can also inhibit carbonate precipitation [27]. Hence, microbialite formation most likely results from the interplay between microorganisms forming complex communities and their metabolic activities under the influence of environmental conditions (e.g. photoperiod, temperature) and local chemistry (ion availability).

The characterization of microbial diversity is thus crucial to further understand microbe-mineral interactions in microbialites. Most diversity studies using molecular methods have focused on marine stromatolites, where Alpha- and Gammaproteobacteria, Cyanobacteria and Planctomycetales appear to dominate [28,29,30,31,32,33,34,35]. In contrast, knowledge about lacustrine microbialites remains much sparser. Firmicutes, Gamma- and Alphaproteobacteria were the most abundant taxa in Lake Van microbialites, but these studies were carried out on 15 year-old dry samples and, hence, probably biased [13]. Recent metagenomic analysis of Cuatro Ciénagas microbialites revealed a complex community where Cyanobacteria, Alpha- and Gammaproteobacteria and Planctomycetales predominated, as in marine microbialites, identifying functions potentially linked to complex redox-dependent activities and the establishment of structured biofilms [21]. Despite these pioneering studies, the precise role in mineralization and biofilm dynamics of many bacterial taxa, but also of the much less studied eukaryotic and archaeal communities, remains to be elucidated.

Understanding the role of microorganisms in stromatolite formation and the environmental conditions promoting it requires extending microbial diversity studies to other systems, including non-hypersaline or freshwater microbialites. Indeed, lacustrine microbialites may be better analogs for several Archaean stromatolites. The fossil 3.5 Ga-old Australian stromatolites likely formed in a caldera lake [36] and the exceptionally preserved 2,7 Ga-old massive stromatolites from Tumbiana also grew under lacustrine conditions [37,38,39]. The alkaline (pH~8.9) Alchichica crater lake in the Central Mexico Plateau is particularly interesting from this perspective. Located at 2300 m above sea level and with a maximum depth of 63 m, it harbors prominent living microbialites down to at least 14 m deep [40]. Conspicuous dry microbialites emerge on the shores due to the 3–5 m lowering of the water level in the past three decades [41]. Alchichica is a monomictic lake, i.e. stratified during most of the year, the oxygenated surface water mixing with deep anoxic water only during the winter season [42]. Hydrochemistry studies show that water is Mg-rich (Mg/Ca = 40), oversaturated with magnesium and calcium carbonates [40,43]. Accordingly, Alchichica microbialites are predominantly composed of hydromagnesite [$Mg_5(CO_3)_4(OH)_2.4(H_2O)$] [40].

Classical morphological observations and preliminary molecular analyses focused on cyanobacteria suggested that Oscillatoriales and Pleurocapsales dominate these microbialites [40]. Here, we applied cultivation-independent molecular approaches to (i) characterize the diversity of microorganisms of the three domains of life, Bacteria, Archaea and Eucarya, in Alchichica microbialites along a 0–14 m depth gradient, (ii) compare the microbial community structure in lake microbialites with that of Alchichica microbialites maintained for two years under controlled laboratory conditions and (iii) identify microbial taxa potentially involved in carbonate precipitation and microbialite formation.

Results

Microbial community fingerprinting analyses of field and aquarium Alchichica microbialites

Field microbialites exhibited different colors depending on the sampling depth (Table 1). Sub-fossil microbialites at the rim of the lake, out of the water, were predominantly white. Submerged, living microbialites close to the surface were dark brown to black, those at 6–8 m depth intensely emerald-green and those at the highest depth sampled (14 m) golden-brown (Figure 1). This suggests that the dominant associated communities and/or their photosynthetic and protective pigments vary according to light intensity. These differences in color were also visible in the samples set on the aquaria soon after collection (Figure S1), though they disappeared with time and, after one year, all microbialite fragments in aquaria showed a similar green color (Figure 2A).

Table 1. Alchichica samples analyzed in this study.

Sample	Origin	Description
AL29	0,08 m	microbialite fragment, black/dark brown
AL31*	0,5 m	microbialite fragment, black/dark brown
AL27	0,8 m	microbialite fragment, black/dark brown
AL43	1 m	microbialite fragment, dark brown
AL36	1,5 m	microbialite fragment, dark brown
AL38	2 m	microbialite fragment, dark brown
AL70	3 m	microbialite fragment, brown
AL67*	4 m	microbialite fragment, brown/dark green
AL64	5 m	microbialite fragment, dark green
AL61	6 m	microbialite fragment, green
AL58	8 m	microbialite fragment, intense emerald green
AL55	11 m	microbialite fragment, intense green/yellowish
AL52*	14 m	microbialite fragment, golden/brownish
AQ1*	Aquarium 1	microbialite fragment
AQ1b	Aquarium 1	aquarium glass wall biofilm
AQ1w	Aquarium 1	water sample
AQ2*	Aquarium 2	microbialite fragment
AQ2b	Aquarium 2	aquarium glass wall biofilm
AQ2w	Aquarium 2	water sample

Samples used for clone library construction are noted with an asterisk. AQ1, aquarium 1; AQ2, aquarium 2.
doi:10.1371/journal.pone.0028767.t001

To rapidly evaluate the complexity of the microbial communities in these microbialites and select representative samples for in-depth analyses, we obtained bacterial denaturing gel gradient electrophoresis (DGGE) fingerprints of 13 samples from different lake depths plus samples from the two aquaria (Figure S2). Cluster analysis of DGGE profiles divided the samples in two major groups. One corresponded to shallow samples (0.5–2 m), whereas the second included deeper (3–14 m) and aquarium samples. This was consistent with the fact that the aquarium fragments analyzed (Figure S1) corresponded originally to 3 m (AQ1) and 6 m (AQ2) depth and suggested that, at least partly, the native bacterial community was maintained in culture. Fingerprints from deeper samples displayed more bands, reflecting either a higher bacterial diversity or the fact that a few phylotypes dominate surface microbialites, masking minor components. The identity of some dominant and characteristic bands was investigated subsequently.

Based on DGGE profiles, we selected the three samples AL31 (0.5 m), AL67 (4 m) and AL52 (14 m), which displayed characteristic profiles and grouped in different clusters (Figure S2). More importantly, they were well distributed along the depth gradient and represented three phenotypic types in terms of color (Figure 1).