Table 1 Alchichica Crater Lake, areas and volumes, cumulative for 10-m intervals

Depth (m)	Area (km^2)	Volume (m^3)
0	2.257	1.05×10^8
−10	1.969	8.43×10^7
−20	1.839	6.52×10^7
−30	1.721	4.74×10^7
−40	1.600	3.08×10^7
−50	1.443	1.55×10^7
−60	1.099	2.48×10^6

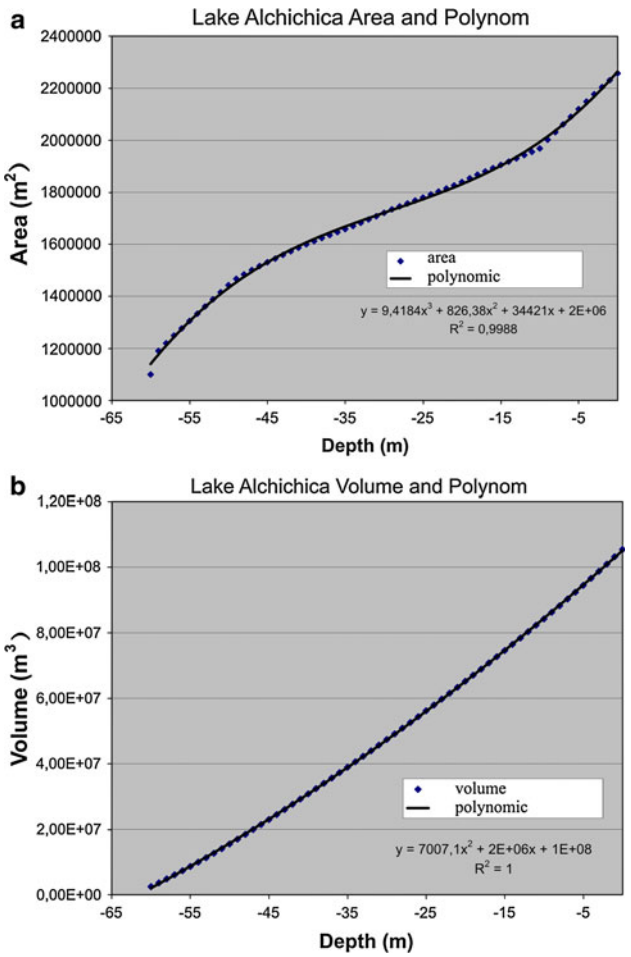

Fig. 3 Lake Alchichica: Graphs showing area-to-depth function (**a**), and volume-to-depth function (**b**)

Sampling for molecular microbiology studies, nucleic acid extraction, gene amplification, and construction of environmental gene libraries

Living microbialite fragments were collected by SCUBA diving along a depth gradient at a single station in the lake from immediately below the surface down to 14-m depth. Samples for microbiology studies were taken with gloves and sterile forceps to minimize the risk of contamination. Subsamples were introduced in Falcon tubes and were fixed in situ in ethanol. They were kept at room temperature during transport and then stored at 4°C until processing. DNA was purified from approximately 300 µl of ground stromatolites using the MoBio Power Soil™ DNA extraction kit (Carlsbad, CA, USA). This was done following the manufacturer's instructions. Duplicate DNA extractions from the same samples were done with the use of a modified classical phenol–chloroform extraction. For the latter, samples were subjected to six freezing/thawing cycles in liquid nitrogen to facilitate cell lysis. Subsequently, 80 µg ml^{-1} proteinase K, 1% SDS, 1.4 M NaCl, 0.2% β-mercaptoethanol and 2% hexadecyltrimethylammonium bromide (CTAB) (final concentrations) were added sequentially. Lysis suspensions were incubated overnight at 55°C. Nucleic acids were extracted once with hot phenol (65°C), once with phenol–chloroform–isoamylalcohol, and once with chloroform–isoamylalcohol, and were subsequently concentrated by ethanol precipitation and resuspended in 10 mM Tris–HCl, pH 8.5. Small subunit rRNA genes were amplified from three selected samples (3, 6, and 8-m depths) by polymerase chain reaction (PCR) using a combination of the bacterial-specific primers B-27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT), and the cyanobacterial specific primers CYA106F (CGGACG GGTGAGTAACGCGTGA) and CYA1380R (TAACG ACTTCGGGCGTGACC). PCR reactions were performed under the following conditions: 35 cycles (denaturation at 94°C for 15 s, annealing at 50–55°C for 30 s, extension at 72°C for 2 min) preceded by 2-min denaturation at 94°C, and followed by 7-min extension at 72°C. SSU rDNA clone libraries were constructed using the Topo TA Cloning System (Invitrogen) following the instructions provided by the manufacturers. After plating, positive transformants were screened by PCR amplification of inserts using flanking vector primers and PCR products partially sequenced using 1492R or CYA1380R (Cogenics, France). Sequences were deposited in GenBank with accession numbers HQ419013 to HQ419058.

Molecular phylogeny analyses

Only high-quality partial sequences (minimum length 700–800 bp) were retained for subsequent phylogenetic analyses. Partial sequences were compared to those in databases by BLAST (Altschul et al. 1997). A multiple alignment of cyanobacterial sequences was carried out using ClustalX (Thompson et al. 1994) and manually edited using the program ED from the MUST package (Philippe 1993). It contained 170 cyanobacteria representative