# 14

# Signatures of habitats and life in Earth's high-altitude lakes: clues to Noachian aqueous environments on Mars

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## 14.1 Introduction

A series of astrobiological high-altitude expeditions to the South American Andean Mountains were initiated in 2002 to explore the highest perennial lakes on Earth, including several volcanic crater lakes at or above 6000 m in elevation. During the next five years, they will provide the first integrated long-term astrobiological characterization and monitoring of lacustrine environments and their biology at such an altitude. These extreme lakes are natural laboratories that provide the field data, currently missing above 4000 m, to complete our understanding of terrestrial lakes and biota. Research is being performed on the effects of UV in low-altitude lakes and models of UV flux over time have been developed (Cockell, 2000). The lakes showing a high content of dissolved organic material (DOM) shield organisms from UV effects (McKenzie *et al.*, 1999; Rae *et al.*, 2000). DOM acts as a natural sunscreen by influencing water transparency, and therefore is a determinant of photic zone depth (Reche *et al.*, 2000). In sparsely vegetated alpine areas, lakes tend to be clearer and offer less protection from UV to organisms living in the water. Transparent water, combined with high UV irradiance may maximize the penetration and effect of UV radiation as shown for organisms in alpine lakes (e.g., Vincent *et al.*, 1984; Vinebrook and Leavitt, 1996). Shallow-water benthic communities in these lakes are particularly sensitive to UV radiation. Periphyton, which defines communities of microorganisms in bodies of water, can live on various susbtrates. While on rocks, they include immobile species that cannot seek low UV refuges unlike sediment-dwelling periphyton (Happey-Wood, 1988; Vincent *et al.*, 1993) or alpine phytoflagellates (Rott, 1988) which both undergo vertical migration. Inhibition of algal photosynthesis by UV radiation has been documented in the laboratory (Häder, 1993) and it has been shown that phytoplankton production is reduced by formation of nucleic acid lesions (Karentz *et al.*, 1991) or production of peroxides and free oxygen radicals (Cooper *et al.*, 1989). Most of the experiments that have demonstrated *in situ* suppression of algal growth by UV radiation have either used artificially enhanced UV irradiance (Worrest *et al.*, 1978) or shallow systems ($< 1$ cm) that lack significant natural attenuation of UV radiation (Bothwell *et al.*, 1993, 1994). Our project is providing the field data that are missing from natural laboratories above 4000 m and will complement the postulation of the effects of UV on life and its adaptation modes (or lack thereof).

The exploration of high-altitude lakes could shed light on early Earth's biological evolution as well. For two billion years, Earth's atmosphere lacked an ozone layer and life was subjected to high UV radiation. These lakes represent an opportunity to observe the evolution of microorganisms in shallow waters that do not offer substantial UV protection. Survival strategies in these lakes might prove to be ancient and could provide a rare look into Earth's past. They could also provide critical information for the search for life on other planets. Their unique environmental analogy to Martian paleolakes of the end of the Noachian era 3.5 Ga ago (De Hon, 1992, Cabrol and Grin, 1995, 1999, 2001, 2002, Cabrol *et al*., 2003; Scott *et al*., 1995; Wharton *et al*., 1995; Newsom *et al*., 1996; Grin and Cabrol, 1997; Ori *et al*., 2000; Malin and Edgett, 2000) allows for a test of the habitability potential of aqueous oases on early Mars and will help to better prepare for future astrobiological missions.

## 14.2 Environmental background

The 2002 expedition investigations took place at the Licancabur lake (LC) and several lower saline lakes (lagunas). Located at 22°50'S and 67°53'W in a tectonically active region, the Licancabur volcano (6014 m) borders both Bolivia and Chile (Figure 14.1). Volcanism is ongoing in the region with two active volcanoes 60 km north and 40 km south of the investigation site.

### *14.2.1 Licancabur summit lake*

The lake (5916 m) is nested in the 200 m diameter summit crater of Licancabur. The volcano was formed during the Early-Pliocene and is classified as latent (Marinovic and Lahsen, 1984) with no recorded activity in recent history (600 to 1000 years). A planktonic fauna was mentioned by a previous archeological expedition (Rudolph, 1955). Divers measured the bottom lake temperature at +6°C (Leach, 1986). Except for these references, the lake's biotic diversity, environment, and survival strategies are undocumented.

We characterized the physical environment (Table 14.1) of LC and focused on elements of the environment that would lend insight to constraints placed on local biology. The stability of LC is of particular interest, since liquid water is uncommon in this section of the Andes. Most lakes above 5200 m on Chile's volcanoes are frozen year-round. At LC, the atmospheric pressure is 480 mb and average air temperature about $-12$°C; precipitation is less than 200 mm/yr and the atmospheric 0°C isotherm lies at 4400 m (Nunez *et al*., 2002). Still, the summit crater contains a $\sim$100 m wide, and $\sim$4 m deep lake which is ice-covered only part of the year. We measured LC

**Figure 14.1.** Simplified geograhical map showing the location of the Licancabur Volcano at the boundary of Chile and Bolivia.

surface water pH (~8.5), temperature (+4.9°C), and total dissolved solids (TDS: 1050 mg/L). The lake waters are two degrees warmer than that predicted by a model of the temperature maximum density for freshwater at this altitude (Eklund, 1983). The temperature and the location of LC atop a young, latent, volcano in an active geothermal setting led us to suspect the existence of a deep magmatic hydrothermal system. We investigated that hypothesis and present new data on the physical and chemical nature of the lake.

The chemical analysis of LC water samples was performed using inductively coupled mass spectrometry (ICP-MS) and ion chromatography (IC). ICP-MS was used to determine elemental abundances across the periodic table to the parts-per-billion (ppb) level. This analysis targeted the elements commonly found in lake fluids near andesitic volcanoes (e.g., Na, Ca, K, Mg, Fe, and Al derived from the dissolution of volcanic glass) and other solutes

**Table 14.1.** *Physical and environmental data from lakes and springs*

| | Thermales hot spring[a] | LB[b] Cold Spring | LB | LV[c] | LC[d] |
|---|---|---|---|---|---|
| GPS Coord. Lat. | 22°46.96'S | 22°48.32'S | 22°47.00'S | 22°47.32'S | 22°50.03'S |
| GPS Coord. Long. | 67°48.15'W | 67°46.34'W | 67°47.00'W | 67°49.16'W | 67°53.00'W |
| Elevation (m) | 4328 | 4340 | 4340 | 4332 | 5916 |
| Length × width (km) | 0.010 × 0.005 | – | 1 × 0.5 | 7 × 3 | 0.1 × 0.09 |
| Maximum depth (m) | 2 | – | 0.5 | 40 | 4 to 10(4) |
| UV flux (W/m$^2$) | 81 | 81 | 81 | 81 | 89 |
| Water temp. (°C) | +36.2 | +17.7 | +14 | +12.9 | +4.9 |
| Air temp. (°C, min/max) | −30/+12 | −30/+12 | −30/+12 | −30/+12 | −40/+3 |
| Atm. pressure (mb) | 600 | 600 | 600 | 600 | 480 |
| pH | 8 | 7.3 | 7.2 | 9 | 8.5 |
| TDS[e](mg/L) | 2120 | 2740 | 22400 | 11 7500 | 1050 |

[a] Spring located between LB and LV.
[b] LB: Laguna Blanca.
[c] LV: Laguna Verde.
[d] Still to be determined.
[e] Total dissolved solids.

common to geothermal fluids (Nicholson, 1993; Varekamp *et al.*, 2000). IC was used to determine anion abundances to the parts-per-million (ppm) level. The goal was to quantify Cl, SO$_4$, and F concentrations, which are elevated in fluids that have been mixed with hot magmatic fluids and gases. Nitrate and phosphate concentrations were also determined using IC to characterize some of the nutrients available to local biology. ICP-MS results show that LC waters are enriched in some major rock-forming elements ([Mg] = 39.6 ppm, [Ca] = 230 ppm, [Al] = 1.75 ppm, [Fe] = 0.902 ppm) with respect to geothermal springs at the base of Licancabur. If it is assumed that the source waters for these features have similar composition, then this enrichment may be representative of increased water–rock interaction as the fluid flowing up to the summit has had more time to react with local lithologies. According to IC, sulfate, fluoride, and chloride concentrations in LC ([SO$_4$] = 4.37 mg/L, [F] = 0.56 mg/L, and [Cl] = 0.54 mg/L) are also higher than those detected in the lower springs and are over 200 times more concentrated than those detected in local snowmelt. Since solute enrichment is not uniform across the analytes in LC, it is unlikely that this chemistry is a result of evaporative concentration alone.

The bulk properties of the lake, as well as the physical and geochemical analyses, lead us toward the conclusion that LC is still an active volcanic lake (low activity) supported by a small geothermal heating term, and may host a diffuse hydrothermal system. More chemical, isotopic analyses and direct measures of the heat flow in the vicinity of the summit lake will be performed in coming years. LC (Figure 14.2) represents an end-member of the physical environment on Earth where lakes and liquid water are stable. As such, it is of considerable interest for astrobiological studies, as fading geothermal flux and evaporating lakes could have been common environments on Mars at the Noachian/Hesperian transition.

### *14.2.2 Laguna Verde and Laguna Blanca*

Two lower hypersaline lakes, Laguna Verde (LV) and Laguna Blanca (LB), and a small warm hydrothermal pond (Thermales) connecting the two lagunas (see Figures 14.2B–D) were also explored. They are located at the foot of Licancabur at 4340 m. The physical environment of the lagunas combines: high UV radiation (40% higher than at sea level) enhanced by the tropical latitude of the site, as well as low atmospheric pressure, low oxygen (58% of $pO_2$ at sea level), high temperature amplitude, low humidity (proximity to the Atacama Desert), and low precipitation ($<200$ mm/yr), see Table 14.1. Because of its salt content (three times that of seawater) LV can stay free of ice at temperatures reaching −25°C (Cabrol *et al*., 2003). LB freezes at −5°C.

The water supply is from influx of cold and warm hydrothermal springs. Compared to LC, the geothermal fluid input through springs is clearly active at the lagunas, especially in Laguna Blanca. LB and LV are joined today by a small channel and the ‘‘Thermales’’ pond. Dataloggers were placed to monitor yearly air, soil, water temperature, UV flux, and relative humidity. A survey of thermal water input to LB covered approximately half of the lake's shoreline. Fifteen individual springs between +12 and +36°C were observed and documented. Surface manifestation of thermal fluid upwelling there is highly dependent on coastal geology along LB and LV and a clear relationship between the lakes, their biological communities, and the local geothermal environment was established (see Section 14.2.2 and Figure 14.4).

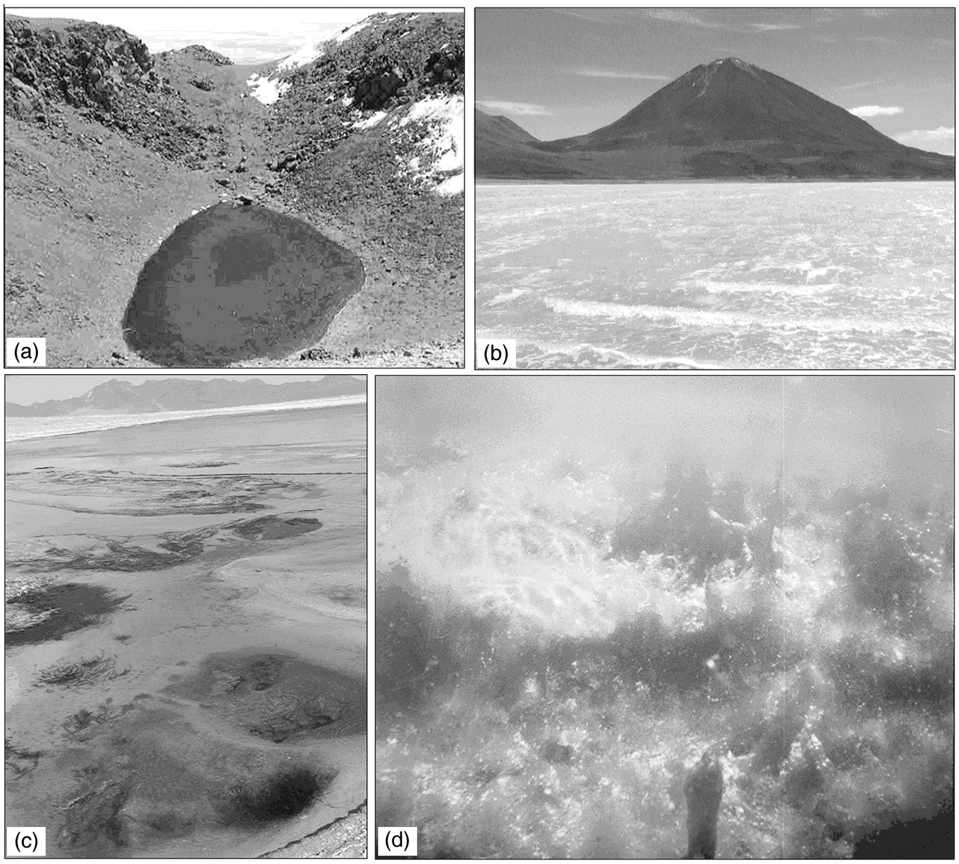

**Figure 14.2.** (a) Licancabur lake 100 m below the summit rim. Paleoshorelines are visible. The lake currently ~100 × 90 m and possibly up to 10 m deep, may have reached 65 m and ~200 m in diameter at its peak. (b) Laguna Verde with Licancabur in the background. (c) Hydrothermal springs in Laguna Blanca and algal mat. (d) Oxygen producing algae in the "Thermales" hot spring. Algae abound in the +36°C water. Credit photographs: Brian H. Grigsby and Nathalie A. Cabrol. (For a color version of this figure, please refer to color plate section).

Most of the brackish lagunas in the Bolivian Altiplano were formed as freshwater lakes and experienced their peak volumes during the early Holocene between 18 000 and 9200 BP, when precipitation reached 400–500 mm/yr (Tauca phase, Messerli *et al*., 1993; Wirrmann and Mourguiart, 1995; Grosjean *et al*., 1995; Baucom and Rigsby, 1999; Sylvestre *et al*., 1999; Vuille *et al*., 2003). Our topographic survey using Global Positioning System (GPS) and Laser Range Finder shows that the two lagunas were originally one large (~20 × 3 km), deep (~55 m) lake which has receded significantly since its formation. The topography, increasing aridity, and local hydrothermal systems at the lagunas have resulted in their evolution from a single large basin (Cabrol *et al.*, 2003) to extremely different lake environments today (pH, temperature, depth, salinity) generating an exceptional diversity of habitats, sometimes only a few meters apart. LB and LV's most ancient terrace belt is stratigraphically correlated with the highest levels of the large paleolake of the wet Holocene episode as shown by $^{14}$C analyses (see Section 14.2.2). The main variable since their formation and the present is the amount of precipitation received per year in this part of the Altiplano.

The environmental conditions shown by these three different lakes might be the closest possible terrestrial analogs to the late Noachian on Mars. Their geosignatures and biosignatures provide detection criteria for the search of sites of astrobiological interest on Mars. Habitats, life diversity, and life survival strategies in environments that provide little protection against UV effects give important information that can test Mars' past habitability potential. Moreover, these lakes provide clues about life's ancient survival strategies on early Earth before the ozone layer was formed. It also provides a test of the limits of life on our own planet.

## 14.3 Present habitats and life

Water and sediment samples were collected at all lakes to document the diversity of life and habitats and understand the impact of environmental stresses. These analyses also contribute to the understanding of the hydrological and algological state of the Andean high lakes (Hustedt, 1927; Rumrich *et al.*, 2000). Several Bolivian lower lakes (mostly in the Unuyi region) were investigated as tracers of modern climate changes (Sylvestre *et al.*, 1999; Servant Vilardy *et al.*, 2000).

### *14.3.1 Diversity*

Our geophysical and chemical analyses show that the three lakes differ considerably from each other. This is reflected in the significant differences of diatom species numbers in the samples: LB has 113 taxa, LV 50 taxa, and LC 22 taxa (Acs *et al.*, 2003). More analyses are ongoing; however, it comes as an unambiguous result that in spite of the high UV radiation and high altitude, even LC shows a species-rich algal flora. Diatom assemblages seem to adjust to enhanced UV radiation up to a certain point. The species identified in our samples show only slight differences in species composition and species numbers compared to the control group

(Reizopoulou *et al.*, 2000). Plankton samples were collected at both lagunas using 10 and 50 μm plankton nets and water samples (all lakes). Periphyton samples were collected from several macroscopically different littoral biotops at LB, small cold springs, Thermales and the central part of the lake, and along the shoreline of LC. The abundance of phytoplankton and the relative abundance of periphytic diatoms were determined by the Utermöhl method (Paxinos and Michell, 2000).

### *14.3.2 Life in the summit lake*

LC is a small alpine lake. Its water is crystal-clear and transparent to the bottom. Its encased location prevents winds that could stir it up. Total dissolved solid (TDS) results lead to its classification as a mesohalobic (moderately saline with intense growth of salt-loving organisms) lake (see Table 14.1). The dominant salt is $CaSO_4$. The freezing point is ~0°C. The geothermal heating term supported by our data might contribute to maintaining the lake's temperature. From an algological standpoint, LC's concentration of Si is adequate; however N and P ($NO_3$, $NO_2$, $PO_4$) concentrations are low, leading to the classification of the lake as ultra-oligotrophic (highly deficient in nutrients with abundant dissolved oxygen). In the phytoplankton, only one *Synechococcus* (Cyanobacteria) colony, three *Chrysophyceae*, one *Euglenophyta*, and two *Chlorophyceae* species were found. The individual number of phytoplankton is low (15 ind/ml). We found a dozen specimens of 1 to 2 *Ciliata* species, and a few cells of a *Hyphomyceta* species. Both observations support the presence of a food web (primary producers, consumers, decomposers) at the summit lake (Figure 14.3).

### *14.3.3 High diatom abnormality rate in Laguna Blanca*

LB is shallow (deepest point $< 1$m) and thus belongs to the littoral zone. It is irradiated to the bottom (euphotic zone) in spite of winds that often stir up the sediment. Its dissolved salt content is high (22 400 mg/l). LB freezes at about −5°C but not to the bottom in spite of the altitude or winter because of the +36°C spring influx of Thermales and several cooler springs. Limnologically, its high NaCl content defines it as brackish. The $SO_4$ and $HCO_3$ content are high; however the concentration of Ca and Mg is not abnormal. N and P are abundant, making the water potentially polytrophic or hypertrophic (large supply of nutrients and low visibility depths). High winds increase the turbidity and the suspended matter content

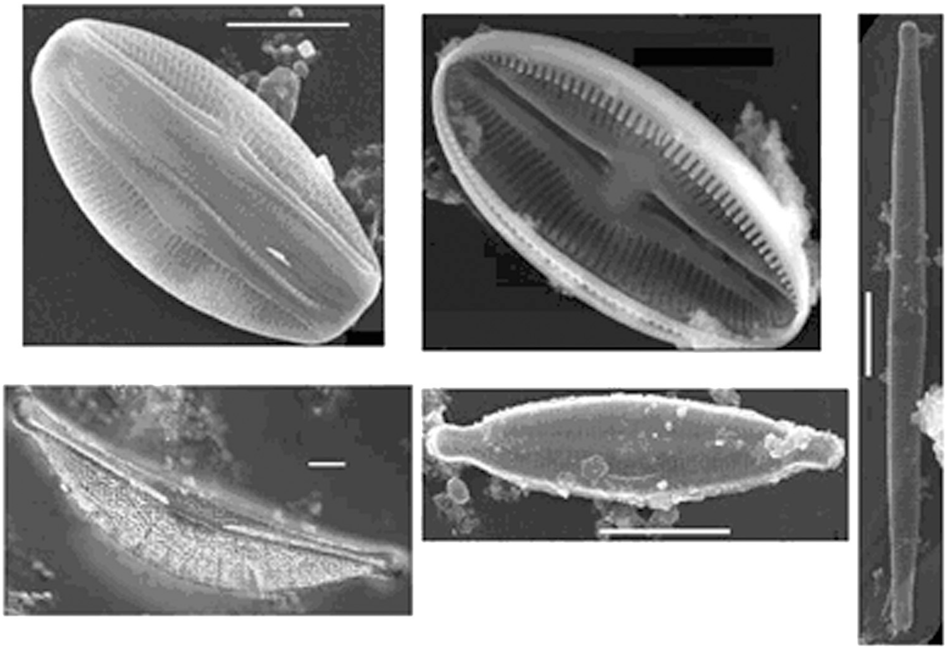

**Figure 14.3.** Micrographs of diatoms in LC. Upper row, from left to right: *Amphora veneta* (SEM) and *Diploneis chilensis* (SEM). Right vertical: *Fragilaria pulchella* (SEM). Lower row: *Amphora platensis* (LM) and *Craticula buderi* (SEM). Scale bars: 10 µm.

reaches 207 mg/l, while Secchi transparency decreases to 20–40 cm. Most of the suspended matter consists of diatom frustules. The high Si concentration of the water favors the development of the huge diatom mass observed. The phytoplankton in LB is poor in euplanktonic elements, both in species and individual numbers. Diatoms living on the sediment float in the water as tychoplanktonic (adapted to living in still water) elements as a result of wind activity.

The number of living cells is extremely high: 82 000 ind/ml. As the species vary considerably in size, the conversion into true biomass is difficult. Moreover, the size of the chloroplasts and central vacuoles is also variable even among individuals of the same species. The primary production of the lake is considerable, allowing its classification as at least mesoeutrophic (moderate levels of organisms, nutrients, and visibility). Because of the stirring effect of the wind, 98% of the floating cells are frustules of dead diatoms. On the basis of the dominant diatoms in the 11 periphyton samples collected, an obvious micro-heterogenity characterizes the different habitats (Figure 14.4) that are at times only few meters apart and have the same

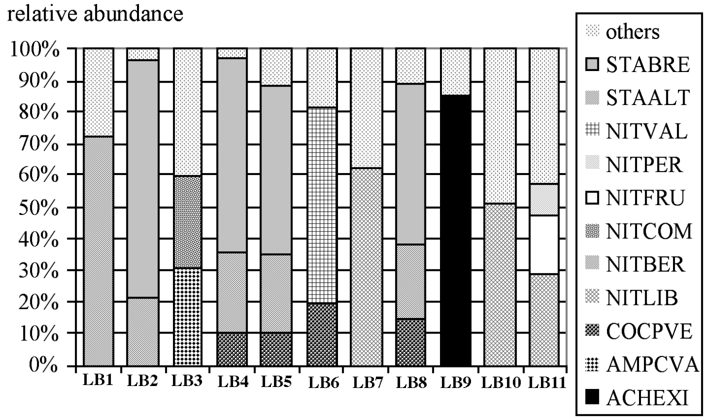

**Figure 14.4.** Distribution of dominant benthic diatoms in Laguna Blanca (species ≥10% relative abundance). ACHEXI: *Achnanthes exigua*; AMPCVA: *Amphora coffaeaformis* var. *acutiuscula*; COCPVE: *Cocconeis placentula* var. *euglypta*; NITLIB: *Nitzchia libetruthii*; NITBER: *Nitzschia bergii*; NITCOM: *Nitzschia communis*; NITFRU: *Nitzschia frustulum*; NITPER: *Nitzschia perminuta*; NITVAL: *Nitzschia valdecostata*; STAALT: *Staurosira altiplanensis*; STABRE: *Staurosira brevistriata*. Sampling sites: LB 1–4: east shore; LB 5–7: north shore; LB 8, 9: Thermales; LB 10, 11: north shore.

5–20 cm water column topping them. These differences could be attributed to, for instance, the distance from warm or cold springs.

The majority of samples were collected at LB, which may explain why most teratological (abnormal) diatoms were found in this lake (Figures 14.5). At LB, the proportion of teratological diatom frustules was found to be 1–2% (normal: 0.1–0.2%). Because of LB's characteristics, the sampling depth was also shallower than average when compared to that of LC and LV. The probability of developing teratological forms amongst diatoms is enhanced by increased UV irradiation, as shown by *Cocconeis placentula* found in LB, which is a UV-sensitive species and for which a wide range of deformities were observed (Figures 14.5A–D). Finally, the flora and fauna are extremely varied in the different biotops. Close to the shoreline, diverse cyanobacterium colonies were sampled. Several macrozoobenthic organisms (*Gammaridae*, *Mollusca* species) also occur and masses of copepods live in the plankton.

### *14.3.4 Hypersaline Laguna Verde*

Though connected to LB, LV has an extremely different environment. It reaches 40 m at its deepest and is surprisingly rough in windy weather,

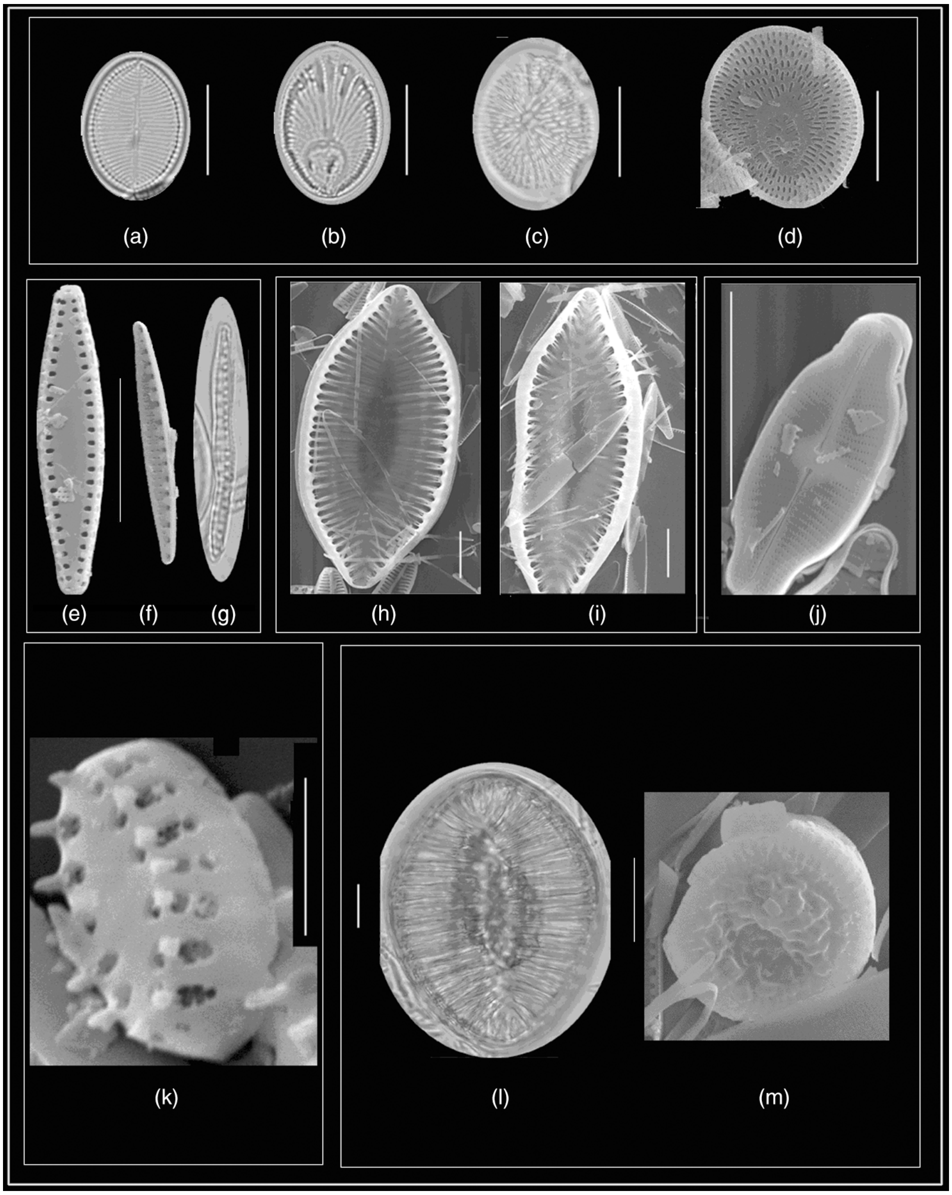

**Figure 14.5.** (a) *Cocconeis placentula*, normal valve, LM (LB and LV); (b, c) Teratological valves, LM; (d) teratological valve (SEM); (e) *Staurosira brevistriata* (LB); (f, g) Normal and teratological *Nitzchia libertruti* (LB); (h, i) Inside views of normal and teratological forms of *Surirella chilensis* (LB); (j) *Achnanthes exigua*, SEM (LB); (k) *Staurosira altiplanensis*, SEM (LB); (l) *Surirella Sella* (LM) living cell with chloroplast found in the central region (LV and LB); (m) *Cyclotella baltica*. First record of the species outside the Baltic Sea and in a lake. All scale bars 10 μm except *Staurosira altiplanensis* (2.5 μm) and *Cyclotella baltica* (5 μm).

with significant waves leading to a decrease in transparency and the color of the water to become turquoise-opaque. In the suspended matter many complete and broken diatom frustules and various size mineral granules were observed. Due to an anomalously high suspended salt content (113 g/l) the freezing point of its water is −25°C. The water TDS value (117 500 mg/l) is three times higher than that of seawater. Moreover, the concentration of Cl, $SO_4$, $HCO_3$, Mg, and Ca ions is considerable. The lake can be classified as continental salt-water. Only one euplanktonic species (*Gymnodinium* sp.) was found in the samples collected with plankton nets. All other species are tychoplanktonic diatoms. The individual number of algae is low (91 ind/ml), classifying the lake as oligotrophic.

Both lagunas yielded unexpected observations and discoveries. We found high numbers of live *Surirella sella* in both LB and LV (Figure 14.5). This species was described mostly from fossil samples, and is rarely mentioned alive (e.g., shallow lake Lipez in Bolivia with high concentration of $NaSO_4$; Hustedt, 1927; Servant Vilardy *et al.*, 2000) and in LV (Servant Vilardy *et al.*, 2000). The species was characterized as an endemic, athalassic (saline) and of a saltwater form. It is abundant in both lagunas.

Only two centric diatom species were found in the lagunas. This is unusual or rare compared with other lakes that usually host 5–10 centric species. One of these species, *Cyclotella baltica*, has only previously been described in the Baltic Sea (Håkansson, 2002), see Figure 14.5. This is the first record of this species both in the southern hemisphere and from a lake.

### *14.3.5 The effect of UV on biomass*

Preliminary examination of surface samples reveals a general decrease in total lipid (waxy oxygenated fats or fatty acides) extracts (TLE) generated per gram dry weight of sediment and per milliliter of water from LC as compared to LB and LV (Table 14.2). UV intensity is ~10% greater at LC but temperature, pH, and alkalinity also vary between these lakes. Further study is thus needed to rigorously establish the limiting effect of each parameter. However, support for the UV hypothesis limiting the biological productivity at the summit lake comes from the analysis of TLE derived from sediments from different depths (with pH, and alkalinity held constant). Sediment taken from under a 50 cm water column had more than three times as much TLE per gram sediment as the sediment taken from under a 5 cm water column (0.800/2.350). The depth difference corresponds to approximately 33% less UV at the sediment–water interface for the deeper sample.

**Table 14.2.** *Characterization of lipids extracted from 2002 expedition samples*

| | Lipids (mg lipid/g) | | | | |
|---|---|---|---|---|---|
| Sample location | TLE[(a)]1 | TLE2 | ATLE[(b)] | Nlip[(c)] | Plip[(d)] |
| LC – water | 0.015 | | 0.015 | 0.007 | 0.008 |
| LC – sediment (shallow) | 0.800 | 0.200 | 0.500 | 0.350 | 0.450 |
| LC – sediment (deep) | 2.350 | 1.300 | 1.825 | 1.850 | 0.500 |
| LB – water | 0.013 | | 0.013 | 0.008 | 0.005 |
| LB – sediment | 1.500 | 10.250 | 5.875 | 1.100 | 0.400 |
| LV – sediment | 0.750 | 0.400 | 0.575 | 0.350 | 0.400 |
| LV – mat | 6.500 | 57.500 | 32.000 | 4.000 | 2.500 |
| Thermales – sediment | 2.050 | 0.100 | 1.075 | 0.350 | 1.700 |
| Thermales – mat | 14.000 | 33.500 | 23.750 | 3.500 | 10.500 |

[a] Total lipid extract;
[b] average total lipid extract;
[c] neutral lipid;
[d] polar lipid.

### *14.3.6 Preliminary assessment of genetic diversity*

Denaturing gradient gel electrophoresis (DGGE) was selected as a fingerprinting technique and sequence analysis of the resulting 16S rDNA bands to carry out a first-order assessment of the genetic diversity of the samples. Although some polymerase chain reactions (PCRs) and primer biases could emerge during the amplification process (Von Wintzingerode *et al.*, 1997) DGGE offers a rapid means of detecting predominant PCR-targeted populations.

The main difference observed through contrast phases and fluorescent microscopy among samples from LC, LB, LV, and Thermales results from their specific populations of cyanobacteria and diatoms species. PCR products were obtained directly from the original DNA without nested amplification, except for the Thermales, LB-2 and LC-1 samples. Only two samples (LC-1 and Thermales) yield nested PCR amplification product using the universal primers combination for archaeal fragments. A variable number of bacterial fragments bands were obtained in the different systems, the total ranging between 1 and 10. The relative intensities of the bands in each lane were used to calculate Shannon's bacterial diversity indices for each sample. Values range from 0 to 1.99. The average Shannon index for bacterial community in three saline lakes studied in the nearby Salars de Llamará, de Atacama, and de Ascotán is 2.21 and 16 bands of bacterial fragments in DGGE gels (Aiken *et al.*, 2002). The 21 main bands (intensity and frequency) were excised, re-amplified, and sequenced. No satisfactory sequence could be recovered from 12 of these bands. The remaining 9 bands produced sequences that could be used for BLAST (Basic Local Alignment Search Tool) analysis. The only amplified archaeal fragments analyzed by DGGE (LC-1) show the occurrence of 4 bands and a Shannon index of 1.32.

Between 42 and 82% of the total band intensity in each lane could not be assigned to known organisms at this time because either the bands could not be re-amplified (8) or the sequences retrieved were of bad quality (1). Some sequences (4) show between 76 and 98% similarity to environmental clones but not to cultured microorganisms, and therefore their physiology could not be assessed. Only four of the sequences have percent similarity values higher than 95% in the 16S rDNA fragments. This allows the assumption that their ecophysiology is similar to cultured organisms. The major contributor in the total band intensity of all samples is unidentified sequences. The second contributor is the group of sequences belonging to the phylum Proteobacteria. In six of the samples $\gamma$-proteobacteria were the most important bands (LB, LB-1, LB-2, Thermales, LV-3, and LC-2), then sequences belonging to $\beta$-proteobacteria in three samples (LB-1, LC-1, and LC-2). The smallest contribution to the phylum is $\alpha$-proteobacteria found in two samples (LB-1 and Thermales). In LV-2 the assemblage was dominated by the Cytophaga–Flavobacterium–Bacteroides (CFB) phylum. Sequences whose closest relative was a member of the genus *Pseudomonas* were recovered in two of the bands (*P. jessenii* and *P.* sp.). They are the major contributors in the total band intensity of the lane for LB, LB-2, LV-3, and contribute in almost the same way with $\gamma$-Proteobacteria in LC-2. One sequence reveals the occurrence of microorganisms related to sulfur oxidizers at the summit lake while another shows low similarity value (80%) with *Acidithiobacillus thiooxidans* (another sulfur oxidizer). Analysis is ongoing to confirm yet unidentified and suspected new species in our first-year samples.

## 14.4 Fossil life

A ~100 km$^2$ field of fossil stromatolites occupies most of the ancient terraces of the former paleolake that originally included LB and LV. Their distribution is stratigraphically discontinuous: the structures are widespread on the oldest terraces, disappear from some intermediate shoreline levels, and reappear on younger ones. These gaps are often associated with sharper stratigraphic transitions (e.g., larger terraced shorelines and thicker deposits), changes in grain size and the nature of material on the deposits.

$^{14}$C analysis was performed on samples of the main terrace and indicates an age of 15 330 ± 210 years. Smaller stromatolites located stratigraphically closer to the current shore are dated 11 210 ± 120 years. These results are consistent with the large paleolake being formed during the Holocene wet climate episode (Messerli *et al.*, 1993; Wirrmann and Mourguiart, 1995; Grosjean *et al.*, 1995; Baucom and Rigsby, 1999; Sylvestre *et al.*, 1999; Vuille *et al.*, 2003). Below this stratigraphic level, and closer to the current active shoreline, the density of fossil structures is scarce. However, the survey of Laguna Verde showed the existence of modern cyanobacterial mat on the present-day shore. Several small (10 cm) round cyanobacterial colonies were observed building domes resembling stromatolites and covering them with cyanobacterial mat.

The short spatial and temporal scale variability in distribution of the stromatolites is associated with an exceptional diversity of forms and sizes ranging from 10 cm to 20 m (Figure 14.6). Both stromatolites (laminated) and thrombolites (clotted structures; Kennard and James, 1986) are observed.

Microscopic examination shows that cyanobacterial filaments formed most of the structure within the laminations. Their morphology shares many characteristics of the Precambrian stromatolites. In addition to cyanobacteria, other layers are mixed and have diatoms and ostrocod shell fragments. Mineralogical variations within the lamination of individual structures are indicative of changes in the environment within short geological periods of time. Future detailed sampling and analysis of the stromatolites along stratigraphic transects through the paleoterraces and at the lamination scale will help understand the magnitude of the changes and their implications for the microorganic communities. In upcoming campaigns the discontinuity of this field at such short temporal scale added to sharp stratigraphic relationships will also allow for the testing of the sensitivity to rapid environmental changes of one of the most ancient forms of life. In less than 15 000 years, precipitation rates decreased from 500 to $\leq$200 mm/yr (Messerli *et al.*, 1993). The extent of the original lake gives a water volume close to 5 km$^3$. Both lagunas now combine only $\sim$0.3 km$^3$. Most of the volume was lost in the past 10 000 years as deduced by our paleobathymetry and $^{14}$C analysis. In spite of these short geological timescale variations and restricted physical environments, cyanobacteria, one of the most ancient terrestrial forms of life, colonized these shores and survived up to the present. Understanding how they survive could provide precious clues for the search for biota on Mars.

Moreover, the textures observed in the samples share many of the features of Precambrian stromatolites, including Mesoproterozoic specimens from

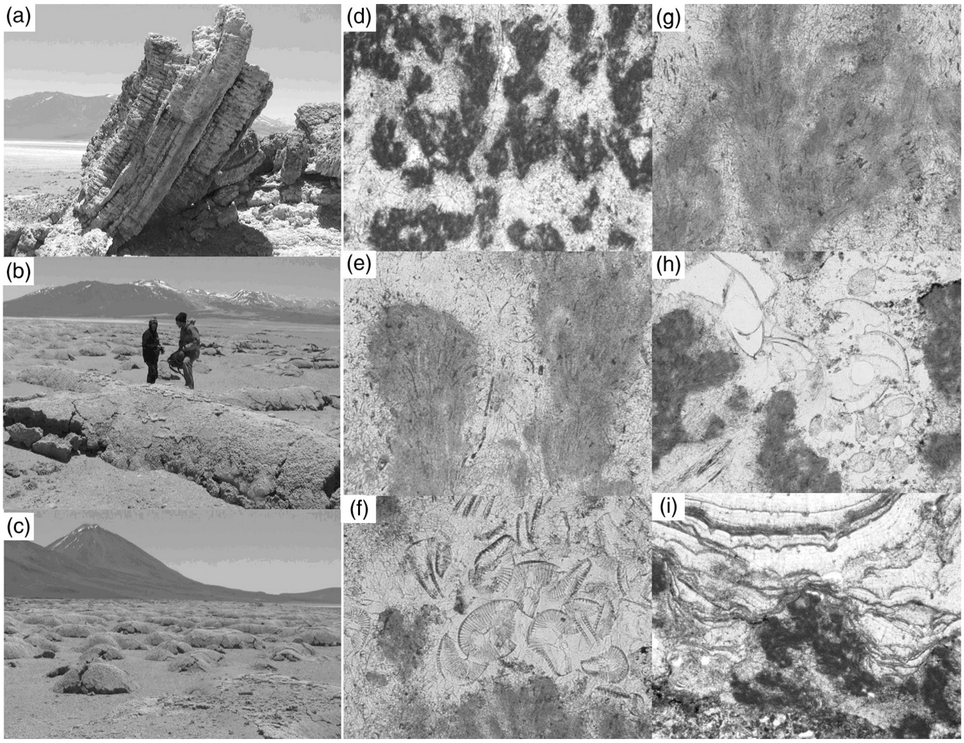

**Figure 14.6.** (a) 5 m collapsed stromatolite (LV); (b) Cross-shaped structure; (c) 50 cm-high domes (Licancabur in the background); D–I: Photomicrographs of a stromatolite fragment. (d) Dendrites (dark gray) consist of micrite with cyanobacterial filaments throughout. Calcite cements fills the area and contains abundant cyanobacterial filaments. Occasionally, dendrites penetrate into the calcite cement layers, and sometimes connect successive dendrite layers. (Scale photo, S: 2.5 mm); (e) Close up of dendritic micrite structures revealing cyanobacterial filaments (black) throughout the structure. These filaments are also found throughout the calcite cement (S: 625 µm): (f) Diatom hash at the termination of additional micritic structures (S: 625 µm); (g) (same as E, S: 625 µm); (h) Ostrocod and diatom hash at the termination of micritic structures (S: 1.25 mm); (i) Fine-scale laminations observed at the edges of the sample (S: 2.5 mm).

the Billyakh Group, Siberia (Bartley *et al.*, 2000) which possess dendritic textures similar to those observed in the lagunas as well as micrometer-scale lamination similar to that found on the edge of the examined dendrites. While the Mesoproterozoic stromatolites formed in a peritidal marine environment and the lagunas stromatolites formed in a shallow lacustrine and arid environment, the morphological resemblance between them suggests that the physical parameters that played a role in forming the specimens at the lagunas may be important to understand the depositional environment of the Mesoproterozoic samples.

## 14.5 Conclusion

A detailed picture of a macro- to microscale diversity in habitats and life is emerging through the study of these extreme lakes, reinforcing the notion of life's ability to colonize every possible niche while under severe environmental stress as revealed by high levels of species deformities. Yearly follow-up expeditions will revisit LC, LV, and LB and other 6000+ m lakes, retrieving environmental and biological data from *in situ* experiments and long-range monitoring. The investigation will focus on the role of UV on productivity, environmental stress, and species malformation shown by our first analyses. UV stations and ELDONET dosimeters will establish clear UV profiles and characterize the impact of UV on biology. Our goal is to better understand if these results point to adaptation-driven mutations (evolution) or to pathways to extinction (limits of life). Ultimately, they will provide new insights into the evolution of early Earth and Mars environments.

The water chemistry at the lagunas and the potential geothermal flux at the summit lake show that water can flow and pond under temperatures that range between −40 and +10°C and reduced atmospheric pressures between 480 and 550 mb). These temperature and pressure ranges are similar to those modeled for the Noachian/Hesperian transition. These lakes harbor abundant microorganic life coping to some extant with high UV radiation. Diatoms-like organisms are unlikely to have ever evolved on Mars as they appeared late in Earth's biological history. However, by their rate of mutation, adaptation, and or extinction, they, and other microorganisms, can provide important clues for the search for life on Mars. Both habitat and life are can leave characteristic geo- and biosignatures that we might learn to recognize on distant Martian shores.

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