**SCHIRMACHER OASIS/LAKE UNTERSEE ANTARCTICA**
**ASTROBIOLOGY EXPEDITION**

Expedition Report
Flag #162

Report by: Richard B. Hoover (FN 2001)
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***Introduction***

The study of life in extreme environments and ecosystems is a key element of Astrobiology. Investigations of life in extreme environments provide valuable testing grounds for Astrobiology instruments and help develop concepts for new missions to search for biomarkers and evidence of past or present life elsewhere in the Cosmos. Understanding the nature, physiology, biochemistry and distribution of microbial extremophiles that inhabit the most hostile environments on Earth may help establish where and how to search for evidence of extraterrestrial life and develop methodologies to differentiate valid biosignatures from bio-mimics or terrestrial bio-contaminants.

Life has been found on Earth virtually that everywhere that water co-exists with an energy source along with a small suite of life-critical biogenic elements (e.g., C, H, O, N, P, S, Na, K, Mg, Ca and Fe). Microbial extremophiles are the dominant life forms that live (and often flourish) in the most extreme environments of our planet. Prokaryotes are by far the most abundant extremophiles that inhabit the cryosphere of Earth (Horikoshi and Grant, 1998). Psychrophilic and psychrotolerant prokaryotes (bacteria and archaea) are the main life forms that inhabit the cold deep sea floor, polar seas, glaciers, and the ice caps and permafrost. Some eukaryotes (e.g. diatoms, penguins, skuas, seals, polar bears, fish, etc.) also inhabit some of these regimes but comprise only a minute part of the biomass. Thermophilic and hyperthermophilic chemolithotrophic archaea and bacteria inhabit hot deep crustal rocks, geysers and deep sea hydrothermal vents providing food for tubeworms, shrimp and other denizens of the deep; halophiles and alkaliphiles live in the desert salt playas, the Great Salt Lake and Mono Lake; acidophiles inhabit acidic fumaroles and acid mine drainage; and radiophiles grow on highly radioactive spent nuclear reactor fuel rods. In 1970, Seckbach and Libby showed that cyanobacteria could grow in hot acid under pure carbon dioxide at high pressure. These life forms provide analogs for life that might be able to find habitable niches in regimes such as acidic droplets in the atmosphere of Venus. Schulze-Makuch *et al.,* (2004) and others have also argued that microbial extremophiles might be able to survive in the atmosphere of Venus.

However, it is well-known that most of the water-rich bodies of our Solar System are Frozen Worlds. Prime targets for Astrobiology include the Polar Caps of Mars, comets, water bearing asteroids and in tidal heated seas beneath the thick frozen crusts of icy moons of Jupiter (Europa, Callisto and Ganymede) and Saturn (Enceladus and Titan). Space Missions over the past few decades have shown that near perihelion the jet-black crusts of comets can become very hot. The surface temperature of comet Halley was found to reach 400 K (127 C) at 0.8 AU. If the gas released by the conductive heating of near surface water-ice and volatiles are produced faster than they can escape through the comet's crust the pressure will increase. In regimes where it exceeds that of the triple point (6.5 millibar), water can exist in liquid state above 0 C. Cyanobacteria, archaea, diatoms and many other psychrotolerant microbial extremophiles are capable of surviving over a wide range of regimes of temperature, pressure, radiation, pH and salinity. These life forms comprise ideal candidates for the types of microbial extremophiles that might be able to survive and exhibit episodic growth on comets (Hoover *et al.* 1986a, 1986b). These microbes should be able to grow within shallow pools in these voids in the ice, crust or near surface rocks or in thin films of liquid water between the mineral grains and ice crystals of water-bearing asteroids or comets (Hoover, 2008) if they have previously been transferred there by accretion of rocks, ice or sediments bearing microbial debris ejected from ancient Earth (or possibly ancient Mars) by impacts of large asteroids or meteoroids. Psychrophilic or psychrotolerant microbes could also inhabit thin films in the permafrost of present day Mars (Paepe *et al.,* 2001) where they may be able to grow in brine channels, cryoconite pools or perhaps even in perennially ice covered lakes in the floor of craters or in glaciers of the Northern Polar Cap of Mars (Wilson, 2003).

Most of the bodies of our Solar System with large amounts of water are frozen worlds. The cryosphere of Earth provides the best terrestrial analogs for life that may someday be detected in these regimes (either living or as cryopreserved remains of ancient ecosystems) by Space Probes, Rovers, of Manned Missions. The NASA Mars Observer and the ESA Mars Express spacecraft have discovered many regions where large bodies of water ice are present and the Phoenix Lander has proven that water ice does exist at the surface of present-day Mars. It is also well established that microbial extremophiles are the dominant life forms in the Earth's cryosphere. Living and cryopreserved microbial extremophiles are abundant in thin films and pools of super-cooled water (cryopegs) that exist in the Siberian permafrost and in cryoconite ecosystems (Hoover and Gilichinsky, 2001). Bacteria, cyanobacteria, archaea and diatoms are present in the deepest explored layers beneath the thick Antarctic ice sheet overlying Lake Vostok (Hoover & Gilichinsky, 2001, Abyzov *et al.,* 2004).

The perennially ice-covered lakes of Antarctica clearly represent the best terrestrial analogs for the environments of the polar caps of Mars or beneath the frozen crusts of the icy moons of Jupiter (Europa, Ganymede, Callisto) or Saturn (Enceladus or Titan). Galchenko et al. (1997) detected oxygenic photosynthesis by cyanobacteria in most of perennially ice-covered Antarctic lakes they explored in the Vestvold Hills and Bunger Hills. Cyanobacterial mats have also been studied from several permanently ice-covered alkaline Antarctic lakes of the McMurdo Dry Valleys, such as Lake Bonney (pH 6-8.5); Lake Fryxell (pH 5.5-9.5and Lake Hoare (pH ~8-9). However, none of these lakes afford the opportunity to study the constraints on life in conditions as extreme as those provided in upper 70 meters of the water column of Lake Untersee (T 0.5 – 0.8 C; pH 11-12.1) above the anoxic trough that was first characterized in 1997 by Wand and his co-workers. In 2007, Pikuta *et al.* provided surveyed the environmental parameters (e.g., pH, Temperature, Salinity, and Radiation) in which extremophiles grow. Although alkaliphilic psychrotolerant microorganisms are known, there are no genera or species of extremophiles yet validated that are capable of simultaneously growing at extremely high pH (>10) and low temperature <10 C. Microbial extremophiles that might survive in cold, hyperalkaline regimes of Earth of our planet are of great interest to Astrobiologists. The *Tawani Foundation 2008 International Schirmacher Oasis/Lake Untersee Antarctica Expeditions* was organized to conduct Astrobiology Research in the perennially ice-covered hyperalkaline Lake Untersee and many of the lakes of the Schirmacher Oasis.

## Tawani Foundation 2008 International Schirmacher Oasis/Lake Untersee Antarctica Expeditions

**OBSERVATIONS:**

*The icy bodies of the Solar System bodies are of great significance to Astrobiology because they could have regimes in which liquid water might exist. Consequently these bodies might harbor living extremophiles or the cryopreserved remains of ancient microbiota that previously flourished. Microbial extremophiles that inhabit the ice-covered lakes, permafrost and glaciers of Siberia, Alaska, Canada, and Antarctica are thought to provide the best terrestrial analogs for the types of life that could inhabit these regimes. These observations led to the formulation of the science hypotheses for international astrobiology expeditions for the exploration and search for microbial extremophiles and ecosystems in the lakes and permafrost of the Schirmacher Oasis and the perennially ice-covered hyperalkaliphilic Lake Untersee in East Antarctica.*

**MISSION STATEMENT:** This International (American, Russian and Austrian) Astrobiology Expedition will assess the species diversity and composition of prokaryotic and eukaryotic microbial extremophiles of the permafrost, cryoconite and cryptoendolithic communities of rocks, ice caves, streams and benthic microbial mats of the lakes of the Schirmacher Oasis and Lake Untersee.

**SCIENCE HYPOTHESES:**

1. The extreme conditions of the physical environments of perennially ice-covered lakes of Antarctica represent the best known terrestrial analogs to extraterrestrial environments relevant to the search for life elsewhere in the Cosmos, particularly the polar glaciers and permafrost of Mars and the icy moons of Jupiter and Saturn.
2. Microbial communities in perennially ice-covered lakes survive by physiological and ecological adaptations that reflect the extreme physical environment.
3. The structure and function of Antarctic lake ecosystems are controlled by the climate, the physical and chemical setting, fluxes of light, nutrients and biogenic elements and biochemical interactions between species of microbial communities.

**PRINCIPAL SCIENTIFIC GOALS:**

**Study of Microbial Extremophiles & Ecosystems of Schirmacher Oasis and Lake Untersee**

The permafrost, lakes, streams, cryoconite pools, cryptoendolithic, ice sculpture and glaciers of the Schirmacher Oasis provide a wide variety of environments and ecosystems in which cyanobacteria, bacteria, archaea, yeasts, diatoms and other microbial extremophiles of interest to Astrobiology may thrive. Lake Untersee, situated only 90 km from the Schirmacher Oasis, is a unique planetary resource. This perennially ice-covered, ultra-oligotrophic lake has sharp vertical gradients of temperature, pH (range ~7-12), dissolved oxygen, and electrical conductivity. The lower regimes of its 100 M deep anoxic trough have the highest methane concentration of any known natural aquatic system. In 2008, V. Samarkin reported (private communication to Hoover) that every liter of water near the floor of the trough contains 0.5

liters of Methane. Dramatic changes in temperature (1.8 C to 5 C) and a sharp increase in the level of hydrogen sulfide in the 50-55 M layer suggest that Sulfate Reducing Bacteria may be responsible. However, the pH is still above 11 at this level and no known bacteria or archaea have been found to grow at this combination of high pH and low temperature. Consequently, this zone of the water column may contain novel genera and species of microbal extremophiles that are new to Science, while the mud and sediments of the methane-rich anoxic trough may harbor novel methanogenic archaea and methane oxidizing bacteria and exotic microbial mats.

A small Reconnaissance Expedition will carry out a fly-over and landing on the Anuchin glacier, in close proximity to the perennially ice-covered and highly stratified hyperalkaliphilic Lake Untersee, which is the principal target for the Primary Expedition that is scheduled to be carried out in November-December, 2008. In the Main Expedition, which will be carried out in November-December, 2008, scientists from the United States, Russia, and Austria will search for new genera and species of psychrophilic alkaliphilic microbial extremophiles and nano-plankton in the cold hyperalkaliphilic waters beneath the permanent ice cover of the oligotrophic hyperalkaline Lake Untersee. They plan to determine the physical and chemical nature of Lake Untersee and measure ATP content in the water column and to conduct molecular biological analyses of the methanogens and methanotrophs. They also plan to deploy a remote meteorological station and use it to measure the climatic conditions at Lake Untersee. These measurements will permit modeling of the physical components affecting microbial ecosystems associated with ice-covered lakes. If conditions permit, dives will be carried out in the shallower regimes of the lake to sample microbial mats. They will also conduct Education Outreach and explore the microbiology, geology, micropaleontology and biomarkers as well as the biodiversity of mat communities, stromatolites, extremophiles and ecosystems of the lakes, streams, rocks and ice of Schirmacher Oasis and Lake Untersee They will assess mechanisms of cold-adaptation in Eubacteria (e.g., cold-shock, cold-adaptive and antifreeze proteins) and explore microbial adaptation and evolution and phage/host ecosystems and the ecological relevance of cryoconite assemblages and glacial systems as microbial refugia and impacts on global carbon budget

**THE 2008 INTERNATIONAL SCHIRMACHER OASIS RECONNAISSANCE EXPEDITION: Feb. 4-14, 2008**

The primary purpose of the 2008 International Schirmacher Oasis Scientific Expedition was to conduct a multidisciplinary collaborative Microbiology and Astrobiology Research program from Russia's Novalazarevskya Station. All research was carried out in accordance with Protocol on Environmental Protection to the Antarctic Treaty. The International Research Team was privately funded by a grant from the Tawani Foundation of Chicago, Illinois and supported by supplemental funding from NASA Headquarters and extensive support from NASA/MSFC and the Von Braun Center for Science and Innovation (VCSI) of the National Space Science and Technology Center (NSSTC) under the Direction of Marty Kress. The Recon Team included Col. James Pritzker, President and CEO of the Tawani Foundation (Expedition Logistics) and Art Mortvedt (Expedition Lead) of Peace of Selby Wilderness, Alaska, as well as scientists from the United States (Richard B. Hoover, NASA/NSSTC, Science Team Lead, and Dale Andersen, SETI Institute) and Russia (Valery Galchenko, Director, Winogradsky Institute of Microbiology, Moscow). These scientists have broad and diverse background in Microbiology, Paleontology, Astrobiology, Meteoritics and Polar Research.

This small Recon Expedition was conducted to prepare for the November-December 2008 Primary Expedition in which an international (American, Russian, and Austrian) team of 15 scientists and educators will search for microbial extremophiles and investigate the constraints on microbial life in the ice and in the cold, hyperalkaline water column and methane-rich anoxic sediments of the perennially ice-covered Lake Untersee - one of the most extreme environments on Earth. They also plan to deploy a meteorological station, dive to explore mat ecosystems and conduct detailed investigations of the water chemistry of Lake Untersee and explore the ice caves and lakes of the Schirmacher Oasis.

The program of Schirmacher Oasis Reconnaissance Expedition was devoted to the scientific exploration of the microbiology of Microbial Extremophiles, cyanobacterial mat communities and ecosystems of perennially ice-covered and subglacial lakes of the Schirmacher Oasis of central Dronning Maud Land of East Antarctica. During the Antarctic summer, glacial ice and snow melts and flows into streams that form lakes and thermokarst ponds and pools. Dr. Rajan Kumar Gupta (2006) and his colleagues studied the algal species diversity of six freshwater streams of the Schirmacher Oasis during the 11th Indian Scientific Expedition to Antarctica (1991-1992). They found the streams of the Schirmacher Oasis to contain abundant diatoms as well as a wide diversity of many genera of cyanobacteria (e.g., *Chroococcus, Chaemosiphon, Lyngbya, Oscillatoria, Phormidium, Plectonema, Nostoc, Calothrix* and *Tolypothrix*). Hoover (2005, 2006, 007) has reported the detection of indigenous morphotypes of representatives of all five orders of cyanobacteria that were found *in-situ* in freshly fractured interior surfaces of the Orgueil CI1 carbonaceous meteorite. It is thought that comets and water-bearing asteroids represent the most probable parent bodies for the CI1 carbonaceous meteorites. Therefore, the Schirmacher Oasis is considered to provide an ideal target for Astrobiology research as an analog for the types of ice and permafrost microenvironments that may exist in outer regimes of water-bearing asteroids and comets and in the ice/water interfaces of icy moons such as Europa, Ganymede, and Enceladus.

The Recon Expedition research team collected samples that are now being used for a detailed optical, Environmental (ESEM) and Field Emission (FESEM) Scanning Electron Microscopy of the size, morphology and species distribution, of benthic and planktonic microbial extremophiles, diatoms, and cyanobacterial mats from the lakes glaciers, meltwater pools, ponds and streams of the Schirmacher Oasis and a cryoconite pool at Lake Untersee,. The samples were documented, roughly characterized and imaged in the field in Antarctica and then brought back to the NSSTC Astrobiology Laboratory and the UAB Microbiology Laboratory of Prof. Asim Bej for detailed study, isolation into pure strains, morphological, physiological, and phylogenetic characterization prior to publication of new species and genera genera encountered in the Peer-Reviewed Scientific Literature. These objectives are directly relevant to the overall goal of understanding the Earth’s Environment and conserving and preserving Lake Untersee and the Schirmacher Oasis. These are precious natural resources that may harbour important new life forms and provide crucial new information relevant to the planetary methane cycle in its impact on global climate change as well as the adaptation of life to harsh environments, genomic stability and horizontal gene transfer in Ecosystems.

Enrichment and axenic cultures have been obtained and the metabolism, physiology, phylogenetics and systematics of several novel strains of bacteria and archaea are now being investigated. Optical and Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy studies have been performed to establish of the morphology, ultramicrostructure, and elemental compositions of the microorganisms found. Several novel strains of aerobes and anaerobes have been isolated and 16S rRNA gene sequencing, DGGE (denaturated gradient gel electrophoresis) and DNA-DNA phylogenetic analysis carried out to determine the taxonomic position of these organisms. The strains found to represent novel genera or species of bacteria or archaea will be fully characterized and deposited in two or more of the recognized International Culture Collections such as the ATCC (*American Type Culture Collection*); DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen*), *Institut Pasteur*, etc. Novel diatoms that may be encountered in sediment cores from Lake Unterseee will be deposited in Diatom Collections, such as that of the *California Academy of Sciences.* Living cyanobacteria, diatoms or other phytoplankton will be deposited with *UTex the Culture Collection of Algae* or the *Provalosi-Guillard National Center for Culture of Marine Phytoplankton*.

New taxa of microbial extremophiles will be described in scientific papers in recognized International Journals such as the *International Journal of Systematic and Evolutionary Microbiology (IJSEM), Extremophiles,* etc. to insure valid publication of novel taxa and wide dissemination of the scientific results of this International Expedition. Members of the 2008 International Schirmacher Oasis Scientific Expedition have previously discovered and described several validated new genera and species of extremophiles from some of the most hostile environments on Earth. These include:

**Alkaliphiles and SRB's from Hyperalkaline Soda Mono and Owens Lakes of Californi**a –
*Spirochaeta americana* (Hoover *et al., 2003*);
*Desulfonatronum thiodismutans* sp. nov. (Pikuta *et al.,* 2003a);
*Tindallia californiensis* sp. nov. (Pikuta *et al.,* 2003b);
*Anaerovirgula multivorans,* gen. nov., sp. nov. (Pikuta *et al.,* (2006a);
*Spirochaeta dissipatitropha* sp. nov. (Pikuta *et al.,* In Press);

**Hyperthermophiles from the Rainbow Deep-Sea Hydrothermal Vent –**
*Thermococcus thioreducens* sp. nov. (Pikuta *et al.,* 2007)

**Psychrophiles and Psychrotolerants from Alaska and Southern Patagonia -**
*Carnobacterium pleistocenium* sp. nov. (Pikuta *et al.,* 2005);
*Trichococcus patagoniensis* sp. nov. (Pikuta *et al.,* 2006b);
*Proteocatella sphenisci,* gen. nov., sp. nov. (Pikuta *et al.,* In Press)

The enormous effort required to isolate, characterize, and publish a validated new genus or species of bacteria is reflected in the fact that the new species *Proteocatella sphenisci* is just now in the final stages of publication at IJSEM, even though this microbe was collected and isolated in Southern Patagonia over eight years ago during the Antarctica 2000 Expedition.
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## THE 2008 INTERNATIONAL SCHIRMACHER OASIS RECONNAISSANCE EXPEDITION – (EXPLORERS CLUB FLAG EXPEDITION – FLAG #162)

The 2008 International Schirmacher Oasis Reconnaissance Expedition was approved by NASA. Dr. Valery Vladimirovich Lukin, Head of Russian Antarctic Expedition (RAE)/Deputy Director, Arctic and Antarctic Research Institute (AARI) in St. Petersburg, Russia also informed us that our Expedition with Russian and American Scientists was designated an Official Russian

Antarctic Expedition. The Recon Expedition Team was delighted to have this Expedition designated and Official Explorers Club Flag Expedition and to be granted the honor of carrying Explorers Club Flag #162 to the Schirmacher Oasis of Dronning Maud Land, Antarctica. This is the same Explorer's Club Flag (#162) that was carried to the Patriot Hills, Thiel Mountains and South Pole during the "*Antarctica 2000 International Astrobiological Expedition*".


**Cdr. James A. Lovell (Co'66) and Richard B. Hoover (FN'01) in the Patriot Hills, Antarctica – Jan. 10, 2000**

Paul Sipiera was Expedition Lead and Richard Hoover served as Science Team Lead. Col. James Pritzker (Tawani Foundation CEO - Polar Logistics), Capt. James A. Lovell (*Gemini 7, Gemini 12 Cdr.; Apollo 8, Apollo 13, Cdr.*) and Scientist Astronaut Owen Garriott (*Skylab 3; Spacelab 1*) played major roles in the tremendously successful Antarctica 2000 Expedition. Twenty meteorites were collected from the Blue Ice of the Thiel Mountains and novel genera and species of psychrophilic and psychrotolerant microorganisms were discovered.

These included the new genus *Proteocatella,* and *Trichococcus patagoniensis,* isolated from a single sample of Magellanic Penguin guano collected at the southern tip of Patagonia. This was the first known bacterium validly published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM) that is capable of active growth at -5 C.

**Magellanic penguins and guano sample at Seno Ottway Colony, Patagonia, Chile-Jan. 4, 2000**


**Flag #162 flys over Paul Sipiera (FN'80)/Richard Hoover tent in Thiel Mountains - Jan. 13, 2000**

## 2008 INTERNATIONAL SCHIRMACHER OASIS RECONNAISSANCE EXPEDITION

We were deeply honored to have the privilege of carrying Flag #162 back to Antarctica during the 2008 International Schirmacher Oasis Reconnaissance Expedition. The Recon Team along with Martin P. Kress (Executive Director of the National Space Science and Technology Center (NSSTC) and CEO of the Von Braun Center for Science and Innovation) gathered on Capetown, South Africa to make final preparations for the Expedition. Marty Kress played a key role in the Planning, Organization, Management and logistics of the 2008 International Schirmacher Oasis Reconnaissance Expedition. He is shown in the photo above at Cape Point, South Africa with Explorers Club Flag #162 shortly before the Team departed on a Russian Ilyushun-76 jet for the Blur One Runway at *Novolazarevskaya* Station near the Schirmacher Oasis, Antarctica. The Schirmacher Oasis of central Dronning Maud Land, Antarctica is 3km wide, 20 km long and oriented in an East-West direction (70$^o$46'04"-70$^o$44'21" S; 11$^o$49'54"- 11$^o$26'03"E). The Russian Antarctic Station *Novolazarevskaya* and the Indian Station *Maitri* are both located in the Oasis. There are over 150 lakes in the Oasis, many of which are perennially covered with ice while others have open water during the mid-summer melt. Shivaji *et. al.* (2004) studied the bacterial diversity of the Schirmacher Oasis and Alam *et. al.* (2006) described the novel species of proteolytic bacterium that they named *Clostridium schirmacherense.* This obligately anaerobic microbe had optimal growth at pH 8 and was isolated from lake sediments.

**Col. James Pritzker, Dale Andersen (FN ‘87), Art Mortvedt (MN ’84), Valery Galchenko and Marty Kress (Executive Director, NSSTC - Front) display Explorers Club Flag #162 at Cape Point, South Africa**

**Art Mortvedt, Richard Hoover, Col. James Pritzker (Front Row), Dale Andersen and Valery Galchenko with Explorers Club Flag #162 at Cape Point, South Africa**


**Team Arrives on IL-76 at Blue One Runway, Novolazarevskaya Base, Antarctica**

**Explorer's Club Flag #162 at Lake Podprudnoye, Schirmacher Oasis, Antarctica**

## 2008 INTERNATIONAL SCHIRMACHER OASIS RECONNAISSANCE EXPEDITION: PRELIMINARY SCIENCE RESULTS

Very little is known of the bacterial diversity of the lakes of the Schirmacher Oasis and the microbial extremophiles of Lake Untersee have never been surveyed. Lake Untersee if 563 m above sea level in the Otto-von-Grubergebirge of central Dronning Maud Land of East Antarctica with a surface area of 11.4 $km^2$ is the largest freshwater lake in east Antarctica. Wand *et al.*.(1997, 2006) have reported that this perennially ice-covered, ultra-oligotrophic lake has sharp vertical gradients of temperature, pH (range 12.1 to 6.9), dissolved oxygen, and electrical conductivity. A portion of the southwestern area of the lake is anoxic below 80 M and exhibits the highest observed maximum methane concentration of any known natural aquatic ecosystem. It is thought that this is produced by methanogenic archaea, and the primary task of the Full-Up Expedition is to explore the microbial communities that may inhabit this exotic environment.

During the February 2008 Recon Expedition, Hoover collected a number of samples from Lake Stancionoye, Lake Glubokoye, Lake Podprudnoye, Lake Zub, and cryoconite ecosystems of wind and melt eroded and sculpted ice formations in the Ice Cave near Novo Ststion and Lake Podprudnoye. These samples were returned to the Astrobiology Laboratory, NSSTC for collaborative studies with Dr. Elena V. Pikuta on the anaerobic extremophiles and several samples were sent to Dr. Birgit Sattler of the University of Innsbruck and to Prof. Asim K. Bej of the Department of Biology, University of Alabama at Birmingham, for the study of the psychrotolerant and psychrophilic aerobic component of the microbial communities and for assessment of the diversity, cold adaptation and pigmentation of these microorganisms.

## PIGMENTED AEROBIC BACTERIA FROM SCHIRMACHER OASIS LAKES

Professor Asim Bej and co-workers at UAB have found several diverse groups of psychrotolerant bacteria with various pigments and isolated them in aerobic cultures on agar media. The pigments may protect these organisms from the intense levels of solar UV radiation prevalent in Antarctica during the summer. Western blot studies of the expression of cold adaptive protein CapB and ice-binding protein IBP using showed positive detection of both or either of these proteins in 6 of 8 isolates. The CapB and IBP protein structure varies greatly in microorganisms. Hence, it is possible that the 2 isolates with negative results may have a different class of these proteins. The expression of the CapB and the IBP in these isolates suggest that these proteins are essential for the survival in the Antarctic cold and subzero temperatures and provide protection from freeze-damage. The Recon samples provided sufficient data to further investigate the rich and diverse biota of psychrotolerant extremophiles in the Antarctic Schirmacher Oasis using both culture-independent and culture-based approaches; and to help understand the mechanisms of cold tolerance.

The results from the culture-based analysis showed the presence of a diverse array of aerobic microorganisms that are taxonomically closely related to *Pseudomonas, Frigoribacterium, Arthrobacter, Flavobacterium,* and *Janthinobacterium.* Some of these microorganisms have been previously reported in water and soil samples of Schirmacher Oasis by Shivaji *et al.* (2004). All these microorganisms were found to develop pigments, which may possibly protect them from high solar radiation and UV damage in Antarctic environment. Work is underway to investigate the species identification of these isolates from samples collected from the Schirmacher Oasis. A more detailed description of the results of this research on the bacteria from the Recon Expedition has been provided by Mojib *et al.* (2008).

## ANAEROBIC EXTREMOPHILES FROM BETTY'S BAY & SCHIRMACHER OASIS

At the NSSTC Astrobiology Laboratory, Richard Hoover has studied the morphology and motility of a large number of anaerobic and aerobic extremophiles in the samples he collected in the Schirmacher Oasis. Dr. Elena V. Pikuta and students have isolated a number of anaerobic microbial extremophiles from the samples collected during the 2008 International Schirmacher Oasis Antarctica Reconnaissance Expedition. The samples showed great diversity of psychrophlic and psychrotolerant bacteria. Six new anaerobic strains have been isolated in pure cultures and have now been partially characterized. Two of them (strains ARHSd-7G and ARHSd-9G) were isolated from a small tidal pool containing guano from the colony of African Penguins *Spheniscus demersus.*

### Exotic Extremophiles Isolated from *Spheniscus demersus* Samples

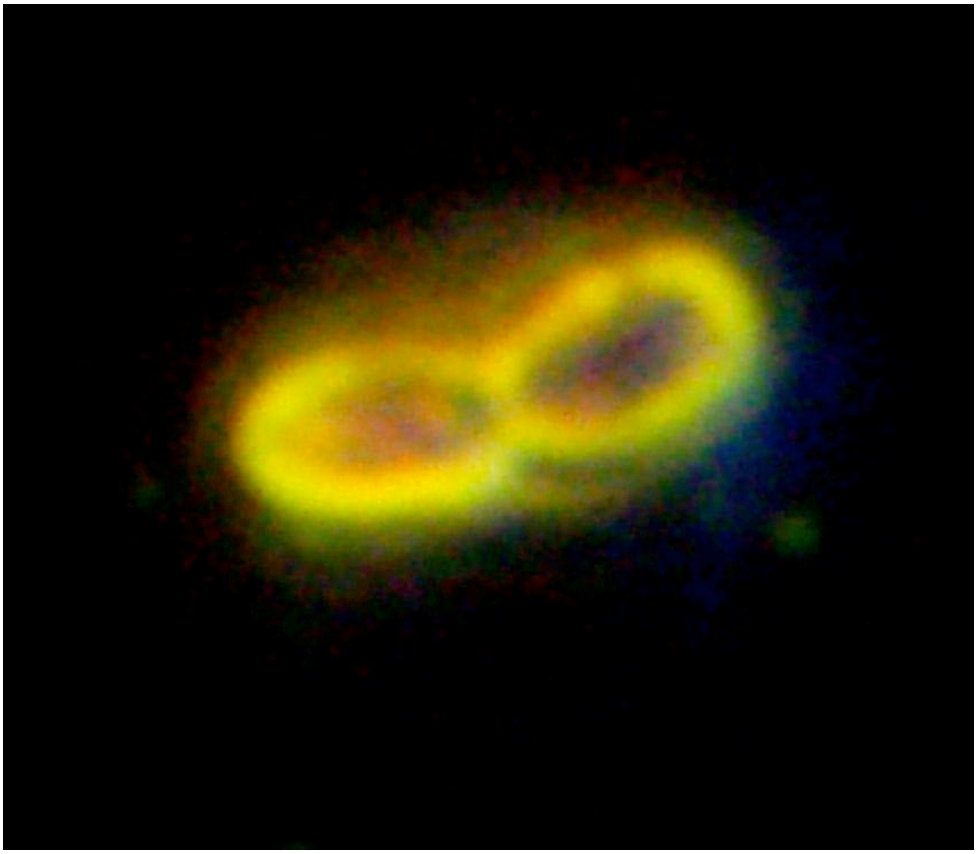
A single sample of guano from the Magellanic Penguin (*Spheniscus magellanicus)* cololected uring the Antarctica 2000 Expedition, yielded a new species of facultative anaerobe, (*Trichococcus patagoniensis* sp. nov.,) that is able to grow at –5 °C and a new genus and new species (*Proteocatella sphenisci* gen, nov., sp. nov.). The extreme productivity of this sample led Richard Hoover to collect samples of guano from the large breeding colony of African Penguins inhabits Stony Point, Betty's Bay (S 34 $^{o}$ 22'26"; E 18 $^{o}$ 53' 43") near the southernmost tip of

South Africa. The African Penguins *Spheniscus demersus* is the only species of 18 penguin species worldwide that breeds around the coast of Africa.

**Colony of African Penguins (*Spheniscus demersus*) at Stony Point, Betty's Bay South Africa.**

On February 2, 2008 (with the assistance of the Park Rangers that protect the Betty's Bay African Penguin colony) Hoover collected samples of guano from a small tidal pool. The pH was 6.8 and the water temperature at the time the sample was collected was 15 C. The samples were returned to the NSSTC Astrobiology Laboratory, Dr. Elena V. Pikuta isolated several interesting novel anaerobes from this sample, including strains ARHSd-7G and ARHSd-9G.

Strain ARHSd-7G was isolated on mineral anaerobic medium with 3 % NaCl, pH 7 and *D*-glucose, it has motile, vibrion shape cells, and is Gram variable. Strain ARHSd-9G grew on anaerobic, alkaline medium with pH 9 and 1 % NaCl at 3°C. The substrate was *D*-glucose supplemented with yeast extract (0.05 %). Cells of strain ARHSd-9G had morphology of straight or slightly curved elongated rods and demonstrated unusual optical effects under dark-field visible light microscopy. The cells were spore-forming, motile by polar flagella, Gram variable, anaerobic and catalase negative. This isolate had a strictly fermentative metabolism and growth was not stimulated by the addition to the medium of any of the following electron acceptors: ($Fe^{3+}$, $NO_3^{2-}$, $SO_4^{2-}$, $SO_3^{2-}$, $S^o$, $S_2O_4^{2-}$). Strain ARHSd-7G was also unable to reduce sulfur compounds to $H_2S$. Preliminary 16S rRNA phylogenetic analysis revealed that strain ARHSd-7G belongs to the genus *Salinivibrio.* It had highest similarity (99.1 %) with *S. costicol* and DNA-DNA hybridization studies are now underway to establish the final taxonomic position of this new isolate.



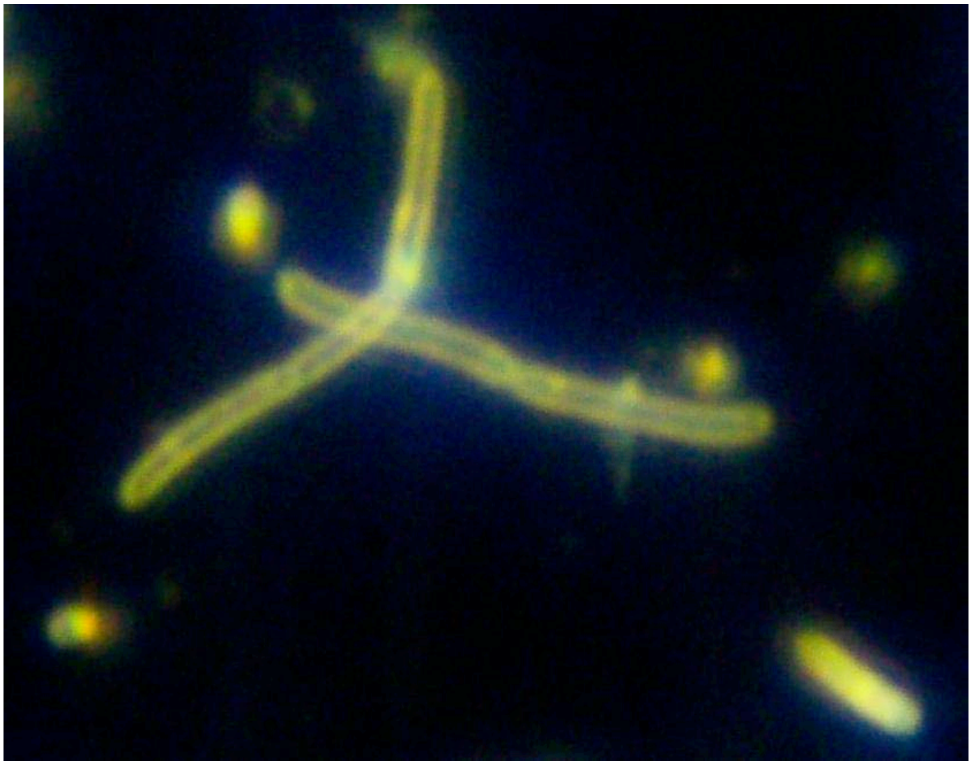
**Cell morphology of non spore-forming dividing vibrions of strain ARHSd-7G; - cells are 0.4x 1.2-1.5 µm.**


**Dark Field Visible Light microscopy image of filament of strain ARHSd-9G shows unusual optical phenomena with intense colors, possibly arising from scattering and interference effects in a thin sheath enveloping the cells.**

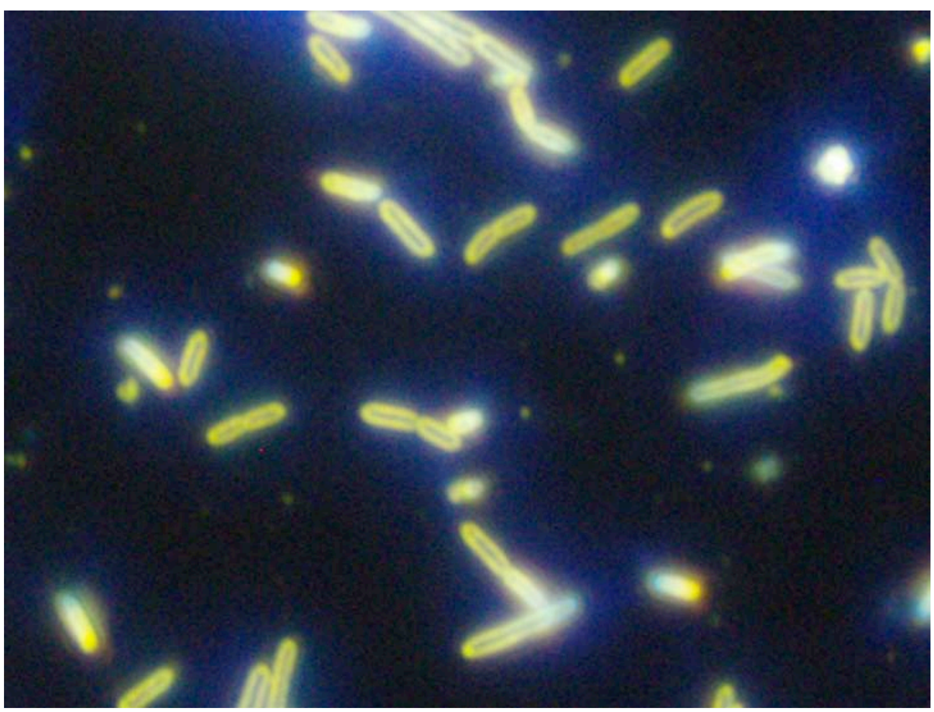
Strain ARHSd-9G is another very interesting microorganism isolated from the *Spheniscus demersus* guano sample. The enrichment culture was grown on alkaline medium with pH 9 and 1 % NaCl on *D*-glucose as a substrate. The pure culture was received from $8^{th}$ dilution on "roll-tubes" with 3 % agar medium. The cell morphology exhibits elongated straight or slightly curved rods. The cells are motile with peritrichous flagella. The cells are 0.4x 3-5 μm and the strain is spore-forming with round spores which are located terminally. Strain ARHSd-9G is strictly anaerobic and catalase negative and grows between 3 and 22 $^{o}$C.

**Anaerobic Extremophiles Isolated from Lake Zub, Schirmacher Oasis**

From the mat sample collected near Lake Zub, the new strain LZ-3 was isolated in pure culture at 3°C. Strain LZ-3 was a gram positive, spore-forming anaerobe that grew on 0.5 % NaCl mineral medium with *D*-glucose as a substrate. The 1x10 μm cells exhibited a distinctive morphology of large rods with rounded ends and size.



**Morphology of strain LZ-22G growing on *D*-glucose at 3 $^{o}$C. Cells are motile with peretrichous flagella and are spore-forming with the oval spores located sub-terminally.**



**Morphology of proteolytic vibrion cells of strain LZV-3P from the ample of green moss collected on the shore of Lake Zub near the Indian *Maitri* Station IN THE Schirmacher Oasis, Antarctica.**

**Anaerobic Extremophiles from Wind Sculpted Ice near Lake Podprudnoye**

Near Lake Podprudnoye, the Recon Team encountered a large number of complex ice formations produced as the ice was sculpted by wind and melting by solar heating.

**Flag #162 at Lake Podprudnoye, Schirmacher Oasis, Antarctica near Wind Sculpted Ice Formations**

These natural ice sculptures contained a large number of small entrained black rocks collected and encased films and pockets of liquid water within the ice forming micro-niches. These are important as they are considered analogs to cryoconite environments where liquid water might and entrapped gasses might be able to remain stable for relatively long periods of time on the glaciers of Mars and perhaps even comets or the frozen moons of Jupiter or Saturn. From cryoconite pools around black tocks in these ice sculptures, the new strain ISLP-22 was isolated in pure culture. The cells of this spore-former had vibrion shape. This culture preferred 0.1 % NaCl mineral anaerobic medium and grew rapidly at 3 $^{o}$C. This strain is now under physiological study and phylogenetic analysis. Similar encased rocks were also abundant in the ice of the beautiful ice cave that is not far from the *Novolazaravskaya* Station.

**Richard Hoover (FN'01) in the Ice Cave near *Novolazarevskaya* Station, Antarctica**

The February 4-14, 2008 Tawani Schirmacher Oasis/Lake Untersee International Reconnaissance Expedition was extremely successful. A great deal of valuable information on Extremophiles of the Oasis was obtained. A number of samples were obtained and they are now undergoing extensive studies in the United States, Russia and Austria. Enrichment cultures of the aerobic strains are being investigated by Birgit Sattler at the University of Innsbruck and the University of Alabama in Birmingham and the anaerobic cultures are under study at the NSSTC Astrobiology Laboratory. Anaerobic enrichment cultures were obtained from almost all of the samples returned, which validated the sample recovery and return methodologies. Several new anaerobic psychrotolerant isolates have been received in pure culture and work with these and other strains from the returned samples is now underway. The purification, characterization, phylogenetic identification and scientific description of the microbial extremophiles found to represent novel taxa will be carried out in near future.

**Explorers Club Flag #162 displayed at the UN International Atomic Energy Agency (IAEA) Headquarters in Vienna, Austria with entire Expedition Team during Meeting to Plan the Full-Up Expedition**

The Recon Expedition was also of great importance to plan the logistics needed to efficiently carry out the Primary Expedition to Lake Untersee that will occur in the November 4 to December 22, 2008 period. Information about physiology and biochemical characteristics and biodiversity of the novel bacteria and archaea obtained from Lake Untersee and the Lakes of the Schirmacher Oasis should contribute our knowledge of life in Extreme Environments and help determine where and how we can best search for evidence of life on other frozen worlds of our Solar System.

## References

Abyzov, Sabit S., Hoover, Richard B. Imura, S., Mitskevich, I. N., Naganuma, T., Poglazova, M. N., and Ivanov, M. V., (2004) "Comparative Results of Using Different Methods for Discovery of Microorganisms in Very Ancient Layers of the Central Antarctic Glacier above Lake Vostok." *Advances in Space Research, Cospar,* **33,** 1222-1230.

Alam, S.I., Dixit, A., Reddy, G.S.N., Dube, S., Palit, M., Shivaji, S. and Singh, L., "*Clostridium schirmacherense* sp. nov., an obligately anaerobic, proteolytic, psychrophilic bacterium isolated from lake sediment of Schirmacher Oasis, Antarctica," Int. J. Syst. Evol. Microbiol. **56**, 715-717 (2006).

Gupta, R. K., Pandey, V. D., Pandey, K. D., and Vyas, D. (2006) Algal Species Diversity of Streams of Schirmacher Oasis, Antarctica. in *Glimpses of Cyanobacteria* (Gupta, R. K., Kumar, M., and Paliwal, G. S., Eds.) Daya Publishing House, Delhi. pp. 231-241.

Helbling, E. W., et al. (1996) Photoacclimation of Antarctic marine diatoms to solar ultraviolet radiation. *Journal of Experimental Marine Biology and Ecology* 204**,** 85-101.

Hoover, Richard B., Hoyle, Fred, Wickramasinghe, N. C., Hoover, Miriam J. and Al-Mufti, S., "Diatoms on Earth, Comets, Europa, and in Interstellar Space," *Earth, Moon, and Planets, 35,* 19-45, 1986a.

Hoover, Richard B., Hoyle, Fred, Wallis, M. K., and Wickramasinghe, N. C., "Can Diatoms Live on Cometary Ice?" in *Asteroids, Comets and Meteors II.,* Proceedings of Meeting at Astronomical Observatory of Uppsala University, June, 1985, (C. I. Lagerkvist, Ed.), pp. 359-352, 1986b.

Hoover, Richard B. and Gilichinsky, David, "Significance to Astrobiology of Microorganisms in Permafrost and Ice," in *Permafrost Response on Economic Development, Environmental Security and Natural Resource Potential,* NATO-ARW held in NOVOSIBIRSK, SIBERIA, 12-16 Nov. 1998. (Roland Paepe, Ed.) Klewer Publishing, New York, 553-580 (2001).

Hoover, Richard B., Pikuta, Elena V., Bej, Asim K., Marsic, Damien, Whitman, William B., Tang, Jane, and Krader, Paul. "*Spirochaeta americana* sp. nov., a new haloalkaliphilic, obligately anaerobic spirochaete isolated from soda Mono Lake in California." *Int J Syst Evol Microbiol* **53**, 815-821, (2003).

Hoover, R. B. and Rozanov, A. Yu., "Microfossils, Biominerals and Chemical Biomarkers in Meteorites." in *Perspectives in Astrobiology,* **Vol. 366,** NATO Science Series: Life and Behavioural Sciences, *IOS* Press, Amsterdam, The Netherlands, 1-18, 2005.

Hoover, R. B., "Fossils of Prokaryotic microorganisms in the Orgueil meteorite," *Instruments, Methods, and Missions for Astrobiology IX,* Proc. SPIE **6309,** 630902:1-17 (2006).

Hoover, Richard B., Pikuta, Elena V., Townsend, Alisa, Anthony, Joshua, Guisler, Melissa, McDaniel, Jasmine, Bej, Asim and Storrie-Lombardi, Michael, Microbial Extremophiles from the 2008 Schirmacher Oasis Expedition: Preliminary Results, *SPIE* **7097,** 70970L1-12, (2008).

Hoover, R. B. (2007). "Comets, carbonaceous meteorites, and the origin of the Biosphere." In *Biosphere Origin and Evolution* (N. Dobretsov, N. Kolchanov, and A. Rozanov, Eds.), Springer, New York, 55-69 (2008).

Horikoshi, K. and Grant, W. D. *Extremophiles: Microbial Life in Extreme Environments.* Wiley-Liss, New York, pp. 1-322. (1998).

Mojib, Nazia, Bej, Asim K., and Hoover, Richard B. Diversity and cold adaptation of microorganisms isolated from the Schirmacher Oasis, Antarctica, *SPIE,* **7097,** (2008)

Paepe, Roland, Hoover, Richard B. & Van Overloop, E., "Patterned Ground as Evidence of Water on Mars," in *Permafrost Response on Economic Development, Environmental Security* *and Natural Resource Potential,* NATO-ARW held in NOVOSIBIRSK, SIBERIA, 12-16 Nov. 1998. (Roland Paepe, Ed.) Klewer Publishing, New York, 581-588 (2001).

Pikuta, E. V. , Hoover, R. B., Marsic, D., Whitman, W. B., Tang, J. and Krader, P. "*Proteocatella sphenisci* gen. nov., sp. nov., a novel psychrotolerant, spore-forming anaerobe isolated from Magellanic penguin guano in Patagonia, Chile", *Int. J. Syst. Evol. Microbiol.* – (In Press).

Pikuta, E. V., Marsic, D., Itoh, T., Bej, A. K., Tang, J., Whitman, W. B., Ng, J. D., Garriott, O. K., and Hoover, R. B., "*Thermococcus thioreducens* sp. nov., a novel hyperthermophilic, obligately sulfur-reducing archaeon from a deep-sea hydrothermal vent." *Int J Syst Evol Microbiol* **57,** 1612-1618, (2007).

Pikuta, E. V., Itoh, T., Krader, P., Tang, J. S., Whitman, W. B. and Hoover, R. B., "*Anaerovirgula multivorans* gen. nov., sp. nov., a novel spore-forming, alkaliphilic anaerobe isolated from Owens Lake, California, USA" *Int J Syst Evol Microbiol,* **56,** 2623-2629, (2006a).

Pikuta, Elena V., Hoover, Richard B., Bej, Asim K., Marsic, Damien, Whitman, William B., Krader, Paul E. and Tang, Jane, **"***Trichococcus patagoniensis* sp. nov., a facultative anaerobe that grows at –5 °C, isolated from penguin guano in Chilean Patagonia," *Int J Syst Evol Microbiol,* **56,** 2055 – 2062, (2006b).

Pikuta, Elena V., Hoover, Richard B., Marsic, Damien Bej, Asim, Tang, Jane, & Krader, Paul, "*Carnobacterium pleistocenium* sp. nov., a novel psychrotolerant, facultative anaerobe isolated from Fox Tunnel permafrost, Alaska." *Int J Syst Evol Microbiol,* **55,** 473-478, (2005).

Pikuta, Elena V., Hoover, Richard B., Bej, Asim K., Marsic, Damien, Whitman, William B., Cleland, D. & Krader, Paul. **"***Desulfonatronum thiodismutans* sp. nov., a novel alkaliphilic, sulfate-reducing bacterium capable of lithoautotrophic growth" *Int J Syst Evol Microbiol* **53,** 1327-1332, (2003a).

Pikuta, Elena V., Hoover, Richard B., Bej, Asim K., Marsic, Damien, Detkova, Ekaterina N., Whitman, William B. & Krader, Paul. **"***Tindallia californiensis* sp. nov.,a new anaerobic, haloalkaliphilic, spore-forming acetogen isolated from Mono Lake in California.".*Extremophiles,* **7,** 327-334, (2003b).

Pikuta, E. V., Hoover, R. B. and Tang, J. (2007). "Microbial Extremophiles at the Limits of Life." *Critical Reviews in Microbiology,* **33,** 183-209.

Shivaji, A., Reddy, G. S., Aduri, R. P., Kutty, R. and Ravenschlag, K., "Bacterial diversity of a soil sample from Schirmacher Oasis, Antarctica," Cell Mol. Biol. (Noisy-le-grand) **50,** 525-536 (2004).

Schulze-Makuch, D., Grinspoon, D. H., Abbas, O., Irwin, L. N., and Bullock, M. A., (2004). "A Sulfur-Based Survival Strategy for Putative Phototrophic Life in the Venusian Atmosphere." *Astrobiology,* **4,** 11-18.

Seckbach, J, and Libby, W. F. (1970). "Vegetative Life on Venus? Or Investigations with algae which grow under pure CO2 in hot acid media at elevated pressures." Space Life Science, **2,** 121-143.

Wand, U., et al. (1997). "Evidence for physical and chemical stratification in Lake Untersee (central Dronning Maud Land, East Antarctica)." *Antarctic Sci.* 9**,** 43-45.

Wand, U., Samarkin, V. A., Nitzsche, H-M, and Hubberten, H-W, (2006) "Biogeochemistry of methane in the permanently ice-covered Lake Untersee, central Dronning Maud Land, East Antarctica." *Limnol. Oceanogr.* 51**,** 1180-1194.

Wilson, A. (2003). "Life in Perennially Ice Covered Lakes on Mars – An Antarctic Analogue. Third Mars Polar Science Conference, 8039.pdf.