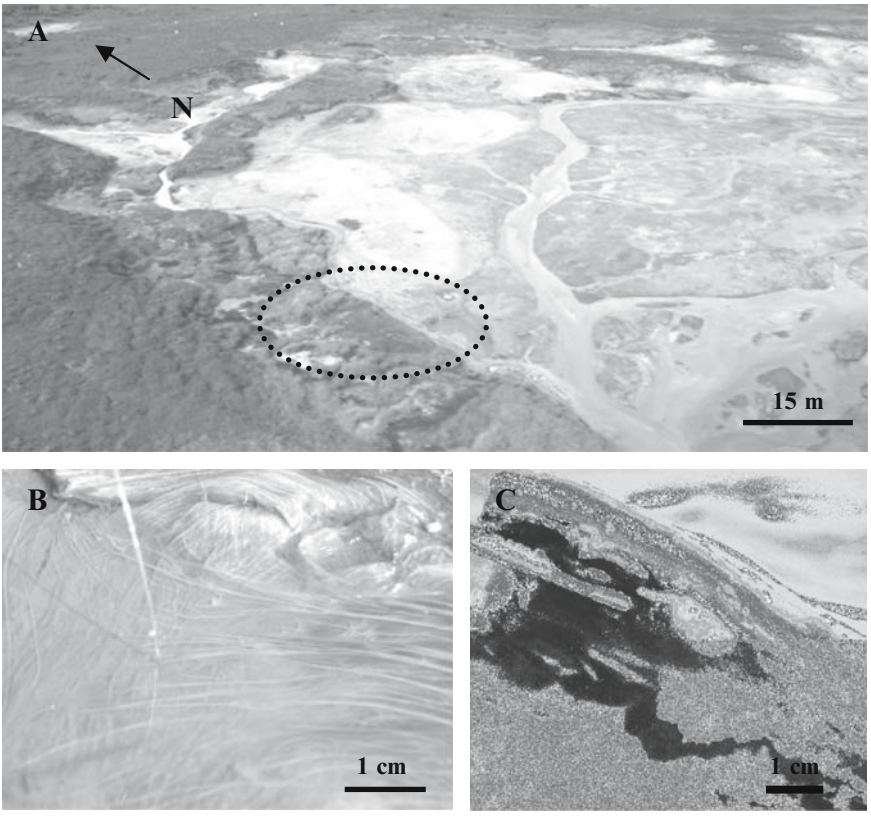

Figure 3. A: Aerial photograph of the Eastern Thermal Field. The circle encloses Thermophile Spring and its outflow channel, the vent appears white, the orientation of the outflow channel turns just beyond the vent, and the dark area is the thick accumulation of microbial mats (photograph provided by C. Romanek). B: Streamers mat fabrics that lie over thickly layered microbial mats. C: Middle layer of a mat with abundant segmented cyanobacteria (positive for chlorophyll-a autofluorescence).

In the mid-to-high temperature regime they colonize the rocky bottom of the outflow channel for at least 4 m. At the mid-temperature portion of the outflow channel they colonize loose particles of black sediment that have accumulated along the bottom of the channel floor. These white streamers are made up of filamentous *Thermothrix spp*. and sulfur reducers (Gorlenko et al., 1987). The subaerial portion of the sinter that surrounds the vent and the sinter islands is coated by pink and green biofilms in the subaerial zone above the water line. These subaerial biofilms are dominated by large cyanobacterial cocci (identified on the basis of chlorophyll-a autofluorescence) and small rods. Thick microbial mats that occupy the outflow channel fluids with temperatures ≤60°C are characterized by long streamers that protrude across the microbial mat surface. These mats are made up of *Chloroflexus spp*., *Oscillatoria spp*., and other cyanobacteria (Gorlenko et al., 1987).

Though Thermophile Spring has continued over the years to host diverse and extensive microbial communities that occupy the entire length of the outflow channel, the potential to preserve biogenic sinter fabrics associated with any of the communities is limited. The morphologically distinctive streamers, for example, are not becoming entombed in the sediment on the outflow channel floor. And though minor amounts of subaerial spicular sinter occur around the vent, most likely forming as evaporative-driven silica precipitates, the system lacks enough mineral accumulation in the subaqueous portion of the outflow channel to preserve biologically influenced sinter.

8. Burliashiy Pool

Burliashiy Pool, a nearly circular pool with many vents, is ~5 m diameter. The pool lies in a topographically low area of the basin and is surrounded and underlain by lake sediments. The turbulence from the vents introduces course grained sediments and weathered diatom frustules that deposit on the bottom of the pool as an unconsolidated layer of sediment. Burliashiy Pool is 90°C and has a pH of ~6. A series of adjacent small pools with active turbulent vents and high temperatures is associated with Burliashiy Pool. In these pools, the lack of benthic microbial mat development or cementation of the sediment through authigenic mineral precipitation leads to the accumulation of an unconsolidated layer of clastic grains. The apparent absence of a benthic microbial community due to the turbulent nature of the vent fluids in these pools and in Burliashiy Pool eliminates the possibility that a lithified sedimentary biofabric would develop in these systems. The sediments also lack the type of cohesion that would have been possible had they been colonized by benthic microbial biofilms. The biosedimentological regime of these hot springs consists of an accumulation of easily disrupted layers of clastic grains in the submerged regions around the effluents.

9. Ochki Pool

Ochki Pool consists of a large, shallow pool surrounded by a relatively thick and laterally extensive lithified siliceous sinter rim (Fig. 4). A series of small vents that protrude through the sinter rim at various distances from the main pool maintain its relatively constant fluid volume, though the water level lies beneath the surface of the sinter rim. Hence, several small vents around Ochki Pool can be accessed by walking to them on top of the lithified sinter. Ochki Pool measures several meters wide and is greater than 10 m long. This entire system lies in an inlet located along the western-most edge of the Central Thermal Field. The range of fluid temperatures in the vents and main pool (40–75°C) and pH (3–6) indicates that the area is fed by several geochemically distinct subsurface aquifers.

Though an extensive amount of sinter has been deposited in the Ochki Pool area in the past, only the rims around the vents and the main pool show any evidence of recent sinter deposition. Spicular and laminar sinter material along the rim of the pool and around the vents has built up primarily as a result of the evaporation and cooling of episodically splashed fluids that wet the sinter surface

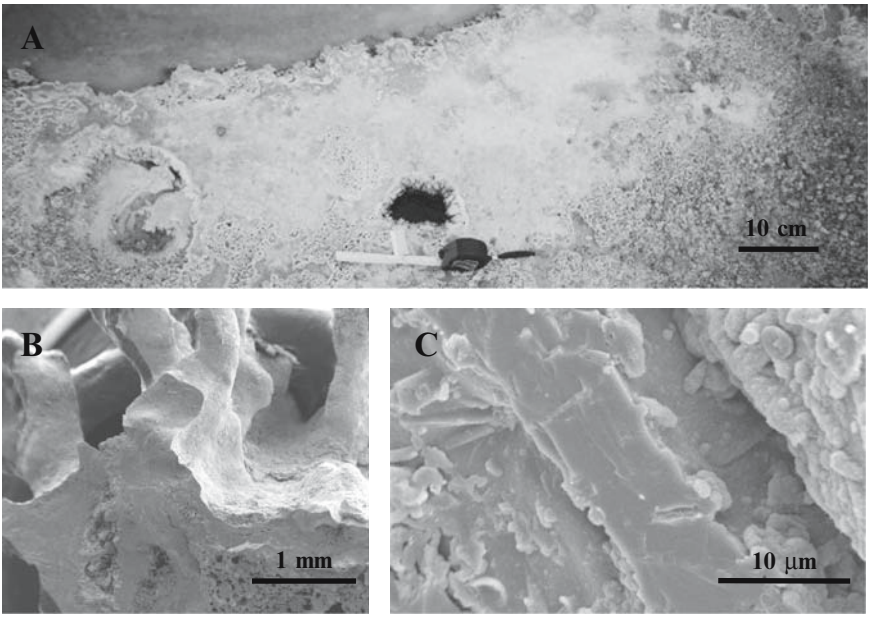

Figure 4. A: Ochki Pool consists of several vents that drain into a large, shallow pool surrounded by lithified sinter and many sinter islands. B: The only sinter actively depositing in this area occurs along the rim of an older sinter horizon within which the vents protrude. C: The massive fabric typically has no obvious signs of biological input that would have resulted from the presence of biofilms on the sinter when it accreted.

but are too infrequent to sustain biofilm growth. Phototrophic growth lines the inner rims of the sinter around a few of the vigorously bubbling vents, but in general, the relatively low water level of quiescent vents and the main pool relative to the height of the exposed horizon of sinter causes the sinter to remain dry for the majority of the summer. The dry sinter deposits exhibit very little morphological evidence of biological influence at the time of its formation (alternatively, see Cady and Farmer, 1996 for examples of high-temperature sinter biofabrics constructed via the silicification of subaerial biofilms on rims of perpetually wetted sinters in the splash zone of active silica-depositing hot springs in Yellowstone National Park). A powder X-ray diffraction (XRD) analysis (2–65° 2θ, step size 0.03° 2θ, scanning rate of 1°/minute, Phillips X'Pert XRD) of the youngest precipitates deposited on the sinter rims at Ochki Pool reveal that it consists primarily of opal-A with minor amounts of kaolinite, cinnabar, and evaporite minerals (XRD data not shown).

10. Zavarzin Pool

Zavarzin Pool, a 4.5 by 2.25 m pool, is situated in the Eastern Thermal Field, several hundred meters from Thermophile Spring. The broad shallow pool is continuously fed by numerous small vents that keep the pool fluid well-mixed; the temperature (~55°C) and pH (6.3) of the individual vents was the same as those of the mixed pool fluid. The bottom of the pool is colonized by a filamentous mat several millimeters thick that traps detrital material as it settles down on it. The edges of the pool, which are not well lithified, are rimmed with thin greenish mats dominated by cyanobacteria (Fig. 5).

Though the trapping and binding of detrital material into the mat generates a recognizable biofabric that could be preserved, it is not becoming cohesively lithified in this subaqueous environment and is unlikely to be buried and retained in the rock record as a primary sinter biofabric. It is worth noting that, should there be a pulse of detrital input, such as from air-borne ash from a distal volcanic eruption, the preservation of organic-rich horizons in the sediments that accumulate within the pool may occur. However, this type of preservation requires an external episodic influx of sediment, a process not inherent to the active depositional regime of the pool today. XRD analysis has confirmed that the pool floor sediments at Zavarzin Pool consist of detrital grains of opal-A and feldspar.

11. K4 Well

K4 Well is an experimental well that was drilled several decades ago and capped. The well head has been leaking extensively for a number of years (G. Karpov, personal communication, 2004–2007). Several distinct chemotrophic biofilms and

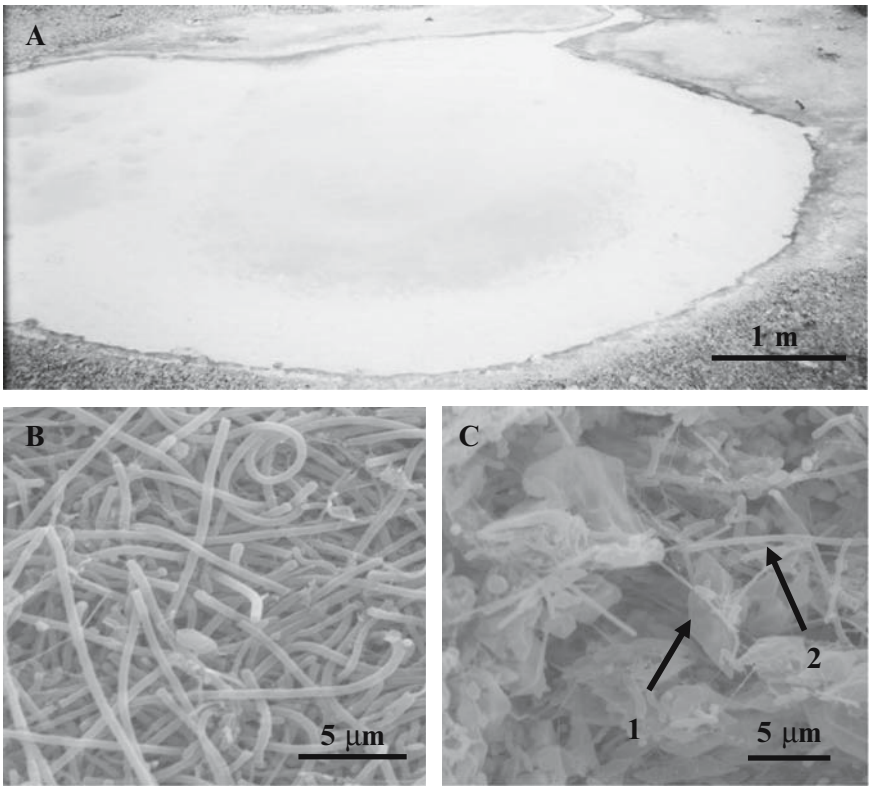

Figure 5. A: Field photo of Zavarzin Pool. B: Filamentous cyanobacteria (positive for chlorophyll-a autofluorescence). C: Mineral grains (1) bound by biological material (2).

photo-trophic mats have developed along the floor of the outflow channel, and their silicification preserves several distinct sinter fabrics along the length of the outflow channel (Goin, 2007). The outflow channel is ~1 m wide and extends ~3.5 m away from the effluent before grading into standing water at the edge of Chloride Lake (Fig. 6). Fluid that sprays out of the well-head at 97°C cools to ~85°C at the beginning of the outflow channel at the base of the well-head. The hydrothermal fluid cools to ~30°C in pools located outside the main runoff channel and at the end of the channel where it encounters Chloride Lake. The pH varies from 6.6 proximal to the well-head to 7.0 near the end of the main runoff channel.

The high-temperature biofacies at K4 Well is dominated by small diameter (less than 0.5 μm) non-pigmented filaments that form short (1–2 mm) pink-to-reddish colored streamers. Where the water temperature in the main channel drops to <75°C, phototrophic non-oxygenic (green pigmented but negative for

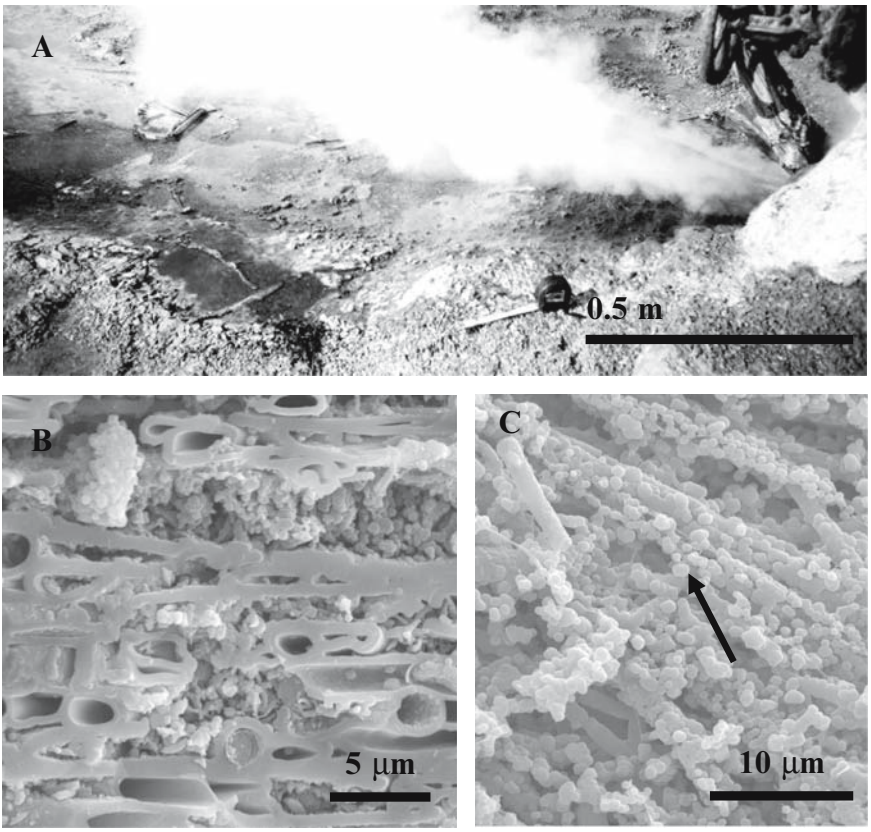

Figure 6. A: Field photo of the K4 Well outflow channel. B: Microfossil casts lie approximately tangential to a horizontally laminated biofabric characteristic of the mid-temperature K4 Well sinter. C: Note the colloidal silica particles (arrow) that coat adjacent filaments.

chlorophyll-a) bacterial filaments ~2μm in diameter dominate the mats. The microbial communities submerged beneath fluids with temperatures <70°C include bacterial morphotypes that exhibit the same characteristics as those of bacterial morphotypes found in the ~75°C fluid and those of filamentous cyanobacteria (positive for chlorophyll-a autofluorescence). The lower temperature biofacies (40–60°C) consists predominantly of cyanobacteria characterized by rods and several different filamentous morphologies. The pools outside the main outflow channel, with temperatures <40°C, contain floating tufts of green-pigmented phototrophic filaments characterized by narrow diameters (0.8μm), though the lack of chlorophyll-a autofluorescence indicates that these filaments are not cyanobacteria.

The silicification of biofilms in the outflow channel at K4 Well preserves sinter biofabrics with a demonstrable biogenic input. The biofabrics preserve information indicative of the biofilm architecture as well as the dominant morphologies of the organisms. The outflow channel at K4 Well represents a system where the processes of biologic input, chemical precipitation, and negligible detrital input result in excellent preservation of biofilm features in the sinter biofabrics.

12. Discussion

The ternary diagram shown in Fig. 7 illustrates how the various processes contribute to the formation of a sinter biofabric in surficial hydrothermal systems. The situation at Thermophile Spring demonstrates that the presence of biology alone is not enough to produce biofabrics; hot spring systems dominated almost

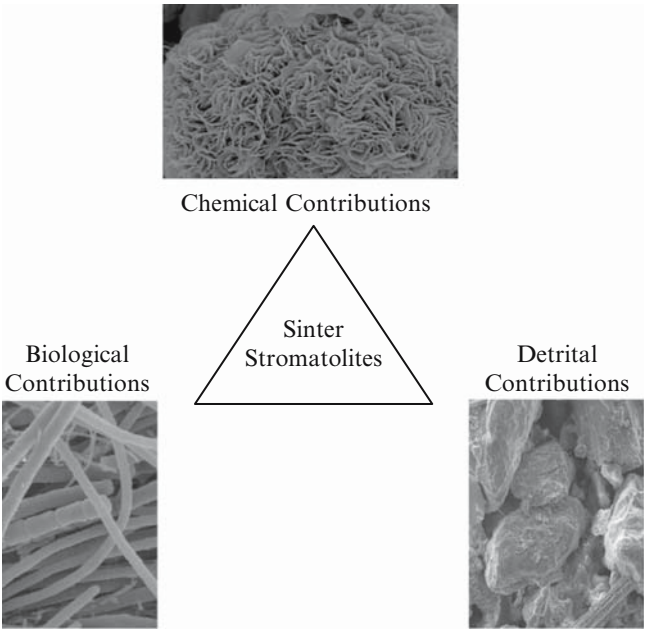

Figure 7. Ternary diagram (modified after Hoffman, 1973) illustrates the main processes that contribute to the formation of hydrothermal sinters. The examples discussed here represent hydrothermal ecosystems dominated by near end-member contributions (i.e., Ochki Pool is dominated by chemical contributions; Thermophile Spring is dominated by biological contributions; and Burliashy Pool is dominated by detrital contributions) and approximate mid-point contributions (i.e., biological and chemical processes contribute to K4 Well sinter formation and biological and detrital processes contribute to Zavarzin Pool sinter deposits).

entirely by biological contributions require a means to convert some aspect(s) of the microbial community into a biofabric. Though hot springs can develop prolific microbial mats and biofilms, the additional contribution of authigenic mineral deposition or the accumulation of detrital debris in association with the microbial communities is necessary to produce a biofabric. Without a biofabric to become lithified or buried and, hence, enter the rock record – one of the main mechanisms of retaining a biological signature of microbially dominated communities (e.g., microfossils, the fabrics of biologically influenced sedimentary structures, chemical fossils, Cady, 2001) – is lost.

Burliashiy Pool and Ochki Pool, the former dominated by detrital input, the latter chemical mineral precipitation, illustrate how difficult it is to form biofabrics in environments dominated by end-member processes other than biological input. In Burliashiy Pool and the adjacent transient pools, the amount of detrital input and the turbulence of the vent activity prevent the development of microbial biofilms and mats on the inner pool surfaces. Biofabrics have not developed in, and adjacent to, Burliashiy Pool because the environment is not physically stable. Biofabric development at the near chemical end-member situation that occurs at Ochki Pool is minimal because the geochemical conditions (low pH, high arsenic concentrations) and relatively calm (i.e., non-splashing, variable, or surging) and low water level in the pool are not conducive to the development of biofilms on sinter substratums. The lack of periodic wetting on the sinter rims around the vents and pool promotes the deposition of opal-A via evaporation, and the lack of well-developed and sustained microbial activity on those surfaces eliminates the potential to preserve distinct biofabrics in the sinter.

Our biosedimentological comparison of the main formation processes involved in biofabric development in Uzon Caldera hot springs underscores the concept that only certain combinations of biological, chemical, and physical processes will lead to the formation and preservation of sinter biofabrics.

Hydrothermal ecosystems like Zavarzin Pool, which receives a significant amount of detrital input in the absence of authigenic mineral precipitation, generates a distinctive binding-and-trapping biofabric on the floor of the hot spring pool. Should the mat/sediment biofabric remain cohesive during post-depositional disruption (e.g., Schieber, 1999; Gerdes et al., 2000; Noffke et al., 2002), the biofabric may be preserved in the rock record. It is worth noting that in hot spring ecosystems with only mixed detrital-biological inputs, the biofabrics may lack any obvious sedimentological or morphological characteristics that would indicate the presence, behavior, or metabolic activity of the populations that contributed to their formation. The link back to the paleoecology would be weak without organic chemofossils (e.g., biomarkers and evidence of biological isotopic fractionation) in the carbonaceous remains of such biofabrics, should they be preserved.

The most easily recognized biofabrics, and those most likely to enter the fossil record, are hydrothermal ecosystems characterized by the growth of microbial biofilms and mats that thrive in mineralizing fluids (e.g., Walter and Des Marais,

1993). In the outflow channel of the K4 Well, silica precipitation occurs in the presence of ubiquitous biofilms that colonize the accretionary sinter surfaces. Seasonal variations in evaporation-driven mineral precipitation lead to seasonal variations in the growth rates of the cyanobacterially dominated microbial communities. The interplay of these factors results in the formation of distinctive stromatolitic biofabrics at K4 Well (Goin, 2007).

In summary, the mixed end-member systems of Zavarzin Pool and K4 Well are characterized by well-developed benthic microbial communities, a fluid medium, and the deposition of detrital grains or the accumulation of authigenic precipitates, all of which are attributes required for stromatolite formation. While a trapping and binding fabric is generated at Zavarzin Pool, degradation of the organic relicts of the microbial communities would leave a collection of detrital grains without a demonstrable biogenic contribution to the sediment fabric. The mineralization of microbial mats and biofilms at K4 Well results in the formation of distinctive biofabrics and enhances the potential to preserve evidence of the biogenic contribution to the fabric, even if the carbonaceous remains of microbial cells or extracellular remains would be removed from the sinter by post-depositional taphonomic processes.

13. Implications

While it is tempting to overlay a mask on the ternary diagram shown in Fig. 7 for the purpose of defining, in a quantitative way, that portion of the plot where biofabrics will form, there are a number of practical reasons why this would be difficult to accomplish for a particular spring, let alone for the variety of hot springs discussed here. Though hot springs often appear to be highly stable, environmental conditions can change rapidly if flow from the effluent is diminished or diverted from the stream channels and runoff plains. Even slight differences in the rate and volume of fluid flow or changes in fluid temperature and composition can alter the apparent stability of any one microbial community in a hot spring ecosystem. Any changes upstream will also result in a variety of changes in the microbial communities downstream. For example, flow and geochemical changes can alter the nature of the hydrodynamic conditions, the flux of nutrients to organisms, the degree of saturation of the hydrothermal precipitates, and the types of interactions that occur within microbial consortia that consist of many populations of organisms and interlopers like viruses and – at lower fluid temperatures – algae and plankton. Microbial communities become susceptible to predation by grazing eukaryotes when fluid flow decreases and water levels drop below the top of the biofilms or microbial mats. Though it is obvious that the behavior of any one microbial population within a community depends upon many factors, how these populations respond to a variety of environmental clues is not fully understood.

As microbiologists, microbial ecologists, geomicrobiologists and geochemists grapple with strategies to categorize, quantify, understand, model, and predict the nature of microbial communities and biogeochemical interactions in natural environments, it is important to remember that we have only begun to study, under laboratory controlled conditions and in natural settings, the various biosedimentological processes that interact to produce biosignatures – such as biofabrics – in the rock record. It is one thing to search for evidence of life as we know it in ancient rocks here on Earth, yet quite another to search for life elsewhere in the universe. Regardless of the remaining challenges, remarkable opportunities lie ahead for those involved in the continued search for life beyond the confines of the blue planet.

14. Acknowledgements

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CYANOBACTERIAL MAT FEATURES PRESERVED IN THE SILICICLASTIC SEDIMENTARY RECORD:
Paleodeserts and Modern Supratidal Flats

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1. Introduction

Up till about 3,850 Ma, planet-sterilising impact events would have made Earth effectively inhospitable to life (Maher and Stevenson, 1988; Sleep et al., 1989). There is no record of the origin of life, but it can be assumed that it began on Earth under extreme conditions: very hot, only trace amounts to no oxygen, possibly saltier oceans than now, higher UV flux, but with a wide range of potential habitats for life, varying from subaerial to deep water conditions (Nisbet, 1995; Nisbet and Sleep, 2001; Westall, 2004). Cyanobacteria, inferred in the rock record from at least 3.5 Ga (Schopf, 2004), were the pioneering oxygenic phototrophs within this framework of early Earth's evolution (Paerl et al., 2000 and references therein) and their further evolution was punctuated by critical biochemical transitions such as the oxygenic atmosphere, which many put at c. 2.3 Ga, others much earlier (cf., Ohmoto, 2004).

By c.2.0–1.8 Ga, all known sedimentary environments were active on Earth, including large hot desert settings (Eriksson and Simpson, 1998; Eriksson et al., 1998) and a large number of researchers agree on an at least partially oxygenic paleo-atmosphere (Ohmoto, 2004). Despite this transformation to a less extreme Earth, extreme environments for cyanobacteria and the mats they form, persisted and occur up till today. In this paper, we will examine the record of microbial mat features preserved within two such siliciclastic (fine sandstones to siltstones) settings, from the c.2.0–1.8 Ga Waterberg Group paleodesert (Kaapvaal craton, South Africa) and modern supratidal flats. In the former, physically separated mat proxy features related to either desiccation or rapid flash-flood events predominate. In the latter, a more complex association of mat proxy features is preserved, concomitant with a more complex cyclical wetting and drying history; crack healing rather than propagation is common, yet fatal desiccation results in analogous features to those from the paleodesert.

2. Microbial Mat Features in Clastic Sedimentary Environments

Microbially-formed structures (cf. stromatolites) are well known from carbonate rocks, particularly of Precambrian age, but features formed from microbial mats in clastic rocks are less well known (e.g., Schieber et al., 2007). Initial biofilms of microorganism clusters and extracellular polymeric substances (EPS) over time become transformed into microbial mats at the clastic sediment-water interface (Schieber et al., 2007). The cyanobacteria are the most successful group in enlarging such biofilms into mats and have a high capacity for biostabilisation on clastic sedimentary surfaces exposed to sunlight (Gerdes, 2007). With time, tough and even leathery mats may form, which provide cohesion to silt and sand grains, thereby helping to bind them and provide resistance to erosion (Schieber et al., 2007).

Such mats and their effects on the clastic sediment within and upon which they grow, leave subtle traces and less evident proxies of their presence within the clastic sedimentary record (Gerdes, 2007). Their wide paleoenvironmental adaptation in modern clastic environments is largely mirrored in the preserved rock record, particularly in that of Precambrian age when predators of mats were absent (Schieber et al., 2007, and references therein). However, in terms of preservation, features directly attributable to microbial mats in terrigenous clastics are either rare or very localized; in contrast, proxy structures resulting from processes such as mat-induced sediment binding, grain agglutination, and chemical compartmentalization of the sediment are common in shallow marine sandstones and offshore shales, especially those of Precambrian affinity (Schieber et al., 2007). These proxy structures largely owe their formation to EPS (Decho, 1990, 2000) secreted by the cyanobacteria and other microorganisms. EPS and microbial filaments make sand and silt cohesive, enabling trapping and binding of clastic particles, which will then respond differently to stress, often behaving more like mud, and forming a host of features (more than 50 are known) generally not expected in sands/sandstones: e.g., desiccation cracks, sand-curls, and flat, pebble-sized rolled-up fragments upon erosion (Fig. 1) (Schieber et al., 2007 and references therein).

The known record of microbial mats in siliciclastic settings extends to c.3.2 Ga (Noffke et al., 2006) and a wide occurrence in Proterozoic siliciclastic sedimentary lithologies probably also reflects the vast epeiric seas of that era (e.g., Eriksson et al., 2005) where low sedimentation rates enhanced the growth of microbial mats. The importance of low sedimentation rates for the transition from an initial and fragile biofilm to a much denser and more robust mat, able to withstand erosive processes, is emphasized by Gerdes (2007); based on experimental work on modern mats, several weeks of non-burial are generally required. However, motile bacteria in well-established mats can easily move upwards through a thin sediment cover (a few millimetres of silt or sand) to re-establish the mat within only a few days; once again, low sedimentation rates are implicit. Terrestrial mat systems have been documented as far back as 1.8 Ga in the fully continental rock record (Eriksson et al., 2000). In this paper, we will briefly describe microbial mat-related features from the oldest known terrestrial example, the

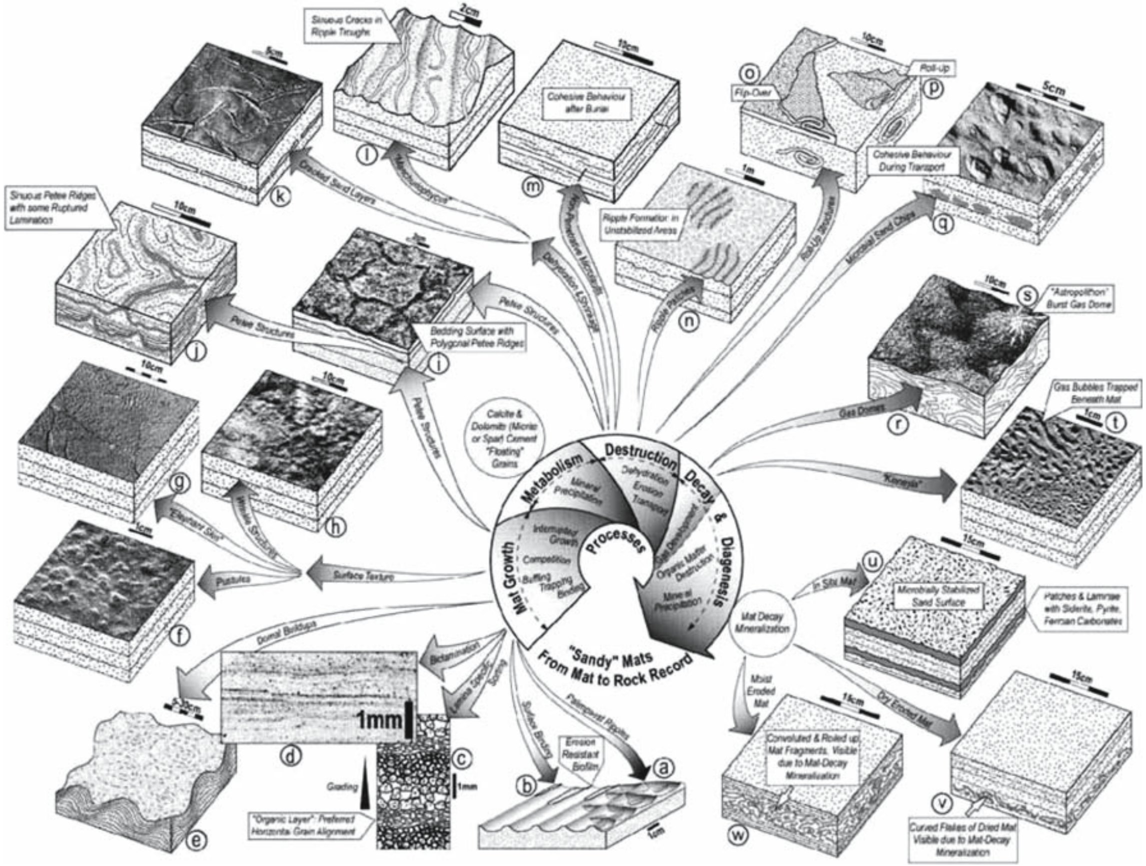

Figure 1. Summary figure of main types of known proxy structures resulting from microbial mat-related processes within sandy sediments, arranged according to genesis of the features. (Reprinted from Schieber, 2004, Fig. 7–9.1. With permission from Elsevier.)

c.1.8 Ga Waterberg Group of South Africa, and from modern supratidal settings, analogous to epeiric sea coastline settings from the Precambrian; both provide examples of the extreme environments where cyanobacteria survive today in the face of metazoan predators in the more favorable settings.

The two chosen environments are superficially comparable: highly saline shallow water pools and temporary inundations are likely, and desiccation is important in both, as will eventual mat destruction be. However, the two extreme settings will also display significant differences in the assembly of mat-related features typically preserved from the two settings, and in their genesis. Our paper attempts to address these facets.

3. The Waterberg Group: Inferred Mat-Related Features and Their Genesis

3.1. GENERAL GEOLOGICAL SETTING

The c.2.06–1.88 Ga (SACS, 1980; Jansen, 1982; Walraven and Hattingh, 1993; Eglington and Armstrong, 2004; Hanson et al., 2004) Waterberg Group is amongst the sedimentary units globally marking the first appearance of red beds (*sensu stricto*) and large erg (sand sea) deposits, at c.1.9–1.8 Ga (e.g. Eriksson and Cheney, 1992; Eriksson and Simpson, 1998); this was a period also marked by large-scale paleoenvironmental changes related to the supercontinent cycle (e.g., Eriksson et al., 2004). Waterberg sedimentation (preserved in a Main and Middelburg Basins; Fig. 2) was mainly fluvial, with lesser alluvial, lacustrine and paleodesert deposits (Callaghan et al., 1991; Simpson et al., 2002, 2004).

The Main Basin is a fault-controlled, continental depository (Callaghan, 1987). The stratigraphy of the Waterberg Group in this basin comprises 11 formations (Fig. 2). Following deposition of marginal, essentially protobasinal Waterberg alluvial deposits (Blouberg in the north; Swaershoek-Sterkrivier-Alma Formations in the south) in the Main Basin, are three correlated pairs of formations with the respective double-sets of formations arranged essentially SW and NE of a synsedimentary fault system (the Vaalwater Fault), running NW-SE across the preserved Main Basin (Fig. 2). The first set of these, comprising the SW Skilpadkop and the NE Setlaole Formations, reflects mainly lithic to arkosic, locally pebbly sandstones and lesser conglomerates, all ascribed to a fluvial braidplain depositional setting (Callaghan et al., 1991). This 450–600 m thick succession is succeeded by the second pair of correlated units, the SW Aasvoelkop and NE Makgabeng Formations; the former is conformably-based with the latter being locally unconformably-based. The Aasvoelkop succession, of basal mudrocks-medial lithic sandstones-uppermost immature pebbly sandstones (with intercalated tuffaceous eruptive beds) is interpreted as the deposits of a through-flow lake followed by fluvial sediments (Callaghan, 1987). The correlated Makgabeng Formation comprises paleodesert deposits containing predominant paleodune sediments, with evidence for an interaction of eolian and wet-desert processes (such as wadi-fluvial, interdune and saline pan influences) within this setting (Simpson et al., 2002, 2004). It is the interdune deposits that contain the microbial mat-related structures discussed in this paper (e.g., Eriksson et al., 2000). This combination of arid and locally/impersistently wetter conditions applies also to the entire Waterberg Group, which is seen as having enjoyed a semi-arid

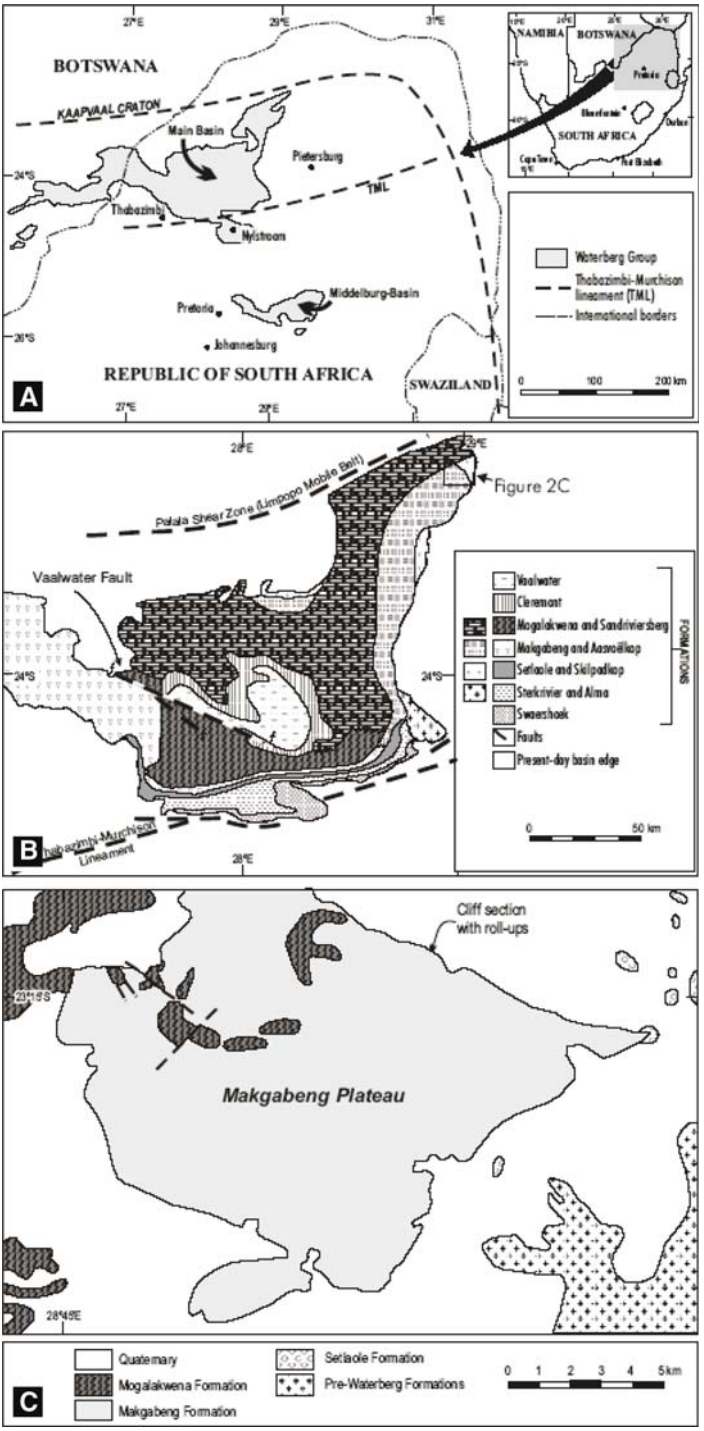

Figure 2. (A) Location of Main and Middelburg Basins of the Waterberg Group in South Africa. (B) Geological sketch map of the Main basin, showing the component formations making up the Waterberg Group; note three pairs of formations arranged NE and SW of the NW-SE orientated Vaalwater Fault. (C) Location of study area with interdune beds and microbial mat features, within the Makgabeng Formation. (Modified after Simpson et al. 2002, 2004.)

paleoclimate (Callaghan et al., 1991; Simpson et al., 2002, 2004). The third set of correlated formations, respectively SW and NE of the Vaalwater Fault, are the Sandriviersberg (1,250 m thick; arenites, granule-rich arenites and conglomerates) and Mogalakwena (similar thickness and lithology) (Fig. 2). A large fluvial braidplain, proximal to the NE, is envisaged (Callaghan, 1987; Callaghan et al., 1991).

Uppermost more mature sandstones of the Cleremont and succeeding Vaalwater Formations complete the succession in the Main Basin, and were deposited across the trend of the Vaalwater Fault, testifying to a smaller tectonic control, lower gradient fluvial systems and significant reworking, with the possibility also of littoral influences (Callaghan et al., 1991). In the much smaller Middelburg Basin, a single formation, the Wilgerivier, reflects western-sourced arenaceous and lesser conglomeratic braidplain deposits (Van der Neut and Eriksson, 1999), which are thought to be coeval with the basal protobasinal formations in the Main Basin.

3.2. GEOLOGY OF THE MAKGABENG FORMATION

The generally mature, fine- to medium-grained quart arenites (and lesser finer rocks as well as pebbly sandstones) of the Makgabeng Formation are interpreted as eolian dune, playa, saline pan and interdune facies within an overall desert setting. The eolian facies (inversely-graded wind ripple strata unequivocally support such an origin) is predominant, both stratigraphically and geographically, with contained cross-bedding patterns suggesting either barchanoid or straight-crested dune bedforms (Eriksson et al., 2000; Simpson et al., 2002). Within the lower portions of these eolian cross-strata are localised, erosively-based massive sandstones which broaden out into sheet sandstones, laid down on the paleodune plinths; their strong resemblance to modern deposits from catastrophic rainfall events resulting in the failure of the front faces of high dunes, suggests an origin through similar processes during Makgabeng deposition (Simpson et al., 2002). Thin (few metres thick) lenses of texturally and mineralogically immature sandstones within the predominant eolianites, up to 5 km in lateral extent and characterised by waterlain structures, are ascribed to an origin as playas, and saline pan deposits also occur locally within the eolian succession (Simpson et al., 2004).

The final Makgabeng facies, comprising thinner (generally <1 m) lenses of quartzose sandstone and minor mudrocks, extending up to about 100 m laterally within the eolianites, bear evidence for wind, current, wave and combined flow ripples, and also exhibit mudcracks and evaporite casts, with trace amounts of evaporate minerals detected through X-ray diffraction (Eriksson et al., 2000; Simpson et al., 2004). These interpreted interdune deposits were allied to the extreme precipitation events inferred above, thus being subject to high-energy flash flood events which would have reworked the desiccation products developed

over long intervals between such high energy aqueous occurrences (Eriksson et al., 2000). It is this facies which contains a suite of sedimentary structures, described next, and ascribed to an origin through microbial mat growth, desiccation and destruction, as longer intervals of shallow water interdune pools and increasing desiccation alternated with extreme rainfall events.

3.3. MICROBIAL MAT RELATED FEATURES IN THE MAKGABENG INTERDUNE DEPOSITS

In addition to the "normal" sedimentary structures (e.g., cross-bedding, planar bedding, ripple marks, evaporite crystal imprints etc. – see Eriksson et al., 2000; Simpson et al., 2004) identified within the interdune facies at Makgabeng, four main groups of inferred microbial mat-related features were also observed:

1. Wrinkle structures (Fig. 3)
2. Sand cracks – from simple to complex-curved forms (Fig. 4)
3. Sand chips and desiccated larger fragments (Fig. 5)
4. Roll-ups (from simple to complex) (Fig. 6)

3.3.1. Wrinkle Structures

These features were observed on a single siltstone bed upper surface, and comprise sinuous flat-topped ridges, which bifurcate and show a measure of parallelism of crest orientations within an overall more honeycomb-like pattern (Fig. 3). Ridge widths are up to about 5 mm, with spacings between ridges up to about 10 mm, and ridge heights are a few millimetres; ridges tend to be steep-sided (Fig. 3).

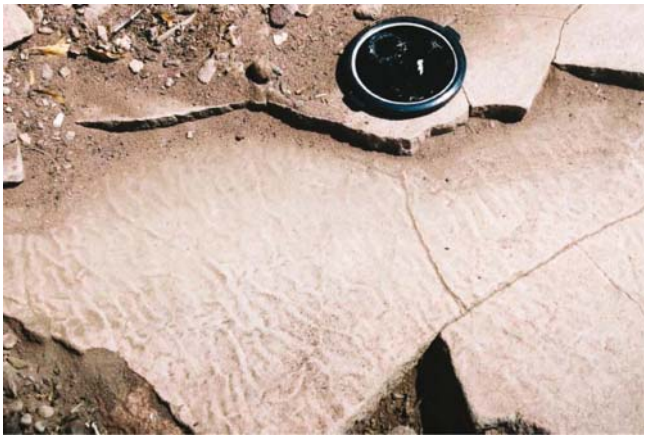

Figure 3. Wrinkle structures preserved on the upper surface of a siltstone bed in the Makgabeng Formation; lens cap for scale. Note bifurcations (splitting) of sinuous flat-topped ridges, and the measure of parallelism of ridge crests within an overall honeycomb-like pattern.

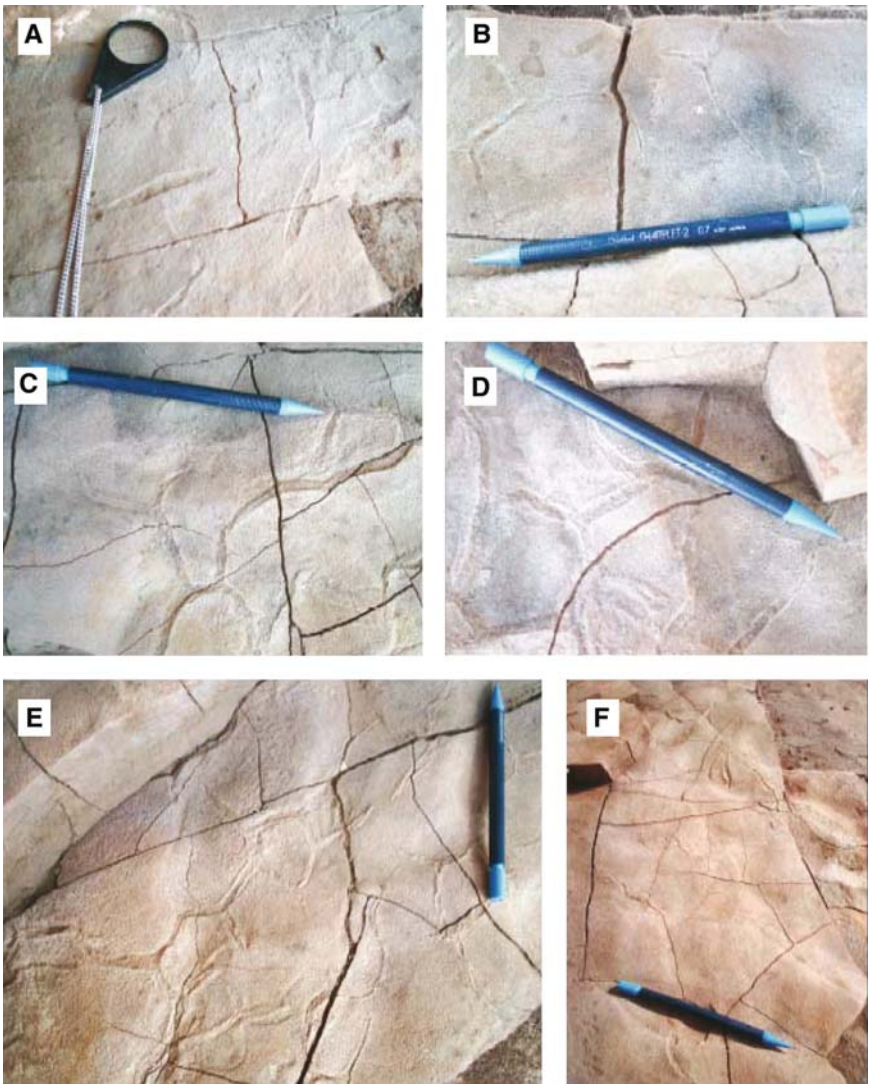

Figure 4. A variety of sand crack types observed within sandstone beds of the Makgabeng Formation. (A) Short, isolated, spindle-shaped cracks (hand lens for scale). (B) Apparent joining up of spindle-shaped cracks to form triradiate ("triple junction") crack patterns (pen for scale). (C) Longer and more sinuous sand crack patterns, to which shorter, triradiate cracks appear to be joined – note the triradiate crack at the pen-tip, which appears to be a separate crack system, adjacent to rather than connected to the longer, curved features. (D) Triradiate crack set, between modern crack in sandstone bed and middle of pen (scale), which appears to be incompletely linked to sinuous, longer crack features. (E) As longer cracks develop, they become even more sinuous, even developing circular patterns (pen for scale). (F) Larger-scale view of rippled upper sandstone bed surface in the Makgabeng Formation, covered by a variety of sand cracks, mainly of higher sinuosity and circular geometry.

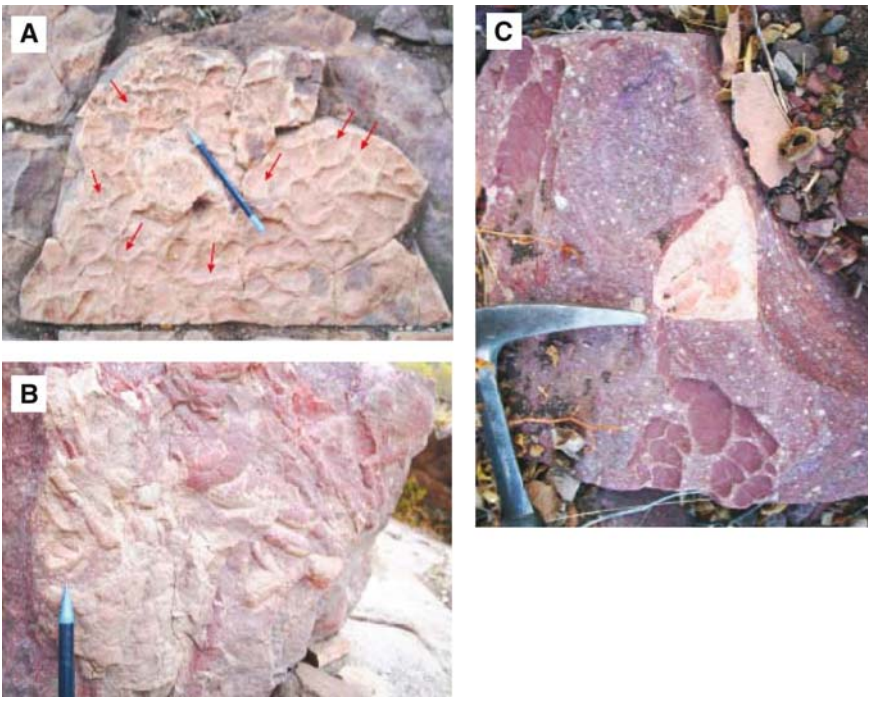

Figure 5. Fragments of detached microbial mat in the sandstone beds of the Makgabeng Formation. (A) Sand chips (several are marked with arrows), reflecting microbially-bound sand still attached to mat fragments which were then transported and partially reworked by flash flood currents before deposition (pen for scale). (B) A set of varied mat fragments, from relatively rounded sand chips to partially rolled-up mat-bound sand fragments, to larger, more irregularly shaped "microbial clasts" (pen for scale). This mixture reflects the irregular energy levels concomitant with desert flash floods. (C) Large (dark and segmented) muddy fragment (below point of hammer; scale) which appears to have suffered desiccation only after physical mat destruction and flash flood deposition had occurred.

These characteristics closely resemble those of "Kinneyia" (Martinson, 1965; Bloos, 1976), which refer to wrinkle structures likely formed beneath a microbial mat and which were then preserved on the upper surface of the underlying, flat siltstone or sandstone beds upon which the mat grew (cf. Porada and Bouougri, 2007). Such structures are known only from the ancient rock record, without modern examples yet found, and are most common within intercalated siltstone/sandstone beds or heterolithic siltstone/mudstone successions deposited within inferred shallow subtidal, intertidal to lower supratidal paleoenvironments (e.g., Häntzschel and Reineck, 1968; Bouougri and Porada, 2002; Noffke et al., 2002; Porada and Bouougri, 2007). Their association to a microbial mat origin is commonly reinforced by the occurrence of what has been called a "microbial mat

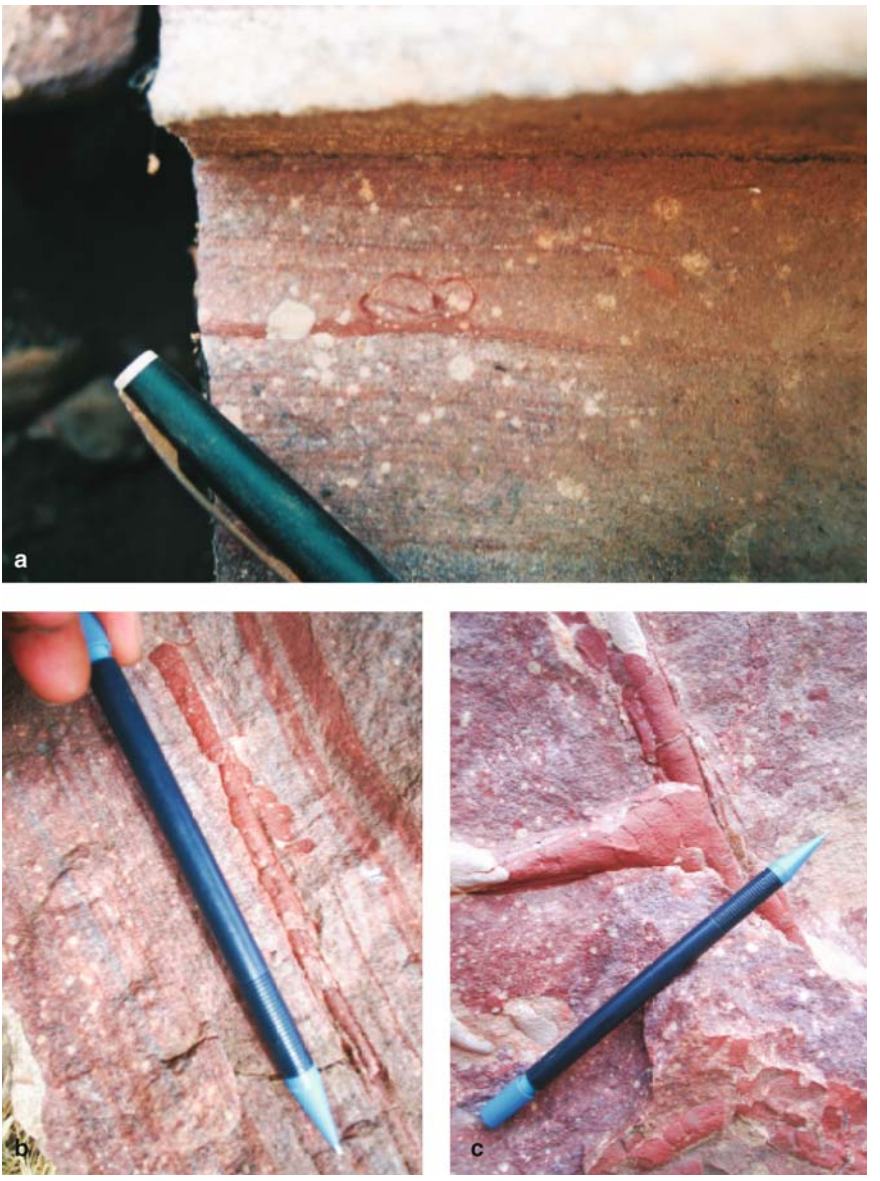

Figure 6. Various roll-up features within the sandstones of the Makgabeng Formation. (A) Thin mudstone layers which have been rolled up for more than a complete 360° revolution, with the example shown having apparently rolled up from both ends (pen for scale). (B) Longitudinal view of roll-up, showing an elongated concentric feature (pen for scale); note partially broken concentric layers, resembling a cigar, near pen-tip. (C) Larger, irregular fragment of mat-bound mud (now mudstone) which became torn and subsequently rolled up along two angles at almost 90° to each other (pen for scale).

facies" comprising a set of associated microbially induced structures, commonly made up of spindle-shaped cracks, longer curved cracks which become sinuous to even circular, and sand chips (e.g., Pflüger and Gresse, 1996; Pflüger, 1999; Porada and Löffler, 2000; Bouougri and Porada, 2002). A very similar association is noted in the Makgabeng interdune beds, with the addition of roll-up structures, as will be described below.

3.3.2. Sand Cracks

A large variety of such sand cracks is found within the Makgabeng interdune facies (Fig. 4), varying from short, isolated spindle-shaped cracks (Fig. 4A), which can then apparently lengthen and join up, forming "triple junction"-like patterns (Fig. 4B), which are sometimes connected to more curved and longer cracks (Fig. 4C). It should be emphasized that not all spindle-shaped cracks join up to form "triple junctions" and that not all of the latter become connected to the longer, sinuous variety. Some of the "triple junction" features appear to connect naturally with the longer sinuous cracks, but others almost appear to reflect separate sand crack systems (compare Fig. 4C, D). As the cracks become more sinuous, even circular patterns may result (Fig. 4E). Many of the thin sandstone beds which predominate within the inferred interdune facies have complex sand crack patterns developed across their upper surfaces, commonly formed on primary current ripples (Fig. 4F).

Seeing that silt and sand lack any cohesion to enable such sediments to undergo cracking, it is necessary that either mud was present to provide the necessary cohesiveness, or that a microbial mat growing on top of such clastic sedimentary beds gave enough cohesion for underlying sand within which the mat was embedded, to crack (e.g., Schieber, 1998; Eriksson et al., 2007). In the absence of any observed mudstone interbeds (see for example in Fig. 4F, that the sand-cracked sandstone bed surface is immediately overlain by a succeeding sandstone bed – at left) or muddy sediment within sandstones (as confirmed by thin section studies) in the Makgabeng examples, a microbial mat binding influence to provide cohesiveness to the sand, is inferred by us.

3.3.3. Sand Chips and Desiccated Larger Fragments

Sand chips (Fig. 5A) reflect eroded mat fragments which can be considered as an end-member in a series of mat destruction features comprising curled crack margins – flipped-over mat edges – rolled-up mat fragments (cf. roll-ups) (Schieber, 2004). They are commonly a few centimetres across and have rounded and plastically deformed shapes, as also observed in Fig. 5A, and can be ascribed to mats providing cohesion for sand beneath the mat, and during high energy erosive events, the mat and its sublayer of sand are broken up into variously-sized fragments (Schieber et al., 2007). These microbially-bound sand clasts can then be transported like other sedimentary particles, and may then display current alignment or even imbrication (Pflüger and Gresse, 1996; Bouougri and Porada, 2002). They are often found associated with larger mat fragments of irregular shapes

and sizes as well as rolled-up mat fragments (also comprising microbially bound sand, mud or silt), reflecting mat-breakup during flash flood events in the present examples (Fig. 5B). Some of these fragments observed in the Makgabeng Formation comprise muddy rather than sandy sediment, and some of the larger such fragments exhibit cracking due to desiccation, which must logically have occurred after deposition of the flat, microbially-bound mud clast (Fig. 5C), as otherwise, such a flat muddy particle would have fragmented along pre-transportational cracks.

3.3.4. Roll-Up Structures

These structures are quite common in the inferred interdune facies of the Makgabeng Formation, and comprise thin mudstone layers which have been rolled up through several full revolutions (Fig. 6A) – it is common knowledge that drying mud cracks and the separate pieces of mud then become curled around their edges. However, for more than a full revolution of such mud through 360°, there must be another factor present to provide the necessary cohesiveness and lateral binding (Schieber et al., 2007) to allow such muddy rolled-up fragments to form – microbial mats provide an obvious answer to this (Eriksson et al., 2000 for discussion of Makgabeng examples). These rolled-up mat fragments with thin (several millimetres) underlying mud layers still attached are broken off mat-bound sediment layers during erosive sedimentation events, analogously to the mat chips and larger desiccated fragments discussed above. The roll-ups commonly form somewhat larger fragments than the sand chips, and vary from long, thin features similar to cigars (Fig. 6B), to more complex features comprising more than one rolled-up fragment at high angles to each other (Fig. 6C).

3.3.5. The Microbial Mat Facies of the Makgabeng Formation

The microbial mat facies of the Makgabeng Formation comprises wrinkle structures, complex patterns of sand cracks, sand chips and larger mat fragments, and rolled-up mat fragments, as discussed above. The wrinkle structures are uncommon and show no observable spatial association with the other features, which occur as two groups, found in separate sandstone-siltstone bed successions within the interdune facies of the study area. The first group comprises the sand cracks, reflecting mat-bound sandstone bed upper surfaces that were preserved *in situ* after desiccation of the mat, and which were preserved in the underlying clastic beds due to cohesiveness supplied to the sand by the living mats. The second group consists of an association of sand chips, larger inferred mat fragments (some desiccated after mat breakup and transport had occurred) and roll-ups.

With the exception of the uncommon wrinkle structures, the other mat-related features are all associated with mat destruction, but of two distinct types: (1) relatively slow mat destruction through exposure and desiccation, to form the sand cracks of various types; (2) relatively rapid mat destruction through flash floods which break up growing mats and their immediate cohesive clastic granular sediment substrate and locally rework them before their deposition within the

flood deposits. In the second group, presumably, desiccation and crack formation would have aided the rapid destruction through flash-flooding. It is hardly surprising that products related to desiccation rather than aqueous settings should preferentially survive within a paleodesert environment. Analogously, aqueous deposits preserved in the Makgabeng Formation preferentially favour those formed through high energy flash flood processes and dune-front collapse due to extreme rainfall events (cf. Simpson et al., 2002, 2004) rather than the low energy playa and interdune pond deposits. Both of the latter would tend to be reworked by either extreme rainfall events or, over a longer period, by desiccation and eolian processes. As expected, eolian dune deposits totally dominate in three dimensions within the preserved Makgabeng Formation.

The microbial mat-related features discussed here thus reinforce the interpretations of the Makgabeng paleodesert made on the basis of the "normal" clastic sedimentary structures (cf. Eriksson et al., 2000; Simpson et al., 2002, 2004); this is in accord with such mat-related features being seen as an additional set of sedimentary structures rather than a fully unique group of features set apart from the physical processes of sedimentation (cf. Noffke et al., 2001). Even though the two extreme silty-sandy environments discussed in this paper, namely paleodeserts and modern supratidal flats, have a significant measure of common influences (viz. desiccation of small ponds, high energy aqueous events such as flash floods in deserts and spring and high tides in suptratidal settings), the microbial mat facies of the two environments exhibit significant differences, as will be seen in the next example discussed below.

4. Microbial Mat Features on Modern Supratidal Flats

4.1. TIDAL FLATS

Tidal flats form in protected coastal areas when strong wave action is absent and tidal range is wide (e.g. Prothero and Schwab, 2004). In arid climate zones, where tidal flats grade into coastal sabkhas, they may form refugia for diverse epibenthic microbial communities which thrive from the subtidal zone through the intertidal zone, with semi-daily inundation, up to the supratidal zone which is flooded during spring high tides and storm surges only. In such environments, microbial mats are best developed in the intertidal and lower supratidal zones, where they tend to form continuous, strongly cohesive layers on the sediment surface. Upslope towards the sabkha, they fade and loose their microbial consortium complexity due to deteriorating conditions, mainly increasing rates of desiccation, salinity and evaporite mineral precipitation.

Tidal flats are very low-gradient and low in relief, and are typically underlain by fine-grained unconsolidated sediment. Influx of sediment is mainly from the sea in the intertidal zone, and from the hinterland in the supratidal zone and sabkha, however, with a wide range of source overlapping. Most favorable for the

establishment and growth of microbial mats are fine-grained sand to silt which possess the best capillarity combined with fastest capillary movement of pore water (e.g., Correns, 1934) and thus can provide mats with optimal capillary water in periods of subaerial exposure (Gerdes and Krumbein, 1987).

Hydrologic conditions on tidal flats are determined by regular or periodic tidal inundations and a continuous downslope flow of groundwater which is recharged episodically by exceptional flooding events and rainfall (e.g., Sanford and Wood, 2001). Upward leakage of groundwater combined with 'evaporative pumping' (Hsü and Siegenthaler, 1969) provide the mats with moisture in the supratidal zone and sabkha. Tidal flats may form in both terrigenous clastic and biogenic-chemical (carbonate and evaporite) coastal depositional systems, whereby climate is an important major control. In temperate humid zones (e.g., southern North Sea; east coast of North America), tidal flats usually are siliciclastic without major precipitation of carbonate and evaporite minerals; in semiarid to arid, subtropical and tropical zones (e.g., Mediterranean coast of southern Tunisia; Persian Gulf), evaporitic mineral formation and carbonate precipitation are of great importance, the latter supporting early lithification of microbial mats (cf. stromatolites).

In this paper, we present examples of microbial mats and related features developed in supratidal zones of siliciclastic tidal flats in (1) the temperate humid zone: Amrum Island, southern North Sea; (2) the subtropical arid zone: Bhar Alouane and El Gourine, Mediterranean coast of southern Tunisia.

4.2. AMRUM ISLAND; SOUTHERN NORTH SEA, GERMANY

4.2.1. Locality and Mats

The western coast and beach (Kniepsand) near Norddorf village on Amrum Island (Fig. 7A) is a type locality of mat-forming microbial communities, arranged in distinctly colored layers consisting chiefly of cyanobacteria and sulfur bacteria. The multilayered microbial mats have been named "Farbstreifensandwatt" (versicolored sand flat) by Schulz (1936). Their occurrence is restricted to the (lower) supratidal zone between a coast-parallel berm and stabilised dunes in the hinterland. Parts of the area are periodically inundated during spring tides, whereas rainfall may episodically provide additional water to the whole area. An important and, over long periods, continuous source of moisture for the mats is from upward leakage of groundwater whose hydraulic head is situated in the hinterland, several meters above the supratidal zone.

In section, the mats typically exhibit three differently colored layers: (1) a greenish surface layer, 2–10 mm thick, consisting of cyanobacteria among which *Microcoleus chthonoplastes*, *Oscillatoria* sp. and *Lyngbya sp*. predominate (Hoffmann, 1942); (2) a pink to red middle layer, 2–5 mm thick, consisting of anoxygenic phototrophic sulfur bacteria (e.g., *Thiopedia rosea*) which coat sediment grains and fill pore spaces; (3) a black bottom layer of variable thickness,

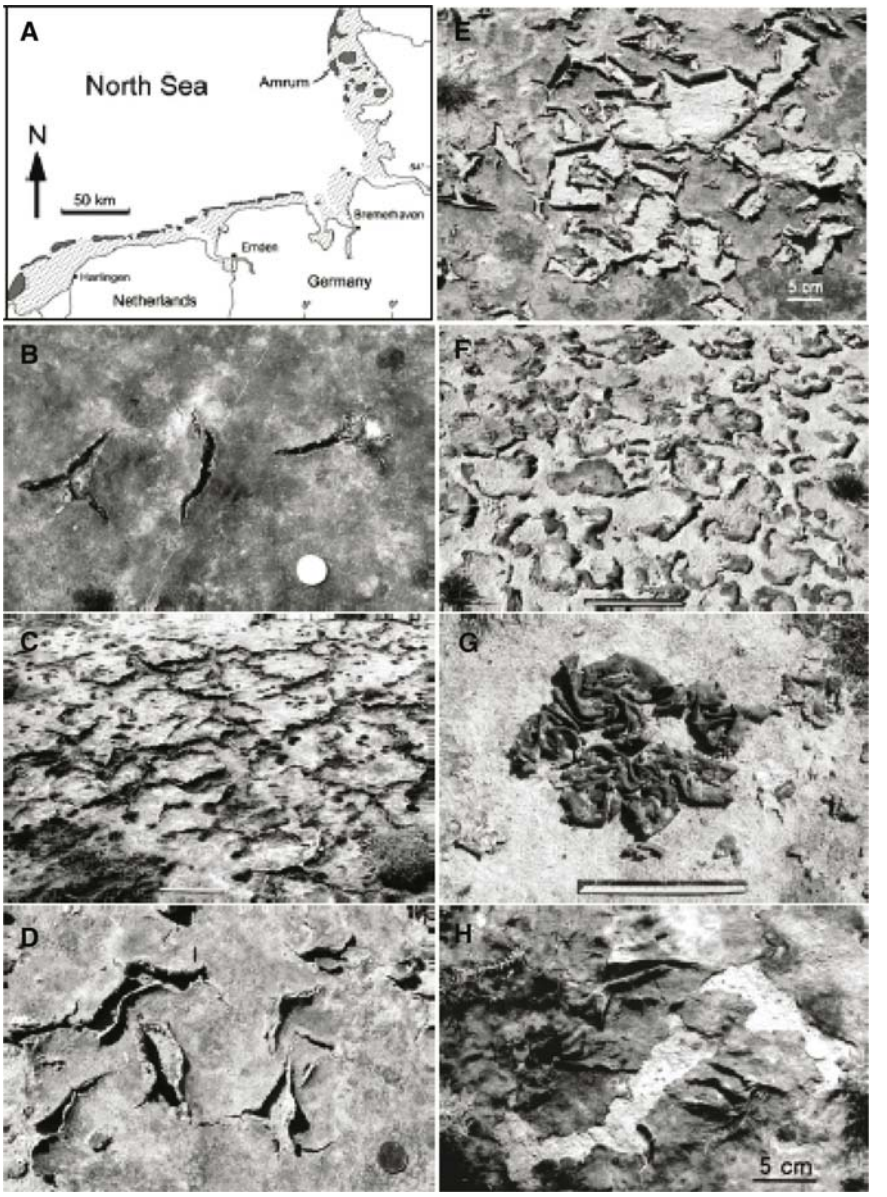

Figure 7. Location and structures related to progressive mat desiccation and shrinkage. All photographs from Amrum Island. (A) Location of Amrum Island within the German Wadden sea area. (B) Initial sigmoidal and tri-radiate shrinkage cracks. Scale (coin) is 2 cm. (C) Polygonal network of shrinkage cracks with upturned margins. Scale bar is 20 cm. (D) Shrinkage cracks with upturned margins. Scale (coin) is 2 cm. (E) Shrinkage cracks with involute margins surrounding irregular to subcircular openings developed in a thin mat. (F) Polygons of detached mat after intense desiccation. Scale bar is 20 cm. (G) Crumpled mat polygon after complete desiccation. Scale bar is 20 cm. (H) Detached mat deformed by tractional forces, e.g., currents or wind.

enriched in iron sulfide and H_2S resulting from the activity of sulfate-reducing bacteria. Similar mats also occur on several of the other islands fringing the Wadden sea area along the North Sea coast from Denmark to the Netherlands (Fig. 7A). Detailed descriptions of their distribution and composition, specifically in respect of Mellum Island, have been given, e.g., by Gerdes et al. (1985a, b, 2000), Stal and Krumbein (1985), and Noffke and Krumbein (1999).

Microbial mats that repeatedly are subaerially exposed, as in the supratidal zone of Amrum Island, tend to develop strongly cohesive, 'felty' layers in the upper photic zone, and a 'leathery' surface. The internal structure of the 'felty' layers is best described as a "condensed fibrillar meshwork" consisting of more or less parallel, "horizontally stretched ensheated filament bundles" (Gerdes et al., 2000, 285) which in neighbouring laminae develop perpendicular orientations, leading to a kind of 'plywood texture' (Fenchel and Kühl, 2000). Bacterial layers below are much less cohesive due to the very uncommon presence of filamentous species. A further component in microbial mats is various EPS which are secreted by coccoid cyanobacteria in great volume, surround the bacterial cells and sediment grains, and partly may exist in an aggregated gel state of high cohesiveness (Decho, 1994; Stal, 2000). Such EPS frequently form a 'leathery' surface film which combines high mechanical resistance with very low permeability, inhibiting the escape of water and even gas rising up from below. Finally, a sedimentary component is introduced into the mat by 'trapping and binding' of detrital grains on the mucilaginous mat surface, and subsequent overgrowth and incorporation of grains into the mat.

4.2.2. Extreme Conditions

Although in the supratidal zone of Amrum Island mats have been more or less continuously observed since the middle of the nineteenth century (e.g., Oerstedt, 1842), they are repeatedly exposed to extreme conditions in two contrasting situations: (1) in hot summers with prevailing eastern winds over longer periods, when spring tides do not reach the supratidal zone and groundwater supply ceases due to lacking recharge by rainfall; (2) in the winter season (between November and April) when strong western winds and storms may raise the water level up to several meters above normal. In both these cases, the mats may be widely destroyed by desiccation or erosion, respectively, but nevertheless recur in the following spring to early summer, thus demonstrating their ability to survive even under extreme conditions.

4.2.3. Structures Related to Subaerial Exposure and Desiccation

Solar irradiation and related evaporation gradually lead to desiccation of the subaerially exposed mat. Thereby, EPS containing >95% water by weight (Sutherland, 1977) act as a buffer for some time, though themselves shrinking in volume with progressive loss of water. At some stage, the contractional force exerted by the shrinkage process overcomes the physical strength of the material and cracking occurs. In the early stages of desiccation, shrinkage and cracking

usually are restricted to the upper, green layer of filamentous cyanobacteria, which during the process gradually becomes detached from the layers below. This happens at the latest when groundwater flow has ceased and capillary water is no longer available.

Shrinkage cracks: Shrinkage cracking may either start at the tip of a small, dome-like elevation and develop a triple junction from which characteristic 'tri-radiate cracks' propagate along the mat surface; or on the crest of a small ridge and then leads to more linear and sigmoidally 'curved cracks' with tapering ends (Fig. 7B). Since the entire mat surface is under contractional stress during desiccation, cracking likely will occur at numerous places simultaneously. This and crack propagation with progressive shrinkage will eventually lead to networks of cracks, typically arranged in polygonal patterns (Fig. 7C).

Upturned and curled crack margins: A further typical feature of shrinkage cracks are their 'upturned margins' (Fig. 7D). Upturning of crack margins results from differential contraction of the shrinking mat layer and EPS film on top. At places where the upper green mat layer is only thinly developed, e.g., towards the outer margins of the mat-covered area, differential contraction may lead to involute or 'curled margins' with more than a full revolution of the mat layer (Fig. 7E). This behaviour is usually combined with a tendency to create irregular to circular openings in the mat, exposing deeper layers of the system.

Fatal desiccation: Once a polygonal network of shrinkage cracks has developed all over the mat, and the upper, green mat layer has been detached from the layers below, cracks increasingly become wider due to further shrinkage of the polygons (Fig. 7F). Finally, the isolated polygons themselves undergo intense shrinkage into irregularly folded and crumpled fragments (Fig. 7G). In these, the completely desiccated mat has remained as a rigid skin, c. 1 mm thick, which can survive transport by wind over considerable distances. Crumpled mat fragments, or pieces thereof, may thus be found embedded in terrestrial sediments far away in the hinterland (see also "meteor paper", Ehrenberg, 1839; Krumbein et al., 2003).

4.2.4. Structures Related to Mat Erosion

Once established on the sediment surface and not seriously injured by desiccation, mats are quite resistant against 'normal' wave action during spring high tides. Shrinkage cracks, however, provide points of attack for erosion, whereas strongly desiccated and detached mats will instantaneously float and become deformed (Fig. 7H). If a mat is locally eroded by current or wave action, 'erosion pockets' may form and develop a rippled surface on the exposed non-cohesive sediment. Vice-versa, if a mat is widely eroded, parts of it may still remain as 'erosion remnants' (see Gerdes et al., 1993; 2000 and documentation therein). According to Gerdes et al. (1993), 'erosion pockets' preferentially develop in the upper intertidal zone where the mat is juvenile and thin, whereas 'erosion remnants' occur towards the supratidal zone where the mat is thicker and tough.

Fragments of eroded mats may survive for some time and be transported by currents and waves to finally be deposited along a trash line or be included in new sedimentary deposits. Such 'mat chips' which may be described as 'organically bound mineral aggregates' (Gerdes et al., 2000) are characterized by high cohesiveness and may attain various shapes ranging from very irregular to well rounded (see 'sand clasts' below).

4.3. TUNISIAN COASTAL SABKHAS

4.3.1. Locality and Mats

The southern Tunisian coast from Djerba Island to the Libyan border is characterized by shallow restricted lagoons and low-gradient tidal flats which grade into wide sabkhas (Fig. 8A). Present climate is semi-arid and precipitation very irregular with episodic catastrophic floods followed by long periods of drought which may extend over several years (Medhioub and Perthuisot, 1981). On many of the tidal flats, microbial mats are developed from the upper intertidal to the supratidal zone and also on the adjoining sabkha if groundwater is present. Thick and mature mats, dominated by filamentous cyanobacteria (e.g. *Microcoleus chthonoplastes*, *Lyngbya* sp.), occur in the upper intertidal zone and in some shallow supratidal ponds; thin mats of dominantly coccoid cyanobacteria (e.g. *Synechococcus* sp.) thrive in the supratidal zone in which precipitation of gypsum also occurs. Mat-related structures observed in supratidal zones are described from Bhar Alouane and El Gourine, and those in sabkha environments from Bou Jemel (Fig. 8A).

4.3.2. Sustaining of Mats Under Extreme Conditions

Mats in the supratidal zone of the southern Tunisian coast are exposed to extreme UV irradiation, high rates of evaporation, and are easily cut off from water supply. Mats surviving under such conditions typically have on top a layer of EPS, containing pigments like scytonemin which protects the underlying cyanobacterial layer from damage caused by UV irradiation (Stal, 2000 and references therein). Due to the pigment-rich layer, supratidal mats in southern Tunisia exhibit a reddish to orange color during summer.

The strongly cohesive and elastic EPS layer on top of the mat, however, has additional effects: (1) it develops small 'photosynthetic domes' (PS-domes) tracing trapped bubbles of oxygen, resulting from photosynthetic processes in the underlying mat (Fig. 8B); PS-domes are soon overgrown and stabilized and contribute to enlarging the mat; (2) it prevents water loss from the underlying mat, due to its low permeability, and any damage of the layer, e.g., by shrinkage cracking, which allows groundwater to rise up and to escape, will in turn induce local bacterial growth and EPS production, and thus eventually lead to repairing of the damage.

As long as traces of water from upward leakage of groundwater are available, mats can thus survive. Drawdown of the groundwater table below the limit of

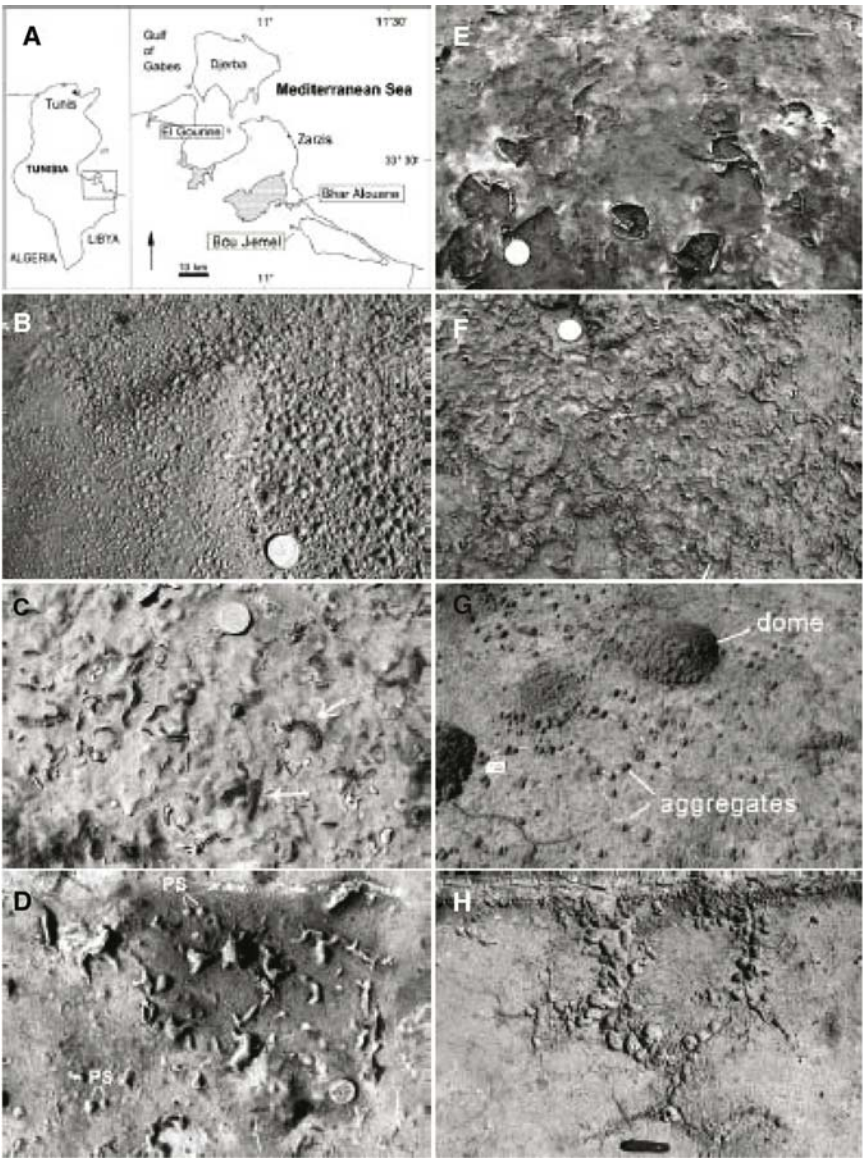

Figure 8. Location and structures related to mats developed in Tunisian supratidal zones and sabkhas. (A) Location of study sites Bhar Alouane (B–D), El Gourine (E, F), and Bou Jemel sabkha (G, H). Scale (coin) is 24 mm in B–F. (B) Photosynthetic domes (PS-domes) trapped below EPS film in thin mat of coccoidal cyanobacteria. (C) Small sigmoidal to curved shrinkage cracks. Arrows indicate cracks with microbial activity riggered by uprising groundwater. (D) Several generations of overgrown shrinkage cracks and PS-domes (PS). (E) Subcircular crack openings with curled margins in thin mat layer. (F) Several generations of overgrown circular cracks. (G) Aggregates and domes of coccoid cyanobacteria and gypsum crystals, related to desiccation cracks. (H) Domes of filamentous and coccoid cyanobacteria arranged along desiccation cracks. Scale is 8 cm.

capillary rise or cessation of groundwater flow, however, will lead to fatal desiccation of the mat within a short time. But even then, mat-forming microbial communities may still remain alive in the sediment below the desiccated mat and provide a potential for mat recreation when moisture returns (G. Gerdes, 2007, personal communication).

4.3.3. Structures Related to Desiccation and Shrinkage

In thin but strongly cohesive mats of dominantly coccoidal cyanobacteria, shrinkage cracks are usually small and occur as isolated features (Fig. 8C); networks of cracks are rarely developed, due to rapid overgrowth and healing. Overgrown cracks remain as small irregular bulges on the mat surface (Fig. 8D).

Very thin mats in which the elastic EPS film undergoes shrinkage, tend to develop more circular openings with involute margins (Fig. 8E). Uprising of water in the fresh openings induces microbial growth and stimulates a process of 'self-healing'. Overgrown margins remain as circular bulges on the mat surface (Fig. 8F). Forming slightly elevated features on the mat, however, they are preferred sites for renewed cracking which, again, will induce microbial growth and overgrowth, and so on. In this way, mats in the supratidal zone may attain more and more irregular surfaces.

Cut off from capillary groundwater, mats rapidly desiccate, become detached from their substratum, and shrink into polygons separated by wide cracks. Dried-up mat fragments may then easily be transported by the wind and be deposited far away from their source, as described above for Amrum Island.

4.3.4. Structures Related to Locally Induced Growth

In the Bou Jemel sabkha, continuous cohesive mats do not occur. Instead, a reddish surface crust is developed, in which orange-pigmented coccoid cyanobacteria are embedded together with gypsum crystals. Where the crust is interrupted by faint desiccation cracks, small 'pop-corn-like' aggregates, arranged along the cracks, are observed (Fig. 8G). They are colonized by abundant biofilms dominated by EPS-enriched colonies of coccoid cyanobacteria, which agglutinate sediment grains and interspersed gypsum crystals and thus form 'clustered aggregates'. It is suspected that the aggregates formed when groundwater uprising along the cracks, locally induced microbial growth together with precipitation of gypsum.

Occasionally, larger domal structures, up to 7 cm across and 2 cm high, are developed. They are hollow and rigid, and have a nodular surface, individual nodules resembling the above aggregates. The domed layer is about 5 mm thick and consists of sediment grains of gypsum agglutinated by coccoid cyanobacteria. In some cases, it is observed that the domes are situated above junctions of polygonal desiccation cracks. As the domes may be described as upward protrusions resulting from local expansion of the surrounding surface crust, it is suspected that they originate from bacterial growth triggered by local water supply.

On a nearby shallow berm which separates the sabkha from an evaporating restricted sound, narrow polygonal desiccation and shrinkage cracks are well

developed. Again, chains of domal structures, partly coalescing into longer bulges, are arranged along the cracks (Fig. 8H) and indicate local growth induced by local water supply. The domes have a smooth surface, are flexible (but brittle when dry), and the involved layer is less than 2 mm thick. Coccoid cyanobacteria and gypsum crystals are dominant in the top level, whereas filamentous *Microcoleus* has constructed a strongly felty fabric below (all microbiological data after G. Gerdes, 2007, personal communication).

4.4. PRESERVATION POTENTIAL AND SOME ANCIENT ANALOGUES

As most of the organic matter produced by the cyanobacteria is soon decomposed and mineralized so that organic components or even carbon are rarely preserved in the ancient siliciclastic record, preservation of mat-related structures is largely dependent on the volume of inorganic material present in the mat or trapped by the structure.

A major process to introduce silt- to sand-sized sediment grains into the mat is 'trapping and binding' as described before. In the same way, clay minerals may be trapped, but formation of authigenic clay minerals by biogeochemical processes (Krumbein and Werner, 1983), e.g., related to bacterial lysis, and possibly microbial trapping of clay minerals from the groundwater (Draganits and Noffke, 2004) may also contribute to the accumulation of clay. Furthermore, formation of iron sulfides and pyrite is not uncommon in peritidal microbial mat systems and results from the degradation of organic material by heterotrophic, sulfate-reducing or methanogenic bacteria (e.g. Berner, 1984; Gerdes et al., 2000). Lastly, microbially mediated precipitation of carbonates is a well-known process (e.g., Gerdes and Krumbein, 1987 and references therein).

The mat-related structures themselves, being either positive or negative features on the mat surface, may act as sediment traps. Obviously, shrinkage cracks may become filled with sediment from currents or eolian action, whereas involute (curled) crack margins may trap grains in their windings. Curled margins have a fairly high preservation potential due to their involute, firm structure and are occasionally found as detached 'roll-up structures' in the ancient record (see Section 3.3.4).

Another mechanism by which some mat-related structures, e.g., domes and bulges on the mat surface, may become preserved is 'filling from below'. This process requires comparatively high hydraulic upward pressure enabling liquefaction of the sediment below the sealing mat so that grains are lifted and moved upwards. As a result, a previously flat surface of a sedimentary layer may be transformed into an irregular surface mimicking the morphological features of the mat.

Of course, preservation of mat-related structures does not depend on the processes listed above only, but also on burial processes and related hydrodynamic conditions, compaction and dewatering etc. Therefore not many of the numerous structures observed with modern mats are clearly identified in the ancient record

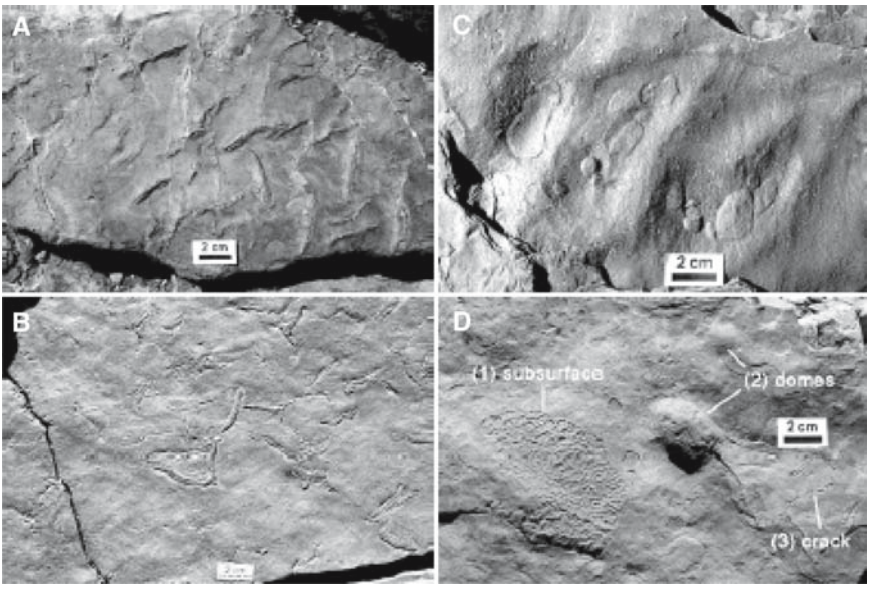

Figure 9. Ancient analogues of structures related to mat desiccation and erosion. All photographs are from the Neoproterozoic (Ediacaran) Vingerbreek Member, Schwarzrand Subgroup of the Nama Group, Farm Haruchas, Namibia. (A) Small sigmoidal, sand-filled shrinkage cracks with tapering ends, preserved on upper surface of sandstone layer. (B) Upper surface of fine-grained sandstone layer exhibiting linear, triradiate and subcircular shrinkage cracks with compacted, involute margins. (C) Upper rippled surface of sandstone bed with 'sand clasts' in ripple troughs. (D) Upper surface of sandstone bed showing features related to a previously existing thin mat: [1] a patch of mat subsurface that was exposed after local removal (erosion) of the mat; [2] domal protrusions of the sandstone bed resulting from 'upward filling' of respective domes developed in the overlying mat; [3] subcircular crack with compacted involute margin.

(see Schieber et al., 2007 for several discussions of this). Among the few that may be considered as reasonable proxies of the former presence of supratidal, thin microbial mats, are: (1) various types of small 'sand cracks' (Fig. 9A); (2) impressions of circular cracks with curled margins (Fig. 9B); (3) 'roll-up structures' representing fragments of curled margins (see Fig. 6); (4) 'sand clasts' and other mat fragments (Fig. 9C); and (5) irregular subsurface structures (Fig. 9D).

Microbial mats themselves are occasionally preserved as thin layers of sericitic argillite with isolated silt-sized grains ('floating grains'), associated with argillaceous siltstone in which individual grains are surrounded by sericite and thus not in direct contact ('coated grains'). In this doublet, the sericitic argillite likely represents the 'felty' upper mat layers including trapped and bound grains, whereas the argillaceous siltstone likely stands for the underlying EPS-rich horizons dominated by coccoid cyanobacteria and/or sulfur bacteria.

5. Discussion

Using the example of the c.2–1.9 Ga Waterberg Group in South Africa, we have briefly investigated typical desert interdune-playa type microbial mat features and accounted for their formation and nature through the conditions implicit in such a setting. For these desert settings, interdune ephemeral lakes or playas are critical for mats to survive, and they may grow episodically after flooding and may remain living as long as groundwater is available. Since they will soon become subaerially exposed, however, shrinkage and cracking will definitely occur. And as the mats likely will not be very thick, curling of crack margins also will be a common feature. Finally, the mat will completely desiccate and shrink into a hard and brittle, tightly folded and crumpled tissue, detached from the substratum. Fragments of this will easily be transported by wind and will break down into smaller pieces. Alternatively, the next desert rainstorm, leading to the beginning of the next mat cycle, will rework these desiccated and rolled up mat fragments into the wadi-fluvial deposits which precede playa formation and the next generation of mat features. As we have identified four such playa-interdune flood deposit complexes, separated by dune deposits, the cycles of mat development were severely interrupted. We cannot determine which species of bacteria may have been responsible for the mats in these ancient (c.1.8 Ga) environments, as only proxy mat structures have been preserved within these Waterberg outcrops.

We feel, for desert settings, that the evolution of the mats was relatively simple, because we are dealing with a unidirectional development, starting with an event (the flooding), followed by evaporation, total desiccation of the surface and finally cessation of groundwater flow. But if flooding events and the following desiccation and mat destruction happened repeatedly, a specific chemistry of the brines (including groundwater) may have developed and respective evaporite minerals (like thenardite, gaylussite, shortite etc.) may have been precipitated during progressive evaporation. Unfortunately, except for trace amounts of evaporate minerals identified by XRD, no such minerals have been preserved in these ancient desert sediments.

In the supratidal zone, the story is much more complex due to much more common periodic (plus episodic) inundations and more or less continuous groundwater flow. Mats can survive for quite long times in such environments and exhibit on their surfaces a kind of "memory" of the various events of drying and wetting. Structures may be manifold, varying from simply flat to blistered, crinkled and highly deformed mat surfaces. There will further be an interplay of microbial growth and evaporitic mineral precipitation, which in combination sometimes may lead to peculiar domal structures. Strangely enough, conspicuous desiccation cracks and networks of such cracks are not really typical of supratidal mats. Rather the cracks are small and soon overgrown. If larger cracks occur, the mat is almost destroyed and may easily be eroded (leading to mat fragments like in the desert setting). Depending on the overall setting, the chemistry may involve carbonate, anhydrite/gypsum, halite, or mainly Fe-sulfides. What is important for both settings, of course, is the grain size of the mat

substratum, which should be in the silt to fine-grained sand range, because of the combined favorable porosity and capillarity.

Comparing the two environments, (ancient) paleodesert interdune-pan settings and (modern) supratidal flats, differences in the set of preserved mat features are noted: in the Waterberg desert, mat proxy features reflect either desiccation-related structures, or those due to rapid physical destruction resulting from flash floods, and these two sets of features are physically set apart in preserved outcrops. Within the modern supratidal flats, incipient destruction of mats due to desiccation is commonly "healed" due to crack infilling and overgrowth features. In these settings a more complex set of mat proxy features is preserved, including dome- and ridge-like positive features, and reflecting also a complex history of repeated alternations of wet and desiccating environmental conditions, thus enabling the "healing" to become a common feature. Once mats become fatally desiccated in suptratidal flats, however, their physical destruction by high and spring tides is ensured and the proxy features thus formed closely resemble their paleodesert counterparts. The desiccation-related cracks formed in mats in both settings have much in common, with the combination of spindle-shaped, triradiate and complex-sinuous cracks being seen in both environments; this reflects a similar effect of desiccation on the (probably analogous) microbial organisms in both cases.

The microbial mat-related features preserved within modern and ancient sedimentary environments are thus both varied and subtle, analogous to the better known physically-formed sedimentary structures, the use of which to build paleoenvironmental models is equally subject to the niceties of interpretation and overlap of features from one setting to another. The facies-specificity of mat-related structures and their physically formed counterparts is thus also open to the subtleties of scientific interpretation.

Within the Waterberg paleodesert, preservation is essentially through incorporation of larger desiccated mat fragments into rapidly formed flash-flood deposits. Without these latter, larger desiccated mat fragments would break up further, and along with earlier smaller fragments would be picked up by the predominant winds, dispersed and incorporated as small, discrete fragments within wind-deposited sediment. Their chances of identification as mat proxies would be very limited. The major control on their potential preservation would thus appear to be climatic, dependent specifically on a succeeding wet desert flash-flood event occurring before desiccated mat fragments had been left to the vagaries of the predominant wind regime for too long. A celestial body such as the planet Mars, where strong wind regimes may have been important in forming surficial sedimentary deposits, may thus make the search for evidence of pre-existing life difficult; preservation of any mat proxies would be largely dependent on wet climatic events occurring when suitably desiccated mat material happened to be present.

In contrast, epeiric marine shorelines where mats can be expected to flourish under relatively low wave energies and enhanced tidal effects, will produce a much larger range of potential mat proxy features to be preserved. Desiccation effects

will be reduced by the common "healing" of cracks. However, once fatal desiccation of mats occurs, the resultant mat fragments will be rapidly dispersed and incorporated into relatively localised littoral clastic deposits, and the different hydrodynamic properties of desiccated mat fragments as opposed to sediment grains, may result in preferential concentration of the former, making identification and preservation more likely. On a planet such as Mars, shallow aqueous deposits reflecting tidal and wave action should be readily identifiable through resultant physically formed sedimentary structures, without recourse to mat proxy features. The problem, as in the rock record on Earth, would be to distinguish lacustrine from littoral marine deposits (e.g., Eriksson et al., 1998). Lacustrine water bodies would tend to become much more desiccated through paleoclimatic variation, with similarities to the Waterberg scenario discussed above. In contrast, shallow marine water bodies should then preserve a much greater range and quantity of mat proxy features. Clastic sediment mat proxies could thus be expected, potentially, to play an important role in indicating evidence for life on a planet such as Mars, as direct preservation of organic matter might have been problematic.

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DECIPHERING FOSSIL EVIDENCE FOR THE ORIGIN OF LIFE AND THE ORIGIN OF ANIMALS:
Common Challenges in Different Worlds

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Abstract The origins of major biological groups contain a series of questions that engage all the natural sciences. Too often the different 'origin' case studies, such as the origins of animals and of life, are treated as separate entities, independent of one another. Viewing 'origin' questions as a whole helps the scientist to appreciate common challenges and then to share possible stratagems. We propose, specifically, that the palaeontologist working on Precambrian fossils should follow a series of nested questions that are outlined within, to guide what questions are valid and how to attain substantial answers to them. Two case studies are used to illustrate this approach: fossil stromatolites and the origins of life; and the Ediacara biota and the origins of animals.

1. Introduction

The deciphering of ancient fossil morphologies can be likened to the poem Jabberwocky from *Through the Looking-Glass and What Alice Found There* (Carroll, 1871) that treads a fine line between being understandable and being incomprehensible. Jabberwocky is littered with nonsense words, arguably, chosen for their sound and metre rather than their definitions. The mind invites us to attach the meanings of words they resemble and then suddenly the whole poem seems to make sense. So as the hunter, having killed the Jabberwocky, exclaims, '*O frabjous day! Callooh! Callay*!" we are invited to translate "O fabulous day! Hooray! Hooray!' but this is clearly not what is said. This is a simple interpretation, though it seems appropriate in context. Alice on reading this poem observes:

> *It seems very pretty, but it's rather hard to understand!" (You see she didn't like to confess even to herself, that she couldn't make it out at all.) "Somehow it seems to fill my head with ideas–only I don't exactly know what they are!*

So it is when considering Precambrian palaeontology and, in particular, attempting to decipher the origins of life on earth in the Archean and the origins of the major animal groups in the Proterozoic. Other interpretations are always possible. Like Jabberwocky, the great strength of Precambrian palaeontology is that there is always the invitation of a different solution, a different approach; this keeps old fossils alive and vigorous. The enigmatic forms of such fossils may be as inviting to us as the words that comprise *Jabberwocky*, giving the appearance of a sensible and coherent whole waiting to be unearthed. Sometimes enigmatic fossils will be esoteric and incomparable to modern forms e.g. *Tribrachidium* from the Ediacara biota 580–543 Ma ago with its unusual three fold radial symmetry that is difficult to match in modern organisms. While at other times, fossil forms will be reassuringly familiar, for example, stromatolite morphology that is largely conserved over geological time. In the Precambrian however, where the majority of planetary processes operated significantly differently, for example: the composition and interaction of the atmosphere and hydrosphere; tectonic rates; the level of meteorite bombardment; and nature of long term solar cycles – just how uniformitarian can we be about our interpretations of these fossils?

We are faced with multiple hypotheses for the affinity of each candidate 'fossil'. To make matters worse these fossils should form a story that is a coherent whole with, for instance, single celled eukaryotes appearing before animals (one type of multi-celled eukaryotes), but this is not always the case. Some scientists would argue that there is no definitive evidence of eukaryotes until c.700 Ma, and they believe there are good theoretical reasons for believing in such a late arrival (Cavalier-Smith, 2006). Whereas, others would argue for the presence of animals as early as 1,500 Ma based on evidence of bilaterian animal trace fossils from the Vindhyan deposits of northern India (Seilacher et al., 1998) and predictions from molecular clocks that have placed divergence time for the Protostome-Deuterostome divergence as far as 1,200 Ma ago with the origin of all animals some time before this (Wray et al., 1996). Clearly these hypotheses are incompatible, but as they deal with separate fields, the origin of eukaryotes and animals respectively, the incongruence rarely meets at close quarters. So what are we to believe?

The traditional scientific approach advocates that in such situations, parsimony must rule. In this methodology, also known as Occam's Razor, the 'simplest solution' is the best and other hypotheses are effectively discarded. This is the 'Phanerozoic' method for distinguishing between hypotheses but should we expect to know what the simplest solution is in a Precambrian world? For instance, when considering the Ediacara biota, is it simpler to place many fossils in separate modern groups, as recently argued by Gehling et al. (2005), or is it simpler for all Ediacaran forms to be fundamentally similar to each other and be placed in one extinct group as advocated by Seilacher (1989); Brasier and Antcliffe (2004)?

The second hypothesis is more epistemologically parsimonious in terms of the number of groups the fossils belong to; i.e. one as opposed to ten or more. However, the first hypothesis invents no groups while the second must invent one, so the first hypothesis is more ontologically parsimonious (in terms of degrees of inference). For parsimony to help us with this problem we must decide whether it is simpler to invent one group or to shoehorn fossils in to ten existing groups. The answer to this question is not known, and perhaps it is irresolvable. When the guiding principle of parsimony can no longer be applied, what are we to do?

Thus, one of the biggest challenges facing Precambrian palaeontology is not a lack of valid, challenging and exciting hypotheses, but a method for distinguishing between them. From the earliest evidence of possible life to the origin of animals, the scientific literature is littered with disputes and controversy. In the following section we discuss common tools and approaches for deciphering Precambrian fossil evidence for the rise of life and the rise of animals on earth. We advocate using a hierarchy of "starting questions" for dealing with fossils from these earliest rocks. This approach will be illustrated using the case studies of fossil stromatolites and the Ediacara biota.

2. Null Hypotheses for Palaeobiologists

Enigmatic fossil groups and the challenge of deciphering their morphology is not uniquely a Precambrian problem, but it certainly predominates in this interval of earth history. Throughout Precambrian time there is a general trend of increasing morphological complexity of the fossils, and this arguably leads to increased confidence when deciphering their biological affinities. This confidence may be misplaced in many instances, however, and we propose that all fossil remains should be treated with equal doubt, from the earliest putative cells to claims of Ediacaran animals. Modern equivalents can be very informative for interpreting much about fossils but we caution that the Cambrian – Precambrian boundary represents the limit to 'biological uniformitarianism' beyond which modern equivalents are of limited use when used in direct analogy. Instead, we emphasise the importance of adopting an abiogenic "starting question" where the burden of proof is towards proving life. If an abiogenic origin can first be rejected, then a prokaryotic affinity becomes the working model, with efforts potentially focused towards demonstrating a higher (eukaryotic) affinity or in the very latest Precambrian possibly metazoan affinity (see Fig. 1). These hypotheses can be viewed as hierarchical, each being carefully refuted before adoption of the next, and thus they are shown in Fig. 1 as nested cones encompassing the expanding biosphere through geological time.

It follows that if we are to accept arguments for early prokaryotic life then the likely abiological mechanism that could have produced the candidate structures must first be rejected – see for instance, the stromatolite case study below. To then accept claims for a eukaryotic affinity we must first reject *plausible*

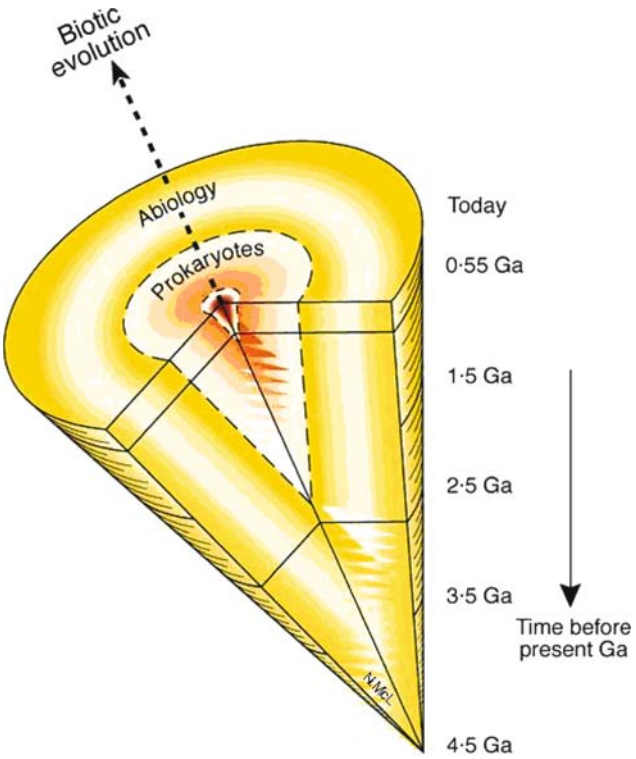

Figure 1. (overleaf) A series of nested cones that represent the starting points for exploring enigmatic fossil morphologies within an expanding biosphere. The outer yellow cone represents the abiotic morphospace, the inner orange cone the expanding prokaryotic morphospace and the central red cone the eukaryotic morphospace. Please note that each layer of the cone represents the likely times that the *starting question* is valid **not** the *origination of a particular group*. Thus the prokaryotic cone appearing at 2.5 Ga does not mean that is when we believe prokaryotes originated but the *approximate* time that it becomes reasonable to start asking if the fossils present are *higher* than prokaryotic in affinity. It does mean, consequently, that we would view claims of eukaryotes before 2.5 Ga as highly questionable. The reader could choose their own dates. The important point is that at any given time the outer cone is the starting point and progress is made by working inwards progressively rejecting hypotheses until an affinity is reached, and no higher claim can be satisfied. (This conical representation was inspired by a diagram of chemical evolution from Williams and Frausto da Silva, 2006.)

abiological mechanisms and demonstrate additional features that could not have been produced by any Prokaryotes (see Brasier et al., 2002). To accept claims of animals we must go one step further, first rejecting an abiogenic origin, then a prokaryote affinity, followed by rejection of single-celled eukaryote affinity before seeking features consistent with a multicelled-Eukarya. Even then the possibility of fungal and plant affinities must still be entertained – see for example the discussion of the Ediacara biota below. This may seem like overkill, but in many

instances it may be rather easy to demonstrate biogenicity, as well as a prokaryote affinity. Take for instance the Ediacaran fossil *Dickinsonia* known from sections in Australia and Russia. It is clearly not abiogenic; the consistency and control of its morphology across a variety of facies, environments and taphonomic regimes speaks against this. The maximum size that *Dickinsonia* can achieve (in excess of a metre in several known specimens) quickly makes a prokaryote affinity very unlikely indeed. Add to this a growth program more complex than anything a prokaryote is known to achieve and we are quickly onto the single celled Eukarya hypothesis. However, not all Ediacaran fossils that have been described as some type of animal can run through this sequence so quickly. For instance the erst-while Ediacaran/Cambrian chondrophorine hydrozoan *Kullingia* (Foyn and Glaessner, 1979; Narbonne et al., 1991) was recently reinterpreted as a scratch mark (Jensen et al., 2002). Thus the creature is not a body fossil and is now thought to have an entirely abiogenic origin. In this way we propose a series of nested cones (Fig. 1) as a theoretical structure within which to consider fossil morphology – this represents the exploration of doubt, in addition to trying to seek confirmation of successive hypotheses for the origins and affinities of a particular fossil.

A large number of criteria have been proposed by many different studies to distinguish between these multiple hypotheses. Table 1 provides a brief summary of these and subsequent case studies. Explore how these have been applied to the origins of life (e.g. stromatolites) and the Ediacara biota.

3. The Origin of Life: The Stromatolite Question

Stromatolites provide the earliest macro-fossil evidence for microbial life on earth. We here adopt a non-genetic definition of a stromatolite *"an attached, laminated, lithified sedimentary growth structure, accretionary away from a point or limited surface of initiation"* (Semikhatov et al., 1979). This definition encapsulates the key morphological and textural characteristics of a stromatolite as seen in outcrop or hand specimen and, crucially, it implies nothing about the relative importance of biotic and abiotic processes in their formation. We have chosen not to adopt a genetic definition of a stromatolite here (e.g. Kalkowsky, 1908; Walter, 1976), because it can be difficult to demonstrate active biological participation in their growth, especially in the early rock record. This challenge has long been recognized, as first explained in the somewhat paradoxical, genetic definition of stromatolites given by Kalkowsky (1908), namely that they are *"organogenic, laminated calcareous rock structures, the origins of which is clearly related to microscopic life, which in itself must not be fossilized"* (translated in Krumbein, 1983). Stromatolites therefore provide an excellent case study to illustrate the application and testing of the "nested cones," outlined above, when examining some of the earliest candidate fossils on earth.

Trends in stromatolite abundance and morphology through time record a complex and changing interplay of physical, chemical and biological processes

Table 1. compilations of key features that have been proposed for distinguishing eukaryotic and prokaryotic fossil morphologies from abiotic artefacts. Criteria marked with a (*) are consistent with biology, but care must be taken that reasoning does not become circular and that biogenicity is not inferred on the basis of these criteria alone.

Question	Abiotic	Prokarya	Eukarya	Notes
Environment conducive to life?	N	Y	Y	Limits of extremophiles? Sedimentary rocks or not?
Are structures discrete? i.e. do not grade into matrix	N	Y	Y	Taphonomic modification?
Are structures hollow?	Y	Y	Y	A hollow tube is not evidence of life
Are there bifurcations or branchings?	Y	Y	Y	Nature of branching, size changes, directionality, fractal?
Do chambers expand with growth?	N/A	N	Y	Could this indicate Eukarya. How could a prokaryote do this?
Is there evidence of nuclei?	N/A	N	Y	Taphonomic difficulties, geochemical corollary – is it a nucleus or a black blob?
*What are the growth curves or stages?	Controlled by physio-chemistry	Y	Y	Population dynamics: Prokarya large populations of similar forms. Eukarya more diversity and potentially recognisable developmental systems
*What is the size of the "fossil?"	No upper or lower limit	Upper limit unknown	Single versus multi celled Eukarya	Single celled Eukarya can be as small as Prokarya and as large as 30–40 cm, overlapping with many multicelled Eukarya. Constraints on surface area to volume ratios of prokaryotes may help to distinguish them from abiotic artefacts
*Can individuals amalgamate during growth?	Y	Y	? fungi, lichens, slime moulds	This is suggestive of Prokarya, but is plausible for many abiogenic mechanism e.g. the growth of crystallites
*Morphological differentiation consistent with biogenic tiering within laminae or aggregates	N/A	Y	Y	Do prokaryotes and eukaryotes do this differently?
*Is there evidence for phototaxis?	N	Y	**N/A**	
*Is there evidence for chemotaxis?	Y	Y		

that are yet to be fully deciphered (see review by Grotzinger and Knoll, 1999). Some first order controls on their abundance have been identified such as the changes in seawater carbonate super-saturation through time (e.g. Riding and Liang, 2005). Stromatolite macro-morphology has also been shown not to be

uniquely biogenic by both numerical modelling studies (e.g. Grotzinger and Rothman, 1996) and recent experimental studies (e.g. McLoughlin et al., 2008). Branched, dendritic stromatolite morphologies can be produced by diffusion limited aggregation processes and more condensed, stratiform to domal forms can be modelled by interface growth equations such as the Kardar-Parisi-Zhang equation (see review by Grotzinger and Knoll, 1999). However, there are some workers who argue that coniform stromatolite morphologies in particular, may be uniquely biogenic, as it is thought that these forms require diffusion up the flank of the cones. This upward diffusion is contended to only take place within a microbial mat (Jogi and Runnegar, 2005). In light of these discussions, the rejection of an "abiogenic null hypothesis" for an individual stromatolite occurrence requires careful testing of the relationships between stromatolite morphology, distribution and depositional environment, in addition to examining any micro-textures and geochemical signatures that may be preserved. Numerous lists of criteria have been proposed for testing the biogenicity of candidate stromatolites, and these are discussed extensively elsewhere (see Hofmann, 2000; Buick et al., 1981). We summarise these criteria in Fig. 2, a flow diagram that can be used to assess the biogenicity of any given stromatolite. Further techniques are emerging that may help investigate stromatolite biogenicity such as high resolution elemental and isotopic analysis at a nanometre scale using NanoSIMS. This gives the opportunity to investigate putative microbial fabrics at a scale never previously obtainable (e.g. Wacey et al., 2008).

Some of the oldest putative stromatolites are described from the 3.4 Ga Strelley Pool Chert of West Australia. These coniform structures, first reported by Lowe (1980), were initially interpreted as biogenic structures, but this interpretation was later rescinded in favour of an abiogenic evaporitic precipitation (Lowe, 1994). The Strelley Pool Chert stromatolites are discussed further elsewhere in this volume (see Wacey et al.) including those from the remarkable Trendall locality, which is notable for the large coniform stromatolites. The structures exhibit a diverse range of coniform and rare columnar morphologies, with significant variation in size, plus examples of putative branching (see Hofmann et al., 1999). A biological origin for these structures has been advanced based largely upon morphological and sedimentological arguments plus rare earth element data suggestive of a shallow marine setting (Van Kranendonk et al., 2003; Hofmann et al., 1999). In a recent study, Allwood et al. (2006) present a shallow-water carbonate platform depositional model for the Strelley Pool Chert to support a phototrophic origin for these stromatolites. Our own field work, undertaken across the whole of the outcrop belt, however, leads us to contest this interpretation. In the West Strelley belt, for example, small unbranched coniform stromatolites are common and do not show strongly depth controlled changes in morphology or distribution (McLoughlin et al., in preparation). We also find a close interrelationship between coniform stromatolites and crystal fan arrays, upon which they can be

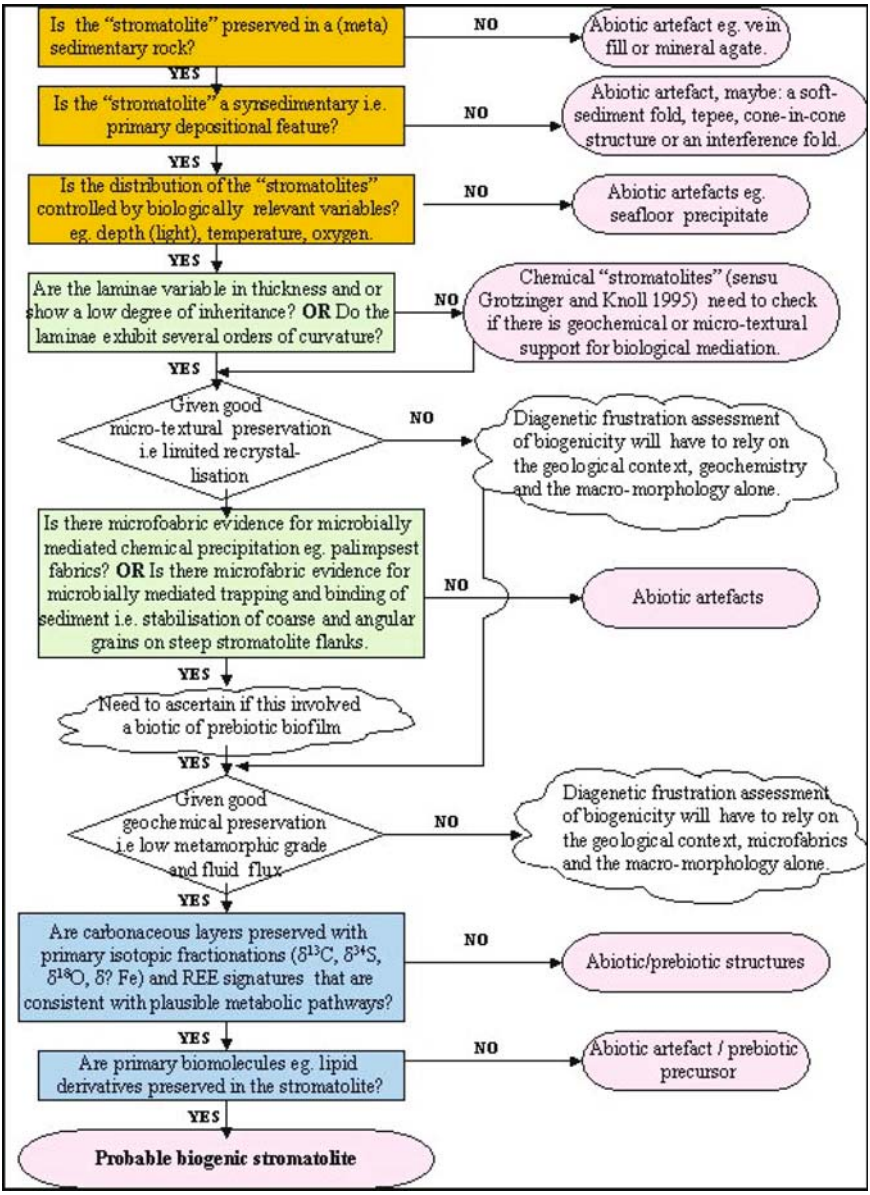

Figure 2. (overleaf) Flow chart summarising the hierarchical series of questions that has been proposed to assess the biogenicity of candidate stromatolites. (Key: Yellow rectangle = decision regarding geologic context; green rectangle = decision regarding biogenic morphology and processing; blue rectangle = decision regarding geochemical evidence for biological processing; pink oval = answer; white diamond = variable; white cloud = additional consideration.)

seen to nucleate, suggesting a strong physio-chemical component to their growth. In the absence of compelling micro-textural and geochemical evidence for microbial mat remains in these stromatolites, we argue that questions remain regarding the biogenicity of at least the simplest, unbranched coniform stromatolites of the Strelley Pool Chert (McLoughlin et al., in preparation). In other words the most probable abiotic null hypothesis has not been rejected in all cases and so demands further testing.

What can confidently be deciphered from the morphology of laminated stromatolites? Firstly, the morphology and distribution of stromatolite build-ups or 'bioherms' can be used as a palaeo-current indicator (Hoffmann, 1967) given that their axes of accretion often inclines towards the sediment source and 'bioherms' may show elongation parallel to the current direction. It has also long been appreciated that a predictable sequence of stromatolite morphologies occurs with changing water depths, as first recognised in Proterozoic carbonate shelves of Northern Canada, and this can be utilised as a relative palaeo-depth proxy (e.g. Hofmann, 1976). In the rare cases where exceptional preservation permits, efforts have also been made to correlate changes in stromatolite morphology with the preserved microfossil communities that they contain. For example, it has been argued that the microstructure and microfossil biota preserved within an outcrop of three vertically intergrading stromatolite morphologies from the Gunflint Chert of Ontario Canada, can account for the gross changes in morphology (Awramik and Semikhatov, 1979). The challenge here, however, is to demonstrate a one to one causal relationship. Namely that the microfossil are actively involved in accreting the stromatolite structure rather than just being passively entombed and are the dominate control on the resultant stromatolite morphologies. This requires the elimination of a multitude of physical and chemical factors that also operate in the sedimentary environment the stromatolite accreted. For instance, changes in current velocity andor seawater carbonate super-saturation may also account for some of the changes seen in stromatolite morphology. In the Bitter Springs Formation, for instance, it has been demonstrated that cyclic changes in stromatolite morphology, in particular the onset of branching, can be attributed to changes in the sedimentary environment rather than the microbial community (Southgate, 1989). In Mesoproterozoic stromatolites from China, it has also been shown that changes in sedimentation rate have a strong control on the community composition, density and orientation of frame building microfossils within the stromatolites (Seong-Joo and Golubic, 1999). In summary, we agree with previous authors that stromatolites hold the potential to act both as "evolutional mileposts" and "environmental dipsticks" if only we can decipher the morphological expression of the physical, chemical and biological controls upon their growth. Like attempting to decipher the poem Jabberwocky, we need to understand the alphabet and grammatical rules that together make up stromatolite morphologies. This enigmatic code will only be cracked by continued numerical and experimental modeling combined with field and petrographic mapping of fossil stromatolites.

4. The Ediacara Biota and the Origin of Animals

The fossils from the most recently defined geological interval of Precambrian time (Knoll et al., 2004), the Ediacaran, are perhaps amongst the most controversial of all. They sit at the critical point just before the Cambrian explosion and as such hold clues to understanding the mechanisms and processes that triggered arguably, the greatest of all evolutionary events. If only it could be decided what the fossils of the Ediacaran rocks actually represented(!) The morphology of many of the forms preserved in the Ediacara biota is reassuringly familiar. These impression fossils have been variously argued to represent the remains of: jelly fish, sea pens (a soft coral group), worms, molluscs, soft bodied sea urchins, and arthropods (see review by Antcliffe and Brasier, 2008). The early classifications of the biota were based upon simple morphological comparisons (e.g. Glaessner, 1984; Jenkins, 1992), and these have not withstood the test of time. Slowly, the pantheon of Ediacaran animals is crumbling. The interpretation of the Ediacara biota is a good example of a Kuhnian paradigm shift that may be nearing completion. It was in a series of classic papers that this paradigm was first challenged on grounds that the forms attributed to so many different groups actually shared a similar fundamental construction that could not be matched in any modern group. This uniquely Ediacaran group was termed the Vendobionta (Seilacher, 1984, 1989; Buss and Seilacher, 1994). Since then it has emerged that some forms previously thought to be jelly fish are actually attachment disks for other organisms, now termed *Aspidella terranovica* (Gehling et al., 2000), while others have been reconsidered, correctly in our view, as microbial communities (Grazhdankin and Gerdes, 2007). The idea that sea pens are represented in the Ediacaran biota has also recently been challenged on developmental grounds, see Antcliffe and Brasier (2007, 2008). The possibility that some of the 'lesser' forms such as some of the disks may be abiogenic in origin has not yet been exhaustively tested (see discussion earlier regarding *Kullingia* and Jensen et al., 2002) though, as noted above, some have recently been relegated to microbial (Grazhdankin and Gerdes, 2007). Seilacher et al. (2003) proposed that many of the forms usually attributed to the Ediacara biota may actually be giant single celled Eukaryotes similar to the foraminiferid group the Xenophyophorea. This is the first time that a non multicelled model has been postulated for the Ediacara biota (with the exception of the early paper by Ford, in 1958, in which *Charnia* was compared to an alga). Whether Seilacher et al. (2003) are correct or not is still open to discussion; nonetheless, their hypothesis is the first explicit testing of the eukaryote null hypothesis and as such is a significant contribution to discussion within the field (again see Antcliffe and Brasier, 2008 for a wider discussion of affinities). If the advocates of an animal affinity for the Ediacara biota are to sustain these arguments, then the hypothesis of single celled Eukarya origin must be continually tested and refuted in each instance. Otherwise, in our view this is the null hypothesis that we must revert to (presuming of course that the abiotic null hypothesis has already been rejected).

So, what of the other forms still hanging on to that crumbling temple of ancient animal taxonomy? Perhaps the two most important forms that have not yet been discussed here are the "Precambrian worm" *Dickinsonia* (e.g. Glaessner, 1984; Gehling, 1991; Gehling et al., 2005) and also the "Precambrian mollusc" *Kimberella* (Fedonkin and Waggoner, 1997; Runnegar, 1992; Gehling, 1991). We note that Seilacher et al. (2003) gave exemption to *Kimberella* in their xenophyophore hypothesis.

This is interesting as many singled celled organisms achieve startling size and complexity, the giant foraminifera not the least. Perhaps *Kimberella* is unrelated to the other Ediacaran forms discussed by Seilacher et al. (2003) but that does not mean that it should be exempt from similar scrutiny. At the current time of writing, there has not yet been a challenge to the '*Kimberella* is a mollusc' hypothesis, and there has been little consideration of a non-metazoan affinity. Yet, it should be remembered that 20 years ago its affinities to the cubozoa (a jellyfish group) seemed assured (Glaessner, 1984).

The interpretation of *Dickinsonia* is perhaps the most controversial of all the Ediacaran organisms. *Dickinsonia* has been translated variously as a 'dipleurozoan' cnidarian (Harrington and Moore, 1956), an annelid (Wade, 1972; Runnegar, 1982; Glaessner, 1984), a platyhelminth (Palij et al., 1979; Fedonkin, 1981), in its own unique phylum (Fedonkin, 1985), a member of the uniquely Ediacaran Vendobionta (Seilacher, 1985, 1989), or even perhaps a subdivision of the Vendobionta the 'Dickinsoniomorphs' of Brasier and Antcliffe (2004). Other workers have been less specific and likened 'the grade of its organisation,' to groups such as 'cnidarian-like' (Valentine, 1992), 'annelidian' (Conway Morris, 1979), and 'xenophyophore-like' or some other giant single celled Eukarya (Seilacher et al., 2003), or as a member of other potentially mystifying groupings of the Ediacaran such as the 'Dipleurozoa' of Dzik and Ivantsov (2002) or the 'Protarticulata' of (Fedonkin, 2003), or a member of the 'Petalonamae' of Pflug (1970, 1972). Recall here the "Jubjub bird" and the "frumious Bandersnatch", not to mention the "borogoves" and the "mome raths," who feature alongside the mysterious Jabberwocky (Carroll, 1871). It makes one wonder what scientists would make of the jabberwocky. Even though the jabberwocky is pure invention, it could hardly attract a wider range of possible affinities than *Dickinsonia*. Such a profusion of taxonomy results when there is no agreed methodology to test between many hypotheses. It *is* possible to narrow the list of hypotheses, and many of these ideas have great merit, but as the profusion of possible affinities continues, scientist seem more and more resigned to the idea that we will never understand what *Dickinsonia* is. As we have said a number of times already in this paper, it is essential to work with multiple hypotheses and test them against each other. But how are we to perform such a test? What criteria can be used? One of the authors (JBA) has argued elsewhere that one possible test that may help to distinguish between these many hypotheses is the consideration of the growth and development of Ediacaran organisms (see Brasier and Antcliffe, 2004; Antcliffe and Brasier, 2007, 2008), and we reiterate the rationale behind this approach once again here.

The standard method for establishing phylogenentic relationships is, of course, cladistics. Like all methods, however, the answers arising from cladistic analysis are only as good as the quality of input (see Felsenstein, 1978; Maddison, 1991; Page and Holmes, 2001 for approaches and problems). That said, we would argue that cladistic analysis provides the only sure way to *approach* the problem of affinities for the Ediacara biota. A major challenge within any cladistic study is to be sure that the features being considered are homologies and not anything else. That is to say, these diagnostic features should be shared as a result of common ancestry (e.g., five digits in reptiles and mammals) rather than any other reason, such as evolutionary convergence (e.g., wings in birds and bats). Curiously, no work known to us has sought to test and establish homologies (using the standard criteria outlined below) between members of the Ediacara biota and any other group of organisms, living or fossil. Hence, no phylogenetic hypothesis, given above, has yet gained support from a cladistic analysis. We recommend that such an analysis should follow the criteria for the establishment of homologies formalised by Patterson (1982). For a particular feature to be considered a homology between two groups, it ideally needs to satisfy the following three criteria. The more of these three it can satisfy, the more confidence there is in any suggested homology. Firstly, the feature should appear and develop in similar ways in the two forms being compared. (Examples of this are the ways that certain embryos share a similar pattern of cell division or the similar way in which the vertebral column develops in mammals, here called 'the ontogenetic criterion'.) Secondly, the derivation of the features should be traceable back through the fossil record – for example, the way in which the hand of a human, a bat and a whale merge into one common structure in their last common ancestor. Hopefully, we can trace the differentiation of such structures through the course of the fossil record (here called 'the phylogenetic criterion'). Thirdly, homologies should have further, associated, homologies that also meet the first two criteria. Hence, a named carpel bone in the 'hand' of a human, bat and whale is surrounded by further carpel bones that also can be satisfied as homologous (here called 'the association criterion'). Could such an analysis help us understand the nature of the ancient and enigmatic Ediacara biota? Problems certainly emerge with the second criterion (phylogenetic) whenever we are interested in the first fossils of a particular group, because we cannot trace the differentiation of features when there are no preceding fossils. Moreover, the third criterion is only useful if we have already satisfied one of the first two criteria. When used alone, we cannot avoid circular reasoning. For the Ediacara biota, therefore, we must place our belief in the analysis of *development* as a methodology that is not just informative for the analysis of homology but also comprises *the homology itself*. In this way, the study of development may be our only direct key to unlocking the phylogeny and evolution of the Ediacara biota. It should be noted that this emphasis upon development is not to be confused with the discredited 'recapitulation' hypothesis of Haeckel (1879), whose biogenetic law made the mistake of comparing the embryo and development of one organism with the adult of another. What we would advocate

is comparison of the embryo and of development in both organisms. Nor, indeed, does it have anything to do with the modern theory of 'heterochrony' (see McNamara, 1990). Both ideas are concerned with understanding how ontogenetic states actually change throughout phylogeny. In the case of 'recapitulation', there follows an inductive extrapolation of such changes backward in time to reveal a hypothetical phylogeny. What we advocate above is something quite different: an understanding of the similarities within the developmental programs of two or more organisms. Development deserves to be highly weighted within any phylogenetic analysis and particularly when dealing with enigmatic fossils and the origin of any major group. The Ediacara biota satisfies both of these criteria, so the need to understand their development can hardly be overstated. This first attempt has now been made to understand the growth and development of some members of the Ediacara biota (Antcliffe and Brasier, 2007, 2008) and has helped to narrow this list of possible affinities for these fossils.

5. Concluding Remarks: "And, has thou slain the Jabberwock?"

Those who investigate the Precambrian fossil record for the rise of life and the major animal groups seek answers to very similar questions: what are the criteria for recognising putative biological structures? How can these criteria be best measured or observed? What new technologies or techniques may aid our progress? Palaeontologists and astrobiologists must also deal with very similar challenges and ask: how uniformitarian can we be about a planet, be it the early Earth or another planet that is truly alien to us?

From the origin of life to the emergence of animals and everything in between, the earliest fossil record is riddled with pitfalls that may lead the unwary palaeontologist to misinterpret their data. We hope to have explained that all structures should initially be considered to be abiogenic until this hypothesis can no longer be sustained. There is always the possibility that a structure is abiogenic because abiogenesis must have existed long before (perhaps 8 or 9 billion years before) processes that we recognise today as biogenic could have developed. Furthermore, abiogenic processes have continued to operate throughout the whole of earth history and the morphospace of abiogenesis remains poorly mapped.

When Precambrian palaeontologists are faced with usual fossil morphologies, there will always be the temptation to erect another Jabberwocky: a mythical beast with uncertain affinities that will reside in our consciousness regardless of how secure the science behind this interpretation may be. So how are such Jabberwock(ies) slain? We must strive to always adopt a critical approach to the enigmatic morphologies we are faced with, and then it will become harder to build new Jabberwockies. But what of the Jabberwockies that already exist? Is there any hope that we may ever really be able to confidently state the affinities of creatures like *Dickinsonia* for instance? Or to establish whether a particular stromatolite is of biogenic origin or not? We believe that there is considerable

reason to be optimistic. New methodologies and techniques are continually presenting themselves, some of which are reviewed above. Finally, we draw some consolation from the fact that enigmatic fossils were not created by Lewis Carol or anyone else. The fossil record is unlike the Jabberwocky, which was written with deliberate obfuscation, so we will never know the express intent of the author, save that to create debate and stimulate a philological frenzy. There is no miscreant author of the fossil record that deliberately set out to confuse us. The fossil record is the product of processes that are understandable in terms of the natural world and given time and the appropriate methodology, there is no reason why they cannot be deciphered.

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MICROORGANISMS IN THE ANCIENT TERRESTRIAL SUBSURFACE – AND IN OUTER SPACE?

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1. Introduction

Microorganisms have been detected in great depth in subterranean environments, such as granite, sediments, permafrost areas, caves, rocks in gold – and uranium mines. Research directed towards exploring intraterrestrial microbial communities is a rapidly growing field and since the appearance of the first book, covering this subject, by Amy and Haldeman (1997), several aspects have been reviewed by Pedersen (2000), Gilichinsky (2002), Stan-Lotter et al. (2004) and Teske and Sørensen (2008). The surfaces on rocky planets and moons, which may be envisaged for the search for extraterrestrial life, can be considered as sterilizing environments, due to the high incidence of ultraviolet radiation, if there is no shielding atmosphere. This applies to Mars, where data about the conditions on the surface have been published (Ronto et al., 2003), and it is likely valid also for other bodies such as Jupiter's moon Europa. However, the subsurface of Mars and the presumed underground ocean of Europa hold great promise, since life may have survived there, protected from the harsh conditions on the surface. Thus, extraterrestrial subsurfaces will likely be probed for signs of life, following the development of suitable methods and instruments. Therefore, results from the often practical and applied microbial research in the terrestrial underground, which deal with storage problems for radioactive waste, aquifers and their cleanup from pollution, or restoration of contaminated sites in mining operations, could have consequences for the planning of missions to outer space in search for organics and life.

New issues have emerged as a result of the subterranean research, and many as yet unanswered questions have been raised: Are microorganisms from geological sites as old as the deposits from which they were isolated or identified? If so, how can this be proven beyond all doubt? And if proven, how could these organisms survive? Which mechanisms would allow them to endure prolonged states of starvation and/or desiccation? Are there specific dormant survival forms other than bacterial endospores? If so, could those be recognized in geological materials,

such as rocks or sediments? Can dormant forms be resuscitated? Are subterranean prokaryotes able to divide almost infinitely slowly (as has been suggested by Fredrickson and Onstott, 1996), which would imply the maintenance of minimal metabolic activity? Answers to these questions will impact the search for life in extraterrestrial subsurfaces.

In this chapter, current evidence for several examples of subterranean microbial life is reviewed, two environments (evaporites and subsurface springs) and their inhabitants are described in more detail, and potential lessons for astrobiological issues are considered.

2. Extent of Intraterrestrial Life and the Availability of Living Prokaryotic Fossils

The number of intraterrestrial microbes varies notably depending on the environments and sites being studied. Whitman et al. (1998) provided estimates for aquatic and soil environments and for the terrestrial subsurface, which probably contributes the major part of all biomass. Values in the range of several thousand up to hundreds of million microbes per millilitre of groundwater or gram of sediment are commonly reported. Although the wet weight of 100 million microbes, which may be present in 1 g of sediment, is only in the range of 100–1,000 µg, the total weight of microorganisms in many square kilometres of seafloor and continental shelve sediments, rock aquifers etc. may reach an impressive number. The carbon content of prokaryotes in intraterrestrial environments was estimated by Pedersen (2000) between 325 and 518×10^{12} kg, who pointed out that the total amount of carbon in intraterrestrial micro-organisms may equal that of all terrestrial and marine plant life together, which is in the order of 562×10^{12} kg (Pedersen, 2000).

The activities of intraterrestrial microbes, let alone their roles in the maintenance and evolution of the geosphere, are only insufficiently known. These issues provide important challenges for future research. The microorganisms in the deep subsurface of the Earth can be considered living fossils, since many of them must have been in their underground environments for very long periods of time and have probably survived in a dormant or nearly dormant state. The age of the microbial populations is currently not known, since accurate methods for dating individual cells are lacking (see below).

The depth of terrestrial subsurface layers, in which microorganisms can be found, is apparently limited just by the temperature, which increases with depth. The current records are in the range of about 3,000–3,500 m, e.g. a thermophilic *Geobacillus* was isolated in a deep South African gold mine from 3,200 m below surface; its temperature optimum was 65°C (Deflaun et al., 2007). Hyperthermophilic archaea can grow at very high temperatures; current record holders are *Pyrolobus fumarii*, which grows optimally at 105°C and survives up to 113°C (Blöchl et al., 1997), and an unclassified archaeon, which was reported to grow up to 121°C (Kashefi and Lovley, 2003). Therefore, in greater depths, archaeal representatives could be expected. Since many of the thermophilic archaea are lithoautotrophs,

they should be well adapted to live in subterranean environments. However, the questions of energy and carbon sources of the subterranean microbial communities are not clarified yet, and suggestions for and against evidence for a hydrogen-driven subterranean biosphere are being discussed (Stevens and McKinley, 1995; Nealson et al., 2005). Alternatively, reduced metals in the surroundings could serve as energy sources; typical microbially mediated redox pairs are manganese (II) oxidizing to manganese (IV), ferrous iron to ferric iron, sulfide to sulfate and methane to carbon dioxide (Madigan and Martinko, 2006).

3. Methods for Detection and Identification

The prokaryotic subterranean population has been analysed by conventional enrichment procedures and plate count methods. However, these approaches generally suffer from the phenomenon of the "great plate count anomaly", as known from environmental sites, since only a small fraction of the existing microbial community can be cultured in the usual types of nutrient media (Amann et al., 1995). In addition, microorganisms from the subsurface were often found to grow extremely slowly, even when appropriate media were used, making enumeration and analysis difficult. Therefore, the amplification of diagnostic molecules, such as the 16S rRNA genes, by the polymerase chain reaction (PCR), which obviates culturing of microorganisms, is being applied widely, and has permitted the detection of novel and unexpected phylogenetic groups in numerous environmental samples (Ward et al., 1990). Other molecular methods, which are increasingly used, are denaturing gradient gel electrophoresis (DGGE; see Muyzer and Smalla, 1998) and fluorescence in situ hybridisation (FISH; Bottari et al., 2006).

Direct light microscopic observations, combined with specific staining methods, are another technique for estimation of environmental microbial content. Staining procedures often use the fluorescent dyes DAPI (4′, 6-diamidino-2-phenylindole), as originally suggested by Porter and Feig (1980) and, more recently, the LIVE/DEAD BacLight™ Bacterial Viability kit (Leuko et al., 2004). These procedures facilitate the enumeration of cells and the latter can also provide judgment about their viability. In some subterranean environments, such as Permafrost, direct electron microscopy of samples has been applied and has revealed dwarf forms and cyst-like cells of non-spore forming bacteria (Soina et al., 2004).

4. Specific Subterranean Environments

4.1. HALOARCHAEA FROM SALT SEDIMENTS

The huge salt sediments on Earth, which were deposited during evaporation of sea water or continental brines, are the origin of evaporitic rocks. Similarly as for other natural environments, progress in methodology has increased greatly the

knowledge of microbial diversity. As recently as 1981, Larsen (1981) described mined rock salts as free from bacteria, although isolations of halophilic microorganisms from ancient salt sediments had occasionally been reported since the early decades of the twentieth century (for references, see Grant et al., 1998; McGenity et al., 2000).

Particularly large salt sediments stem from the Permian and Triassic ages (280 to 192 million years ago); the discovery of viable microorganisms, which have likely survived in fluid inclusions in the halite, is of special interest to astrobiology. Grant (2004) thoroughly discussed the problems associated with the isolation of culturable microorganisms from ancient halite, verification of their presumed antiquity, similarities of their genes to those of extant microbes and their mechanisms of longevity in the absence of resting stages, such as spores.

Our group isolated from alpine Permian rock salt, which was collected from the salt mine in Bad Ischl, Austria, a haloarchaeon, which, based upon polyphasic taxonomic data, was recognized as a novel species and named *Halococcus salifodinae* (Denner et al., 1994). This was the first isolate from ancient rock salt, which was formally classified and deposited in several international culture collections. Two independently isolated strains, 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 numerous properties, including the characteristic morphology of tetrads arranged in large clusters (Fig. 1); in addition, rock salt samples were obtained eight years later from the same site 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 archaea, which belong to the same species, occur in geographically separated evaporites of similar geological age.

Another halococcal isolate from the Bad Ischl salt formation, which differed from the previously described strains, was subsequently identified as a novel species and named *Halococcus dombrowskii* (Stan-Lotter et al., 2002). *Hc. salifodinae* and *Hc. dombrowskii* have so far not been found in any hypersaline surface waters, or any location other than salt mines. Recently, a series of non-coccoid strains was 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 *Halobacterium salinarum* NRC-1, from which the whole genome sequence is known; however, other properties were different and consequently, a novel species was created, *Halobacterium noricense* (Gruber et al., 2004). Figure 2 shows the two species of the genus *Halobacterium*. A single rod-shaped *Halobacterium* isolate from 97,000 year old rock salt in the US was described by Mormile et al. (2003) and deemed to resemble *Hb. salinarum* NRC-1. From the Permian-aged Salado formation in New Mexico, a strain of a novel genus, *Halosimplex carlsbadense* (Vreeland et al., 2002) was isolated. Table 1 contains a list of the formally classified isolates from alpine rock salt, the Salado formation and a strain from a British salt mine.

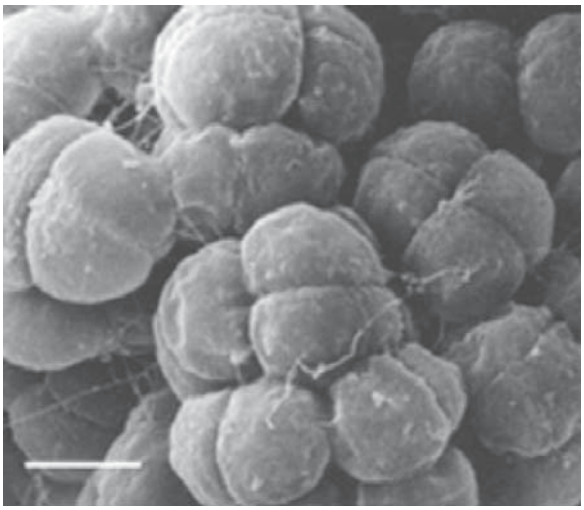

Figure 1. Scanning electron micrograph of *Halococcus salifodinae* Br3 DSM 13046. Bar, 0.5 µm. (Photograph taken by G. Wanner.)

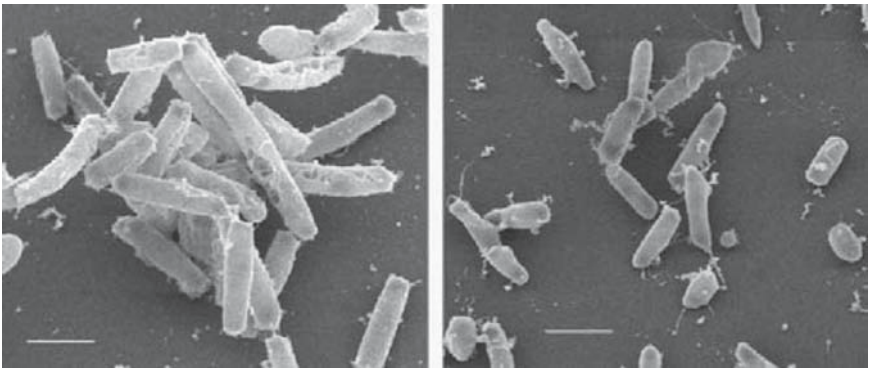

Figure 2. Scanning electron micrograph of *Halobacterium salinarum* NRC-1 (left panel) and *Halobacterium noricense* DSM15987^T (right panel). Bars, 1 µm. (Photographs taken by C. Frethem.)

Although 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 even up to 10^4 CFUs per g of Permian salt of the Salado formation in the USA (Vreeland et al., 1998) – the reports showed that viable haloarchaeal isolates were obtained reproducibly by several groups around the world. Taken

Table 1. Validly described haloarchaeal isolates from Permo-Triassic rock salt and salt mine brine.

Organism, strain	Type strain (^{T}); catalogue numbers	Origin	Reference
Halococcus salifodinae BIp JCM9578^T	DSM8989^T ATCC51437^T	Rock salt (lumps) Bad Ischl, Austria	Denner et al., 1994
Halococcus salifodinae BG2/2 Berchtesgaden, Germany	DSM13045	Salt drill core	Stan-Lotter et al., 1999
Halococcus salifodinae Br3	DSM13046	Brine in salt mine, Cheshire, England	Stan-Lotter et al., 1999
Halococcus salifodinae N1	DSM13070	Rock salt (lumps) Bad Ischl, Austria	Stan-Lotter et al., 1999
Halococcus salifodinae H2	DSM13071	Rock salt (lumps), Bad Ischl, Austria	Stan-Lotter et al., 1999
Halococcus dombrowskii H4 NCIMB13803^T	DSM14522^T ATCC BAA-364^T	Rock salt (lumps), Bad Ischl, Austria	Stan-Lotter et al., 2002
Halobacterium noricense A1 NCIMB13967^T	DSM15987^T ATCC BAA-852^T	Salt drill core, Altaussee, Austria	Gruber et al., 2004
Halosimplex carlsbadense	ATCC BAA-75^T JCM 11222^T	Permian salt, New Mexico	Vreeland et al., 2002

together, the data support the hypothesis that the halophilic isolates from subterranean salt deposits may be the remnants of populations which inhabited ancient hypersaline seas; in addition, they provide strong evidence against the notion that the recovered strains could be the result of laboratory contamination, since the isolates were obtained independently from different locations.

Besides isolation of viable haloarchaea, PCR procedures were applied to rock salt from Bad Ischl, Austria. The salt was dissolved under sterile conditions, filtered through membranes (pore size 0.22 μm) and the residue on the filter was subjected to DNA extraction (Radax et al., 2001). Nucleotide primers, which were specific for archaeal 16S rRNA genes, yielded a single PCR product of the expected size, following separation of molecules by agarose gel electrophoresis. 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; about 37% of approximately 130 analysed clones had sequence similarity values of less than 95% when compared with established haloarchaeal species and the remaining clone sequences were 98–99% similar to isolates from rock salts of various ages, including cultured haloarchaea from British, Polish and Thai salt mines and with uncultured phylotypes (Stan-Lotter et al., 2004; Radax et al., 2001; McGenity et al., 2000). In a similar

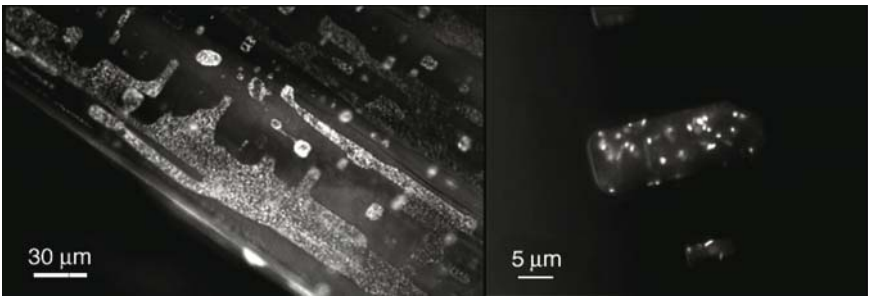

Figure 3. Localisation of pre-stained haloarchaea in fluid inclusions of artificial halite. Cells were stained with the LIVE/DEAD BacLight kit prior to embedding. Low (left panel) and high (right panel) magnification of *Halobacterium salinarum* NRC-1 cells. Cells were observed with a Zeiss Axioskope fluorescence microscope.

experimental approach, using halite samples ranging in age from 11 to 425 millions of years, Fish et al. (2002) found haloarchaeal sequences and, in the older samples, also evidence for bacterial 16S rRNA genes which were related to several bacterial genera. These data suggested the presence of a probably very diverse microbial community in ancient rock salt.

During simulation of halite formation in the laboratory by drying salty solutions which contained microorganisms, the cells accumulated within small fluid inclusions (Fig. 3). The haloarchaea can be prestained with the fluorescent dyes of the LIVE/DEAD kit (Fendrihan et al., 2006), which indicated the viability status of a cell; this procedure also greatly improved the visualization of cells within crystals. 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. From such experiments it appeared that the cells always accumulated in the fluid inclusions; there were no stained cells within the mineralic halite (Fig. 3).

4.2. CRENARCHAEA AND BACTERIA FROM SUBTERRANEAN SPRINGS

Thermal springs, delivering 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. Although the environments may vary greatly in mineral composition, extent and depth, springs provide an access to explore the hidden biosphere, which would be difficult to achieve otherwise.

A culture independent molecular analysis of microbial communities on rocks and in the water of a moderately thermal spring (46°C) in the Central Alps

near Bad Gastein, Austria, was performed. Four hundred fifteen clones were analyzed (Weidler et al., 2007); about 130 were found to be affiliated with 14 bacterial operational taxonomic units (OTUs) and about 280 with 4 archaeal OTUs. The majority of the cloned archaeal 16S rRNA gene sequences belonged to the so-called soil-freshwater-subsurface (1.1b) crenarchaeal group, according to DeLong (1998) and Jurgens et al. (2000); other representatives belonged to the freshwater-wastewater-soil (1.3b) group, except one clone, which was related to a group of uncultivated *Euryarchaeota*. These findings supported recent reports that *Crenarchaeota* do not only exist in high-temperature environments (DeLong, 1998; Bintrim et al., 1997; Schleper et al., 2005), but are probably wide-spread in rather temperate ecosystems. Most of the bacterial sequences were related to the *Proteobacteria* (*α, β, γ* and *δ*), *Bacteroidetes* and *Planctomycetes*. One OTU was allied with *Nitrospina* sp. (*δ-Proteobacteria*) and three others grouped with *Nitrospira*. Since *Crenarchaeota* have been implicated recently in the nitrogen cycle (Treusch et al., 2005), the spring environment was also probed for the presence of one of the key enzymes, the ammonia monooxygenase subunit A (*amoA*) gene. Sequences were obtained which were related to crenarchaeal *amo*A genes from marine and soil habitats. The data suggested that nitrification processes are occurring in the subterranean environment and that ammonia could possibly be an energy source for the resident communities (Weidler et al., 2007).

Probing biofilms in the spring with fluorescently labeled oligonucleotides directed against portions of the 16S rRNA genes of bacteria revealed numerous morphologies, most notably single cells and chains of bacteria (Fig. 4, left panel); similar hybridisation experiments were performed with archaea-specific sequences and showed mainly short rods or coccoid cells (Fig. 4, right panel) of uncultured archaea; similar archaeal morphologies were reported recently from a Siberian spring by Hatzenpichler et al. (2008), who discussed in their paper also the early evolution of nitrogen cycling.

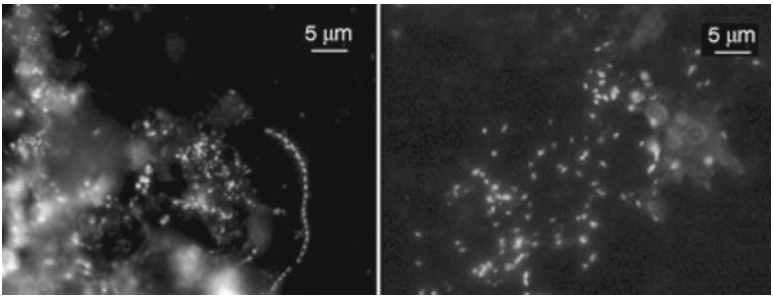

Figure 4. FISH analysis of biofilm from a deep subsurface thermal spring. Probes labeled with the fluorescent dye Cy3 were used, which were specific for eubacteria (left panel) or archaea (right panel); fluorescence was observed with a Leica DM4000 B microscope.

5. How Old Are Microorganisms in the Subsurface?

The results presented above provided evidence for an extensive biodiversity of extremely halophilic archaea in salt sediments, which are thought to have been deposited about 280 to 192 million years ago, and in brines, which are associated with the deposits. The salt sediments can be viewed as remnants from ancient hypersaline oceans. The fluid inclusions in Permian rock salt were found to contain cations and anions in a similar composition as today's sea water (Horita et al., 1991). Dating of the salt deposits by sulfur-isotope analysis (ratios of $^{32}S/^{34}S$ as measured by mass spectrometry), in connection with information from stratigraphy, indicated a Permo-Triassic age for Alpine and Zechstein deposits (Holser and Kaplan, 1966). This estimate was independently confirmed by the identification of pollen grains, which possessed distinct morphological features and could be assigned to extinct plants, in the sediments (Klaus, 1974). While there is no direct proof that viable haloarchaea have been entrapped in rock salt since its sedimentation, it would also be difficult to prove the opposite, namely that masses of diverse microorganisms entered the evaporites in recent times (see also McGenity et al., 2000 for further discussion). Especially for the Alpine deposits, an influx of meteoric waters containing microorganisms is rather improbable, because these sediments have been folded up and are located at altitudes of 1,000 m or higher for at least the last 100 million years (Einsele, 1992). Besides, the Alpine salt deposits are covered by layers of dolomite, limestone, marl, clay and other rocks; most of them are water-impermeable, and thus have contributed to the preservation of the salt deposits during the ice ages with their heavy precipitation.

If a Permo-Triassic age is postulated for the haloarchaeal isolates, then it becomes necessary to explain the biological mechanisms for such extreme longevity. Grant (2004) and Grant et al. (1998) suggested several possibilities, such as the formation of resting stages other than spores – since archaea are not known to form spores –, or the maintenance of cellular functions with traces of carbon and energy sources within the salt sediments, which would imply an almost infinitely slow metabolism. At this time, there are no methods available to prove directly a great prokaryotic age, whether it be a bacterium or a haloarchaeon. The mass of an average prokaryotic cell is only about 10^{-12} g (picograms); it is composed of about 3,000 different biomolecules, some of which are present at femtogram levels at best; therefore, no current dating procedures can be applied.

The deep surface thermal springs in the Central Alps are located in igneous rocks (basalt, granite etc.). These are the predominant solid constituents of the Earth, formed through cooling of molten material, as described by Pedersen (http://www.gmm.gu.se/groups/pedersen/research.php). Igneous rocks are too hot when formed to contain life of any kind. Therefore, if prokaryotic life is found in igneous granitic rocks, it must have entered after cooling and fracturing of the rock mass. From geological studies it has become clear that groundwater at depths of about 500 m in such rocks can be very old and ages of 10,000 years are not unusual (Pedersen; http://www.gmm.gu.se/groups/pedersen/research.php).

The water of the thermal springs in the Central Alps is known to circulate with an interval of at least 3,000 years (see Weidler et al., 2007).

6. Considerations for Astrobiology

The interest in subterranean microbial life has increased considerably since the discovery of bacteria-like microfossils in the Martian meteorite ALH84001, together with polycyclic aromatic hydrocarbons and low temperature carbonate globules (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 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.

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 – or may have existed in the past – in similar subsurface salt deposits on other planets or moons. This notion becomes all the more plausible in view of the detection of halite in extraterrestrial materials: the SCN meteorites (Gooding, 1992), which stem from Mars (Treiman et al., 2000), were found to contain halite. Quite recently, the data from the US rovers Spirit (http://www.msnbc.msn.com/id/5166705/) and Opportunity (http://www.missionspace.info/news/merupdate/saltwater.html) suggested the formation of at least some Martian deposits from concentrated salt water. 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 were inspected days after being collected (Zolensky et al., 1999). Another line of evidence for the existence of extraterrestrial salt was provided by the Galileo spacecraft; its onboard magnetometer detected fluctuations that are consistent with the magnetic effects of currents flowing in a salty ocean on the Jovian moon Europa (McCord et al., 1998).

The examples presented here imply that much of prokaryotic life on Earth is indeed adapted to a subterranean lifestyle. This suggests that a planet with a lifeless surface may hide life deep beneath its surface, provided there is liquid water and energy available; alternatively, microbes may be present in a dormant state.

The search for life in the solar system and beyond is a goal of several space agencies in the twenty-first century (Foing, 2002). Current planning of the European Space Agency includes the ExoMars concept, which consists of a mobile rover capable of drilling into the surface of Mars at least 2 m deep and of probing for traces of organics and biomolecules (Vago and Kminek, 2007). In addition, return samples from Mars or other celestial bodies might become available.

Other well-characterized samples may stem from meteorites, which might 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 – i.e. proof, that any detected substances are not stemming from Earth – is severe, and this will likely spawn applications to terrestrial samples as well.

7. Summary

The deep terrestrial subsurface harbors large microbial communities which have not yet been fully explored. Evidence from geological findings suggests that certain prokaryotes may have remained viable in sediments and subterranean water for thousand and perhaps millions of years. Considering the harsh environments on other planets and moons as well as in interplanetary space, 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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EVIDENCE OF ANCIENT MICROBIAL LIFE IN AN IMPACT STRUCTURE AND ITS IMPLICATIONS FOR ASTROBIOLOGY:
A Case Study

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1. Introduction

The search for present and past life on Mars has drawn major attention from the scientific community, as well as from national and international space agencies. A major reason for focusing the search for life on Mars is that, apart from being the closest planetary body of major astrobiological interest, Mars may have shared a number of environmental features with Earth during the early phases of planetary history. The atmosphere was denser and probably similar to the Earth's atmosphere in composition, and liquid water was present on the surface (Squyres et al., 2004), either as a stable water body or as frequent flooding events of short duration (Segura et al., 2002). A number of geological processes occurred on the hydrologically active surfaces of both planets early in their history, including intense volcanism and frequent meteorite bombardment, which would have led to the widespread distribution of hydrothermal systems on early Earth and Mars (e.g., Farmer, 1996). Hydrothermal deposits are of particular interest as targets in the search for fossil (and extant) life on the red planet (e.g., Farmer, 1998; Nealson, 1999; Newsom et al., 2001; and references therein), since life on Earth either originated or adapted relatively early to thermal environments (Stetter, 1996). In addition, hydrothermal systems are capable of preserving biosignatures indicative of microbial life (chemofossils, organosedimentary structures, silicified microfossils, biomarkers, etc.) (e.g., Walter and Des Marais, 1993; Farmer and Des Marais, 1993; Simoneit et al., 1998; Renaut and Jones, 2000; Konhauser et al., 2003). Collectively, these findings suggest that a conservative search strategy for evidence of life on Mars could be carried out with the use of the same principles that have been applied to Precambrian paleontology on Earth (Schopf and Klein, 1992; Walter, 1999).

Because early Mars and early Earth in many ways resembled each other, it is reasonable to argue that ancient-Earth studies will aid in the search for life on Mars even though the nature of the problems encountered may differ somewhat. The main problem with early-Earth studies is the dearth of ancient sedimentary rocks and the extensive amount of alteration and metamorphosis of the rocks that did survive on the surface of our tectonically active planet. The surface of Mars, on the other hand, is most likely well preserved because of the lack of plate tectonics, and in some cases, it probably represents some of the most ancient contiguous strata in the solar system. Nevertheless, when an ancient and well-preserved rock from the surface of the Earth is identified, and the search for traces of life is initiated, the problems encountered are similar to those that will present themselves in the search for traces of biologic activity in martian rocks.

Assuming that life did emerge on Mars, the question is where to look for it. Although it may be relatively easy to locate regions that show evidence for a hydrological past, such as sedimentary rocks (clastics, calcareous sediments, evaporitic rocks, BIFs, and phosphorites) and hydrothermal deposits, a key factor will be the ability to identify deposits that accumulated in environments that could have supported life and preserved microbial biosignatures (e.g., Cady et al., 2003).

Possible sedimentary traps and paleoenvironments on Mars have been discussed, for example, by Komatsu and Ori (2000) and Farmer and Des Marais (1993). On Earth, paleobiological repositories include carbonaceous cherts, carbonates, and phosphorites. Such rocks either have not been identified or they are not likely to exist in reasonable quantities at the surface of Mars today. On the other hand, possible clastic and evaporitic sedimentary deposits that would have formed in fluvial and lacustrine environments have been observed at several locales on Mars (e.g., Cabrol and Grin, 1999, and references therein; Squyres et al., 2004).

Hydrothermal systems, which are often active mineralizing environments with high preservation potential, have been suggested as promising targets in the search for life on Mars. Microbial preservation has been extensively studied in modern hydrothermal ecosystems (e.g., Cady and Farmer, 1996; Renaut et al., 1998; Konhauser et al., 2001), and ancient hydrothermal deposits have been shown to contain fossilized microorganisms (e.g., Walter, 1996; Rasmussen, 2000; Westall et al., 2001; Logan et al., 2001).

1.1. IMPACT-INDUCED HYDROTHERMAL SYSTEMS

The formation of an impact crater is a short-lived catastrophic event that occurs when an asteroid or comet impacts on the surface of a planetary body (Shoemaker, 1963; Grieve, 1987; Melosh, 1989; Toon et al., 1997; Grieve and Therriault, 2004). The cratering process can be divided into three major stages (Gault et al., 1968): (1) contact and compression, (2) excavation, and (3) modification. The first two stages induce a shock deformation of the bedrock and cause intense fracturing on

a micrometer to kilometer scale. These events are followed by the modification stage where the gravitational collapse of the transient crater causes a complex interaction of a series of concentric and radial faults, all of which provide excellent fluid pathways for the subsequently formed impact-induced hydrothermal system. Geochemical studies of impact structures have demonstrated that the temperature range of impact-induced hydrothermal systems can be favorable for thermophilic microbial communities (Osinski et al., 2001; Kirsimäe et al., 2002; Naumov, 2002; Hode et al., 2003; Versh et al., 2005), and that these systems can remain active for thousands of years (Abramov and Kring, 2004; Jõeleht et al., 2005).

Impact-induced hydrothermal systems (Newsom et al., 1986, 2001; McCarville and Crossey, 1996; Cockell and Lee, 2002), therefore, represent target areas of interest for sample return missions. Impact craters on Mars are easy to identify from orbit, and any associated hydrothermal mineral assemblages should be localized to a relatively narrow ring around the impact structures (Newsom et al., 2001; Hode et al., 2003).

Here, we report evidence for microbial biosignatures in an ancient impact-induced hydrothermal system in Siljan, Sweden. A variety of mineralized (some carbonaceous) features similar to partially mineralized hyperthermophilic biofilm remnants that occur in modern hot springs were discovered. These findings support the hypothesis that impact-induced hydrothermal systems may be favorable targets in the search for evidence of fossil life, a discovery that may have implications for the search for ancient life on Mars.

2. The Siljan Impact Structure

The Late Devonian (Bottomley et al., 1978; Reimold et al., 2005) Siljan Impact Structure, located in central Sweden (Fig. 1a), is the largest known impact structure in Western Europe with a present topographic expression of approximately 80 km. The target sequence consisted of a Proterozoic basement covered by Paleozoic sedimentary rocks of Ordovician to early Devonian age. Though the exact thickness of the sedimentary cover at the time of impact is unknown, Grieve (1988) estimated that the maximum amount of erosional unloading was 1 km. The Siljan Impact Structure still resembles a typical impact ring structure with a central uplifted peak; the central 30 km wide region of fractured, brecciated, and shocked Proterozoic igneous rock is surrounded by a peripheral trough comprised mainly of Paleozoic sedimentary rock.

Throughout the impact structure are traces of a post-impact hydrothermal system, as evidenced by the presence of hydrothermally precipitated mineral phases that include quartz, calcite, fluorite, pyrite, galena, and sphalerite (Hode et al., 2003; Komor et al., 1988a, b; Valley et al., 1988). Hydrothermal quartz occurs exclusively as vein and breccia filling in the crystalline basement inside the central uplifted region, whereas calcite, fluorite, and sulphide minerals are present in fractures and veins in the down-faulted sedimentary rocks that consist mainly

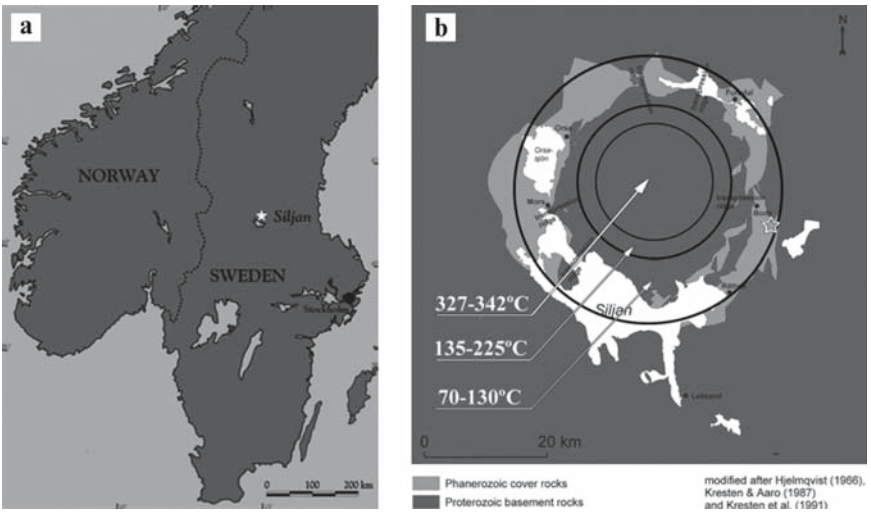

Figure 1. (a) (Modified after Hode et al., 2003). Map showing the location of the Siljan Impact Structure located in Dalarna, south central Sweden. (b) Schematic overview of the Siljan Impact Structure with projected isotherms that outline three main fluid temperature regimes of the impact-induced hydrothermal system. The isotherms and the sedimentary rocks (marked as light grey in the figure) that lie parallel to the ring-shaped structure are preserved even after 1 km of erosional unloading due to the severe downfaulting (Fig. 2a) in the perimeter around the central uplift caused by the impact event. The star in Fig. 1b indicates the location of the Jutjärn Quarry from which the limestone sample described in this case study was collected.

of carbonates (Fig. 2a) in the eastern part of the structure (Hode et al., 2003). The formation temperature (Fig. 1b) of the hydrothermally precipitated minerals ranged from 342°C for the earliest formed quartz in the central part of the structure (Lindblom and Wickman, 1985) to below 75°C for minerals precipitated in the outer regions of the crater (Hode et al., 2003). A general calcite-fluorite-pyrite-galena-sphalerite-quartz paragenetic sequence for the low temperature hydrothermal minerals was proposed by Hode et al. (2003).

An important consideration when evaluating a purported biosignature is to determine whether the paleoenvironment in which it was found could have supported life. Thermo-philic and hyperthermophilic microorganisms thrive in hydrothermal systems where fluid temperatures remain below the boiling point (Stetter, 2006). Fluid inclusion microthermometry, which in this study included a pressure correction for 1 km erosional unloading, indicates that the formation temperature of the hydrothermal calcite ranged between 76°C and 110°C (Hode et al., 2003), a temperature interval that overlaps with the temperature range for hyperthermophiles (≤80°C to 100°C at 1 atm, Stetter, 1996, 2006). The mineral assemblage within the veins and oil-bearing fluid inclusions indicates that the

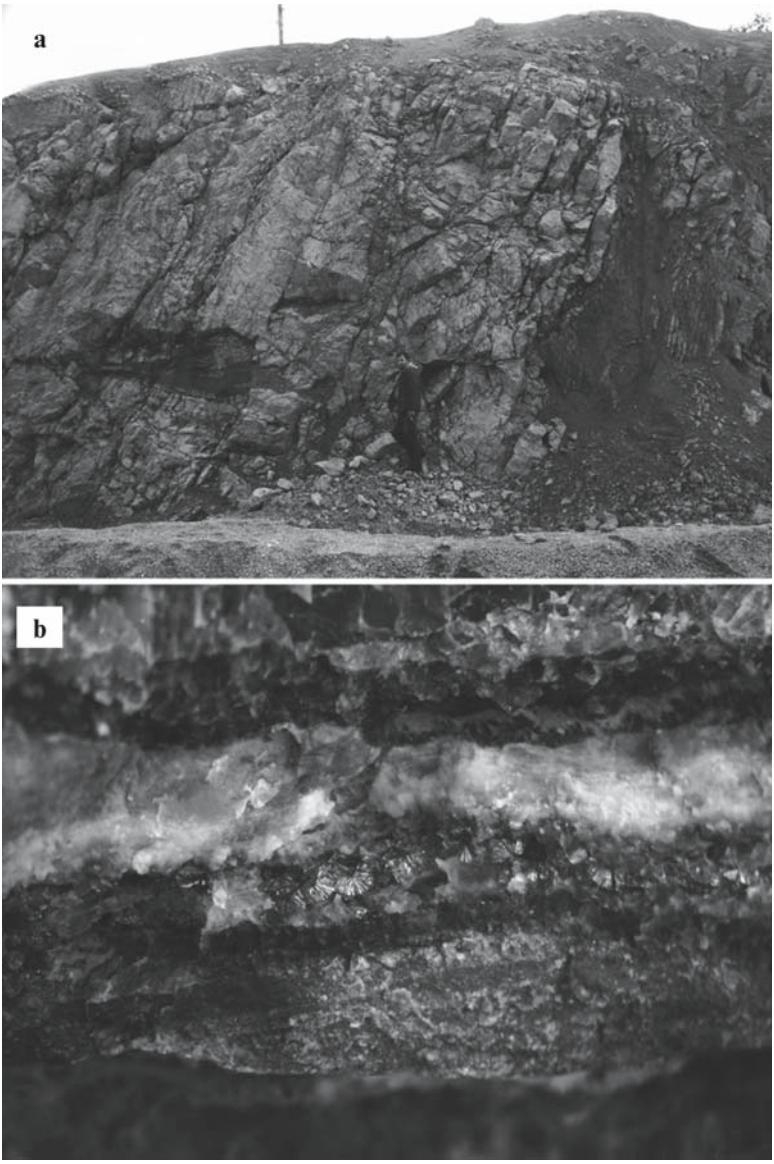

Figure 2. Sample site in the Jutjärn Quarry in Dalarna, Sweden. (a) Downfaulted Ordovician limestone in the Jutjärn Quarry on the eastern side of the Siljan Impact Structure. (b) (Modified after Hode et al., 2003.) Close-up of the hydrothermal calcite vein that was investigated in this study.

circulating hydrothermal solutions were anoxic and rich in hydrocarbons (Vlierboom et al., 1986). The occurrence of metal sulphides in the veins of the Siljan Impact Structure is consistent with a scenario in which reduced metal ions could have acted as electron donors, possibly together with molecular hydrogen. The geochemical characteristics of the hydrothermal fluid are similar to those reported for fluid associated with the 1,640 Ma McArthur River (also known as HYC) lead-zinc ore deposits and host sediments in Northern Territory, Australia, which are hypothesized to have supported subsurface anaerobic chemolithotrophic communities (Logan et al., 2001).

3. Materials and Methods

The findings of this case study are based on the results of an extensive submicroscopic study of one hand sample (#S39-01b-01, Figs. 2b and 3), which is part of a larger sample set of rocks associated with the Siljan Impact Structure that was collected by T. Hode (Hode et al., 2003; Hode, 2005). Sample #S39-01b-01, a piece of limestone collected from exposed outcrop (Fig. 2a) at the active Jutjärn Quarry, contains calcite-filled veins (Fig. 3) that precipitated at 90–110°C (determined by micro-thermometry with a pressure correction for 1 km erosional unloading).

The Jutjärn Quarry is located a few kilometers outside the central uplift of the im-pact structure in an area that was most likely located below the impact melt sheet when the hydrothermal system was active. The current surface features, hydrothermal veins, and mineral assemblages were exposed after ~1 km of erosional unloading and are, thus, remnants of a deep reaching impact-induced hydrothermal system. The calcite vein from which the case study sample was collected cross-cuts the tilted layers of downfaulted sediments, indicating that the vein post-dates the impact event. Since no regional thermal event has occurred after the impact and no traces of hydrothermal mineralization induced by the Caledonian orogeny (~400 Ma) have been reported in the area, the hydrothermal mineral assemblages could only have been formed by the residual heat from the actual impact event.

The sample was sawn into serial sections that were prepared in three different ways: one serial section was chelator-etched with 2% (m/v) EDTA for 30 minutes, another was acid-etched with 30% (v/v) HCl for 30 seconds, and a third "control split" was not etched. Splits of the etched sections, fractured to fit on specimen holders for SEM analysis, were individually washed in distilled deionized water prior to and after etching. Immediately after the water-rinsed specimens dried, they were gold coated. Splits of the non-etched control sample were washed in distilled deionized water twice prior to gold coating.

The specimens were investigated with an Hitachi S-4300 field emission scanning electron microscope (FE-SEM) at the Swedish Museum of Natural History, and a FEI Sirion FESEM at Portland State University, both of which were equipped with an Oxford Instruments EDS detector.

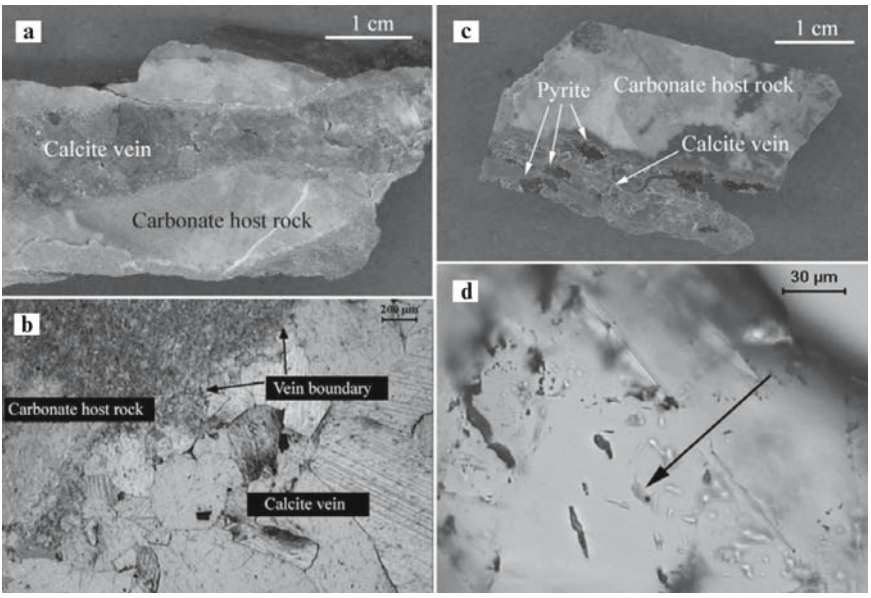

Figure 3. Hand specimens and thin sections of the samples investigated. (a) Sawn hand specimen with calcite vein cross-cutting the limestone matrix. (b) (Modified after Hode et al., 2003.) Microphotograph shows the boundary between the calcite vein and the carbonate matrix. The layer of calcite crystals adjacent to the wall of the vein contain smaller crystals than those formed toward the center of the vein, an observation consistent with mineral precipitation from cooling hydrothermal fluids. (c) Photograph of a doubly polished thin section used for fluid inclusion microthermometry. Pyrite crystals (shown with arrows) indicate a reducing fluid environment. (d) Photomicrograph showing oil-rich fluid inclusions. Microthermometry indicated that the minimum formation temperature of the calcite crystals ranged between 90°C and 110°C after pressure correction, and a salinity of 0–2.4 eq wt% NaCl. The formation temperature confirms that the mineral assemblage originated from hydrothermal fluids and not from later-stage groundwater fluids (see also Fig. 2b). The oil in the Siljan Impact Structure is present either in fluid inclusions or in cracks and pore spaces in the rock.

Rutherford backscattering (RBS) element analysis was performed on the specimens with the use of a nuclear microprobe located at the Nuclear Microprobe Facility at the Lund Institute of Technology, Sweden (Malmqvist et al., 1993). All of the RBS data were analyzed with the SIMNRA software developed at the Max Planck Institute of Plasma Physics. An RBS spectral plot represents the energy loss of the accelerated alpha particles after they scatter from nuclei in the sample. The alpha-particle beam size was set to approximately 5 µm for the experimental analyses made during this study.

Incoming alpha particles that undergo RBS loose a portion of their energy due to the transfer of momentum that occurs during their collision with a target nuclei and atomic electrons encountered prior to and after the collision. The amount of energy lost by a backscattered alpha particle during an elastic scattering event

depends on the mass of the sample nucleus encountered and the depth at which the scattering event occurs in the sample. The measured yield (i.e. the relative number of counts) from a specific element in the sample depends on the probability that the projectile will collide with it (i.e., a function of the effective size or scattering cross section of the nucleus) and on the concentration of the element in the sample.

An RBS spectrum thus represents the total number of backscattered alpha particles (y-axis) plotted as a function of their respective energies (x-axis). As discussed in the next section, the SIMNRA analysis of the data not only revealed which elements are present in the sample, but also illustrated the relative proportion of these elements at the point analysis site.

4. Results

A comparison of FE-SEM photomicrographs of etched vs. non-etched specimens revealed that biogenic-like features were partly encased in the hydrothermal minerals that precipitated within the impact-induced fractures (Figs. 4, 5, 6a, 8). Biogenic-like features associated with calcite crystals and calcite/pyrite grain boundaries included bundles of thread-like objects (Figs. 4 and 5) and stretched, curved films (Figs. 4, 6a, and 8).

The montage of SEM images of the vein shown in Fig. 4 provide a spatial context for the various biogenic-like features observed in the specimen. Though several hydrothermal assemblages of various temperature regimes and mineral facies were investigated for similar structures, only the low-temperature calcite-filled veins contained evidence of biofilm-like remnants.

Evidence that the film-like feature shown in Fig. 6a is calcified is illustrated by a comparison of the Rutherford backscattering (RBS) spectra shown in Figs. 6b, c: the position of the slopes that represent Ca in each spectrum start at 1,700 KeV. In other words, Ca was encountered at the immediate surface for both points where analyses were performed. If the film was not calcified, then the incoming alpha particles would have lost a portion of their energy as they penetrated through the film before they encountered the underlying carbonate, and the positions of the slopes that represent Ca in both spectra would have been located at different energies.

The depth sensitivity of the RBS element analysis revealed that the C content of the curved film-like feature shown in Fig. 6a is approximately three times as high as it is in the underlying carbonate and in the adjacent calcite grain. Spectral modeling with the SIMNRA program, which can provide a rough estimate of the individual components of the total spectrum, revealed that the higher C concentration in the curved film-like remnant (Fig. 6c) does not extend into the calcite grain situated beneath it (i.e., if it did, then the summation spectrum shown in Fig. 6c would be flat, as it is for

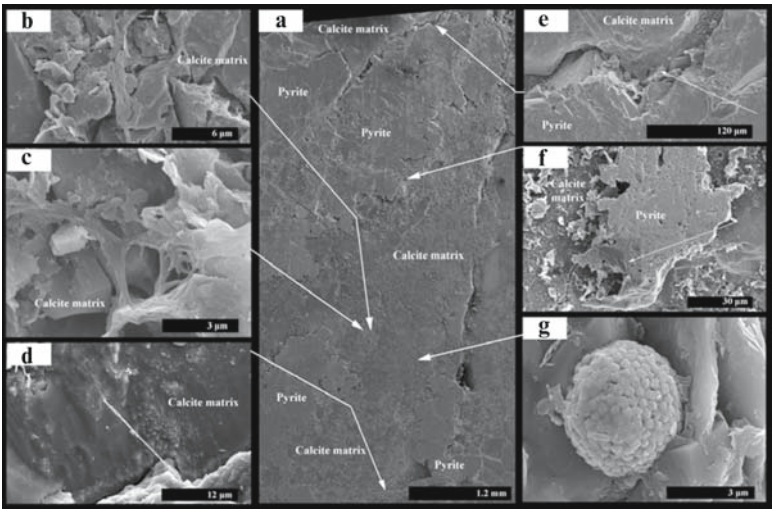

Figure 4. Montage of SEM micrographs that illustrate the relative distribution of various microbial-like features within the calcite vein. (a) Overview image of the investigated EDTA-etched calcite vein. Pyrite assemblages comprise the topographical highs since etching removed the top few tenths of micrometers of the surrounding calcite. Arrows indicate where the various features showed in Figs. 4b–g are located. All of the biogenic-like features were exposed by etching, an indication that they are located within the mineral matrix and are not later-stage contaminations. The crack along the right side of the image is the center of the vein. The overview image is a montage of three low-magnification SEM images. (b) A bundle of threads exposed by etching (close-up and further description of the feature is shown in Fig. 5c). (c) Microbial-like feature with a threaded and torn polymer-like structure. It is partly embedded in the calcite matrix, which indicates that it was trapped when the crystal was formed, and lies within the same calcite crystal as the feature shown in Figs. 4b and 5c. (d) Thread-like feature attached and stretched between pyrite (topographical high) and the calcite matrix (close-up shown in Fig. 5a). (e) Sheet-like feature (indicated by arrow) exposed after etching is located at the border between a pyrite crystal and the calcite matrix. See Fig. 6 for further discussion. (f) Sheet-like feature (indicated by arrow) wrapped around the edge of a pyrite aggregate. The feature is fully pyritized and no evidence for carbonaceous matter could be found. Note how the etching has removed the surrounding calcite leaving the pyrite exposed as topographical highs. See Fig. 8 for further discussion. (g) A pyrite framboid located inside the calcite matrix. Pyrite framboids are often found in reducing hydrothermal systems rich in carbonaceous matter (e.g., McKinley et al., 2000).

the summation spectrum that represents the adjacent calcite grain (Fig. 6b). Figure 7 shows the simulated SIMNRA spectral curve superimposed on the spectrum of the point analysis of the film-like feature (Fig. 6c). The simulated total spectrum was generated by summing the simulated spectral contributions of Ca, C, and O in the sample (Fig. 7). The dashed line in Fig. 7 indicates the shape of the simulated carbon curve of the spectrum from the point analysis of the adjacent calcite grain (Fig. 6b). The difference in

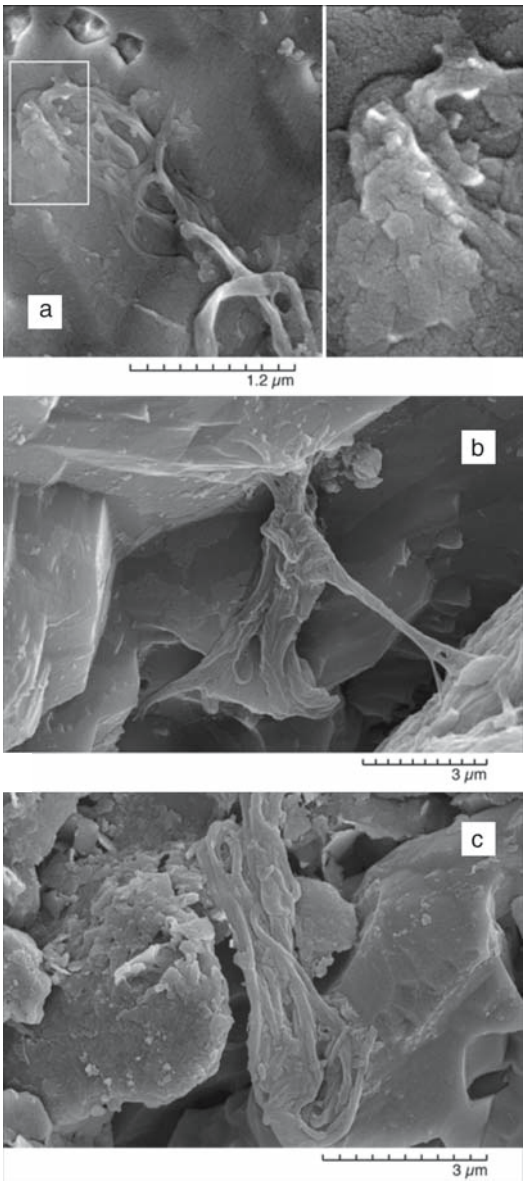

Figure 5. SEM photomicrograph images that illustrate the thread and polymer-like objects associated with the fossilized biofilm remnants. The features could not be remnants of fibrous illite as the EDS analysis indicated the absence of K and Al. (a) Threads partly embedded in a calcite matrix. The white rectangle marks the enlarged area on the right-hand side of the image, showing detail of the partly embedded threads. (b) A twisted film with threads attached to a calcite crystal (upper left corner) and clay fragment (lower right corner). (c) A bundle of threads situated on top of calcite and clay. The threads are broken on the lower right portion of the bundle, which indicates that they are brittle and partially mineralized.

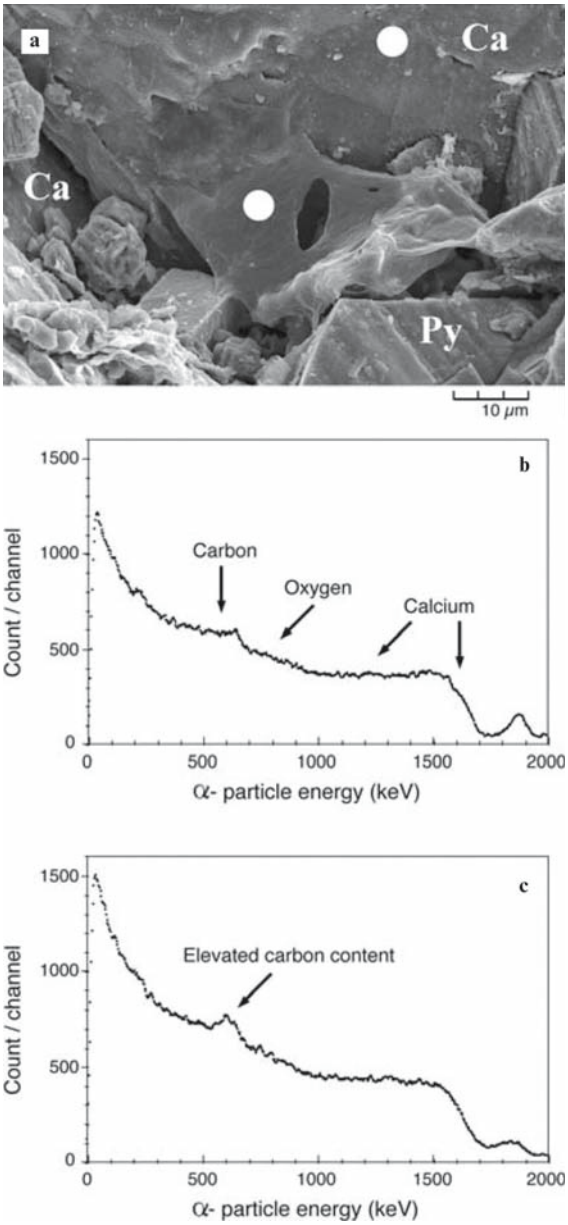

Figure 6. Photomicrograph and Rutherford backscattering (RBS) spectra of a purported microbial feature (also shown in Fig. 4e) and surrounding calcite grains. (a) SEM image that shows a smooth though curved film-like feature stretched between calcite (Ca) and pyrite (Py) crystals. Filled white circles indicate points of analysis. (b) RBS spectrum of the calcite grain (Ca) around and beneath the upper portion of the curved film-like feature (filled white circle to the upper right in Fig. 6a indicates point of analysis). The x-axis shows the energy of the backscattered alpha particles, which is determined by the cross-section (size) of the target nuclei and depth in the sample at which the scattering event took place. The y-axis shows the number of counts at a given energy. Carbon, oxygen, and calcium peak slopes are labeled in the spectrum (see also Fig. 7). (c) RBS spectrum of the curved film-like feature indicates an elevated (3x) carbon content (filled white circle to the lower left in Fig. 6a indicates point of analysis).

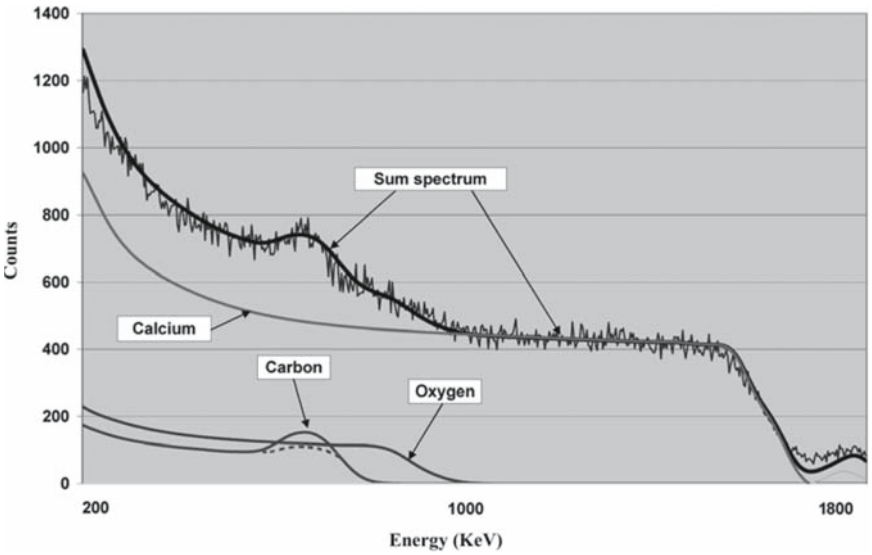

Figure 7. Simulated RBS spectrum superimposed on the acquired spectrum (rugged line) from the point-analysis of the film-like feature (Fig. 6c). Each acquired RBS spectrum represents the total number of backscattered alpha-particles (y-axis) as a function of the energy (x-axis). Note: The diagram is slightly truncated on the x-axis (starts at 200 KeV). The energy of the backscattered alpha particles depends on the cross section of the nuclei from which the alpha particles scattered, and the depth in the sample at which the scattering event took place. The simulated total spectrum (indicated "sum sectrum") in the figure was generated by summing the simulated spectral contributions of Ca, C, and O in the sample (see lables in the figure). The dashed line indicates the shape of the simulated C curve of the spectrum from the point analysis of the adjacent calcite grain (Fig. 6b). The difference in height of the dashed versus solid curve of the simulated C spectra corresponds to a concentration difference of about three times that relative to the amount of C in an adjacent carbonate grain.

height of the dashed versus solid curve of the simulated individual C spectra corresponds to a concentration difference of about three times.

Possible biogenic features with similar morphological traits as those shown in Figs. 4b–e, 5, and 6 were also found along calcite/pyrite grain boundaries, as shown in Figs. 4f and 8. A pyritized biogenic-like structure was found wrapped around and along the edge of an aggregate of vein-filling pyrite grains. Fine-grained calcite crystals, which appear in petrographic thin section to have precipitated before the pyrite (Fig. 3c) grains that filled the remaining pore space, were removed during the etching process. The absence of any of the biogenic-like features in the non-etched specimens indicates that the biogenic-like features were embedded within the calcite precipitates that formed during post-impact hydrothermal activity (Figs. 9 and 10).

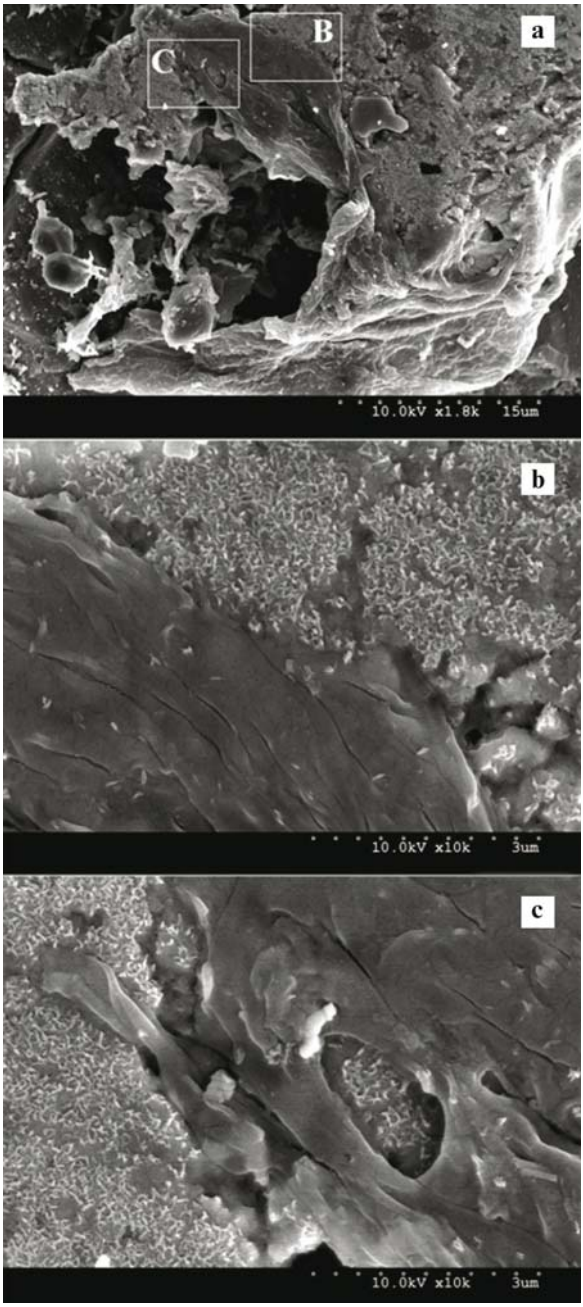

Figure 8. A pyritized biogenic-like structure. (a) The film-like feature is wrapped around and along the edge of an aggregate of vein-filling pyrite grains. Calcite removed during etching around the pyrite aggregate revealed that the curved film-like feature was not introduced into the sample preparation procedure prior to etching. White rectangles mark the areas shown in Fig. 8b, c. (b) SEM micrograph reveals that the film-like feature is partly embedded in the aggregate pyrite matrix. (c) Detail that shows an elliptical hole in the film-like feature, which indicates that it must have been flexible enough to tear and contract slightly prior to mineralization.

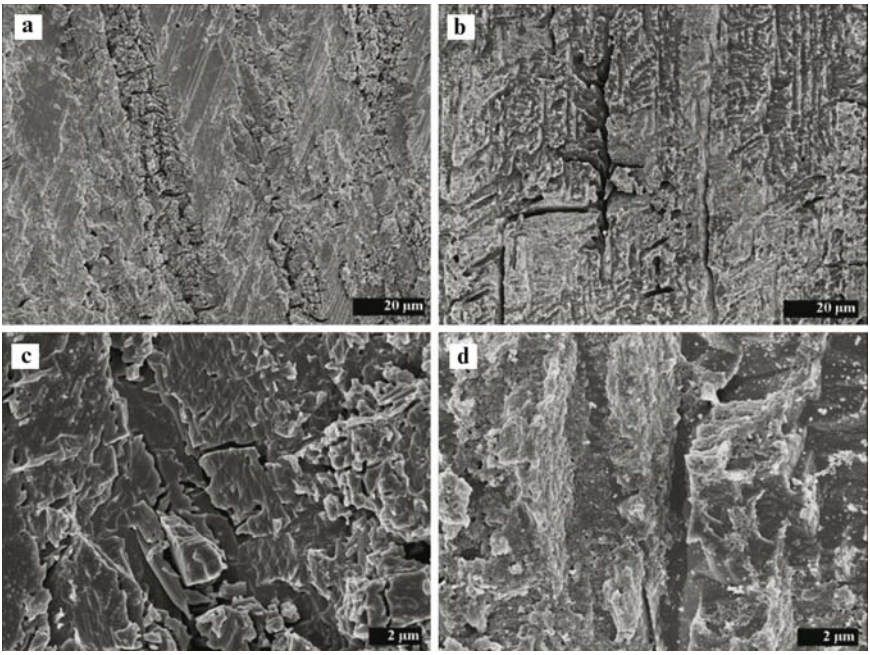

Figure 9. Series of SEM images that illustrate the effects of the etching protocols used in this study. (a, c) Non-etched specimen vs. (b, d) EDTA-etched split of the investigated calcite vein at different magnifications. The images are coupled in pairs (a & b, c & d) to show the effects of the EDTA-etching. (a) Freshly sawn calcite crystal surface of a non-etched split of the calcite vein. (b) EDTA-etched split of the calcite vein. Image shows an etched calcite crystal similar to the non-etched surface shown in Fig. 9a. Note how the etching is more severe in fractures and along edges, which creates a distinct etched topography. (c) Detail of non-etched area shown in Fig. 9a. (d) Detail of EDTA-etched area shown in Fig. 9b. Note how the EDTA-etching has caused the "crumbly" appearance of the surface in contrast to the crystal surface shown in Fig. 9c.

5. Discussion

The biogenic-like features, revealed by etching the hydrothermal calcite that filled an impact-induced fracture in sedimentary limestone of the Siljan structure, were found to consist of bundled or isolated "threads" (Fig. 5) and curved films (Figs. 6a and 8). The diameters of the threads vary, especially where they bifurcate near the attachment points. The thickness of a single thread rarely exceeds 0.3 µm, and some threads (not shown) extend several tens of micrometers between attachment points. These features were not visible with an optical microscope, which could be due to their small diameter, the lack of sufficient contrast between the partly calcified biogenic-like remnants and the limestone matrix, and the absence of extensive alteration of the organic matter, which could have enhanced its visibility in standard petrographic thin sections.

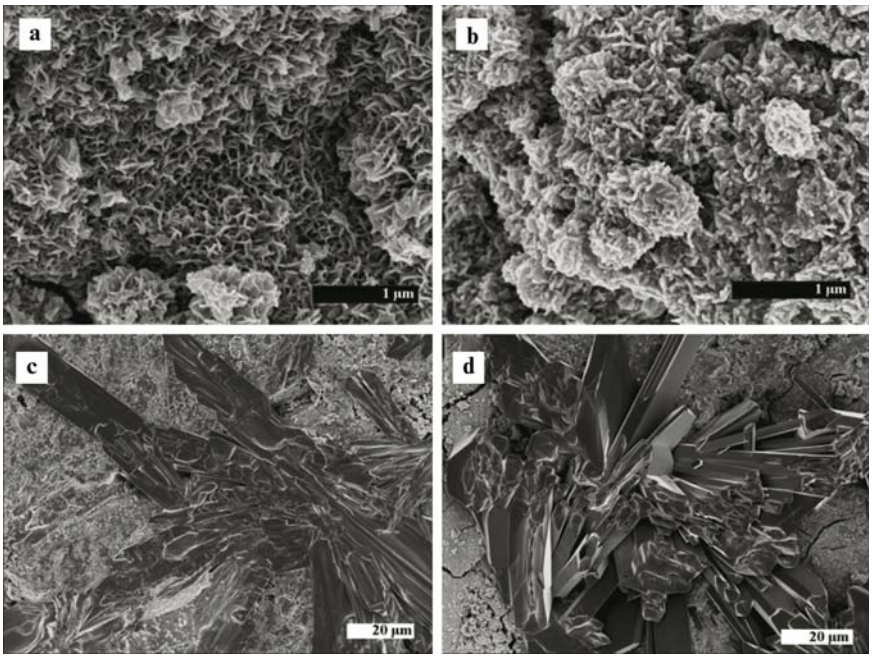

Figure 10. Series of SEM images that illustrate the effects of the etching protocols used in this study (continued from Fig. 9). (a, c) Non-etched specimen vs. (b, d) EDTA-etched split of the investigated calcite vein at different magnifications. The images are coupled in pairs (a & b, c & d) to show the effects of EDTA-etching of the calcite. (a) Detail of the non-etched split that shows an unusual surface texture on a calcite precipitate. Scale bar equals 1 µm. (b) EDTA-etched equivalent of the area shown in Fig. 10a. The original appearance of the precipitates shown in Fig. 10a is still present in the EDTA-etched sample, but it is evident that the etching has "attacked" the unusual precipitates, which results in their shorter and "thicker" appearance. Scale bar equals 1 µm. (c) Pyrite rosette on a freshly sawn non-etched surface of the calcite vein. Scale bar equals 20 µm. (d) Pyrite rosette of an EDTA-etched split of the calcite vein. The etching has exposed the pyrite rosette by removing the surrounding calcite. Note that the etching did not produce any visible artifacts on the surfaces of the pyrite rosette. Scale bar equals 20 µm.

As shown in Figs. 6a and 8, the curved films were flexible and attached to grains at discrete positions, and they appear to have contracted somewhat prior to mineralization. The curved film shown in Fig. 6a stretches nearly 30 µm between attachment points and is partly embedded in the calcite grain on the left. The lower right-hand side of the film is draped over underlying crystals and elongate objects, and wrapped up around the underside of the objects below. Though we found that the film was partly mineralized, the elliptical shape of two holes within it indicates that it must have been flexible enough to tear and contract slightly prior to mineralization. Similarly, the pyritized sheet-like structure shown in Fig. 8 has elliptical holes (Fig. 8c), which indicates the same type of flexibility

as the feature shown in Fig. 6a. The pyritized sheet-like structure also wraps around the edge of a pyrite crystal in a flexible manner.

The embedded nature of several of the features indicates that they are not artifacts of the etching procedure, which is also supported by the presence of fully pyritized films (Fig. 4f) and threads (not shown). In addition, the distinct attachment points on mineral surfaces indicate that they were not passively embedded in the mineral matrix, but rather represent remnants of actively attached microbial extracellular polymeric substances (EPS). However, though it is likely that the organisms that produced the thread-like biofilm remnants lived within the hydrothermal system, the possibility exists that such objects were flushed along with the fluid from other parts of the system. The authors are not aware of any examples of allocthonous organic remains that display the morphological characteristics of the film-like structures with distinct attachment points revealed only by etching.

Several of the morphological attributes of the film-like structures match those of modern partly mineralized hyperthermophilic biofilms that form on sinter surfaces and among debris that accumulates at the bottom of active, near-boiling hot spring pools and outflow channels (Fig. 11). These remnants of modern hyperthermophilic biofilms reveal the flexible nature of the biofilm matrix, which is stretched and contracted between attachment points (Fig. 11a). In some cases, as shown in Fig. 11b, distinct threads that serve to attach the film to the substratum bifurcate and appear to splay into bundles of threads, which ultimately connect to the curved sheet-like biofim matrix remnant.

Demonstrating the biogenicity of ancient biofilm-like objects requires linking morphological (e.g., cell remnants and extracellular polymeric substances) and chemical (e.g., isotopes, biomarkers, biominerals) evidence indicative of microorganisms or microbial activity (Cady, 2001; Cady et al., 2003). Evidence of microbial activity can include the presence of extracellular polymeric substances (EPS) that form when organisms attach to surfaces during the process of biofilm formation (Westall et al., 2000, 2006; Westall and Southam, 2006). Hyperthermophilic biofilms develop on actively accreting mineral surfaces in modern hot springs and display key features that are likely characteristic of subsurface biofilms. The flexible nature of the extracellular matrix of the biofilms allows for resistance to even the most deleterious effects of fluid movement (Fig. 11c). We have observed in modern hot spring settings that the extracellular matrix of hyperthermophilic biofilms is much more recalcitrant than the microbial inhabitants that excreted the EPS, especially during the earliest stages of mineralization, as evidenced by the common occurrence of EPS remnants sans microbial cells in modern hot spring deposits (Fig. 11).

5.1. MINERALIZED EPS

Based on our analyses and a comparison of our findings with modern hyperthermophilic biofilm remnants, we propose that the threads and curved films are the mineralized remains of microbial biofilms. We have also found that this

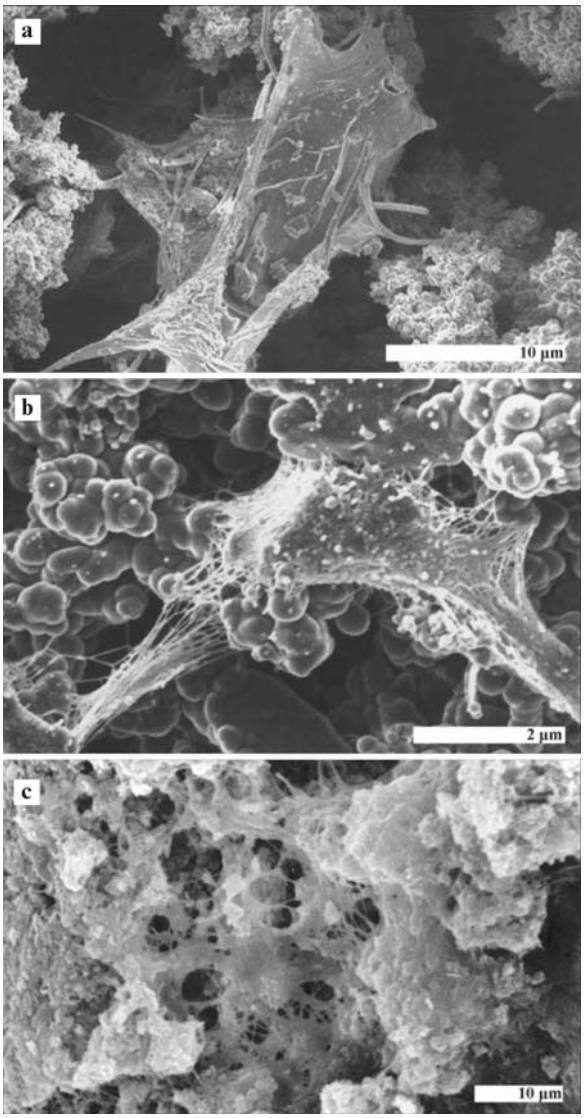

Figure 11. Examples of remnants of modern hyperthermophilic biofilms collected from near-boiling hot springs, Yellowstone National Park, USA. The biofilm remnants consist primarily of extracellular polymeric substances (EPS) that give enough coherency and flexibility to the biofilm matrix to allow it to remain attached at several places to the mineral substratum even after some amount of contraction and fluid-movement induced disruption. (a) Note the attachment points of the extracellular biofilm matrix on the substratum. The flexible biofilm matrix has also curled back around itself along some of its edges. (b) Biofilm matrix attached to underlying substratum with several convergent and distinct "threads" characterized by a range of thicknesses. For this particular microbial community, the EPS was attached at numerous points by polymers that bifurcate and become more numerous toward the center of the biofilm. (c) An SEM micrograph reveals the same distinct stretched, torn, curved-film characteristics displayed by the ancient biofilm remnants discovered in the hydrothermal veins in the Siljan Impact Structure.

particular combination of specimen preparation and analysis techniques, which includes specimen etching, FE-SEM, and Rutherford backscattering analyses, is a particularly effective means by which to reveal microbial biosignatures preserved within hydrothermal minerals that precipitated as fracture filling mineral assemblages. Though the curved and flexible biofilm-like remnants appear to have formed and become preserved *in situ*, the filamentous-like remains appear to have been transported some distance, as evidenced by the twisted curvi-linear nature of the thread-like objects that have been twisted around one another. In any case, the fact that the objects are embedded in the hydrothermal minerals that fill the calcite veins underscores the importance of impact-related hydrothermal systems as possible paleobiological repositories of subsurface biofilm remnants.

As noted below, we found no evidence that would indicate that the biofilm-like objects were produced abiologically (e.g., García-Ruiz et al., 2002; Holm and Charlou, 2001) or that they represent calcified insoluble residues of oil.

Our hypothesis, that impact-related hydrothermal deposits are important paleo-biological repositories that will preserve evidence of allocthonous and autocthonous microbial biofilm remains, is supported by the following key findings:

1. The features are structurally and texturally complex, and display morphological attributes known only to be produced by living microbial communities. Experiments designed to produce biological-like objects abiologically have not resulted in the production of curved films with flexible structures and multiple attachment points such as those presented here (J.M. García-Riuz, personal communication, 2005). The bifurcation of threads at attachment points, the curved and flexible morphology of the films, the elliptical perforations in films stretched between attachment points, the morphological fidelity of the objects exposed by etching; all of these attributes support our hypothesis that the features are remnants of objects produced by living microbial communities that thrived as thin microbial biofilms on mineral precipitates within the impact-induced fractures.
2. The mode of occurrence and the partly embedded nature (e.g., Fig. 5a) of the features indicate that they formed while the hydrothermal system was active. No mineral alteration or secondary infilling of the fracture post-hydrothermal fluid activity has occurred (Hode et al., 2003), nor is there any evidence of such biogenic-like features in the control (non-etched) sample, which would be consistent with the accumulation of such features having grown on the surface of the samples (i.e., in the field or the laboratory). The fact that the biogenic-like features were partly-to-fully mineralized (calcite or pyrite) indicates that the features originated in a reducing and actively mineralizing environment, which eliminates the possibility that the biogenic-like features were produced by modern endoliths.

3. RBS analysis indicated that the calcified biogenic-like features are enriched in carbon relative to the surrounding mineral matrix. The fact that enhanced concentrations of carbon were found in association with objects that display morphological characteristics like those of biofilm remnants is only consistent with a biological origin for the features. The anoxic and reducing character of the hydrothermal fluids that circulated through fractures in the impact structure (i.e. evidenced by the mineral assemblage within the veins) would have enhanced the preservation of organic remains that eventually converted to the kerogenate breakdown products of a biofilm matrix during mineralization.
4. The geological setting, while the hydrothermal system was active, could have supported a hyperthermophilic community such as those known to produce extensive biofilms on mineral surfaces in hot springs (e.g., Cady and Farmer, 1996). The 90–110°C fluid was anoxic, reducing, and rich in organics, and reduced sulphur phases (Hode et al., 2003). It may also have supported an ancient subsurface hyperthermophilic community. Isotopic evidence suggests that Pb and Zn were leached from surrounding granitic rocks (Johansson, 1984) and deposited as galena and sphalerite as they entered the limestone. Together with ferrous iron, these reduced metal ions may have acted as electron donors. Molecular hydrogen could also have served as an electron donor for a subsurface chemolithoautotrophic microbial community (e.g., Stevens and McKinley, 1995). Modern subsurface hydrothermal systems that display similar chemical and physical properties are known to support chemolithoautotrophic communities (e.g. Takai et al., 2002; McKinley et al., 2000; Stevens and McKinley, 1995).

5.2. IMPLICATIONS FOR MARS SAMPLE RETURN

The results presented in this case study indicate that any sample returned from Mars should be investigated for the presence of biofilm remnants. Our findings confirm the relevance of searching impact-induced hydrothermal deposits for evidence of microbial life on Mars. Impact-induced hydrothermal deposits have been shown to be exposed at the surface after an impact (Osinski et al., 2001; Newsom et al., 2001), which means that it would be possible to access the outer regions of impact structures where life could have resided (Hode et al., 2003). Given the availability of necessary microanalytical tools and a sample preparation procedure designed to reveal traces of life trapped inside hydrothermal mineral matrices, it is likely that traces of microbial life, if it has existed on Mars, could be revealed in even a very limited sample set from the red planet. For example, confocal (Schopf et al., 2006) microscopy and Raman spectroscopy (Schopf et al., 2005) can reveal preserved cells in a transparent mineral matrix, and as shown here, Rutherford backscattering and SEM can reveal preserved traces of biofilms in mineral etched samples.

6. Summary

We have shown that ancient impact-induced hydrothermal systems are favorable sites for the preservation of remnants of biofilm-forming microbial communities. At least two possibilities exist. First, an impact-induced hydrothermal system can supported a subsurface biofilm-forming community in the extensive fracture system through which hydrothermal fluids circulated. Mineralization of microbial biofilm remnants would be expected under such conditions. Second, the eventual infilling of the fractures by mineral precipitates can entrap any remnant biofilms, whether autochtonous or allocthonous. Our findings confirm the relevance of searching impact-induced hydrothermal deposits for evidence of ancient microbial life on Mars (Newsom et al., 2001).

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PHYLOGENOMIC DATING AND THE RELATIVE ANCESTRY OF PROKARYOTIC METABOLISMS

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1. Introduction

At present, little is known about when major groups of microorganisms and their metabolisms appeared on the Earth. The fossil record provides some limited information, however this information can be contradictory or ambiguous. For example, lipid biomarkers suggest the presence of Cyanobacteria at ~2.7 Ga (Ga = billions of years ago; Brocks et al., 2003). In contrast, the first unambiguous cyanobacterial microfossils are not seen for another 700 million years (Hofmann, 1976; Tomitani et al., 2006), after the rise in atmospheric oxygen at ~2.32 Ga (Bekker et al., 2004). There are major changes in the mass-dependent and mass-independent fractionation of sulfur isotopes at ~2.4 Ga (Canfield and Raiswell, 1999; Farquhar et al., 2000). While this could been interpreted as the origin of widespread mesophilic sulfate reduction (Blank, 2004), it can also been interpreted as a continued presence of sulfate reducers but a change in sulfate concentrations and/or rates of sulfate reduction (Ohmoto et al., 1993; Habicht et al., 2002). The changes in the mass independent fractionation can be also interpreted as a change in atmospheric photochemistry (Farquhar et al., 2000). Lastly, the extensive record of carbon isotopic fractionation in organic carbon has long been interpreted as evidence of a diverse biosphere containing photosynthesis as far back as 3.5 Ga (Schidlowski, 1988). However, abiotic synthesis has recently been shown to produce carbon isotopic fractionations in the range of phototrophic bacteria, calling into question the biological interpretation of early Archean carbon isotopic signatures (McCollom and Seewald, 2006).

Although the past decade has seen major advances in our understanding of earliest record of life on Earth (Brasier et al., 2002), one of the biggest outstanding challenges in the field of Astrobiology is our lack of a deep comprehension of how microorganisms co-evolved with the Earth through time. This is due to a lack of detailed understanding of both the early rock record and how microorganisms evolved. This challenge will have to be overcome if we are to fully appreciate changing planetary habitability through time and if we are to design the most effective strategies for searching for life on other bodies in the solar system.

Phylogenomic dating (Blank, 2004, 2009a) is a method that has the potential to shed some light upon both the ancient rock record and microbial evolution.

It combines well-resolved phylogenies derived from whole genome sequence data, the inferred evolution of metabolic and physiological traits, and patterns seen in the rock record to identify new age constraints for the origin of major prokaryotic groups (i.e., clades). This method is also potentially useful in the constructing and testing of causal biological hypotheses of major transitions in the early geologic record. Such transitions include changes in the cyanobacterial fossil record, sulfur isotopic fractionation, carbon isotopic fractionation, and the sudden rise in atmospheric oxygen. This paper outlines the methodology behind phylogenomic dating. It also discusses the many of the challenges associated with its implementation and how these challenges might be addressed in the future.

2. Prokaryote Phylogenomics

The first requirement for phylogenomic dating is a well-resolved phylogenetic tree – this provides the backbone for all subsequent analyses and inferences. In the past, phylogenetic trees of prokaryotes have been largely constructed using the 16S (also called small subunit, or SSU) ribosomal RNA gene (Woese, 1987). SSU rDNA was instrumental in articulating the three-domain structure of the tree of life (Woese et al., 1990). Soon after, rDNA studies identified a large number of fundamental bacterial lineages, or divisions (Pace, 1997). At the same time, the resolution of the branching relationships between these divisions was poor, with the phylogeny often collapsing into a polytomy. Also, phylogenies of other genes (such as RNA polymerase) sometimes conflicted with the rDNA tree (e.g., Klenk et al., 1999).

There are multiple reasons why a single gene tree (like the 16S rDNA tree) may lack resolution or may conflict with other single gene trees (Eisen, 2000; Gribaldo and Philippe, 2002). Single genes typically lack sufficient characters needed to resolve distant relationships (Brown et al., 2001). Single genes may lack sufficient slowly evolving characters to resolve deep-branching relationships. Even worse, they may have too many fast-evolving characters that can exacerbate systematic error such as long branch attraction (LBA). Long branch attraction or lack of phylogenetic signal can lead to incongruent or unresolved topologies and are a significant concern in prokaryote phylogenetics, given the ancient divergence times for major lineages (Gribaldo and Philippe, 2002). Lateral gene transfer (LGT) is the horizontal movement of genetic material between distantly related lineages (in contrast to the "typical" vertical movement of genetic material between ancestors and descendants). Lateral gene transfer can introduce genes with different histories into the recipient genome, producing phylogenic trees that are incongruent with other genes in the genome. The frequency of LGT may also play a role in the overall topology of the bacterial tree. It has been proposed that organisms that frequently exchange DNA by LGT may cluster together in trees, leaving the basal lineages to be populated by organisms with lower rates of LGT

(Gogarten and Townsend, 2005). Fourthly, ancient gene duplications followed by loss can also produce phylogenetic incongruence in a process known as lineage sorting.

Resolving the phylogenetic history of prokaryotes is a significant, on-going challenge (e.g., Gupta and Griffiths, 2002). Fortunately, the ever-growing abundance of genome sequences is providing the data that will ultimately contribute toward a more comprehensive understanding of the evolutionary history of prokaryotic genes and genomes. Many approaches have been developed for constructing prokaryote phylogenies using genome data. One uses presence/absence data of orthologous protein families or protein domains (Snel et al., 1999; House and Fitz-Gibbon, 2002; Wang and Caetano-Anollés, 2006). Another uses rare genomic changes, such as the positioning of shared overlapping genes (Luo et al., 2006), genome rearrangements, and mobile genetic elements (Boore, 2006). Yet another uses the presence of shared insertions and deletions within proteins (Gupta, 1998). An increasingly common approach is to use concatenated sequences of highly conserved genes (e.g., Daubin et al., 2002; Ciccarelli et al., 2006; Barion et al., 2007). The most highly conserved genes in a genome are often involved in critical cellular functions such as the processing of information (RNA, DNA, and protein). Genes in a concatenated dataset can be analyzed individually using a "supertree" approach (Sanderson et al., 1998), or trees can be calculated with the entire concatenated dataset using a "supermatrix" approach (de Queiroz and Gatesy, 2006).

Early genomic phylogenies constructed with presence/absence of orthologous genes (Fitz-Gibbon and House, 1999; House and Fitz-Gibbon, 2002) generally agreed with those constructed using a supermatrix approach (Hansmann and Martin, 2000; Brown et al., 2001). However there were some differences, particularly within the Euryarchaeota, a major clade of the archaeal domain of life. The presence/absence phylogenies placed the methanogens *Methanococcus* and *Methanothermobacter* sister to *Archaeoglobus* to the exclusion of the Pyrococci, while the concatenated analyses placed them with the Pyrococci to the exclusion of *Archaeoglobus*. A study using both gene content trees and concatenated ribosomal proteins (Slesarev et al., 2002) found a similar pattern with the hyperthermophilic methanogen *Methanopyrus*. Their gene-content tree placed *Methanopyrus* with *Methanococcus*-*Methanothermobacter*-*Archaeoglobus* while their concatenated analysis placed it with *Methanococcus*-*Methanothermobacter*-Pyrococci. Subsequently, a more exhaustive orthologue presence/absence analysis showed that methanogens and *Archaeoglobus* formed a large monophyletic group (House et al., 2003). The authors questioned previous phylogenies showing non-monophyly of methanogens, but acknowledged that clustering of this group in presence/absence trees could be an artifact of having 81 shared gene groups possibly correlated with a methanogenic lifestyle (64 having unknown function, 11 involved in methanogenesis; Fitz-Gibbon and House, 1999; House et al., 2003). This study also observed the anomalous Thermoplasmas branching before the basal Crenarchaeota and Euryarchaeota split – a placement they claim is likely

caused by highly reduced genomes. Given these potential biases, the supermatrix approach may be more desirable than the presence/absence approach.

At the present time, supermatrix analyses employing large numbers of taxa and different sets of genes appear to be converging upon a "core" archaeal tree. One study (Brochier et al., 2005; Gribaldo and Brochier-Armanet, 2006) analyzed a set of concatenated transcriptional proteins (5,809 characters) and a set of ribosomal proteins (2,213 characters). Both produced trees that were identical except for the placement of *Methanopyrus*. In the transcriptional set, *Methanopyrus* branched deep (agreeing with the 16S rRNA tree; Burggraf et al., 1991), however in the translational set *Methanopyrus* branched higher up with *Methanococcus* and *Methanothermobacter*. The authors proposed that the deep placement was due to LBA to long branching outgroups, and favored the higher placement of *Methanopyrus*. A larger supermatrix study (Blank, 2009a) used a different mix of operational genes (involved in metabolism and processes such as cell division and DNA repair) and information processing genes (15,220 characters). The trees constructed with this dataset agreed with the archaeal "core" tree. It was, however, noted that bootstrap support for the basal branching of *Methanopyrus* depended upon the sets of genes analyzed (e.g., metabolic vs. informational) and the tree reconstruction method used. Nevertheless, most topologies supported *Methanopyrus* branching deep with moderate to high support. Only some analyses placed *Methanopyrus* higher up, and these topologies tended to have weaker support than the basal branching position. *Methanopyrus* does have a number of physiological traits agree with a deep placement (hyperthermophily; lipids thought to be primitive; Hafenbradl et al., 1993). Regardless, the difficulty of accurately placing this lineage is due to the inherent problems associated with rooting and with long branches (Philippe and Laurent, 1998). Ultimately the issue will not be resolved unless another organism can be found that breaks up this long branch.

Although a "core" tree appears to be emerging for the archaeal domain, this is not necessarily the true tree or the strict organismal tree. Rather, it is an average tree that likely contains conflicting signal from lateral gene transfer, long branch attraction artifacts, lack of phylogenetic signal, and lineage sorting (Susko et al., 2006). The complexity hypothesis (Jain et al., 1999) proposes that the genes predominantly reflecting vertical evolution and the "core" are involved in information processing, while genes that experience more horizontal evolution are involved in metabolism. Indeed, upon the archaeal "core" appears to be superimposed a more complex history involving metabolic genes with vertical, horizontal, and convergent evolutionary components (Blank, 2009a).

Although initial studies conveyed hope for being able to identify a similar "core" for the bacterial domain (e.g., Brown et al., 2001), later studies soon reported unresolved phylogenies or well-supported conflicting phylogenies (Teichmann and Mitchison, 1999; Hansmann and Martin, 2000; Brochier et al., 2002; House and Fitz-Gibbon, 2002; Ciccarelli et al., 2006). The controversy over the shape of the bacterial tree extends not just to branching relationships between mesophilic divisions, but to whether the root lies in the hyperthermophilic bacteria

Aquifex and *Thermotoga* (Bocchetta et al., 2000; Brown et al., 2001; Di Giulio, 2003) or in the mesophilic Planctomycetes (Brochier and Philippe, 2002). The most recent analyses with large sets of taxa do re-affirm support for the thermophilic origin of the bacteria (Teeling et al., 2004; Barion et al., 2007), although there have long been questions about the deeply branching nature of thermophilic bacteria (Woese et al., 1991).

The reasons behind the inability to resolve the bacterial "core" are unclear. Some have hypothesized that high rates of LGT preclude resolution (Doolittle, 1999; Gogarten et al., 2002). Undoubtedly, LGT has played an important role in bacterial evolution, particularly in metabolic genes (Boucher et al., 2003; Bauer et al., 2004; Ma and Zeng, 2004; Mussmann et al., 2005; Pál et al., 2005). However, the extent to which lateral transfer has involved the highly conserved "core" genes is unclear and controversial (Daubin et al., 2003). Recent studies show that although LGT is difficult to distinguish from other phylogenetic causes of incongruence and therefore to enumerate, most bacterial genes appear to evolve vertically rather than horizontally (Lerat et al., 2003; Kunin et al., 2006; Susko et al., 2006). For example, an analysis of 205 gene families in the γ-Proteobacteria (Lerat et al., 2003) identified only two cases of LGT. Using the same dataset, however, and a different approach of statistical tests to compare all possible topologies, about 10% of the genes (18/205) were identified as candidates for LGT (Susko et al., 2006).

Clearly, if there is a bacterial "core" tree it will be a challenge to find. It will require performing analyses on individual genes in the supermatrix to identify and remove taxa and/or characters that contribute to phylogenetic incongruence whether by LGT, LBA, lack of phylogenetic signal, or lineage sorting. Luckily, not all genes appear to experience LGT at the same rates. It is also likely that not all microorganisms experience LGT to the same extent. For example, lateral transfers appear to be most likely between organisms that share ecological niches (Zhaxybayeva et al., 2004). Large concatenated datasets are not immune to LBA (e.g., Sánchez-Baracaldo et al., 2005). Consequently, measures should be taken in both supermatrix analyses and individual gene analyses to test for LBA (such as "long branch extraction"; Siddall and Whiting, 1999; Pol and Siddall, 2001). Taxa that show LBA behavior should either be removed or additional taxa added to break up the long branch. For divergences that occurred billions of years ago, resolving deep branching relationships will require analyses with large numbers of slowly evolving characters/genes (Keeling et al., 2005). Also multiple phylogenetic methods should be used, including those that take into account rate heterogeneity among sites with a discrete gamma distribution parameter (Bergsten, 2005).

3. Compartmentalization

Compartmentalization was first proposed by Mishler (1994) as a way to improve resolution of deep-branching phylogenetic relationships. In this method, a global

analysis is first performed on the data matrix to identify well-supported monophyletic groups (the compartments). Next, detailed phylogenetic analyses are performed within each compartment to obtain the best local/ingroup topologies. Two advantages of this approach is that it reduces LBA caused by the presence of outgroup taxa (Bergsten, 2005) and it maximizes the number of characters that can be used to construct the phylogeny (because taxa within a compartment share more characters/genes than taxa between compartments). Once the best topology is obtained within a compartment, the tree is rooted with a closely related outgroup and a constraint tree is generated. Poorly supported relationships are collapsed and the constraints from all compartments are coalesced into a single constraint tree. Finally, global analyses are performed while invoking the constraint statements.

Compartmentalization is uniquely suited for genomic data and deep phylogenic questions. Different sets of genes can be chosen for global and local levels, to maximize the amount and type of characters needed to resolve the phylogenetic problem at hand. The first large-scale implementation of Compartmentalization used a supermatrix of 53 taxa and 38 genes from bacterial genome sequences (Blank, 2002). The dataset included a mix of information processing genes (17), cellular processing genes (11), and metabolic genes (10) – in total 36 amino acid sequences, the small subunit rRNA gene, and the large subunit rRNA gene. Global analyses using all 17,750 characters were first performed using all taxa with maximum parsimony. (Analyses of mixed datasets are currently at a computational disadvantage. At present, maximum likelihood can only be used on pure amino acid or nucleic acid data. Bayesian methods can be used with partitioned mixed datasets, but are so computationally intensive they require significant pruning of taxa to the extent that they lose meaning. It is well established that phylogenetic resolution requires adequate representation of taxa to break up long branches. These factors effectively limit analyses of large, mixed datasets to, what could be considered less savory, parsimony and distance methods.) As expected, the initial global tree was poorly resolved, particularly at the deepest levels, with 6 tree islands and 13 steps to the next tree island (Fig. 1).

Next, branch lengths were measured. Branches with $\lambda > 0.100$ ("long branches", where λ = branch length divided by the total number of characters) included the Chlamydias, Spirochaetes, Mycoplasmas, and the ε-Proteobacteria. Taxon excision experiments showed that random pairs of these taxa were always monophyletic, often branching in locations different from analyses with a single long-branching taxon – classic LBA behavior. Large concatenated datasets are clearly not free from systematic error introduced by mutational saturation, and can result in highly supported incorrect topologies, often regardless of the tree reconstruction method (Jeffroy et al., 2006).

There are many ways of compensating for potential systematic error in concatenated phylogenies. One is to remove taxa that exhibit mutational saturation and/or LBA artifacts, and another is to remove fast-evolving positions. In our initial study, we did both. Global trees calculated without the long branching taxa had

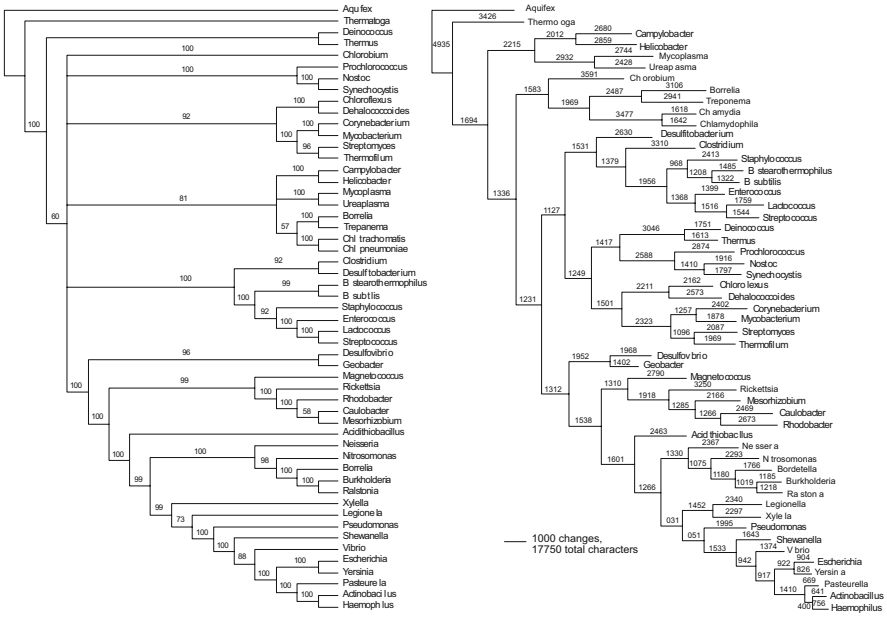

Figure 1. Global analysis using MP with 38 concatenated genes and 53 bacterial taxa. Left: cladogram showing relationship between clades and bootstrap support of nodes. Right: phylogram of one of the best trees showing branch lengths. *Aquifex* was the chosen outgroup based on analyses using archaeal taxa and concatenated information processing genes (not shown).

marginally better resolution (Fig. 2a), with 4 tree islands with 18 steps to the next best island (Fig. 2a). Trees with a more conserved dataset of 18 genes and 10,826 characters (identified by examining pairwise distances between taxa) had even better resolution, with 3 tree islands and 68 steps to the next island (Fig. 2b).

Next, local analyses were performed on the four monophyletic groups identified in the global analyses – the Cyanobacteria, High G + C Gram Positives (HGC, or Actinobacteria), Low G + C Gram Positives (LGC, or Firmicutes), and the Proteobacteria (for details see Blank, 2002). Analyses were performed using different subsets of genes, and with taxon excision experiments to identify potential LBA behavior. Next, the trees were rooted using neighboring outgroups and constraint trees constructed in MacClade (Maddison and Maddison, 2005; Fig. 3a).

A variant of Compartmentalization involves inference of ancestral sequences of compartment clades. To do this, the taxa in the compartment and the closest outgroup are selected in PAUP* (Swofford, 2001), the best ingroup topology obtained by Compartmentalization is enforced using a backbone constraint, and the ancestral sequence at the basal node is calculated using the Show Ancestral States command. The taxa in the compartment are then replaced by HTU sequences in the final global analyses. This approach may shorten overall branch

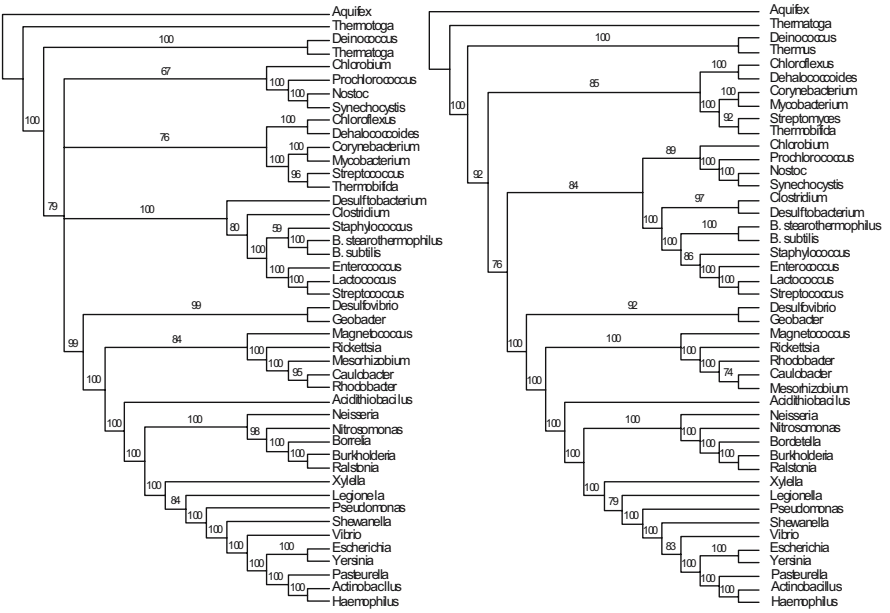

Figure 2. Global analyses using MP without long-branching taxa. Left (a) was performed with all 38 genes; right (b) was performed using the more conserved set of 18 genes.

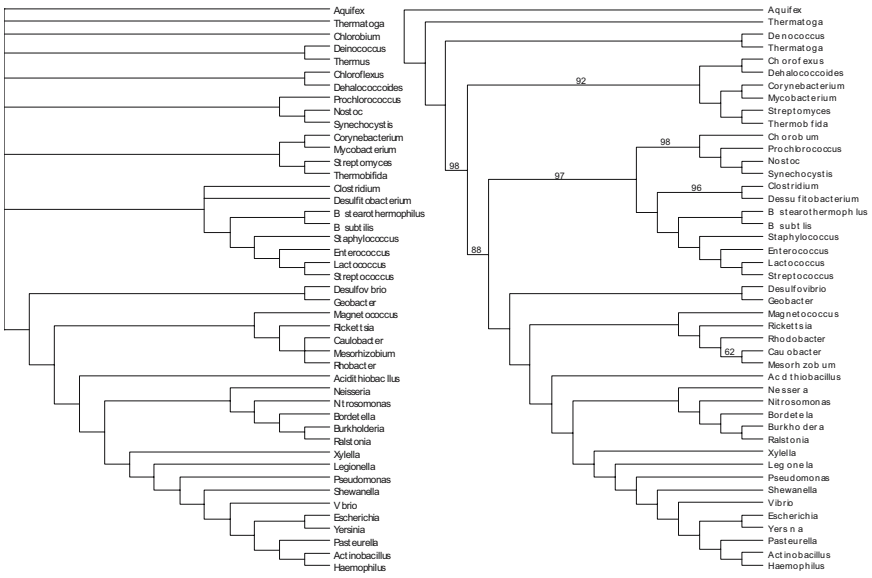

Figure 3. Constrained MP analyses. Left (a) was the constraint tree merging the results of each of the compartments; right (b) was the tree constructed while invoking the backbone constraint.

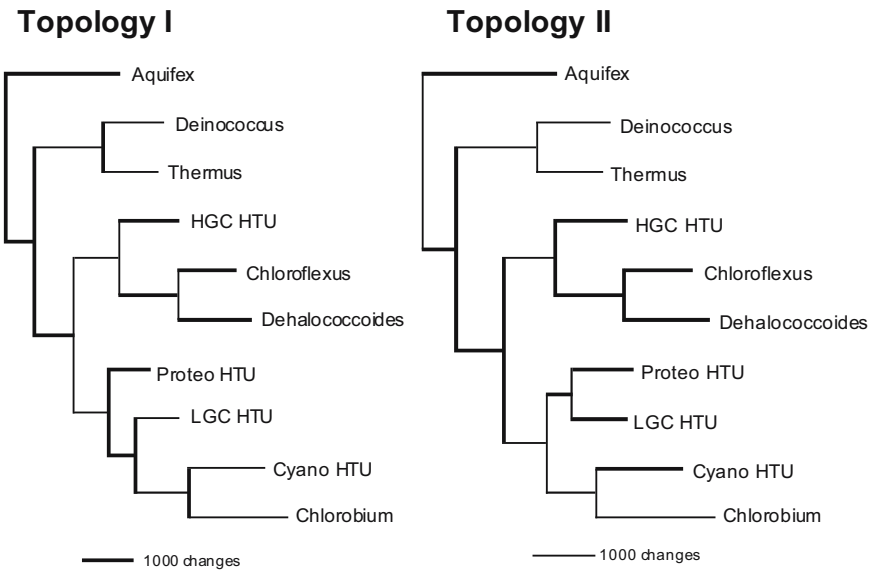

Figure 4. Trees generated by HTU analyses using MP showing the two related topologies observed.

lengths and improve the ability to resolve deep-branching relationships. In all HTU analyses, Proteobacteria, LGC, Cyanobacteria, and *Chlorobium* formed a monophyletic group (Fig. 4). However this monophyletic group had two alternative rootings, resulting in topologies I and II. Most HTU analyses using different taxa and sets of genes, produced topology I (this same topology was also seen in Figs. 2b and 3b).

With the character sets, taxa sets, and methods of analysis employed, Topology I (Fig. 4) appears to be leaning toward a "core" bacterial tree. This core is hypothesized to reflect the shared evolutionary history of highly conserved, essential proteins involved in the construction of the bacterial cell. Note, however, that initial global analyses did not immediately reveal this topology. It required identification and removal of taxa with long branches that were shown to exhibit LBA behavior. It also required choosing the most highly conserved genes – reinforcing the need for slowly evolving characters to resolve deep-branching relationships. Whether this topology is the true "core" topology for the bacterial domain or not will require analyses with additional taxa that not only adequately sample evolutionary diversity in the bacterial domain, but that also break up long branches that contribute to phylogenetic artifacts. Independent confirmation may also require analyses of an overlapping or different set of highly conserved genes.

4. Ancestral State Reconstruction of Prokaryotic Traits

Methodologies for ancestral state reconstruction (ASR) are some of the most contentious in systematic biology. Nevertheless, ASR can be useful in generating hypotheses regarding the evolution of traits – metabolic, ecological, or morphological – in important clades of interest (Maddison and Maddison, 2005). Such evolutionary hypotheses are ultimately testable: by constructing phylogenies of the genes underlying traits and by assessing particular time intervals in the rock record for biosignatures, lipids, and/or microfossils. For microorganisms, ASR can help to determine which traits are ancestral, and which traits are derived (Blank, 2004; Sánchez-Baracaldo et al., 2005).

For example, Fig. 5 shows the distribution aerobic respiration in the Euryarchaeota and the Crenarchaeota (Blank, 2009a). Aerobic respiration does not appear to be an ancient trait in these groups, rather anaerobic respiration appears to be basal in the archaeal domain (Woese, 1987; *contra* Castresana and Saraste, 1995). Aerobic respiration can be traced to the ancestor of five clades: (1) the Thermoplasmatales, (2) halophilic archaea, (3) Sulfolobales, (4) *Thermoproteus neutrophilus-Pyrobaculum spp*., and (5) in either the ancestor to *Caldivirga-Thermocladium* or the ancestor to the Thermoproteales. Genomes in the *Thermoproteus neutrophilus* and *Pyrobaculum spp*. were shown to have both cytochrome oxidases and quinol oxidases, and therefore the ancestor to this group was inferred to have obtained both of these genes, and therefore likely lived in an environment with a local presence of oxygen. Phylogenetic analyses showed that these genes were closely rerelated and share a common ancestor, confirming this hypothesis. *Thermocladium* and *Caldivirga* are sister taxa capable of microaerophilic aerobic respiration and the *Caldivirga* genome contains a quinol oxidase. *Thermofilum* was formally described as an obligate anaerobe (Zillig and Reysenbach, 2001), however its genome also contains a quinol oxidase. Phylogenetic analysis (not shown; Blank, 2009a) shows that these two genes are closely related and shared a common ancestor. This suggests that the ancestor to *Thermofilum-Caldivirga-Thermocladium*, i.e., the ancestor to the Thermoproteales, was aerobic.

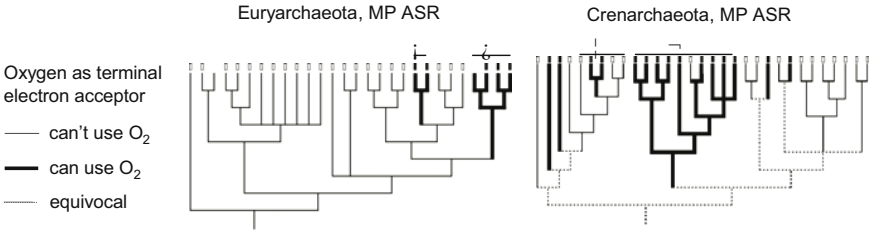

Figure 5. ASR of the character aerobic respiration on the two major lineages of cultured Archaea, the Euryarchaeota (left) and the Crenarchaeota (right).

ASR starts with an input tree, for example a "core" tree developed using genome data. Next, traits are coded into characters and character states (these can be discrete unordered characters such as presence/absence of aerobic metabolism, discrete ordered characters such as freshwater/brackish/marine/hypersaline, or quantitative characters such as a optimal growth temperature). This is readily accomplished using programs such as MacClade (Maddison and Maddison, 2005) or Mesquite (Maddison and Maddison, 2006). Lastly, ancestral states are inferred given the tree, the character matrix, and the optimality criterion (parsimony, likelihood, and Bayesian). There is contentious debate as to the appropriate choice optimality criterion (e.g., Cunningham et al., 1998). Parsimony is frequently used given its simplicity, but does not take into consideration branch length and performs best when the rates of character change are low (Schluter et al., 1997). Likelihood or Bayesian methods are sometimes preferable, particularly since the error in state reconstructions can be readily estimated. Nevertheless, these methods are prone to estimation errors in branch lengths, and there are some types of characters (such as ecological characters) where parsimony may be a preferred criterion (Hardy, 2006).

There are a number of critical requirements for the accurate reconstruction of ancestral character states (Hardy, 2006). These include (1) a resolved starting tree, (2) an accurate tree, (3) dense taxonomic sampling, (4) adequate knowledge of the presence or absence of the traits of interest, and (5) choice of optimality criterion. ASR can be sensitive to the presence of polytomies, or collapsed branches, in the tree because phylogenetic reconstruction algorithms treat them as unresolved ("soft" polytomies), whereas in ASR they are often treated as multiple speciation events ("hard" polytomies; Maddison and Maddison, 2005). Thus, it is desirable to either resolve the polytomy if possible (by excluding or including taxa or characters) or to perform ASR on all possible trees underlying the polytomy. Because the inference of ancestral character states uses the relationships (and often the branch lengths) of the tree in the reconstruction, an accurate tree is essential. Ideally for prokaryotes this would be a supermatrix tree thought to be the "core" tree of the group of interest.

Dense taxonomic sampling is also essential for accurate ASR (Salisbury and Kim, 2001). There are two fundamental reasons for this: it produces a better starting phylogeny and it better samples the diversity of traits within the group. Although the number of genome sequences is increasing rapidly, few are available for most groups of free-living prokaryotes. ASR studies, therefore, must rely heavily on trees constructed with single genes (most often 16S rDNA) that have much less resolving power than multiple genes. The study of character evolution in the Cyanobacteria exemplifies this problem (Sánchez-Baracaldo et al., 2005). Here, a supermatrix of 36 genes from 14 genomes was used to calculate a core cyanobacterial tree. To better sample the broad diversity of taxa in the Cyanobacteria, 71 additional taxa were added to the core tree using single gene data (all taxa had 16S rDNA; 34 had *rpo*C). Because 16S and *rpo*C contain limited phylogenetic signal, the final tree had many collapsed branches (although it was significantly better resolved than previous studies). Thus, prokaryote ASR studies must strike

a balance between increasing taxon sampling and decreasing phylogenetic resolution. In the Cyanobacteria study, ancestral character states were inferred using Fitch parsimony in MacClade (Maddison and Maddison, 2005), implementing the "soft" polytomies option. Populations of sub-trees with no polytomies have also been examined using a broader diversity of optimality criteria (Blank and Sánchez-Baracaldo, in preparation). However, these sub-trees perforce contained much fewer taxa.

Inadequate knowledge of prokaryotic traits may be another significant issue in the reconstruction of ancestral character states. For one, cultures can lose traits over time. In the Cyanobacteria, cultures have been shown to lose important traits such as heterocysts, hormogonia, akinetes, and sheaths (Wilmotte, 1994; Hoiczyk, 1998; Meeks et al., 2002). Morphologies of cells and colonies have been shown to change in cultures of *Nostoc*, *Anabaena*, and *Aphanizomenon* (Gugger et al., 2002; Meeks et al., 2002). It is also widely appreciated that microbial cultures undergo mutational, morphological, and physiological changes while in culture (e.g., Riley et al., 2001). Such changes could potentially inflate the number of inferred character gains and/or losses and result in incorrect estimation of the ancestral state.

Inadequate knowledge of the traits of prokaryotes may also be an issue in ASR. When new microbial taxa are described, the cultures are tested for optimal growth temperature, the ability to use a range of electron acceptors and donors, and whether it can grow aerobically or anaerobically. Characterizations may also report optimal salinity, pH, and any unique traits. Yet a handful of recent papers have shown that sometimes we know little about the physiology of even "well-understood" microorganisms. One surprise was that many thermophiles, even the heterotroph *Thermotoga* and several methanogens, were found to be able to reduce iron (Vargas et al., 1998; Bond and Lovley, 2002). Another surprise was that many "strict anerobes", including some sulfate-reducers, iron reducers, and a Gram-positive acetogen, can not only tolerate but also reduce oxygen (Lemos et al., 2001; Baughn and Malamy, 2004; Lin et al., 2004; Das et al., 2005). This illustrates that our knowledge of the traits of cultured prokaryotic taxa is imperfect. Such an underestimation of traits could lead to an over-estimation of the numbers of gains and losses of the traits and an incorrect inference of the ancestral state.

Lastly, our knowledge of microbial biodiversity is incomplete. It is well known that over 99% of microbial taxa are uncultured, either because they have yet to be cultured, do not grow in isolation, or are "unculturable" (Amann et al., 1995). The process of culturing itself selects for strains that grow well under artificial laboratory conditions and these strains are likely not dominant members of the natural community. Also, there is no doubt that significant extinction has occurred in the microbial world over the last 4 billion years. Thus, the traits of most extant uncultured and extinct microbial lineages are fundamentally unknowable.

Given our incomplete knowledge of microbial traits and the uncertainties in the ASR itself, it is of paramount importance that any hypotheses of character evolution be tested. For metabolic traits, this can be relatively straight forward by

constructing phylogenetic trees of the genes underlying the traits. If no statistical incongruence is found with the character reconstruction, confidence can be placed on the ASR. Such tests require the availability of the appropriate gene sequences, and preferably whole genomes. Lastly, the hypotheses of character evolution should be tested by analyzing the rock record at various age intervals for isotopic biomarkers, trace element biomarkers, lipids, and/or microfossils that might either support or refute the predicted transitions in character states.

5. Phylogenomic Dating

This last section discusses how ASR can be used to identify age constraints in major microbial groups, focusing on a recent character study of the archaeal domain of life (Blank, 2009a, b). Here, a supermatrix of 38 proteins, the SSU rRNA, and the LSU rRNA gene was constructed using 20 archaeal genome sequences. Analyses were performed on the entire matrix, on functional categories of genes, and on individual genes using parsimony, distance, likelihood, and Bayesian methods. As discussed above, the placement of *Methanopyrus* was problematic, although most trees showed it to be the basal branch in the Euryarchaeota.

Given only 16 euryarchaeal and 4 crenarchaeal genomes, a number of additional lineages were added using 16S rDNA to adequately sample taxonomic diversity. Weakly supported branches were collapsed into polytomies. Next, a number of metabolic, habitat, and physiological traits were coded into characters using MacClade, and the ancestral character states inferred using maximum parsimony.

Five candidate clades were inferred to have aerobic ancestors (Fig. 5). This was supported by BLAST results, showing that these clades had terminal oxidases most similar to other taxa in the clade. Next, the inferred habitats of the ancestor were examined. In the Sulfolobales, Thermoplasmatales, and *Thermoproteus neutrophilus-Pyrobaculum spp*. the inferred ancestors lived in habitats that were too hot and/or too acidic for Cyanobacteria (a local source of O_2) to exist. Therefore these three clades must have originated after 2.32 Ga, when oxygen appeared in the atmosphere (Bekker et al., 2004; darker arrows in Fig. 6). The halophilic Archaea, in contrast, have niches that overlap with Cyanobacteria (although halophilic Cyanobacteria branch peripherally in the tree; Garcia-Pichel, 1998; Sánchez-Baracaldo et al., 2005). Thus, it is possible, although not certain, that the halophilic archaea arose after 2.32 Ga (lighter arrow in Fig. 6). Lastly, because *Thermofilum*, *Caldivirga*, and *Thermocladium* are all extreme thermophiles, their aerobic ancestor must have originated before 2.32 Ga.

These "oxygen age constraints" provide valuable information about the antiquity of additional traits in the archaeal domain. Many traits were inferred to be ancient: sulfur reduction, hydrogenotrophic methanogenesis, autotrophy, heterotrophy, and hyperthermophily. Many traits were inferred to have arisen after atmospheric oxygenation: aerobic respiration, nitrate reduction, hydrogen

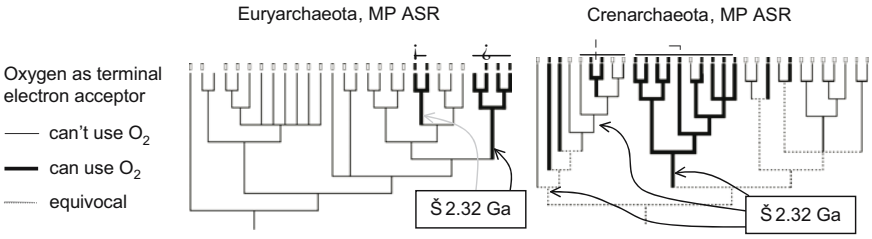

Figure 6. Age constraints placed upon the Euryarchaeota and Crenarchaeota trees. The darker arrows point to clades where the confidence in the age constraint is higher.

oxidation, sulfate and thiosulfate reduction, sulfide oxidation, and hyperacidophily. Traits that were not found to be ancient: mesophilic methanogenesis, anaerobic methane oxidation, thermophily and mesophily in the Crenarchaeota, and halophily.

As outlined above, the accuracy of these hypotheses depend upon the accuracy of the starting tree. The starting tree contains several polytomies given the inclusion of rRNA sequences. With more genomes, the resolution of this tree should improve and so should the ASR. Next, the hypotheses depend upon taxonomic sampling. As more genome sequences become available, future studies will include a better sampling of taxonomic diversity without compromising the resolution of the tree. The hypotheses also depend on our imperfect knowledge of traits. As our knowledge of prokaryote physiology improves, so should ASR studies. Lastly, as more genome sequences are completed our knowledge of the diversity of traits increases as we identify the presence or absence of genes that underlie the traits.

At present, there is scant evidence regarding the antiquity of Archaea in the geologic record. Archaeal microfossils lack unique morphological synapomorphies, and are indistinguishable from most bacterial microfossils. Lipid biomarker studies suggest archaeal lipids are absent in the Archean era (Brocks et al., 2003), but present in later Mesoproterozoic rocks (Logan et al., 2001; Dutkiewicz et al., 2003; Li et al., 2003). These lipids, however, are in most archaeal taxa and so provide little information on the age of specific archaeal taxa. Lastly, carbon isotopic fractionation at 2.7 Ga suggests the presence of methanogens (and methanotrophs; Hayes et al., 1992). Yet methanogens are widespread in the Euryarchaeota, so this also provides little age information. In contrast, phylogenomic dating provides new insights on the antiquity of many archaeal metabolic processes. Ultimately, phylogenomic dating may provide a novel way to determine the age of many microbial processes and to reconstruct ancient biogeochemical cycles. At the same, given the difficulties associated with prokaryote phylogenetics and ancestral state reconstruction, the hypotheses that are generated using phylogenomic dating must be carefully tested. Ground-truthing with the rock record will be essential.

6. Acknowledgements

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Biodata of **Roberto Barbieri** and **Barbara Cavalazzi** of ***"Fossil Microorganisms at Methane Seeps: An Astrobiological Perspective"***

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FOSSIL MICROORGANISMS AT METHANE SEEPS: AN ASTROBIOLOGICAL PERSPECTIVE:
Astrobiology of Methane Seeps

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Abstract The recent detection of methane in the martian atmosphere has stimulated a debate on its source, including speculations on a possible biological origin as in the Earth's atmosphere, where methane is present as a trace gas and is mostly produced by life. Large amounts of methane seepage flows from the subsurface are documented on Earth since the lower Paleozoic by the formation of authigenic carbonate deposits. Methane-derived carbonates also precipitate in the modern continental slopes throughout the world with a great variety in size and shape, and document a still active methane advection from deep sources. The interest of seep carbonates in an astrobiological perspective relies on their relationship with microbiological communities that inhabit the methane seep ecosystems and establish the base of their food chain. They also might represent terrestrial analogues for martian environments and possible models for microbial life on other planets.

1. Introduction

Exciting new discoveries in terrestrial extreme environments are widening the physicochemical limits of life on Earth, and therefore enabling an understanding of the potential spectra of conditions in which life can be present. The expanding limits of the environments of life on the edge may also help to answer the key question of how to recognize life, if life were ever to be met somewhere outside of our planet. A recent remarkable finding that documents the ability of life to survive in seemingly hostile conditions, involving both complex animal biota and microorganisms, is given by the dense beds of living clams and bacterial mats found in 2005 in a deep-sea site off the Antarctic Peninsula (Domack et al., 2005a). In this site, which is permanently covered by more than 10,000 years old superficial ice shelves (Domack et al., 2005b), a chemosynthetic-interpreted ecosystem sustained by low temperature fluid flow (cold seepage) is the first ever recorded from the Antarctic.

Methane seeps, as well as hydrothermal vents, are of great interest as extreme environments because the macroinvertebrates living there have developed efficient mutualistic partnerships with specific microbial consortia adapting to a range of conditions in which biochemical processes are largely driven by archaea and bacteria (Levin, 2005). Metazoa-microbe mutualisms would, therefore, be a successful strategy for further development along with new possibilities for life under extreme physical and chemical stresses (Hickman, 2003). On Earth, chemosynthetic generated (non-hydrothermal) geologic bodies have been known at least since the Silurian Period (Barbieri et al., 2004) and can preserve significant parts of ecosystems that are generated by hydrogen sulfide and hydrocarbons, especially methane. The enormous amounts of methane delivered to the atmosphere every year largely have geological sources (Kvenvolden and Rogers, 2005), including seeps, volcanic activity, and gas hydrates. It is believed that some periodic destabilization of natural gas hydrates throughout geological time, with episodic CH_4 release, may have influenced the Earth's climate (Kennett et al., 2003) and the distribution of seep ecosystems (Van Dover et al., 2006). At least some of this methane should have been originated from the activity of methanogenic archaea (methanogenesis), which have a high adaptability at extreme environments, including the conditions present on early Earth. Inorganically produced methane, such as that which is formed through serpentinization reactions related to hydrothermal processes, was recently found to sustain unique ecosystems. In the Lost City hydrothermal field (Kelley et al., 2001), near the Mid-Atlantic Ridge, for example, elevated (relative to seawater) methane concentrations support methane-consuming and methane-producing archaean populations (Kelley et al., 2005).

The record of methane, one of the major gas components at cold seeps, in the atmospheres of solar system bodies, including Europa, Titan, and especially Mars (Formisano et al., 2004; Krasnopolsky et al., 2004; Mumma et al., 2005), has revived interest in this biogenic tracer. Methane abounds in the solar system, however, its detection from small and localized spots, such as those recently reported from Mars and Titan, agrees with the presence of consortia of methanogenic bacteria similar to the ones described on Earth in cold seep ecosystems and based on methane for their biogeochemical processes. New insights into methane-dominated environments may help us to understand primordial life on Earth, and by analogy, in other parts of the solar system.

The likelihood that life, if present on Mars, is microbial, along with the expression of earlier wet periods that might have left some fossil records, have enhanced astrobiological interest in cold seep ecosystems, thanks to the fossilization potential of their microbe-mineral interactions. The focus of the present paper is to discuss these astrobiological perspectives and their controversies in light of the latest data from the ongoing European Space Agency (ESA) and National Aeronautics and Space Administration (NASA) missions to Mars, Titan, and Europa.

2. Modern and Fossil Methane Seep Ecosystems

The ecosystems sustained by methane-rich fluids (see the recent review by Levin, 2005) have diverse biota that produce enormous biomasses (Sibuet and Olu, 1998; Olu-Le Roy et al., 2004) and have been recognized from relatively shallow marine, down to hadal environments (Fujikura et al., 1999). Macroinvertebrate chemosymbiotic groups at cold (methane) seeps include mussels, clams, vestimentifera, gastropods, polichaetes, and nematodes as distinctive faunal components, which are partially shared with hydrothermal vent communities at different taxonomic levels (Van Dover et al., 2006). Some of these communities have a symbiotic relationship with methane-consuming and sulfide-oxidizing bacteria, whereas other parts obtain their nutrition from the direct utilization of the microbial biomass. The food web is, therefore, based on these chemoautotrophic primary producers. Methanotrophs belonging to specific archaeal clusters (ANME-1, ANME-2, and ANME-3) and the sulfate-reducing bacteria *Desulfovibrio* and *Desulfococcus-Desulfosarcina* clusters have been extracted from different anoxic environments associated with gas hydrates (Hinrichs et al., 1999; Meyerdierks et al., 2005; Treude et al., 2005; Zhang et al., 2005). Moreover, large communities of sulfide-oxidizing, filamentous bacteria – including *Beggiatoa*, which is amongst the largest of the prokaryotes (Jannasch et al., 1989), *Thioploca*, *Thiothrix,* and *Arcobacter* – typify the seep ecosystems, where they indicate areas of active gas seeping. Mats produced by the thiotrophic bacteria *Beggiatoa*, in particular, represent an interface separating oxic and anoxic environments at cold seeps. The authigenic carbonates, which make up most of the seep deposits (Ritger et al., 1987), only precipitate within anoxic environments (e.g., beneath the *Beggiatoa* mats), where the oversaturation of Ca^{2+} and CO_3^{2-} ions is largely determined by bacterial-induced kinetic constraints. The hydrothermal-generated carbonate bodies of the Lost City hydrothermal field (Kelley et al., 2001) are similar to methane seeps in many respects. In particular, they host macro- and microorganisms, which consume or generate methane in their metabolic processes (Kelley et al., 2005).

In ancient methane seeps the shelled megafaunal component represents a readily discernible diagnostic feature and include chemosymbiotic bivalves belonging to lucinids, bathymodiolids, thyasirids, vesicomyids, and solemyids that develop dense, low-diversity fossil assemblages. Lucinidae, which engage in a well known symbiosis with chemoautotrophic bacteria (Schweimanns and Felbeck, 1985; Fisher, 1990), have a large fossil record for this strategy of life, which makes this group remarkable from a geological perspective. The oldest known lucinid with morphological features that are similar to recent members of this group is *Ilionia prisca*, a Silurian species for which a bacteria-symbiotic feeding strategy is hypothesized (Liljedahl, 1992). The worldwide abundance of hydrocarbon seeps in the geologic record (Campbell, 2006) represents a useful condition for studying chemosymbiotic relationships through time; however, this is limited by the substantial changes that occurred throughout the Phanerozoic to the macroinvertebrate component of the seep fauna, especially for bivalve and brachiopod groups (Little et al., 2002).

These changes, and the consequent reduced phylogenetic relationships between modern and ancient macroinvertebrates, make it rather difficult to have a paleoecological analysis that is based on a uniformitarian approach (Campbell and Bottjer, 1995; Barbieri et al., 2004). Additionally, it is unclear as to whether the seeming resistance to the extinction of the seep macrofauna (Tunnicliffe, 1992; Kiel and Little, 2006) is a genuine feature or is rather dependent on the incompleteness of the fossil record. Unlike most of the modern and fossil seep ecosystems, some of them lack the typical (and diagnostic) macroinvertebrate assemblages. For example the Miocene-aged deposits of Santa Cruz, California (Aiello et al., 2001) consist of low magnesium calcite with associated foraminiferal and diatom tests. The activity of methanotrophs (as anaerobic methane oxidizers) and sulfate-reducing bacteria seems to be, therefore, the common factor that is shared by modern and fossil cold seep environments. High alkalinity conditions promoted by the biogeochemical processes of these microbial communities lead to the formation of calcium carbonate deposits (Ritger et al., 1987), the geological expression of the cold seep environments, past and present. The precipitation of a variety of limestone thin crusts, just beneath the seafloor surface, up to several meters-high limestone bodies (chemoherms and chimneys) packed with seep megafaunas, depends on the intensity and persistence of the seepage (Teichert et al., 2005; Campbell, 2006).

3. Microbial Evidences at Methane Seep Ecosystems

The presence and role of microorganisms in seep habitats are documented by various types of microbial mats, in which sulfide-oxidizing bacteria grow at or just below the water-sediment interface, and host a variety of faunal groups (Robinson et al., 2004). *Beggiatoa*, for example, that develop dense and thick mats, can be visually recognized because they are white (colorless) or have yellow-orange pigmentation (Prince et al., 1988; Levin, 2005). Other thiotrophic communities with higher diversity (including *Thioploca* and *Thiomargarita*) exhibit a gray color (Lichtschlag et al., 2006). The patchy distribution of these mats in a seep habitat is largely dependent on the extent and duration of the reduced fluid (H_2S) emissions.

The source of carbon in a seep ecosystem is strongly imprinted in the carbon isotopic composition of the tissues of invertebrate and microbial communities, and the authigenic carbonates. The rather negative $\delta^{13}C$ excursions measured on both biological and geological components (Campbell et al., 2002) of seep environments come from the microbial oxidation of hydrocarbons (oil and methane), which are the main carbon source. On average, biogenic methane is more depleted in $\delta^{13}C$ than thermogenic methane, and can reach extremely negative $\delta^{13}C$ values: less than −100‰ (PDB) (Schoell, 1988). $\delta^{13}C$ values as low as −69‰, as measured in Cenozoic high Mg-calcite (Campbell et al., 2002), represent a permanent signature in the geological record and are a useful diagnostic feature for documenting methane seep activity.

The microbial community structure and activity at methane seep sites is complex and is not only limited to the seafloor. Methanotrophs are also distributed in the overlying water column, as is the case in the Black Sea, where archaea and bacteria have been detected in both anoxic and oxic waters above seep sites (Durisch-Kaiser et al., 2005). Archaeal and bacterial communities are also found to occur consistently in deep marine sediments that are associated with methane hydrates (Inagaki et al., 2006). The recent record of microbial CH_4, from fluid inclusions detected in the low-temperature hydrothermal silica of the early Archean-aged Dresser Formation in the Pilbara Craton, Western Australia (Ueno et al., 2006), is the oldest evidence of the activity of methanogenic microbes at an early (and probably Mars-like) stage of the evolution the Earth's atmosphere.j

Methanotrophic symbiont-bearing macroinvertebrates, such as certain living mussels (Childress et al., 1986), or other taxa with methanotrophic food sources, such as certain polychaete species (Fisher et al., 2000), are strongly depleted $\delta^{13}C$. The strongest depletions (values around −110‰), however, have been measured in lipid biomarkers as archaeol, the most common ether lipid in archaea, hydroxyarchaeol, and crocetane, derived from methanotrophic archaea and other microbial consortia (Hinrichs et al., 1999; Treude et al., 2005). Conversely, in the Gulf of Mexico, the isotopic compositions of lipid biomarkers typifying sulfur-oxidizing bacteria (*Beggiatoa*) associated with gas hydrates (Zhang et al., 2005), and of bulk *Beggiatoa* mats (Sassen et al., 1993), are slightly less than −30‰, and indicate that they used carbon from a non-methane origin. In methane seeps of the Californian continental margin, however, lower values (around −50‰) from *Beggiatoa* mats have been measured (Orphan et al., 2001).

4. Microbial-Induced Fossils, Fabrics and Minerals of Seep Deposits

Primary among the geobiological interactions involved in the preservation of fossils (Bottjer, 2005) are those performed by microbes in a wealth of environmental conditions, whereby they promote mineral dissolution and diagenesis, and authigenic mineral precipitation (Ehrlich, 1998). Microbiologically induced mineralization, whether passively or actively promoted, have profoundly influenced the composition of the lithosphere and the fossil record as a whole. Although still underestimated, the mineral by-products of the interaction between microorganisms and physico-chemical environments (in the sense given by Lowenstam, 1981) have provided the Earth's surface with the most common type of fossils: microbes and microbial-derived structures.

The preservation potential of cold seep ecosystems throughout the geologic record is mostly determined by authigenic carbonate precipitation induced by microbes in anoxic conditions. The resulting complex carbonates contain accumulations of clams, tube worms, brachiopods, and other seep-related megafaunal components, and can also store microbial remains and other products of these

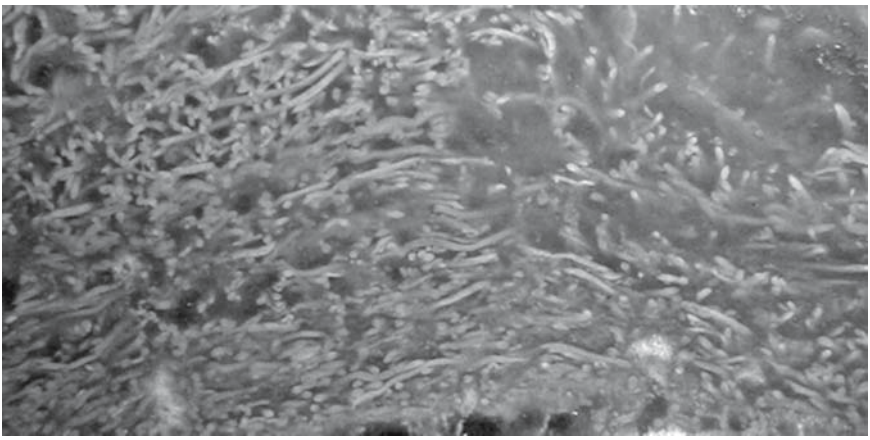

Figure 1. Reflected light micrograph of a polished surface. Large filaments interpreted as giant *Beggiatoa* embedded in a microsparite groundmass from Miocene-aged seep carbonates, northern Italy. Average diameter of the filaments is 90–100 μm.

chemosynthetic ecosystems. A compelling example is the extraordinarily well-preserved mats of giant *Beggiatoa*-type filaments from Miocene-aged seep carbonates of northern Italy (Fig. 1). The preservation of these unique filamentous microbes relies on early, microbially-mediated diagenetic events that produced aragonite cement fringes around the giant filaments (Barbieri et al., 2001; Peckmann et al., 2004; Barbieri and Cavalazzi, 2005).

The groundmass of seep deposits, however, generally consists of authigenic micrite and microcrystalline calcite forming crusts, mounds, and chimneys that are strongly depleted in $\delta^{13}C$. This authigenic micrite may often consist of microbial clotted fabrics (Peckmann et al., 1999, 2001). Other carbonate (high-Mg calcite, aragonite, and dolomite) textures are variously interpreted as the product of microbial activity, they include botryoids, peloids, rhomboids, spheroids, dumbbell-shaped structures in the hollow cores of minerals (Terzi et al., 1994; Savard et al., 1996; Cavagna et al., 1999; Peckmann et al., 1999, 2001; Aharon, 2000; Peckmann and Thiel, 2004; Barbieri and Cavalazzi, 2005). Early diagenesis is often related to the above textures. Radial fibrous, sparry and scalenoedral cements, which are typical early diagenetic products in the development of a seep-carbonate body, have been interpreted as products of microbial processes. Because of their early precipitation, these cements can also favor the preservation of delicate microbial morphologies (Fig. 1).

Morphologies interpreted as mineralized biofilms have been described in Cenozoic seep deposits, and include the coating of the dolomitic matrix (Peckmann et al., 1999) and membranous structures incorporating mineral and clastic grains (Shapiro, 2004; Barbieri and Cavalazzi, 2005). Their negative $\delta^{13}C$ values further enable an interpretation as biofilms produced by methanotrophic bacteria.

Stromatolite morphologies may be present in fossil (Kelly et al., 1995) and modern seep (Greinert et al., 2002) ecosystems. Submillimeter-scale microcolumnar buildups have been described as microstromatolites in the laminated infill of veins crosscutting the Devonian mounds of Hamar Laghdad (Anti-Atlas, Morocco), where $\delta^{13}C$ values suggest relationships with hydrocarbon-rich fluids (Cavalazzi et al., 2007).

Apart from the case of the giant *Beggiatoa* mentioned above, preserved bacterial cells and colonies are comparatively rare in seep deposits. Bacteriomorphs as cocci, rods, and filaments are limited to rock portions in which early carbonate (or other mineral) precipitation has allowed their preservation. Cocci and rods have been described in a Pliocene seep of northern Apennine (Cavalazzi and Barbieri, 2006) and in the Cretaceous Tepee Buttes, Colorado (Fig. 2) (Shapiro, 2004), whereas intertwined and closely packed filamentous structures make up well-developed microbial mats in the Devonian mounds of Anti-Atlas, Morocco (Fig. 3) (Cavalazzi, 2007; Cavalazzi et al., 2007).

Bacterial clusters are often coated by amorphous membranes, which are interpreted as EPS (extracellular polymeric substances). The EPS are more frequently recovered as fossils than cells by themselves. The higher fossilization potential of EPS biofilms results from their abundance (cell colonies are usually embedded in protective biofilms that greatly stabilize the microbial environments) and a better resistance to degradation than single cells. The high affinity of EPS for calcium (and other) ions should also favor mineralization, and consequently, calcium carbonate precipitation (Altermann et al., 2006).

Together with generic biofilm textures, more architecturally complex structures have been described from fossil seeps. In the Silurian hematite-rich carbonate

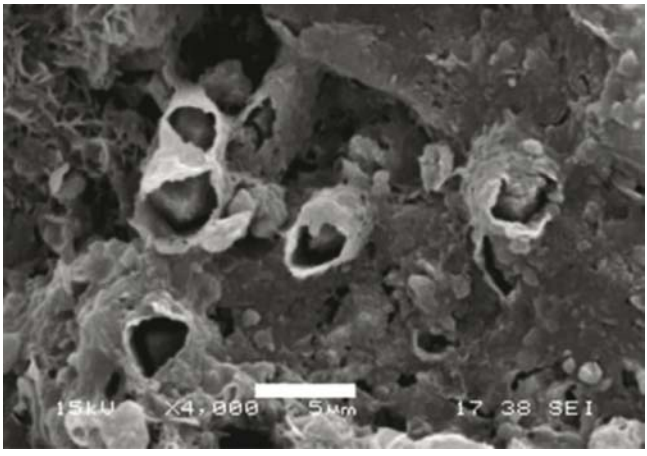

Figure 2. Scanning electron microscope (SEM) image of fossilized sheaths containing calcite crystals in the centers from Cretaceous seep carbonates, Colorado. (Image reprinted with permission from Shapiro, 2004.)

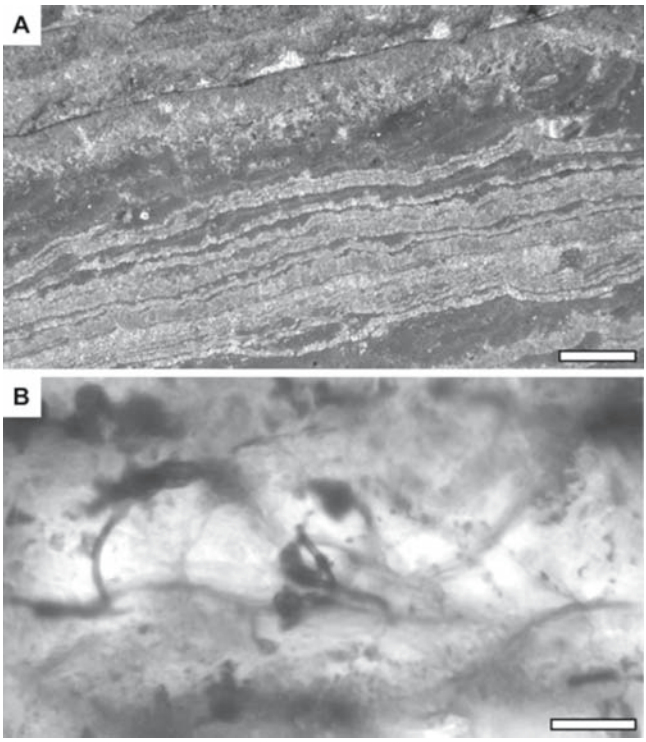

Figure 3. Transmitted light micrographs from a petrographic thin section of laminated carbonate infilling veins cutting a Devonian-aged paleoseep, Anti-Atlas, Morocco. (A) Stromatolitic fabric with thin interlayers made up of intertwined filaments. Scale bar: 5 mm. (B) Details of filaments with an average diameter of 3–5 µm. Scale bar: 30 µm. (Images reprinted with permission from Cavalazzi et al., 2007.)

deposits near Khenifra, Morocco, packed with the remnants of the most ancient-known seep derived ecosystem (Barbieri et al., 2004), early mineral (hematite) replacement let to the preservation of complex, three dimensional structures (Fig. 4) that are interpreted as the product of *Beggiatoa*-like mats. Morphological analogues of these microbially induced alveolar textures have been described from Pliocene-aged, seep carbonate accumulations (Barbieri and Cavalazzi, 2005), and other fossil settings, such as the Carboniferous-aged Panther Seep Formation in New Mexico (Chafetz et al., 1993), and the stromatolites of the Miocene-aged Monterey Formation in California (Williams, 1984). The modern analogues include microbial mats of modern lagoons (cyanobacteria) (Bauld et al., 1993; Sprachta et al., 2001), and deep marine settings (sulfide-oxidizing chemotrophic *Beggiatoa*) (Williams and Reimers, 1983). Because of their recurrence through time in microbially derived systems that were developed in different environmental settings, along

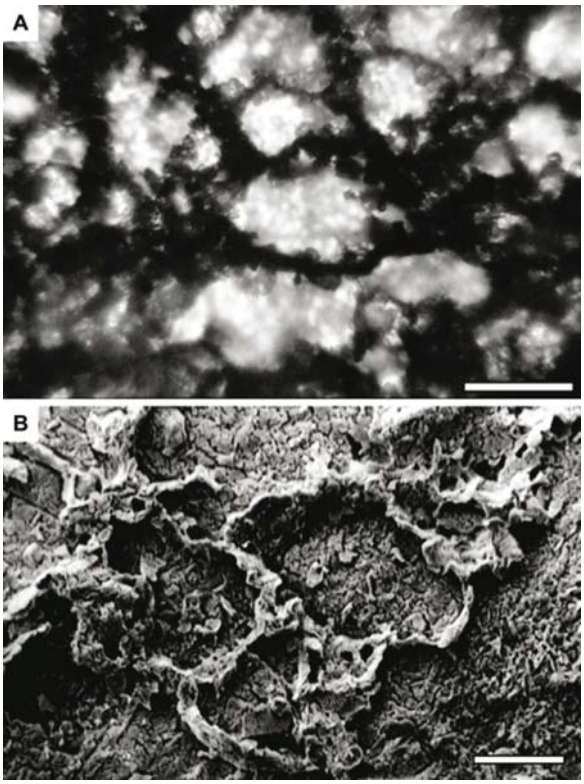

Figure 4. Three dimensional alveolar structures of inferred microbial origin from Silurian seep carbonates, central Morocco. The frame is made up of hematite and the infill is calcium carbonate. (A) Transmitted light micrograph. Scale bar: 10μm. (B) Scanning electron microscope (SEM) image of HCl-etched rock surface. Scale bar: 10μm. (Image B reprinted with permission from Barbieri et al., 2004.)

with their fossilization potential, these alveolar morphologies deserve a remarkable amount of interest.

Useful tracers for documenting the relationships between methane seeps and microbes in the geologic record are lipid biomarkers (chemofossils). In fossil seeps these complex organic compounds include $\delta^{13}C$-depleted archaeal isoprenoids, hopanoids, and non-isoprenoidal lipids (Peckmann and Thiel, 2004) derived from contributions by various microbiotic sources.

Sulfide minerals, especially authigenic pyrite, are distributed within microfractures and intergranular spaces (Cavagna et al., 1999), scattered in seep-carbonates and locally forming pyrite rims across the length of corroded surfaces (Peckmann et al., 2001; Campbell et al., 2002). Pyrite occurs as grains, rods, palisade-like, regular framboids (from a few micrometers to several tens of microns), concentric layers, and represents a precipitation product related to the metabolism of sulfate

reducing bacteria in anoxic seep sediments (Cavagna et al., 1999; Peckmann et al., 2001; Peckmann and Thiel, 2004; Sassen et al., 2004; Chen et al., 2006). Other iron sulfides, such as the ferromagnetic greigite, accumulate by anaerobic magnetotactic bacteria in methane-derived carbonates and associated microbial ecosystems (Reitner et al., 2005).

Barite, arranged in banded or layered, porous deposits, is another important, although rare, seep-related authigenic mineral. It occurs as a concretion, mound, and chimney (Koski and Hein, 2003), and represents a useful record for documenting past and present seep activity. The amount of barite precipitation depends on the seepage intensity, on the available sulfate and barium, and on biogeochemical processes governed by the microbial oxidation of methane (Aloisi et al., 2004).

Bio-induced iron minerals, such as hematite, may provide a characteristic reddish pigmentation in the micritic seep deposits (Barbieri et al. 2004) as a possible consequence of the mineral dispersion of this Fe oxide, previously accumulated via intracellular biomineralization, by bacterial cell lysis.

5. Astrobiological Perspectives

5.1. MINERALOGY OF MARS AND THE PROBLEM OF CARBONATES

The question of the type of elements and minerals comprising the surface of Mars is of extreme interest for the reconstruction of the geological evolution of the planet (Baker, 2006). The mineral composition of the Martian surface may provide clues to deciphering the question of possible life on Mars. The recent missions of the NASA's exploration rovers *Spirit* and *Opportunity* and ESA's *Mars Express* have produced a wealth of new data about its surface composition by using a variety of instruments, including different types of spectrometers (e.g., Christensen et al., 2004a, b; Bibring et al., 2005). The most exciting discovery of astrobiological interest is the mineralogical evidence for the (past) presence of water on the Mars surface, provided by hematite-rich grains and hydrated sulfate minerals (gypsum, jarosite, and kieserite) of a possible evaporite origin (Gendrin et al., 2005). From an astrobiological point of view, it is somewhat disappointing that carbonates have thus far only been detected in low abundance, with concentrations of approx. 1 weight% in several martian meteorites (Gooding, 1992) and 2 to 5 weight% in the martian dust (Bandfield et al., 2003). Based on our terrestrial experience, calcium carbonate is common biological byproduct and its paucity, combined with high sulfur content in surface rocks and soils, is explained by the presence of acidic waters, such as possible ancient martian oceans with acidic environments (Fairén et al., 2004). They may have prevented carbonate accumulation. Small carbonate concentrations, however, may also have other explanations, such as ultraviolet photodissociation and acid-fog weathering (Bandfield et al., 2003). Mars might also have had a thicker and denser CO_2 atmosphere during the

Noachian era (about 4 billion years ago), which has been stored as carbonate accumulations (Kahn, 1995). Apart from basin-wide sedimentary processes, carbonate-bearing rocks might be present on Mars as the product of local processes, as in hydrothermal precipitation (Griffith and Shock, 1995), and perhaps might not have been detected remotely yet. Hydrothermal carbonates are plausible as geological products when considering the presence of effusive vulcanite on Mars that may have produced geyser and hot spring phenomena (Walter and Des Marais, 1993). In summary, although only small amounts of calcium carbonate have been detected in martian dust and meteorites, perhaps larger carbonate accumulations will be discovered by subsurface investigations.

Most published scientific research papers agree on the abiotic nature of the bacteriomorphs from the carbonate globules described in the famous martian meteorite ALH84001 (McKay et al., 1996), however, these globules deserve some interest since they could have precipitated as a consequence of early aqueous processes on Mars (Scott, 1999; Kargel, 2004). The zoned organization of the globules is indicative of compositional variations and resembles the zoning that characterize rhombs and spheroids described from Mg-rich, terrestrial cold seep carbonates (Cavalazzi and Barbieri, 2006), as a consequence of ambient chemistry changes. Since seep carbonates can entrap micrometer-scale fluid inclusions during precipitation, direct information on ambient fluids (including methane) and organic molecules, if any, might be gained through this type of rock (Parnell et al., 2002).

5.2. METHANE ON MARS (AND ELSEWHERE)

Most of the terrestrial methane has a biogenic origin that is derived from the activity of methanogenic archaea (Kvenvolden and Rogers, 2005). Marine and continental clathrates (also known as gas hydrates) stock the largest methane amounts ever detected, which probably originate through abiotic (thermal) and biotic (microbial) processes (Kvenvolden, 1993), in which the microbial decomposition of organic material by deep-earth methanogens leads to the production of methane gas. Methane release from clathrates in the atmosphere through time depends on their dissociation, which is driven by fluctuations in the sea level and bottom water temperature (Dickens, 1999, 2003) with environmental effects registered in the sedimentary record. On Mars, unlike on Earth, vast deposits of clathrates are believed to occur as carbon dioxide clathrates because of the abundance of atmospheric CO_2 and H_2O, both in the atmosphere and beneath the surface in the form of water ice (Hansen et al., 2005). CO_2 clathrates would be present, therefore, especially at the south polar surface, in a way similar to the terrestrial clathrate-bearing permafrost (Kargel and Lunine, 1998; Kargel, 2004). The recent detection of methane in the martian atmosphere, obtained using Earth-based spectrometers and the spectrometer onboard the ESA's Mars Express spacecraft (Formisano et al., 2004; Krasnopolsky et al., 2004; Mumma et al., 2005),

has redoubled the interest for possible methane-related biological activity on Mars and other bodies of the solar system, such as Europa and Titan. Although methane is a potential tracer of biological processes, on Mars it would also have been formed by other (abiotic) processes, including magmatic/hydrothermal outgassing and cometary/meteorite impacts (Wong et al., 2003; Krasnopolsky et al., 2004). Recently, the near surface hydration of olivine and pyroxenes (serpentinization) has also been proposed as a possible way for the martian abiotic production of methane (Oze and Sharma, 2005). A similar process of methane formation has been described through the reaction between upper mantle rocks (serpentinized peridotites) and ocean waters in submarine hydrothermal fields (Kelley et al., 2001). Regardless of the source of martian methane, however, its supply is assumed to be continuous or very recent because of the limited residence time of methane in the atmosphere (evaluated at about 340 years) due to the photolytic breakdown by the UV radiation (Krasnopolsky et al., 2004). The detected methane and water vapor association in the atmosphere of Mars and the large variations of methane abundance that changes with the location are both potential indicators of possible releases from clathrate destabilization (Formisano et al., 2004; Max and Clifford, 2005), if any is present. Such a subsurface, possibly deep source of methane, is compatible with the hypotheses of autotrophic methanogenesis that was potentially developed by chemosynthetic ecosystems on Mars and Europa (Boston et al., 1992; McCollom, 1999). In ice-covered planetary bodies, such as Europa and Callisto, methanogenesis and the reduction of sulfur compounds has been hypothesized as a possible, although unlikely, energy source for life (Gaidos et al., 1999). The above scenarios necessarily require that ancient or still extant microbial life in the subsurface environments of Mars (and other bodies) have stored methane for later release. A possible origin of the martian methane from an ancient (extinct) biosphere is debated. The supporters of a martian subsurface biosphere – similar to the one established on Earth, for example, by methanogenic archaea in permafrost (Wagner et al., 2002) – speculate on the presence of methanogens in permafrost aquifers and of methane and carbon dioxide clathrates that would have stored fossil methane that was developed on early Mars (Pellenbarg et al., 2003; Max and Clifford, 2005). Other estimates, based on expected low biomass production by methanogenesis (Krasnopolsky et al., 2004), assume that methane on Mars cannot originate from an ancient biosphere. Whether it formed biotically or abiotically, this does not inhibit the possibility of methane to be the fuel of biogeochemical processes that consume methane anaerobically as in the terrestrial cold seeps.

The present-day harsh conditions of the martian regolith, due to factors such as intense cosmic radiation, dryness, and daily temperature excursion, combined with geothermal gradient and surface gravity that is much lower than terrestrial ones, might potentially favor a deep biological activity at depths down to 10–12 km (Kargel, 2004). In the case that it derives from mechanisms that are related to seepage, such a deep biosphere can in fact reach surface (or near

surface) locations, with detectable localized spots that are similar to the ones described on Earth. The interest in seep mechanisms, together with the obvious relationship with methane, relies on the variable methane concentrations detected from localized spots in the terrestrial methane seeps, which apply to methane as is detected on Mars. Moreover, low-temperature minerals of seep deposits can contain fluid inclusions in which methane might be preserved and (assuming with the adequate analytical techniques) detected.

6. Conclusion

The recent discovery of methane in the martian atmosphere, coupled with unequivocal evidences of the activity of liquid water on its surface, has revived interest in the possibility of life on Mars. Methane seep carbonates, that are known in the geological record since the Palezoic and precipitate in modern continental slopes, can be considered a prime astrobiological target because (i) they are chemically precipitated deposits with the advantage of preserving potential traces of life; (ii) they represent good repositories for microbiologically derived features; (iii) in terms of size they may develop different types of geological bodies that are often recognizable as anomalous deposits in the rock record; (iv) they have obvious and detectable relationships with methane. Well-developed microbial features include morphologies, biominerals, chemofossils, and stable isotope signatures. The genesis and patchy distribution of the terrestrial methane seep deposits agree with the distribution of methane as has been recently depicted on the surface of Mars. The paucity of carbonate-rich sediments found to date on the martian surface, as well as the lack of carbonate outcrops, do not appear to be major limiting factors for considering methane seep carbonates a target for astrobiology. For example, one would expect greater amounts of carbonates in older (and therefore buried) successions, for which only a drilling exploration would provide solid information. Also, a patchy distribution for martian carbonates cannot be excluded *a priori* at the present stage of knowledge, and it might imply limitations for a clear detection of these mineral components based on remote sensing instruments, with negative consequences for field recognition and sampling during future landing missions. Future issues in a thorough investigation of methane seep ecosystems and their geological products should therefore consider their potential as possible terrestrial analogues for methane-derived deposits on Mars. For this purpose, the integrated investigation of the microbiological-mineral interactions of methane-seep limestones associated with the assessment of their spectral and geochemical characteristics would set the base for the identification of martian outcrops. This approach can be best performed by investigating extant seeps where the influence of microbial processes on the precipitation can be directly established. In addition, similar comparative studies extended to the fossil counterparts can establish a link with unique, ancient textures containing microbial imprints.

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Biodata of **Nunzia Stivaletta** and **Roberto Barbieri,** authors of the chapter ***"Endoliths in Terrestrial Arid Environments: Implications for Astrobiology"***

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ENDOLITHS IN TERRESTRIAL ARID ENVIRONMENTS: IMPLICATIONS FOR ASTROBIOLOGY

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Abstract Microbial life in hot and cold desert environments inhabits endolithic niches. The endolithic microorganisms include bacteria, fungi and lichens. To protect themselves from the inhospitable conditions, such as high UV radiation, dryness, and rapid temperature variations, microorganisms migrate into fractures or in pore spaces where the necessary nutrient, moisture, and light are sufficient for survival. Examples of endolithic communities are well documented from the Negev Desert, Antarctica and the Artic regions, and the Atacama Desert. The most common substrates are porous, crystalline sandstones with calcium carbonate cements and sulfate (gypsum) and other evaporite mineral crusts. The detection of sulfate on the Martian surface has sparked off considerable interest in the astrobiological potential of the evaporite deposits of continental environments, which may potentially host (or may have hosted) endolithic microorganisms.

1. Introduction

Fossil endoliths are known in the rock record back to the Late Proterozoic. The oldest example of fossil endoliths occurs in silicified pisoids of the Eleonora Bay Formation in East Greenland that contain organically preserved cyanobacteria resembling *Hyella gigas* (Campbell, 1982; Knoll et al., 1986). Wierzchos and Ascaso (2002) for the first time documented the fossilization of cryptoendolithic microfossil communities in sandstones from a cold desert environment, the Ross Desert on Antarctica. Since extremely cold and dry sites may be considered terrestrial environmental analogues of Mars, the endolithic communities have remarkable astrobiological significance and can be expected in Martian surface and rocks (Wynn-Williams and Edwards, 2000; Wierzchos and Ascaso, 2002), in which strong limiting factors for life include the instability of superficial liquid water and the intense solar (UV) radiation.

Lithobionthic microbial life in terrestrial ecosystems can flourish on the rock surface (epiliths), at the rock-soil interface (hypoliths) and inside the rocks (endoliths). The endolithic mode of life includes several different ecological niches: chasmoendoliths live in cracks or fracture in rocks, euendoliths penetrate actively soluble carbonate and phosphate substrates and cryptoendoliths occupy

pre-existing fissures and structural cavities in the rocks, such as the pore spaces between grain boundaries or spaces produced and vacated by euendoliths (Golubic et al., 1981). Endolithic strategies are performed by bacteria, fungi and lichens. Some microorganisms are partially epilithic and partially endolithic (e.g. lichens), whereas others penetrate carbonate substrates, as euendoliths, and colonize preexisting structural cavities (Golubic, 1981). Endolithic and hypolithic microorganisms inhabit regions where high ultraviolet radiation, aridity, and huge daily temperature range typify the environment, such as in deserts. In such inhospitable conditions, the endolithic microorganisms migrate into fractures or in pore spaces where the necessary nutrient, moisture, and light are sufficient for survival. Examples of endolithic communities have been described from the Negev Desert (Friedmann et al., 1967), Antarctic (Friedmann and Ocampo, 1976) and Artic regions (Omelon et al., 2006a) and the Atacama Desert (McKay et al., 2003; Wierzchos et al., 2006).

2. Microbial Life in Arid Environments

In arid environments microorganisms must be able to withstand rapid desiccation and high levels of ultraviolet radiation. The Atacama Desert (Fig. 1), the driest desert on Earth, represents a paradigmatic example that offers the possibility of testing the ability of microorganisms to survive in extremely dry conditions (Dose et al., 2001; Navarro-Gonzãles et al., 2003; McKay et al., 2003; Maier et al., 2004; Drees et al., 2006; Warren-Rhodes et al., 2006; Wierzchos et al., 2006; Connon et al., 2007). The only inhabitants in hypersaline continental lakes of arid environments, such as sabkhas, are extremely halophilic organisms, including halophilic Archaea (halobacteria), halophilic cyanobacteria, and green algae (Grant et al., 1998). Invertebrates, such as the brine shrimp *Artemia salina* may locally be abundant. When waters approach saturation (30% NaCl), however, halobacteria, the most halophilic biota (Rodriguez-Valera et al., 1981), dominate.

In sites with limited or scarce water the microorganisms live either inside porous rocks (endolithic mode life) or below the translucent rocks partially embedded in the soil (hypolithic mode life), where they take advantage of the standing moisture just below the surface or between single grains or minerals. The extracellular polymeric substances (EPS), which are important for cell aggregation and protection, have also a beneficial role by reducing water loss of single cells (Potts, 1994; Gerdes, et al., 2000). Besides tolerating the desiccating conditions and extreme temperatures, microorganisms inhabiting mineral soils of hot and cold deserts are subjected to osmotic stress due to the high salt concentrations from accumulated sodium, calcium, magnesium, chloride, sulfate and nitrate; to prevent the loss of cellular water under high osmolarity in hypersaline conditions, halophiles generally accumulate high solute concentrations within cytoplasm (Madigan et al., 2003). The UV radiation flux of arid environments can also be lethal for microorganisms. Microbes are vulnerable to radiation, particularly at an

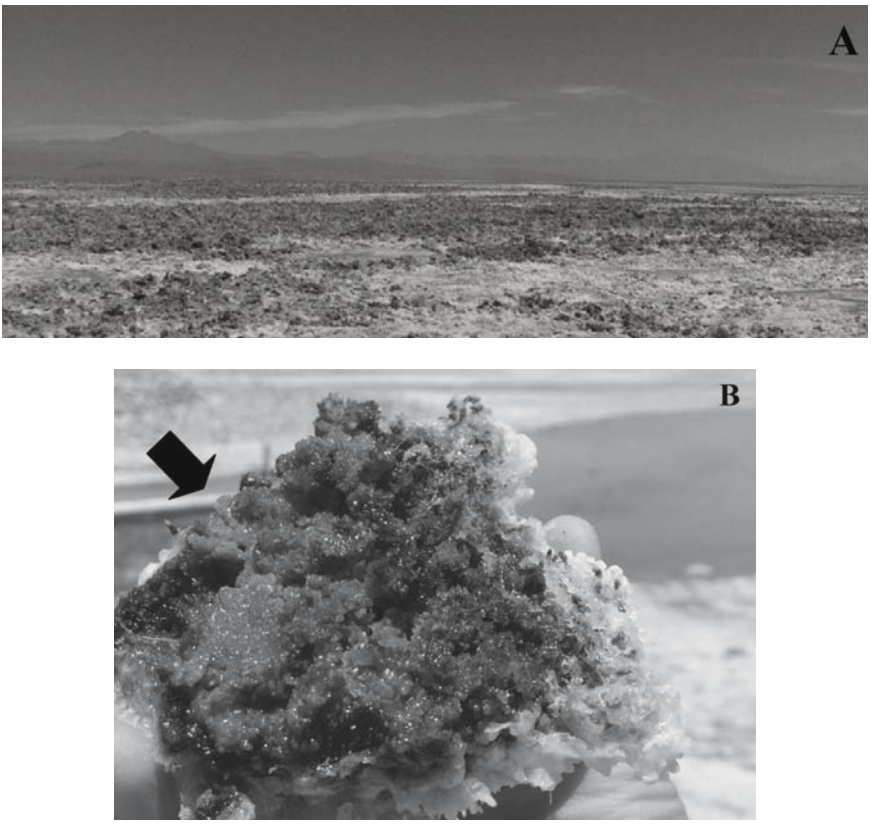

Figure 1. Atacama Desert (Chile). (A) Panoramic view of Laguna Chaxa (Salar de Atacama). Note the salt crusts due to the intense evaporation of the ephemeral water. (B) Endolithic microorganisms (arrow) beneath a salt crust in Laguna Chaxa.

early growth stage, when screening pigments have not yet been developed. These pigments help protect cells from the chemical damage of proteins, DNA and membranes (Cockell, 2000). For example, carotenoids (e.g., *B*-carotene) absorb the UVA (320–380 nm) and the UVC radiations range (<280 nm), which are lethal to DNA that absorbs at 254 nm (Wynn-Williams et al., 2002).

In the Yungay area, located in the driest belt of the Atacama Desert, evaporite (halite) minerals have been recently found colonized by cyanobacteria identified as *Chroococcidiopsis* morphospecies and associated heterotrophic bacteria (Wierzchos et al., 2006).

This colonization is selective and seems to be dependent on the mineral composition of the particles that make up a given surface. In the case of halite, colonization occurs just a few millimeters beneath the surface between distinct halite crystals. The hygroscopic nature of halite, which retains water when the relative

humidity of air is more than 70–75% (Wierzchos et al., 2003), appears to sustain microbial colonization. If the surface consists of quartz grains, however, no colonization has been observed beneath and between grains, as reported by McKay et al. (2003) on the basis of four years of climate observations in the Atacama Desert.

2.1. ENDOLITHIC LIFE IN COLD DESERTS

In spite of their limited water availability, cold temperature, strong winds, and large variations in solar radiation input, cold deserts harbour endolithic microorganisms. Antarctica is characterized by extreme climatic conditions, with low humidity and precipitation during the winter (<10% RH; relative humidity) (Horowitz et al., 1972), which make the continent relatively inhospitable for the development of biological communities. In proper niches, however, microbial life can thrive and endolithic microbial communities have been intensively studied in the Antarctic region (Friedmann and Ocampo, 1976; Friedmann, 1982; Friedmann et al., 1988; Nienow et al., 1988a, b; Banerjee et al., 2000; Wierzchos and Ascaso, 2001, 2002; Ascaso and Wierzchos, 2002, 2003; de los Rios et al., 2003, 2004; de la Torre et al., 2003; Hughes and Lawley, 2003; Wierzchos et al., 2004, 2005; Villar et al., 2005).

Endoliths occupy habitats beneath and between porous and translucent rocks and minerals (Fig. 2). Rock porosity provides interstitial spaces for microbial colonization and translucence enables photosynthesis to take place (Friedmann, 1982).

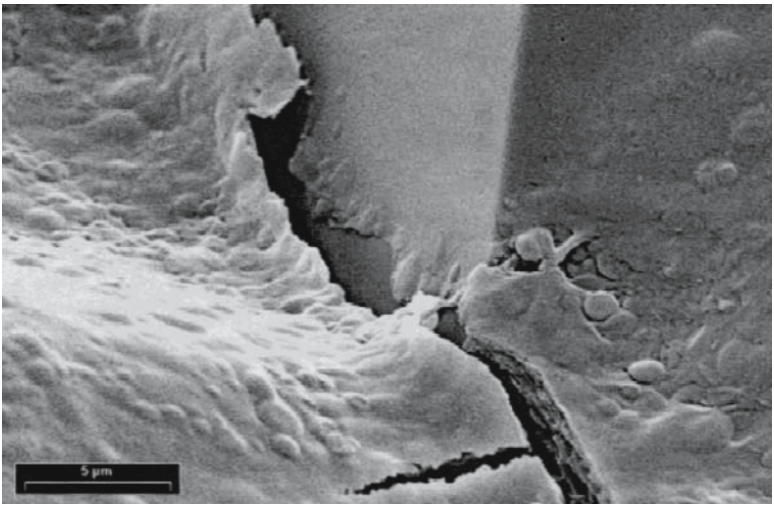

Figure 2. Scanning electron (SEM) micrograph of Beacon Sandstone, McMurdo Dry Valley (Antarctica), showing cells of Chroococcidiopsis embedded in an exopolysaccharide matrix on quartzite crystal. (Image reprinted with permission from Banerjee et al., 2000.)

Friedmann (1980) reported that the water content of sandstone colonized by endolithic microorganisms is represented by 0.1–0.2% by weight. Because of the low atmospheric humidity, much of the snow sublimes with little moisture penetrating the upper soil horizon (Cowan and Ah Tow, 2004). As calculated by Friedmann et al. (1987), have estimated that metabolism is possible in endolithic microbial communities for less than 1,000 h per annum, based on the assumption that the lower limit for endolithic metabolism is between −6°C and −8°C (Vestal, 1988).

Friedmann and Ocampo (1976) first reported the presence of endolithic communities in the pore space between quartz grains of Beacon Sandstone in the Dry Valley (Antarctica). Cryptoendoliths have been originally studied by microscopy and laboratory culture methods (Friedmann et al., 1988; Hirsch et al., 1988; Siebert and Hirsch, 1988; Siebert et al., 1996), and only recently their study has been combined with phylogenetic tools (de la Torre et al., 2003). Friedmann et al. (1988) identified two dominant community types of endoliths: lichens (fungal hyphae with the green algae symbiont *Trebouxia*) and cyanobacteria (*Chroococcidiopsis* or *Gleocapsa* species). Refractory to cultivation, most of these autotrophs have been mainly described by morphology (Friedmann et al., 1988). Studies of heterotrophic bacteria associated with lichens were instead based on laboratory cultivation (Hirsch et al., 1988; Siebert et al., 1996). Microscopy studies performed *in situ* have documented the presence of microbial fossils within Antarctic sandstone rocks in the McMurdo Dry Valleys Desert, where scanning electron microscope techniques enabled the identification of living and decaying endolithic communities (Ascaso and Wierzchos, 2002; Wierzchos and Ascaso, 2002). A phylogenetic study based on the analysis of 1,100 individual 16SrDNA clones of lichens and cyanobacteria (de la Torre et al., 2003), has documented that clones fell into 51 groups (phylotypes) with >98% rRNA sequence identity (46 bacterial and 5 eucaryal). In the lichen-dominated community, three phylotypes accounted for over 70% of the clones: the fungus *Texosporium sancti-jacobi* (29%), the green algae *Trebouxia jamesii* (22%) and a chloroplast related sequence (22%). In the cyanobacteria-dominated community, cyanobacterial phylotypes (mostly belonging to the *Leptolyngbya-Phormidium-Plectonema* group) make up over 30% of clones sequenced. Heterotrophic bacteria phylotypes represented nearly 60% of the tested clones, falling in two major groups: the α-proteobacteria and the *Thermus-Deinococcus* group.

In the Antarctic rocks traces of past life have been reported in form of geophysical and geochemical bioweathering patterns (Friedmann and Weed, 1987; Sun and Friedmann, 1999). The surface of the Beacon Sandstone (Beacon Supergroup, Victoria Land) shows characteristic pattern of exfoliative weathering caused by the oxalic acid secreted by microorganisms that colonized the porous rocks. This weak acid can leach the iron compounds coating the quartz crystals and produce a snow-white zone. The weathering process causes exfoliation and loss of biomass. After an exfoliation event the microorganisms grow deeper into the rock and a new siliceous crust forms on the rock surface. The alternating

processes of crust formation and exfoliation produce a characteristic mosaic with several millimeters deep relief. The formation of trace fossils of microbial colonization can therefore be preserved in the geological record. Wierzchos et al. (2003) have documented that some minerals of Antarctic rocks are biologically transformed, such as the Fe-rich biogenic minerals in the form of iron oxyhydroxide nanocrystals, whereas biogenic clays are deposited around chasmoendolithic hyphae and bacterial cells.

In the Arctic region a great microbial diversity has been documented without any documented biomineralization (Omelon et al., 2006a, b). Such lack of evidence contrasts with the remarkable data collected from similar environments of the Antarctic Dry Valleys because of the warmer and wetter conditions of the Arctic summer period, with consequent longer periods of metabolic activities. Erosion rates, however, might be responsible for habitat destruction, as there may not be enough time for biomineralization (Omelon et al., 2006a, b).

2.2. ENDOLITHIC LIFE IN HOT DESERTS

Cryptoendolithic algae from hot, semiarid lands and deserts were first described from the Negev Desert (Friedmann et al., 1967). Cryptoendoliths of hot desert were then documented in North America (Friedmann, 1972; Bell et al., 1983, 1986) and South Africa (Critchley et al., 1987). The most common substrate are porous, crystalline sandstone and, less often, limestone. The first report of hot desert cryptoendoliths identified the coccoid cyanobacteria *Chroococcidiopsis* and *Gleocapsa* (Friedmann, 1971, 1980). Bell et al. (1986) and Critchley et al. (1987) revealed a rich flora including chlorophytes. Green algae are usually represented by coccoids or sarcinoids, in which *Coccomyxa*, *Fasiculochloris* and *Friedmannia* prevail. Tests on the effect of water stress in *Chrooccoccidiopsis* and *Chroococcus* cells isolated from rocks revealed that *Chroococcidiopsis* strains were not particularly resistant to low water potential, whereas desiccated cells of *Chroococcidiopsis* restarted photosynthesis within five minutes after rewetting (Potts and Friedmann, 1981). The tolerance to desiccation showed by these microorganisms and their ability to quickly activate their metabolism in response to sufficient liquid water or vapor concentration enables survivorship in arid environments. The cryptoendolithic microhabitat may provide sufficient moisture for the survival of microorganisms. Most of the green algae react to rewetting with rapid production of a motile phase with consequent expansion of the colonized area (Bell, 1993). Friedmann and Galun (1974) pointed out that the increased thermal load of the rocks could probably lead to frequent dew formation on the rock surface. This water may be taken via capillary action. Water may not stay in liquid state, but once inside the rock matrix, it may be retained in the form of vapor and, in conjunction with the lack of convection within the rock, loss to the outside can be delayed. Desert lichens can use atmospheric vapor (>80% RH, relative humidity) as a water source (Lange, 1986). Generally, the ability to utilize water vapor varies

widely for green algae and cyanobacteria (Lange et al., 1986). For example, cryptoendolithic cyanobacteria from the sandstones of the Negev Desert (Potts and Friedmann, 1981; Palmer and Friedmann, 1990) photosynthesize only at very high matric water potential (>6.9 Mpa, 90% RH, relative humidity, at 20°C).

The most crystalline sandstones contain calcium carbonate as a crystal cementing material and it is likely that endoliths solubilize this material. The solubilization of rock cementing materials, freezing and thawing, and possibly pressure exerted by algal growth within the rock air spaces may contribute to exfoliation of rock (Friedmann and Ocampo-Friedmann, 1984; Bell et al., 1986). Once exposed, the algae probably dry and blow away. In case of available liquid water before they die under surface conditions, they might be drawn into the rock matrix.

Sulfate minerals in the form of gypsum crusts can be common in arid environments, such as salt lakes. Translucent gypsum crusts can host endolithic communities (Fig. 3) and retain microbial signatures (Oren et al., 1995; Douglas and Yang, 2002; Hughes and Lawley, 2003; Dong et al., 2007; Ionescu et al., 2007). Gypsum and other sulfates can provide microenvironments that protect these microorganisms from exposure to extreme temperature, UV flux, and desiccation, yet they are sufficiently translucent to allow the photosynthesis (Friedmann, 1982).

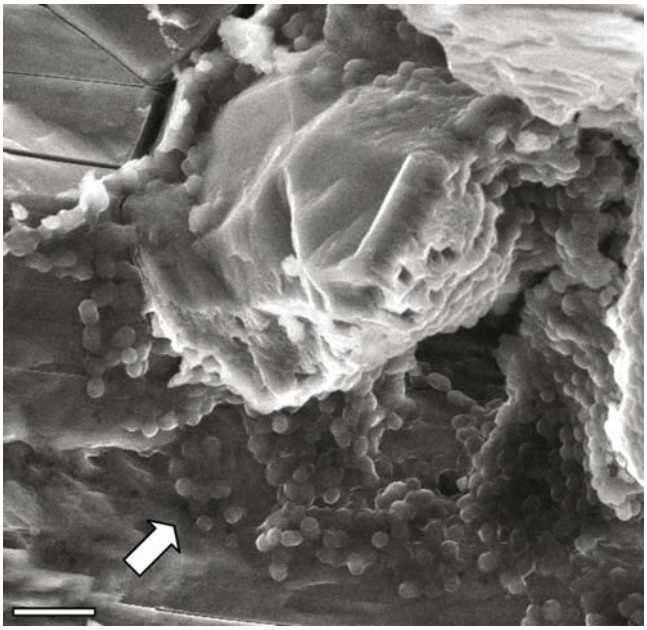

Figure 3. Environmental scanning electron (ESEM) micrograph of endolithic microorganisms in a gypsum crust along the border of the Chott el Jerid (Southern Tunisia). Endolithic bacterial cells embedded in extracellular polymeric substances (arrow). Scale bar: 10 ∝m.

The rapid sealing provided by the evaporite precipitation in arid and saline areas, such as continental and coastal sabkhas, seem to be a useful condition for testing the preservation potential of microbial signatures in the geological record (Barbieri et al., 2006). Because of their wide diffusion, evaporites can be ideal for the reconstruction of types of microbial signatures and their preservation over geological times. Evaporite minerals have also the advantage of preserving fluid inclusions that can retain information of the environment of mineral precipitation, as well as biotic remains. In inclusions from Permian halite cements sampled in the Salado Formation (New Mexico), for example, spores of the halotolerant bacterium *Virgibacillus* have been recovered (Vreeland et al., 2000; Satterfield et al., 2005).

3. Endoliths as Models for the Search of Martian Life

In environments characterized by harsh conditions, such as in hot and cold deserts, several studies have shown the survival of microorganisms in endolithic niches (Friedmann, 1967; Palmer and Friedmann, 1990; Potts and Friedmann, 1981 Nienow et al., 1988a,b; Vestal, 1988; Oren et al., 1995; Wierzchos and Ascaso, 2001, 2002; Hughes and Lawley, 2003; Onofri et al., 2004; Wierzchos et al, 2006; Dong et al., 2007; Pointing et al., 2007; Walker and Pace, 2007). Because the Antarctic Dry Valley and the Atacama Desert are considered the most hostile lithic environments for the microbial life on the Earth surface (Dose et al., 2001), the recovery of cryptoendolithic microorganisms in rocks from both these regions is a compelling example of survival of microorganisms in extremely dry conditions. These environments are therefore considered to be a good terrestrial analogue for Mars (McKay et al., 1992; Navarro-Gonzãles et al., 2003). Cryptoendolithic mode life might occur in Mars environments when the Mars surface became progressively drier and colder (Friedmann and Koriem, 1989).

Recent data from the European Space Agency's Mars Express and NASA's Mars Exploration Rover missions have documented the presence of hydrated sulfate deposits on the surface of Mars (Squyres et al., 2004; Vaniman et al., 2004; Langevin et al., 2005) in environmental settings interpreted as analogues to terrestrial continental sabkhas (McLennan et al., 2005; Gendrin et al., 2005). Furthermore, halite has been identified in mineral assemblages of SNC meteorites arising from Mars (Gooding, 1992; Bridges and Grady, 2000; Sawyer et al., 2000; Treiman et al., 2000) suggesting that the evaporite rocks might be relatively common on Mars surface. This finding has sparked off considerable interest in the astrobiological potential of the evaporite salts (Krumbein et al., 2004; Barbieri et al., 2006; Wierzchos et al., 2006), which can be considered a good target for the investigation of terrestrial analogues of Martian environments. Evaporite mineral precipitation can provide protection from cosmic radiation and allow certain life forms, for example dormant bacterial spores, to survive in salt fluid inclusions for calculated time intervals of more than 100 million years

in terrestrial and Martian conditions (Kminek et al., 2003). Inhabitants of hypersaline and arid environments include stress resistant microorganisms, such as the cyanobacterium *Chroococcidiopsis*, which has been proposed as pioneer microorganism for the terraforming of Mars (Friedmann and Ocampo-Friedmann, 1995).

The study of Earth's analogues of potential extraterrestrial environments is a prerequisite for astrobiology and planetary exploration. The settlement of how and where life survives in the most extreme terrestrial conditions, as well as the agents involved in its preservation processes and delivery to the fossil record, and lastly, the combined analytical techniques for its study and recognition, are all crucial in the development of strategies for future planetary missions in the search for life. In particular, the Mars Sample Return mission, based on an international collaboration between ESA, NASA, and other space agencies, is planning to bring Martian rock samples back to Earth in the next decade. For a success of this pioneering mission, which is also designed to answering the question of life on Mars, preparatory studies on terrestrial near-surface environments are required. In-depth analysis of the biota with endolithic mode of life, such as the ones that inhabit certain terrestrial extreme environments may help for finding the best techniques for their recognition. Because Martian near ground surfaces have the advantage of easy sample collection, microbial communities with endolithic strategies should have a special interest for astrobiology.

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MAGNETOTACTIC BACTERIA AND THEIR POTENTIAL FOR TERRAFORMATION

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This paper is focused on magnetotactic bacteria and their possible contributions to the terraformation of Mars or other planets. The potential for terraformation is mainly based on their ability to carry out aerobic or anaerobic respiration with either nitrate or ferric iron, to fix carbon dioxide in the dark using the energy released through the oxidation of inorganic chemicals such as thiosulfate, and to use molecular nitrogen for cell growth. Furthermore, the magnetic assisted taxies, could help magnetotactic bacteria in their navigation toward optimum growth conditions, when a magnetic field is present.

1. Introduction

Magnetotactic bacteria (MTB) are prokaryotes whose specific functional characteristic is magnetotaxis, the orientation and swimming along the Earth's geomagnetic field lines (Blakemore, 1975). Magnetotaxis is determined by the presence inside the cell of particles named magnetosomes. The discovery of MTB stimulated interest among microbiologists, physicists, engineers, geologists, chemists (Schüler and Frankel, 1999) and today the subject has become a *bona fide* field of research in microbiology (Bazylinski and Frankel, 2004).

The aim of this paper is to shortly review the present state of research on MTB in order to put forward that the structure and the functions of different MTB should be important in the process of terraformation. This focus on their possible role in the terraformation of Mars or other planets should take into account that other types of microorganisms, for example photoautotrophic and/or extremophiles are already better known as the most important candidates for terraformation (Nienow et al., 1988; Friedmann et al., 1993; Nussinov et al., 1994; Haynes and McKay, 1992; Hiscox, 2000b). However, the so far known particularities of MTB argue that they have potential to participate to the process of terraformation.

Several comprehensive and authoritative reviews on MTB have been published (Blakemore, 1982; Frankel et al., 1998; Mann et al., 1990; Stolz, 1993; Schüler and Frankel, 1999; Bazylinski and Frankel, 2004; Frankel and Bazylinski, 2006; Stephens, 2006; Bazylinski et al., 2007) and their reading is highly recommended for a larger and deeper view on MTB. Terraforming "a process of planetary engineering, specifically directed at enhancing the capacity of an extra-terrestrial planetary environment to support life" (Fogg, 1995) evolved from a science-fiction story to a scientific domain (Sagan, 1961, 1973; Averner and MacElroy, 1976; Badescu, 2005; Jukes, 1991; McKay et al., 1991; Haynes and McKay, 1992; Hiscox and Thomas, 1995; Hancox, 1999; Nussinov et al., 1994; Hiscox, 2000a; Birch, 1992; Fogg, 1989, 1993, 1998; Zubrin and Wagner, 1996; Zubrin and McKay, 1997; McKay and Marinova, 2001; Marinova et al., 2000; Gerstell et al., 2001; Popoviciu, 2006).

2. Magnetotactic Bacteria – General Considerations

2.1. MORPHOLOGY

The morphology of single celled MTB ranges from spirilla, vibrioids, cocci, rods to ovoid. The reports on multi-celled magnetotactic prokaryote come with details about the life cycle of an isolated from a saline lagoon (Rodgers et al., 1990; Keim et al., 2004).

2.2. MAGNETOSOMES

Magnetosomes are intracellular bodies which in *Magnetospirillum* strains (*M. magnetotacticum* or *M. gryphiswaldense*) consist of magnetic iron mineral particles enclosed within a membrane about 3–4 nm thick (Gorby et al., 1988). The size and morphology of magnetic crystals are species specific and uniform within a single cell. For example in *M. gryphiswaldense* the dimension of magnetosomes is around 45 nm (Schüler, 2004). In many MTB strains the iron mineral particle consists of magnetite (Fe_3O_4), whereas in several MTB from marine, sulfidic environments, the iron mineral particles consist of greigite (Fe_3S_4). However, there are few reports concerning MTB that produce both magnetite and greigite (Bazylinski et al., 1995; Kasama et. al., 2006; Lins et al., 2007). The size of magnetite or greigite crystals found in different strains is between 35–120 nm which is within the permanent single-magnetic-domain (SD) size range for both minerals (Butler and Banerjee, 1975).

In the last few years the study of the proteins found in magnetosome membrane created special interest because it was expected that at least some of these proteins would enable the processes of mineral formation of nanocrystals to be regulated by biochemical pathways (Schüler and Baeuerlein, 1998; Schüler and

Frankel, 1999; Matsunaga et al., 2000; Schüler, 2004; Grünberg et al., 2004; Bazylinski and Frankel, 2004; Frankel and Bazylinski, 2006; Tanaka et al., 2006).

2.3. BIOLOGICAL SIGNIFICANCE OF MAGNETOSOMES

The orientation and active swimming of MTB along the Earth's geomagnetic field lines is called magnetotaxis, which is determined both by the presence of magnetosomes and the ability to perform active movements. Dead cells containing magnetosomes also align along the geomagnetic field lines whereas alive and swimming MTB with no magnetosomes do not align. In Earth's geomagnetic field (around 0.05 mT) cells are neither attracted nor pulled towards either geomagnetic pole (Bazylinski and Frankel, 2004). It is proposed that in natural environments magnetotaxis enables the cells to locate and maintain an optimal position in water columns or in sediments, with respect to their main metabolical needs: molecular oxygen and other nutrients (Frankel et al., 1997), all in all allowing them to keep their headings as they swim in the face of the disorienting Brownian buffering by the medium (Bazylinski et al., 2007). Bazylinski and Frankel (2004) claimed that the term magnetotaxis is in fact a misnomer because in contrast with a true tactic response MTB swim neither up nor down a magnetic field gradient. They proposed the term magneto-aerotaxis to better explain the interaction between magnetotaxis and aerotactic sensory mechanisms (Frankel et al., 1997). In this respect, Smith et al. (2006) showed that magnetic, wild-type cells swimming in an applied magnetic field migrate more quickly away from the advancing oxygen than either wild-type cells in a zero field or nonmagnetic cells in any magnetic field. According to the authors, the key benefit of magnetotaxis is an enhancement of the bacterium's ability to detect oxygen, not an increase in its average speed for moving away from high oxygen concentrations. Bazylinski and Frankel (2004) developed the new concept of magnetically assisted taxis, claiming that it is possible and perhaps likely, that there are other forms of magnetically assisted taxis in response to sulfide, redox or light gradients. This is why, in dynamic water columns MTB appear to be associated with peaks in dissolved and particulate iron that were also present at oxycline. Peaks of particulate Fe(III) are often observed immediately above marine chemoclines due to the upward flux of Fe(II) into oxygenated waters (Murray et al., 1995; Zopfi et al., 2001).

Another possible function of magnetosomes is in iron homeostasis and detoxification (Mann et al., 1990). In a mutant of *M. gryphiswaldense* the loss of capability to form magnetosome was accompanied by a higher sensitivity against elevated concentrations of iron in the growing medium (Schübbe et al., 2003). This might be indicative of a contribution of magnetite formation to iron homeostasis and detoxification (Schüler, 2004).

Urban (2000) favors the hypothesis that magnetosomes formation primarily affects the cell density and the cell's response to gravity and that magnetotactic behavior is only a secondary response. He showed that *M. magnetotacticum*

placed in microgravity conditions was impaired in their ability to orient towards the magnets. It has also been speculated that magnetosomes can play a role in redox and pH control (Gorby et al., 1988; Mann et al., 1990).

2.4. ECOLOGY

Although MTB were isolated from different habitats such as ponds and lakes, sewage-treatment ponds, rivers, estuaries salt marsh and seas (Mann et al., 1990; Bazylinski et al., 1988) their ecology and distribution in sediments and stratified water columns is not very well known. In an early report, Stolz (1992) showed that whereas magnetotactic cocci could be detected only in the oxic and microoxic zone, diverse morphotypes were abundant both in the microoxic and anoxic zone in the presence of up to 2 mM sulfide. Therefore it can be concluded that the distribution of different MTB is determined by different optima in sulfide and oxygen gradients. This behavior could be important in terraformation for building up microcosms in which MTB should be able to reversibly move from the oxic to the anoxic zone in search of optimum conditions for growth and multiplication.

Another study investigated the vertical distribution of magnetite- and greigite-producing MTB (Bazylinski et al., 1995). Generally, more magnetite producers were found at and above the oxic–anoxic transition zone (OATZ), whereas more greigite-producing MTB were located in the anoxic sulfidic zone. Similar observations were reported for a stratified water column of a brackish water pond (Bazylinski and Moskowitz, 1997).

Another paper (Petermann and Bleil, 1993) showed that the majority of MTB were found in the anoxic zone (in the upper 10 cm of South-Atlantic deep-sea sediments), where nitrate was available, suggesting that most MTB might reduce nitrate as the terminal electron acceptor.

Although these studies have indicated that MTB are major constituents of microbial communities in certain zones of aquatic habitats, the biogeochemical interactions controlling their occurrence in stratified sediments have remained poorly understood. In the studies of microcosms performed by the Flies et al. (2005), in all samples analyzed, the occurrence of MTB was restricted to a narrow layer in the upper sediment located closely to the OATZ. As most of the cultivated MTB strains are known to behave as typical microaerophiles, it was surprising that in all microcosms most MTB were detected in the suboxic zone immediately below the OATZ. Maximum numbers were between 9.7 10^5 and 1.5 10^7/cm^3, thus accounting for at least 1% of the total cell numbers in this region. The maximum MTB numbers in their study were considerably higher than MTB numbers estimated for environmental samples (10^3–10^4 MTB/cm^3) (Blakemore et al., 1979; Blakemore, 1982) but were in the same range as previously reported for other laboratory enrichments (Blakemore et al., 1979; Spring et al., 1993; Moench and Konetzka, 1978; Petersen et al.,

1989). It has also been suggested that in microcosms and possibly in natural ecosystems, MTB can exists in a resting or metabolically inactive state which occurs in the absence of higher concentrations of organic substrates (Flies et al., 2005), which represent an important aspect for terraformation. If MTB can exist in a resting or metabolically inactive state, then we could speculate that MTB together with other bacteria having resting or metabolically inactive states (but able to recover) could have potential in terraformation by switching off to an inactive state while the nutrients (organic and/or inorganic) are not available in appropriate concentrations and by switching on as nutrients become available.

As already suggested (Flies et al., 2005), further studies regarding *in situ* measurements of metabolic activity of MTB in undisturbed sediments from marine and freshwater habitats and the interactions between MTB and other (micro)organisms are urgently required, before actually using MTB for terraformation of Mars or other planets.

Little is known about the relationships between MTB and other microorganism on Earth. Bazylinski et al. (2000) described some magnetic protists which may possible ingest MTB. If the iron from ingested magnetosomes can be used by the protistan cells as an iron source, then these protists could play a role in iron cycle by making it available to other microorganisms that consume them. These putative trophic relations could be useful for terraformation.

2.5. MAGNETOTACTIC BACTERIA AND EXOBIOLOGY

The chemical and microscopic analyses of Martian meteorite ALH 84001 caused a great surprise to the scientific community because it contains magnetite crystals whose origins, either biotic or abiotic, remain an open question (Barber and Scott, 2002; Buseck et al., 2001; Taylor et al., 2001; Thomas-Keprta et al., 2002; McKay et al., 2003; Weiss et al., 2004). McKay et al. (2003) also suggested that more studies of ALH84001, extensive laboratory simulations of non-biological magnetite formation, as well as additional studies of MTB on Earth are required to further address this question.

3. Potential Role of Magnetotactic Bacteria for Terraformation

Terraforming as defined by Fogg (1995) is “a process of planetary engineering, specifically directed at enhancing the capacity of an extra-terrestrial planetary environment to support life”. The initial stage in terraformation is ecopoiesis, “the fabrication of an uncontained anaerobic biosphere on the surface of a sterile planet” (Fogg, 1995) while the ultimate stage for terraformation would be to create an uncontained planetary biosphere emulating all the functions of the biosphere of the Earth (Fogg, 1995, 1998).

For now, Mars is considered one of the best candidates for terraformation. However, at present, the Martian surface environment is effectively sterilizing for all forms of terrestrial organisms (Hiscox, 2000b), being very different from the picture concerning the ultimate goal of terraformation.

Mars is a telluric planet, smaller than Earth which receives 43% of the sunlight that reaches Earth. The Martian day is almost equal to a terrestrial one and the gravity is 38% of the terrestrial one. The present-day magnetic field is about 1/800th of Earth's and it is not uniform distributed. The atmosphere has a very low pressure (0.8% of the terrestrial one) and consists mostly of carbon dioxide (95%). Temperatures vary between −75°C and +25°C, with an average of −60°C. Chemical analyses of Martian rocks and of Martian meteorites show that they have almost the same composition as the terrestrial ones, with one exception: iron is much more abundant on Mars than on Earth. The primary rocks contain ferrous iron (Fe^{2+}) whereas the regolith (the upper layer) contains ferric iron (Fe^{3+}) in the form of hematite, jarosite and goethite.

The properties of the nowadays Martian environment: low atmospheric pressure and temperature, extremely low molecular oxygen concentration and thus the absence of ozone layer, the instability of liquid water, strong UV irradiation, and the absence of organic matter (Hiscox, 2000a, b; McKay et al., 1991; Haynes and McKay, 1992; Birch, 1992; Zubrin and Wagner, 1996; McKay and Marinova, 2001) would preclude the survival and growth of terrestrial organisms. In order to allow ecopoiesis to occur (Fogg, 1995; Nussinov et al., 1994; Zubrin and Wagner, 1996; Zubrin and McKay, 1997; Gerstell et al., 2001; Badescu, 2005) the following modifications are needed (Fogg, 1998):

1. Mean global surface temperature must be increased by 60 K.
2. The mass of the atmosphere must be increased and atmospheric composition must be altered to increase its molecular oxygen and nitrogen fractions.
3. Liquid water must be made available.
4. The surface UV and cosmic ray flux must be substantially reduced.

These tasks could be achieved by planetary engineering, the application of technology for the purpose of influencing the global properties of a planet (Fogg, 1995). According to the most important papers in the field, Mars' planetary engineering would be achieved by releasing a certain quantity of gaseous carbon dioxide from the planet's polar caps, permafrost or regolith, in order to trigger a runaway greenhouse effect that would dramatically change the Martian environment. This planetary engineering can be done by various means: the use of orbital mirrors or nuclear explosions and/or by decreasing the planetary albedo etc. (Fogg, 1995; Nussinov et al., 1994; Zubrin and Wagner, 1996; Zubrin and McKay, 1997; Gerstell et al., 2001; Badescu, 2005). After successful planetary engineering, Mars would have a dense CO_2 atmosphere, acceptable temperatures, an ozone layer and a hydrographic network with rivers, lakes, seas, and even a boreal ocean. During the first phases of terraformation, the Martian biosphere would be dominated by photoautotrophic microorganisms (Haynes and McKay,

1992) which are able to use solar light to synthesize organic compounds from carbon dioxide. There are already proposed cyanobacteria such us *Chroococcidiopsis* which is capable of surviving in a large variety of extreme conditions: exceptional aridity, salinity, high and low temperature (Friedmann and Ocampo-Friedmann, 1995). On Earth, *Chroococcidiopsis* is particularly common in regions with desert pavement morphology, living beneath translucent pebbles which act both as a moisture trap and UV shield. According to the same group (Friedmann et al., 1993) another candidate is *Matteia*, the desiccation-resistant cyanobacterium that can dissolve carbonate rock. Furthermore, *Matteia* has the ability to fix atmospheric nitrogen when nitrogen compounds are not available from the surrounding environment. It has been suggested that this organism might be used to liberate carbon dioxide on Mars as part of a biogeochemical carbon cycle.

In the following, we shortly review the metabolic versatility of MTB and we put forward the idea that MTB could participate to the process of terraformation together with photosynthetic and/or extremophillic microorganisms, which have been already proposed for this task (Nienow et al., 1988; Friedmann et al., 1993; Nussinov et al., 1994; Haynes and McKay, 1992; Hiscox, 2000a, b).

3.1. CARBON METABOLISM

The majority of known MTB use organic acids (succinate, pyruvate, lactate etc.) as carbon source (Blakemore, 1982; Mann et al., 1990; Heyen and Schüler, 2003; Moisescu et al., 2005; Smith et al., 2006) being able to grow on complex or rather simple media (Blakemore, 1982; Mann et al., 1990; Smith et al., 2006).

The ability of some MTB to use carbon dioxide as the sole carbon source for the synthesis of cell components and multiplication could be important for terraformation. The recent discoveries concerning the biochemical and genetic details of their autotrophy show that MV-1 and MV-2 are chemolitoautotrophs that use the Calvin-Benson-Bassham pathway (Bazylinski et al., 2004) whereas the strain MC-1 seems to fix carbon dioxide via a reverse Krebs cycle (Williams et al., 2006). The growth of MV-1 and MV-2 strains with $S_2O_3^{2-}$ and radiolabeled ^{14}C bicarbonate ion (HCO_3^-) showed that cell carbon was derived from HCO_3^-/CO_2. Apart from the importance of these findings in understanding carbon metabolism in MTB, they could also reveal a possible role for MTB in the process of terraformation. Chemolitoautotrophy enables MTB to fix carbon dioxide in the dark using the energy released through the oxidation of inorganic chemicals such as thiosulfate, making them interesting candidates even for the early stages of terraformation.

3.2. OXYGEN METABOLISM

MTB behave as typical microaerophilic organisms, preferring to grow at low oxygen levels, but their respiratory metabolism is one of the less studied metabolic processes. Bazylinski and Blakemore (1983) found that *M. magnetotacticum*, the

first MTB isolated and grown in pure culture, can grow either in aerobic or microaerobic conditions, but not under strict anaerobic conditions, with nitrate as final respiratory electron acceptor. Tamegai et al. (1993) found a membrane bound novel ccb-type cytochrome *c* oxidase that seems to function as the terminal oxidase in microaerobic respiratory chain. In addition, the same group (Yamazaki et al., 1995) found a cytochrome cd_1-type nitrate reductase which has also Fe(II) nitrite oxidoreductase activity. It was proposed that it could function as an Fe(II) oxidizing enzyme for magnetite synthesis under anaerobic conditions (Fukumori et al., 1997). Short and Blakemore (1986) showed that magnetotactic cells of *M. magnetotacticum* strain MS1 extruded protons when ferric iron is added to an anaerobic suspension of cells. The same treatment applied to nonmagnetic cells (*M. magnetotacticum* strain ANM-1A) does not result in the extrusion of protons. The authors concluded that the results are consistent with iron reduction as a terminal site in the electron transport chain (Short and Blakemore, 1986). Guerin and Blakemore (1992) further showed that iron respiration in this strain can sustain bacterial growth and multiplication as well. Thus is seems appropriate to think that this strain, as well as other MTB, could use three terminal respiratory electron acceptors: oxygen, nitrate and iron which could be an advantage in real life on other planets as it is on Earth.

3.3. NITROGEN METABOLISM

MTB are versatile also with respect to nitrogen metabolism, the fixation of molecular nitrogen (Bazylinski and Blakemore, 1983; Bazylinski et al., 2000) and denitrification being performed by different strains. There are two types of denitrification, assimilatory and disassimilatory nitrate reduction, both being important for terraformation. Assimilatory nitrate reduction allows cells to use extracellular nitrate as a source of nitrogen for biosynthetic purposes whereas disassimilatory nitrate reduction will eliminate nitrogen gas in the atmosphere. The latter can be done by some MTB as well as by other bacteria such as *Pseudomonas denitrificans*, *Thiobacillus denitrificans* etc., which can be an important factor for terraformation. The use of nitrate as the terminal electron acceptor in anaerobic respiration (nitrate respiration) by some MTB could enable them to act, together with other denitrifying bacteria, for terraformation by increasing the atmospheric N_2 level. It seems probable that Mars has significant nitrate reserves (equivalent to as much as 300 mbar of gaseous N_2) which could be mobilized by denitrification. Denitrification should be very intense during the first phases of terraformation, until the atmospheric oxygen level would increase enough to inhibit the process at aerobic sites and forcing nitrate-respiring bacteria to withdraw to anaerobic niches.

In a more advanced stage of terraformation, when atmospheric nitrogen level would surpass a certain limit (5 mbar), nitrogen fixing bacteria including cyanobacteria and some MTB (Bazylinski and Blakemore, 1983; Bazylinski et al.,

2000) could begin fixing nitrogen, thus making it available for various organisms and trophic chains.

3.4. SULFUR METABOLISM

MTB would also take part in the sulfur cycle. In nature, sulfur is mainly found under three forms: compounds of the sulfate ion (SO_4^{2-}), compounds of the sulfide ion (S^{2-}) and elemental sulfur (S^0). Sulfates are very abundant on Mars and some MTB can reduce the sulfate ion to sulfide. This biochemical process would form sulfydric acid and sulfides. Other bacterial species, such as strain MC-1, can oxidize sulfides and thiosulfates and producing elemental sulfur. The sulfide ion can be used by anoxygenic photosynthetic bacteria, including facultative anoxygenic cyanobacteria, as the electron source for cell metabolism enabling them to reduce carbon dioxide during the synthesis of organic compounds.

4. Conclusions and Future Prospects

In the new environment created by ecopoiesis, allowing autotrophic microorganisms and/or extremophilic ones to growth and multiply, MTB could play roles in carbon, oxygen, nitrogen, iron and sulfur cycles on Mars or on other planets (Fig. 1). MTB have several particularities that argue for their potential in the terraforming process, the following being the most important:

1. The ability of some MTB to fix carbon dioxide in the dark using the energy released through the oxidation of inorganic chemicals such as thiosulfate;

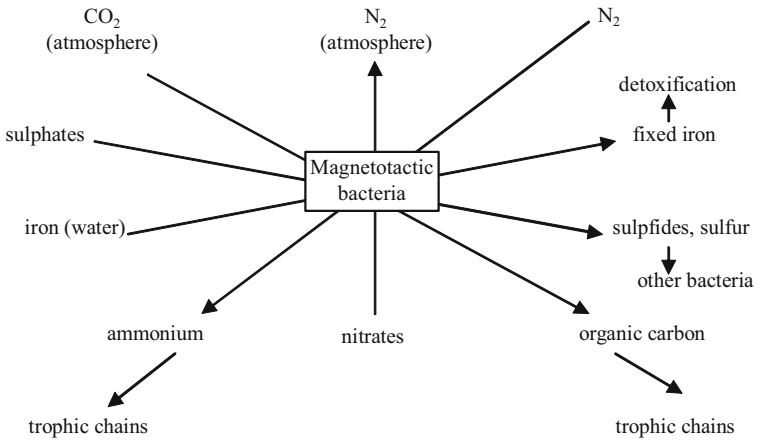

Figure 1. The importance of magnetotactic bacteria for terraformation (for details see text).

these chemolitoautotrophic MTB could be a primary source of organic carbon once molecular oxygen, even in limited concentrations, is available.

2. The ability to carry out aerobic or anaerobic respiration with either nitrate or ferric iron. The use of nitrate as the terminal electron acceptor in anaerobic respiration (nitrate respiration) by some MTB could enable them to work together with other nitrate respiring bacteria during terraformation of Mars and other planets. Nitrate respiration should be very intense during the first phases of terraformation, until the atmospheric oxygen level would increase, inhibiting the process at aerobic sites and forcing nitrate-respiring bacteria to withdraw to anaerobic sites.
3. MTB together with other types of microorganisms could contribute to a nitrogen cycle on Mars, carrying out two important tasks: a constant percentage of N_2 in the atmosphere (by denitrification) and the availability of this macro element to ecosystems (by nitrogen fixation).
4. MTB could consume ferric iron which, at high concentrations, is toxic for living organisms. At a neutral pH, the solubility of ferric iron is very low, but for each pH unit less, its solubility increases 1,000 times. During the first stages of terraformation of Mars, due to the CO_2 atmosphere, Martian waters would be acid and ferric iron would dissolve causing problems to living cells. MTB could have a contribution in solving these problems by fixing the iron in the form of solid magnetite or greigite.
5. Magneto-aerotaxis, as well as other magnetic assisted taxies, could constitute specific advantages of MTB in their navigation toward optimum growth conditions during the process of terraformation of planets with a global magnetic field similar to that of Earth. MTB could keep this advantage even on Mars, in the regions having a local magnetism of 100 to 600 nT (http://mgs-mager.gsfc.nasa.gov; http://denali.gsfc.nasa.gov/terr_mag/index.html). In those regions containing large iron deposits in the crust, one can develop microcosms where MTB could use magneto-aerotaxis or other magnetic assisted taxies to reach the appropriate concentration of nutrients. The experiments concerning microcosms are important for terraformation as it seems rational that microcosms could be used to start up ecosystems on Mars or others planets, once ecopoiesis is established.

The improvement of our knowledge concerning the biology of MTB, including their relationship with biotic and abiotic factors, is needed for the use of MTB in the terraformation of Mars or other planets. Furthermore, genetic modification of MTB could increase their potential for terraformation by improving their relationship with autotrophic and extremophilic microorganisms as well as making them more robust to face adverse physical and chemical conditions.

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PALEONTOLOGICAL TESTS: HUMAN-LIKE INTELLIGENCE IS NOT A CONVERGENT FEATURE OF EVOLUTION

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1. The Planet of the Apes Hypothesis

I taught a course called "Are We Alone?" at the University of New South Wales for a few years. The most popular lecture was "The Great Drake Equation Debate" – half a dozen "experts" would sit at the front of the crowded lecture theater defending their estimates for the various terms in the Drake Equation (an equation created by Frank Drake to estimate the number of civilizations in the Milky Way with whom we might communicate via radio telescopes). The first terms of the equation are astronomical. How many stars are in our galaxy? – most experts agreed – about 300 billion. What fraction of those stars are orbited by "Earth-like" planets? – estimates ranged from ~100% to ~0.1% depending roughly proportionally on how specific "Earth-like" was interpreted to be. Then came the more contentious biological terms: What fraction of these Earth-like planets would harbor life? I defended a relatively high probability (~10%) based on how rapidly biogenesis occurred on Earth (Lineweaver and Davis, 2002). We argued back and forth about how probable or improbable the steps of molecular evolution were, that led to life on Earth – and whether there were places on Earth where life could still be emerging. We all learned a lot about biochemistry, autocatalytic cycles and hydrothermal vents. However, the most contentious term was: Once there is life of any kind, what is the probability that it will evolve into a human-like intelligence that can build and operate radio telescopes? (We define intelligence this way not out of some geeky technophilic perversity but because posed this way, we have the ability to answer the question by searching for other telescopes with our telescopes. So far, no signals from intelligent aliens have been identified, Tarter, 2001.)

In the "Great Drake Equation Debate" most of the invited experts assumed that once life got started it would get smarter and smarter until one day, it would hit upon the idea of building a radio telescope. Most students also subscribed to this "stupid things get smarter" model of animal evolution and believed it to be a universal trend. I call this idea the Planet of the Apes Hypothesis.

The movie *Planet of the Apes* (1968) is set in the future after a catastrophic nuclear war between *Homo sapiens*. The surviving humans have lost the ability to

speak and have to forage in the wild. Meanwhile, three species of apes (chimps, gorillas and orangutans) learn to speak English, ride horses, farm corn, shoot rifles, and in general begin to act like hairy Victorian humanoids with human-like intelligence. They move into the recently emptied "intelligence niche" and turn into the "functional equivalent of humans" (to use Carl Sagan's term, Sagan, 1995a). On the Planet of the Apes, human-like intelligence is so adaptive that it is a convergent feature of evolution – species are waiting in the wings to move in and occupy the intelligence niche.

G.G. Simpson in "The Nonprevalence of Humanoids" (1964) articulated the case that humans (or any given species) were a quirky product of terrestrial evolution and therefore we should not expect to find humanoids elsewhere. Thus stupid things do not, in general acquire human-like intelligence. The evidence we have tells us that once extinct, species do not re-evolve. Evolution is irreversible. This is known as Dollo's Law (Dollo, 1893; Gould, 1970). The re-evolution of the same species is not something that happens only rarely. It never has happened. Simpson also argued that biologists, not physicists, can best judge this issue because the problem is one of evolutionary systematics, not deterministic physics.

Whether there is a trend in the fossil record indicating that stupid things tend to get smarter, is an important and controversial issue in which the discussion has become polarized into two camps. In one camp are the non-convergentists (mostly biologists) who, after studying the fossil record, insist that the series of events that led to human-like intelligence is not a trend, but a quirky result of events that will never repeat themselves either on Earth or anywhere else in the universe. In the other camp are the convergentists (mostly physical scientists) who believe that stupid things get smarter and that intelligence is a convergent feature of evolution here and elsewhere. See Lineweaver (2005) for more on the protagonists in this debate.

Is there any real evidence for the Planet of the Apes Hypothesis? Is human-like intelligence a convergent feature of evolution? Should we expect to find extraterrestrials with human-like intelligence? Despite the lack of direct evidence, we would like to assemble and evaluate the best indirect evidence for or against the idea that life (terrestrial or extraterrestrial) evolves towards human-like intelligence. The study of the evolutionary trends in the paleontological record of life on Earth is probably the most relevant evidence and that is what is critically examined here.

2. Frank Drake, Carl Sagan and Ernst Mayr

In 1960 Frank Drake conducted the first radio search for extraterrestrial intelligence. He is the Director of the SETI (Search for Extra Terrestrial Intelligence) Institute's Center for the Study of Life in the Universe. SETI work would seem much more promising if there has been an evolutionary trend among terrestrial life forms towards higher intelligence.

On a flight to a conference I asked him: "Frank, why do you think there are intelligent aliens who have built radio telescopes? What do you think is the strongest evidence for the idea that such human-like intelligence is a convergent feature of evolution?" Frank's answer went something like this:

> The Earth's fossil record is quite clear in showing that the complexity of the central nervous system – particularly the capabilities of the brain – has steadily increased in the course of evolution. Even the mass extinctions did not set back this steady increase in brain size. It can be argued that extinction events expedite the development of cognitive abilities, since those creatures with superior brains are better able to save themselves from the sudden change in their environment. Thus smarter creatures are selected, and the growth of intelligence accelerates. We see this effect in all varieties of animals – it is not a fluke that has occurred in some small sub-set of animal life. This picture suggests strongly that, given enough time, a biota can evolve not just one intelligent species, but many. So complex life should occur abundantly. (Drake, 2006)

During the flight Frank referred me to the debate between biologist Ernst Mayr and planetologist Carl Sagan (Sagan, 1995a, b; Mayr, 1995a, b). Sagan articulated the concept of "functionally equivalent humans":

> when we're talking about extraterrestrial intelligence, we are not talking – despite Star Trek – of humans or humanoids. We are talking about the functional equivalent of humans – say, any creatures able to build and operate radio telescopes. (Sagan, 1995a)
>
> We are not requiring that they follow the particular route that led to the evolution of humans. There may be many different evolutionary pathways, each unlikely, but the sum of the number of pathways to intelligence may nevertheless be quite substantial. (Sagan, 1995a)
>
> other things being equal, it is better to be smart than to be stupid, and an overall trend toward intelligence can be perceived in the fossil record. On some worlds, the selection pressure for intelligence may be higher; on others, lower. (Sagan, 1995b)

To which Mayr replied:

> Sagan adopts the principle "it is better to be smart than to be stupid," but life on Earth refutes this claim. Among all the forms of life, neither the prokaryotes nor protists, fungi or plants has evolved smartness, as it should have if it were "better." In the 28 plus phyla of animals, intelligence evolved in only one (chordates) and doubtfully also in the cephalopods. And in the thousands of subdivisions of the chordates, high intelligence developed in only one, the primates, and even there only in one small subdivision. So much for the putative inevitability of the development of high intelligence because "it is better to be smart." (Mayr, 1995b)

To which Sagan re-replied:

> Mayr argues that prokaryotes and protista have not "evolved smartness." Despite the great respect in which I hold Professor Mayr, I must demur: Prokaryotes and protista are our ancestors. They have evolved smartness, along with most of the rest of the gorgeous diversity of life on Earth. (Sagan, 1995b).

Hold those poetic horses Carl! You're in Mayr's territory. By "prokaryotes and protists" Mayr is referring to extant organisms and their ancestors, **after** they diverged from our lineage. These ancestors and their lineages have continued to exist and evolve and have not produced intelligence. All together that makes about 3 billion years of prokaryotic evolution that did not produce high intelligence and about 600 million years of protist evolution that did not produce high intelligence.

3. The Fossil Evidence for an Overall Trend Towards Intelligence

The comments by Drake (2006) and Sagan (1995b) about the fossil record showing evidence for a trend toward increasing vertebrate encephalization are references primarily to the work of paleoneurologist Harry Jerison (Jerison, 1973, 1975, 1991). Jerison has been measuring the volume of modern and fossil animal skulls for several decades (Jerison, 1975). Jerison introduced the concept of Encephalization Quotient (E.Q.), which is the ratio of brain weight to some power (~2/3 or ~3/4) of the body weight. E.Q. is arguably the most objective way to compare the intelligence of different groups of encephalated animals (Jerison, 1955, 1963, 1973).

4. Interpretation Problem #1: Selection Bias: Choosing E.Q. Because We Have the Highest E.Q.

Every species has some unique feature – a feature that makes it different from its closest living relatives and from its ancestors. To make Fig. 1, Jerison first identified our unique feature (high E.Q.) and then plotted it as a function of time. Looking backward at the history of any existing extreme trait will produce a similar apparent trend. The evolutionary history of a feature (identified as extreme today) is almost guaranteed to look something like Fig. 1. Therefore, we cannot consider

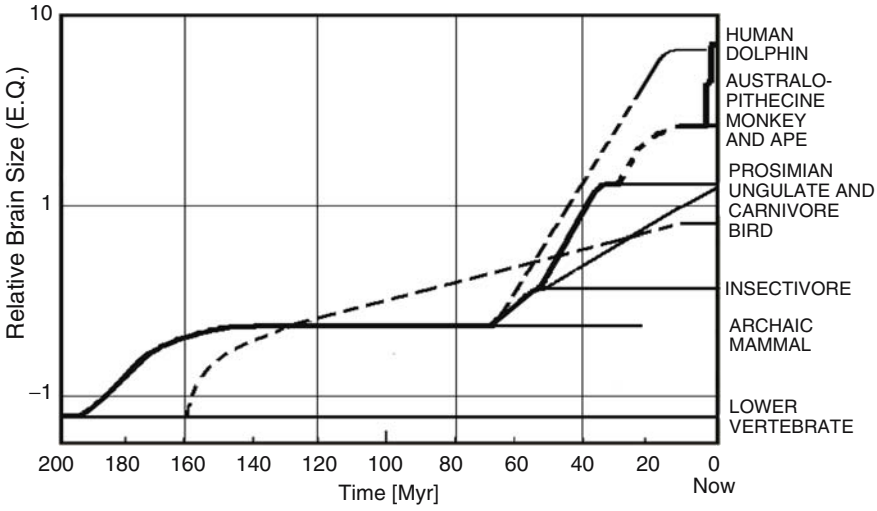

Figure 1. The evolution of relative brain size in groups of vertebrates over the past 200 million years (adapted and updated from Jerison, 1976, p. 96; Jerison, 1991, fig. 17). This plot seems to show an evolutionary trend towards increasing relative brain size (=E.Q. = Encephalization Quotient) and is probably the most definitive evidence for such a trend. Average living mammal E.Q. is defined as 1. The broken lines indicate gaps in the fossil record. Variation within groups is not shown. The lineage that led to humans is drawn thicker than the other lineages.

the trend seen in our lineage to be a general trend representative of any other lineages. If you choose a feature because of its current extreme nature, it is no surprise that it had to get that way and that its evolution will display a trend. But this trend has no claims to being representative of life in general.

Another example may make selection bias more obvious. Elephants have longer noses than their living relatives. Thus, when we focus on this unique feature and plot the sizes of the noses of its living relatives and of their evolutionary ancestors (Fig. 2), we find that in the series of progressively earlier ancestors, noses get progressively shorter. This is a selection effect that has nothing to do with a general tendency that can be extrapolated to the rest of biology. Increasing nose size is not a general feature of evolution. It is something that occurred in the lineage that led to elephants. After diverging from this lineage the N.Q. (nasalization quotient) of most groups stayed constant.

Another example: Humans have big brains with extremely small olfactory lobes. A similar analysis of the evolution of our olfactory lobe size would lead to the conclusion that the shrinkage of olfactory lobes is a generic trend. Such a conclusion would be misguided. By selecting an extreme outlying feature of an extant organism (whether big or small, short or long, hard or soft – anything as long as it is an outlier) the evolution of that feature in that organism's ancestors is very likely to show a monotonic trend that leads up to the outlier (Fig. 3).

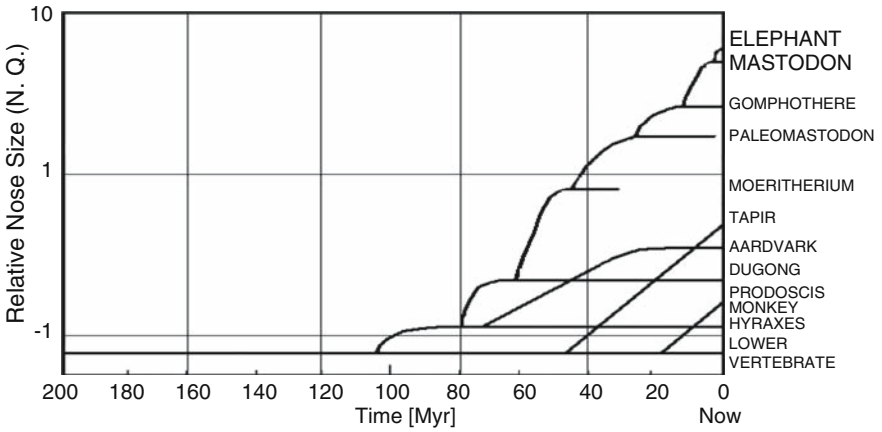

Figure 2. The evolution of relative nose size (=N.Q. = Nasalization Quotient, ratio of nose length to body length) over the past 200 million years. Notice the apparent trend in the data as, over time, N.Q. reaches its ultimate value in extant pachyderms. Notice also that once the direct lineage that led to elephants is ignored, most of the species do not have an increasing N.Q. A few do (tapirs, aardvarks, proboscis monkeys) and such exceptions are discussed in "Interpretational Problem #3." This preliminary plot is meant to illustrate a point, and should not be taken as more than a crude representation of a specious trend in N.Q. that has been largely ignored and poorly quantified by paleontologists. (See Poulakakis et al., 2002 and Benton et al., 2005 for more detail.)

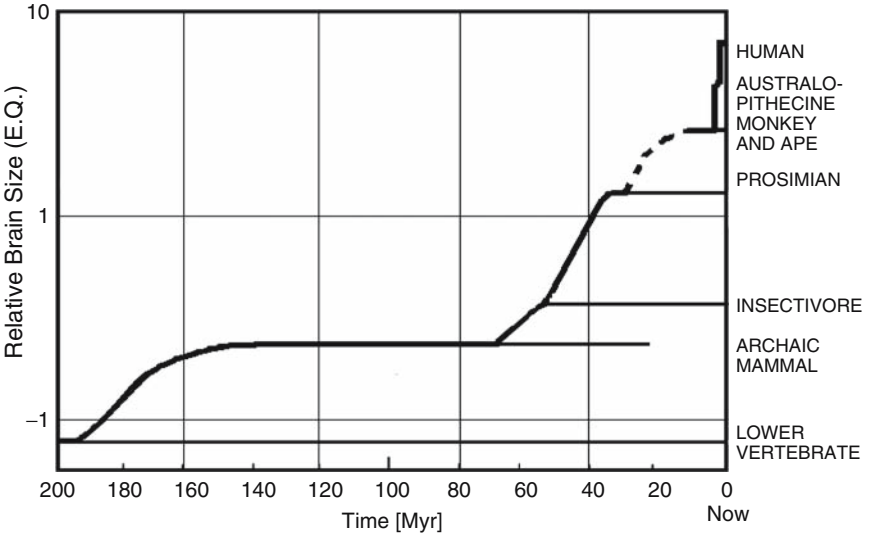

Figure 3. Same as Fig. 1 but we have removed the three lineages (dolphins, birds and ungulates/carnivores) with increasing E.Q. after diverging from our lineage. The lineage that leads to us provides no evidence of a generic trend towards increasing E.Q. because it has been selected to have that property. Notice that all of the increase in E.Q. is on the lineage that leads to humans. After diverging from us, all lineages shown here (lower vertebrates, archaic mammals, insectivores, prosimians, monkeys and apes) stayed at approximately the same E.Q. The lineages that remain flat represent the vast majority of all the species that evolved from the low E.Q. group shown in the lower left at 200 million years ago. The stasis of the E.Q. of the vast majority of species is evidence for an absence of selection pressure towards higher E.Q. The lineages whose E.Q. did increase after diverging from our lineage (dolphins, birds and ungulates/carnivores) are discussed in "Interpretational Problem #3."

5. Interpretational Problem #2 Selection Bias: Non-democratic Line Assignments

In Fig. 1 some lines represent one species (dolphin, humans) while other lines represent thousands of species (archaic mammals) or tens of thousands of species (lower invertebrates). If our goal is to fairly represent what has happened during evolution then each line should represent an independent cladistic group: order, class or genus – something democratic. However, having some lines for single species and other lines for thousands of species is a biased non-representative form of gerrymandering. If one is looking for a trend, one needs to consider all the data, not just the data that supports the trend one wants to show. If the evolution of the E.Q.s of all species were plotted, some would go up, some would go down and the vast majority would stay the same. This variation is not shown in Fig. 1.

6. Interpretational Problem #3: Non-independence of "Convergence" on High E.Q.

Consider Fig. 1 again. After diverging from our lineage, the increasing E.Q. of birds, dolphins and ungulates/carnivores **does** seem to be evidence for the trend in

the fossil record toward intelligence that Drake and Sagan were referring to. About 310 million years ago the last common ancestor of birds and humans had a small brain (E.Q. < 0.1). And 310 million years later birds and humans have bigger brains (E.Q. ~ 0.8 and E.Q. ~ 8 respectively). About 85 million years ago the last common ancestor of dolphins, humans and ungulates/carnivores had a small brain (E.Q. ~ 0.2). And 85 million years later humans and dolphins have a big brain with E.Q. ~ 8 and ungulates/carnivores have an E.Q. ~ 1. The E.Q. in all three lineages "independently" got bigger. Can we extrapolate this independent convergence on high E.Q. to the evolution of extraterrestrials?

Simon Conway-Morris (2003, 2005) has documented many cases of evolutionary convergence in evolution – both marsupial and placental mammals converged on saber-toothed carnivores (thylacosmilids and placental cats). The ability to fly evolved in insects, pterosaurs (reptiles), birds and bats (mammals). Conway-Morris and other authors have cited N independent examples of the origin of the eye, where N is some largish but indeterminate number.

The common ancestor of these "independent" eye inventors did not have easily identifiable eyes, but it almost certainly did have proto-eyes of some sort. The supposed independence of these convergences is undermined by the ~3.5 billion years during which the creatures were biochemically and genetically identical, and during which they evolved their proto-eyes. They shared the same genes, the same genetic machinery of gene expression, regulation, inhibition and activation that controlled the genetic exploration of morpho-space and the ability to tinker with the structure of the head and the placement and framing of photoreceptors. Many shared the same head and brain. The supposedly independent evolvers of eyes, share the same basic biochemistry and photoreceptor proteins and the same plasticity (West-Eberhard, 1989) that enabled and constrained the morphological, preservable features that are superficially different enough to be called "independent".

When considering convergence, a basic principle is often ignored: the extent of convergence cannot be larger than the extent of divergence from the common ancestor. With all terrestrial life having a common origin, one must first quantify the degree of divergence of two groups before one can discuss their convergence. For the species that converged on eyes, this divergence could only take place during the relatively brief fraction of their existence as independent organisms. Roughly speaking, and depending on the eyes, the organisms were independent for about 500 million years but shared a common ancestor for about 3,500 million years. Thus they were independent divergers for only ~10–20% of their existence (~500 Myr/4,000 Myr ~ 12%) and were identical for ~80–90% of their existence. In other words, the common ancestor of the independent eyes had, during ~3,500 million years, already evolved the complex biochemical pathways for photoreception. In many detailed and fundamental biochemical and genetic ways, the purported "independent" originators (although they were phylogenetically isolated) were working in the same workshop, with the same tools and the same materials with the same set of genetic regulatory skills.

Similarly, the common ancestor of dolphins and humans who lived ~85 million years ago had a head, a small brain and ~500 million year common history of

regulatory genes that tinkered with the characteristics of that brain. It had the same, (or very similar) biochemical neural pathways and genetic plasticity and constraints that dolphins and humans are still endowed with. This 500 million year history produced a finite number of correlated non-independent ways to adapt to environmental challenges. In other words, there were a finite number of highly evolved toggle switches that could be successfully tinkered with. Among the two thousand species of Laurasiatheres that diverged from our lineage 85 million years ago with the dolphins and carnivores/ungulates, all had heads and all had a similarly constrained potential for modification. There were thousands of species, undergoing a variety of environmental challenges, and their ability to adapt to these challenges was provided by hundreds or thousands of shared toggle switches. We would expect some apparent convergences. What I am calling genetic "toggle switches" is more articulately and authoritatively described as "conserved core processes", "facilitated variation" and "invisible anatomy" by Kirschner and Gerhart (2005).

Similar arguments can be made about the increased nasalization quotients in tapirs, aardvarks and proboscis monkeys (Fig. 2). They too converged on large noses. Once noses were present and a common genetic tinkering apparatus had become available from a long shared history, nose sizes increased and decreased but mostly (like brains) just stayed the same size.

Three and a half billion years of identical evolution comes with much biological baggage and many constraints and it is these limited choices that are largely responsible for the apparent convergence on big brains. What Drake, Sagan and Conway-Morris have done is interpret correlated parallel moves in evolution as if they were unconstrained by shared evolution but highly constrained by a universal selection pressure towards intelligence that could be extrapolated to extraterrestrials. I am arguing just the opposite – that the apparently independent evolution toward higher E.Q. is largely constrained by shared evolution with no evidence for some universal selection pressure towards intelligence. If this view is correct, we cannot extrapolate the trends toward higher E.Q. to the evolution of extraterrestrials. If the convergence of dolphins and humans on high E.Q. has much to do with the 3.5 Gyr of shared history (and I argue that it has everything to do with it) then we are not justified to extrapolate this convergence to other extraterrestrial life forms that did not share this history. Extraterrestrials are related to us in the sense that they may be carbon and water based – they may have polymerized the same monomers using amino acids to make proteins, nucleotides to make a genetic code, lipids to make fats and sugars to make polysaccharides. However, our "common ancestor" with extraterrestrials was probably pre-biotic and did not share a common limited set of genetic toggle switches that is responsible for the apparently independent convergences among terrestrial life forms.

We can use Fig. 4 to identify trends in the evolution of life, or convergences on some specific feature, whether it be E.Q., N.Q., olfactory lobe size or eyeballs. First we randomly select a few of the ~60 branches shown. Then we determine if two or more of them have independently evolved the feature of interest. For

example, human-like intelligence probably depends on the existence of heads. Thus, we want to know if the tree of life shows any convergence towards heads. If heads were a convergent feature of evolution one would expect independent lineages to evolve heads. Our short twig on the lower left labeled "Homo" has heads, but heads are found in no other branch. Our two closest relatives, plants and fungi, do not seem to have any tendency toward evolving heads. The evolution of heads (encephalization) is therefore not a convergent feature of evolution. Heads are monophyletic and were once the possessions of only one quirky unique species that lived about 600 or 700 million years ago. Its ancestors, no doubt possessed some kind of proto-head related to neural crests and placodes (Wada, 2001; Manzanares and Nieto, 2003).

Drake (2006) stated that "[intelligence] is not a fluke that has occurred in some small sub-set of animal life." However, Fig. 4 shows that intelligence, heads, even all animal life or multicellular life, may well be a fluke that **is** a small sub-set of terrestrial life. One potential problem with this conclusion: It is possible that existing heads could have suppressed the emergence of subsequent heads. Such suppression would be difficult to establish.

7. Interpretational Problem #4: The Tenuous Link Between High E.Q. and Human-Like Intelligence

About 600 million years ago, two kinds of metazoans, protostomes and deuterostomes, diverged from each other. Both evolved separately for ~600 million years and were very successful. Today there are about a million species of protostomes and about 600,000 species of deuterostomes (of which we are one). We consider ourselves to be the smartest deuterostome. The most intelligent protostome is probably the octopus. After 600 million years of independent evolution and despite their big brains, octopi do not seem to be on the verge of building radio telescopes. The dolphinoidea evolved a large E.Q. between ~60 million years ago and ~20 million years ago (Marino, 2004). Thus, dolphins have had ~20 million years to build a radio telescope and have not done so. This strongly suggests that high E.Q. may be a necessary, but is not a sufficient condition for the construction of radio telescopes. Thus, even if there were a universal trend toward high E.Q., the link between high E.Q. and the ability to build a radio telescope is not clear. If you live underwater and have no hands, no matter how high your E.Q., you may not be able to build, or be interested in building, a radio telescope.

8. A Universal Intelligence Niche?

Life has been evolving on this planet for ~4 billion years. If the Planet of the Apes Hypothesis is correct and there is an intelligence niche that we have only recently

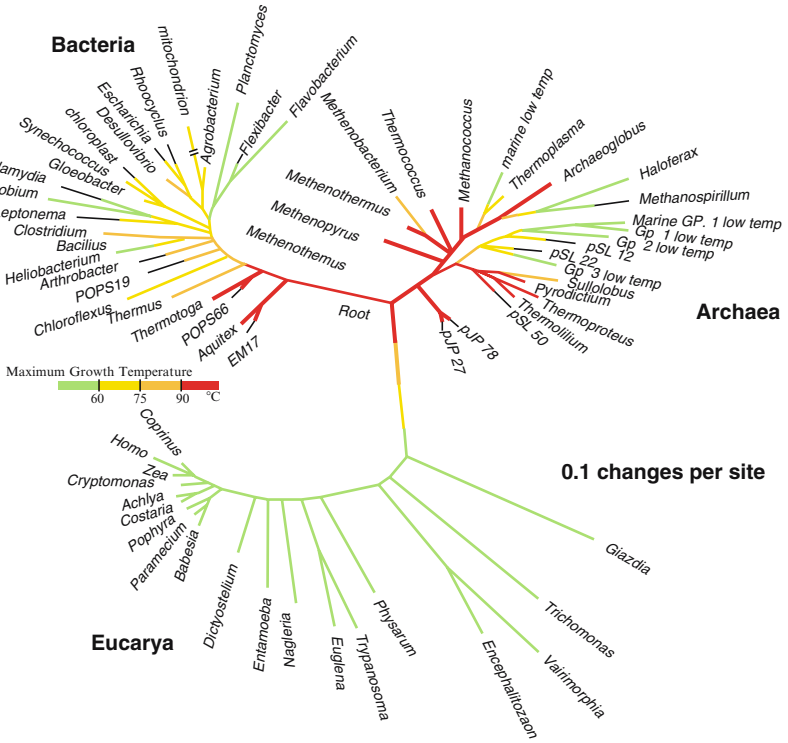

Figure 4. Phylogenic tree of terrestrial life based on the 16s subunit of ribosomal RNA. The last common ancestor of all life is at the center of the tree (labeled "Root"). The distance from the root to the end of each branch corresponds to the same amount of time – roughly 3.5 or 4.0 billion years. Because the ticking of the 16s molecular clock is not exactly uniform, the distances from the root to the ends of the branches are not the same length. Among the Eucarya in the lower left are the three twigs of complex multicellular life: Coprinus (representing fungi), Homo (humans, representing animals) and Zea (corn, representing plants). The common ancestor of fungi, animals and plants lived ~1.5 billion years ago (Hedges et al., 2004). Thus, the 200 million year time frame shown in Figs. 1 and 2 corresponds to the last ~2 mm of the twig labeled "Homo". (diagram from Lineweaver and Schwartzman, 2004 based on Pace, 1997). This RNA tree should be compared to Fig. 6.

occupied – Who occupied it 2 billion years ago, or 1 billion years ago or 500 million years ago? Stromatilites? Algae? Jellyfish?

Sagan defines "the functional equivalent of humans" so narrowly (creatures able to build and operate radio telescopes) that only one species on Earth belongs to it – and then assumes that it is so broad that many aliens will fit into it (Fig. 5). He is postulating an imaginary group of species with only one species in it. Most biologists refuse to take the idea of such an imaginary group seriously. In studying the variety of life on this planet, they see that general groups with only one species in them are self-contradictions that do not exist – probably not a sound foundation upon which to build our hypotheses about extraterrestrial life.

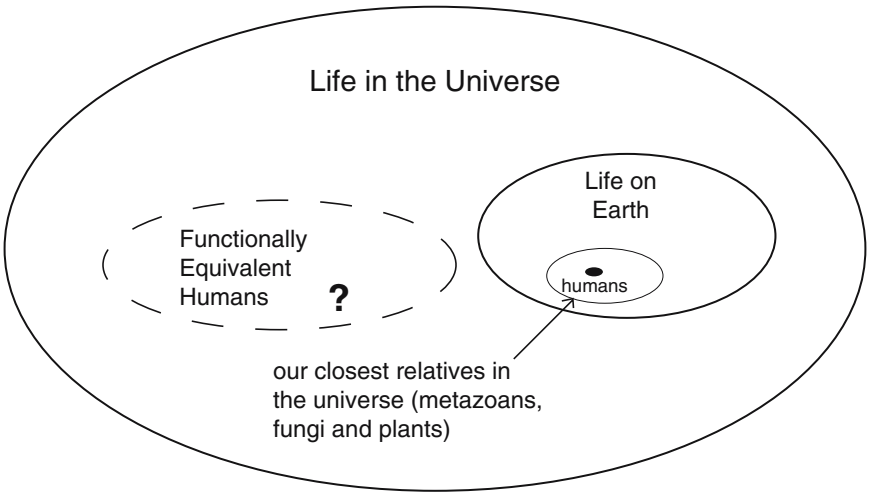

Figure 5. Consider the subsets of life in the Universe. We know that humans (black dot) are a subset of Life on Earth and we know that metazoans, fungi and plants are our closest relatives on Earth (small circle around the black dot). Sagan and others postulate an imaginary group of "functionally equivalent Humans" to which we (and some aliens) belong but none of our closest relatives do.

It seems unreasonable to define intelligence so narrowly that only Homo sapiens have it on Earth (among the ~100 million species that have ever lived) and then imagine that the human-like intelligence niche is so generic that even life forms very different from ours (not sharing 3.5 billion years of evolution) would evolve into it.

Any given species that has evolved on the Earth will have its closest relatives here on Earth. Thus, if we consider humans to be unique and alone on Earth, then humans are a fortiori unique and alone in the Universe. We are more closely related to the life forms with whom we have shared 3.5 billion years of common ancestors than we will be with any alien evolved independently on another planet. Our closest relatives, genetically, physiologically and mentally are here on this Earth.

9. Conclusion

The search for extraterrestrial intelligence is a search for ourselves. And therein lies its strength and weakness. Knowing that we are searching for ourselves gives us the strength and motivation to explore and find our place in the Universe. The weakness is the labyrinth of dead-ends created by our natural sense of self-importance and by our bias about what our place should be. Figure 6 is an older tree than Fig. 4 and displays more obviously what we want to believe about ourselves.

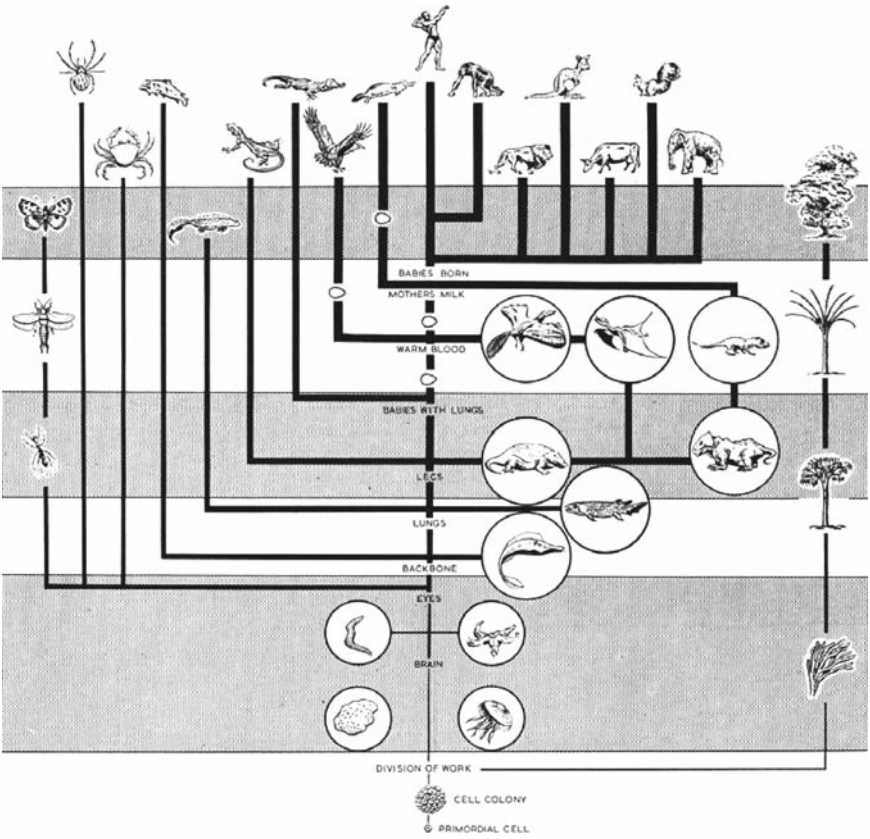

Figure 6. The Schwarzeneggerization of Life. A muscle-bound man stands as the end product of a linear progression – the Great Chain of Being – a ladder of life that leads to male Caucasian weight lifters. One can create such an apparent linear trend out of the crooked phylogenetic branch of **any** species. Looking back from any particular species we will find the evolution of the traits of that particular species. However, precisely because we can construct such a figure from the lineage of **any** species, such a construction should not be construed as a general linear trend applicable to all life. The simple appeal of this figure is a good example of how easy it is to believe that the important events and the major transitions in evolution that led to us, are important events for all organisms (Smith and Szathmary, 1995). The problems with this view are detailed in Gould (1989). The prevalence and recurrence of this mistaken interpretation of evolution needs to be avoided as we try to use terrestrial evolution to give us hints about the evolution of extraterrestrial life (figure from Gatland and Dempster, 1957). This homo-centric tree should be compared with Fig. 4.

If human-like intelligence were so useful, we should see many independent examples of it in biology. We could cite many creatures who had involved on independent continents to inhabit the "intelligence niche". We can't. Human-like intelligence seems to be what its name implies – species specific.

I have argued that the fossil record strongly suggests that human-like intelligence is not a convergent feature of evolution. The evidence is indirect and suggestive, but it is, I think, the best we have. Despite this evidence, I am a strong supporter of SETI – because I may be wrong about how the evidence is best interpreted, and because SETI is relatively cheap science. SETI is the exploration of new parameter space with new instruments – a proven recipe for scientific discovery. However, we do not need to misinterpret the fossil record to justify this inspiring research.

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COSMIC LIFE FORMS

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Abstract We propose that the first principle of biology is a useful guide in exploring cosmic life forms. Moreover, it determines the basic prerequisites of life in material-independent form. Starting from the Bauer principle (BP), we made explicit its content, and found that the Bauer principle is mediated by virtual interaction (VI) which generates biological couplings (BC) opening up an enormous realm of biologically spontaneous reactions. With the help of biological couplings, it becomes possible that the organism self-initiate systematic investment of work ΔW against the equilibrium, which would otherwise necessarily be approached on the basis of the given initial state and the laws of physics. Therefore, the essence of life can be formulated as the following: the Bauer principle (BP) is manifest in virtual interactions which generate biological couplings leading to investment of work ΔW that generates thermodynamically uphill processes increasing extropy Π ($\Delta\Pi > 0$); compactly, $BP \rightarrow VI \rightarrow BC \rightarrow \Delta W \rightarrow \Delta\Pi$. We point out that generation of lawful algorithmic complexity is a fundamental characteristic of life (Grandpierre, 2008). Applying the Bauer principle for the Sun, we found that the Sun is a living organism. We are led to recognize a cosmic life form in stellar activity cycles. Then we generalized the Bauer principle and found new kinds of cosmic life forms like the microscopic, intermittent and hidden life forms. We found that the first principle of biology is able to be manifest in the whole universe through virtual interactions. This result led us to recognize a new cosmic life form present in the vacuum that we call universal life.

1. Introduction

Recently astrobiology has become main foci of modern science. In 1996, the Astrobiology program was added to NASA's lexicon. (Dick and Strick, 2004, 19) "With the advent of the means to explore space, the prospect of developing a truly universal science of biology now seemed possible for the first time." (ibid., 2) Similarly as the research of stellar physics plays a significant role in understanding our Sun, the research of cosmic life is of fundamental importance for the scientific understanding of what life is. We point out that being imaginative in exploring cosmic life forms will be facilitated if helped by exploring the most universal aspects of biology. If we base our exploration of cosmic life forms to

the most general principle of biology, a whole list of yet unimagined cosmic life forms will become closer to us. It appears the universe itself can offer much wider perspectives for exploring the nature of life. This means that we do not consider life as being restricted to protein-based life forms; yet the basis of defining general life is specified by the first principle of biology: the Bauer principle (see below).

2. Life Forms Are Manifestations of the Biological Principle

Life on Earth shows extreme variability in forms and behavior. A physical object like a falling stone falls always in the same manner from the Pisa tower. In contrast, living organisms can behave very differently even within the same conditions. Moreover, living organisms show a behavior profoundly divergent from the physical one. *We define physical behavior as the one governed by the laws of physics, with the given initial conditions* (boundary conditions included). Similarly, *we define biological behavior as the one governed by the Bauer principle, with the given initial conditions*. The difference between biological and physical behavior can be demonstrated by an extended Galileo experiment in which a living bird dropped from a height follows a trajectory characteristically different from the trajectory determined by the free falling stone.

At present, the theoretical description of the most general laws of biological behavior seems to be unavailable. In the last decades, the general belief has been that all phenomena of any systems are determined by bottom-up laws of physics, ultimately, the action principle, governing the material building blocks of the given system. Nowadays the general view of scientists is that biological laws do not exist, but if they did, they would be mere byproducts of physical laws, and the reason for the different behavior of living organisms lies in their intractable complexity (Vogel and Angermann, 1988, 1). At variance with these widespread views, theoretical biology as an exact science has been founded by Ervin Bauer on the basis of the universal and invariable characteristics of living organisms (Bauer, 1935/1967).

Recently, Popa (2004, 170–172) presents a whole list of material-independent signatures of life. Such signatures are, for example, the recovery of energy lost by the living organism in performing work on itself, as internally controlled by specific mechanisms; that life forms use this energy to control their internal entropy level; the target-oriented nature of energy transduction, which is related to couplings that must exceed a certain minimal negentropic level in order to occur. As we will show here, the common characteristics of all life forms are rooted in the existence of the biological principle.

3. The Biological Principle Acts on Possibilities Left Open by Physics

The bottom-up approach of physics starts from material building blocks plus physical laws. Yet it is insufficient and incompetent in a biological context to produce a model that explains such elementary biological processes as the bending

of a finger. There are not physical equations that can determine the time-dependent behavior of my finger which I will intend to bend in the next moment, even if it would be possible to give all the positions of the elementary particles in the initial state. Actually, there is more to nature than elementary particles plus physical laws. Besides complexity, biological behavior also enters to the scene.

In physics, any problem can be regarded as definite only if the boundary conditions representing the connection of the system are given; otherwise the differential equations cannot be solved. These conditions in physics are usually external. In contrast, in living organisms the changes initiated within the organism by the living organism itself govern behavior. This means that in biology the internal and time-dependent conditions are decisive. The same body can behave very differently within the same conditions.

It is a general view that life can perfectly well emerge from the laws of physics plus accidents (cf. Gell-Mann, 1995). Indeed it seems that physics can describe any phenomenon by boundary conditions (describing the initial state) plus the laws of physics, with the qualification that the source of all occasional physical indetermination is chance. Actually, any physical state can be reached from a previous state with the help of chance. Nevertheless, biological behavior shows a remarkably consequent character that profoundly differs from the physical case, as the example of a living bird dropped from the Pisa tower indicates. The characteristic property of the trajectory of a living bird dropped from a height is that it regains, approximately, its original height. In general, biological behavior leads to the regeneration of the distance of the organism from thermodynamic equilibrium.

Thermodynamic systems are defined as consisting of statistically independent subsystems (Landau and Lifshitz, 1959). Now the Second Law of thermodynamics tells us that all isolated thermodynamic systems will develop towards equilibrium (ibid., section 8). Systems in thermodynamic equilibrium have independent, separable subsystems and so they manifest chance (e.g., thermal fluctuations) and necessity (the systems consisting of a large number of separable subsystems are governed by the determinate laws of physics). They cannot show *organized* changes, since their interactions are statistically independent and chaotic (ibid., section 1).

"Thermodynamics is the study of the macroscopic consequences of myriads of atomic coordinates, which, by virtue of the statistical averaging, do not appear explicitly in a macroscopic description of a system" (Callen, 1960, 7). In terms of complexity science, the random interactions of independent subsystems have no *lawful algorithmic complexity* representing the algorithmic complexity of the laws of nature (in the followings, shortly: algorithmic complexity), since their effects can be averaged out. In contrast, living organisms manifest an extremely high algorithmic and genetic complexity. Therefore the – let us use that term for the moment in a biological context – "subsystems" of living organisms do not form a pure thermodynamic system, and so their interactions cannot be averaged out to thermodynamic parameters like temperature or entropy only. In respect of biological behavior, living organisms are not thermodynamic systems. In living

organisms, after averaging out all statistically chaotic interactions, something remains, and this something has a fundamental importance in understanding biological organization. It seems inevitable to allow that the non-randomness of living organisms' subsystems is directly related to their observed, profoundly non-physical behavior. Actually, living organisms do not have subsystems comparable to the ones of a thermodynamic system, since biological organization extends from the level of the whole organism downwards to the level of molecules and beyond. This means that systematic dependences exist between the entities existing at the molecular, submolecular and supramolecular levels of biological organization. These systematic dependences represent *systematic* interactions and couplings.

It seems to be clear that if a systematic coupling exists between the subsystems in a way that determines the behavior of these subsystems, we indeed leave the realm of physical systems and enter to the field of cybernetics. It is important to keep in mind that the behavior of living organisms is much subtler governed than cybernetic machines. The non-random mechanical couplings between the components make it possible to show definite functions manifested in refrigerators and airplanes. Actually, the behavior of living organisms is also characteristically non-random. Their mechanical couplings (like that of the bones of an athlete) are originated in subtle biological couplings, determining the contraction of its muscles. These subtle, non-random biological couplings act between the myosin and ATP molecules, between the muscular cells and the global organism of the athlete. At the deepest level, biological couplings are related to couplings between thermodynamically downhill (exergonic) and uphill (endergonic) biochemical reactions. (Green and Reible, 1975; Purves et al., 1992, 1, 137) For the sake of precision, we note that thermodynamically downhill processes are defined here on the global level with the thermodynamic state variable extropy, while endergonic and exergonic reactions are qualified at the level of individual biochemical reactions.

The basic fact of life is the avoidance of thermodynamic equilibrium, which corresponds to death. Living organisms live by utilizing their nonequilibrium energies. Their functions require high-level forms of energy at their input and low-level forms of energy at their output. Thermodynamic aspects of living organisms are accompanied by equilibration or downhill processes. In order to avoid equilibrium, living organisms must continuously realize thermodynamically uphill processes compensating the downhill ones. Life in this respect is the consequent activity against thermodynamic equilibrium. Therefore, living organisms have a fundamental characteristic in compensating the equilibration downhill processes by uphill ones. The regular appearance of uphill processes may seem as contradicting the Second Law, but only when ignoring the simultaneous downhill processes. Most of these downhill processes also serve in useful biological roles, for example, dissipating "low quality" thermal radiation. This dissipation is required to balance the incoming high quality energy; and the low quality (e.g. lower temperature) of the output thermal radiation offers a net gain of useful

energy for the organism. Definitely, only with the help of biological couplings between the subsystems can the organism make its biological behavior so different from the physical.

4. Formulation of the Bauer Principle in Elementary Sentences

Regular compensation of equilibration processes with uphill ones requires a systematic work on the internal structure of the organism. In order to initiate uphill processes, regenerating nonequilibrium structures, gradients and potentials, living organisms must be able to work continuously against the thermodynamic equilibrium that otherwise ultimately would be reached given the actual instantaneous state of the organism on the basis of physical laws. This simplified chain of thoughts points towards the Bauer principle. The Bauer principle in its full form tells that "T*he living and only the living systems are never in equilibrium, and, on the debit of their free energy, they continuously invest work against the realization of the equilibrium which should occur within the given outer conditions on the basis of the physical and chemical laws*." Bauer had shown that this is the first principle of biology, since all the fundamental phenomena of life can be derived from it (Bauer, 1935/1967, 51).

Let us formulate this compact definition in elementary statements. Requirement (a) tells that living systems are never in equilibrium. Requirement (b) tells that on the debit of their free energy content, they continuously invest work against the realization of the equilibrium which should occur within the given outer (initial and boundary) conditions on the basis of the physical and chemical laws. We can break requirement (b) into (b1) requiring continuous and self-initiated work investment ΔW in order (b2) to initiate a behavior differing from the one determined by the laws of physics and chemistry. In our understanding, (b1) and (b2) tells that the investment of work ΔW must be thermodynamically uphill. Moreover, (b2) tells that if the considered system has elementary constituents with coordinates x_i, their spatial coordinates R have to differ in time from the one expected on the basis of physical and chemical laws, given the initial conditions. This means that the spatial trajectory of the constituent parts differ from the physical one by an amount $\Delta R(x_i, t)$. It is not allowed to simplify the Bauer principle to its requirement (a), or misinterpret it as requiring only the "avoidance of thermodynamic equilibrium". As our detailed analysis clearly shows, only the simultaneous fulfillment of all the three requirements (a), (b1) and (b2) is equivalent with the Bauer principle.

It is usual to consider that in physically spontaneous processes entropy can only increase. Actually, when a piece of matter exists in a colder/hotter environment, its entropy S will decrease/increase in the equilibration. Moreover, the free energy is defined through the change of the chemical potentialψrelative to the standard state corresponding to $T\psi = 298.16\,\mathrm{K}$ and $p\psi = 1\,\mathrm{atm}$ (Haynie, 2001, 81).

Therefore, the change of the entropy ΔS (and ΔG, the Gibbs free energy) of the system is not always a good indicator of thermodynamically downhill processes occurring within the considered system. Instead, thermodynamically downhill or equilibrating processes of physico-chemical systems can be characterized by the decrease of extropy Π, the distance from equilibrium (Martinás and Grandpierre, 2007) of the system ($\Delta\Pi < 0$). We define thermodynamically uphill processes here as processes in which the extropy of the system increases, $\Delta\Pi > 0$. Extropy is measured relative to the environment; therefore it always decreases in equilibration or downhill processes.

Systems receiving positive extropy flow from their environment, like self-organizing physical systems, or like living organisms, can manifest structure formation. In terms of extropy, one can formulate the Bauer principle as requiring an investment of work ΔW in order to initiate uphill processes $\Delta\Pi > 0$ compensating the equilibrating processes $\Delta\Pi < 0$ occurring in the system.

Now let us consider how the Bauer principle applies to physical self-organizing systems. Self-organizing physical systems like Benard-convection cells in a fluid heated from below have constant energy supply (through incoming energy flow from below) and extropy supply (they receive higher quality energy at their input and release lower quality energy at their output) and so their distance from thermodynamic equilibrium can be constant. The permanent transformation of higher quality energy into lower quality energy can be described as an extropy flow through the system maintaining the structure and internal organization in the cell balancing the downhill process of radiated heat. For such systems, the change of extropy within the system can be practically zero, $\Delta\Pi \sim 0$, *without any investment of systematic work by the Benard cells themselves*. Instead, their behavior is described by the laws of physics. This means that Benard cells do not fit the (b1) and (b2) requirements of Bauer principle.

We define a process as *thermodynamically spontaneous* if it occurs spontaneously, without any non-thermodynamic influence or intervention. Equilibrating processes occur by themselves, they are thermodynamically spontaneous. In comparison, *we define a process as biologically spontaneous if it occurs spontaneously in the presence of biological couplings*. Active transport regenerating a gradient is an uphill process; it cannot occur spontaneously in thermodynamics but can occur spontaneously in biology in the presence of suitable conditions and biological couplings. Now let us compare the range of physically spontaneous and biologically spontaneous processes. Although physical spontaneity is wide-ranged, including spontaneous emission, spontaneous absorption or spontaneous energy focusing at the wheel of a breaking car, biological spontaneity is much more wide-ranged, since it includes an astronomically rich realm of uphill processes which cannot occur spontaneously in thermodynamics. Therefore, systematic work investment also cannot occur spontaneously in thermodynamics. On the other hand, systematic work investment is a basic characteristic of living organisms required by the first principle of biology.

Complexity enters into the scene because *systematically directed useful work* is possible only by systems having a significant rate of algorithmic complexity. This is why machines require delicate planning and realization of a task-solving procedure having an algorithmic complexity. All machines serve some need or function. To obtain biologically useful, thermodynamically uphill work, living organisms must have extremely large algorithmic complexity. The first principle of biology holds that biologically useful work is exerted spontaneously in any part of the system in such a way as to promote the biologically optimal range, which corresponds to the characteristic distance of the organism from equilibrium.

Let us consider a simple example. A burning candle does not invest work on the debit of its free energy content. It does not have algorithmic complexity content in its structure. It does not fulfill requirements (b1) and (b2), therefore it cannot be regarded as living.

5. On the Nature of Biological Couplings

We indicated that biological couplings, in general, connect nonequilibrium energies. "Reactions that consume energy [endergonic reactions] can occur in living organisms only because they are coupled to other reactions that release it [exergonic reactions]" (Purves et al., 1992, 1). All biological transport is based on biological couplings (Harvey and Slayman, 1994). Biological coupling can occur due to chemical coupling with metabolic reactions or by coupling physical processes to chemical processes like energy or electron transfer, isomerizations, chemical bond-breaking or formation (Sundström, 2007). Ultimately, chemical bonds can be explained by quantum electrodynamics. The basic field of quantum electrodynamics corresponds to three basic types of actions: a photon goes from place to place, an electron goes from place to place, and an electron emits or absorbs a photon (Feynman, 1985, 84–85). These basic actions correspond to radiative energy transfer, linear energy transfer and light emission and absorption, respectively. Besides radiative and linear energy transfer, fluorescence (or Förster) resonance energy transfer, proton coupled energy transfer, and many-body phenomena like energy transfer through delocalized collective excitations (Dahlbom et al., 2002) also play important role in biological organization.

We find it of basic importance that biological organization always starts from the level of the organism/cell; the overall biological viewpoint breaks down into partial processes, into an organized system of more and more partial functions at the lower and lower level of organizational hierarchy, similarly as in the case of the more closely known overall reactions of metabolism, photosynthesis and respiration (Crofts, 2007, 17). In order that all these individual reactions, contributing to more and more global functions could sum up into the global level biological viewpoint, all these partial functions at the many levels of hierarchy

must be cohered. The mechanism securing the extremely fine tuning of all these partial functions must be more subtle than the biological processes themselves. We propose that the mechanism beyond the exquisite fine tuning of all these partial processes is governed by the most subtle process possible to realize in physics: by virtual interactions.

Actually, virtual interactions are governed in physics by the action principle (Feynman and Hibbs, 1965). Definitely, virtual interactions in living organisms must be governed by a separate, biological principle. We propose that biological couplings are realized by virtual interactions governed in living organisms by the biological principle.

In this way, we found that the fundamental requirements of the Bauer principle, when formulated as $\Delta W \rightarrow \Delta \Pi$, can be extended not only to $BC \rightarrow \Delta W \rightarrow \Delta \Pi$, but still further. Biological organization is initiated by the Bauer principle (BP) as manifested in virtual interactions VI, and so we can write it formally as $BP \rightarrow VI \rightarrow BC \rightarrow \Delta W \rightarrow \Delta \Pi$. Describing the complexity aspects of biological organization, we find that the deepest level of complexity of the Bauer principle is manifested in virtual interactions determining biological couplings, and these coupling processes determine the biochemical reactions representing a time-dependent series of reaction networks representing algorithmic complexity.

6. A Classification of Cosmic Life Forms

It seems that "All living organisms depend on external sources of energy to fuel their chemical reactions" (Purves et al., 1992, 1). We found that the first principle of biology, the Bauer principle corresponds to self-initiated work of the organism; and this work requires energy. We point out that this requirement can be helpful in exploring cosmic life. Within cosmic conditions, in principle, two types of living organisms can exist, both of which must obey the Bauer principle. The difference between them is that a living organism that belongs to the first class is supplying the required energy for internal work W directly from internal energy sources under its own control. A living organism of the second class has its own internal energy sources, but on relatively long timescales, it cannot indefinitely manage without external energy sources. Certainly, living organisms depending on external energy resources need to actively explore their spatial environment; that is, they must have the ability to change their place to obtain the required energy for internal work W. The basic forms of changing place are growth and locomotion, corresponding to plants and animals.

In contrast, living organisms of the first category, which have their own internal energy sources, are not obliged to growth or locomotion, for they can regulate their access to their own internal energy sources. In comparison, a machine with an accumulator does not invest work by its own initiation, since all the work it makes is prescribed in its program which is given to it externally.

Moreover, machines work in a way corresponding to the laws of physics plus the input conditions. Therefore, machines with accumulators do not qualify as living organisms, since they do not fulfill requirements (b1) and (b2).

7. On the Living Nature of the Sun

Now let us consider whether the Sun fulfils the Bauer criterion or not. Definitely, the Sun is a nonequilibrium system, fulfilling requirement (a). Regarding requirement (b1), we note that the systematic regeneration of solar activity in the solar cycles involves a systematic work investment. The generation of the activity forms, their quasi-cyclic regeneration during the whole lifetime of the Sun definitely fulfill requirement (b1). Regarding requirement (b2), it may seem that the Sun is overly complex, and because of this unfathomable complexity it is not possible to determine whether the behavior of solar activity corresponds to physical behavior or not. Moreover, the boundary conditions of the Sun (e.g. because of planetary motions) are continuously changing. Therefore, it seems that it is not easy to apply the conditions of the Bauer principle. We can overcome this difficulty if we find that physically unexpected phenomena show up systematically and regularly in the Sun. Actually, fundamental aspects of solar physics like solar structure and evolution are determined by the so-called Standard Solar Model (SSM). Remarkably, solar activity is missing from the SSM, and it does not follow from it. Although some consequences of solar activity like diffusion are already included into the SSM, solar activity still today represents an enigma (Grandpierre, 1996, 1999, 2004; Grandpierre and Agoston, 2005, and more references therein). Regarding these considerations, on the Bauer principle we can realize that the Sun is a living organism, because it initiates a systematic work for an activity-regenerating activity that seems to differ definitely from the corresponding physical behavior, given the same initial conditions.

Definitely, the term *systematic* work refers to the lawful algorithmic complexity content of the related processes. Let us consider now some complexity aspects of solar activity.

"The prime cause of the solar cycle is a quasi-periodic oscillation of the solar magnetic field" (Ossendrijver and Hoyng, 2001). Electromagnetic field has an unlimited potential to represent complex forms. Electromagnetic fields can vary from place to place both spatially and temporally, and their complete description may require an astronomically large amount of data. In stars like the Sun, these complex structures are related to filamentary structures, current sheets, plasmoids, etc. Remarkably, all these structures can form spontaneously within stellar interiors (Grandpierre, 2004; Grandpierre and Ágoston, 2005).

A whole list of fundamental facts showing the life-like nature of the Sun has already been advanced (Grandpierre, 1996, 1997, 1999, 2004; Grandpierre and Agoston, 2005). The Sun shows an organized spontaneous macroscopic activity

that is known as solar activity. Actually, solar activity is governed by the solar magnetic field; that is, it is a self-initiated activity. Solar activity has an extremely complex nature with respect to the wide variety of its forms (flares, sunspots, flocculi, coronal mass ejections, spicules, prominences, etc.), and its temporal and spatial scales. Solar activity has been shown to manifest a kind of *information* (Consolini et al., 2003).

Remarkably, the Sun has practically infinite degrees of freedom. This basic fact offers a new, wider perspective by which to consider the complex behavior of the Sun. The fact that solar activity has been present in the Sun for billions of years is, as we point out, an unusual condition for a physical system. Normally, one would expect that a thermodynamic system continuously dissipating energy and mass into its environment, like the Sun, equilibrates on its thermal timescale. Indeed, the Second Law of thermodynamics tells that any system without internal constraints storing energy in forms inaccessible to dissipation should approach thermodynamic equilibrium on the dissipation timescales. The dissipation timescale of thermal energy in the Sun is the Kelvin timescale and its magnitude is around 30,000 years. Nevertheless, solar activity regenerates the global magnetic field cyclically on a timescale of 11 years, and this cyclic activity has been going on in a timescale of 5 billion years. The problem is not only that there should be a mechanism regenerating thermal differences. In order for the Sun to be able to regenerate its cyclically disappearing magnetic field, cyclically changing sign and regenerating every 11 years (22 years if the polarity of the field is taken into account), the mechanism regulating the vectorial velocity space and magnetic field space must work systematically and apply in each cycle fine tuning.

We point out that in the real Sun the actual magnetic and velocity fields are highly complex. Definitely, on the basis that magnetic fields are governed by the Maxwell equations and hydrodynamic flows are governed by the laws of hydrodynamics, one would expect that they develop quasi-independently. Since the process generating magnetic field works repeatedly, and because fine-tuning is required in order to match the extremely complex velocity fields to the extremely complex magnetic field, we are led to assume the presence of a lawful fitting mechanism that acts from cycle to cycle. The consecutive and systematic variation of the field occurred already a hundred million times. Again, the hundred-million-times repeated exquisitely sophisticated co-operation of physically extremely improbable events presents a definite difference from the behavior one would expect merely on the basis of the initial conditions plus the laws of physics, fulfilling both requirements of the Bauer principle (b1) and (b2).

The fitting of the complex velocity and magnetic fields involves time-dependent internal boundary conditions that support regeneration of the magnetic activity. We propose that the fine tuning of such extremely complex fields cannot be repeated hundred million times requires without a rule or a law. It is a formidable task to modify the magnetic field and the velocity field in the whole body of the Sun from point to point just in a way that regenerates the magnetic activity forms. The solution of this task represents a significant amount of algorithmic

complexity. We are led to propose that solar activity represents algorithmic complexity. Algorithmic complexity is the characteristic of man-made machines and living organisms. Since the Sun is not a man-made machine, our proposal leads to the conjecture that the Sun is a living organism. Indeed, if the Sun represents an algorithmic complexity in its activity forms governed by the magnetic field, then the information content corresponding to the algorithmic complexity of the magnetic field's variations governs solar activity. Now it is a widely accepted view that living organisms can be defined as natural systems governed by information (see e.g. Roederer, 2003; Ben Jacob et al., 2006). Now since solar activity is governed by its information content corresponding to its lawful algorithmic complexity, the Sun is a living system.

8. Experiments Suggested Testing the Living Nature of the Sun

We suggest that terrestrial plants absorbing photon flux emitted by the Sun can serve as suitable measuring devices. Photons by their very nature are suitable to manifest information since light is the par excellence carrier of information. We are wondering how can the possibility that light emitted by the Sun carries information escape due attention – other than that of Tribus and McIrvine (1971), who suggested that the Sun emits information at the rate of 10^{38} bit s^{-1} in the form of light? If solar photons carry information, and if the Sun is a living organism, than solar photons can carry information about a cosmic life form, including biologically useful information arising from the Bauer principle. Certainly, during the hundreds of million years, biological life on Earth has already figured out how to utilize the astronomically huge flow of biologically useful information reaching the Earth from the Sun. In that way, terrestrial cells did not have to start from scratch, from the physical level of algorithmic complexity. Biogenesis on the Earth seems to be facilitated enormously by the information flow present in solar radiation carrying an enormous flux of algorithmic, and, perhaps, still deeper level of complexity.

And if so, then plants could react sensitively to deprivation of sunlight. In accordance with this expectation, tomatoes grown outdoors would be found to have better biological effects than tomatoes grown in greenhouses. We propose an experiment to grow tomatoes in solarium light and compare their biological effects with control tomatoes grown outdoors.

9. Life Forms Bridging Up the Gap Between Life and Non-life

Now we make a further step in exploring cosmic life forms by asking whether life can be continuous with the apparently inanimate world, as many scientists suggested (e.g. Nature, Editorial, 2007). We all know that highly organized life can

be manifest only when suitable conditions are present. Yet there are strong arguments telling that there is no sharp boundary between life and non-life. For example, quanta in the double-slit experiment are able to orientate themselves according to the situation as a whole and behave correspondingly (Grandpierre, 2007). Therefore, it seems that quanta conduct their behavior not only according to the laws of physics but also according to the situation as a whole. We attempt here to bridge the apparent gap between living organisms and quanta with the help of a series of steps generalizing the Bauer principle, replacing the requirement of systematic investment of work by some less restrictive conditions that can actually correspond to forms of cosmic life.

Let us try to approach the most general life form by recognizing the special properties of life as we know it on Earth and try to look at what we find if we remove these special properties from the concept of life. First of all, the difference between animals and plants is that animals are able to move. Usually, plants are motile, but are able to govern their shapes (as the Sun, too, regarding its activity forms).

The difference between a physical object and a living organism is that the living organism can select an endpoint for the action principle, like a living bird when dropped from a height, in contrast to a fallen stone which must follow the law of free fall. The fallen stone follows the least action principle, while the living bird follows the most action principle securing the maximum available distance from equilibrium. The selection of the endpoint for the most action principle produces an input for the first principle of physics securing the least action to be consumed. (Grandpierre, 2007) In order that an organism can move its parts like an animal or change its forms as a plant, it must be able to select an endpoint and govern its whole macroscopic structure towards reaching the selected state. In plants and animals, the conditions are such that they are able to realize such hierarchical organization from the global to the microlevel, continuously. It seems to be possible that there are systems in which the conditions necessary for realizing a selected macrostate through organizational processes across all hierarchical levels of organization are not present continuously. In such systems, endpoint selection cannot be realized continuously, but intermittently, or only occasionally. Microscopic and intermittent life may be present in the inorganic world in the form of occasional realization of the most action principle in microscopic processes. Hypothesizing microlife has a definite advantage of allowing life to be continuous with the inanimate world, since microlife in a physical environment without any forms of available free energy content can lead the same result as the least action principle. Clearly, if all the available free energy is zero, the maximum usable energy is identical with the minimum of it. This interpretation may explain the origin, nature and working mechanism of the least action principle, by the same token.

We may add that microlife can lead through relatively long time scales they can produce observable macroscopic consequences in geology and astrophysics. This kind of life form may be referred to as microlife at large or hidden life.

Microlife at large is different from macrolife in that macrolife organisms manifest biological behavior in their macroscopic changes like activity forms or locomotion, while microlife at large show variations only on geological or astronomical time scales.

Exploring cosmic life forms we are led to an unexpected and surprising result. This result tells that the universe may be full with cosmic life forms: stars with stellar activity cycles, intermittent life, microlife can populate the universe from cosmic clouds until stellar surfaces. If so, life can be truly a universal phenomenon, in a more full sense of the word as suspected until now.

10. On the Origin of the Anthropic Principle of the Universe

In the last decades, the fine-tuning of the fundamental constants of physics led to the wide ranged discussion of the anthropic principle (cf. Davies, 2006). We propose here a simple explanation for the fine-tuning of the fundamental constants. According to this proposal, the fundamental constants and laws of physics are in a certain sense secondary in comparison to the biological principle.

We indicated that within living organisms, it is the biological principle that acts first, and the physical principle acts only after the approximate range of biologically selected end states are determined. Considering cosmic life forms it is of importance to keep in mind that the biological principle is universal, similarly to the physical principle. Therefore, the biological principle has a fundamental cosmic aspect. If the biological principle acts first in the cosmic context, then all the material properties of the universe have to fit to biology. Our argument indicates that the thesis of the anthropic principle telling that fundamental constants of physics must fit to the existence of life is a corollary of our thesis telling that the universe is fundamentally alive and so biology is the control theory of physics.

11. On the Living Nature of the Universe

As the observations show, the distribution of matter is favorable for the organization of matter into cosmic clouds, for the birth of the Solar System and the life on Earth. The appearance of life and humans from a gravitationally contracting cosmic cloud seems to imply an increase of algorithmic complexity. We argue that such an increase of algorithmic complexity can be regarded as an important sign indicating the living nature of the universe.

We argue that our universe consists not only from elementary particles and forces, but also from the laws and first principles of nature *governing* interactions. A basic difference between forces and the laws of nature is that forces are local and instantaneous entities, while the laws of nature governing their evolution are universal. We propose that the laws and first principles of nature connect all

material systems of the universe into a unified whole. Now if the biological principle selects endpoints that are input elements to the first principle of physics, then the universe becomes unified as a biological system.

We indicated that the first principle of biology acts through virtual interactions realizing biological couplings that determine the material processes. Now if virtual interactions are ultimately controlled by biological interactions, then the vacuum has to have a fundamentally biological nature. We suggest that in this sense the vacuum qualifies as a living organism. By our argument, the biological vacuum qualifies as the ultimate cosmic life form. This cosmic life form can be referred to as universal life.

We point out that the exact definition and theoretical derivation of these cosmic life forms from the Bauer principle makes it possible to work on finding their observational signatures.

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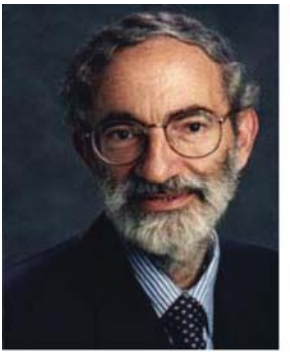

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ASTRONOMICAL AND ASTROBIOLOGICAL IMPRINTS ON THE FOSSIL RECORDS: A REVIEW

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1. The Common Frontier of Astronomy and Astrobiology

Both astronomy and astrobiology share a common frontier. Vertiginous progress in instrumentation such as novel microanalytical tools to study extraterrestrial materials, including those collected in space return missions, and availability of long ice cores and other fossil archives providing detailed records of the past terrestrial environment, can give deeper insights into the origin and history of life on Earth. The early stage of the Sun and other space-palaeoclimate conditions are relevant to the emergence of life on Earth.

The record of Earth's condition in the past is studied by different scientific communities involved in space palaeoclimate research. A set of data derives from historical observations of the solar surface. Other data are based on laboratory studies of matter derived from the surface of planets, the Moon, meteorites and comets (Pepin et al., 1981), which contain imprints due to past space weather conditions, such as implanted ions and radionuclides produced by nuclear reactions induced by high-energy cosmic rays. A final set of data derives from terrestrial archives, including tree rings, ice and marine sediment cores, corals, lake varves, manganese nodules and other crusts that grow slowly at the bottom of the ocean. All these systems contain a detailed record of proxies revealing Earth- and space-climate conditions in the past. Such information can be retrieved with advanced instrumentation, such as high sensitivity analyzers of stable and long-lived isotopes.

We shall focus our attention on space weather as a factor that is relevant for the origin and evolution of life on Earth. Then we will review possible changes in

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the evolution of life in general. Moreover, we will discuss possible clues contained in the fossil records of past life on Earth including some aspects of evolution, especially of humans, that may be due to space palaeoclimate. In general, the fossil record of the ancient Sun and of space palaeoclimate will yield insights into how our ecosystem may have evolved.

2. The Impact of Space Climate and Weather on Living Systems

During the early stages of the study of the origin of life (Oparin, 1953; Ponnamperuma and Chela-Flores, 1995) not enough attention was paid to the correlation between chemical evolution of Earth materials and variability of the early Sun (Messerotti, 2004) or remote events taking place in our galactic neighbourhood. Today, a meaningful study of the factors that may have led to an early onset of life on Earth begins to be possible due to the advent of a significant fleet of space missions and the possibility of performing experiments in the International Space Station (ISS). Our review lies within the scope of astrobiology (the study of the origin, evolution, distribution and destiny of life in the universe) and astronomy. Both disciplines should search analogous objectives, as we shall endeavour to illustrate with a few examples in this short review. Preliminary modelling of the Sun does not allow useful extrapolations into the distant past in order to study in detail the influence of solar physics on the emergence and early evolution of life on Earth (Jerse, 2006).

Electromagnetic and particle radiation that originate from the Sun, and from other space sources external to the solar system, are continuously impinging upon the Earth environment at different time scales and in a broad range of energies (Messerotti, 2004). The long-term evolution of the physical state of the space environment is referred to as *space climate*, whereas the short-term evolution is defined as *space weather (SpW)*. The interplay between the impinging energy carriers and the relevant impacts at the planetary level is determined by the complex physical couplings among the galactic, the solar and the terrestrial environments and the processes occurring therein. For instance, high-energy particles that originate from galactic sources, known as galactic cosmic rays (GCR), interact with the Earth atmosphere and generate showers of secondary particles such as muons and neutrons. It should be remarked that the flux of the GCR at the Earth depends on: (a) the position of the Sun in the Galaxy, since during its revolution around the galactic centre our star crosses environments richer or poorer of GCR sources on a time scale of 225 million years; (b) the activity level of the Sun. This contribution to the GCR flux is due to higher solar activity producing denser and faster solar wind. Hence, the particle flux is continuously accelerated by the star, which carries the solar magnetic field and fills up the interplanetary space by defining the region of space confined by the interstellar wind (heliosphere). When the solar wind is denser, it acts as a more efficient shield to the GCRs. Consequently, a lower GCR flux can reach the Earth; (c) the

strength of the Earth magnetic field, which acts as a further shield. Other materials reaching the Earth include meteorites, asteroids, comets and cosmic dust.

We can understand general trends of the influence of space climate and weather on the evolution and distribution of life. An important factor for understanding fully the origin and evolution of life on Earth is the evolution of the Sun and our galactic neighbourhood. We consider the constraints that present knowledge of our own star and its galactic environment imply for the emergence and evolution of life on Earth. This, in turn, will provide further insights into what possibilities there are for life to arise in any of the multiple solar systems that are known to date. Fortunately, the particles that have been emitted by the Sun or other galactic sources in the past have left a record in geologic samples in small bodies of the solar system in the Hadean (4.6–3.8 billion years before the present, Ga BP) and Achaean (3.8–2.5 Ga). In small bodies the geologic data has not been lost by metamorphism, as it has happened on the Earth. It is generally agreed that the latter period corresponds to the emergence of life, but we cannot exclude possible earlier dates for the onset of life on Earth.

The difficulty encountered in the simultaneous study of astrobiology and SpW is not insurmountable. Fortunately, considerable information can be retrieved from observations of extraterrestrial samples, either meteorites, or lunar material. Similarly, it is possible that we could retrieve bioindicators of the imprint that our galactic environment may have left on the fossil record of life on Earth. We will consider the fossils that represent an imprint of anomalous conditions in our environment since the Proterozoic. We have studied with special attention the records that may give some information on the factors favourable for life. Such data may be retrieved from the Sun during a period when fossils of animals were not available, during, or at the end of the Achaean. Such imprints are available in the upper layer of the lunar surface, on its regolith.

3. The Possible Role of Space Palaeoclimate in Mass Extinctions and Planetary Evolution

As suggested in the previous section, solar climate during the first Ga of the Earth was radically different. The earliest relevant factor was excessive solar-flare energetic particle emission, a phenomenon that has been recorded in meteorites (Goswami, 1991). These extraterrestrial samples provide information on events that took place during this early period after the collapse of the solar nebula disk. Gas-rich meteorites have yielded evidence for a more active Sun. A considerable number of young stars with remnants of accretion disks show energetic winds that emerge from the stars themselves. Similar ejections are still currently observed from our Sun. For this reason it is believed that some of the early Solar system material represented by meteorites could have retained the record of such emissions.

Information on the energetic emission of the Sun during this period can be inferred from data on X ray and UV emission (larger than 10 eV) from pre-main-sequence stars. We may conclude that during pre-main-sequence period,

solar climate and weather presented an insurmountable barrier for the origin of life anywhere in the Solar system. In the Hadean, conditions may still have been somewhat favourable, especially with the broad set of UV defence mechanisms that are conceivable. The high UV flux of the early Sun would, in principle, cause destruction of prebiotic organic compounds due to the presence of an anoxic atmosphere without the present-day ozone layer (Canuto et al., 1982, 1983). Some possible UV defence mechanisms have been proposed in the past, such as atmospheric absorbers and prebiotic organic compounds (Margulis et al., 1976; Sagan and Chyba, 1997; Cleaves and Miller, 1998).

Inversions of the Earth's geomagnetic dipole represent a well-established geochronological framework. The most recent of these inversions, referred to as the Matuyama–Brunhes (M–B) transition, has been dated to about 780 ka ago.

During a geomagnetic reversal, the dipole field strength is believed to decrease by about an order of magnitude. During this time, galactic cosmic rays can more easily penetrate into the Earth's atmosphere and thus increase the production of cosmogenic isotopes, such as ^{10}Be. Evidence has been presented for enhanced ^{10}Be deposition in the ice at 3,160–3,170 m, interpreted as a result of the low dipole field strength during the Matuyama–Brunhes geomagnetic reversal. If correct, this provides a crucial tie point between ice and marine core records (Raisbeck et al., 2006).

4. Traces of Space-Climate Events in the Geologic Record

The solar corona is the outermost region of the Sun's atmosphere. Its expansion induces a flux of protons, electrons and nuclei of heavier elements (including the noble gases). These interplanetary particles are accelerated by the high temperatures of the solar corona, to high velocities that allow them to escape from the Sun's gravitational field. The wind contains approximately five particles per cubic centimetre moving outward from the Sun at velocities of 3×10^5 to 1×10^6 m s^{-1}; this creates a positive ion flux of just over 100 ions cm^{-2} s^{-1}, each ion having an energy equal to at least 15 eV. The solar wind reaches the surface of the Moon modifying its upper surface or regolith. We have considerable information on the lunar regolith thanks to the Apollo Missions.

In the years 1969–1972 these missions retrieved so much material and made it available to many laboratories that influenced much of our preliminary understanding of the origin of life on the early Earth. These missions gave an opportunity for detailed studies of isotopic fractionation of the biogenic elements on the surface of the Moon. In general terms, the preliminary understanding that the Apollo Missions added to the work that was available at the time on meteorites was related to the fractionation of H, C, N and S on the lunar surface. In fact, the preliminary hint that was relevant for the origin of life was that the distribution range of ^{32}S/^{34}S appears to be narrower than the isotopic ratio of hydrogen, carbon or nitrogen. For this reason, it was suggested that the fractionation of S isotopes would be the most reliable parameter for estimating biological effects

(Kaplan, 1975; Chela-Flores, 2007). Deviations of $^{32}S/^{34}S$ from meteoritic values discovered on the Moon by the Apollo missions can be understood by the fact that the solar wind modifies its structure leaving a tell-tale signal of how it changes over geologic time, since the Moon is an inactive body being modified only by the impacts of meteorites and asteroids.

Much more recently, the Genesis Mission was NASA's first sample return mission sent to space. It was the fifth of NASA's Discovery missions. Genesis was launched in the year 2001 with the intention to bring back samples from the Sun itself. Three years later, after crash-landing, the probe was retrieved in Utah, USA. Genesis collected particles of the solar wind on wafers of gold, sapphire, silicon and diamond. The amount of stardust collected by Genesis was about 10^{20} ions, or equivalently, 0.5 mg. Preliminary studies indicate that contamination did not occur to a significant extent. The objective is to obtain precise measures of solar isotopic abundances. By measuring isotopic compositions of oxygen, nitrogen, and noble gases we would have data that will lead to better understanding of the isotopic variations in meteorites, comets, lunar samples, and planetary atmospheres. This will lead to a deeper understanding of the early Solar system, and hence an additional opportunity beyond fossils for a closer approach to the mystery of the origin of life on Earth by being able to assess properly potential biomarkers that may be suggested from the point of view of biogeochemistry.

There will be also attempts to use Accelerator Mass Spectrometry (Tuniz et al., 1998) to detect a long-lived radionuclide of solar wind origin, for example such as ^{10}Be and ^{26}Al (Jull and Burr, 2006).

The Moon is depleted of volatile elements such as hydrogen, carbon, nitrogen and the noble gases, consistent with the fact that the most widely accepted theory of its formation is the impact of the Earth by a Mars-sized body during the accretion period. Exceptionally though, volatiles are abundant in lunar soils. The lunar surface evolved during the heavy bombardment period, adding material with a different composition to the Sun, and not derived from the Sun: The variability of ^{14}N and ^{36}Ar in grains (single mineral and glass) from a lunar soil were measured by laser extraction, to study the origin of trapped nitrogen in the lunar regolith (Wieler et al., 1999). The ratio of N is very uniform relative abundances of Ar, Kr, and Xe trapped from the solar radiation observed in mineral grains from the same soil. This strongly suggests that, on average, some 90% of the N in the grains has a non-solar source. This seems to suggest that the non-solar N has not been trapped by ion implantation.

Ions from the solar wind were known to have been directly implanted into the lunar surface (Kerridge et al., 1991). This component was detected during the Apollo missions. The isotopic composition of the noble gases in lunar soils has been established as being subsequent to the formation of the Moon itself.

The production of a long-lived radionuclide on the moon can provide information about the flux of galactic and solar cosmic rays in the past. This can also be done on meteorites from the moon (Gnos et al., 2004).

To gain further insights into the early Solar system, evidence has been sought for a predominantly non-solar origin of nitrogen in the convenient source of information that is represented by the lunar regolith. This search suggests that,

on average, some 90% of the N in the grains has *a non-solar source*, contrary to the view that essentially all N in the lunar regolith has been trapped from the solar wind, but this explanation has difficulties accounting for both the abundance of nitrogen and a variation of the order of 30% in the $^{15}N/^{14}N$ ratio. The origin of the non-solar component remains a puzzle, but it presumably must have changed its isotopic composition over the past several billion years. The Moon regolith presents a very challenging geological phenomenon. It consists of a very large number of grains with a rich history regarding their exposure to the Sun. Two parameters are useful in the systematic study of the lunar regolith: firstly, its '*maturity*' namely, the duration of solar wind exposure and, secondly the '*antiquity*', namely, how long ago the exposure took place.

For the maturity parameter a useful way to measure it is in terms of the abundance of an element from the solar wind that is efficiently retained. The element nitrogen is a good example. (Alternatively solar noble-gas elements can be used.) Both antiquity and maturity have been used to learn about the evolution of the early Solar system, especially the ancient Sun, the knowledge of which is needed for a comprehensive understanding of the problem of the origin of life on Earth. The exposure age to galactic cosmic rays produce certain nuclides in amounts proportional to the time the sample spends at the topmost part of the surface (some 2 m). The contrast between the known low abundance of a certain nuclide and the one induced by cosmic rays produce an indicator of antiquity. The antiquity parameter has been discussed in detail (Kerridge, 1975). A related question is the search for live ^{244}Pu (half-life = 81 Ma) that is expected to be present in the interstellar medium (ISM) from ongoing nucleosynthesis. The use of resonant ionization mass is capable of detecting extremely low levels of this isotope that may have accreted onto Earth from the ISM (Ofan et al., 2006).

5. Evidence in the Geologic Record of Extinctions by Impact of an Asteroid or Comet

5.1. EXTINCTIONS DUE TO EXOGENOUS SOURCES

Evidence for impact from the geologic boundary between the Cretaceous and Tertiary periods (the so called K/T boundary) is the Chicxulub crater in the Yucatan Peninsula in Mexico, and the global distribution of an anomalous iridium (Ir) layer (Keller and Stinnesbeck, 2000). In addition, impact ejecta such as pressure-shocked mineral grains support the meteorite impact hypothesis. Perhaps the better discussed evidence for the K-T impact at 65 Ma (million years ago) was the Ir-abundance coinciding with the geologic evidence of mass extinction. In spite of the very abundance of Ir in well-studied meteorites, the Ir-rich deposit may alternatively be interpreted as volcanic ejecta. In other words, the possibility remains that the layer could instead have been produced by volcanic Ir-rich eruptions.

The Permian period gave way to the Triassic at about 251 Ma. At that time the Earth experienced its greatest mass extinction known to us. Ninety percent of

all marine species, including the trilobites, disappeared, while on land pervasive extinctions opened the way for the rise of the dinosaurs. But despite the magnitude of mass extinction its cause is a source of controversy (Kerr, 2001).

A new analysis of rock that marks the Permian-Triassic (P-T) extinction now suggests that it was caused by the hypervelocity impact of an asteroid or comet similar to the one thought to have led to the extinction of dinosaurs at the K-T boundary (Kaiho et al., 2001). There is some evidence for some catastrophic event that gave rise to the P-T extinction. Paleontologic evidence seems to suggest that a single event may have been responsible for the P-T transition. One such possibility shall be discussed in the next section.

Noble gases such as helium and argon apparently were trapped in molecular cages of carbon (fullerenes). This hypothesis follows the extraction of the gases from rocks at the P-T transition (Becker et al., 2001). Analyses of these gases show that their isotopic compositions are analogous to those found in meteorites, and are not typical of the Earth-bound abundances. This is some evidence that a major impact may have delivered the noble gases to Earth at the time close to the period when the extinctions did take place. Indeed, this suggestion provides an indicator for a P-T impact that is analogous to the earlier theory of the impact at the K-T boundary, an event that we saw above to have been supported by the Ir-data.

Fullerenes are also candidates for indicators of impact. Previous work by others showed that they are present in rock at the K-T boundary (Heymann et al., 1994). Together these findings suggested that fullerenes are the product of the high pressures and temperature generated in the collision and are impact markers like iridium. That prompted Becker and her colleagues to look for the compounds in rock at the P-T boundary at the in South China, and in southwest Japan and reported the detection of fullerenes in boundary rock, but not in similar rock a few centimetres to meters above, or below the boundary. However, it should be kept in mind that fullerenes can be produced by, for instance, forest fires.

In the case of the K-T mass extinction shocked quartz was detected, (i.e., crystals containing distinctive lamellae made only in the extreme pressures of large impacts). Shocked quartz has not been identified with the same certainty at the P-T transition, but the noble gas indicators may offer additional evidence. Fullerenes can trap gas atoms. When the gases trapped in fullerenes from P-T-boundary rocks was analyzed (Becker et al., 2001), it was found that the abundance of helium-3 was significantly enhanced above what it was immediately above or immediately below the boundary. The ratio of helium-3 to helium-4 was typical of meteorites. Besides the ratio of argon-40 to argon-36 in boundary fullerenes is likewise analogous to that of meteorites.

5.2. EXTINCTIONS DUE TO ENDOGENOUS SOURCES

The warming caused by volcanoes through carbon dioxide emissions would not be large enough to cause mass extinctions by itself. That warming, however, could set off a series of events that may have led to mass extinction. During the P-T extinction 95

percent of all species on Earth became extinct, compared to only 75 percent during the K-T when a large asteroid apparently caused the dinosaurs to disappear.

Volcanic carbon dioxide would cause atmospheric warming that would, in turn, warm surface-ocean water. Normally, the deep ocean gets its oxygen from the atmosphere at the poles. Cold water there soaks up oxygen from the air and because cold water is dense, it sinks and slowly moves equator-ward, taking oxygen with it. The warmer the water, the less oxygen can dissolve and the slower the water sinks and moves toward the equator (Kump et al., 2005).

The constant rain of organic debris produced by marine plants and animals, needs oxygen to decompose. With less oxygen, fewer organics are aerobically consumed. In the Permian, if the warming from the volcanic carbon dioxide decreased oceanic oxygen, especially if atmospheric oxygen levels were lower, the oceans would be depleted of oxygen. Once the oxygen is gone, the oceans become the realm of bacteria that obtain their oxygen from sulphur oxide compounds. These bacteria strip oxygen from the compounds and produce hydrogen sulphide. Hydrogen sulphide kills aerobic organisms.

Humans can smell hydrogen sulphide gas, the smell of rotten cabbage, in the parts per trillion range. In the deeps of the Black Sea today, hydrogen sulphide exists at about 200 parts per million. This is a toxic brew in which any aerobic, oxygen-needing organism would die. For the Black Sea, the hydrogen sulphide stays in the depths because our rich oxygen atmosphere mixes in the top layer of water and controls the diffusion of hydrogen sulphide upwards. At the end-Permian, as the levels of atmospheric oxygen fell and the levels of hydrogen sulphide and carbon dioxide rose, the upper levels of the oceans could have become rich in hydrogen sulphide catastrophically. This would kill most the oceanic plants and animals. The hydrogen sulphide dispersing in the atmosphere would kill most terrestrial life. Another aspect of this extinction is that hydrogen sulphide gas destroys the ozone layer. Once this process has started, methane produced in the ample swamps of this time period has little in the atmosphere to destroy it. The atmosphere becomes one of hydrogen sulphide, methane and ultra violet radiation.

Biomarkers of photosynthetic sulphur bacteria in deep-sea sediments were recently reported in shallow water sediments of an age comparable to the P-T transition (Grice et al., 2005). These bacteria live in places where no oxygen exists, but there is some sunlight, as it may have happened at the end of the Permian. Confirming the evidence for these microorganisms would suggest hydrogen sulphide to have been the cause of the mass extinctions. The question remains however whether the extinction may have been the effect of another underlying process.

6. Are There Possible Traces of Catastrophic Space-Climate Events in the Hominid Fossil Record?

The role of cataclysmic events in the evolution of life on Earth has been discussed in recent times. Tobias has suggested the possible causal connection between large impacts such as the Vredefot impact structure in the Free State (it is the latest

World Heritage Site to be listed in South Africa). Its reconstructed diameter of 250–300 km was made by a projectile estimated at 10–15 km in diameter, which collided with the Earth at 2.1 Ga (Tobias, 2005).

This impact coincided with two significant events in the evolution of life on Earth, namely the oxygenation of the atmosphere and the first appearance of the eukaryotes. Although Tobias attempts to make a causal connection between the large impact and these two events, an approach that he calls catastrophism, there are alternative explanations as discussed by others: in the case of the oxygenation of the atmosphere (Abelson, 2007), and the first appearance of the eukaryotes (Chela-Flores, 1998). But even if the alternative explanations are maintained, what is true is that the Vredefot impact illustrates the major implications that extraterrestrial events, not only SpW, as illustrated in this paper, but even planetary sterilizations going back into the Phanerozoic and extending back to the Hadean.

Tobias insists that milder environmental impacts might have been relevant in the evolution that led to *Homo sapiens*. For instance, about 2.6–2.5 Ma marked climatic changes in Africa that were associated with uplift of its southern and eastern parts. The ensuing cooler and dryer weather was accompanied with significant changes in the paleontological record:

(a) Extinction of the small-brained hominids *Australopithecus africanus*
(b) The earliest appearance of *Homo* of the species *Homo habilis*
(c) The first signs of the enlargement of the hominid brain, as compared with the smaller brains of the australopithecines

The possibility of a supernova explosion near the Solar system has been discussed for a long time (Ruderman, 1974; Reid et al., 1978; Ellis and Schramm, 1995). Such a nearby supernova explosion can be confirmed by the detection of radioisotopes on Earth that were produced and ejected by the supernova. A measurement of a well-resolved time profile of the ^{60}Fe concentration in a deep-sea ferromanganese crust showed a significant increase 2.8 Ma (Knie et al., 2004). The amount of ^{60}Fe is compatible with the deposition of ejecta from a supernova at a distance of a few 10 pc. The well-defined time of the supernova explosion makes it possible to search for plausible correlations with other events in Earth's history. Other possible radionuclides for tracing supernova explosions are 182Hf (8.9 Ma) (Vockenhuber et al., 2004), 244Pu (81 Ma) (Winkler et al., 2004).

The profile of the ^{60}Fe concentration in the deep-sea ferromanganese crust has been considered in terms of the environmental changes that were relevant for *Homo* evolution (a-c) According to the authors (Knie et al. 2004), at the time of the supernova explosion there was an increase of the cosmic radiation of a few percent that lasted for some thousand years. They claim this might have triggered climate change in Africa, causing significant developments on hominid evolution. This effect would in any case be superimposed on other phenomena causing climate change, such as

tectonic activities (like those that gave rise to the Great Rift Valley in Africa), as well as other global phenomena.

Besides, this event could have a significant effect on the ozone layer. Hence it may have had an effect on the natural UV filter that led to the present Earth biota. Improved tools for detailed modelling of atmospheric chemistry have been developed to calculate ozone depletion, and advances have been made also in theoretical modelling of supernovae and of the resultant gamma-ray spectra. In addition, we now have better knowledge of the occurrence rate of supernovae in our galaxy and of the spatial distribution of progenitors to core-collapse supernovae. The results of two-dimensional atmospheric model calculations estimates (Gherls et al., 2003) are interesting in this respect, since they take as input the spectral energy distribution of a supernova, adopting various distances from Earth and various latitude impact angles. In separate simulations there is an estimate of the ozone depletion that is due to cosmic rays. These calculations suggest that for the combined ozone depletion from these effects roughly to double the "biologically active" UV flux received at the surface of the Earth, the supernova must occur at 8 pc. Based on the latest data, the time-averaged galactic rate of core-collapse supernovae occurring within 8 pc is 1.5 Ga.

In principle, high-energy galactic cosmic rays could be also responsible for genetic changes related to human evolution. Some groups have been searching for discrepancies in the production rate of stable cosmogenic radionuclides, such as ^{21}Ne and radioisotopes with different half-lives such as ^{10}Be, ^{26}Al and ^{53}Mn that might indicate time variation in the galactic cosmic-ray flux within the solar system. The existing data do not support major variations in cosmic ray intensity within the past 5 million years, crucial period for the evolution of the *Homo* species (Moniot et al., 1983). A huge asteroid collided with the Earth in South East Asia around 780,000 years ago, with devastating environmental effects. Magnetic properties of rocks formed at that time show that the impact of the extra-terrestrial body might have caused the Brunhes-Matuyama magnetic reversal. Tonnes of tektites – obsidian-like pebbles produced by the fusion of sediments during the impact – were launched into the air and scattered all over South-east Asia and Australia. Some scholars connect this environmental disaster to the introduction of advanced Acheulean-like technology in Asia during this period.

More recently it has been suggested that a comet or asteroid exploded over North America 13,000 years ago. This event wiped out a Stone Age culture known as Clovis, as well as the mammoth and the mastodon. This event may have caused a major shift in the climate, the well-known Younger Dryas cooling event. The cooling produced may also have affected humans in Europe and Asia (Firestone et al., 2007). A detailed analysis of the sediments corresponding to 13 ka ago reveal a high concentration of extraterrestrial (ET) markers such as glass-like beads, soot and fullerenes, materials that are absent in other layers of the stratigraphy. The glassy beads could only be produced by melting carbon at 4,000°C. Electron microscope analyses show the glassy spherules are reach in micro-diamonds. Diamonds are produced in the interior of the Earth by compressing carbon at the pressure of several gigapascals.

These conditions could be produced on the surface of the planet only by the impact of a massive extraterrestrial body.

Finally, we should mention that the evolution and dispersion of the *Homo* species during the last 2 million years was strongly conditioned by the variable climate of Earth, driven by changes in the Earth's orbit around the Sun as proposed by Milankovitch in the 1920s. Three variations of the Earth's orbit are considered, eccentricity, obliquity and precession, which affect the quantity of sunlight hitting the Earth's surface. They are the main cause of the ice ages during the Pleistocene, characterised by periods of about 100,000, 40,000 and 20,000 years, respectively, as confirmed by the ice fossil record in sea sediments.

7. Beyond the Sun: Influence of Our Galactic Environment on Life on Earth

In this section we shall discuss two possible sources of SpW factors that may have influenced the evolution of life on Earth and such factors may be reflected in the fossil record: gamma ray bursts (GRBs) and cosmic rays.

Firstly, GRBs are powerful explosions that produce a flux of radiation detectable across the observable Universe. These events possibly originate in distant galaxies, and a large percentage likely arises from explosions of stars over 15 times more massive than our Sun. A burst creates two oppositely directed beams of gamma rays that race off into space. If a GRB were to take place within the Milky Way we would have to consider the possibility of mass extinctions comparable to the other known sources, such as the meteoritic collision with the Earth (cf., the next section), or a singular abundance of sulphur in the atmosphere due to the causes that are reviewed below. Mass extinctions have eliminated a significant fraction of life on Earth. For example, the most severe extinction of the past 500 million years occurred in the Late Permian (Erwin, 1994). The large masses of the first stars suggest that they may have produced supernovas at the end of their relatively short lifetimes. Such events may in principle be detectable as GRBs at very large red shifts, which may be detectable with the SWIFT satellite (Hartmann, 2005; Markwardt et al., 2005).

A number of other astrophysical objects also produce GRBs, such as quasars and neutron stars. Quasars were also forming at around $z \sim 6$, so part of the challenge is to identify the proper GRB source (Xu et al., 2005). GRB, together with meteoritic collisions, or an atmosphere that has gone through a transition unfavourable to the Earth biota are three likely causes that need to be discussed together, as we have attempted to do in the present review.

The Ordovician is the second oldest period of the Palaeozoic Era, thought to have covered the span of time between 505 and 440 million years before the present Ma BP. The late Ordovician mass extinction took place at approximately 440 Ma BP may be at least partly the result of a GRB. Due to expected depletion of the ozone layer arising from the incoming energetic flux, the solar ultraviolet radiation that is normally shielded would give rise to a severely modified ecosystem.

It is known that all marine animals suffered mass mortalities during the Late Ordovician Mass mortalities at the close of the Cambrian and late in the Ordovician resulted in the unique aspects of the Ordovician fauna.

The Swift mission, launched in November 2004, contributes to determine recent burst rates. During evolution of life certain events triggered large-scale extinctions. We consider one of the most remarkable possible candidates. The Late Ordovician extinction created new opportunities for benthic and planktonic marine fauna. Biological radiation during post-Ordovician glaciation led to many new taxa typical of the Silurian. GRBs within our Galaxy have been repeatedly suggested to be a possible threat to life on Earth (Thorsett, 1995; Scalo and Wheeler, 2002; Melott et al., 2004).

Some effects similar to those due to a nearby supernova should be expected. GRBs are less frequent than supernovae, but their greater energy output results in a larger region of influence, and hence they may pose a greater threat. It is likely (Melott et al., 2004) that in the last billion years (Ga), a GRB has occurred close enough to have dramatic effects on the stratospheric ozone, leading to detrimental effects on life through increases in solar ultraviolet (UV) radiation, which is strongly absorbed by ozone. A major question has been the timescale for atmospheric chemistry: most of the GRB influence comes in seconds or minutes as compared to months for the case of supernovae.

There is no direct evidence that such a burst activated the ancient extinction. The conjecture is based on atmospheric modelling (Thomas et al., 2005). The main conclusion to be derived from these calculations is that gamma-ray radiation from a relatively nearby star explosion, hitting the Earth for only ten seconds, could deplete up to half of the atmosphere's protective ozone layer. Recovery could take at least five years.

With the ozone layer damaged, ultraviolet radiation from the Sun could kill much life on land and near the surface of oceans and lakes, and disrupt the food chain. Nevertheless it is important to recall, as we shall do in the next two sections based on the fossil record that there are two other competing theories for mass extinctions during earlier geologic periods, such as the suggested Ordovician mass extinction.

A related issue of SpW besides GRBs is whether cosmic rays may have left their imprint in the fossil record. To answer this question we may recall some recent research related to the rationalization of observed cycles in the fossil diversity (Kirchner and Weil, 2005).

As the Earth's solar system travels around the centre of the Milky Way galaxy, it also wobbles up and down from the galaxy's disc. U.S. scientists found that these swings take about 62 million years to complete – thus, may expose the Earth to higher doses of dangerous cosmic ray that may also cause mass extinctions. One complete orbit around our galaxy takes the solar system about 225 million years to complete. So, we go through about four of these cycles above and below the galactic plane during one orbit around the galaxy. (The galactic plane as the plane that is contained within the equator of the Milky Way galaxy, with the centre of the galaxy being the origin of this galactic coordinate system.) The

modulation of the cosmogenic nuclide production expected from the galactovertical motion of the solar system was evaluated earlier (Vanzani et al., 1987). The time distribution of ^{10}Be concentration predicted by the model appears to be consistent with the data of deep-sea sediments.

The translation between the northern and southern sides of the galactic plane happens due to mass and gravity. When the solar system is on the northern side of galactic plane, the galactic mass located in the southern part uses its gravity to pull the solar system back down. Similarly, the northern galaxy mass, through the gravitational force, displaces the solar system from the southern side. A large amount of fossil data that covered an era of over 500 million years has been published (Sepkoski, 2002). Further studies suggest that living things on the Earth have been at their greatest risk of extinction every 62 million years or so for the past 542 million years (Rohde and Muller 2005).

This suggests that living in the south side of the galactic plane of the Milky Way may be safer for humans and all living things here on the Earth. Cosmic rays strike the Earth on their travels from a large cluster of galaxies in the direction of the Virgo constellation (Medvedev and Melott, 2007). Our own galaxy is moving toward the Virgo constellation in the northerly direction. So, when the solar system is on the north side of the Milky Way's plane, we are being bombarded by more cosmic rays from the Virgo constellation. The more cosmic rays that hit the Earth, the more that these energetic particles could possibly cause various problems such as changes in weather and climate, damage to DNA within humans and other animals, and mass extinctions. This work suggests that mass extinctions may very likely correspond to peaks in cosmic rays when the Earth is at its maximum northerly distance from the galactic plane.

8. Discussion and Concluding Remarks

The main thesis that we have maintained in this work is that solar activity, space climate and astrobiology should be brought within a unified framework that would include contributions from other disciplines relevant to the past of environments in the solar system. This approach naturally leads us to the suggestion of exploiting instruments and methods from somewhat dissimilar sciences (astronomy and astrobiology) with a unified objective (Messerotti and Chela-Flores, 2007a, b).

We have attempted a preliminary comprehensive discussion of how research in the conditions of the early Sun combine with observations in several disciplines giving insights into the factors that lead to the emergence of life in a given Solar system: biogeochemistry, lunar science, cosmochemistry, chemical evolution, palaeontology, palaeoanthropology, palaeoecology, geochronology and oceanography, amongst others. These considerations are necessary for a holistic approach to understand the conditions that allow life to emerge and evolve elsewhere in the universe.

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DO IMPACTS REALLY CAUSE MOST MASS EXTINCTIONS?

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The great tragedy of science—the slaying of a beautiful hypothesis by an ugly fact.

Thomas Henry Huxley

For every problem, there is a solution that is simple, neat, and wrong.

H.L. Mencken

Abstract For the past 28 years, the trendy "bandwagon" in the geosciences has attempted to explain most mass extinctions by extraterrestrial impact events. However, the past decade of research has shown no significant evidence of impacts at any mass extinction horizon except for the Cretaceous-Tertiary boundary at 65 Ma. In fact, numerous paleontologists have even questioned whether the Cretaceous-Tertiary impact was as important as once supposed. Alleged impact horizons at the other major mass extinctions have proven to be of the wrong age or the wrong size. By contrast, there were many major impacts (especially in the late Eocene) that had no effect on life whatsoever, further falsifying the impact hypothesis as a general explanation. Explaining all the major mass extinctions by huge mantle-derived flood basalt eruptions has also failed, as such eruptions occur at only three of the extinction horizons. Attempts to find a general explanation that explains all mass extinctions are usually unsuccessful, because there are no common signals at every mass extinction. Each event shows a distinct and different pattern. Currently, the trendy model postulates high carbon dioxide and low oxygen in the atmosphere, although it only works for the Permo-Triassic, Triassic-Jurassic, and possibly Paleocene-Eocene events, and no others, and it has not fared well in preliminary testing.

1. Introduction

Before 1980s, the topic of mass extinctions was relatively unstudied and poorly known, with only a handful of papers published by paleontologists, and almost no testable hypotheses about their causes. But in the 1980s, mass extinction and its causes became the hottest topic not only in paleontology, but spilling over to earth sciences and even astrophysics. The crucial trigger for this change was the

discovery in 1978 (published by Alvarez et al., 1980) of abnormally high levels of iridium at the Cretaceous/Tertiary (K/T) boundary near Gubbio, Italy. Alvarez et al. (1980) interpreted this discovery as evidence of an impact of a 10-km asteroid, which supposedly caused dust clouds that darkened and cooled the earth, and wiped out the non-avian dinosaurs, ammonites, and many other creatures that did not survive into the Cenozoic.

The appeal of this hypothesis was immediate, because it offered ways to test whether it had occurred, and what its effects might be, and also because it postulated a catastrophic mechanism that brought geologists and astrophysicists into a research area normally reserved for stratigraphers and paleontologists. The "K/T impact bandwagon" then got rolling, with many different scientists tackling the problem, and within a decade there was a scientific literature of several thousand papers, as well as numerous trade books. Soon the impact advocates were seeking evidence of iridium and other impact products in nearly every other mass extinction horizon, and claiming that they had found it in many of them (reviewed below).

Along with the "bandwagon" of impact advocates claiming they had found the cause for this or that mass extinction was another parallel argument. In 1984, Raup and Sepkoski (1984, 1986) made the claim that mass extinctions were periodic, occurring approximately every 26 million years. They based this on what they interpreted as major pulses of extinction in their database of fossil marine families and genera. As Raup (1986) details, astronomers jumped the gun on this idea, publishing explanations ranging from periodic comet showers (Davis et al., 1984), the oscillation of the solar system through the galactic plane (Rampino and Stothers, 1984; Schwartz and James, 1984), an unknown Planet X (Whitmire and Jackson, 1985), and an undetected companion star to the Sun dubbed Nemesis (Whitmire and Jackson, 1984). Most of these papers were written based on the preprint of Raup and Sepkoski (1984), before it had even undergone peer review, let alone much critical testing from the paleontological community.

Unfortunately for this exciting hypothesis, several ugly facts have completely discredited the periodicity model (see review in Prothero, 2004a, 94–95). No evidence for Nemesis or Planet X has ever been found, nor any evidence tying comet showers or the motion through the galactic plane to mass extinctions. Many statisticians challenged the statistical robustness for the periodicity model. The taxonomic basis for much of the "periodicity" has been debunked, since many of the "extinction peaks" of Raup and Sepkoski (1984) are not real, or are nowhere near the 26 million year spacing required by the model. Most importantly, the periodicity model requires some sort of regular external forcing factor in the environment (comets, Nemesis, Planet X), so each mass extinction horizon should show a common signal of its causation. As we'll see below, they don't.

After a decade of controversy, Stanley (1990) proposed a model that represents the general consensus among paleontologists for why extinctions appear spaced by at least 20 million years. According to his hypothesis, the "aftermath" world of major mass extinctions is inhabited by a low diversity of "weedy"

opportunistic survivor species, which are extinction-resistant and persist for a long time after the event. It takes a full 10 to 20 million years after a major extinction for life to recover its former high diversity, complete with vulnerable ecological specialists that might be prone to the next mass extinction. If there were a major impact too soon after a mass extinction, it would have little or no biotic effect.

For most scientists, the periodicity hypothesis is long dead. However, some do not give up. Rohde and Muller (2005) claim to have found a 62-million-year extinction periodicity in the long-discredited Sepkoski database. Firestone et al. (2007) recently postulated that the late Pleistocene megafauna of North America was wiped out by impact, although their model does not explain which so many large mammals (especially bison) survived while others did not. In addition, it does not address a whole host of other problems (Pinter and Ishman, 2008). Other elements of the mass extinction debate are still widely accepted among those who do not keep up with the paleontological literature or attend the professional meetings. Due to its high public profile and "sexy" nature, the impact hypothesis for the K/T extinction is still widely believed among the public, and among non-paleontologists. The idea that an impact killed the dinosaurs is hot enough to make the cover of *Time* magazine, whereas most of the public knows nothing else about the K/T extinction or any other extinction (including the much larger Permo-Triassic extinction). Most of the trade books and now even many of the textbooks repeat the idea from the 1980s that the K/T extinction, and most of the other major mass extinctions were caused by impacts. But research on these topics has not stood still, even if it no longer dominates the spotlight as it did in 1980s. A lot of work has been completed on the detailed record of the major mass extinction horizons, and the past decade of research results have not gone favorably for the impact advocates (Prothero, 1999, 2004a, b; Keller, 2005; Lucas, 2005; White and Saunders, 2005; Morrow, 2006; Ward, 2007).

In this paper, I will review the recent evidence for the impact hypothesis, and the current evidence for the causes of the major mass extinctions.

2. Did an Impact Kill Off the Dinosaurs?

This is still the most widely accepted notion, with polls revealing that a majority of geoscientists and astronomers believing it to be true. Largely because of the intense publicity associated with the Alvarez et al. (1980) discovery and its aftermath, it has entered the consciousness of many scientists who do not keep up with the details of the paleontological and stratigraphic literature, or attend professional meetings that discuss the latest developments in this topic. In their favor, the K/T impact is the only one that has produced a crater of the right age, Chicxulub in the Yucatan Peninsula of Mexico.

But throughout the entire K/T impact debate over the past 28 years, there has always been a strong resistance to overly simplistic ideas about the K/T extinction. At first, skeptics challenged the reality of the impact, but even after

Chicxulub crater was found in 1990, this did not solve the entire problem. As I reviewed in my recent book (Prothero, 2006, Chapter 2), understanding the K/T extinction has always been complicated by the fact that at least two other significant events are also occurring at the end of the Cretaceous. One is the eruption of the Deccan traps in eastern India and Pakistan, one of the largest eruptions in earth history. The Deccan eruptions yielded over 10,000 km^2 of lava with thousands of flows totaling over 2.4 km in thickness. Such eruptions undoubtedly produced huge amounts of volcanic gases and ash that could have had catastrophic effects on the environment. The other is a major drop in sea level, causing huge inland seas to regress and dry up, which could have had major effects on many kinds of marine life.

An even more serious complication is that the evidence of the fossil record must be consistent with the predictions of the impact hypothesis. Most non-paleontologists have heard about the K/T impact model, and know that the dinosaurs vanished about this time, and have no further interest in finding out whether the evidence actually fits the impact model. But from the very beginning, paleontologists were the principal skeptics of the K/T impact hypothesis, largely because they did not see evidence in the fossil record that all the K/T victims died out abruptly right at the iridium horizon. Instead, they found evidence that the extinction was much longer and more protracted than could be explained by a single impact. Although some of this has since been explained as an artifact of the incompleteness of the fossil record and the Signor-Lipps effect (Signor and Lipps, 1982), not all of it can be so explained. As a distinguished panel of British paleontologists (MacLeod et al., 1997) showed, most of the marine organisms that were alive in the later Cretaceous were either in decline and extinct long before impact, or sailed right through the events of the K/T boundary with little or no effect. Only the coccolithophorid algae and possibly the planktonic foraminifera show much of an effect consistent with the impact model. By contrast, the benthic foraminifera, diatoms, dinoflagellates, and radiolaria show almost no effect, so the extinction in the microfossils was minimal. More importantly, the major victims of the Late Cretaceous (such as inoceramid and rudistid bivalves) were long gone before the end of the Maastrichtian, and even the ammonites were in a long-term decline, with only a few taxa surviving to near the end of the Cretaceous. By contrast, corals, brachiopods, nautiloids, echinoids, and most bivalves and gastropod showed only a minor extinction, or none at all, at the end of the Cretaceous (MacLeod et al., 1997). The marine reptiles were also apparently declining through most of the Campanian and Maastrichtian, so there is no direct evidence that mosasaurs or plesiosaurs actually witnessed the impact. Teleost fish, on the other hand, showed a 90% survival across the K/T boundary.

Even more revealing is the pattern of land communities. Although there is a change in the pollen flora and a "fern spike" right at the iridium anomaly on land, most other land organisms were little affected (Archibald, 1996). The non-avian dinosaurs did vanish, but there is still much debate as to whether their absence

from the final 3 m of section below the iridium horizon is clear evidence as to whether they actually vanished before the impact, or survived to the end of the Cretaceous but are not fossilized. Much more striking is the evidence of nearly every other land vertebrate taxon. Most groups, including the crocodilians, champsosaurs, turtles, amphibians, birds, bony fish, insects, and mammals, sailed through the K/T boundary with minimal extinction or no effect whatsoever. This evidence alone falsifies most of the more extreme K/T impact scenarios, such as the "acid rain" hypothesis, which argues that the world was bathed in sulfuric acid created when the Chicxulub asteroid vaporized a lot of buried sulfate from gypsum in the Yucatan subsurface. As Weil (1984) pointed out, this notion can easily be ruled out by the survival of nearly all Cretaceous frog and salamander species, which are very sensitive to even slight changes in the acidity of their environment and even now are declining due to human-induced acid rain. The survival of crocodilians as large or larger than many non-avian dinosaurs rules out the idea that small creatures could hide from the impact, but the larger ones were wiped out. Impact advocates have argued that most of the survivors could have hid from the harsh conditions by aquatic adaptations or by living underground (Robertson et al., 2004). But this still does not adequately address the problem, since Late Cretaceous sharks were wiped out, while bony fish did just fine. In addition, the largest crocodilians were too large to be protected underground or underwater and still survive in a world as hellish as the K/T impact advocates propose.

Thus, the public perceptions of the causes of the K/T extinction are out of touch with the last decade of research on the topic. Most of the recent books (Archibald, 1996; Keller and MacLeod, 1996; Officer and Page, 1996; Hallam and Wignall, 1997; Dingus and Rowe, 1998; Hallam, 2004) on the topic are critical of the K/T impact as the sole or even most important explanation. Even more revealing is the response of paleontologists after 27 years of the controversy. Brysse (2004) polled the vertebrate paleontological community, the scientists who know the terrestrial evidence the best. Of those surveyed, 72% felt that the K/T extinctions were caused by gradual processes followed by an impact. Only 20% felt that an impact was the sole cause.

3. What About Other Impacts?

In the heady days after the initial discovery of the iridium anomaly, many scientists were seduced by the idea that impacts caused many or most mass extinctions. In 1989, Digby McLaren told a stunned audience at the International Geological Congress in Washington, DC, that all mass extinctions were caused by impacts, *whether or not there was evidence of an impact in the fossil record*. Raup (1991) also suggested that all mass extinctions might be caused by impacts. With untestable statements like these, why bother gathering data at all? Impacts occurred and extinctions occurred, therefore impacts caused all mass extinctions. End of discussion.

The classic counter-example of the importance of impacts occurred in the late Eocene. This was also a time of significant extinction, although it is not one of the "Big Five" mass extinctions recognized by Raup and Sepkoski (1984, 1986). When the impact "bandwagon" first began rolling, the Alvarez-Asaro Berkeley lab group (Alvarez et al., 1982; Asaro et al., 1982) and other lab groups (Ganapathy, 1982; Glass et al., 1982) looked for and found iridium anomalies "near" the Eocene-Oligocene boundary, declared that impacts had caused the Eocene extinctions, and considered the case closed. The discovery of tektites in late Eocene marine rocks further strengthened the case for impact, eventually leading to the discovery of huge buried impact craters beneath Chesapeake Bay and on the Atlantic continental shelf at Tom's Canyon (Poag et al., 1992; Poag, 1997, 1999) and the Popigai crater in Siberia (Masaitis et al., 1975; Bottomley et al., 1997). Since then, further detailed study has shown that both the Chesapeake and Popigai craters were huge (90–100 km in diameter), only slightly smaller than the Chicxulub crater.

But paleontologists were unconvinced from the very beginning, since they already knew that the Eocene-Oligocene extinctions looked nothing like the K/T event. For most of the 1980s and 1990s, much more intensive research focused on documenting the evidence at the end of the Eocene (Prothero and Berggren, 1992; Prothero, 1994, 2006; Prothero et al., 2003). There was a pulse of extinction of tropical organisms at 37 Ma due to oceanographic cooling, followed by the impact events at 35.5 Ma, followed by another big pulse of cooling as the first Antarctic ice cap formed in the early Oligocene, 33 Ma. The impacts occurred in the middle of the late Eocene, associated with only a few extinctions in some radiolaria (Maurrasse and Glass, 1976), and no significant extinction in any other group.

Such a striking non-effect of two impacts nearly as large as Chicxulub throws a real monkey wrench into the assertions of the impact advocates. Poag (1997) showed just how different the impact model looks when the Eocene craters are considered. The original "kill curve" of Raup (1991) was fit to the Chicxulub data point, and suggested that craters as small as 50 km in diameter might cause 40% species extinction (Fig. 1). But fitting the curve to the non-extinctions in the late Eocene and Chicxulub gives a very different result. Under this constraint, the "kill curve" suggests that only the largest impacts (only Chicxulub, in this case) had an effect. Any impact crater smaller than 150 km in diameter had virtually no effect.

Some impact advocates have tried to salvage the late Eocene impact-extinction story by postulating that Chesapeake and Popigai had long-term effects and climatic perturbations that eventually led to extinction (Poag, 1999; Vonhof et al., 2000; Coccioni et al., 2000; Poag et al., 2003; Fawcett and Boslough, 2002). However, these explanations predict opposite effects. The impacts should have produced a global cooling event due to the debris clouds (Vonhof et al., 2000; Fawcett and Boslough, 2002), but the marine isotopic evidence shows that in reality a slight warming occurred (Poag, 1999; Poag et al., 2003). In addition, the impacts were at least 2–3 million years before the early Oligocene extinctions, and no mechanism has been proposed that allows an impact to trigger events over such long time scales.

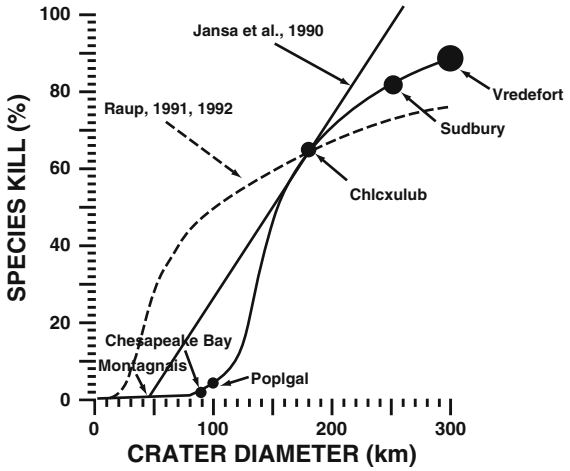

Figure 1. Poag's (1997) modifications of the Raup (1991) "kill curve" showing that only impacts producing craters much greater than 100 km in diameter are capable of producing mass extinction. (From Poag, 1997.)

The overstatements of the impact advocates have prompted a detailed study of the impact and extinction record. Alroy (2002) and Prothero (2004b) both showed that there was no correlation between any of the many well-documented Cenozoic impacts and the extinction events of fossil mammals. The end-Triassic extinctions were once touted as impact caused (Olsen et al., 1987, 2002a, b). But the iridium anomaly near the Triassic-Jurassic boundary (TJB) is only 285 ppt, compared to the 6,300 ppt at the K/T boundary at Gubbio. In addition, the supposed culprit, Manicuoagan Crater in Canada, has turned out to be 14 million years too old (Keller, 2005). Instead, the recent work on the Triassic-Jurassic boundary has shown that the extinctions were spaced out over many different pulses spanning the later Triassic. The apparent "mass extinction" at the end of the Triassic was actually an artifact of compiling large low-resolution databases, where every last occurrence of a fossil is treated as occurring at the end of the binned interval, when in actuality their last occurrences were spaced throughout the interval (Tanner et al., 2003; Lucas and Tanner, 2004). As Lucas and Tanner (2004, 37) wrote: "two hundred years of fossil collecting failed to document a global mass extinction at the TJB, yet another 20 years of literature compilation and the 'compiled correlation effect' did."

In recent years, attention has shifted away from the K/T event to the "mother of all mass extinctions," the Permian-Triassic event, in which 95% of marine species may have died out (Hallam and Wignall, 1997; Benton, 2005; Erwin, 2006; Ward, 2006a, b). This event was so profound that the seafloor communities were completely reorganized, from the crinoid-brachiopod-tabulate-rugosid-bryozoan-dominated "Paleozoic fauna" to the mollusk-echinoid dominated "Modern

fauna" that has prevailed since the Triassic (Sepkoski, 1981). Once the abruptness of the Permian extinction became apparent (Bowring et al., 1998; Jin et al., 2000), it was natural that someone would try to find evidence of impacts. Becker et al. (2001) claimed to find evidence of fullerenes at the Permo-Triassic boundary, but this evidence has been disputed (Farley and Mukhopad-hyay, 2001; Braun et al., 2001), as have other claims for evidence of a Permo-Triassic impact (Koeberl et al., 2004; Zhou and Kyte, 1988). The supposed Bedout impact crater in Australia (Becker et al., 2004) has not proven to be the right age or size or have the right kind of shocked quartz grains (Glikson, 2004; Wignall et al., 2004), nor have the alleged shocked quartz grains in Antarctica (Retallack et al., 1998) convinced the scientific community.

Finally, the Devonian extinctions have sometimes been blamed on impacts as well (McGhee, 1996, 2001), with impact debris reported from a number of Late Devonian sections. Two of the largest Late Devonian craters (Alamo and Woodleigh) reach 44–65 km in diameter (Glikson et al., 2005), yet the Alamo impact is at least 5 million years older than the late Frasnian extinction, and the Woodleigh impacts occurred in the middle of the Famennian, so no impact crater has dates that coincide with the major extinctions at the end of the Frasnian or the end of the Famennian (Keller, 2005). However, the Siljan crater is dated at 377 ± 2 Ma (Reimhold et al., 2005), close to the Frasnian-Famennian boundary of 376 Ma, so it potentially coincident with this extinction event. Unfortunately, the crater diameter is only 65–76 km, too small to be the principal "killer" in the Late Devonian extinction.

In summary, most of the recent evidence has shown loud and clear that impacts do *not* cause most major mass extinctions. In fact, there is no impact crater associated with any mass extinction except the K/T boundary, and even that is not the sole factor involved (the Deccan traps and global regression occurred at the same time as the impact). In contrast to the views voiced by McLaren in 1989 and Raup in 1991 cited above, impacts seem to have little to do with mass extinctions. The earth and its biosphere are apparently more resilient to the effects of an impact that was once widely believed.

4. If Not Impacts, What Causes Mass Extinctions?

In recent years, scientists have moved beyond naïve and simplistic impact scenarios, and looked at the detailed records of several mass extinctions to test new hypotheses of their causes. One of the most popular alternatives is the volcanic hypothesis (Rampino and Stothers, 1988; Courtillot, 1999; Courtillot and Renne, 2003). Indeed, the Permian extinction is associated with the huge Siberian trap eruptions, the largest in earth history, and the late Triassic extinctions seem to be associated with the eruption of the Central Atlantic Magmatic Province (CAMP) basalts. As discussed above, the K/T extinction coincides with the Deccan traps in India and Pakistan, the second largest eruptions in earth history.

However, the rest of the record is less convincing. There were small eruptions known as the Viluy traps in the Late Devonian, but their volume was too small and their ages too uncertain to associate them with the Late Devonian extinctions (Keller, 2005). The eruption of the North Atlantic Magmatic Province at 61 and 56 Ma did not cause any extinctions. The Ethiopian and Yemen traps, originally blamed for the Eocene-Oligocene extinctions (Rampino and Stothers, 1988), are now dated between 29.5 and 31 Ma, in the middle of the late Oligocene, when there were no extinctions of consequence. The massive Columbia River flood basalts dated between 15.3 and 16.6 Ma caused no extinctions, either. In fact, they occurred at a peak of North American mammalian diversity (Prothero, 2004b). Once again, the attempt to blame all mass extinctions on a single common cause falters.

Instead of common causes, many scientists now see each mass extinction as a complex interaction of many different causes (Bambach et al., 2004; White and Saunders, 2005; Keller, 2005; Morrow, 2006). The most striking features of the great Permian extinction are the carbon isotopic events, which lead scientists toward hypotheses involving oceanic circulation, anoxia, hypercapnia (excess carbon dioxide poisoning), and rapid global warming (Knoll et al., 1996; Huey and Ward, 2005; Ward, 2006a, b). By contrast, the Devonian extinctions occurred in several pulses, with global cooling a prominent feature of many of them (Hallam and Wignall, 1997; Keller, 2005). Kump et al. (2005) proposed that there is a major release of hydrogen sulfide (suggested by the sulfur isotopes) at the Late Devonian, Permo-Triassic and Cenomanian-Turonian extinction in the Cretaceous. However, these sulfur isotopic signals have not yet been seen in the Ordovician, K/T or Eocene extinctions, so they are not a general cause for mass extinction.

Currently, one of the few attempts to infer a link between mass extinctions and impacts has suggested that *both* a major impact and simultaneous major volcanic eruptions are needed to cause mass extinctions (White and Saunders, 2004; Keller, 2005; Arthur, 2006). This is certainly possible for the K/T extinction, but the lack of evidence for impacts at the end-Permian and end-Triassic extinctions are no longer supportive of this hypothesis. Alternatively, Arthur (2006) argues that some extinctions might be caused when major flood basalt eruptions intrude through carbonate-evaporite sequences which release more toxic volatiles. By contrast, intrusions through chemically inert basaltic oceanic crust or continental crust release few poisonous gases and apparently cause no extinctions. Retallack and Jahren (2008) argued that in the case of the Permian extinction, the critical factor may have been the intrusion of the Siberian traps and other volcanics through coal-bearing strata, which would have released a lot of light carbon and explain the unusual isotope signal

The latest "hot idea" is that some of the major mass extinctions (especially the Permo-Triassic and Triassic-Jurassic extinctions, and possibly the Paleocene-Eocene thermal event) might be caused by unusually high carbon dioxide and/or low oxygen in the atmosphere, forming a suffocating super-greenhouse climate

(Ward, 2007). This notion is still too new to evaluate here, although it has already failed some of the first tests (Holtz, 2007).

5. Conclusion

Mass extinction theory has come a long way since the initial simplistic impact theories of the 1980s. Today, only the K/T extinction shows evidence of impact, and there is no strong evidence for an impact causing any other mass extinction event. In contrast, the mismatch between major impacts (such as the late Eocene and other Cenozoic impacts, or Manicouagan impact in the late Triassic) and mass extinction shows that only the largest impacts, possibly coupled with flood basalt eruptions, is capable of causing a mass event in the history of life. Massive flood basalt volcanic eruptions occurred in three mass extinctions (K/T, Permian, Triassic), but not in the others, while many other major eruptive events have no correlation with extinctions. The current thinking is that the release of sulfides from the ocean floor, and/or the balance of oxygen and carbon dioxide in the oceans and atmosphere (with our without contribution from major volcanic eruptions) are more critical in the major extinctions that happened at the end of the Devonian, Permian and Triassic.

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IRRADIATION OF ICY COMETARY ANALOGS: ITS RELEVANCE IN REFERENCE TO CHEMICAL EVOLUTION AND THE ORIGIN OF LIFE

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1. Why are Comets so Interesting for Chemical Evolution?

The study of comets has, with good reason, intensified over the past few years. Comets are minor bodies in our solar system which are almost as old as the solar system itself. They provide unique information about chemical evolution processes that occurred at early stages of the solar system. Besides that, they are dynamically connected with other small objects all over the solar system (Weissman and Levison, 1996). In addition, within the scenario of the origin of life, Chamberlin and Chamberlin (1908) were the first to suggest that extraterrestrial material could have played an important role in terms of having contributed to the organic matter on the Earth, influencing abiotic synthesis and biological evolution. Later, J. Oró (1961) reworked this hypothesis, implying a specific role for comets as raw material providers for chemical evolution on Earth, and, since then, this line of research has been widely investigated, thus reinforcing this idea (Hartman et al., 1985; Negrón-Mendoza et al., 1994; Oró and Lazcano, 1997; Oró and Cosmovici, 1997; Irvine, 1998, Colín-García et al., 2008 *in press*). This means that, not only local processes, such as UV irradiation and electrical discharges on the primitive atmosphere, were important in triggering necessary reactions on the early Earth, but extraterrestrial contributions were also of great significance.

The putative contribution of impacts could be paradoxical. Clear evidence of impact events in the past can be observed on the surface of our moon; those events have been extrapolated to understand what occurred on Earth (Chyba and Sagan, 1992). Those impacts could have contributed organic material that was necessary for prebiotic synthesis on the early Earth. Noble gases, for example, were carried mainly by cometary impacts (Owen and Bar-Nun, 2001). On the other hand, comets and asteroids could have annihilated forms of life during various extinction events (Cockell and Bland, 2005).

Extraterrestrial prebiotic molecules (such as aldehydes, CN-containing compounds, etc.) may have played an important role, as raw material, in the origin and evolution of life on Earth (Oró, 1961; Chyba et al., 1990; Bernstein et al., 1997). The contribution of comets in reference to organics depends on two main factors: first, the mass contribution and, second, the survival capacity of carbon containing compounds, while entering the atmosphere, and on impact.

The flux of cometary material to the early Earth has been estimated between 10^5 to 3×10^6 kg per year (Chyba et al., 1990). According to Svettsov (2002), bodies of 1 km size could have reached the surface and have contributed largely to the influx of cometary organics to the surface of the Earth. However, major bodies of hundreds of kilometers in diameter had to be completely evaporated in a dense atmosphere.

There are several approaches to the study of comets: observational, theoretical modeling, and experimental simulations. This review intends to bring attention to some experimental models that reflect the processes that may occur on cometary ice and that may have had a direct impact on the synthesis of bioorganic molecules on Earth. The main interest in this paper is to provide a general perspective in terms of data obtained through the high-energy irradiation of cometary icy analogues. A brief summary of some cometary characteristics is presented in the following paragraphs. To obtain a full perspective about what comets are, their properties, and their origins, see, for example, Delsemme (1977).

2. What are Comets?

Comets are small bodies with diameters of a few kilometers (1–15), with masses of the order of 10^{15} to 10^{18} g (Lazcano-Araujo and Oró, 1981) that can be detected only when they approach the Sun. When this happens, the surface volatiles sublimate, creating an atmosphere of dust and gas which is referred as the coma (Stern, 2003). Due to their small sizes, comets do not have much gravity, and, thus, their coma largely expand from their surfaces. Deep impact, Stardust and Giotto missions have made a great contribution for a better understanding of cometary nuclei.

There are severals models of comets. The early model of a "dirty snowball" proposed by Whipple in the 50s has been modified since that time. This model suggests that comets are made basically of water ice and rock particles (Ehrenfreund and Charnley, 2000). There are some others models which describe the structure and composition of comets; for example, there is the fractal and the primordial rubble pile proposed by Donn (1990). According to the first model, a comet is formed by small cometesimals (around 100 m) weakly bound to each other, forming a porous structure with internal void spaces. The second model is similar, but, in it, the cometesimals are closely packed together by evaporation and ice freezing (Weissman 1986).

Another model suggests that comets are made of icy, rocky, and organic components (Delsemme, 1983). Greenberg (1998) proposed that up to 30% of a

comet's mass may be water ice, 26% silicates, 23% refractory material, and 9% carbonaceous molecules. Recent observations have shown that cometary ice is predominantly made of water; in fact, water-rich ice is an important component of dense molecular clouds, and is common throughout the solar system; this is followed by CO and CO_2 and by small quantities of organic constituents. It is important to note that water is a common molecule in the interstellar medium and, in general terms, in astrophysical environments (Ehrenfreund and Charnley, 2000); this is corroborated in comets, where water ice constitutes the major component in which the others are diluted (Gerakines et al., 2001). The Deep Impact mission on Comet 9P/Tempel 1 revealed the presence of H_2O, C_2H_6, and HCN (Disanti et al., 2007). Other authors, such as Mumma et al. (2005), quantified eight parent volatiles (H_2O, C_2H_6, HCN, CO, CH_3OH, H_2CO, C_2H_2, and CH_4) in the same comet (Tempel 1) using high-dispersion infrared spectroscopy. The results obtained suggest that the volatile ice in Tempel 1, and in most Oort Cloud comets, originated in a common region of the protoplanetary disk. There could still be a number of undetected products in comae due to their low concentrations and high vapor pressures (Cottin et al., 2001).

2.1. THE ORIGIN AND NATURE OF COMETS

Modern assumptions derived by geochronology and chemical data agree that approximately 4.6×10^9 years ago, a portion of a dense interstellar cloud collapsed to create the Sun and the solar nebula that, eventually, formed the planets, satellites, comets, and asteroids in our solar system (Stern, 2003). In this way, in the outermost regions of the solar system, where temperatures are very low, silicate and carbonaceous materials behave as condensing surfaces for atoms and molecules from the environment; cometesimals were, thus, formed basically from silicate particles covered by organic materials and ices (Kossacky et al., 1997). As a result, they acquired a covering icy grain mantle (Ehrenfreund et al., 2001). Molecules contained in the presolar nebula could have been conserved into comets soon after they formed (Hartman *et al*. 1985). In fact, comets and asteroids are considered relicts that can help us to understand the formation and origin of our solar system (Irvine, 1998).

The formation of comets includes not only nucleation, but also the slowly accretion of interstellar grains into icy bodies (Hudson and Moore, 1999). While some of these cometesimals could have been stored in the exterior of the planetary region, close to Neptune's orbit, others were thrown to outer regions, in the Oort Cloud (Fernández, 1999). According to this, there are at least two known big reservoirs for comets: the Oort Cloud and the Kuiper Belt (Amici et al., 2000). Neither of these is directly detectable, but they have been deduced from the orbital properties of comets (Gladman, 2005).

The study of the Oort Cloud has been considered of enormous significance in reference to understanding the formation of the solar system. This region is a

spherical cloud that surrounds the planetary region of our solar system at a distance of 10^4–10^5 AU from the Sun. The name was given to honor the Dutch astronomer Jan Oort, who first proposed its existence in 1950 (Oort, 1951). Oort's estimations suggested that the population of cometary nuclei in this region could be of 1.9×10^{11}. He also pointed out that these kinds of comets were formed in the planetary region and then ejected by planetary perturbations (Neslušan and Jakubík, 2005). This implies a change in temperature during cometesimal formation at ~100 K and during their ejection into the cloud at ~20 K (Delzeit and Blake, 2001); that is, the cold memory of comets is related to the period of their formation. Comets in the Oort Cloud are weakly bound to the Sun, and any small disturbance can change their orbits, causing their injection to the inner solar system. Comets here extend their orbit up to 10,000–20,000 AU from the Sun; they have long orbits and, as a consequence, are long-period comets (Harold et al., 2001; Stern, 2003). Comets Halley, Hyakutake, and Hale-Bopp are part of the Oort Cloud family.

A second population of comets has been detected; they have shorter periods and their orbits are within the elliptic. This observation suggests a second source region at distances of between 40 and 50 AU. It would be the Kuiper Belt, named after the Dutch astronomer Gerald Kuiper, who suggested the existence of such a reservoir beyond the orbit of Neptune. Since then, around sixty cometary bodies have been detected in planetary orbits.

Cometesimals remain in their orbits, either in the Oort Cloud or in the Kuiper Belt, until an alteration by gravitational effects occurs. This causes their orbits to alter in such a way that their perihelions move closer to the Sun, leading to passages through the inner solar system. These passages cause the vaporization of ice and the appearance of the object as a comet (Wood and Chang, 1985). In general, a comet can be considered an object of low density, formed at low temperatures, with an internal structure that may have been preserved since its origin (Davidsson and Gutiérrez, 2004).

2.2. THE DEBATE ABOUT THE PRISTINE NATURE OF COMETS

For many years, comets were considered fully pristine and the most intact material that has been preserved in the solar system. It was thought that comets were held in stasis during their storage in the Oort Cloud or the Kuiper Belt during 4.6 billion years. Now, new results and understanding has changed that perception. Cometary ices have been at least partially processed (Ehrenfreund and Schutte, 2000). Nonetheless, it is widely held that, in fact, comets reflect, at some degree the composition of interstellar grains (Strazzulla, 1997; Ehrenfreund et al., 2001; Szopa et al., 2003).

There are essentially three phases during which alteration of comets can occur: formation, residence in the reservoirs, and, a very important one, passage through the inner solar system, (Kochan et al., 1998). Irradiation of those bodies

starts since the comets are formed from interstellar grains; UV photons and cosmic ions had already interacted with those grains (Hudson and Moore, 1999; Strazzulla, 1999). Later, during their storage in the reservoirs mentioned, their surfaces were continuously irradiated by cosmic rays. All of this exposure alters the chemical and physical properties of those bodies (Hudson and Moore, 1999).

Comets have experienced ionizing particle flux due to cosmic rays and UV photon flux. Donn (1976) suggests that accumulated irradiation doses in the outer layers of comets could be enough to polymerize the simple volatile original ice. It has been also suggested (Strazzulla and Johnson, 1991) that comets exposed to background particle radiation in the Oort Cloud obtain another web of nonvolatile material which leads to the formation of a "crust." All of these irradiations alter the chemical and physical properties of cometary ices. synthesizing new molecules and destroying others. As a consequence, the observed molecules during the passages of comets near the Sun are the result of, among other things, the irradiation of ice material at low temperatures (Hudson and Moore, 1999). Many of the observed products in the comae of comets are not the result of direct sublimation of the ice in the nucleus, but evidence of a continuous transformation.

It has been recognized that many processes contribute to the evolution of material in those bodies (Strazzulla and Moroz, 2005). Energy sources include particles, ions, and photons (interstellar radiation field); ionizing radiation mainly includes cosmic rays (mostly H^+ and He^+), solar wind particles and magnetospheric particles, and gamma rays (Johnson, 1990). The processes that result from those interactions are thermal, collisional and radiation processing (Stern, 2003). Almost all of these processes have been modeled in the laboratory. Besides that, comets may undergo modifications such as stratification, resulting from the phase change of the icy component produced by solar heating (Henrique et al., 1999; Capria et al., 2003). Actually, the nucleus is a structure with stratified ice, not only in terms of density, but also in temperature and porosity (Ehrenfreund et al., 2002).

It is essential to note that neither the origin nor the degree of alteration of cometary material is well understood (Ehrenfreund and Charnley, 2000). This is because more information is needed in order to obtain a complete picture of comets.

3. Irradiation of Cometary Ices in Laboratory Experiments

Ample lists of authors have devoted their efforts since the early 60s, when cometary simulation experiments began, to understand the processes and the evolution that comets experience. A Russian group did pioneering work; their samples (of sand and water) were deposited onto a cold finger, and then exposed to a strong light (see Kochan et al., 1998 for references). In 1961, Oró proposed that comets could have been carriers of organic material; then, with experimental data, he reinforced the hypothesis of the importance of comets in reference to the origin of life. Since

that time, studies and experimental designs have become greatly diversified, based on new discoveries and information obtained from observation, theoretical modeling, and experimental work.

Increasing knowledge of the composition, dynamics, and component interactions has been reflected in the quality and quantity of existing data on comets. Experimental work has focused on the major constituents of comets: ice and, in some cases, the refractory material. Kossacky et al. (1997), for example, studied the thermal evolution of cometary analogs following the structural behavior of samples made of water ice, carbon dioxide, and dunite. Among their interesting results are the stratification of the samples into well-defined layers of varied chemical composition and cohesion.

Ices may be investigated in pure or mixed solutions by three experimental approaches: following thermal processing, changes in structure and mobility, and by irradiation (with high energy electrons, ions, protons, and UV light) and its effects (*i.e*. desorption, sputtering). All of these factors are fundamental and have close relationships to one another. However, for better comprehension of the entire phenomenon, it is necessary to isolate each one from the others. Irradiation is fundamental to understand the evolution of comets. Among the effects produced by irradiation on frozen targets are fundamental ones: sputtering, structural and morphological alterations, and the synthesis of new molecular species (Strazzulla and Palumbo, 2001).

Many of these experiments relate not only to comets, but also to other objects in the solar system, where ice is an important component. There are different approaches to the study of the irradiation of ices. Many research groups are devoted to the study of irradiation of thin ice films (see, for example, the works of Greenberg, Strazzulla, Moore and Hudson). Other research groups analyze samples obtained by spraying suspensions of water, silicates and other components in liquid nitrogen (Kochan et al. 1998). Bulk irradiations were carried out by Colin-Garcia et al., 2008. They studied the radiation chemistry of HCN, one of the most important and highly versatile constituent of cometary ices.The behavior of over-irradiated water dominant HCN ice mixtures underline the importance of radiation-induced reactions as an energy source in extraterrestrial scenarios, like comets or other icy objects. Even, when low temperature is a limiting variable for diffusion-controlled reactions, it is evident that reactions occur and produce many organics relevevan. in prebiotic chemistry

3.1. THE IMPORTANCE OF VARIABLES: PURE ICE VS. ICY MIXTURES

Irradiation studies related to cometary analogs are diverse. For example, the composition of the sample to be analyzed, the temperature of irradiation, and the kind of radiation employed are features that can be monitored and modeled. For this reason, experiments can be performed with pure or mixed ices. Evidently, the

selection of the components to be incorporated into the model depends on the pursued objectives. Studying simple ice may help us to understand basic processes and to elucidate the reaction mechanism of species involved. On the other hand, icy mixtures contribute to the recreation of more complex interactions, and are closer to reality, but isolating phases is not easy.

3.1.1. Models Related to Pure Ices

As previously stated, most of the experiments that have been conducted used water ice as the main component. The reason for this is that cometary nuclei are composed mostly of water ice (Ehrenfreund et al., 2002). Moreover, water ice is the most abundant icy component in astrophysical environments and in planetary systems; many bodies in the solar system (Kuiper Belt objects, satellites, the nuclei of comets, and some planetary rings) are composed of abundant amounts of water ice (Zheng et al., 2006).

A whole body of research has been conducted in order to understand the processing of water ice by energetic particles and ultraviolet photons. Zheng et al. (2006) present a good review of this theme; they indicate that an ample range of conditions has been explored. The variables include temperature, film thickness, type of radiation employed, and, in some cases, the observed products.

Many authors have studied the irradiation of pure icy water systems with ions of different energies. The first research studies were those of Brown et al. (1978, 1980a, 1980b) who studied films of different thicknesses made of water at different temperatures (15–110 K) irradiated with He^+ and H^+. Other authors have employed the same particles, although the ion energy was different (Bar-Nun et al., 1985; Shi et al., 1995b; Moore and Hudson, 2000; Baragiola et al., 2003; Leto and Baratta, 2003; Gomis et al., 2004a; Gomis et al., 2004b; Baragiola et al., 2005; Loeffler et al., 2006). Many other radiation sources have been tried; this includes F^+ (Cooper and Tombrello, 1984), Ne^+ (Bar-Nun et al., 1985; Christiansen et al., 1986; Shi et al., 1995a); N^+ (Christiansen et al., 1986; Shi et al., 1995b; Orlando and Sieger, 2003); Ar^+ (Christiansen et al., 1986; Baragiola et al., 2003; Orlando and Sieger, 2003; Loeffler et al., 2006); Ar^{++} (Letto and Baratta, 2003; Gomis et al., 2004b); O^+ (Shi et al., 1995a; Shi et al. 1995b; Gomis et al. 2004b); UV radiation (for example, Matich et al., 1986; Gerakines et al., 1995; Leto and Baratta, 2003) and electrons (Christiansen et al., 1986; Kimmel and Orlando, 1995; Sieger et al., 1998; Orlando and Sieger, 2003; Pan et al., 2004, Baraggiola et al., 2005; Zheng et al., 2006).

Despite the wide set of conditions of raw materials and the variarity of experimental conditions explored, the final products in those simulations are common. These include products such as H_2, O_2, H_2O_2 (Bar-Nun et al., 1985). The presence of some of these products could be temperature dependent; for example, Moore and Hudson (2000) found no H_2O_2 production at 80 K, but the product was identified at 16 K when water ice had been irradiated under the very same conditions. Other species, such as radicals are rarely reported; an exception is the work of Gerakines et al. (1995), in which the authors mention the presence

of HO_2 and OH. The lack of this kind of information could be a consequence of the technical difficulties in reference to the detection of this kind of species (that are extremely reactive) at low temperatures and while irradiation is occurring.

The molecular products formed coincide with those observed for the radiolysis of liquid water (H_2, O_2, H_2O_2). The radiolysis of water has been widely discussed by many authors; for a detailed treatise, see Draganič and Draganič (1971), Spinks and Woods (1990), and references therein. When a sample is irradiated, the first effects are excitations and irradiation events. It has been pointed out that radicals and ions that are produced are condition-dependent. For example, the formation of the HO_2 radical is dependent on the presence of oxygen and the source of radiation. It is common when irradiating with particles of high Linear Energy Transference (LET). The abundance of other species is dependent on pH; this is the case with e^-_{aq} and $H^{\bullet}$, which can interconvert from one into the other. The presence of these intermediates can finally modify the yield of formation of the products. On the other hand, there are other processes that can be followed through sputtering (whose main consequence is mass lost) and amorphization.

Other efforts are directed at the irradiation of other components, such as carbon monoxide. In this context, Loeffler et al. (2005) performed the irradiation of CO, comparing the effect of different sources of energy, UV and ions, and found that the main product in both experiments was CO_2. Hudson and Moore (2001) affirm that, in general terms, the species produced by ionizing and UV irradiation are almost the same, but there are differences in terms of yields; that depends on the rate of energy deposition (Linear Energy Transfer, LET).

Brucato et al. (1997a) performed an experiment in which CO_2 was irradiated, using the technique of ion implantation (H^+). This resulted in the production of carbonic acid (H_2CO_3), which is an indicator of the incorporation of ions implanted into parent molecules to generate new ones.

3.1.2. Multi-component Systems

Mixed molecular ices are so common, not only in cometary nuclei, but also in grains in the ISM, planets, and satellites (Ehrenfreund and Charnley, 2000). Interstellar and cometary ices include not only pure ice, but also non-polar (dominated by CO, N_2, O_2 and CO_2) and polar (dominated by H_2O, and containing CO, CO_2 and CH_3OH) ices (Bernstein et al., 1997). As can be seen, carbon monoxide can be included in the group of non-polar or polar ices, depending on the presence of other components: water and its particular characteristics make the difference.

The volatile fraction should include mixtures in different proportions and combinations and other materials such as minerals and amorphous carbon. All of these ingredients can be combined and tested in different proportions, temperatures, and by employing different sources of energy. For this reason, the number of experimental simulations is vast. There are experiments in which the volatile fraction is the sole component; in others, besides this, other constituents are involved.

There are a lot of examples which reveal the significance of the synthesis of new molecules by irradiation; molecules that are produced match the signatures observed. Laboratory astrophysics is mainly devoted to the characterization of interstellar molecules and cosmic dust; this allows the comparison of laboratory spectra with astronomical data (Altwegg et al. 1999).

The next paragraphs do not pretend to be exhaustive, but are presented to illustrate the ample assortment and nature of the material formed from irradiation of simple ices.

Carbon monoxide is the second most abundant type of ice after water in the ISM. This and carbon dioxide are important molecules. CO is commonly dispersed through the solar system and the ISM (Hudson and Moore, 2001). In fact, many of the observed lines that contain H_2O ice also contain CO (Bernstein et al., 1997). Hudson and Moore (1999) have shown the formation of CO_2 and methanol during ion irradiation of water and carbon monoxide mixtures. The source of energy is not a constraining factor in the synthesis of methanol, which is formed both from ion (Hudson and Moore, 1999) and UV irradiation (Schutte et al., 1996) of the same mixture. In reference to H_2O:CO mixtures, ion irradiation produces different molecules, many with increased volatility in respect to the raw material, others with less volatility, and some with intermediate volatility (Strazzulla, 1997). Hudson and Moore (1999) propose that the $H^{\bullet}$ and $OH^{\bullet}$ produced from the radiolysis of water are added to CO to form HCO, H_2CO, HCOOH, and CH_3OH. They also point out one characteristic that has to be taken into account: the yields. In addition, they found higher yields compared to other previous experiments. This demonstrates that ice composition and other characteristics are critical factors in laboratory simulations.

Methane, the most reducing form of carbon, has been extensively studied. Hudson and Moore (1997) irradiated icy mixtures of methane and water at 16 K, and identified the formation of methanol and ethane; the first one was also formed when irradiating water, but, this time, with another molecule, acetylene (Hudson and Moore 1997).

Strazzulla and Baratta (1992) demonstrated that the irradiation of isolated volatile carbon molecules (benzene, butane and methane) or their mixtures with water ice produced a refractory residue. This means that the production of an organic crust formation on comets through irradiation was possible, and the evolution of the residue as a function of irradiation dose was also followed. At low doses (10 eV/C-atom) they observe the conversion of the frozen film into a solid; but, at higher doses (10–25 eV-C-atom), they observed properties of polymer; at ever-higher doses, the compounds changed to an amorphous carbon film.

More than one or two single component models have been studied. In another approximation, Bernstein et al. (1997) simulated the photo processing of polar and non-polar ices. The experiments simulating non-polar ices included irradiation of CO mixtures with different amounts of O_2, N_2, and CO_2. These experiments produced CO_2 N_2O O_3, CO_3, HCO, H_2CO, and, maybe, NO and NO_2. It is thought that hot O atom production dominates the reaction mechanisms;

in this system, the destruction of N_2 is difficult. On the other hand, the radiolysis of polar ices with H_2O, CH_3OH, CO, and NH_3 generated small molecules, such as H_2, H_2CO, CO_2, and CH_4, as well as the formyl radical (HCO). After photolysis, the warming produces complex species, such as ethanol, formamide, acetamide, isonitriles, and/or nitriles, amides, ketones, and polyoxymethylene. So, the differences in initial composition and polarity are clues to the production of more complex molecules.

Cottin et al. (2001) irradiated a mixture containing H_2O, CO, CH_3OH and NH_3 with both UV and proton sources. They identified the presence of hexamethylenetetramine (HMT) in organic residues. For the first time, HMT was detected after proton irradiation of an interstellar or cometary ice analog. In fact, it was thought that this molecule was a characteristic signature of UV processing.

Probably one of the most significant examples of evidences of important compound synthesis is the work of Kobayashi et al. (1995). They reported that, when irradiating propane (methane or carbon monoxide) in the presence of water and ammonia, and after an acid hydrolysis, amino acid production was detected by ion chromatography.

In reference to this point, the importance of the production of more refractive materials, when irradiating simple icy molecules, is fundamental in explaining the emission of other molecules, for example CO (Brucatto et al., 1997b) and in order to explain the abundance of detected molecules. This means that the rate of production and the stabilization of species in the irradiated samples need to be considered.

As has been revealed in those representative examples, organic material is formed when irradiating simple ices. Other experiments with bulk irradiation of simple molecules (H_2O, HCN) reveal the formation of complex organic compounds, which can be used as raw materials for the synthesis of bioorganic compounds. Among the products detected after the gamma irradiation of a frozen solution of HCN were CO_2, ammonia, urea, some simple amines, an oligomeric material that upon hydrolysis yields amino acids, carboxylic acids and other products such as adenine. (Colín-García et al., 2008).

In addition, most of the material formed is consistent with those supposed to be in the nucleus of a comet; many studies are focused on matching the signatures obtained from studies *in situ* and those done on the basis of observation. But production is not the most important feature; the volatility, stability, and reactivity of raw compounds and those formed by irradiation need to be considered. So, experiments of thermal stability and observations of change in the structure in ice and other components have to be considered for a better understanding of phenomena occurring in comets and in all the icy surfaces in the interstellar medium.

4. Comets and the Origin of Life

According to the Oparin-Haldane Hypothesis, the origin of life could be explained through chemical and physical processes. Thus, biological evolution was preceded by a period of chemical evolution during which formation and

organization of bio-organic compounds accomplished (Oró et al., 1990; Negrón-Mendoza and Albarrán, 1993). Many scenarios have been proposed to explain the origin of life on Earth, and the role that comets may have played has been extensively recognized (Oró, 1961; Oró 2000). Comets and asteroids can be considered to have been beneficial for prebiotic synthesis in mainly two ways: as carriers, bringing organics and other components and as fuels, providing the energy necessary for the synthesis of more complex molecules (Whittet, 1997; Lyons and Vasavada, 1999; Pierazzo and Chyba, 1999).

Earth was formed in a particular region of the solar nebulae; because of this, it was depleted of molecular material. Consequently, the molecules and organic materials that comets brought to the primitive atmosphere may have been important for the emergence of life on Earth (Chyba and Sagan, 1992; Owen and Bar-Nun, 2001).

The organic contribution of comets to the early Earth may have been crucial because cometary impacts could have delivered organics to the surface of the early Earth. The contribution of those bodies is noteworthy in the case of some molecules, especially CN^- bearing molecules, such as HCN, that are abundant through the universe and were probably scarce on Earth due to its high volatility.

5. Concluding Remarks

Despite the fact that comets are minor bodies in the solar system, they constitute an important link between molecules present in the interstellar clouds and, those that existed when the solar system formed. Their study is one of the most challenging objectives in the field of modern planetary science. Besides that, there is a theory that suggests that comets could have contributed to the prebiotic synthesis of molecules on the primitive Earth.

A variety of sources of energy are crucial for the processing and evolution of ice in comets and oher bodies. The form of deposition of this into the sample is different for each type of source. Evidently, the importance of a source is a complex combination of abundance, availability, and effectiveness. In reference to UV radiation, the most abundant source of energy in regions close the Sun, its effects could be restricted to the surface of comets. For other radiation sources such as ions, the interaction could be deeper and, as a consequence, the effects are not confined to a small layer on the surface, but, instead of that, they may be distributed.

The real and net contribution of each source of energy is not well understood nowadays. In fact, two key sources are considered as the main contributors to the modification of ice throughout the solar system: cosmic radiation (ions) and UV radiation. This is why, most of the time, the icy laboratory samples are studied irradiating with ions and UV radiation.

Evidently the complex nature of comets demands an integral study of those bodies. It this review, only one aspect has been considered, the generation of

compounds from the irradiation of ices. There is a great deal of evidence that irradiation of simple ices produce a large amount of organic molecules. Many of the substances identified through IR detection techniques, match the signatures obtained from those of observations. However, not only the production of different compounds is important, but also the stability of molecules, the process of loss in mass (sputtering), the change in structure of the ice, and thermal annealing. It is difficult to follow all of the effects at the same time. However, many efforts are being carried out for the better understanding of comets.

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THE BIG BANG AT TIME ZERO

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Abstract The cosmic background radiation left over from the Big Bang approximately 14 billion years ago is the oldest of all fossils. The Big Bang at time zero is the most important of all boundary conditions for the very possibility of life in the Universe. In the Big Bang singularity, space and time do not exist, so causality cannot be operative. This leads us to conclude that the Big Bang was an uncaused event.

1. Introduction

On October 3, 2006, for the second time only, a Nobel prize in physics was awarded for experimental observational work in cosmology, to John Mather and George Smoot, for detailed study of the relic cosmic background radiation left over from approximately 400,000 years after the Big Bang (Kaku, 2006), which was the beginning of time itself. The first time that a Nobel prize in physics was awarded for experimental observational work in cosmology, was in 1978, to Arno Penzias and Robert Wilson, for their detection in 1965 that a relic cosmic background radiation left over from the Big Bang actually *existed* in the first place (Penzias and Wilson, 1965). That such a cosmic background radiation would be left over from a Big Bang was, in fact, predicted by the physicists George Gamow, Ralph Alpher, and Robert Herman in 1948 (Gamow, 1952).

It was not always clear that there *was* a Time Zero, or a beginning of time. For example, in the 1940s, the 1950s, and the 1960s, when two different theories of the Universe, the Big Bang Theory (Weinberg, 1994) and the Steady State Theory (Hoyle, 1975), were in competition, the first theory stating that the Universe had a beginning time billions of years ago and the second theory stating that the Universe was eternal and had no beginning time, it was not clear at all that either theory could be proved. Scientists worried about how one would prove that the Universe and time itself began with a certain event billions of years ago. However, with the discovery of the cosmic background radiation by Penzias and Wilson (Singh, 2005), this problem was solved, and it was convincingly demonstrated that the Universe did indeed begin in a huge explosion of matter, energy, space, and time approximately 14 billion years ago. The enduring characteristics

of the Big Bang that encompass the properties of space-time and the laws of electromagnetism and gravity remain unchanged from then until now. What this example points out is that the laws of science, at least the laws of physics and chemistry, do not change even over billions of years of time and over billions of parsecs of space.

The cosmic background radiation can be viewed even today as the static 'snow' seen occasionally on television screens and can be heard on radios as the static noise accompanying and between stations. The cosmic background radiation left over from the Big Bang is the oldest of all fossils.

2. From the Big Bang to Astrobiology

The Big Bang at time zero is the most important of all boundary conditions for the very possibility of astrobiology in the Universe. Apparently, at time zero, from a singularity of infinite temperature and density, the Universe was born from an explosion of matter, energy, space, and time (Hawking, 1988, 2001, 2002; Weinberg, 1994). This event occurred roughly 13.7 billion years ago. About a hundredth of a second after time zero, the Universe cooled to a temperature of a hundred trillion degrees Kelvin. About a hundred seconds after time zero, the Universe cooled down further to a temperature of one trillion degrees (Adams, 2002).

When the Universe was about 400,000 years old and its temperature had further cooled down to three thousand degrees, protons, neutrons, and electrons combined for the first time to produce the first atoms of hydrogen and helium only. Hundreds of millions of years later, when the first stars formed, atoms of heavier elements were formed by fusions of hydrogen and helium in the interiors of such stars where temperatures averaged about ten million degrees. The heavier elements were spit back out into the cosmos via cosmic rays and mass ejecta from supernova explosions of such stars after they had burned up all of their nuclear fuel (Hartquist and Williams, 1995).

The heavier elements in the cosmos proceeded to form primitive inorganic compounds in the following manner: Carbon reacted with hydrogen to produce methane. Nitrogen reacted with hydrogen to produce ammonia. Hydrogen and oxygen reacted to produce water. Carbon and oxygen reacted to produce carbon dioxide. Phosphorous and oxygen reacted to produce phosphate. Sulfur and hydrogen reacted to produce hydrogen sulfide. Carbon, oxygen, and hydrogen reacted to produce formaldehyde. Other simple primitive inorganic compounds were formed in similar chemical reactions.

About four billion years ago, at least here on the planet Earth, the above types of primitive inorganic compounds, continued to undergo chemical reactions to form amino acids, nucleobases, sugars, and lipids. Between four billion years ago and a few hundred million years later, on the primitive Earth, amino acids, nucleobases, sugars, phosphates, and lipids underwent further chemical evolution (Calvin, 1969) to form the first proteins, nucleic acids, polysaccharides,

and complex lipids, which subsequently organized themselves into entities called *cells* which possessed the emergent property of assemblies of molecules that we call being *alive* (Morowitz, 1992) and that were capable of further undergoing biological evolution (Oparin, 1938; Seckbach, 2004). The rest of the story is biological history (Darwin, 1859). It would be very strange indeed if there were not life forms spread throughout the Universe. After all, we Earth based life forms inhabit just one rock orbiting around an average yellow star a quarter of the way in from the rim of a typical spiral galaxy in a local cluster, surrounded by billions of other galaxies, and billions and billions of other planets. Among all of the billions of galaxies in the Universe, there undoubtedly are other life forms. However, sentient life forms may be rare in the Universe (Ward and Brownlee, 2000).

3. From Special Relativity to General Relativity

The Newtonian Universe, which held sway in the minds of scientists for three centuries, was perfectly Euclidean and infinite in extent. Within this sensorium, space was perfectly uniform with no space being different from any other space, and time also was perfectly uniform, with time differing not at all from one location in the Universe to any other place in the universe. The Newtonian Universe looked very much like a Universe which could have been eternal and essentially changeless over a large scale, a Universe with no beginning and no end.

However, when James Clerk Maxwell completed the main development of classical electrodynamics with his famous four equations describing static and changing electric and magnetic fields, and it was found that exactly one speed for the electromagnetic radiation that we call light fell out of these equations, an impasse in the fitting together of classical electromagnetics with classical mechanics began to be noticed. The impasse was this: Maxwell's equations predicted a single precise speed of light independently of the state of motion of the inertial reference frame in which the measurement of the speed of light might be taken. This fact was in distinct contradiction to Newtonian mechanics where the speeds of material objects depended very much on the state of motion of the inertial reference frame in which the measurement of the speed of such material objects might be taken.

This vexing conundrum in physics was successfully resolved in the year 1905 by Albert Einstein who convincingly demonstrated that the electrodynamics of bodies moving with constant velocities are correctly described by his Special Theory of Relativity (Einstein, 1905). The main results of this theory are as follows: Space and time are *not* uniform everywhere and everywhen as in the Newtonian Universe. In fact, space shrinks in the direction of forward motion, such space shrinking to zero as an object's motion approaches the speed of light. Also, time slows down for objects in motion, such time slowing to a complete stop as an object's motion approaches the speed of light. In addition, the mass of a

material object increases with the material object's motion, such mass increasing to infinite mass as the material object's motion approaches the speed of light.

The most famous equation associated with Einstein's Special Theory of Relativity is the formula $E = mc^2$ or energy equals mass times the speed of light squared. The reality of this equation was brilliantly illuminated by J. Robert Oppenheimer and his colleagues at Alamogordo, New Mexico in the year 1945 by the explosion of the first fission uranium-based atomic bomb.

The reason that the Special Theory of Relativity is called special is that it pertains to the electrodynamics of objects traveling at a *constant* velocity, as an object would travel through empty space devoid of gravitational fields. Thus, the Special Theory of Relativity is a theory of space and time especially simplified, and hence the word "Special".

Albert Einstein, who in large part overthrew the Newtonian Universe which had lasted for 300 years, then went on to reconstruct the Universe anew in his General Theory of Relativity where there *are* gravitational fields and where the velocities of material objects are *not* constant but rather where the velocities of material objects are subject to accelerations and deaccelerations. Thus it became clear that whereas the Special Theory of Relativity was a theory of space, time, and motion, the General Theory of Relativity was going to be a theory of space, time, motion, and gravitation (Einstein, 1916).

Einstein started out by noting that it was impossible for an observer inside a rocket ship without windows to tell whether the ship was stationary in a gravitational field or was accelerating through open space without a gravitational field. This realization Einstein called the Principle of Equivalence because it pointed out the fundamental equivalence of the inertial and gravitational mass of matter, which equivalence had been known but previously never really understood before Einstein's happy thought experiment.

Einstein then realized that a mass in free fall in a gravitational field did not actually experience a gravitational force if the inertial reference frame was taken to be centered on the mass itself during its free fall in the gravitational field. Thus, gravitation (Misner et al., 1973), instead of being looked upon as a force, could more accurately be seen as a bending or curvature of space-time around the source of a gravitational field. As the renowned physicist John Wheeler put it, "Matter tells space how to curve and space tells matter how to move."

The main results of the General Theory of Relativity, which was published in 1916, are these: Space and time (or, to be more accurate, space-time) is a property of the distribution of matter-energy. In fact, the space and time of the Universe is a property of the distribution of matter-energy in the Universe. As the curvature of space, that is, the gravitational field increases, time slows down. At an infinite curvature of space, as found at the Big Bang singularity or as found in present-day black holes of galactic, stellar, or even smaller sizes (Novikov, 1995), time comes to a complete stop. In other words, in the Big Bang singularity and in a black hole singularity time does not exist.

4. From Classical Mechanics to Quantum Mechanics

In the Newtonian Universe, the distribution of matter in a material object and the distribution of energy in a physical system can vary in a continuous manner, smoothly increasing or decreasing from zero matter and energy to the highest level of matter and energy in the particular system under study (Marion, 1965). However, in the quantum mechanical revolution of the first quarter of the twentieth century it was found that matter came in discrete bits called atoms or particles and that energy also came in discrete bits called *quanta*.

The quantum mechanical revolution began when Max Planck studied the spectrum of black body radiation and found that he had to invent a new physical constant h as a parameter of the size of energy quanta to obtain a match between black body theory and black body experimental results. Then Albert Einstein showed that light waves are actually composed of discrete bits of energy called photons and where the energy of a photon was given by the equation $E = h\nu$ where E is the energy, h is Planck's constant, and ν is the frequency of the photon. Consequently it was shown that not only do waves of light have particle-like properties but also that particles of matter such electrons *also* have wave-like properties. This quantum mechanical wave-particle duality at first seemed quite strange indeed but physicists by now have gotten used to it.

The next step in quantum mechanics came with the discovery of Werner Heisenberg that for any particle whatsoever, the exact position and the exact momentum of a particle could never be precisely determined at the same time. The absolutely unavoidable uncertainty of the state of the particle was described by the equation $h \leq \Delta p\, \Delta x$ where h is Planck's constant, Δp is the uncertainty in the particle's momentum, and Δx is the uncertainty in the particle's position.

The next step in the evolution of quantum mechanics was taken when Erwin Schrodinger discovered the equation which correctly describes the wave function ψ associated with a particle.

Finally, the last step in the quantum mechanical revolution was taken by Max Born who correctly interpreted the wave function ψ by saying the absolute value of ψ squared was a function describing the probability distribution of finding the said particle at a particular location in space.

The thing about quantum mechanics that physicists found initially to be a shock was that quantum mechanics showed that there was a certain amount of indeterminism unavoidably built in to the world. In other words, there was a certain amount of acausality built in to reality. For example, it was subsequently shown that in all physical systems, there were always random quantum fluctuations occurring to a certain degree. Even in a perfect vacuum, so-called *virtual* particles are constantly flickering into and out of existence. This had been demonstrated experimentally in what is known as the *Casimir Effect*. Vacuum quantum fluctuations are possible as long as they do not violate an alternative form of the Heisenberg Uncertainty Principle.

5. The Planck Length and the Planck Time

Although classical physics has been superceded by the more inclusive theories of general relativity and quantum mechanics, and although these two theories of modern physics have passed every experimental and observational test to which they have been subjected, still there is currently no common consensus among physicists of how to combine the two theories, both of which must form some part of an ultimate theory of the Universe as we approach closer and closer to time zero of the Big Bang. In any case, it is generally agreed by an overwhelming consensus of physicists that as we approach a size dimension called the Planck length defined as $l_P \equiv 10^{-35}$ m (Peacock, 1999), the theories of general relativity and quantum mechanics begin to break down. Also, as we approach a time dimension after time zero called the Planck time defined as $t_P \equiv 10^{-43}$ seconds (Peacock, 1999), the theories of general relativity and quantum mechanics again begin to break down. To get closer to understanding the initial Big Bang singularity at a time earlier than 10^{-43} seconds after time zero, when the Big Bang was only 10^{-35} m in diameter, in the absence of a unified theory of general relativity and quantum mechanics, we have to project what those theories imply by extrapolation to time zero and to diameter zero of the said Big Bang singularity. Those theories imply that *at the Big Bang Singularity, space and time come to a complete stop*. That is, space and time themselves originated only at the moment of the Big Bang explosion. There was no space and there was no time before the Big Bang explosion. Even the word "before" loses all meaning in this context. How can there be a "before" when time does not exist? Clearly there cannot be a "before" to the Big Bang at time zero. Neither can there be any "place" before the Big Bang at time zero for a place implies a location in space, but there was no space before the Big Bang at time zero.

6. Space, Time, and Causality

As the great eighteenth century philosopher Immanuel Kant (1781) pointed out in his classic book *Critique of Pure Reason*, space and time are modes of apperception without which we are hard pressed to conceptualize anything whatsoever. Also, all mechanisms by which we understand how anything in the Universe works, namely causality, also take place only within the ground works of space and time. Even to the extent that quantum mechanics has displayed a fundamental acausality or indeterminism in the course of events that take place in the Universe, particularly at a micro level, even such quantum mechanical acausality or indeterminism takes place within space and time. Even a vacuum requires space, and quantum mechanical vacuum fluctuations thus take place in space and time. Even a so-called false vacuum requires space. A Higgs field, or for that matter (pardon the pun), any field whatsoever requires space.

7. The Big Bang at Time Zero

So, what can we say about the very first of all possible states – the Big Bang at time zero? If we wish to be scientific, we have to admit that at time zero of the Big Bang, no space and no time existed. There was no "before" to this initial state. "Before" is something which only takes place in time. When time does not exist, an instant and eternity have no meaning. When time does not exist, causality also cannot be operating because causality itself has no meaning. For the above reasons, about the Big Bang at time zero, we conclude the following: *The Big Bang was an uncaused event*.

8. Acknowledgment

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Biodata of **Koichiro Matsuno**, the author of ***"Molecular Imprints of Reaction Network: Living or Non-Living"***

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MOLECULAR IMPRINTS OF REACTION NETWORK:
Living or Non-living

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1. Introduction

Atoms are relics of the Big Bang and the subsequent supernovae explosions, according to the currently accepted cosmology. From this perspective, any atoms present on any planetary systems including our Earth are atomic imprints of the earlier events of the Big-Bag cosmology. At the same time, DNA molecules retrievable from dinosaurs frozen in stones found on the surface of the Earth are also molecular imprints of the organisms going into extinction by now, while living creatures inhabiting there constantly feed upon material resources made out of atoms or atomic imprints. Moreover, if a DNA molecule retrieved from a recent fossil is put in an appropriate ribosome, it can restart the protein synthesis already programmed on the DNA sequence. This observation then raises a serious question of how one could distinguish molecular imprints between living and non-living.

Molecular imprints are quite relative in their implications and context-dependent with regard to how they are related to living things. The present vague aspect surrounding molecular imprints is, however, not necessarily disadvantageous in clarifying the issue of how living things could emerge in the cosmological context. Rather, molecular imprints will turn out advantageous as an analytical tool in introducing those material units that may have the capacity of searching for the material contexts to be fitted in on their own (de Duve, 2005). This is a theme to be explored in the present chapter. One question unique to molecular imprints is whether they are a record of something to be read out by the human external observer or a memory of something to be deciphered by a material body internal to whatever material organization.

2. Molecular Imprints: Record or Memory?

Molecular imprints as a remnant of an organism of remote past age preserved in the Earth's crust is a record to be read out by the paleontologist. Such a record is anthropocentric in that only the paleontologist is competent enough and responsible for providing the reading frame required for the deciphering. In contrast, molecular

imprints as part of a living organism carry the memory to be read out by the very organism, as being part of the whole from within. Memory takes participation of the reading frame of material origin for granted. It assumes intervention of a certain material processor for deciphering what is stored in the form of memory. The processor is by no means a monopoly of the human external observer. In short, the memory stored in the computer hard disk is specific only to the central processing unit, that is, CPU, and not to the computer scientist.

A carbon atom in the molecule of carbon dioxide in the air can be identified and recorded as such by the physicist. There is nothing inconvenient in the present physical recording of atoms and molecules. However, molecule registered as a record differs from molecule functioning as a memory. Molecule as a memory is a material token passed over from past events and processed at the present moment by the material body that can read it as such. Carbon dioxide in the air is a material token to be taken in by a photosynthetic plant having the capacity of assimilating it to the own body. Anabolism on the part of photosynthetic plant assumes the memory such that carbon dioxide in the ambient can be the material resource to feed upon. In parallel, carbon dioxide in the air is a molecular imprint having the memory, or equivalently the capacity, that it can be assimilated into photosynthetic plants.

Assimilation of small molecules into the larger material organization such as an organism is unquestionably a material process. However, it has not been well worked out in the traditional scheme of physics. A main difficulty with it resides within the methodological stipulation unique to the latter. To be sure, physics is competent in coping with a wide variety of material processes in terms of the notions that are memory-free. The wavefunction is a typical example of demonstrating how effective the memory-free state attributes could be in the endeavor of describing what the molecule is all about. The state attributes such as the phase-space coordinates of the wavefunction can easily be frozen and registered in the record by the physicist. Memory attributes, if any, are thus necessarily marginalized by the stipulation that the state attributes should underlie the deciphering of them. On the other hand, once such a strict stipulation dismissing the memory attributes is lifted, a new perspective may be in sight. One clue toward this directive comes from the practicing of biochemistry.

Biochemical processes demonstrate how versatile and ubiquitous the process of material assimilation or anabolism could be in the material realm. Underlying the process of assimilation are molecular enzymes that are quite substrate-specific and unidirectional in regulating the involved chemical reactions. In fact, the origin of molecular assimilation met in the practice of biochemistry could be taken almost equivalent to the origin of living things. This association of the origin of molecular enzymes with the origin of living things however does not help us to perceive how molecular enzymes could have come into being. Replacing molecular enzymes simply by living things does not clarify the issue. A more pressing agenda is whether memory-specific molecular assimilation could get started even in the absence of molecular enzymes that biochemistry is at home with. Only when

the process of molecular assimilation can be demonstrated to take place without the help of molecular enzymes of biological origin, one may be able to conceive of how molecular enzymes or living things could emerge on the material basis. This should be an issue to be settled empirically or experimentally, more than anything else.

3. Molecular Assimilation Without Enzymes

A most simple and straightforward example of molecular assimilation is an indefinite growth of the population of the molecules of like kind as feeding upon whatever molecular resources, though necessarily limited in both spatial and temporal extensions. What is proceeding there is to assimilate the available molecular resources into the molecules of like kind. An exponential growth of the population of the molecules of like kind is a typical example. In fact, if an autocatalytic molecule is available, an exponential growth of the population of such molecules could be likely if the conditions are of a right kind. Rather, what should be sought here is a sort of conditions enabling an exponential growth of the population of a certain chemical species without importing an autocatalytic molecule already synthesized in an explicitly completed form elsewhere (Wächtershäuser, 2006). One candidate for fulfilling this requirement must be the conditions similar to the ones supporting the likelihood of hydrothermal circulation of seawater on the floor of the ocean of the Earth.

Chemical reactions occurring in the reaction solution while visiting both hot and cold regions repeatedly in a cyclic manner are peculiar in that the reaction products are constantly converted into the reactants with the aid of the physical means of making the circulation of the reaction solution between the hot and the cold possible. The products synthesized in the preceding cycle can be made the reactants in the succeeding cycle. In particular, the reactants can gain thermal energy driving various synthetic reactions while visiting hot regions. In this setting, once a reaction product in the current cycle happens to be similar to the reactant in a previous cycle, the over-all reaction will turn out network-catalytic in assisting the synthesis of the product of like kind through the reaction cycle. Hydrothermal circulation of the reaction solution serves as a physical means for the realization of such a reaction cycle. An exponential growth of the population of the reaction product, at least initially, will be a straightforward consequence. This consequence can be established even in the absence of a self-sufficient autocatalytic molecule to start with if the conditions of a right kind are available. For instance, elongation of oligomers in a hot region and hydrolysis of those oligomeric products in the cold environment, when combined together through repeated circulations, can induce an exponential growth of the population of the oligomers if their residence time in the hot region is so limited as to prevent their thermal decompositions. Network-catalytic reactions conditioned by the repeated cycles of heating and quenching come to sustain an exponential growth of the reaction product as a form of molecular assimilation.

Significant to the occurrence of an exponential growth of the reaction product is the material capacity of sensing, searching for and cultivating the necessary resources available in the environments in an exhaustive manner. In fact, the exponential growth of the reaction product is a demonstration of molecular assimilation to an extremely enhanced extent in the sense that the rate of assimilation per unit time is in proportion to the amount of those molecules already assimilated. Since the available resources are not unlimited, exponential assimilation is context-dependent in that the resources available in the accessible environments are exhaustively explored. Molecules participating in the exponential assimilation can thus carry with themselves the memory of the accessible environments.

What remains to be seen here is how to ascertain on an experimental basis the present likelihood of molecular assimilation whose growth is exponential in time at least over a limited time interval, even in the absence of molecular enzymes. We then constructed a flow reactor simulating hydrothermal circulation of seawater through hot vents in the ocean (Matsuno, 1997). Earlier review of the experimental work will appear in Matsuno (2008), some of which is reproduced in the following.

4. Molecular Imprint of Hydrothermal Circulation

When the aqueous solution including only glycine, the simplest amino acid molecule of all, was run in the flow reactor circulating the fluid across the temperature gradients between 230°C and 0°C repeatedly with the cycle time of roughly 1 minute, we observed an exponential growth of diglycine and triglycine over a limited time interval at least initially (Imai et al., 1999). The exponential assimilation or synthesis of both oligomers can unquestionably serve as the molecular imprints of the flow reactor we attempted. The upper limit of the exponential assimilation that could eventually be reached in due course of time is unique exclusively to the experimental setup we prepared, that is to say, the environment accessible to the chemical reactions proceeding there. The exponential assimilation would eventually turn the growing molecules into the molecular imprints of the environments to be exploited for the sake of their exponential growth.

What is more, the shakeup of the molecular imprints of exponential assimilation would also become inevitable, that is definitely history-dependent. In the case of the reaction solution starting with only glycine monomers, for instance, we observed the takeover of the exponential assimilation by tetraglycine after the preceding synthesis of diglycine and triglycine had been saturated. The emergence of tetraglycine is history-dependent in the sense that it emerged only after the synthesis of diglycine and triglycine had been saturated and by no means vice versa. The process of exponential assimilation is intrinsically evolutionary and irreversible in its operation. When a certain chemical species gets involved in an exponential explosion of its population, the species that actually grows exponentially is the one having the largest growth rate among the contenders. All of the

other alternatives are wiped out in the process. However, the exponential growth of that species cannot survive indefinitely. Once the growth reaches a saturation level for whatever reasons, this level can now prepare a refreshed stage for a new species to start up an alternative exponential growth. The exponential growth of tetraglycine actually started after the growth of both diglycine and triglycine had been saturated.

In a similar vein, when we attempted the reaction solution comprising both glycine and alanine initially, the dimer that first appeared was glycylalanine, and then followed by the emergence of alanylglycine (Ogata et al., 2000). Once alanylglicine appeared in the reaction solution, the exponential assimilation into alanylglycine got started as dissecting the pre-existing glycylalanine in which alanylglycine effectively functioned as a molecular enzyme decomposing glycylalanine once synthesized. The sequence of the takeover of exponential assimilation was from glycylalanine to alanylglycine, but not vice versa, with no exceptions. When the synthesis of glycylalanine reaches its saturation level, the succeeding fate of glycylalanine could be at least two-fold. One is to utilize the dimer of glycylalanine as a unit for further exponential synthesis with other monomers and oligomers, and one more alternative is to dissect the dimer into monomeric units as the resources for an alternative exponential synthesis of de novo oligomers. Our experimental observation of the synthesis of alanylglycine actually revealed the case that the appearance of a molecular enzyme carrying a protease-like capacity sets a refreshed condition for starting up the takeover by a de novo exponential assimilation. As a matter of fact, a set of emerging molecular enzymes like alanylglycine can be stabilized in the reaction system once they become an indispensible member of the currently prevailing exponential assimilation. Alanylglycine as a molecular enzyme can certainly function as a molecular imprint memorizing how the vicissitudes of exponential assimilation could have been in place so far.

The demonstration of how exponential assimilation gets started and alternated in the flow reactor simulating hydrothermal circulation of seawater through hot vents rests upon the interplay between endergonic and exergonic reactions. Peptide synthesis is endergonic, while the cooling of the reactants transferred from the hot vents is exergonic. The endergonic products, once quenched rapidly in the cold environments, can be stabilized there without suffering further exergonic decompositions. Rapid quenching tends to accomplish the exergonic decomposition of the endergonic products only incompletely as leaving behind those endergonic derivatives that may still maintain the endergonic remnants of chemical synthesis internally.

What is peculiar to the synthesis of endergonic derivatives in the hydrothermal circulation is the incremental increase of the amount of energy supplied to the ongoing endergonic reactions every time the reactants visit the hot regions or their neighborhood. The energy accumulated in the endergonic derivatives can increase as the frequency of visiting the hot regions increases. For instance, endergonic synthesis of tetraglycine from glycine monomers can be made possible

only after the reaction solution including glycine visits the hot regions repeatedly. Synthesis of tetraglycine from monomeric glycine cannot be accomplished instantaneously during a very short visit to the hot region only once. To the contrary, tetraglycine can be formed only after following the intervening prior stages of synthesizing diglycine and triglycine as endergonic derivatives that could remain meta-stable at least temporarily during the repeated cycles of heating and cooling.

Hydrothermal circulation round the hot and the cold provides a physical means for exploring the energy required for completing endergonic reactions step by step incrementally as repeating the cycle. In this regard, one more serious test for examining the plausibility of step-by-step accumulation of the energy required for endergonic reactions may come from the experimental likelihood of implementing the prebiotic citric acid cycle in the absence of molecular enzymes.

5. Endergonic Derivatives and the Citric Acid Cycle

The citric acid cycle constitutes the inner-most core of the whole network of metabolic pathways found in any biological organisms. The prebiotic significance of the likely occurrence of the citric acid cycle may be found in the evolutionary emergence of the cycle in the absence of molecular enzymes of biological origin (Morowitz et al., 2000). When the issue of the evolutionary likelihood of the citric acid cycle is examined, the question of which could have been first, either the reductive cycle or the oxidative counterpart, would become inevitable. However, we shall not address this question in a directly confronting manner. Rather, what concerns us here is an experimental likelihood of starting up the network chemical reactions comprising various carboxylic acid molecules constituting the citric acid cycle in prebiotic conditions. A case study we shall focus upon is an experimental possibility of preparing the stage for the oxidative citric acid cycle.

Every step of chemical reactions round the citric acid cycle is either endergonic or exergonic (Smith and Morowitz, 2004). The highest energy barrier for running the oxidative citric acid cycle resides in the endergonic reaction pathway from L-malate to oxaloacetate requiring the energy as much as 29.7 kJ/mol. Although the second steepest energy barrier is found in the endergonic pathway from citrate to isocitrate, the energy required for crossing over the barrier is about 13.3 kJ/mol that is less than half of the energy required for the pathway from L-malate to oxaloacetate. The contemporary citric acid cycle drives both the endergonic and exergonic reactions along the cycle with the aid of biological enzymes and coenzymes in the normal thermal ambient. However, such enzymes and coenzymes could not have been available in the prebiotic setting. If the evolutionary emergence of the citric acid cycle is a matter of concern, an evolutionary scheme implementing the cycle in the absence of biological enzymes would have to be worked out. At this point enters the evolutionary opportunity such that hydrothermal circulation of the reaction solution going through the hot and the cold

repeatedly in a cyclic manner may play a positive role, especially in preparing endergonic derivatives as a means of accumulating the energy required for the endergonic reactions step by step incrementally.

In order to experimentally examine the evolutionary potential latent in the hydrothermal circulation, we ran the flow reactor for the reaction solution comprising all of the eight major kinds of carboxylic acid molecules constituting the citric acid cycle, that is, oxaloacetate, citrate, isocitrate, alpha-ketoglutarate, succinate, fumarate, L-malate, and pyruvate serving as both the energy and carbon sources to the cycle (Matsuno and Nemoto, 2005). The temperature gradient traversing the hot and the cold in the flow reactor was implemented over the crossover region between 120°C and 0°C, in which the reaction products made at 120°C were rapidly transferred and quenched at 0°C in a cyclic manner. The observation we made revealed an increase, though slight, of the concentration of oxaloacetate in time going along with the flow-reactor operation, indicating that the endergonic reaction transforming L-malate into oxaloacetate was certainly operative (Matsuno, 2006).

Rapid quenching of the reaction products from 120°C to 0°C in a repeatedly circulating manner can provide the energy as much as 29.7 kJ/mol required for the endergonic reactions. This observation is consistent with the synthesis of phosphodiester bonds for making the 3′–5′ linkaged oligonucleotides out of monomeric nucleotide molecule of AMP requiring the energy about 22 kJ/mol, through hydrothermal circulation between 110°C and 0°C (Ogasawara et al., 2000). Likewise, we also observed the synthesis of pyrophosphate bonds requiring about 29 kJ/mol in the reaction of making ADP and ATP from AMP and trimetaphosphate serving as the phosphate source, in the flow reactor circulating the reaction solution between 100°C and 0°C (Ozawa et al., 2004). Needless to say, the peptide synthesis requiring about 11 kJ per bond was made possible in the flow reactor simulating hydrothermal circulation of seawater through the hot vents (Imai et al., 1999).

When these observations are integrated together, we come to recognize that it may be possible to have the products from endergonic reactions requiring the energy of order of 30 kJ/mol even by means of hydrothermal circulation alone. However, the resulting reaction products as the molecular imprints of the underlying hydrothermal environments are not robust enough evolutionarily. Once those molecular imprints happen to be removed to the other places far away from their birthplace near the hydrothermal vents on the ocean floor, they would soon lose most of the capacity of energy transductions imputed to the presence of hydrothermal environments. No energy input for supporting the molecular imprints could be conceivable once they are detached from the birthplace. In this regard, the oxidative citric acid cycle might play a significant role.

The reaction from isocitrate to alpha-ketoglutarate along the circular reaction pathway of the cycle is exergonic as releasing the energy as much as 20.9 kJ/mol, though the reaction pathway from L-malate to oxaloacetate is endergonic. In addition, the succeeding reaction from alpha-ketoglutarate to succinate is also

exergonic as releasing 36.4 kJ/mol. More specifically, on conditions that the supply of pyruvate serving as both the energy and carbon sources is guaranteed by whatever means, one turn of the reaction pathways round the oxidative citric acid cycle is effectively exergonic as releasing the net energy of 49 kJ/mol. This energy transduction may suggest a likely evolutionary scenario such that once the citric acid cycle is put in place; the cycle could serve as the primary candidate for the energy supplier if pyruvate is already available. The role of hydrothermal circulation toward the operation of the citric acid cycle would have to be at most catalytic and not energetic, as making the energy input to the cycle from the hydrothermal system superfluous. Then, an issue may arise with regard to whether there could have been available any further evolutionary opportunity to the possible takeover of catalytic capability by other than the operation of hydrothermal circulation.

6. Carbon Flow; Anabolic and Catabolic

Evolutionary likelihood of starting up the citric acid cycle in the absence of molecular enzymes of biological origin could have been substantiated if pyruvate may become available by whatever means. The prebiotic synthesis of pyruvate could in fact be likely in the vicinity of hydrothermal vents (Cody et al., 2000; McCollom et al., 1999). The prebiotic citric acid cycle conceivable in the hydrothermal environments can be characterized by two kinds of carbon flow. One is the intake of carbon into the cycle that is anabolic, and another is the takeout of carbon from the cycle that is catabolic. Between these two flows, catabolic carbon atoms in the form of carbon dioxide are statistically independent with each other if the originating carbon dioxide molecules are different. The outgoing catabolic carbon flow measures the extent to which carbon atoms already assimilated into the anabolism may remain and survive in the inside. One figure of merit in this regard is the catabolic rate of carbon per carbon atom, residing in the reaction cycle of circulating carbon flows as in the case of the citric acid cycle. The catabolic rate of carbon gives a quantitative estimate of how many times one carbon atom in the assimilated body can be alternated per unit time by new ones entering in the form of anabolic flow from the outside. Equivalently, the catabolic rate of carbon also measures the turnover rate of carbon as counting how many times one carbon atom in the assimilated body can be replaced by new ones entering from the outside.

One significant aspect of the turnover rate of carbon as focusing upon the catabolic carbon flows, in which each carbon dioxide molecule behaves almost independently with others statistically, is found in the observation that the anabolism proceeds toward minimizing the turnover rate (Matsuno, 1978). The underlying reasons are quite simple and straightforward in that the most prevailing material elements residing in the anabolic assimilation are the ones that can minimize the turnover rate, because of the statistical independence of the outgoing carbon atoms among themselves. Those material elements with the greater turnover rate

cannot compete with the ones with the smaller turnover rate in the material assimilation and in the resulting accumulation.

Once anabolism gets started, a new evolutionary directive may be in sight in the form of minimizing the turnover rate of carbon exclusively on the material ground. At the same time, there might also happen to raise a new impasse if the turnover rate actually vanishes in the limit of its minimization. No turnover would come to imply no anabolism and accordingly no chances for biological organizations. One alternative for preventing the turnover rate from actually vanishing is the presence of temperature gradients supporting the occurrence of both anabolic and catabolic activities, since it is the very occurrence of temperature gradients which could make possible the process of material assimilation in the first place, as met in the hydrothermal environments. Unless the physical approach to the heat reservoir at the lower temperatures is blocked off, material assimilation could be likely. The pressing issue in this regard must be what sort of low-temperature heat reservoirs could be available and accessible to the process of material assimilation proceeding within the thin crust of the surface of the Earth. This perspective begs the question on the nature and classification of the low-temperature heat reservoirs accessible to the surface of the Earth.

Insofar as only the thin atmosphere surrounding the surface of the Earth is focused upon, gas molecules in the air are almost in thermal equilibrium with each other at the normal ambient temperature. If the organization of material assimilation is in thermal equilibrium with the surrounding atmosphere, the photons going back and forth through the interface between the two would eventually come to be balanced in the form of radiation field in thermal equilibrium. There is no net emission or absorption of photons by the organization. This absence of net photon flow through the interface, however, meets severe counterfactual evidence.

ATP hydrolysis as a basic material means of biological energy transduction driving respiration and motor activity among many others is under the condition that the energy, once released, has to be dissipated with no chances of being struck back and counteraction, as differing from the case of radiations in thermal equilibrium. Both ATP synthesis and hydrolysis are constantly running down the energy landscape in an irreversibly unidirectional manner. Although the atomic substrates of ATP molecules including hydrogen, carbon, oxygen and phosphor are usable repeatedly countless times, the flow of energy is totally different. That is strictly unidirectional from the source to the sink with no exception. The real question with ATP hydrolysis is where it could find and make an access to the sink at low temperatures that can absorb the used and dissipated energy. If the dissipation were to take place in the form of infrared photons being in thermal equilibrium with the radiation field of the ambient atmosphere, there would be no chances of being totally absorbed by the ambient atmosphere, since the counteraction from the ambient atmosphere would also have to be counted equally. The energy of the photons carrying the dissipated energy must be much less than that of infrared photons.

One clue for figuring out where the sink for ATP hydrolysis could be located is to estimate the energy of photons being emitted from the hydrolysis. ATP hydrolysis proceeding in actomyosin complexes as a functional unit of muscle contraction releases energy as much as 29 kJ (7 kcal)/mol over the time interval of order of 10 milliseconds. If the energy release takes place continuously over the interval, the average photon energy can be of order of 10^{-19}erg, that is equivalent to the radiation energy at temperature roughly 1 mK. In other words, ATP hydrolysis would come to search for the sink that can absorb the microwave photons whose frequency is of order of 10^8 Hz, otherwise proper functioning of ATP hydrolysis would be jeopardized because of the difficulty in finding the appropriate sink (Matsuno, 1999). At this point enters the cosmological constraint. In fact, thanks to the ever-lasting cosmic expansion, outer deep space looks transparent to the microwave photons of frequency around 10^8 Hz though it remains opaque to far infrared photons constituting the cosmic microwave background specified by temperature at 2.725 K or frequency around 10^{11} Hz (Matsuno, 2006).

Carbon assimilation or anabolism characterized by the minimization of its turnover rate is constantly under the cosmological constraint guaranteeing the presence of the sinks for dissipated photons. The empirical peculiarity of cosmic expansion leaves outer space transparent even to a tiny amount of photons if the emitted photons are the microwave photons in a limited frequency range, say, around 10^8 Hz. This microwave transparency originating in the cosmic expansion does not, however, apply to the photons constituting the cosmic microwave background in the frequency range centered around 10^{11} Hz. The microwave receiver, wherever located in the cosmological context, can certainly detect noise signals imputed to the cosmic microwave background. Receiving such noise signals is equivalent to being susceptible to radiations from the heat reservoir maintained at 2.725 K. Henceforth, there should be no likelihood for dissipating energy exclusively in an irreversible and unidirectional manner in the form of the photons whose frequencies happen to coincide with those constituting the cosmic microwave background.

The cosmic microwave transparency open to the frequencies far below 10^{11} Hz, on the other hand, provides one decisive condition on how the process of carbon assimilation into the supporting reaction cycles could have emerged and evolved since then on the planet Earth. If only those molecules present in the thin crust of the atmosphere surrounding the surface of the Earth are focused upon, they can participate in the thermal motion specified by the ambient temperature. It would then be inconceivable to expect the occurrence of biological organizations only from the motion of the molecules in thermal equilibrium with their immediate environments. Inorganic materials such as stones and rocks on the surface of the Earth can be in thermal equilibrium with the ambient locally either spatially or temporarily, or both unless the ambient conditions are disturbed significantly. To the contrary, however, biological organisms and their precursors constantly defy the asymptotic approach to thermal equilibrium with their immediate ambient in contact because of its intrinsic capacity of exploring the sinks for the dissipated

energy on the cosmological scale. One decisive practical means for exploring the sinks on the cosmological scale here on the planet Earth could have been to utilize ATP hydrolysis as a source of energy driving a wide variety of biological functions and to dispose of the consequential waste with the use of the cosmic microwave transparency.

In short, participation of ATP molecules in biological organizations requires at least two different classes of low-temperature heat reservoir. One is the normal thermal environment on the surface of the Earth serving as a low-temperature heat reservoir toward the sun light or the geothermal heat driving the ATP synthesis. One more class of low-temperature reservoir is for absorbing the necessarily dissipated energy precipitated from ATP hydrolysis delivering the energy for driving various biological functions. The thermal environment toward ATP synthesis in general or the temperature gradient applied to it in particular must be different from that toward ATP hydrolysis; otherwise the operation of both ATP synthesis and hydrolysis may be disturbed on the basis of thermodynamics in the presence of temperature gradients. An ATP molecule as an end product of synthesis cannot be the end product of its decomposition through hydrolysis at the same time as sharing and experiencing the same temperature gradient. Nonetheless, ATP hydrolysis takes the normal thermal environment on the surface of the Earth to be one end of the source for generating and maintaining the necessary temperature gradient. The cosmic microwave transparency serves as a physical means for substantiating the necessary low-temperature reservoir required for the proper operation of ATP hydrolysis without interfering with ATP synthesis on the planet Earth.

7. Concluding Remarks

As much as atoms are the material imprints of both the startup of the current Big-Bang cosmology and the succeeding supernovae explosions, bio-molecules can be the molecular imprints of the ever-lasting cosmic expansion. This perspective invites us to figure out the role of quantum mechanics, which furnishes whatever material organization, either non-biological or biological, with its stability, under a new light (Davies, 2004). The stability of an atom can be fathomed quantum mechanically even without referring directly to the details of its emergence through the historical development on the cosmological scale. In the similar vein, the stability of bio-molecules in general and molecular enzymes in particular should also be sought in quantum mechanics, though in a bit different manner compared to the case of atoms and non-biological molecules. The difference resides in the fact that bio-molecules could be substantiated only through constantly disposing of the dissipated energy with use of the cosmic microwave transparency. The underlying functional unit is a heat engine processing both energy intake and takeout in a continuous fashion, in which the cosmic microwave transparency takes care of the takeout of the used energy.

The likelihood of the occurrence of a heat engine in quantum mechanics now suggests at least two possibilities. One possibility is that the heat engine is merely a secondary derivative, fabricated from the quantum already established in the non-biological realm, and one more alternative is that the heat engine belongs to a new class of quantum that has not yet been worked out in the realm of traditional physics. Between these two alternatives, the one which could be likely is the instance of quantum as a heat engine. A principal reason behind is that the occasion of taking advantage of the cosmic microwave transparency is fundamentally irreducible on the cosmological scale and there should be no chance of expecting to derive it from something else that may remain more fundamental in the current cosmology.

Quantum as a heat engine just happens to be irreducible as much as the cosmic microwave transparency is. This shared and common irreducibility makes bio-molecules taken as the molecular imprints of the ever-lasting cosmic expansion also to be the molecular imprints of the supporting heat engine. In addition, the molecular imprints carry with themselves the memory to be read out by the built-in operating system, that is nothing other than the processor identified with a quantum as a heat engine.

The material unit or imprint employed for referring to a quantum can be either a record or a memory of the vast historical events experienced by it. At this point, physics has been quite competent in deciphering the quantum as the material record of past events. In contrast, biology is striving toward focusing upon the functional processor for letting the molecular imprints be read out as a memory of the past events.

Quantum as a heat engine thus provides one pathway through which one may naturally reach biology while being anchored at the stronghold of physics at the same time.

Biology is astronomical even from its very start in taking advantage of the cosmic microwave transparency. Bio-molecules are the molecular imprints of the quantum as a heat engine whose heat reservoir positioned at the lower temperature side happens to be the ever-expanding universe itself.

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THE ALH84001 CASE FOR LIFE ON MARS

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Abstract The Martian Meteorite ALH84001 provided the most recent and controversial chapter in the search for life on Mars. The bold claims of possible traces of Martian biological activity within the meteorite, stirred countless debates regarding some of the basic principles of life, and gave rise to the golden age of Space Exploration that we are witnessing. Regardless of the final verdict, the ALH84001 case for life on Mars exemplifies the importance, the complexity and the excitement associated with the field of Astrobiology, and the quest for the search of life beyond our own planet.

1. A Place in History

The popular belief that Mars may be the nearest inhabited world has persisted for centuries. Early observers of Mars saw through their telescopes oceans and continents (Secchi, 1863; Flammarion, 1892), vegetation (Lowell, 1894), and even complex structures that could only be the making of intelligent civilizations (the famous canali, Schiaparelli, 1878). These interpretations were widely accepted by the public, and readily assimilated by the fiction literature, but met strong opposition within the scientific community. At the turn of the twentieth century, theoretical calculations already indicated that temperatures on the Martian surface were likely well below freezing (Wallace, 1907). At these temperatures the white Martian poles were probably composed of frozen carbon dioxide, rather than water and the dark patches on the Martian surface were likely deserts, not forests. After decades of efforts directed towards the mapping of the Martian surface, the interest of astronomers then turned to determining the composition of Mars' atmosphere and surface. Soon it was established that Mars had a very thin atmosphere mainly composed of CO_2, and that the surface of the planet was extremely arid (Kaplan et al., 1964). As new discoveries unraveled, the surface of Mars was slowly becoming more and more inhospitable.

As the era of ground-based observations of Mars declined, the era of robotic exploration slowly rose. *Mariner 4* (1965) was the first successful robotic mission to Mars. After the first close up images of the Martian surface were

transmitted by the small spacecraft, an unexpected picture of the planet unfolded: No oceans or continents, no vegetation, and worst of all, a dry and heavily cratered surface suggesting a long-lasting, violent past. On December 2, 1971 a descent module successfully landed for the first time on the surface of Mars. *Mars 2* became the first man-made object to land on another planet. However, contact with the lander was lost within seconds. At the same time, *Mariner 9* was quietly orbiting Mars, and conducting a systematic mapping of the surface. Olympus Mons was the first feature to emerge, Hellas and Argyre followed. But the most revolutionizing discovery brought up by *Mariner 9* were the images of networks of channels and tributaries that strongly resembled runoff channels and dry riverbeds. Mars was suddenly brought back to life. If there was, or had been, water on the surface, then organisms could have also been present.

The great success of *Mariner 9* represented a strong push to what arguably became the most ambitious mission ever sent to Mars: The *Viking* landers. This mission remains to date as the only life detection experiment conducted outside our planet. Each of the *Viking* modules consisted of an orbiter and a lander. The landers were equipped with television cameras, meteorological instruments and a miniature laboratory to carry out sophisticated biological experiments. *Viking 1* landed on Chryse Planitia on July 20, 1976. *Viking 2* landed at Utopia Planitia on September 3, 1976. Both landers started to provide outstanding images and data immediately after touchdown. The long awaited biological experiments also seemed to work according to plan, however they provided confusing results. They had been designed to unambiguously detect organisms thriving in the surface soil, but the results of the experiments were difficult to interpret. After years of debate over the *Viking* results (Klein, 1999), the overall scientific consensus is that the landers did not provide any hint of Martian life, albeit some voices still argue heartily that there is indeed evidence for life hidden in the data. Independently of anyone's position, after the *Viking* mission it became clear that the surface of Mars is extremely severe and harsh, and likely not habitable for life-as-we-know-it. Planetary scientists began to recognize the need to better understand the limits of life on Earth, before we attempt to search for life somewhere else. Mars terrestrial analogues such as the Polar Regions or the hyper-arid deserts became the new ground for Astrobiology during the 1980s and early 1990s. These analogue studies provided a wealth of data that increased vastly our understanding of the limits of life on Earth, and allowed to draw realistic limits on the habitability of Mars and the Solar System.

With the 1990s came a renewed interest on Martian exploration. A new armada of landers and orbiters has been sent to the planet over the past 10 years, and three ambitious missions are scheduled between now and 2013; one of them, the Phoenix lander, is right on its way to the planet as these lines are written. This renewed interest on Mars owes to a body of literature and scientific research built up during the two decades after the *Viking* mission, but also owes to a single event that shocked the public and the scientific community on August 6, 1996:

> "A team of NASA and Stanford scientists will discuss its findings showing strong circumstantial evidence of possible early Martian life, including microfossil remains found in a Martian

meteorite, at a news conference scheduled for 1:00 p.m., August 7, at NASA Headquarters, 300 E. St. SW, Washington, DC."

Public note from NASA HQ, August 6, 1996

Ten days latter a manuscript entitled "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001" appeared in the journal *Science* detailing the different lines of evidence for past Martian life found in the small meteorite. Immediately an unprecedented scientific and public debate burst over these 1.93 g of Mars. The debate became increasingly heated over the months and years that followed the announcement, as it is expected over a question that transcends science, and percolates into the very foundations of our society. While for some the debate is still open, many already speak of the rise and fall of ALH84001. In any case ALH84001 is forever linked to the history of Martian exploration and there are important lessons to be learned from it.

2. The ALH84001 Case for Life on Mars

The Martian meteorite ALH84001 (Fig. 1) was launched into interplanetary space after an impactor hit the surface of Mars around 16 million years ago. It landed on the Antarctic continent around 13,000 years ago, where it was discovered by an American expedition in the Allan Hills region in 1984. ALH84001 is primarily a volcanic, silicate-rich rock composed mainly of orthopyroxene, and smaller amounts of chromite, olivine, pyrite, apatite and Si-rich glass. In their seminal paper, McKay et al. (1996) proposed several lines of evidence for biological activity present in the meteorite: (1) carbonate globules within the meteorite with textures similar to bacterially induced carbonate crystal bundle precipitates, (2) the presence of complex organic compounds, specifically polycyclic aromatic hydrocarbons (PAHs), (3) the coexistence of iron-oxides, iron-sulfides and carbonates, (4) ovoid and bean shaped structures that resemble fossilized ancient microbes and (5) magnetite particles that could have formed through controlled bio-mineralization processes. The authors pointed out that none of their single observations was itself conclusive for the existence of past life on Mars. Each of the observation had reasonable alternative non-biological explanations, but the totality of their observations considered collectively, particularly in view of their spatial associations, was claimed to constitute evidence for relic biological activity on Mars. Since the work was published many scientific and popular articles have focused on the possible evidence of life on Mars. We will resume here the most relevant literature on that respect.

2.1. CARBONATE GLOBULES

ALH84001 contains secondary carbonate minerals in the form of globules from 1 to 250 μm across, that have radiogenic Rb-Sr ages of 3.9 ± 0.04 billion years (Borg et al., 1999) (Fig. 1). This is a distinct feature of ALH84001, since other

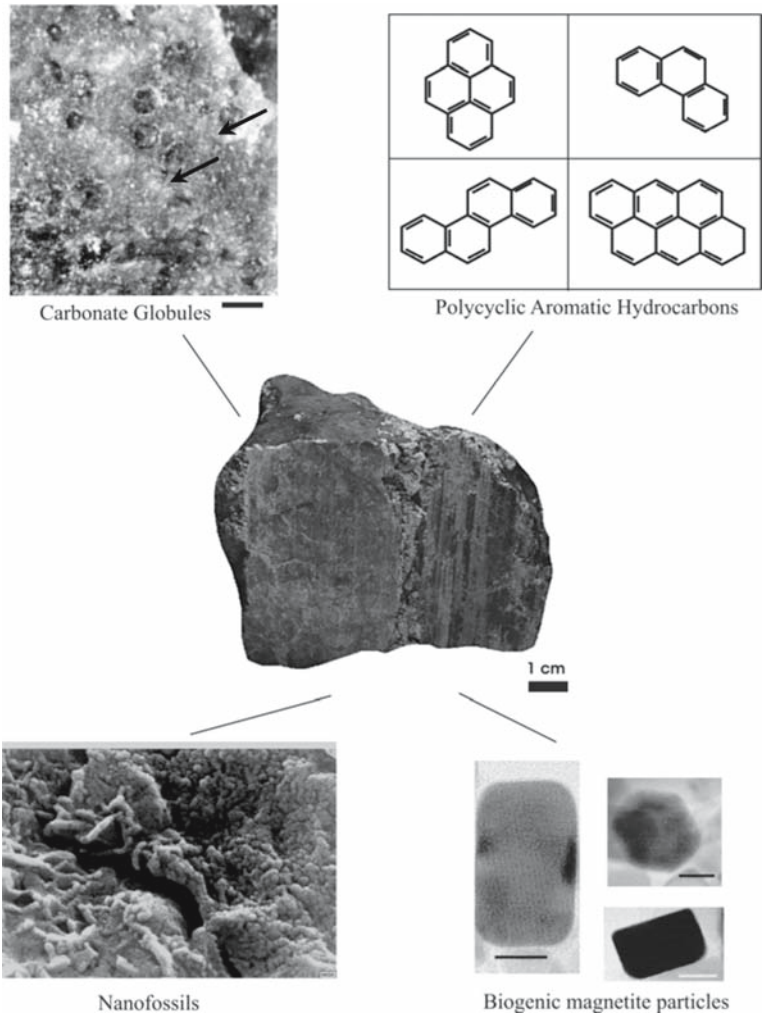

Figure 1. Evidence of Martian life suggested by McKay et al. (1996). *Center*: ALH84001 after removal of some samples (Photo Credit: NASA/JSC). *Top left*: carbonate globules (examples marked by arrows) in the meteorite. Scale bar is 200 μm (Photo Credit: Jacek Wierzchos and Carmen Ascaso). *Top Right*: structure of some of the PAHs identified in the meteorite. *Bottom Left*: bacteria-shaped-objects <100 nm in length (Photo Credit: NASA/JSC). *Bottom Right*: magnetite crystals that resemble those synthesized by magnetotactic bacteria on Earth. Scale bars are 20 nm. (From Thomas Keprta et al., 2000.)

SNC meteorites (Shergottite, Nakhlite, Chassigny), contain only trace carbonate phases. The conditions in which the carbonate globules formed and their thermal history have been a subject of heated debate, as they are central to the discussion surrounding the evidence of life in ALH84001. The maximum temperature at which life has been found on Earth is 121ºC (Kashefi and Lovley, 2003), and if

the carbonate globules formed at substantially higher temperatures, then the life hypothesis would be seriously undermined. Petrographic and electron microprobe studies (Mittlefehldt, 1994; Scott et al., 1997, 1998) suggest that the carbonates formed at temperatures in excess of 500ºC, whereas the stable oxygen isotope data indicate that the carbonates formed between 0ºC and 80ºC (Romanek et al., 1996), a range of temperatures compatible with life. The magnetic properties of pyroxene grains within the meteorite seem to reflect primary features of the ancient Martian magnetic field. These paleomagnetic imprint can be erased at temperatures >200ºC, which indicates that the rock could not have been substantially heated after its formation, and thereby supporting a low temperature origin of the carbonate globules (Kirschvink et al., 1997). However, thermochronometry analyses indicate that the meteorite may have been shocked multiple times, reaching peak temperatures of 400°C in localized areas (Min and Reiners, 2007). A large number of studies based on a variety of techniques have followed one another since 1996, arguing for and against a low temperature origin of the carbonate globules (i.e. Harvey and McSween, 1996; Warren, 1998; Golden et al., 2001; Kent et al., 2001). However, none of these studies has provided conclusive evidence for the conditions in which the globules formed and evolved. Currently there seems to be a consensus that the carbonates did form at temperatures below 300ºC. This temperature is still incompatible with life as we know it, but being an upper-end value it cannot be used to rule out the biogenic hypothesis either.

2.2. COMPLEX ORGANIC COMPOUNDS

Polycyclic aromatic hydrocarbons (PAHs) were detected in the interior of ALH84001 at concentration in the parts per million range (McKay et al., 1996). PAHs are complex organic compounds with two or more fused aromatic rings (Fig. 1). On Earth, PAHs form as a result of the incomplete combustion of organic materials during industrial and other human activities, and also through diagenesis of organic compounds. McKay et al. (1996) only found a few different types of PAHs in the meteorite, in close association to the carbonate globules. This was taken as indicative of diagenetic alteration of microorganisms accumulated within the ALH84001. The controversy around this finding centered on whether these organic compounds formed on Mars, or represent terrestrial contamination from the Antarctic ice where ALH84001 was found. McKay et al. (1996) showed that the content of PAHs was minimum on the surface of the meteorite and increased towards its center, with a tendency to accumulate around the carbonate globules. Furthermore, ALH84001 is depleted in PAHs near its fusion crust, the edge of the meteorite that was melted during its passage through the Earth's atmosphere (Clemett et al., 1998). This suggests that the PAHs were already in the meteorite when it landed on Earth. On the other hand, positive Carbon-14 analyses indicates that a large portion of the organic carbon present in the meteorite is terrestrial contamination, despite a significant percentage

(~8%) has no Carbon-14 signature and is too old to be terrestrial. This carbon component could be an inorganic carbonate phase or a high molecular weight organic component, but it still remains uncharacterized (Jull et al., 1998; Becker et al., 1999). Steele et al. (2007) conducted spectroscopy and microscopy analyses of carbonate globules in ALH84001 and the Bockfjorden volcanic complex (BVC), Svalbard. The authors identified macromolecular carbon in association with the carbonate globules in both samples, which they linked to the uncharacterized organic phase. Based on thermodynamic calculations, the authors postulated a non-biogenic origin associated to the primary development of the globules. Small amounts of amino acids, which are nearly identical to amino acids found in Antarctic ice, are also associated to the carbonate globules (Bada et al., 1998), indicating that the rock has in fact been contaminated to a certain degree with specific terrestrial organic compounds. Yet, other meteorites with no indigenous PAHs also found in Allan Hills, are clean of terrestrial PAHs contamination (Clemett et al., 1998), and the heterogeneous distribution of PAHs and their relatively large concentrations within the rock compared to the Allan Hills ice, are also hard to reconcile with contamination processes.

2.3. COEXISTENCE OF IRON-OXIDES, IRON-SULFIDES AND CARBONATES

ALH84001 also contains nanophase magnetite (Fe_3O_4) and pyrrhotite ($Fe_{1-x}S$, x = 0 to 0.2) crystals in rims surrounding the carbonate globules (McKay et al., 1996). Although both types of minerals are known to form individually through both biotic and abiotic processes, McKay et al. argued that simple inorganic precipitation models could not explained their coexistence. Life, however, operates under disequilibrium conditions and often favors the co-precipitation of minerals that could not otherwise occur simultaneously. Their close association to the carbonate globules was taken as further evidence for the intervention of life in the formation of these minerals. On Earth, some bacteria are known to co-precipitate intracellular iron-oxide and iron-sulfide particles, a biologically controlled mineralization process that can take place under anaerobic conditions (Blakemore, 1982; Petersen et al., 1986; Bazylinski and Frankel, 2003). Yet, Anders (1996) quickly pointed out that some C-chondrites, a different type of meteorites, also have a similar suit of mineralogies, which were clearly formed under abiotic conditions. The same author proposed and alternative, non-biological processes that could explain these seemingly incompatible mineralogies.

2.4. FOSSILIZED ANCIENT MICROBES

The images of putative fossilized nano-bacteria inside ALH84001 were perhaps more appealing to the public and the press, but turned into a major source of

controversy within the scientific community. McKay et al. (1996) reported ovoid and elongated forms ranging in size from 20 to 100 nm in longest dimension (Fig. 1). These forms were similar to terrestrial nanobacteria and fossilized filamentous bacteria found in calcite concretions, travertine and limestone (Folk, 1992, 1993), a very suggestive argument in favor of their biotic origin. Using control samples the authors ruled out any possible artifacts associated to sample preparation, and the same analyses conducted in other meteorites recovered from Allan Hills did not show any of the structures, which ruled out a possible terrestrial origin. Bradley et al. (1997) found that similar bio-morphs were lamellar growth steps on pyroxene and carbonate crystals, and their segmented surface merely artifacts due to sample preparation. Accepting that argument, McKay et al. (1997) replied that it did not apply to the entire suit of bio-morphs in ALH84001, and some of them could still be remnants of Martian microorganisms.

But it was the size of these bio-morphs, 100 times smaller than the smallest known organism on Earth, what made the whole argument unconvincing for many. Soon the debate turned into the minimum size requirements for life, an issue that had not received much attention prior to 1996. The main critics of the biogenic hypothesis argued that something that small could not contain all the molecules necessary for the basic cellular activity. Before ALH84001, the concept of nanobacteria was barely accepted in the scientific community. But the images presented by McKay et al. (1996) stirred a debate that lead into the meeting of a National Academy of Sciences (NAS) panel of experts in microbiology to discuss the size limits of life. Before the panel of experts released their conclusions, a number of publications related to nanobacteria had already seen the light. In 1998, Uwins et al. reported detection of living colonies of nano-organisms on Triassic and Jurassic sandstones and other substrates. These nanobes have cellular structures similar to Anctinomycetes and fungi, but their diameters range from 20 to 100 nm, and are composed of C, O and N. Ultrathin sections revealed membrane-like structures and different staining techniques indicated the presence of DNA. Kajander et al. (1998) claimed to having isolated nanobacteria from blood and blood products. The authors observed growth in culture plates seeded with samples that had been filtered through 0.07 μm pores, and estimated a lower size limit for the nanobacteria of 80 nm in diameter. Kajander and Ciftçioglu (1998) also reported the culture of nanobacteria and the partial characterization of a nanobacterial ribosomal RNA. These results were later debated by Cisar et al. (2000) who argued that the observed bacterial growth, were in fact inorganic precipitates, and that the isolated RNA was likely a contaminant from the reagents used in the experiments.

Taking into account these precedents, and considering theoretical constrains about the minimum amount of biomolecules required for the basic living processes, the consensus of the panel of experts dictated that a sphere of 200 nm in diameter was the minimum volume required for a single-cell organism. With its 20–100 nm proportions, the putative bio-morphs in ALH84001, and some of the reported terrestrial nanobacteria, didn't seem to make the cut. However, new discoveries seem to call for a redefinition of the theoretical size limits of life.

Baker et al. (2006) extracted nanoarchea ranging in length from 193 to 299 nm, from naturally occurring biofilms in an acidic environment. These sizes fall dangerously close (and some below) the theoretical limit of 200 nm. The conservative estimates of the size limit of life may not be conservative anymore, and as new discoveries continue to lower this limit, the concept of Martian nanobacteria is likely to gain new thrust.

2.5. BIOGENIC MAGNETITE PARTICLES

Perhaps the most contentious discussions erupted around the magnetite crystals in ALH84001 (Fig. 1). They were claimed by McKay et al. (1996) to represent a biomarker for life on Mars. The original argument was based on the single-domain crystals, purity, and lack of structural defects of the magnetite grains. This idea was extended by Thomas-Keprta et al. (2000) who argued that 25% of the magnetite crystals in ALH84001 conformed to six properties, which constitute a robust Magnetite Assay for Biogenicity (MAB): (1) narrow size range, (2) restricted width to length ratios, (3) chemical purity, (4) few crystallographic defects, (5) crystal morphology, and (6) elongation along only one of the possible rotation axes of a regular octahedron. Magnetotactic bacteria, a widespread type of aquatic prokaryotes on Earth, synthesize intracellular magnetite crystals, called magnetosomes, which typically meet all properties in the MAB. The magnetite crystals in the magnetotactic bacteria appear aligned in chains, a highly unstable configuration that is achieved by means of cytoskeletal microfilaments and proteins (Scheffel et al., 2006; Komeili et al., 2006). Comparative studies of morphological and structural defects between magnetite crystals from magnetotactic bacteria and from ALH84001 also seemed to support the biogenic origin of the later (Taylor et al., 2001), and single-domain magnetite crystals aligned in chains in ALH84001 were eventually found by Friedmann et al. (2001), who argued that they would be nearly impossible to produce inorganically and were consistent with a biological origin.

These claims were vigorously disputed. Barber and Scott (2002) noted that solid-state diffusion as a result of carbonate decomposition during impact heating could result in magnetite nano-crystals similar to those found associated to the carbonate globules. Thomas-Keprta et al. (2002) responded by pointing out that the heat necessary to decompose iron carbonates and form magnetite was not present and would require a homogenization of all magnetic dipoles. Instead, they observed considerable heterogeneity in the ALH84001 carbonates inconsistent with significant heating. Replicating the microscopy and crystallographic analyses conducted by Thomas Keprta et al. (2000, 2001, 2002), Golden et al. (2004) presented a detailed electron microscopy work on magnetite crystals extracted from ALH84001, and compared them to magnetite crystals from the magnetotactic bacteria strain MV-1. The authors concluded that the shape of the [111]-elongated magnetite crystals in ALH84001 is not identical to that from the bacterium MV-1,

an argument that undermines the biogenic hypothesis. Bell (2007) conducted shock-recovery experiments with naturally occurring siderite, and obtained magnetite crystals with a similar composition, size and morphology as those found in ALH84001. The shock temperatures required for siderite composition were >470ºC, somewhat in excess of the 300ºC upper limit mentioned above for the formation of the carbonate globules. The author argued that local thermal excursions within the meteorite could account for the high temperatures required by this process, without substantially altering the bulk temperature of the rock.

While several authors have concluded that the magnetite crystals in ALH84001 cannot be taken as evidence for biological activity, it is important to note that some results supporting the biogenic hypothesis remain undisputed. Particularly intriguing is the claim by Friedmann et al. (2001) that chains of magnetite particles are present in ALH84001 in close association to the carbonate globules. The authors used backscattered-SEM to study undisturbed, carbonate-rich fragments of the meteorite, and imaged chains of Single-Domain magnetite crystals (Fig. 2). Auger Electron Spectroscopy and Energy Dispersive X-ray Spectroscopy showed that the particles in the chains contained a heavy element. Due to the limited resolution of these techniques, the exact chemical composition of the particles could not be determined, but based on indirect evidence the authors interpreted this heavy element to be Iron and concluded that the particles forming the chains where in fact magnetite. There are two main results published by Friedmann et al. (2001) that have not yet been disproved: (i) small crystals (ca. 50–100 nm) composed of (possibly) Fe and O occur in chain-like structures in the outer rim of the carbonate globules in the ALH84001, and (ii) these chains are not the result of terrestrial contamination. The only known process that has been shown to originate similar structures is magnetosome formation in the magnetotactic bacteria. Moreover, in the same paper the authors identified five properties of the chains that may constitute a possible biosignature, namely: uniform crystal size and shape; gaps between crystals; orientation of elongation along the chain axis and flexibility of the chain. Friedmann et al. (2001) also reported electron-dense regions surrounding the chains. The electron dense regions were consistent with the magnetosomal matrices reported by Taylor and Barry (2004) and the electron-dense regions surrounding terrestrial magnetofossils reported by McNeill (1990). Barber and Scott (2002) argued that magnetite crystals growing on ledges and kink sites on microfractures could align in chains, however the authors failed to support their claim with any form of evidence.

Of all the original evidences for ancient life on Mars put forward by McKay et al. (1996), the carbonate globules, the presence of indigenous organic compounds and the occurrence of nanobacteria like structures and of minerals that resemble bacterial precipitates on Earth, remain plausible, albeit disputed. The conditions in which the carbonate globules formed are likely to remain highly speculative, given the lack of basic data required to build up realistic geochemical scenarios. PAHs, however Martian they might be, do not represent a good indicator of biological activity, and can hardly be considered evidence for life in

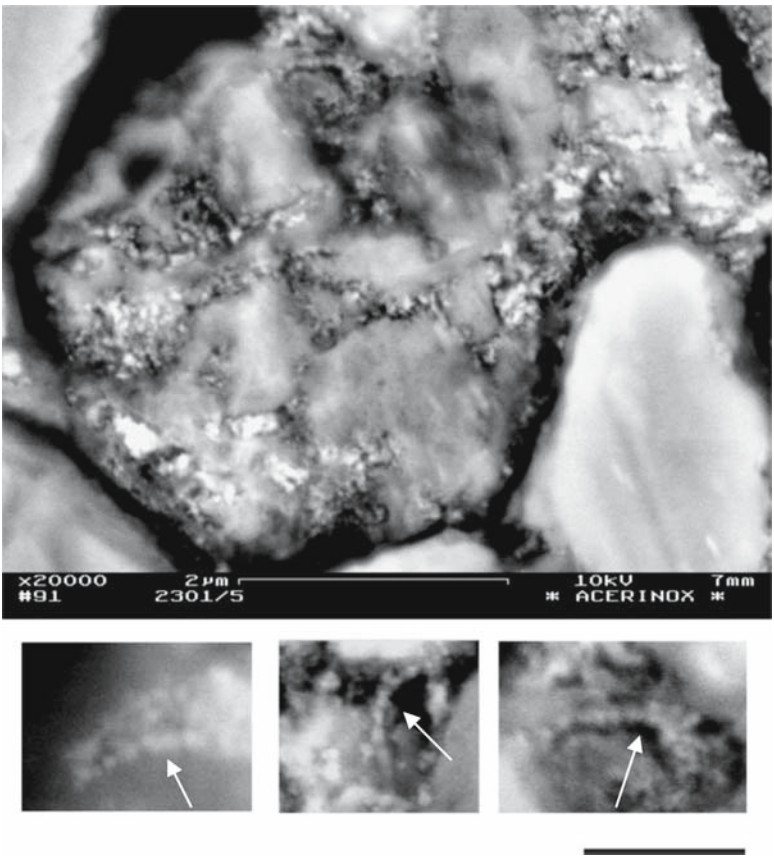

Figure 2. *Top*: Backscattered Scanning Electron Microscope (SEM-BSE) image of the magnetite-rich area around a carbonate globule. Brighter tonalities are indicative of elements with high atomic weight, and darker tonalities are indicative of lighter elements. *Bottom*: three SEM-BSE images of chains of particles (arrows) containing a high atomic weight element. Similar structures were interpreted as fossil chains of magnetosomes, analogue to those synthesized by terrestrial magnetotactic bacteria (By Friedmann et al. 2001). Scale Bars is 1 µm. (Photo Credit: Jacek Wierzchos and Carmen Ascaso.)

themselves, given their widespread existence in the universe. But the putative presence of nanobacteria and of fossil chains of magnetosomes do represent important contributions of ALH84001 to the search for life beyond our planet, and are still considered by some as indicators of past biological activity on Mars.

3. Martian Nanobacteria

The main argument against the nature of the bacteria-like structures in ALH84001 was their size, well below the theoretical limit proposed by the NAS panel of experts. However, estimating the theoretical size limit of life is not without problems.

As the experts in the panel pointed out: "Commonly used methods of measuring cell size have inherent uncertainties or possibilities of error. Perhaps more important, most cells found in nature cannot be cultivated. Thus, ignorance about biological diversity at small sizes remains large".

The lower limit in size for a free-living cell is set by the necessity of being large enough to house the required biological molecules to perform its basic functions (Koch, 1996). Because terrestrial life is our only model, our estimates on the lower size limit of life are therefore based on the minimum requirements of terrestrial microorganisms. However, the cell machinery of terrestrial organisms is a result of evolution and natural selection of traits that are not necessarily fully optimized in terms of size (Koch, 1996). Not just size but also cell shape is important; an efficient surface/volume ratio is likely the reason that many bacteria are rod-shaped, filamentous, vibrios, or fusiforms. These shapes increase the surface/volume ratio because a sphere has the least surface for the volume enclosed. An organism inhabiting a nutrient depleted environment, would be the least limited by diffusion of resources into the cell if it is as small as possible in cross-section, even if that entails being longer (Koch, 1996).

In the past years a large amount of literature related to nanobacteria has accumulated. Most of that research is carried out within the medical community, where it is hypothesized that an extremely small self-replicating particle, that is ubiquitous in living terrestrial organisms, may be responsible for several types of urologic pathologies (i.e. Akerman et al., 1997, Wood and Shoskes, 2006, Shiekh et al., 2006). While these claims are disputed, research carried out in natural samples is providing a second line of evidence to the existence of nano-organisms, and further suggests that they may be more widespread and complex than previously thought (Folk, 1992, 1993; Uwins et al., 1998; Sommer et al., 2002; Miyoshi et al., 2005; Baker et al., 2006). Typically these deceptive forms of life seem to be characterized by their small size (80–500 nm), very slow growth rates, their capability to induced mineral precipitates, and their adaptability to extreme environmental conditions, including temperatures of up to 90ºC, starvation, extremes of pH and oxidizing agents, and high doses of gamma radiation (Björklund et al., 1998; Wood and Shoskes, 2006).

Accepting the existence of nano-organisms does not imply that the putative fossils in ALH84001 are indeed remnants of past Martian life, however this would remain as a possible explanation. The conditions on the Martian surface have been intolerable to life likely for several billion years, particularly due to the high doses of radiation that bath the surface. ALH84001 was ejected from the Martian subsurface, and while radiation is not a major concern for organisms living underneath the uppermost layers of soil, starvation, extremely low temperatures, hyper-aridity and oxidizing agents also contribute to make life on Mars a difficult challenge, even in the subsurface. While the surface of Mars appears relatively stable and in equilibrium at large scales, things may be different at the microscale. Atmospheric photochemistry, transient dew and fog events, meteorite fallout, or daily radiation cycles are but a few processes that can induce a small disequilibrium in the conditions of the surface soil. Life feeds on disequilibrium and a form

of life that is able to adapt its metabolism to these frequent and short-lasting events may be better suited to survive in these extreme environment. Because of their large surface/volume ratio, extremely small organisms may be more efficient at gathering resources from the environment in short periods of time. The transition from resistant (i.e. spore like) to active stages may also be more effective if the required morphological changes are small.

The notion of nanobacteria is an intriguing and interesting one, not only with regard to the search for life on Mars, but also with regard to life in our own planet. When samples are returned from Mars, there will be a battery of biological assays lined up to search for evidence of life. Nanobacteria will arguably be searched for too, and it is therefore necessary to unambiguously verify the existence of nanobacteria, and to establish a consensus in the scientific community. In that respect extreme environments such as the hyper-arid Atacama Desert or the Antarctic Dry Valleys, which are also important Martian analogs, may be ideal candidates to search for nanobacteria, and if present, to understand their distribution, diversity and abundance.

4. Fossil Chains of Magnetosomes as Biomarkers on Mars

The use of fossil chains of magnetosomes as extraterrestrial biomarkers is also an important contribution of the research conducted around ALH84001. These biominerals could in principle resist billions of years of oxidative conditions on the Martian surface and near subsurface, and their intrinsic characteristics could be used to assess their biogenicity.

On Earth two general types of magnetotactic bacteria can be distinguished based on the type of minerals they synthesize: the iron-oxide type that mineralize crystals of magnetite (Fe_3O_4) and the iron-sulfide type that mineralize crystals of greigite (Fe_3S_4) (i.e. Bazylinski and Frankel, 2000). Magnetite producing magnetotactic bacteria have optimized their magnetotactic response by maximizing the magnetic moment per atom of ion of their intracellular magnetic crystals. This has resulted in defect free, nearly stoichiometric precipitates, with morphologies that increase the magnetic SD stability field (Witt et al., 2005). However, there seem to be more diversity in magnetosome morphology among greigite-producing magnetotactic bacteria, for example several particle morphologies have been observed within a single cell (Pósfai et al., 1998b). Among this type of magnetotactic bacteria, transition metals other than Fe are incorporated in the magnetosomes, which often show defects in their crystal lattice (Pósfai et al., 1998a, b). Furthermore, greigite magnetosomes occasionally contain a mixture of transient, non-magnetic, iron sulfide phases that likely represent mineral precursors to greigite; these non-magnetic crystals are still aligned in chains and fall within the magnetic SD size range (Pósfai et al., 1998a, b).

Taking the biochemistry and ecology of all known magnetotactic bacteria on Earth as a reference, then the evolution of magnetotaxis on Mars at least

required the presence of liquid water habitats and a planetary magnetic field. It has been established by orbiter measurements that Mars possessed a magnetic field early in its history (Acuña et al., 1999). Additionally, recent missions with landers and orbiters have provided evidence of aqueous sedimentation or aqueous alteration on the Martian surface, a finding consistent with models of liquid water near the surface (i.e. Squyres et al., 2004; Bibring et al., 2005), coexistent with the active Martian magnetic field.

4.1. A UNIVERSAL MAGNETIC ASSAY FOR BIOGENICITY

As mentioned above, Thomas-Keprta et al. (2002) proposed a MAB, including six distinctive properties displayed by bacterial magnetosomes, that allow to distinguish them from inorganic magnetic particles, including defect-free crystals, consistent shapes and aspect ratios, elongated crystal morphologies and chemical purity. Yet some magnetite-producing bacteria synthesize magnetosomes with crystallographic defects similar to that of synthetic magnetite crystals (Devouard et al., 1998; Taylor et al., 2001), and shapes and aspect ratios of magnetosomes from different strains of magnetotactic bacteria differ from one another, and do not always show elongated morphologies (Buseck et al., 2001). Among the greigite-producing magnetotactic bacteria, magnetosomes often lack the chemical purity and present crystallographic defects (Pósfai et al., 1998a, b). These exceptions question the universality of the MAB proposed by Thomas-Keprta et al. (2002) and suggest that different criteria are needed to distinguish between biogenic and non-biogenic nanophase magnetic iron minerals.

It is important to note that organisms lacking several traits listed in the previous MAB, are still able to efficiently move along magnetic field lines. This indicates that compositional purity, defect-free crystals and elongated crystal morphologies are not strictly necessary for the purpose of magnetotaxis. These traits are highly desirable from the point of view of magnetic optimization, since they maximize the saturation magnetization of the particles and thus the degree of efficiency when aligning with the geomagnetic field lines, yet they are not necessary for magnetotaxis itself. If these traits are typical, but not universal, among magnetite-producing magnetotactic organisms on Earth, and do not generally apply to greigite producing ones, then the tenet that bacterial magnetic precipitates can be unambiguously identified by the MAB needs to be reconsidered.

There are however two essential properties common to all magnetotactic bacteria that may constitute a universal MAB: (1) the use exclusively of magnetic SD crystals and (2) the arrangement of these crystals in chains or chain-like structures. These traits are not fortuitous but obey to universal physical laws, independent of environmental or biological factors. A chain of magnetic SD crystals has a net magnetic moment that results from the addition of each crystal's magnetic moment within the chain (Blakemore, 1982; Bazylinski et al., 1995; Dunin-Borkowski et al., 1998). Smaller magnetic particles are unstable because of thermally

induced fluctuations of their magnetic moment, whereas larger particles have a low net magnetic moment due to magnetic domain formation. In either case, the resultant chain would have a null or very low magnetic moment. This suggests that microorganisms analogous to terrestrial magnetotactic prokaryotes, if ever present on Mars, likely developed a similar orientation mechanism to magnetotactic prokaryotes on Earth. Support for this idea comes from phylogenetic studies, which have shown that different species of MB belong to different phylogenetic lineages, suggesting that magnetotaxis in prokaryotes evolved separately more than once throughout life history (DeLong et al., 1993), yet always based on chains of magnetic SD particles. Additionally, in any given population of fossil chains of magnetosomes, likely some traits concerning the shape, crystallography and composition of the magnetosomes listed in the previous MAB, may be present, and could then be considered as additional evidence to support the biogenic origin of the magnetic crystals. Fossil chains of magnetic single-domain particles are therefore an ideal biomarker and ought to be searched for in returned samples from the Martian surface such as ancient sedimentary deposits formed in standing waters (McKay et al., 2003).

5. Summary

The paper presented by McKay et al. (1996) did not only inspire a large number of studies aiming to prove or disprove the evidence of life hypothesis, but it also stirred new debates about life on Earth and opened new avenues for the search of life outside our planet. Together with other technological and scientific innovations in the 1990s, it precipitated the foundation of the NASA Astrobiology Institute (NAI), and inspired a renewed interest in the exploration of Mars, which was practically forgotten since the Viking mission in the 1970s. Of the different lines of evidence for life in ALH84001 suggested by McKay et al. (1996), the presence of fossil nanobacteria and of chains of magnetic single-domain particles remain plausible albeit controversial. Irrespectively of their origin in ALH84001, these structures ought to be considered as important biomarkers and therefore be searched for in samples returned from Mars in the future.

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PRESERVATION WINDOWS FOR PALEOBIOLOGICAL TRACES IN THE MARS GEOLOGICAL RECORD

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1. Introduction: A New Perspective on the Mars Sedimentary Record

For years, the Mars robotic missions have provided different evidences that Mars had an active hydrologic past which involved the emergence of distinctive sedimentary systems and its corresponding weathering sources. Minor geomorphic features to regional-scaled structures have been used to inferthat sedimentary systems such as deltaic, fluvial, lacustrine or marine-like environments (Malin and Edgett, 2000) to have occurred sometimes in Mar's history (Carr, 2006). In this context, the information obtained by geomorphological interpretations have inferred those physical conditions – e.g. hydrological activity, water energy or climatic evolution- that were in equilibrium with the landforms (Baker, 2001). In recent times, new instrumentation aboard the different planetary missions to Mars (e.g. IR specs in the Mars Oddyssey, Mars Express and MRO, or APXR and Mössbauer specs of MERs) have shed light in the mineralogical and geochemical composition of some ancient materials.

Both Orbiter and Rover explorers have recognized that the two main age-differentiated Mars terrains have differential mineralogy and geochemistry (Poulet et al., 2005; Bibring et al., 2006). In the oldest Noachian areas (age older than 3.8 Ga), the Mars Express orbiter has detected phyllosilicates concentrated in layered terrains (Michalski and Noe Dobrea, 2007), currently covered by younger deposits of lavas and other sediments. On the other side, Late Noachian to Hesperian younger terrains (3.8–3.0 Ga) are composed of sulfates, some of them bearing iron (Squyres et al., 2004; Morris et al., 2004; Fernández-Remolar

et al., 2005). These mineralogies have been used as indirect evidences in inferring the hydrogeochemical processes occurring on the Mars surface, which have resulted of the interaction between climate, hydrosphere and geosphere of Mars. On Earth, phyllosilicates are formed during diagenesis and metamorphism in a diverse range of marine and subaerial environments. Thus, the clays found on Mars, if sedimentary (Michalski and Noe Dobrea, 2007), denote high rates of weathering that could be easily driven by CO_2-saturated meteoric waters (Francois and Walker, 1992; Franck et al., 1999) under warm conditions. Analogous aqueous acidification through CO_2 hydration to H_2CO_3 (Orr et al., 2005) has been induced for early Earth water masses by CO_2 saturation (Corcoran and Mueller, 2004). Such an scenario would fit a early development of potential habitats on Mars characterized by atmospheric enrichment on carbon dioxide, carbonate lacking and formation of phyllosilicates under acidic conditions though weathering.On the other hand, the hydrated sulfates bearing ferric iron precipitate under strong acidic brines (pH < 3) are sourced in the sulfide weathering by oxygen-rich meteoric waters and/or anaerobic iron oxidizers (Amils et al., 2007; Fernández-Remolar et al., 2008b). As a result, an association of different iron-bearing sulfates as copiapite, jarosite and schwertmannite co-occurs with any other sulfates like gypsum or epsomite formed by cations sourced in the silicate dissolution.

Later on, the occurrence of Hesperian to Amazonian outflow channels and great catastrophic landforms suggest that Mars had some post-Noachian planetary events of thermal reactivation and transient water masses (Carr, 2006) when great iced terrains – clathrate-rich deposits permafrost and glacier-like systems – were warmed up. Such episodic massive release of water took place under a declining atmosphere that was transitionally recovered through the volatile replenishment (Baker, 2001). As a consequence, different highland lacustrine and lowland sea-like regions were created or reactivated during one or several episodes where the climatic conditions were warmed up.

If life arose on Mars, it should have adapted and evolved to the long-term climate evolution that is driven by the inner planetary activity. Moreover, preservation of biological information produced by the Mars life into the Mars geological record must have occurred according to fossilization processes that depend on the crust diagenetic geochemistry, which also emerges from atmosphere, crust composition, hydrological activity and heatflow. According to the Mars geological record uncovered along different planetary missions, we propose some preservation windows in which primary biological information may have been recorded in the form of one or several fossil states, ranging from pure organic compounds to resistant mineralized remains. The following preservation windows are considered to have preserved paleobiological entities:

- Early Noachian phyllosillicate deposits (e.g. Mawrth Vallis)
- Fluvial to lacustrine or marine deltaic-like Early Noachian materials (e.g. Holden Crater)
- Hydrothermal-associated deposits (e.g. Holden Crater)

- Late Noachian to Hesperian sulfate to hematitic basaltic sands (e.g. Meridiani Burns Fm)
- Rock coatings (e.g. Gusev)

To these five preservation windows two more can be added in relation to subsurface regions and rock coatings. The main reason is whatever surface conditions reigned on the Mars surface, its subsurface counterpart may have been more stable, concerning temperature and shielding against radiation. Moreover, environmental conditions are easier to control by microbes in the subsurface as it has been shown concerning to temperature and pH (Gómez et al., 2004; Fernández-Remolar et al., 2008a). Under these favorable circumstances, biogeochemical cycling can operate during the long and cold episodes that followed the benign climate inferred for the Early Noachian age. Subsurface areas associated to volcanic centers with hydrothermal activity are exposed to high mineralization rates that are an essential parameter for morphological conservation. Finally, Mars has developed along its long history fluvial and desertic systems in which boulders of sedimentary bars or pavemented soils are covered by complex thin films composed by oxides, sulfates, carbonates and weathering silicates (Potter and Rossman, 1977; Giorgetti and Baroni, 2007). Microbial endoliths and endolithic structures (Golubic and Schneider, 2003), not discussed in this work, should be added to these geobiological entities of great importance for searching for life on Mars.

2. Taphonomic and Organic Chemistry-Related Processes Involved in Preservation

Paleobiological remains and fossils are currently concerned as real biological entities, but are not life entities. On the contrary, they result from the interplay and succession of several geo-biological processes that occur before, during and after burial, and which are recorded additionally to the primary remain (Fernández-López, 1991, 1995; Brocks and Summons, 2005). As a consequence, fossil entities record not only some information concerning to its biological origin, but also all those processes that have played any role in generating the preserved entities. A good example is the organics obtained in sedimentary rocks that come from the multi-way degradation of primary biomolecules under different thermal and compositional conditions along the rock diagenesis (Brocks and Summons, 2005). In this sense, exposition of biomolecules to iron- and sulfur-rich environments has a strong imprint in the final geopolymers that are associated with iron and sulfur (Sinninghe Damsté and de Leeuw, 1990). Therefore, the fossilization process can follow complex pathways that involve preservation before and after definitive burial, fossilization phases known and biostratonomy and fossildiagenesis, respectively (Fernández-López, 1991, 1995). Obviously, the final fossil state will be the result of all these stages and can be as simple as a fast and in-situ burial, which is the best case for the preservation of chemical fossils.

The parameters involved in the formation of the preserved entities (fossils and/or any paleobiological trace) are countless (Farmer and Des Marais, 1999). They range from molecular processes (Banfield et al., 2005) currently driven by microbes to planetary-scaled events such as sea level global changes (Fernández-López, 2007). These planetary events are ruled by macrotectonic to long-term climatic changes affecting global biogeochemical cycles (Brasier, 1992) that are drivers of preservation in simple parameters as e.g. redox potential to preserve organics.

Whatever processes drive fossil preservation, all micro and macro mechanisms are sustained on some physicochemical parameters which are essential in the final record of the biological information. These parameters are hydrodynamics (diffusion vs. advection), temperature (biogeochemical reaction, mineralization and recrystallization rates), redox potential (oxidation processes), and ion concentration (mineralization), which dominance or co-occurrence can diversify the paleobiological record into different preservation windows. Obviously, exceptional fossil preservation – e.g. the so called conservation deposit fossil-lagerstätten as Burguess Shale (Conway Morris, 1990; Seilacher, 1990) – result from the positive concurrence of all these parameters; but from the interplay of all parameters will emerge the diversity of preservation windows that enrich the geological record on Earth. In the next section some ancient and modern terrestrial analogs will be considered to exemplify different kinds of preservation windows that can be expected to occur in the extensive geological record of Mars (Fig. 1).

Oxygen availability and redox potential are two elements that rule the molecular preservation of life. However, it should be noted that low oxygen fugacity does not mean low redox potential, which can be essential to understand preservation pathways on Mars. Although biotic and abiotic oxidation destroys most of low resistant biomolecules (e.g. sugars, proteins and nucleic acids), a minor part can be transformed to macromolecular humic complexes and geopolymers precursing kerogen and bitumen. On the other hand, more resistant fatty acids and lipids are transformed to geolipids and hydrolyzed hydrocarbons, but maintain the original structure that enable an easier identification concerning to its biological origin (Brocks and Summons, 2005). In any case, it has to be noted that not only redox, and other primary factors, but temperature during diagenesis is an essential factor to maintain preserved the molecular traces of life. Indeed, organic matter that can suffer thermal destruction under metamorphism, show a distinctive preservation under high-temperature extreme areas that are close to hydrothermal centers (Brocks and Summons, 2005). On the contrary, when the thermal and redox history of the preserving remains converge in a positive way, exceptional conservation of biopolymers occurs and some of them can be amplified using current molecular biology techniques (Logan et al., 1993).

A final thing to consider as essential to understand the processes involved in preserving biology is time. Indeed, rock aging, currently known as diagenesis,

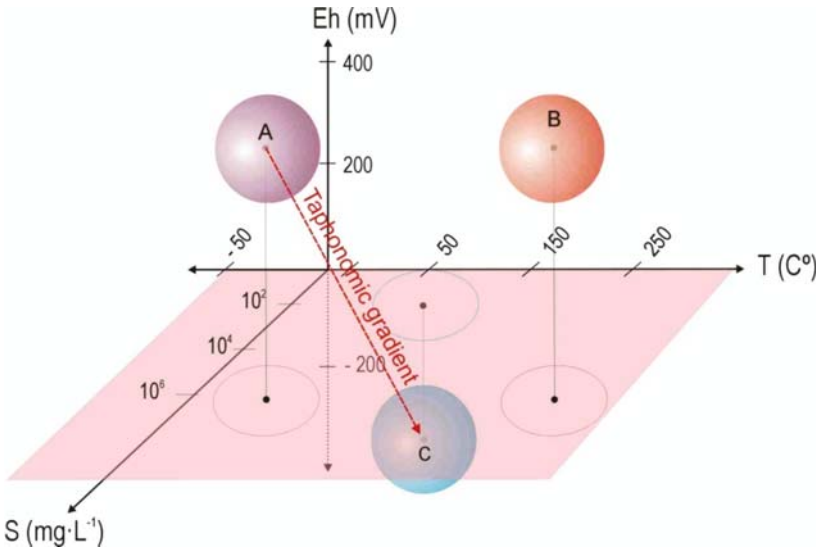

Figure 1. Theoretical representation of preservation windows (A–C) displayed in a three-dimensional space defined by essential environmental parameters for preservation of biological information such as temperature, Eh and ion concentration (named as S in mg L^{-1}). (A) represents ion-enriched medium temperature and high Eh conditions compatible for acidic to neutral environments where paleobiological structures are preserved under a fast mineralization. Same S and Eh records changing to high temperature conditions (B) would be a window for hydrothermal areas where high mineralization rates are also accounted. (C) corresponds to medium-temperature and low-concentrated fluids under reducing conditions that favor a net preservation of organics. Note that some taphonomic gradients emerge according to changes in environmental parameters as observed in Río Tinto for Eh, pH and ion concentration (see Fig. 2).

encloses strong changes in several parameters that are drivers in selecting some paleobiological entities. In this sense, oxidant replenishment in sediment fluids produces changes in the hydrochemistry exposed to meteoric waters that produces the complete oxidation of organic matter (Fernández-Remolar and Knoll, 2008) to heavy carbon-bearing compounds of complicated determination.

3. Preservation in Terrestrial Analogs

Some terrestrial habitats dated from Archean to modern ages have been claimed as feasible analogs of different Mars potential habitats that have emerged over time (Benison and LaClair, 2003; Benison, 2006). Such a statement is based on a methodological background that considers the terrestrial life inhabiting Earth

regions as the unique reference to detect life in other regions of the Universe. Moreover, given that there is not a conceptual base to define Life, the terrestrial nature has currently provided the source to infer what particular life inhabites a defined area considered as a Mars analog. On the other hand, as one of the main connections between life and habitable region as water, it can be deduced that water is the main factor that characterizes a Mars potential habitat. As a result, water, as the exchange matrix for matter and energy used by life, and life itself are intrinsically linked in a search for extinct or extanct non-terrestrial living beings on Mars.

Many other environmental parameters such as pysichochemical or hydrogeochemical produce differentiation between environments, which will also cause characteristic imprints on preserving paleobiological traces. Although some terrestrial analogs are inferred through the geological record, they bear information to unlock some essential questions to uncover the paleoenvironmental conditions that could have been linked to the emergence of life on Mars, assuming both planetary sytems had an analogous volatile inventory during early stages of evolution (Grady and Wright, 2006). Therefore, very early geohistorical terrestrial analogs such as Isua metasedimentary sequences older than 3.7 billions of years (Rosing et al., 1996; Fedo, 2000) are of great importance to trace back those driving forces that might have impeled the possible emergence of life on Mars. Later on, the geological record of subsequent Archean environments (Pilbara and Barberton Archean deposits younger than 3.7 billion of years) are reference systems to determine those mechanisms driving the planetary divergence between Mars and Earth (Grady and Wright, 2006). Table 1 displays an equivalence between some Mars potential habitats and their terrestrial analogs, including environmental parameters involved in preservation.

As showed in Table 1, combined studies of ancient and modern environments will improve acquiring knowledge not only about that processes involved in recording paleobiological information, but also those involved in long-term preservation. Therefore, to unlock the aging processes that favor preservation of some paleobiological over other entities it is necessary to appeal to analogous modern environment. In a few cases the geological record of ancient and its corresponding modern equivalent coexist within the same area as seen in the Río Tinto fluvial basin (Fernández-Remolar et al., 2005; Fernández-Remolar and Knoll, 2008).

All environments described in Table 1, as others not mentioned here, deserve a detailed analysis in order to have a perspective for the preservation evolutionary changes occurred on Mars since its earliest evolutionary stages. This would demand indeed an extensive work dealing on characterization of the taphonomic processes driving preservation of any paleobiological entities that can be potentially produced in each analog. On the contrary, some interesting analogs having a Mars real counterpart are herein described in order to provide an idea how the research on the preservation in Earth analogs is essential to drive the exploration for extinct life on Mars.

Table 1. Correspondence between Mars potential habitats and its terrestrial counterparts.

	Terrestrial analogs	
Some Mars potential habitats	**Environmental analogs**	**Parameters for preservation**
Fluvial to lacustrine/marine Noachian environments in geomorphic and sedimentary units as fluvial to deltaic sedimentary systems as observed in Holden Crater and others (Cabrol and Grin, 2001; Grant et al., 2007)	Fluvial to deltaic environments of Archean sedimentary systems which substrate is frequently weathered to be exposed to the atmosphere (i.e. Moodies Group in South Africa)	Fast decreasing of redox potential in the water column and high silica and iron availability from mildly acidic conditions buffered by a CO-rich and aggressive atmosphere, which chemical attack would decrease over time as carbon budget was sequestered in form of carbonates. Lack of atmospheric oxygen would produce strong redox gradients to reducing conditions in the water masses, but shallow oxidizing conditions cannot be discarded maintained by the oxidant production in surface
Late Noachian to Early Hesperian acidic environments recorded as sulfate and oxide-iron rich deposits (Squyres et al., 2004)	Modern and ancient Acidic extreme environment-sand its underground conterparts (Rio Tinto, ephemeral acidic saline lakes. Permian deposits) (Benison, 2006; FernándezRemolar et al , 2005)	Oversaturation in ferric ionic complexes through leaching under low pH and high redox changing to lowredox and quasi-neutral pH subsurface conditions (Fernández-Remolar et al., 2008b), which will create strong a phonomic gradients
Hydrothermal and related-sedimentary systems associated to tectono magmatic complexes, shield volcanoes impactors. Some silica-rich deposits recently discovered in Gusevare supposed to be mineral evidences for hydrothermal activity (Prof. R. Arvidson, personal communication, October 2007). Tharsis, Elysium, Nili Fossae or Terra Tyrrhena (Varnes et al., 2003; Schulze-Makuch et al., 2007). Sedimentary-like environments associated to subaqueous hydrothermal complexes (finegrained sulfidic to silica-rich)	Acidic to neutral silica enriched hot springs and hydrothermal fluids (i.e. Kilauea and Manua Kea volcanic region, Yellowstone hydrothermal system. Iberian Tectono Magmatic Complex) (Lewis et al., 1997; Leistel et al., 1998). Study of Archean hydrothermal deposits are also essential to understand long-term preservation processes to be present on Mars (Kiyokawa et al., 2006)	Fast mineralization rates under moderate to high temperature fluids enriched in Si, S, Fe and other ions leached from the rock substrate and from magmatic sources (S sources). In origin, material leaching will be destructive but mineral re-precipitation will preserve paleobiological traces
Rock coatings and varnishesoriginated as thin films on weathered rocks exposed to fluids, volcanic fog and atmosphere(atmospheric sprays)	Fine water lamina sourced in aqueous environments (Rio Tinto terrace deposits, upper Permian to lower Triassic fluvial and lake deposits), coating of boulders affected by subaqueous volcanism (Moore and Clague, 2004), but extposed to fog, dew or snow in deserts (Death Valley, Atacama desert) (Kuhlman and McKay, 2007) and volcanic centers (Kilaueavolcano) (Schiffman et al., 2006)	High mineralization rates induced specifically in briny lacustrine and fluvial systems (i.e. Rio Tinto acidic brines) but also favored under weathering rates (acidicfog)

3.1. PRESERVATION IN FLUVIAL AND DELTAIC TO MARINE ARCHEAN DEPOSITS

Early terrestrial habitats are likely the most suitable approaches to the early Mars environments (Figs. 2A, B). During the Archean, the upper part of the Terrestrial crust, lacking terrestrial flora, was directly exposed to CO_2-driven atmospheric weathering on basaltic-like primary rocks, which induced high physicochemical alteration (Hessler and Lowe, 2006). As a result, detrital sediments were massively transported to marine environments through transition sedimentary systems like deltaic.Moreover, the aggressive chemical weathering introduced silica into solutions (Hamade et al., 2003) in greater proportion than the subaqueous geothermal systems. On the other hand, some oxidative processes might have occurred (Ohmoto, 2004) impeled by photochemical pathways affecting the ferrous iron in the form of aqueous complexes that were sourced in hydrothermalism. Such oxidation likely resulted in the production of ferric deposits as banded iron formations and red beds. Interestingly, there is no evidence of carbonates before 3.5 Ga (Grotzinger, 1994) despite a high concentration of CO_2 in the early atmosphere on Earth, which suggests some acidification mechanisms prevented the massive production of these minerals.

Strong redox gradients, as well as fast silicification and ferruginization are mechanisms expected to favor preservation of organics and paleobiological morphologies, respectively (Walsh and Westall, 2003). An exceptional case to be mentioned is the preserved microbial mats occurring in deltaic tidal flat deposits of the 3.2 billion-of-year Mesoarchean Moodies Group in Barberton (Noffke et al., 2006), South Africa (Figs. 2C, D). In this case, high sedimentation rates added to strong redox gradients along the water column might have favored reducing conditions in shallow areas inhabited by the microbial mats. Moreover, abundance of silica in sediment porewater likely increased the preservation potential of microbial remains by fast mineralization of the deposits. Although organics would have prevailed in these conditions, late metamorphic processes related to a combining increase of sediment pressure and temperature (Brocks and Summons, 2005) have favored the organic destruction.

Analogous Mars environments can be found in shallow deltaic deposits that occur elsewhere infilling crater lacustrine basins and different-sized impact craters of Noachian age (Fasset and Head, 2005).

3.2. PRESERVATION IN ENVIRONMENTS LINKED TO HYDROTHERMAL ACTIVITY

Hydrothermal activity has been present on Mars since early Noachian to recent times. Such a process has been recognized through the volcanism (Fig. 3A) that affected the crust water mobilization in the form of lacustrine and fluvial systems (Cabrol and Grin, 2001; Varnes et al., 2003; Schulze-Makuch et al., 2007), as well

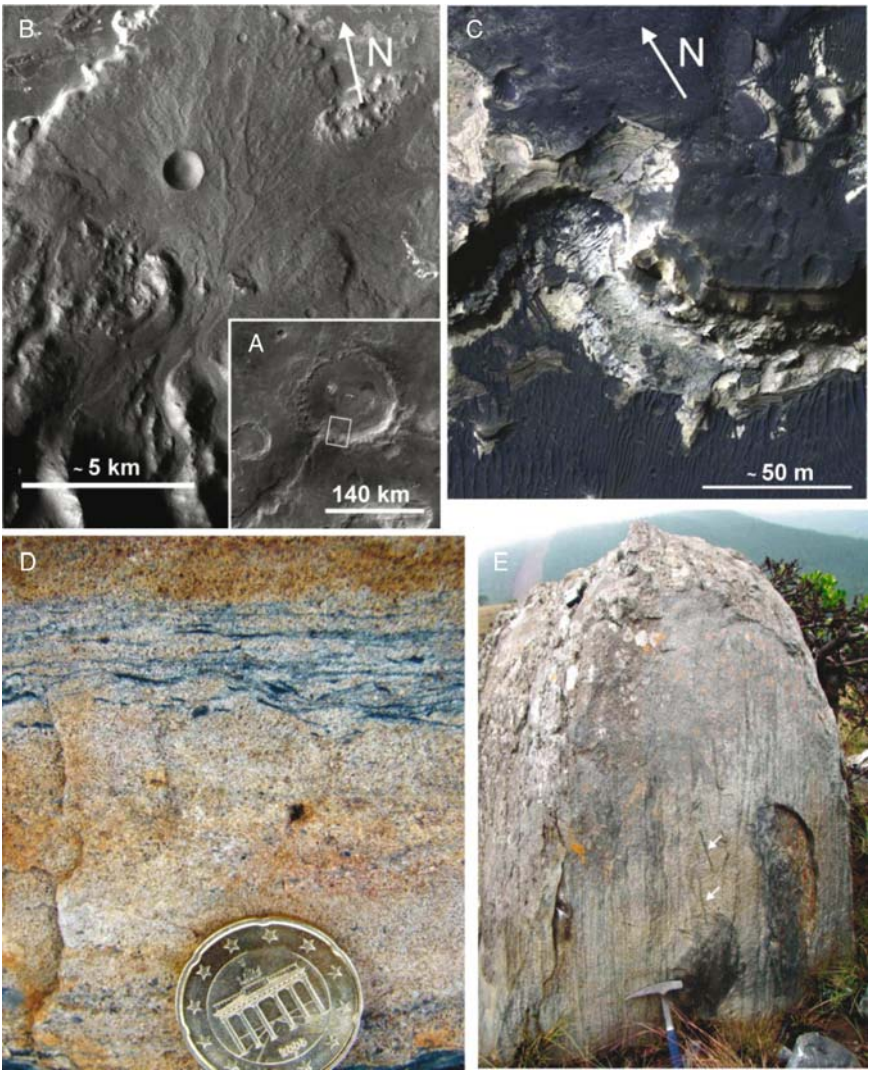

Figure 2. Noachian deltaic deposits (Grant et al., 2007) infilling the Holden Crater in Mars (A–C) and preserved microbial mat structures in Archean deltaic materials (D–E) at Barberton Greenstone Belt, South Africa. (A) Mars Orbiter Camara (MOC) wide angle image of the Holden Crater area where a deltaic structure occurs (white rectangle). (B) THEMIS Visible Image V17376003 (Themis Public Data Releases, Planetary Data System node, Arizona State University at http://themis-data.asu.edu) showing the deltaic structure filling the crater. (C) High Resolution Imaging Science Experiment (HiRISE) image PSP_001468_1535 (supported by the NASA/JPL/University of Arizona at http://hirise.lpl.arizona.edu), onboard the Mars Reconaissance Orbiter (MRO) unlocking the deltaic-like sequence that were sedimented inside the crater. (D) detail and (E) outcrop images of preserved microbial mats occurring in deltaic siliciclastic materials of the 3.2 Ga Archean Moodies Group of South Africa.

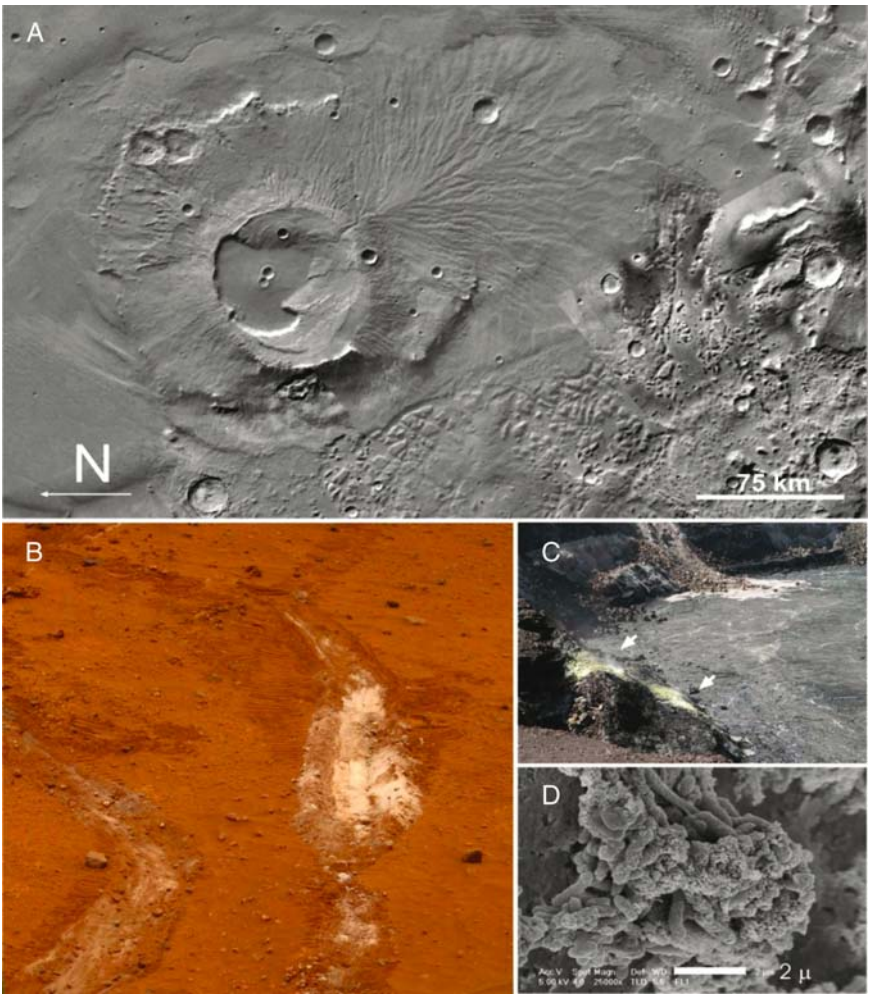

Figure 3. Context image of the Apollinaris Patera shield volcano (A) showing geomorphic structures related to caldera collapse, sediment infilling and water activity as fan-channeled pattern emerging southwards out from the crater, as well as a strong volcano erosion operated by gullies. Such a volcanic building was likely the scenario of hot fluid production displayed as geysers, volcanic chimneys or any other geothermal phenomenon (image was composed using the PIGWAD GIS Mapping system, courtesy USGS Astrogeological Research Program at http://astrogeology.usgs.gov). (B) Image PIA09491 silica-rich soil uncovered by Spirit at Gusev Crater that might be interpreted as a consequence of geothermal mineralization of silica-enriched fluids or a strong leaching on basaltic precursors inducing a secondary silica enrichment (courtesy of NASA/JPL-Caltech). (C) Crater caldera of the Kilauea volcano (Island of Hawaii) showing geothermal activity that produce sulfur and silica rich deposits around (withe arrows) (credited by Professor Raymond Arvidson and Thomas Stein). (D) Silicified bacili-like microbial structures in sinter deposits of the Rotokawa Geothermal Field in New Zealand. (Credit: Dr. Richard Schinteie.)

as silica-rich deposits discovered recently in the Gusev Crater (Professor Raymond Arvidson personal communication shown in Fig. 3B), which likely originated under acidic hot fluids.

On Earth, geothermal systems are often associated with volcanic centers (Fig. 3C), but also with hot springs that form minor structures which detection depends on the resolution of remote sensing instruments boarded in the orbiters. These geological systems introduce high silica and sulfur fluxes that induce high mineralization rates of a very resistant mineral-complexes as opaline silica currently forming sinters (Schinteie et al., 2007). Such a fast mineralization induces instantaneous silicification of living microbes (Jones et al., 2001; Walker et al., 2005) that facilitates preservation of organics over time. In some cases, silicification in geothermal systems produces exceptional replica of the microbial components (Fig. 3D) taking part in the community. Interestingly, silica preservation in some geothermal systems as the Parakiri Stream sourced in the Rotokawa Geothermal of New Zealand (Schinteie et al., 2007) occurs under acidic condition, as will be described in the following section. On the other hand, hot spring carbonate deposits have been also described to preserve biological information (Kazue, 1999). Although carbonates are pervasive on the surface regions of Mars, geothermal systems driven by CO_2-rich solutions could be present in some areas of the planet. This is monsistent with the TES identification of very low concentrations of carbonate in the Martian dust (Bandfield et al., 2003). Moreover, some Mars meteorites have provided carbonates as a part of the rock forming mineral assemblages (Bridges et al., 2001), which suggest that these mineralogies were formed in some Mars environments.

3.3. PRESERVATION IN ACIDIC ENVIRONMENTS

Late Noachian to Early Hesperian surface environments on Mars were probably ruled by global acidic conditions as inferred through the sulfate and oxide materials occurring in different areas such as Meridiani (Fig. 4A), Gusev, Valles Marineris, and North Polar regions. However, acidic environments have long been considered incompatible to life and related to highly contaminated areas (Blowes et al., 2005) given that some acidic solutions are sourced in mine tailings resulting from mining operations (Davis et al., 2000; Blowes et al., 2005). However, recent studies on the Earth geological record of ancient and modern acidic environments have identified natural systems (Fernández-Remolar et al., 2005; Benison, 2006).

Preservation in modern and ancient acidic environments have been reported from the Tyrrel Lake at nothwestern Victoria and other lacustrine areas of Western Australia (Benison and LaClair, 2003), acidic mine drainage of Indiana (Brake et al., 2002) and Río Tinto (Fernández-Remolar et al., 2007; Fernández-Remolar and Knoll, 2008). Combination of studies on modern and ancient environments are essential to understand long-term preservation in acidic environments. Interestingly, in Rio Tinto modern and ancient sediments dating back to more

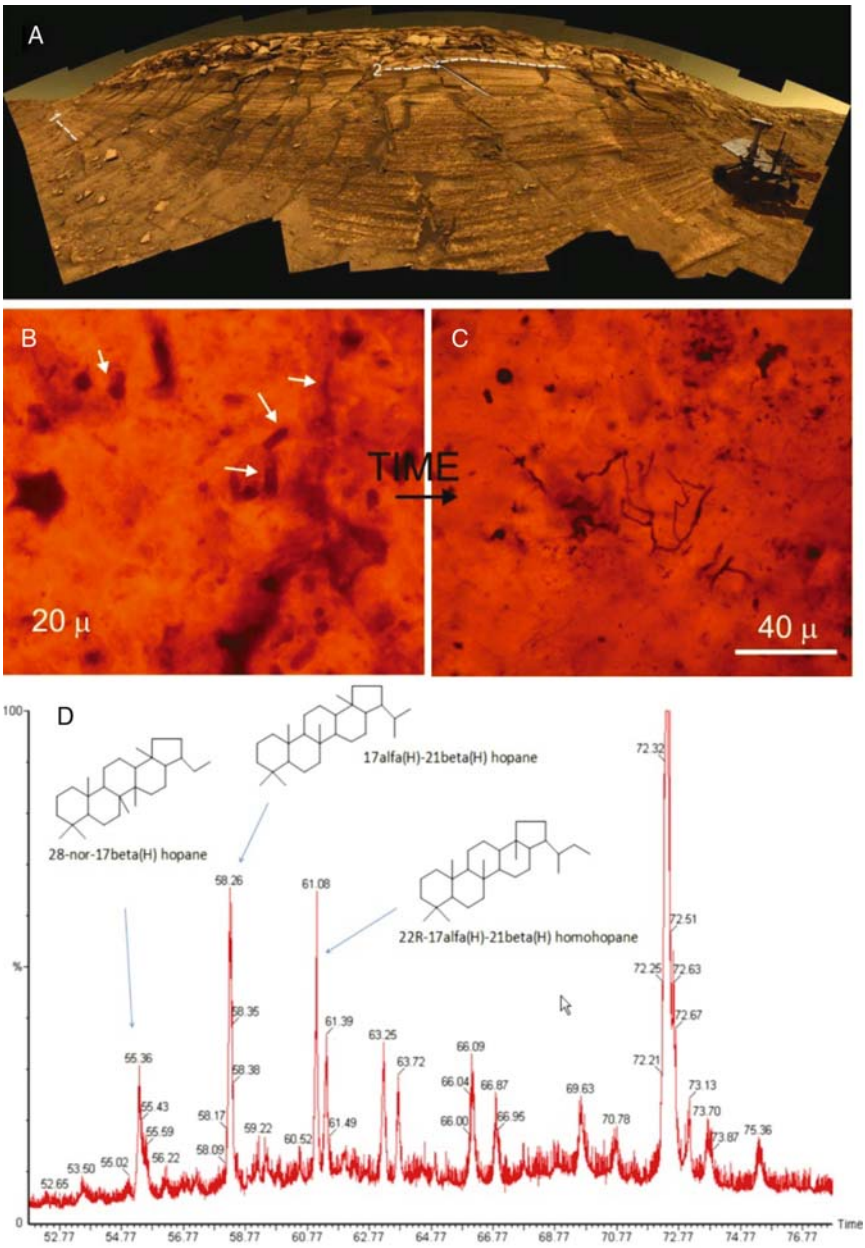

Figure 4. Burns Formation at the Burns Cliff in the Endurance Crater of Meridiani (A), Pan cam image PIA03241 resulted of a false color composite mosaic (courtesy of NASA/JPL-Caltech). Whattanga and Wellington sedimentary boundaries (Grotzinger et al., 2005), showed as 1 and 2, respectively; grained-sulfates and the hematites in concretional structures (blueberries). Both mineralogies have been identified as mineralogies giving some clues concerning to the acidic conditions of

than 6 million years coexist in the same area (Moreno et al., 2003; Fernández-Remolar et al., 2005), allowing an intregrative study concerning the syngenetical and postsedimentary taphonomic processes over time (Figs. 4B–D). Moreover, subsurface sampling from the Río Tinto underground regions has allowed sampling of the aquifers sourcing the surface acidic solutions (Fernández-Remolar et al., 2008a).

Physichochemical and geobiological analysis of the Río Tinto subsurface areas have determined very different environmental conditions when compared to the surface acidic environment, which are characterized by neutral and reducing conditions with lower concentration in ions (Fernández-Remolar et al., 2008b). Under these new environmental circumstances geopolymers and specifically geolipids of different origin (Fig. 4D) are preserved. On the contrary, the organics detected in the Río Tinto acidic sulfate deposits should have a long-term preservation if protected against the meteoric and diagenetic solutions as demonstrated by Aubrey et al. (2006), who reported organics from jarosite mineralogies in the Panoche Valley (California) with an age of 40 million of years.

Preservation of sulfates on some Mars deposits (Fig. 4A) like jarosite (a very soluble mineral phase) suggests that late meteoric and diagenetic solutions only partially remobilized the sulfate and the iron to form hematite concretions. If this interpreation is true, and assuming that life emerged sometimes on Mars, the Burns Formation could be expected to bear organic and paleobiological structures in the sulfate rich units (Fernández-Remolar et al., 2008c).

3.4. PRESERVATION IN THIN FILMS COVERING ROCKS

The water history of Mars suggests that rock coatings, desert varnishes and weathering rinds should be present in any sedimentary deposit or embedded inside alteration materials derived from ancient and recent aqueous activity. Spirit MER has indeed provided direct evidences of rock coatings of unequivocal aqueous alteration (Haskin et al., 2005). In this sense, location and analysis of rock coatings of several ages can be essential to trace back the climatic and environmental history (Dorn and Dickinson, 1989; Liu and Broecker, 2000) of the planet since its origin. In fact, lamina accrection and substrate weathering currently work under seasonal climatic regimen that results from the periodic water availability and thermal conditions. Such information can obvioulsy be essential to determine the ancient to recent surface and subsurface environmental water patterns that took place on Mars.

Figure 4. (continued) Meridiani Mars area explored by the Opportunity (B) microbial remains embedded in a cryptocristalline matrix composed of SO_4 and Fe^{3+}-bearing mineralogies sampled n modern deposits of Río Tinto (Spain). (C) 2.1 million years goetithe layer showing preserved filaments inside having cryptocristalline habit (Río Tinto, Spain). (D) Organic association preserved in neutral and reducing underground areas of the Río Tinto system where hopanoids are present.

In desert varnishes thin aqueous films contacting the rock surfaces are currently oversaturated in ionic species such as silica, manganese or iron remobilized from the rocky substrate (Perry et al., 2006), which favors microbial activity (Kuhlman et al., 2006) and later preservation to organic traces when the conditions are favorable (Perry et al., 2006). In rock coatings and weathering rinds oversaturation under aggressive acidic or alkaline conditions is also favorable for inducing high mineralization rates to preserve from microbial structures to organics. As a result, a complex mixing of different mineralogies such as iron and manganese oxides, sulfates, carbonates, opaline silica and different phyllosilicates (Potter and Rossman, 1977) occurs as laminae enveloping weathered rocks.

Several environmental conditions besides aridity areas can be imprinted in the surface rinds. Some volcanic emissions centered in geothermal activity produce SO_2-rich acidic fog which acts on volcanic tephra to induce the formation of silica and sulfate laminae (Schiffman et al., 2006). Ancient fluvial deposits are currently composed of conglomeratic materials which pebbles may show coatings depending on the climatic conditions that originated them. The Triassic Buntsandstein fluvial deposits in association to contemporaneous and older lacustrine and aeolian desert-like sedimentary materials, contain rounded pebbles that are covered by iron-rich coatings recording paleoclimatic information of great interest (Fig. 5A). The Río Tinto geological record dating back from Tertiary also shows iron rich coatings which origin is undoubtly associated to seasonal activity of acidic environments (Fig. 5B) and with clear traces of biological activity (Figs. 5C, D).

The importance of these microdeposits as recorders of modern and ancient biological activity, which can be easily detected in many planetary regions of Mars, cannot be overemphsized. As showed by Kuhlman et al. (2006), rock varnishes, as many other film coating environments, are microhabitats inhabited by diverse microbial communities having up to 10^8 microorganisms per gram of varnish lamina. Such a microbial activity can be traced through biochemical and organic compounds (Perry et al., 2006) that can be the base for the development of an exploration strategy for searching for life on Mars.

4. Conclusions: Mars Preservation Windows and Strategy for Planetary Exploration

Integrative research on preservation of biological information in terrestrial analogs is essential for building a consistent strategy to search for extinct life on Mars. From the scientific point of view, any exploration strategy developed for this compelling objective has to deal with the diverse Mars geological record which shows different preservation potential of biology depending on the paleoenvironment and diagenetic processes that have conformed it. As a result, different paleobiological entities may have potentially persisted and detection demands distinctive explorative procedures, sampling techniques and instrumentation (Farmer and Des Marais, 1999).

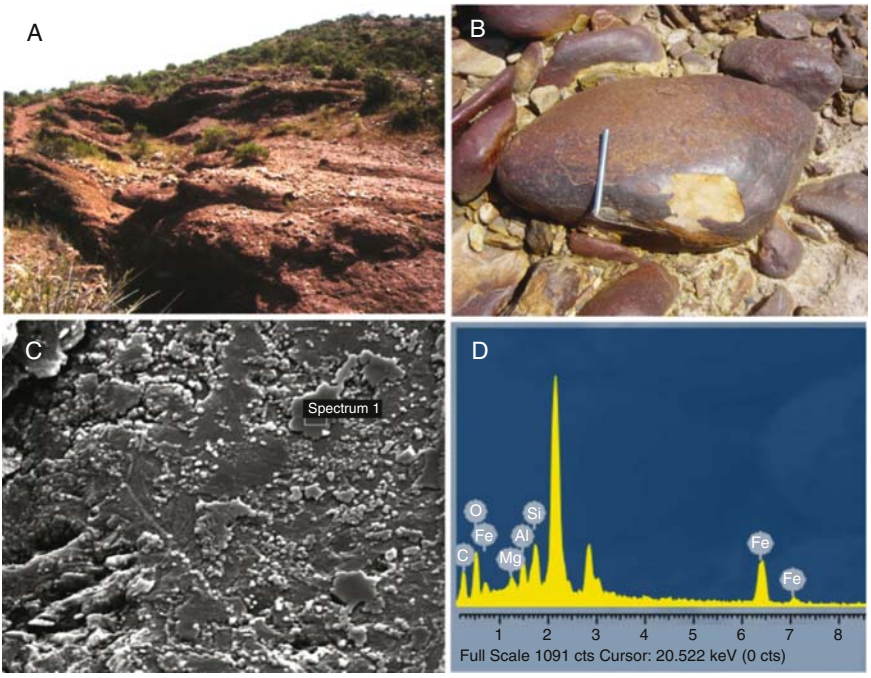

Figure 5. Iron-rich coatings on boulders (A) embedded in fluvial Iberian Permotriassic deposits (250 million of years), and (B) inside young Río Tinto terrace materials (1,000 years old). (C) SEM image showing microbial patches covering coated boulders and (D) EDAX microanalysis (spectrum 1 in (C)) showing a carbon and iron enrichment as expected for microbial films associated to watery environments enriched in iron.

Therefore, the application of the preservation windows concept can be of great utility to define a specific explorative strategy based on the preservation potential of a given geological unit. The Río Tinto Mars analog can be claimed to illustrate shortly this assumption (Fernández-Remolar et al., 2008c). As mentioned above, whereas the Rio Tinto surface environment favors preservation of morphologies and organics in iron- and sulfate-rich materials, organics are only preserved in the reducing subsurface; however, preservation is reset to simple preservation of morphologies when all these materials are exposed to a 2-million-of-year diagenesis. Under these varying conditions, the detection of extinct life on Mars would require different methodologies going from optical to analytical instrumentation which application strongly drives the exploration strategy of a given area. Finally, the differential preservation observed in those surface and subsurface Río Tinto environments strongly support that automated drilling instrumentation will be essential to sample subsurface regions that can have recorded some traces of extinct life. Same underground areas protected against conditions that may have induce the destruction of biological information through oxidizing, acidic or any other extreme conditions.

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SUMMARY, FINAL COMMENTS AND CONCLUSIONS

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This volume describes and discusses the oldest, extinct microorganisms from the depth of Earth and possible microbes from the upper spheres above Earth. Even though spacecraft or space Lander vehicles have yet to detect life traces outside of Earth, it is well possible that a record of past life or even currently living forms will be found on the surface or in subsurface areas of some extraterrestrial places.

"Fossil" according the Encyclopedia Britannica means a remnant, impression, or trace of an animal or plant of a past geologic age that has been preserved in the Earth's crust. Fossils are thus mineralized or preserved remains or traces of various organisms, such as microorganisms, plants and animals. Paleontology investigates fossils across geological periods, their formation, and the evolutionary relationships between taxa. When searching for fossil remains, one finds remnants ranging from microscopic single cells (often in large masses forming structures such as stromatolites), plants including petrified wood, and animals: mammals, fish, snakes, turtles, birds, up to gigantic animals such as elephants, mammoths or dinosaurs. Fossils are found ubiquitously in land and marine environments. The common presence of fossilized sea creatures high up in the mountainsides was considered by some people as a proof of the Great Flood described in the Bible or similar stories that appear in folklore worldwide. Fossil fuel is also consists of a class of ancient biological material occurring within the crust of the earth. Usually fossils mainly consist of portions of organisms that were already partially mineralized during life, such as the bones and teeth of vertebrates. Such compounds can be used as biomarkers to specifically detect certain groups of organisms. The age of the rock strata which contain fossils can be determined by radiometric dating method. Some fossil specimens can be estimated to have originated billions of years ago.

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The **Stromatolites** (Burns et al., 2008) are the oldest fossils found on Earth, and they were formed mostly by massive colonies of cyanobacteria and other prokaryotes, now found in Precambrian rocks. The layered communities were responsible of precipitation of minerals, now mainly preserved as limestone. The structures are characterized by thin, alternating light and dark layers. The fossil cyanobacteria cells from Western Australia, dated from 3.5 billion years ago, are probably the oldest known fossils. Even older are the marine sediments and pillow lavas discovered in Greenland in which evidence of life has been observed, and these were determined to be 3.85 billion years old.

A "**Living Fossil**" is an informal term for any living species of organism that appears to be identical to a species otherwise only known from fossils. Such species have survived major extinction events and seemingly have not changed during their very long evolutionary history. For example, an alga, plant or animal once thought to be extinct and was found living in modern times. Some living specimen were identical to fossils dating from 400 million year ago, and such an organism is therefore considered a "living fossil".

Astrobiology (see further) is a multidisciplinary science investigating the origin of life, evolution, life in extreme environments, search and distribution of life on Earth and beyond, paleontology, physiology of radiation resistance, and more.

An understanding of the factors that enabled Earth to become so successfully colonized is important when we wish to learn more about the possibility that similar (or even different) life forms may exist elsewhere in the Universe. We now have a reasonably good understanding of the development of Earth as an ecosystem during the last two-and-a-half to three billion years. However, the question when did life originate on planet Earth remains still open, and the processes that led to the formation of early life on Earth are still enigmatic. The early events that led to the colonization of our planet by a diversity of microorganisms during the first half a billion years since living cells first appeared, remain elusive.

Our views on life on Earth in the earliest times are mainly based on the following factors: geochemical signatures (especially on stable isotope studies), limited fossil evidence, study of microbial life as it exists today, and on theoretical considerations based on our understanding of the physical and chemical properties of our planet during the first billion years of its existence. Stable isotope studies of the oldest rocks has pushed the date of the earliest appearance of life back as far as 3.85 billion years ago (Mojzsis et al., 1996; Schidlowski, 1988). On the other hand, when until recently it was assumed that microfossils with the morphology of several types of present-day cyanobacteria could be safely dated to 3,460 million years ago, the age and nature of these fossils of "Precambrian microbial mats" have been challenged in recent years (Brasier et al., 2002, 2006; Wacey et al., 2008).

Theoretical considerations to reconstruct the nature of early life on Earth have always been based on our (ever changing) views about the chemical composition of early Earth: presence or absence of oxygen, possible abundance of

organic building blocks according to the Oparin-Haldane 'prebiotic soup' hypothesis, etc., and on thermodynamic constraints. Engelbert Broda's 1975 book on "The Evolution of Biogeochemical Processes", presented a for the time convincing scenario based on the "prebiotic soup" model. Since that time our understanding of the conditions on early Earth has greatly increased. Especially we know much more about the diversity of hyperthermophilic and other extremophilic microorganisms that inhabit our present-day planet. A number of very different possible scenarios for the origin of life and the nature of the first cells have been proposed in the past decades (see e.g Horgan, 1991; Peretó, 2005). The possibility that some kind of hyperthermophilic chemolithotroph may have been the first type of living organism has become an attractive alternative in our thinking. This recent concept is rather more accepted by the scientific community than a mesophilic heterotrophic fermentative bacterium swimming in a rich organic broth.

The understanding of the true nature of the first organisms on Earth will of course have a profound impact on our views how subsequently planet Earth, and possibly other planets in the universe as well, were colonized by life and as result became modified by the activity of living organisms.

A more recent development is the approach to reconstruct the order in which different types of metabolism (aerobic, anaerobic, fermentation, respiration, different kinds of chemolithotrophy and phototrophy) appeared, is 'phylogenomic dating'. Here both 16S rRNA genes and other slowly evolving genes are used as 'molecular clocks' in an attempt to reconstruct the history of life on Earth. The chapter by Blank in this volume presents a state-of-the-art overview of this intriguing field. The pioneering work of Carl Woese has shown that modern biomolecules are historical documents that can be used to learn even about the most distant past (Woese, 1987, 1994). However, the 'phylogenomic approach' still cannot be used for absolute dating of events in the early history of life on Earth. Therefore, calibration by means of other, independent methods will remain necessary. As long as the implications of the results obtained by such other methods it remains to be contested. Also the sequence information present within modern biomolecules will not yield a coherent picture of what happened on our planet over 3.5 billion years ago.

We clearly have learned a great deal since Oparin and Haldane proposed their theories about the origin of life in the 1920s. New information from disciplines as diverse as geology, paleontology, isotope geochemistry, microbiology, biogeochemistry, molecular biology and bioinformatics has contributed greatly to the discussion, and much of the current knowledge can be found in the preceding chapters. But unfortunately, in spite of all this information, we are still far removed from a generally accepted scenario of what kinds of life were found on Earth in the first half a billion years since it first appeared, and what kind of life we may expect to find elsewhere in the Universe.

Now all the major space agencies are planning new missions to the Moon, to the planets of our Solar System and to its outer boundaries, we have thought

it timely to devote this volume to different aspects illustrating the above statements under the title "From Fossils to Astrobiology". The main conclusion that becomes obvious while reading the chapters in this book is that multiple disciplines, including geophysics, study of solar activity, space climate and astrobiology should be brought within a unified framework that takes the fossil evidence from earlier stages of life on Earth into account. This book was intended to provide a framework for disentangling the microbial and multicellular fossil record, interpreting its possible implications for the existence of life elsewhere.

Answering the question whether there is life in the Solar System beyond our own planet should be viewed as a complement to the astronomical approach for the search of evidence of the later stages of the evolutionary pathways towards intelligent behavior. This is possible with the ongoing SETI (Search for Extraterrestrial Intelligence) project using radio telescopes and other astronomical instruments.

Since we are able to overcome terrestrial gravity and to send spacecrafts to explore the solar system, the direct search for life elsewhere is becoming an inherent part of space exploration and one of the major approaches of astrobiology. Although only a few extraterrestrial planetary bodies have been explored in detail so far, from the data already collected one can distinguish different categories of targets of major interest for astrobiology in our solar system.

There are extraterrestrial environments where life, either extinct or extant, may be present. These places are characterized by conditions in the past that had been compatible with the development of complex prebiotic processes, and had existed for a period long enough to allow the emergence of life (or compatible with the importation of living system from other places), followed by habitable conditions. One of the main parameters driving habitability on a planetary body is the presence of liquid water. Mars, like Earth, very likely had large bodies of liquid water on its surface for long period of times – several hundreds of millions of years – in its early history. In spite of its evolution and drastic changes, Mars may have preserved traces of extraterrestrial bio-signatures, due to its lack of strong tectonic activity.

If life was – or still is – present on Mars, those traces may be recovered today from its subsurface. This makes the red planet the most attractive body in the solar system for searching for extraterrestrial life. But there are other places in the solar system where liquid water is probably present. This is the case for three out of the four Galilean satellites of Jupiter: Ganymede, Callisto and Europa. This is also the case for Titan, the largest satellite of Saturn, being the only satellite of the solar system having a dense atmosphere and the only planetary body having atmospheric condition close to that of the Earth. Evidence for the presence of an internal water ocean is dramatically supported by the recent observations of the shift of surface features on the satellite, observed by the Cassini-Huygens mission. This mission has also revealed the unexpected properties of another satellite of Saturn, Enceladus, ten times smaller than Titan. Surprising gigantic plumes of water ice particles and gas, together with organic molecules have been observed by the Cassini instruments coming from the south polar region of Enceladus.

This strongly suggests the presence of a large liquid water reservoir in the internal structure of this small satellite, together with an active organic chemistry. Although we have so far no direct evidence for the existence of these internal oceans, the most interesting cases are those of Europa and Enceladus, since if they exist, their internal liquid water reservoir may be in contact with rocky materials, facilitating redox reactions that provide chemical energy to sustain prebiotic processes as well as energy for living systems.

There are also extraterrestrial environments that show today similarities with the primitive Earth, before the emergence of life. The study of such environments is of tremendous importance since most of the conditions that were present on the early Earth have disappeared today, been erased by geological processes and by life itself. To understand the processes that allowed the origin of life on our planet, and verify our theories on chemical evolution leading to life, we need to check these concepts in a realistic environment. The availability of planetary bodies having analogies to the early Earth offers such an essential opportunity. This is, again, the case for Titan, which, thanks to the many observations from the Cassini-Huygens mission, looks now more and more like an evolving primitive Earth, with methane on Titan playing the role of water on Earth, and water ice that of silicates. And finally, there are extraterrestrial planetary bodies where a complex organic chemistry is occurring, the study of which offers a way to study and understand the general processes of complexification of matter in the universe, in a real planetary environment. This is the case for comets, but also for the two satellites of Saturn, again, Titan and Enceladus.

Thus, there are several locations of great interest in the solar system for looking at prebiotic processes or searching for bio-signatures, especially in the outer solar system: Europa, Titan and even Enceladus are among those important targets. Although the Cassini-Huygens mission is far form ended, and is officially extended until 2010, and likely to be extended-extended beyond, the scientific community is already working on a new mission to the Saturn system, able to explore Titan's surface with dedicated instruments from a balloon and surface probes, and Enceladus from surface probes.

However, from what we already know today about the habitable bodies, it seems likely that the Earth is the only place in the solar system where macroscopic life is present. To search for extraterrestrial macroscopic life, we probably have to look outside the solar system. The discovery of extrasolar planets opens new possibilities in that field. Within the last decade, almost 300 exoplanets have been detected. We have now to identify and develop efficient tools to search for clear signs of biological activity on these far planetary bodies. With our current technology it is out of question to search for fossils, but we should be able soon to search for atmospheric biomarkers. Searching for evolutionary biosignatures during the exploration of the Solar System is an important objective in our search for deeper insights into life on Earth. Finding traces of life in any of the candidate-sites that are known to space geophysicists, as we have pointed out above: Europa, Enceladus, Titan, or Mars, would add arguments towards the

improved understanding of life on Earth, as we know it today. The study of fossil evidence of life on Earth can contribute considerably to the exploration of outer space, and that is one of the major messages of this book.

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