



**Figure 3. Phylogenetic distribution of bacterial, cyanobacterial and eukaryotic SSU rRNA gene sequences in Alchichica microbialites.** In the specific panel for cyanobacteria, the phylogenetic distribution of cyanobacterial clones retrieved with universal bacterial primers (B) or with specific cyanobacterial primers (C) is shown for comparison. Sample names and origins are explained in Table 1. Non-Latin names correspond to Candidate Divisions; Deino-Thermus, *Deinococcus/Thermus* group. doi:10.1371/journal.pone.0028767.g003

exclusively identified in field samples, 23 in the aquaria and 10 in both field samples and aquaria (Figure S3). The composition of the deeper samples AL67 and AL52 was similar, with high proportions of Rhodospirillales and Rhodobacterales, whereas Rhizobiales were scarce in them but more abundant in the shallowest sample AL31. The most abundant Rhodobacterales OTU, AlphaOTU65 (34% and 24% of AL67 and AL52 sequences, respectively), was relatively close to members of the metabolically versatile genus *Rhodobacter*. Many *Rhodobacter* species are sulfur-oxidizing photosynthesizers and, in the context of the lake, AlphaOTU65 might actually correspond to anoxygenic photosynthesizers. Moreover, many Rhodospirillales (e.g. *Rhodospirillum*), represented by the abundant phylotypes AlphaOTU20 and AlphaOTU21, and Rhizobiales (e.g. *Rhodomicrobium*), are also anoxygenic photosynthesizers [48]. In contrast, the vast majority of Gammaproteobacteria phylotypes likely have heterotrophic metabolisms. However, some might be photosynthetic; for example the Chromatiales GammaOTU06 (Figure S4), related

to environmental sequences from the Mexican alkaline lake Texcoco [49], suggesting an adaptation to these particular alkaline environmental conditions.

Chloroflexi (green non-sulfur bacteria) are typically anoxygenic photosynthesizers, although an increasing number of non-photosynthetic lineages (*Anaerolineae*, *Caldilineae* and *Dehalococcoides*) has also been characterized [50]. Likely phototrophic Alchichica representatives were ChloroOTU1 and ChloroFOTU2, related to *Chloroflexus* and *Chlorothrix*, though probable heterotrophic OTUs related to *Anaerolinea* and other environmental Chloroflexi were more diverse (Figure S5). In contrast to their low proportion in gene libraries (Figure 3), DGGE analyses suggested a high abundance of Chloroflexi in Alchichica microbialites. Such difference may reflect a negative bias in the general primers used for gene library construction, as already noted in the study of Ruidera stromatolites [19,50]. In fact, seven of the most intense DGGE bands from Alchichica field samples (bands A, B, C, D, G, K and M; Figure S2 and Table 3) were