Microbial biodiversity in saline shallow lakes of the Monegros Desert, Spain

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Abstract

The Monegros desert contains one of the largest sets of inland saline lakes in Europe constituting a threatened landscape of great scientific and ecological value with large number of reported endemisms. We analyzed bacteria, archaea and microbial eukaryotes from 11 saline lakes in winter and spring by rRNA gene fingerprinting and sequencing covering large salinity (2.7-22.1%) and temperature ranges (1.5-35.3°C). The highest ecological diversity (Shannon-Weaver index) was found in protists and the lowest in Archaea. Eukaryotes showed higher ecological diversity at intermediate salinities, whereas Bacteria and Archaea did not. The genetic diversity was broad and with remarkable novelty. The highest novelty was found in Archaea at the lowest saline concentrations, whereas for bacteria and protists no differences were observed along the gradient. Euryarchaeota of the enigmatic group DHVEG-6 and phylotypes distantly related to well-known haloarchaea were present in several sites. Recurrent presence of bacterial phylotypes distantly related to *Psychroflexus* and *Cryomorphaceae* initially isolated from polar marine habitats, was observed. Saline lakes contained chlorophyta, among other new groups, substantially different from green algae previously reported in marine or freshwater. The great scientific and ecological value found for macroorganisms can be extended to the idiosyncratic microbes inhabiting such unique habitat in Europe.

Introduction

Saline lakes usually occur in endorheic drainage basins which approximately cover 1/10 of the Earth's surface area (Waiser and Robarts, 2009). Inland saline lakes represent about 5% of modern drylands (Bryant, 1996), are numerous, and are distributed worldwide in semi-arid or arid areas (Williams, 1996). They account for a similar proportion of world water (about 0.008%) than freshwater lakes (0.009%) from humid areas (Ramsar Convention Secretariat, 2010). Some of the best-known inland saline lakes occur in Australia (e.g., Bowler 1986, Tweed et al. 2011), the southwestern parts of the US (Lines, 1979; Senger et al., 1987; Stafford et al., 2008), western China (Wen and Zhi-Hui, 1999), southern America (Svensen, 2003), and Africa (Nissenbaum, 1980; Mees 1999). In Europe, inland salt lakes are rare and threatened, with probably the highest number present in Spain, where small shallow saline lakes are found in Tertiary depressions related to salty geologic materials (Comín and Alonso 1988).

The Monegros desert area (NE Spain) contains one of the largest sets of inland saline lakes in Europe constituting a unique landscape of great scientific and ecological value. The uniqueness of these saline wetlands (locally named ***saladas***) has been documented in geologic, hydrologic, mineralogic and edaphic studies (Pueyo-Mur and Inglés-Urpinell 1987, Samper-Calvete and García Vera, 1998, Mees et al 2011). Playa-lakes and other saline depressions in Monegros are very dynamic environments providing habitats for rare and threatened plants and animals, and many studies have unveiled a large number of endemisms mainly of invertebrates, vascular

plants, lichens and bryophites (Braun-Blanquet and Bolòs, 1958; Pedrocchi 1998; Casas et al., 1992; Santamaría et al., 1992; Baltanás, 2001; Conesa et al., 2011) with presence of active photosynthetic benthic microorganisms in magnesium sulfate rich areas (Guerrero and Witt, 1992). Although, the genetic identity and novelty of microbial extremophiles (i.e., bacteria, archaea, and protists) of this area remains largely unexplored.

Only very recently the genetic diversity of microbial communities inhabiting saline lakes is being unveiled (Bowman et al., 2000; Humayoun et al., 2003; Demergasso et al. 2004; Jiang et al., 2006; Wu et al., 2006; Maturrano et al. 2006) and detailed studies have been carried out in different areas of the world such as the USA (Dillon et al. 2009, Parnell et al. 2010), Mongolia (Pagaling et al. 2009), Iran (Makhdoumi-Kakhki et al. 2012), Australia (Narasingarao et al. 2012), and the Andean altiplano (e.g., Demergasso et al. 2008), among others. Microorganisms inhabiting saline environments have interesting enzymes of potential biotechnological interest (Oren, 2002). Nonetheless, the gap existing between the current knowledge of the microorganisms isolated in culture and the true microbial diversity in saline systems is still big (Oren et al. 2009). High salinity water bodies also contain an unexpected large genetic diversity and novelty of protists (Triadó-Margarit and Casamayor 2013) and are an excellent source of new culturable microorganisms, but we are still far to understand how many different microbial species exist in these environments, what are their functions and adaptations, and which environmental conditions promote the highest and the lowest diversity for each life domain. Here, we present the first study of the planktonic microbial biodiversity from the three

domains of life inhabiting several salt lakes in the Monegros Desert area. Very few reports in the literature study the three microscopic groups simultaneously. Thus, it was of additional interest to compare how the genetic diversity and novelty behave in each domain along the salinity gradient.

Materials and methods

Description of the Monegros area

The Monegros desert is located within the semiarid Central Ebro Basin, NE Spain (41º 42'N, 0º 20'W), and includes an endorheic area (~400 km^2) with 149 scattered saline depressions (Fig. 1, and see an example in Fig. 2), some of them recently protected within the Ramsar Convention. The basins are excavated on gypsum-rich bedrock, range in size from < 2 to 239 ha, and have a close connection with the two main aquifers in the area. The upper aquifer develops in lutitic limestones with interleaved gypsum layers, and the lower aquifer contains gypsum and limestone with intercalations of lutite (Samper-Calvete and García-Vera, 1998). Some of the depressions host playa-lakes. The mean annual temperature in the area is 14.9 ºC with cold winters and hot summers, and frequent and predominant NW winds. Rainfall shows a high inter-annual and seasonal variability. Both the annual low rainfall (mean = 337 mm yr^{-1}) and high evapotranspiration rates (mean = 1408 mm yr^{-1}) produce one of the highest mean annual water deficits in Europe (Herrero and Snyder, 1997). The hottest period is also the dry season, from June to September, and the wet season extends from October to May. The hydrological changes in playa-lakes are conditioned by the high-evaporation/low-rainfall regime (Fig. 3). Epehmeral brines occur even in periods of hydric deficit, due to the proximity of a permanent saline water table. The water depth of Monegros playa-lakes is shallow for most of the year, (Fig. 3) making the plankton communities in these ecosystems very sensitive to changes in the atmospheric temperature. The percentage of playa-lake area covered by water is highly variable even in the same season (Castañeda and Herrero, 2005). Frequently, the dry and persistent wind displaces the sheet of water and spreads it

creating ponds prone to salt precipitation and desiccation. Brines are of Cl-SO_4-Na-(Mg) type for all sites but Lake Chiprana, which exhibits higher SO_4 content. More detailed information on the geochemistry of the Monegros system can be found in Pueyo-Mur (1980).

Sampling and environmental data

We studied 14 salt sites located on the left side of the Ebro river at a mean elevation of 328 m a.s.l. and one additional site ~13 km away, Lake Chiprana, an hypersaline permanent lake located on the right side of the Ebro river (Fig. 1). Three sites were sampled in La Playa lake, i.e., the eastern margin (site 3) and two ponds at the southwestern margin (sites 1 and 4) due to its large size (239 ha) and heterogeneous water distribution. The salt lakes were visited in Winter 2004 (December, 21-22) and Spring 2005 (May, 24-25). Overall, 21 samples were analyzed (Table 1). Temperature and pH were measured with a portable digital pH meter (Orion 290, Thermo Electron Co., USA). Salinity was measured by a hand refractometer (Atago S-28E, Japan). Water samples were collected in plastic bottles and kept in an icebox until further processing. Samples for chlorophyll *a* analysis (1 L) were filtered through 47 mm diameter Whatman GF/F glass fiber filters. The filters were placed in aluminum foil and kept frozen. Chlorophyll *a* concentration was determined in acetone extracts by spectrophotometry (UV-2401PC, ultraviolet-visible Spectrometer, Shimadzu) as previously reported (Bartrons et al. 2012). Total microbial cells were fixed in the field with formaldehyde (4% final concentration) and 5 mL were filtered on 0.2 μm GTTP membranes. Cells concentration was determined by epifluorescence microscopy using the DNA-specific dye 4′, 6-diamidino-2-phenylindole (DAPI) in a Zeiss

epifluorescence microscope.

Nucleic acid analyses: DGGE, clone libraries and 16S rRNA sequences analyses

For DNA analyses water samples were pre-filtered in situ through a 40 mm pore-size net, to retain large zooplankton and algae, and 300–500 ml were subsequently filtered on 0.2 μm pore polycarbonate membranes (47 mm diameter). The membranes were stored in lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.75 M sucrose), enzymatically digested and phenol extracted and purified (Hervàs et al., 2009). DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol) and precipitated with ethanol. The extracted genomic DNA was used as target in the PCR to amplify the 16S and 18S rRNA genes with universal primers for bacteria, archaea and protists, respectively (see more details in Casamayor et al. 2002). The genetic analysis was based on denaturing gradient gel electrophoresis (DGGE) separation of rRNA gene segments amplified by PCR, and sequencing of excised bands as previously reported (Casamayor et al. 2001). Around 800 ng of PCR product was loaded for each sample. The gels were stained with the nucleic acid dye SybrGold for 45 min, and visualized with UV. High-resolution images were analyzed using the gel plotting macro tool of the NIH-Image software package version 1.62 (National Institute of Health, USA). After background subtracting, the intensity of each band was measured integrating the area under the peak and was expressed as percent of the total intensity in the lane, and the Shannon-Weaver index was calculated for each sample and each microbial domain. The error measured among replicates was < 4 %. Bands were excised from the gels, re-amplified and purified for sequencing as reported (Casamayor et al., 2001). Sequencing was carried out using external facilities

(http://www.macrogen.com).

DNA sequences analyses. The rDNA sequences were manually inspected for sequencing errors with BioEdit (Hall, 1999) and checked for chimera detection with UCHIME (Edgar et al. 2011). Sequences were further processed with Mothur v1.12.0 (Schloss et al., 2009). Next, the sequences were automatically aligned with SINA in SILVA (Pruesse et al., 2012), imported into the SSU Ref NR 108 database in ARB (Ludwig et al., 2004) and inserted in the optimized ARB tree keeping the overall tree topology by using the Parsimony Interactive tool. We explored the rRNA gene novelty of the dataset by BLAST identity search against GenBank sequences (search Oct 2012). The identity of each single sequence was related both to the closest environmental match (CEM), and to the closest cultured match (CCM) available in GenBank.

Nucleotide sequences accession numbers. Sequences were deposited in GenBank with the accession numbers shown in Table 2.

Results

In the present study 21 water samples were analyzed and the different physicochemical and biological parameters measured are shown in Table 1. These water bodies were shallow with water depths < 15 cm in most of the cases. The only exception was Lake Chiprana (depth > 1 m at the data that we were sampling). The

water temperature ranged between 1.5 °C in winter and 35.3 °C in spring, and the salinity concentrations ranged between 2.7 and 22.1% (w/v). Temperature strongly changed in the same sampling site between sampling dates as expected for regions with a continental climate. Chlorophyll *a* varied up to 70-fold among sites, between 0.8 µg L^{-1} (Muerte, winter) and 55.5 µg L^{-1} (Salobral, winter), and again substantial differences were observed between sampling dates at the same sampling site. Bacterial numbers changed up to two orders of magnitude (0.12 to 40 x 10^6 cells mL^{-1}) among sites. Cells numbers however did not significantly correlated (Spearman's rank correlation) with any of the environmental variables tested such as lake area, altitude, temperature, salinity, or chlorophyll *a* concentrations ($p>0.05$). We found, however, a significant positive relationship between total cells counts and the bacterial Shannon-Weaver index H' (Spearman's rho = 0.62, $p<0.05$). A strong negative and significant correlation was also observed between the eukaryal index H' and temperature (Spearman's rho = -0.69, $p<0.01$), suggesting more diverse eukaryotes in the coldest samples. Additional correlations between different parameters can be found in Table S1.

Microbial diversity along the salinity gradient

In this study we explored changes for bacteria, archaea, and protists in both ecological diversity (Shannon-Weaver index H', based on the number and intensity of the DGGE bands) and genetic diversity (novelty level based on BLAST search comparison against the ribosomal gene sequences previously reported in GenBank). We did not observe a decrease in microbial ecological diversity as salinity increased (Fig. 4). The highest diversity index was found for microbial eukaryotes (H'= 2.80),

and the lowest for Archaea (H'< 0.90). Overall, at intermediate salinity concentrations (i.e., salinities around 10%) both the highest and the lowest ecological diversity were found for protists (best fit curve R^2=0.262) and archaea (best fit curve R^2=0.205), respectively. Bacterial diversity, in turn, increased the H' value up to c. 20% salinity concentrations (best fit curve R^2=0.222). After excision and sequencing of the DGGE bands, the identity of the different microorganisms was analyzed by BLAST search (see detailed information in Table 2, and a view on the community composition and diversity indices from each site in Table S2). Each sequence was compared with its closest relative in database (both to the closest cultured match – CCM –, and to the closest environmental clone – CEM –) and those sequences with < 97% identity ("species level" identity threshold) were identified. Interestingly, the novelty distribution was consistently different in each of the three Domains (Fig. 5). In Eukarya and Bacteria, the median genetic diversity values were ≥98% for both cultured and environmental matches with quite similar sequence identity distribution. Conversely, the Archaea median identity values were 92% for the matches with cultured archaeal strains, and <96% with previously reported environmental counterparts (Fig. 5, lower boxplots). We analyzed the novelty level along the salinity gradient to unveil which salt lake conditions may contain the largest number of novel species. Interestingly, the highest novelty was found for Archaea at the lowest saline concentrations, whereas for bacteria and protists no differences were observed along the gradient (Fig. 6). In all the cases, novel sequences (i.e., <97% identity) were found all along the gradient suggesting that any of the shallow lakes examined might contain substantial genetic novelty.

Finally, we explored the taxonomic composition of the different assemblages

related to the novelty level after combining dispersion plots of both the closest environmental match (CEM) and the closest cultured match (CCM) available in GenBank (Fig. 7, and see more details in Table 2). In such plot we defined "the highest novelty section" as the area of the plot that contained microbial phylotypes matching <97% identity to both CEM and CCM. Overlapping this area, we highlighted two additional regions in the plot, i.e., "the cultured gap section" for phylotypes that showed >97% with CEM and <97% with CCM (i.e., microbes poorly represented in culture collections but previously detected in environmental surveys anywhere else), and "the environmental gap section" for those represented in cultured collections but previously not detected in environmental rRNA genes surveys (i.e., <97% with CEM and >97% with CCM). The taxa located on the right upper corner section of the plot were considered phylotypes of limited novelty.

Within the highest novelty section we found representatives of the three domains of life. Thus, for Archaea we exclusively found Euryarchaeota both within the largely unknown group DHVEG-6 (Deep Sea Hydrothermal Vent Euryarchaeotic Group 6) spread in several of the salt lakes, and phylotypes distantly related to well-known haloarchaea genera (*Halobacteriaceae*) such as *Halomicrobium*, *Natronomonas*, *Halonotius*, and *Halorhabdus*, also present in several of the sites examined (Table 2). One phylotype of Thermoplasmata (94% identity in the 16S rRNA gene) was observed in Lake Chiprana very distantly related to any cultured counterpart (<75% identity). Bacteria of the highest novelty category were fewer and less novel than Archaea, with counterparts of the genera *Psychroflexus* (*Bacteroidetes*), *Halomonas* (Gamma-*Proteobacteria*), and interestingly distant uncultured members of the family *Cryomorphaceae* (phylum *Bacteroidetes*). Finally,

for microbial Eukarya we found highly new phylotypes within the green algae (*Trebouxiophyceae* and *Chlorophyceae*), Fungi (*Chytridiomycota*-like), Ciliates (Euplotes), Stramenopiles (Pedinellales), and Alveolata (Colpodelli). The "cultured gap section" was represented by archaeal *Halobacteriaceae*, bacterial *Cryomorphaceae*, Burkholderiales and putative new species of *Psychroflexus,* and green algae (*Chlorophyceae*). In the "environmental gap section" we only found *Flabellula* (Amoebozoa) and *Lotharella* (Cercozoa) as individuals that successfully develop in the laboratory but are seldom detected in nature.

Discussion

Worldwide distributed saline lakes show a large diversity of geology, hydrology, and compositional variety (Eugster and Hardie 1978) with a well-established scientific interest for ecological studies (Larsen, 1980; Hammer, 1983; Melack, 1986). Inland saline lakes are extremely responsive to changes in climatic conditions and although hold important ecological, economic, and cultural values, they are threatened worldwide by diversion and pollution of their inflows, introductions of exotic species, and economic development with change in land uses. One of the greatest threats to these ecologically valuable habitats is therefore human activity, especially the anthropogenic inputs of freshwater (Williams 2002). In Monegros, the extreme aridity was historically the reason for a low agricultural pressure in the area, granting the conservation of inland saline lakes. The human pressure was modest until the middle 20th Century (Domínguez et al., 2013) but strongly increased in the last years by the Common Agricultural Policy of the

European Union and by the irrigation program with water diverted from the Pyrenees that may lead to the disappearance of salt resistant organisms as already happened in conterminous irrigated areas (Guerrero and Witt, 1992; Valero-Garcés et al., 2000). Several studies have shown that saline lakes are important reservoirs of largely unseen microbial biodiversity with high phylogenetic richness and novelty. The highest value of microbial novelty captured in these environments shows important gaps both in culturing and in sequencing efforts for inland saline water bodies. Thus, more detailed investigations in these environments are justified, as well as the development of active conservation strategies to preserve microbial biodiversity in areas often considered of reduced environmental interest for macroorganisms but very relevant for the microbial world (Barberán and Casamayor 2011), biotechnological applications (Oren, 2002), and exobiology studies looking for the potential strategies of life on other planets (Kunte et al. 2002).

Most saline lakes are defined by endorheic drainage basins in dry areas of the world. Given the scarcity of water in arid lands, temporary water has an ecological significance much greater than in wet regions. The temporary flooding episodes of the largest playa-lakes in Monegros are closely related to both weather conditions (Castañeda and Herrero, 2005) and groundwater fluxes (Samper and García Vera, 1998; Castañeda and García Vera, 2008), and determine the natural salinity concentration dynamics of these ephemeral lakes. Salinity ranges from <1% after autumn rains to 20% and even higher in early summer before the water sheet gets dry, being intermediate salinities the most commonly found in the area. Aquatic systems with intermediate salinities and inland salt lakes have not received much attention by

microbial ecologists (Demergasso et al., 2008 and references therein) as hypersaline coastal areas (salinities >20%) have received, despite the fact that ponds with intermediate salinities show relatively high levels of heterotrophic activities (Gasol et al., 2004), accumulation of photosynthetic picoplankton (Estrada et al., 2004), and a large unknown fraction of microbial diversity (Demergasso et al., 2008, Triadó-Margarit and Casamayor 2013, and present work). Another important characteristic of saline lakes is the significant seasonal variability in microbial diversity, richness, and the specific genera recovered. Little overlap has been reported in the bacterioplankton communities across three seasonal samples in the Salton Sea, a warm polymictic, eutrophic, endorheic saline lake in the Sonoran Desert of southeastern California, USA (Dillon et al. 2009), and a very temporally heterogeneous microbial community has been also observed in Lake Tebenquiche, Atacama Desert, northern Chile (Demergasso et al. 2008). This variability has been also seen in the hypersaline virioplankton from Lake Tyrrell, Victoria, Australia (Emerson et al. 2012).

From the general ecological principles it can be established that a more extreme environment is expected to be less species rich (Frontier, 1985). We tested this hypothesis after comparing how OTUs richness changed along the salinity gradient for the three domains of life simultaneously (Fig. 8) using the Monegros dataset and our own published data from similar studies carried out in saline lakes of the Atacama desert (data from Demergasso et al., 2004), and in Mediterranean multipond coastal solar salterns (Santa Pola, Alicante, Spain, Casamayor et al., 2002). We did not observed a consistent decrease in the number of OTUs as salinity increased for any of the domains and any of the three geographical regions explored. The comparison also

showed that DGGE band richness did not decrease above 15% salinity, and that OTUs richness was at the same level or even larger in eukarya than in bacteria and archaea. Recently, we have highlighted that the genetic diversity in planktonic protists inhabiting inland and coastal highly saline water bodies is high, far beyond the few groups traditionally considered as high salinity-adapted (Triadó-Margarit and Casamayor et al. 2013). In addition, the novelty level was high in Monegros saline lakes for phylotypes related to distantly related groups such as green algae, fungi, ciliates, stramenopiles, and alveolates. The case of *Chlorophyceae*, an extremely large, important and well-known group of green algae distinguished mainly on the basis of ultrastructural morphology, is worth to mention. Traditional taxonomy studies had shown large abundance and high richness with thousands of species reported in freshwaters (Margalef, 1983) and, for instance, most of the 18S rRNA gene sequences recently obtained in several alpine oligotrophic lakes are closely related to cultured counterparts (Triadó-Margarit and Casamayor et al. 2012). However, saline lakes apparently contain quite abundant and new chlorophyta substantially different from any other green algae previously reported either in freshwater or in the sea. This case exemplifies how far we are still for a complete characterization for the microbial species present in the environment even for well-characterized groups, and for obtaining a reasonable estimation of those environmental conditions that determine the diversity distribution for most of these microbial groups. We can gather the same conclusion for *Halobacteriaceae*, a well-known group of halophilic archaea with several cultured representatives that, on the basis of the 16S rRNA gene sequence identities, is not fully covered at the genus level yet. Interestingly, the saline lakes of Monegros are prone to the development of a new

group of halophilic archaea within the enigmatic group DHVEG-6, initially reported from deep sea hydrothermal vents and without cultured representatives. Novel major lineages of Archaea have been recently reported from hypersaline microbial communities (Ghai et al. 2011, Narasingarao et al. 2012), indicating that still large archaeal diversity remains to be investigated from hyperhaline environments. Finally, a noteworthy finding for the Bacteria domain was the recurrent presence in most of the saline lakes studied of phylotypes related to *Psychroflexus* (identity range 94-98%). Recurrent presence of *Psychroflexus*–like populations has been also noticed in a previous study carried out in the Andean altiplano (Demergasso et al. 2008). *Psychroflexus* is a genus of psychrophilic *Bacteroidetes* initially isolated from Antarctic sea ice and with specific features in the genomic content and cellular biochemistry for adaptations not only to low temperatures but also to the dynamic sea ice environment with salinities varying highly between low concentration due to thaw to high concentrations due to salt extrusion into brine channels (Bowman 2008). Another members of the psychrophilic *Bacteroidetes* found in Monegros and in the Andean Plateau (Demergasso et al. 2008) were related to *Cryomorphaceae* initially isolated again from polar marine habitats in the Antarctica (Bowman et al. 2003). Apparently, the dynamic saline regime and the continental climatic conditions of Monegros desert, and of other deserts with saline ponds such as Atacama, may explain the successful adaptation of species within the *Psychroflexus* and the *Cryomorphaceae* clades to inland shallow saline lakes in desert regions.

Overall, the present investigation shows that any saline lake in Monegros desert was susceptible to contain a substantial microbial novelty and that these environments

hold new groups of microbes that deserve to be brought into culture for detailed ecophysiological studies. The discrepancy between microorganisms retrieved from nature both by molecular and by pure culture methods is usually attributed to the difficulties for microbiologists to find suitable conditions to tame microbes that are abundant in nature. In the case of saline lakes, probably the discrepancy is also due to the limited exploration of inland environments with intermediate salinities by microbial ecologists and traditional microbiologists. Most probably, the great scientific and ecological value found in Monegros for animals and plants can be extended to the idiosyncratic microbes inhabiting such unique landscape in Europe.

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FIGURES LEGEND

Fig 1. Geographical location of the Monegros desert endorheic area within the semiarid Central Ebro Basin, NE Spain. The system includes 149 scattered saline depressions excavated on a gypsum-rich bedrock (grey spots on the map). The basins investigated in the present study are highlighted.

Fig 2. Aerial picture of one of the shallow saline lakes studied, Lake Salineta, surrounded by dry farming lands.

Fig 3. Averaged water depth, rainfall (P), and evapotranspiration (ET0) monthly values estimated from weekly data recorded in eight playa-lakes in Monegros from March 1993 to June 1997. Maximum water depth recorded along the period: 51 cm (La Playa, Dec 1994). The figure illustrates how this area is typically under a high-evaporation/low-rainfall regime producing one of the highest mean annual water deficits recorded in Europe.

Fig. 4. Best curve fit for the distribution of diversity (Shannon-Weaver index) along the salinity gradient for Eukarya (R^2=0.158), Archaea (R^2=0.205), and Bacteria (R^2= 0.234).

Fig. 5. Boxplots showing the distribution of the percentages of identity against the closest environmental match (CEM) and the closest cultured match (CCM) in databases (GenBank search, Oct 2012) for all tree microbial domains of life.

Fig. 6. Distribution of the rRNA genes sequence novelty along the salinity gradient for the three domains of life. Highly novel sequences (<97% identity) were found all along the gradient.

Fig. 7. Taxonomic assignment for the phylotypes found in each domain of life dissected by a combined dispersion plot of the closest environmental match (CEM) and the closest cultured match (CCM) available in GenBank (search Oct 2012). See accesion numbers in Table 2.

Fig. 8. Changes in the richness of bacterial, archaeal and eukaryal OTUs (DGGE bands) along the salinity in Monegros desert salt lakes (present study), Atacama desert salt lakes (Chile, data from Demergasso et al., 2004), and Mediterranean coastal solar salterns (Santa Pola, Alicante, Spain, data from Casamayor et al., 2002).

Supporting Information

Table S1. Correlograms visualization for correlation matrices (corrgram function in R) between different environmental and diversity parameters measured in this study. * $0.05 \leq p \leq 0.1$; ** $p \leq 0.05$; *** $p \leq 0.01$.

Table S2. Microbial community composition and diversity indexes for the different microbial groups from each site.

Table 1. Data from the salt lakes analyzed in the present study for winter **W** (December 2004) and spring **S** (May 2005) water column samplings. T: temperature; Cells concentration $x10^6$ (SD < 10%); nd: not determined.

Salt lake	Coordinate UTM ED50	Area (ha)	Altitude (m a.s.l.)	Season	Salinity (%)	T (°C)	Chl *a* (µg·L^{-1})	pH	Total cells mL^{-1}
Camarón	30TYL2702587484	45.1	327.9	W	12.3	5.6	21.7	9.5	2.17
				S	17.1	27.2	1.4	nd	0.92
Guallar	30TYL3187487901	15.0	335.8	W	9.3	7.8	14.9	8.7	nd
				S	6.5	27.1	11.1	nd	8.33
La Playa (site 1)	30TYL3450789388	239.9	325.1	W	10.2	1.5	15.6	8.2	25.50
				S	6.9	19	36.3	nd	11.30
La Playa (site 3)	30TYL3607188993			W	5.3	3.9	2.8	8.2	1.51
				S	5.6	35.3	3.3	nd	0.36

La Playa (site 4)	30TYL3456789472			S	12.8	22.1	6.3	nd	nd
Muerte	30TYL2904187130	18.2	332.1	W	14.1	5.2	0.8	9.1	2.83
Pez*	30TYL2912484604	9.3	330.2	S	22.1	nd	nd	nd	nd
Piñol	30TYL2944287974	15.7	335.2	W	10.1	1.9	18.8	9.3	1.37
				S	9.8	19.7	3.9	nd	1.77
Pito	30TYL3833388632	80.5	320.3	S	4.8	25.2	1.4	nd	1.10
Pueyo	30TYL3645088612	27.0	326.7	S	3.8	34.3	3	nd	0.12
Rollico	30TYL2615185874	40.8	320.6	W	7.1	6.5	1.2	9.4	1.63
Salineta	30TYL3721396429	23.2	325.1	W	13.8	1.3	7.2	7.7	3.22
				S	19.3	28.3	3.4	nd	38.50
Salobral	30TYL3865498426	15.3	323.6	W	8.1	0.9	55.5	9.7	13.20
				S	12.3	24.6	11.7	nd	40.40
Chiprana	30TYL3610069606	162.0	138.6	W	2.7	10.2	1.1	9.7	4.07

* Groundwater

Table 2. Accesion numbers and BLAST searchs for the closest environmental match (CEM) and cultured match (CCM) for Bacteria, Archaea and Eukarya.

Bacteria

Tax.	acc.	Affiliation - ARB taxonomy	bp	Site	Samp-ling	acc. CEM	% id. CEM	acc. CCM
Actinobacteria; Actinobacteria; Micrococcales; Microbacteriaceae								
	AM085961	Arthrobacter_3	460	Guallar	W	HM126928	99.6	NR_043879 / DQ4735
	AM085989	Arthrobacter_3	461	Playa-4	S	HM127067	99.6	AB583921
	AM085976	Arthrobacter_3	461	Salobral	S	JX243051	99.4	HQ256840
	AM085954	DS001	511	Chiprana	W	FJ948217	96.9	AM945590
Bacteroidetes; Cytophagia; Cytophagales; Cyclobacteriaceae; Algoriphagus								
	AM085967	Algoriphagus_1	490	Pito	S	JX222713	98.2	HQ401024
	AM085987	Algoriphagus_1	490	Pueyo	S	JQ229578	99.0	JN967625
Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae								
	AM085957	Cryomorphaceae_O	478	Playa-3	W	AY701417	93.1	JF488640
	AM085984	Cryomorphaceae_O	478	Playa-4	S	AY580694	98.7	HQ675251
Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Psychroflexus								
	AM085949	Psychroflexus	536	Camaron	W	FJ844050	97.8	NR_044410 / EU1357
	AM085980	Psychroflexus	540	Guallar	W	EU722692	99.8	HQ534335
	AM085981	Psychroflexus	520	Muerte	W	FJ844036 / FJ844058	100.0	EU874390
	AM085991	Psychroflexus	523	Pez	S	FJ844052	96.4	FM865890
	AM085977	Psychroflexus	523	Pez	S	FJ844040	95.6	HQ534335
	AM085964	Psychroflexus	524	Piñol	S	EU722703	99.6	EU874390
	AM085983	Psychroflexus	533	Playa-1	W	HM128275	99.8	FM865894
	AM085982	Psychroflexus	533	Playa-1	S	HM128275	99.8	NR_044410 / EU1357
	AM085990	Psychroflexus	523	Playa-1	S	FJ844052	96.4	NR_044410 / EU1357
	AM085973	Psychroflexus	526	Playa-3	S	FJ844036 / FJ844058	99.8	AB381940
	AM085956	Psychroflexus	533	Playa-3	W	FJ844065	99.6	CP003879
	AM085971	Psychroflexus	530	Playa-4	S	CU467437	97.2	NR_028854 / AF5134
	AM085953	Psychroflexus	520	Salineta	W	EU725597	99.8	CP003879
	AM085952	Psychroflexus	540	Salineta	W	FJ844028	98.1	FM865890
	AM085974	Psychroflexus	528	Salobral	S	HM127889	94.7	NR_028854 / AF5134

Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae								
AM085962	Roseovarius_3	506	Camaron	S	HM126967	98.4	EU346498	97.8
AM085979	Roseovarius_3	508	Pez	S	GU212606	99.2	JQ675542	98.0
AM085993	Roseovarius_3	508	Playa-1	S	GU212606	99.2	AM990868	99.2
AM085968	Rhodobacterales	504	Pito	S	EF123342	98.4	HM031999	97.8
AM085959	Loktanella_2	506	Salobral	W	EF491281	99.4	GU061018	99.2
AM085966	uncultured	501	Pito	S	EU283263	99.6	DQ659414	99.8
AM085986	uncultured	501	Playa-3	S	JN639305	99.6	NR_044514 / EU512919	99.6
Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; GKS98								
AM085988	GKS98 freshwater	529	Playa-1	S	HM127055	98.7	AB599842	96.4
AM085969	GKS98 freshwater	529	Playa-1	S	JN532633	98.3	AB470444	96.8
Proteobacteria; Gammaproteobacteria; Alteromonadales; Idiomarinaceae; Pseudidiomarina								
AM085992	Pseudidiomarina	528	Playa-1	S	AY375107	99.6	FJ581032	99.6
AM085978	Pseudidiomarina	528	Pez	S	AY375107	99.6	NR_043732 / DQ342238	98.3
Proteobacteria; Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Halomonas								
AM085985	Halomonas_1	547	Muerte	W	HM128259	99.8	EU308331	99.5
AM085960	Halomonas_1	547	Piñol	W	HM127948	99.8	EU880513	99.5
AM085955	Halomonas_1	525	Chiprana	W	HM128259	97.9	JN903896	97.9
AM085951	Halomonas_1	554	Camaron	W	HM128043	98.5	JN903896	98.5
AM085950	Halomonas_1	549	Camaron	W	HM128135	96.5	JN903896	96.5

Archaea

Tax.	acc.	Affiliation - ARB taxonomy	bp	Site	Samp-ling	acc. CEM	% id. CEM	acc. CCM	% id
Euryarchaeota; Halobacteria; Halobacteriales; DHVEG-6									
	AM071509	DHVEG-6	505	Camaron	W	AJ969789	98.02	AB518739	7
	AM071501	DHVEG-6	503	Camaron	S	AF477917	95.42	AB260042	7
	AM071499	DHVEG-6	514	Playa-1	W	AF477917	95.52	AB518736	7
	AM071504	DHVEG-6	505	Playa-4	S	EU731065	99.6	AB197163	8
	AM071496	DHVEG-6	485	Salineta	W	AF477917	91.75	HQ675778	8
	AM071503	DHVEG-6	467	Pito	S	EU731145	88.65	AB260045	7
Euryarchaeota; Halobacteria; Halobacteriales; Halobacteriaceae									
	AM071506	Halomicrobium related	491	Playa-3	S	JQ033965	94.07	HQ425054	9
	AM071495	Natronomonas	509	Camaron	W	HQ110061	95.68	NR_028172 / AF071880	9
	AM071502	(Halolamina related)	496	Piñol	S	HQ400534	94.94	CP002988	9
	AM071508	Halonotius	509	Salineta	S	HQ157628	98.04	HM159607	9
	AM071497	Halonotius	507	Salineta	W	AM947464	97.83	AY498641	9
	AM071505	Halorhabdus	487	Playa-4	S	EF459703	95.7	JN196513	9
	AM071500	Halonotius	507	Camaron	S	FJ172053	94.08	JX188267	9
	AM071507	Halonotius	507	Playa-3	S	FN391188	94.67	JQ068944	9
Euryarchaeota; Thermoplasmata; 20a-9									
	AM071498	cluster 20a-9	441	Chiprana	W	AF477914	94.09	HE802582	7

Eukarya

Tax.	acc.	Affiliation - ARB taxonomy	bp	Site	Samp-ling	acc. CEM	% id. CEM	acc. CCM	% id. CCM	origin of the closest match
Amoebozoa; Lobosa; Tubulinea; Leptomyxida; Flabellula										
	AM087465	Flabellula	581	Salineta	S	FN598467	84.72	EU852652	97.75	marine
Archaeplastida; Chloroplastida; Chlorophyta										
	AM072917	Trebouxiophyceae	515	Piñol	W	EU143982	98.26	AY197626	98.64	n.a.
	AM072932	Trebouxiophyceae	508	Salineta	W	EU143982	92.35	AY197626	92.75	n.a.
Archaeplastida; Chloroplastida; Chlorophyta; Chlorophyceae										
	AM072939	Chlorophyceae	513	Pito	S	FJ157337	95.33	JN903984	91.68	inland lagoon - brackish-saline
	AM087462	Chlorophyceae	506	Piñol	S	FJ157337	97.63	JN903984	93.73	inland lagoon - brackish-saline
	AM087464	Chlorophyceae	484	Playa-4	S	FJ157337	97.52	FN824396	93.63	inland lagoon - brackish-saline
	AM072934	Chlorophyceae - Dunaliella	516	Salineta	S	AM179821	99.42	EF473745	99.61	coastal lagoon - hypersaline
	AM072933	Chlorophyceae - Dunaliella	508	Salineta	S	AM179821	99.41	JQ712983	99.61	inland lake - hypersaline
	AM087467	Chlorophyceae - Dunaliella	492	Salineta	S	AM179821	98.37	JQ712983	98.58	inland lake - hypersaline
Opisthokonta; Fungi										
	AM072921	Chytridiomycota Incertae	479	Playa-1	W	JQ689418	94.57	NG_017174 / AY635838 / HQ440100	93.14	inland lake - freshwater
	AM072920	Chytridiomycota Incertae	479	Salobral	W	JQ689418	96.04	NG_017174 / AY635838 / HQ440100	94.8	inland lake - freshwater
Alveolata										
	AM072916	Protalveolata Colpodelli	524	Muerte	W	AB695489	96.66	AY234843	96.66	Freshwater lakes
Alveolata; Ciliophora; Intramacronucleata; Conthreep; Oligohymenophorea; Scuticociliatia										
	AM072935	Cyclidium	502	Playa-3	S	JQ692043	99.2	AJ767059	99.6	coastal salt pan
Alveolata; Ciliophora; Intramacronucleata; Litostomatea; Haptoria										
	AM087461	Didinium	476	Piñol	S	JN090883	97.9	U57771	97.48	inland lake - brackish- saline
	AM087463	Didinium	465	Playa-4	S	JN090883	97.83	U57771	97.39	inland lake - brackish- saline
Alveolata; Ciliophora; Intramacronucleata; Spirotrichea; Euplotia; Euplotes										
	AM072936	Euplotes	498	Playa-3	S	GU385618	94.09	EF094971	94.11	marine
	AM087466	Euplotes	503	Salineta	S	GU385637	98.01	EF094971	98.2	marine
Alveolata; Ciliophora; Postciliodesmatophora; Heterotrichea										
	AM087460	Heterotrichea	449	Camaron	S	AM084307	99.77	DQ168806	100	n.a.
	AM072937	Heterotrichea	449	Playa-3	S	AM084307	99.77	DQ168806	100	n.a.
	AM087468	Heterotrichea	450	Salineta	S	AM084307	99.77	DQ168806	100	n.a.

Rhizaria; Cercozoa; Silicofilosea; Chlorarachniophyta									
AM072938	Lotharella	499	Salobral	S	HM997335	91.5	JF826444	99	marine
Stramenopiles; Bicosoecida; Cafeteriidae									
AM072923	Cafeteria	486	Playa-3	W	FN598459	98.34	GU170211	98.55	spring - hypersaline
Stramenopiles; Chrysophyceae									
AM072914	Chromulinales	487	Muerte	W	JQ782092	98.97	EF043285	98.97	diverse
Stramenopiles; Dictyochophyceae; Pedinellales									
AM072930	Pedinellales	507	Muerte	W	FN690666	95.01	AB097408	95	sea ice
AM072918	Pedinellales	466	Piñol	W	JF698788	97.42	AB097408	97.42	Beaufort sea
AM072928	Pedinellales	517	Rollico	W	FN690666	94.32	AB097408	94.2	sea ice