**Dynamics and ecological distributions of the Archaea microbiome from
inland saline lakes (Monegros Desert, Spain)**

Mateu Menéndez-Serra¹, Vicente J Ontiveros², Xavier Triadó-Margarit¹, David
Alonso² and Emilio O Casamayor^{1*}

¹Integrative Freshwater Ecology Group, and ²Theoretical and Computational
Ecology Group. Centre of Advanced Studies of Blanes (CEAB), Spanish
Research Council (CSIC), 17300 Blanes, Catalonia, Spain.

Running title: Archaea microbiome in inland saline lakes

*Corresponding author. Emilio O Casamayor. CEAB-CSIC, Accés Cala St
Francesc, 14. E-17300. Blanes (SPAIN). Phone: +34 972 336 101, Fax: +34
972 337 806. E-mail: casamayor@ceab.csic.es

Keywords: archaea, inland saline lakes, community ecology, DPANN
Woesearchaeota, Pacearchaeota, halophiles, 16S rRNA gene

Abstract. We characterized the rich Archaea microbiome of shallow inland lakes (Monegros Desert, NE Spain) by 16S rRNA gene tag sequencing covering a wide salinity range (0.1-40% w/v) along three years. Up to 990 OTUs (<97% identity) were detected allocated in 14 major archaeal phyla and heterogeneously distributed along the salt gradient. Dynamics and idiosyncratic ecological distributions were uncovered for the different phyla. A high genetic richness was observed for Woesearchaeota and Pacearchaeota (>370 OTUs each), followed by Halobacteria (105), Nanohaloarchaeota (62) and Thermoplasmata (19). Overall, the distribution of genetic richness was strongly correlated with environmental niche amplitude, but not with occurrence. We unveiled high occurrence for a very rich Woesearchaeota assemblage, and an unexpected positive correlation of Pacearchaeota abundance with salinity at >15% dissolved salt content. The estimated dynamic behaviour (temporal “turnover” rates of presence/absence data) unveiled Thaumarchaeota and Halobacteria as the most dynamic groups, and Aenigmarchaeota and Thermoplasmata as the most stable. The DPANN Pacearchaeota, Woesearchaeota, and Nanohaloarchaeota showed intermediate rates, suggesting higher resilience to environmental perturbations. A rich and dynamic Archaea microbiome was unveiled including unseen ecological traits for relevant members of the still largely unknown DPANN group, supporting a strong ecological differentiation between Pacearchaeota and Woesearchaeota.

37 Introduction

38 Inland lakes in endorheic areas are very dynamic ecosystems globally distribute
39 in semi-arid and arid regions of the world (Williams 1996). Most shallow inland
40 lakes show wider ranges of salinities, environmental conditions, and salt
41 compositions than coastal areas (Williams 1996). Numerous microbial ecology
42 studies have been carried out on diverse terrestrial saline environments in all
43 the continents (North- and South-America (e.g., Dillon *et al.* 2009; Demergasso
44 *et al.* 2008), Australia (Narasingarao *et al.* 2012), European Mediterranean
45 areas (Gasol *et al.* 2004; Ventosa *et al.* 2014), Asia (Wu *et al.* 2006), Africa
46 (Cavalazzi *et al.* 2019), Antarctica (Williams *et al.* 2014), just to mention a few
47 among many others). The role of salinity as a physiological stressor has also
48 promoted the study of idiosyncratic ecophysiological adaptations along salinity
49 gradients in simplified ecological communities (Pedrós-Alió *et al.* 2000).

50 Terrestrial saline aquatic environments (both inland and coastal) are very good
51 natural model systems and have been traditionally associated to highly
52 specialized planktonic organisms. At the most saline ends, dominance of a few
53 groups has been reported, i.e., *Haloquadratum waslbyi*, *Salinibacter ruber* and
54 the recently uncovered Nanohaloarchaea (Ventosa *et al.* 2015; Narasingarao *et*
55 *al.* 2012; Ghai *et al.* 2011). At intermediate salinities, different studies show high
56 levels of heterotrophic activity (Gasol *et al.* 2004), planktonic algae
57 accumulation (Estrada *et al.* 2004), and presence of largely unknown organisms
58 (Demergasso *et al.* 2008; Triadó-Margarit and Casamayor 2013). Among them,
59 inland lakes are prone to contain new groups of Halobacteria and poorly
60 characterized uncultured Archaea (Narasingarao *et al.* 2012; Casamayor *et al.*
61 2013).

Several studies in the last decade have improved the picture on the Archaea domain, with higher diversity and environmental distribution than historically considered (Auguet *et al.* 2010; Rinke *et al.* 2013). There are however important gaps still on the current knowledge of the Archaea ecology even in apparently well studied habitats where archaea were predominant organisms, such as high saline systems. Recently, the monophyletic superphylum DPANN has been shown to contain a large number of widespread taxa most of them missing cultured counterparts, such as Nanohaloarchaeota, Pacearchaeota, Woesearchaeota, Aenigmarchaeota, Micrarchaeota, Diapherotrites, Parvarchaeota, and Nanoarchaeota (Rinke *et al.* 2013; Castelle *et al.* 2015, Ortiz-Alvarez and Casamayor 2016). Because of the difficulties in culturing and the still limited application of both powerful single cell genomic analysis (Dal Molin and Di Camillo 2018) and metagenomics approaches (Narasimarao *et al.* 2012) in archaea, the biology and ecology of many of these groups are still unknown. In addition, the detailed study of temporal dynamics in archaeal ecological communities has been rarely carried out (Galand *et al.* 2010, Hugoni *et al.* 2013) missing substantial information on the ecology of the third domain of life.

Within this context, we explored community patterns and temporal dynamics of the aquatic archaeal microbiome in the Monegros Desert (NE Spain). The Monegros ecosystem contains one of the largest sets of inland saline lakes in Europe and constitutes a unique landscape of great ecological value, easy to reach and sample (Casamayor *et al.* 2013). Physico-chemical parameters strongly change with time in the same aquatic spot offering a wide repertoire of heterogeneous environmental conditions within a very narrow geographic

distance of a few kilometers. A preliminary study already evidenced the high microbial novelty present in Monegros, including novel archaea (Casamayor *et al.* 2013). In the present study, we aimed to unveil temporal dynamics, ecological patterns and environmental preferences for the most unknown Archaea covering different inter-annual dry and wet periods. We added a quantitative temporal dimension to the analysis by applying a community ecology method based on temporal changes in the absence/presence of local populations. This method applies a mathematical estimation of “colonization” and “extinction” rates (Ontiveros *et al.* 2019) showing different ecological dynamics for the different groups. The range of habitat gradients occupied and the estimated dynamical behavior, unveiled community level traits and patterns unseen to date for some of the most intriguing uncultured archaeal groups.

Experimental Procedures

The Monegros Desert is an endorheic platform (c. 400 km²) located in the Central Ebro Basin (NE Spain, 41°42'N, 0°20'W, 328 m a.s.l.), with 149 scattered depressions ranging in size from < 2 to 239 ha, some of them hosting hypersaline wetlands. This is one of the most arid regions in Europe (mean annual rainfall 350 mm, mean annual temperature 14.9 °C, and mean reference evapotranspiration 1255 mm yr⁻¹, Faci González and Martínez-Cob 1991), and unique for the combination of geological, edaphic, mineralogical and hydrological characteristics (Pueyo Mur and Inglés Urpinell 1987; Samper-Calvete and García-Vera 1998; Mees, Castañeda del Álamo and Ranst 2011).

We carried out monthly sampling in 14 different ponds (Table 1) covering different dry-wet periods with a total of 128 water samples analysed (see details in Table S1). Conductivity, temperature, pH, dissolved oxygen and redox potential were measured in situ using an HQ40d multiparameter meter (Hach, Loveland, CO, USA). For chlorophyll *a* (Chl *a*) analysis 1L water was filtered through 47-mm-diameter Whatman GF/F filters (0.7 µm nominal particle retention), stored in the dark, and kept frozen. Chl *a* concentration was determined in acetone extracts by spectrophotometry (UV-2401PC, ultraviolet-visible Spectrometer; Shimadzu) as previously reported (Bartrons, Catalan and Casamayor 2012). Salinity was measured in situ by a hand salinity refractometer (Atago S-28E, Japan). The water column height was measured at the deepest part of the ponds. Substantial changes in water column height, temperature, salinity and other environmental variables were observed both among ponds and inside the same pond along the hydrological period. Detailed information about environmental parameters and sampling date and sites is available in Supplementary Table S1.

DNA extraction and sequencing

For DNA analyses, water samples were pre-filtered in situ through a 50 micron-pore-size net, to retain large zooplankton and algae, and then 100–500 mL were subsequently filtered on 5 and 0.2-micron pore-size polycarbonate membranes (47 mm diameter), respectively. The 0.2 µm PC membranes were stored in lysis buffer (40 mM EDTA, 50 mM Tris, pH 8.3, 0.75 M sucrose), enzymatically digested and phenol extracted (phenol–chloroform–isoamyl alcohol; 25:24:1, v/v/v) as previously reported (Ferrera *et al.* 2004). DNA

purification and concentration was carried out with Amicon® Ultra 4 Centrifugal Filter Units – 100 000 NMWL (Millipore). DNA extracts were quantified using Quant-it assays in a Qubit™ fluorometer (Invitrogen). Sequencing with the Illumina 2x250 MiSeq platform was carried out on PCR amplicons of the 16S rRNA gene (V4 region, primers set F515-R806, Caporaso *et al.* 2010) according to genomic core facilities, protocols and methods of the RTSF-MSU (Michigan State University, USA) (<https://rtsf.natsci.msu.edu/>).

Bioinformatic processing and genetic analyses

Raw sequences were processed using UPARSE (Edgar 2013). The total number of sequences prior to quality filtering was 14,445,263 and 70.6% of them had $\geq$ Q30. After merging of read pairs, the dataset was filtered by setting a maximum number of expected errors of 0.25 (Edgar and Flyvbjerg 2015), and chimeric reads were detected and filtered out by UCHIME following both de novo and reference-based analyses (against the ‘Gold’ reference database available for ChimeraSlayer). Filtered sequences were clustered into Operational Taxonomic Units (OTUs) at 97% identity. The taxonomic assignment was carried out with SINA aligner v.1.2.11 (Pruesse, Peplies and Glöckner 2012), using the SILVA 128 database (Gurevich *et al.* 2013). OTUs were filtered by their alignment quality score and sequences classified as mitochondria and chloroplasts were excluded. A final filtering by library size keeping only samples with $> 10,000$ reads was carried out. The resulting OTU table consisted of 7,241 prokaryotes OTUs (6251 bacterial OTUs and 990 archaeal OTUs), corresponding to a total of 3,374,654 reads (of which 54,807 were assigned to Archaea) for 128 samples. Data was normalized by rarefying

the reads of all samples to a minimum threshold of 10,000 reads/sample. For community related analysis, rarefaction at the same depth was repeated 100 times in order to avoid the loss of less abundant OTUs, and the resulting rarefactions were unified on an average rarefied OTU table.

Genetic distances between OTUs were calculated using the “calc_distmx” command in the USEARCH pipeline. Reference sequences were included to properly differentiate Candidatus Pacearchaeota (i.e., GenBank accession numbers MFWP01000008, MNVW01000021, MNVX01000029, and MNVY01000002, Anantharaman *et al.* 2016) from Candidatus Woesearchaeota (accession number MNWW01000056, Nawrocki *et al.* 2018). The resulting distance matrix was visualized by Non-Metric Multi-Dimensional Scaling (NMDS) analysis run with MetaMDS functions in Vegan package. We estimated genetic diversity by calculating the nucleotide diversity using the R package “Pegas” (<https://cran.r-project.org/web/packages/pegas/index.html>). The phylogenetic tree shown in supplemental Fig. S1, is an ARB maximum parsimony tree provide by default (<http://www.arb-home.de/>) with 16S rRNA gene amplicon sequences added using the tool “add species to existed tree / ARB-parsimony tool interactive”, providing only very rough information because of the short read length.

The complete raw data set is available in GenBank under BioProject record ID PRJNA429605, and GenBank accession numbers for highlighted OTUs are shown in Supplemental materials (Table S2, Fig. S1).

Statistical analysis and calculation of colonization and extinction rates

Statistical analyses were run in R (<http://www.r-project.org>). Species richness, evenness, and diversity indexes were calculated using “Vegan” package version 2.4-5 (Oksanen *et al.* 2016). The environmental amplitude (range of environmental conditions occupied) for the different archaeal lineages, was calculated according to salinity, pH, redox potential, dissolved oxygen, and Chl *a* data, weighting its variance with the relative abundance of the lineage. We avoided the use of variables that co-varied with salinity such as conductivity (Spearman, rho 0.98 and p value < 0.001) and temperature (Spearman, rho 0.50 and p value < 0.001). Spearman’s Rank order correlation coefficient was used to test monotonic associations between numeric variables.

The estimated temporal “turnover” rates were calculated for each archaeal lineage based on the absence/presence of local populations for consecutive sampling times (“extinction / colonization” rates, respectively). “Colonization” and “extinction” rates were calculated using the “island” package on R (<https://CRAN.R-project.org/package=island>; Ontiveros *et al.* 2019) and provide an effective view on the dynamic behavior of the different archaeal populations in time (“turnover” rate). The “turnover” rates were expressed as day⁻¹.

Results

Archaeal abundance and diversity

The relative abundance of Archaea in the 16S rRNA gene amplicon mixture ranged between 0.01 and 50% of total prokaryotes. Archaea abundance consistently increased along the salinity gradient, mainly at salinities > 20%

(w/v), reaching the highest relative abundances at the highest salinity end (Fig. 1). For salts concentrations <20%, relative abundances were < 5% in all cases.

The genetic richness of the Archaea planktonic microbiome was notorious. We observed the presence of 14 different lineages, with occurrences (percentage of samples in which the group was detected) between 96% and 0.8% (Table 2). As a whole, the OTUs richness was higher for Woesearchaeota (402 OTUs) and Pacearchaeota (376 OTUs), followed by Halobacteria (105 OTUs) and Nanohaloarchaeota (62 OTUs). The highest values of abundance in the amplicon mixture were found for both Nanohaloarchaeota (24%) and Halobacteria (22%). Woesearchaeota and Pacearchaeota did not reach relative abundances >5% for any of the samples, although they were widely distributed being present in >87% of the total dataset. Halobacteria was also found in a large percentage of samples (82%), followed by Nanohaloarchaeota (49% occurrence). Minor groups were Thermoplasmata, Thaumarchaeota and Aenigmarchaeota, among others (Table 2). Only six reads (1 OTU) matched methanogens (i.e., Methanomicrobia, genus *Methanohalophilus*).

Archaeal assembly along the salinity gradient

We selected the seven most abundant lineages and with occurrences >15%, for a more detailed downstream analysis. These groups showed a distinctive distribution along the salinity gradient (Fig. 2). At salinities below 15%, the most abundant groups were mainly Woesearchaeota, and in one case we detected noticeable concentrations of both Thermoplasmata (3% relative abundance in Lake Muerte on January 2013, salinity 5.8%, temperature 7.8°C; OTU 316 in Table S2) and Pacearchaeota (2% relative abundance in Lake Salobral on July

2013, salinity 3.8%, temperature 29.6°C). Conversely, at > 15% salinity the archaeal assemblages were mostly dominated by Nanohaloarchaeota (up to 24% in Lake Salineta, October 2013, salinity 40%, temperature 18.8°C), Halobacteria, and Pacearchaeota. Finally, Thaumarchaeota (ammonia-oxidizing archaea, AOA) showed a maximum abundance of 0.23% at marine like salinities (close to 3% w/v) and were mostly detected at salinities up to 10% (8 OTUs, accession numbers MH028008 to MH028015) indicating a strong selection by local environmental conditions on this functional group.

Overall, salinity was the environmental variable which explained most of the variations in the relative abundances of the different archaeal populations and not major effects were observed for the additional environmental/biological parameters measured ($p > 0.05$ for pH, redox potential, dissolved oxygen, Chl *a* concentration, and bacterial diversity). We found a strong correlation between salinity and relative abundances of Pacearchaeota (Spearman, ρ 0.59, p value < 0.001) and Woesearchaeota (Spearman, ρ 0.65, p value < 0.001). This correlation was also significant for Nanohaloarchaeota (Spearman, ρ 0.36, p value < 0.01) and Halobacteria (Spearman, ρ 0.41, p value < 0.001). The robustness of the correlations increased when the whole salinity gradient was divided into the three main salinity ranges based on both physicochemical and biological parameters (Por 1972; Kushner 1978; Imhoff 2001), i.e., <5% (saline), 5-15% (hypersaline), and >15% (highly hypersaline), and some unseen trends were subsequently unveiled (Fig. 3). Nanohaloarchaeota and Halobacteria showed stronger correlations (c. ρ 0.70) at the most saline end, whereas the relationship was non-significant at <15% salinity. Woesearchaeota only showed a significant positive relationship at salinities <5%. Conversely,

relative abundances of Pacearchaeota showed an unexpected positive correlation with salinity in the highly hypersaline range (>15% salinity, Fig. 3). These results unveiled that these two highly unknown taxa tend to behave differently in front of salinity. Candidatus Pacearchaeota and Woesearchaeota were the richest Archaea taxa in Monegros and showed the highest genetic variation along the salinity gradient in comparison with the remaining Archaea populations (Fig. 4). Despite the fact that both had been grouped within the Deep-sea Hydrothermal Vent Euryarchaeota Group 6 (DHVEG-6) in former taxonomic arrangements, Pacearchaeota and Woesearchaeota appeared clearly separated from each other in the NMDS ordination according to its nucleotide sequence composition (Fig. 4).

A closer look to the most saline end of the gradient (>35% salinity) showed that Halobacteria at these salinities were spread in 12 already known genera, whereas most of the Nanohaloarchaeota were only distantly related to any previously reported counterpart (supplemental Fig. S1, with available individual accession numbers shown). Interestingly, two of the most abundant taxa in the most saline sample (Lake Salineta, October 2013, 40% salinity) were the Halobacteria OTU 320 (97% identity to *Halonotius pteroides*) and the Nanohaloarchaeota OTU 289 (100% identical to Candidatus *Haloredivivus* G17 AGNT01000024) previously found in Santa Pola solar salterns in the Spanish Mediterranean coast (Ghai *et al.* 2011), aprox. 450 km away from Monegros.

Archaeal ecology and dynamic behaviour

Community ecology analyses provided new insights and unveiled unseen ecological trends for the different Archaea groups. As a whole, group occurrence was strongly correlated with OTUs richness (Spearman, ρ 0.96, p value < 0.005), i.e., the highest occurrence was found for the richest groups. In addition, the distribution of genetic richness (i.e., nucleotidic diversity) for the seven major archaeal phyla was strongly correlated with environmental amplitude (Spearman, ρ 0.89, p value < 0.05 , Fig. 5, panel B) but not with lake occurrence (Fig. 5, panel A). Aenigmarchaeota had the highest environmental amplitude (i.e., larger range of habitat gradients occupied) despite the fact that only 5 OTUs were detected for this phylum, and showed the lowest occurrence (19%). Conversely, Nanohaloarchaeota, Halobacteria and Thaumarchaeota had the bulk of their abundances within a restricted environmental niche (Fig. 5, panel B), despite having a medium-high occurrence (between 35% and 85%). The analysis of OTU richness vs. evenness also showed interesting trends (Fig. 5, panel C); Pacearchaeota and Woesearchaeota showed the highest richness and evenness, whereas at the other end, Aenigmarchaeota, Thermoplasmata, Thaumarchaeota, and Nanohaloarchaeota were low richness-low evenness groups. Halobacteria was the only group with low richness-high evenness structure (i.e., relatively low number of OTUs and with similar population density). Finally, the estimated dynamic behaviour (“turnover” rates) for the main archaeal lineages showed again important differences among groups (Fig. 5, panel D). The most dynamic groups (the highest “colonization/extinction” turnover) were Thaumarchaeota and Halobacteria. The most stable taxa (the lowest turnover) were

Aenigmarchaeota and Thermoplasmata. The DPANN groups Pacearchaeota, Woesearchaeota, and Nanohaloarchaeota showed intermediate turnover rates between the two ends.

Discussion

Comprehensive studies carried out in the last years have provided a better picture on the Archaea ecology and diversity (e.g., Auguet *et al.* 2010; Rinke *et al.* 2013). There are however important gaps still in the current knowledge of the environmental Archaea microbiome. For instance, the widespread DPANN group has been recently arranged (Castelle *et al.* 2015b; Spang, Caceres and Ettema 2017), but little is known on the ecology, dynamics, and environmental preferences of these archaea. Our study added relevant information analysing the ecological niche, range of environmental preferences, and temporal dynamics of uncultured DPANN and other relevant archaea, using a detailed temporal survey in a high dynamic set of inland salt lakes.

We observed higher archaeal relative abundance at the most extreme salinity end, mostly of Halobacteria (superphylum Euryarchaeota) but also of the DPANN Nanohaloarchaeota. The importance of Halobacteria (haloarchaea) at extreme salinities have been recurrently reported (Oren 2002). However, the role and distribution of Nanohaloarchaeota has been unseen for a long time (Narasimarao *et al.* 2012). Its recent detection in diverse hypersaline environments, has arisen a high interest for both plankton and benthic Nanohaloarchaeota populations (Podel *et al.* 2014; Andrei *et al.* 2015; Cowan *et*

al. 2015; Ventosa *et al.* 2015). In our study, we observed similar relative abundances of Nanohaloarchaeota and Halobacteria at the most saline end, although with substantial differences in community structure and community ecology dynamics. Nanohaloarchaeota showed a more limited environmental breadth, being less abundant than Halobacteria at lower salinities, and lower colonization rates. More studies are needed to confirm whether or not this observation indicates a higher specialization of the DPANN Nanohaloarchaeota to the most extreme saline conditions compared to a more versatile behavior of Halobacteria. Recent discoveries on a symbiotic lifestyle for Nanohaloarchaeota (Hamm *et al.* 2019) indicate that it is necessary to apply an integrated view on the whole microbial community (including bacteria and eukarya) to better understand Nanohaloarchaeota ecology.

A symbiotic/parasitic lifestyle has also been proposed for the other two important DPANN groups (in terms of relative abundance and occurrence) in the Monegros Archaea microbiome: Woesearchaeota and Pacearchaeota. Woesearchaeota and Pacearchaeota (both formerly named Deep-sea Hydrothermal Vent Euryarchaeota Group 6, DHVEG-6) have been recently proposed as candidate phyla belonging to the DPANN superphylum (Castelle *et al.* 2015a). Since then, they have been uncovered as major components of the archaeal assemblages in a large repertory of environments, and the need of a better understanding of their biology, ecology and distribution has stimulated research in recent years (Castelle *et al.* 2015a; Ortiz-Alvarez and Casamayor 2016; Liu *et al.* 2018). The lack of an unequivocal phylogenetic split between Woesearchaeota and Pacearchaeota has led in some cases to overlapping analysis (Lipsewers *et al.* 2018; Liu *et al.* 2018) and blurred ecological

differentiation between them. The environmental niches of these two phyla are known to be large and they are widely distributed in saline aquatic environments, mainly in sediments, microbial mats and plankton, but also in hot springs and freshwater (Robertson *et al.* 2009; Kan *et al.* 2011; Pachiadaki *et al.* 2011). Pacearchaeota has only been rarely detected in marine plankton (Castelle and Banfield 2018), which matches the observed trend in Monegros where higher relative abundances of Pacearchaeota were found at higher salinities. In the studied saline lakes, these two phyla showed the highest OTU richness, evenness, sites occurrence and extinction rates among Archaea. All these traits suggest an ecological strategy based on fast colonization but low persistence along the whole salinity gradient for the different OTUs shaping these two groups. As previously mentioned, new insights in the metabolisms of DPANN archaea brought by metagenomics and single-cell genomics, suggest that small genomes, small cell sizes, and limited metabolic variability are common characteristics. Thus, the lack of core biosynthetic pathways has led to conclude that most of the DPANN archaea depend on other microorganisms – as a symbionts or episymbionts – to carry out basic metabolic functions (Castelle and Banfield 2018). In a recent study in alpine lakes, relative abundances of Pacearchaeota and Woesearchaeota were observed to be positively correlated with phylogenetic diversity of bacterial communities, respectively (Ortiz-Alvarez and Casamayor 2016). This correlation was however not observed in Monegros. If this correlation is expected to be consistent and somehow related to potential bacterial symbionts, probably their high occurrence along the salinity gradient and their large environmental amplitude may have masked the detection of this potential signature in Monegros.

374 Certainly, this point deserves further and more detailed studies considering
375 more integrated analyses of the whole microbial community.

376 For the first time, our study quantifies the in situ dynamic behavior of uncultured
377 archaea. This new perspective needs of experimental tests to be further
378 substantiated. The more “dynamic” taxa were Thaumarchaeota (limited to
379 salinities below 10%, in agreement with previous studies, Fernandez-Guerra
380 and Casamayor 2012) and Halobacteria (with the bulk of its abundance at
381 extreme salinities), and showed their optimal within a restricted environmental
382 niche suggesting these are groups with a higher cell sensitivity to salinity
383 changes. Conversely, the DPANN groups Pacearchaeota, Woesearchaeota,
384 and Nanohaloarchaeota showed intermediate turnover rates. Pacearchaeota
385 and Woesearchaeota showed the widest environmental breadth, the highest
386 richness, and the highest genetic variability. These results, when contextualized
387 in a highly dynamic system with quick changes in community composition
388 mainly driven by changes in salinity (Triadó-Margarit *et al.* 2019), suggest a
389 higher resilience to environmental perturbations.

390 Overall, the study carried out in inland salt lakes of Monegros showed a rich
391 and dynamic Archaea microbiome including relevant members of the still largely
392 unknown DPANN group. We unveiled ecological properties for some of the
393 most enigmatic DPANN members as Nanohaloarchaeota, Pacearchaeota,
394 Woesearchaeota, and Aenigmarchaeota, and provided further evidences to
395 support strong ecological differentiation between Pacearchaeota and
396 Woesearchaeota. Further studies both in situ and in combination with traditional
397 microbiological approaches, should help to improve our still very limited

398 understanding on the biology and ecology of the monophyletic superphylum
399 DPANN, and on the ecology of inland saline systems in general.

400

401

402 **Acknowledgements.** Funding was provided by grant BRIDGES, CGL2015-
403 69043-P from the Spanish Office of Science (MINECO) and European funding
404 (ERDF) to EOC and DA. MMS was funded by the Spanish FPI PhD
405 scholarships program (BRIDGES-MINECO).

406

407 **Conflict of Interest**

408 The authors declare no conflict of interest

409 **References**

- 410 Anantharaman K, Brown CT, Hug LA *et al* (2016) Thousands of microbial
411 genomes shed light on interconnected biogeochemical processes in an
412 aquifer system. *Nat Commun* 7:13219.
- 413 Andrei, A.Ş., Robeson, M.S., Baricz, A., Coman, C., Muntean, V., Ionescu, A.,
414 et al. (2015) Contrasting taxonomic stratification of microbial communities
415 in two hypersaline meromictic lakes. *ISME J.* 9: 2642–2656.
- 416 Auguet JC, Barberan A, Casamayor EO (2010) Global ecological patterns in
417 uncultured Archaea. *ISME J* 4: 182-190
- 418 Bartrons, M., Catalan, J., and Casamayor, E.O. (2012) High Bacterial Diversity
419 in Epilithic Biofilms of Oligotrophic Mountain Lakes. *Microb. Ecol.* 64: 860–
420 869.
- 421 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D.,
422 Costello, E.K., et al. (2010) QIIME allows analysis of high-throughput
423 community sequencing data. *Nat Meth* 7: 335–336.
- 424 Casamayor, E.O., Triadó-Margarit, X., and Castañeda, C. (2013) Microbial
425 biodiversity in saline shallow lakes of the Monegros Desert, Spain. *FEMS*
426 *Microbiol. Ecol.* 85: 503–518.
- 427 Castelle, C.J. and Banfield, J.F. (2018) Major New Microbial Groups Expand
428 Diversity and Alter our Understanding of the Tree of Life. *Cell* 172: 1181–
429 1197.
- 430 Castelle, C.J., Wrighton, K.C., Thomas, B.C., Hug, L.A., Brown, C.T., Wilkins,
431 M.J., et al. (2015) Genomic expansion of domain archaea highlights roles
432 for organisms from new phyla in anaerobic carbon cycling. *Curr. Biol.* 25:
433 690–701.
- 434 Cavalazzi et al. (2019) The Dallol Geothermal Area, Northern Afar (Ethiopia)-An
435 Exceptional Planetary Field Analog on Earth. *Astrobiology* 19(4):553-578
- 436 Cowan, D., Ramond, J.-B., Makhalanyane, T., and De Maayer, P. (2015)
437 Metagenomics of extreme environments. *Curr. Opin. Microbiol.* 25: 97–102.
- 438 Dal Molin, A. and Di Camillo, B. (2018) How to design a single-cell RNA-
439 sequencing experiment: pitfalls, challenges and perspectives. *Brief.*
440 *Bioinform.* bby007: 1-11
- 441 Demergasso, C., Escudero, L., Casamayor, E.O., Chong, G., Balagué, V., and
442 Pedrós-Alió, C. (2008) Novelty and spatio-temporal heterogeneity in the
443 bacterial diversity of hypersaline Lake Tebenquiche (Salar de Atacama).
444 *Extremophiles* 12: 491–504.
- 445 Dillon, J., M. McMath, L., and L. Trout, A. (2009) Seasonal changes in bacterial
446 diversity in the Salton Sea. *Hydrobiologia.* 632: 49-64
- 447 Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial
448 amplicon reads. *Nat Meth* 10: 996–998.
- 449 Edgar, R.C. and Flyvbjerg, H. (2015) Error filtering, pair assembly and error
450 correction for next-generation sequencing reads. *Bioinformatics* 31: 3476–
451 3482.
- 452 Estrada M, Henriksen P, Gasol JM *et al.* Diversity of planktonic
453 photoautotrophic microorganisms along a salinity gradient as depicted by
454 microscopy, flow cytometry, pigment analysis and DNA-based methods.
455 *FEMS Microbiol Ecol* 2004;**49**:281–93.
- 456 Faci González, J.M. and Martínez-Cob, A. (1991) Cálculo de la
457 evapotranspiración de referencia en Aragón. Diputacion General de

- Aragon pp. 115.
- Ferrera, I., et al. (2004) High-diversity biofilm for the oxidation of sulfide-containing effluents. *Appl. Microbiol. Biotechnol.* 64: 726–734.
- Fernández-Guerra A, Casamayor EO (2012) Habitat-Associated Phylogenetic Community Patterns of Microbial Ammonia Oxidizers. *PLoS ONE* 7(10): e47330.
- Galand P, Gutiérrez-Provecho C, Massana R et al (2010) Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay Microbial Observatory). *Limnol Oceanogr* 55: 2117–2125
- Gasol, J., Casamayor, E., Joint, I., Garde, K., Gustavson, K., Benlloch, S., et al. (2004) Control of heterotrophic prokaryotic abundance and growth rate in hypersaline planktonic environments. *Aquat. Microb. Ecol.* 34: 193–206.
- Ghai, R., Pašić, L., Fernández, A.B., Martín-Cuadrado, A.-B., Mizuno, C.M., McMahon, K.D., et al. (2011) New Abundant Microbial Groups in Aquatic Hypersaline Environments. *Sci. Rep.* 1: 135.
- Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. (2013) QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29: 1072–1075.
- Hamm JN, Erdmann S, Eloë-Fadrosch EA et al. (2019) Unexpected host dependency of Antarctic Nanohaloarchaeota. *Proc Natl Acad Sci* **116**:14661 LP-14670.
- Hugoni M, Taib N, Debroas D et al. (2013) Structure of the rare archaeal biosphere and seasonal dynamics of active ecotypes in surface coastal waters. *Proc Natl Acad Sci* **110**:6004 LP-6009.
- Imhoff JF (2001) True marine and halophilic anoxygenic phototrophic bacteria. *Arch. Microbiol.* 176: 243–254
- Kan, J., Clingenpeel, S., Macur, R.E., Inskeep, W.P., Lovalvo, D., Varley, J., et al. (2011) Archaea in Yellowstone Lake. *ISME J.* 5: 1784.
- Kushner DJ (1978) Life in high salt and solute concentrations. In: Kushner DJ, editor. *Microbial Life in Extreme Environments*. London: Academic Press; pp. 317–368
- Lipsewers, Y.A., Hopmans, E.C., Sinninghe Damsté, J.S., and Villanueva, L. (2018) Potential recycling of thaumarchaeotal lipids by DPANN Archaea in seasonally hypoxic surface marine sediments. *Org. Geochem.* 119: 101–109.
- Liu, X., Li, M., Castelle, C.J., Probst, A.J., Zhou, Z., Pan, J., et al. (2018) Insights into the ecology, evolution, and metabolism of the widespread Woese archaeal lineages. *Microbiome* 6: 102.
- Mees, F., Castañeda del Álamo, C., and Ranst, E. van (2011) Sedimentary and diagenetic features in saline lake deposits of the Monegros region, northern Spain. *Catena* 85: 245–252.
- Narasimharao, P., Podell, S., Ugalde, J.A., Brochier-Armanet, C., Emerson, J.B., Brocks, J.J., et al. (2012) De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities. *ISME J.* 6: 81–93.
- Nawrocki, E.P., Jones, T.A., and Eddy, S.R. (2018) Group I introns are widespread in archaea. *Nucleic Acids Res.* 414–414.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Henry, M., and Stevens, H. (2016) *vegan Community Ecology Package* version 2.4-1 September 2016.
- Ontiveros VJ, JA Capitan, R Arthur, EO Casamayor, D Alonso. (2019) Colonization and extinction rates estimated from temporal dynamics of

- ecological communities: The island R Package. *Methods in Ecology and Evolution* 10 (7), 1108-1117
- Oren, A. (2002) Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. *J. Ind. Microbiol. Biotechnol.* 28: 56–63.
- Ortiz-Alvarez, R. and Casamayor, E.O. (2016) High occurrence of Pacearchaeota and Woesearchaeota (Archaea superphylum DPANN) in the surface waters of oligotrophic high-altitude lakes. *Environ. Microbiol. Rep.* 8: 210–217.
- Pachiadaki, M.G., Kallionaki, A., Dählmann, A., De Lange, G.J., and Kormas, K.A. (2011) Diversity and Spatial Distribution of Prokaryotic Communities Along A Sediment Vertical Profile of A Deep-Sea Mud Volcano. *Microb. Ecol.* 62: 655–668.
- Pedrós-Alió C, Calderón-Paz JI, Gasol JM. (2000) Comparative analysis shows that bacterivory, not viral lysis, controls the abundance of heterotrophic prokaryotic plankton FEMS Microbiol Ecol. 32:157-165.
- Podell, S., Emerson, J.B., Jones, C.M., Ugalde, J.A., Welch, S., Heidelberg, K.B., et al. (2014) Seasonal fluctuations in ionic concentrations drive microbial succession in a hypersaline lake community. *ISME J.* 8: 979–990.
- Por FD (1972) Hydrobiological notes on the high salinity waters of the Sinai Peninsula. *Mar. Biol.* 14: 111-119
- Pruesse, E., Peplies, J., and Glöckner, F.O. (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28: 1823–1829.
- Pueyo Mur, J.J. and Inglés Urpinell, M. (1987) Magnesite formation in recent playa lakes, Los Monegros, Spain. *Geol. Soc. London, Spec. Publ.* 36: 119 LP-122.
- Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J.F., et al. (2013) Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 499: 431–437.
- Robertson, C.E., Spear, J.R., Harris, J.K., and Pace, N.R. (2009) Diversity and stratification of archaea in a hypersaline microbial Mat. *Appl. Environ. Microbiol.* 75: 1801–1810.
- Samper-Calvete, F.J. and García-Vera, M.A. (1998) Inverse modeling of groundwater flow in the semiarid evaporitic closed basin of Los Monegros, Spain. *Hydrogeol. J.* 6: 33–49.
- Spang, A., Caceres, E.F., and Ettema, T.J.G. (2017) Genomic exploration of the diversity, ecology, and evolution of the archaeal domain of life. *Science* 357:563
- Triadó-Margarit, X. and Casamayor, E.O. (2013) High genetic diversity and novelty in planktonic protists inhabiting inland and coastal high salinity water bodies. *FEMS Microbiol. Ecol.* 85: 27–36.
- Triadó-Margarit X, JA Capitan, M Menendez-Serra, R Ortiz-Alvarez, VJ Ontiveros, EO Casamayor, D Alonso. (2019) A Randomized Trait Community Clustering approach to unveil consistent environmental thresholds in community assembly. *ISME J* 13: 2681–2689
- Ventosa, A., Fernández, A.B., León, M.J., Sánchez-Porro, C., and Rodríguez-Valera, F. (2014) The Santa Pola saltern as a model for studying the microbiota of hypersaline environments. *Extremophiles* 18: 811–824.
- Ventosa, A., de la Haba, R.R., Sánchez-Porro, C., and Papke, R.T. (2015)

558 Microbial diversity of hypersaline environments: A metagenomic approach.
 559 *Curr. Opin. Microbiol.* **25**: 80–87.
 560 Wu QL, Zwart G, Schauer M, Kamst-van Agterveld MP, and Hahn MW (2006).
 561 Bacterioplankton Community Composition along a Salinity Gradient of
 562 Sixteen High-Mountain Lakes Located on the Tibetan Plateau, China. *Appl*
 563 *Environ Microbiol.* 72: 5478–5485
 564 Williams, W.D. (1996) The largest, highest and lowest lakes of the world: Saline
 565 lakes. *SIL Proceedings, 1922-2010* **26**: 61–79.
 566 Williams, T. J., et al. (2014) Microbial ecology of an Antarctic hypersaline lake:
 567 genomic assessment of ecophysiology among dominant haloarchaea.
 568 *ISME J* 8: 1645-1658
 569
 570

571 **Data Accessibility**

572 DNA sequences. The complete raw data set is available in GenBank under
 573 BioProject record ID PRJNA429605.

Tables

Table 1. Range of environmental parameters measured in the different salt lakes in Monegros

Table 2. OTU richness, occurrence and abundances for the archaeal lineages found in Monegros

FIGURE LEGENDS

Fig 1. Relationship between archaeal percentage in the prokaryotic 16S rRNA PCR amplicon mixture and salinity (% w/v) for the complete dataset analyzed.

Fig 2. Relative abundances of the main archaeal lineages along the salinity gradient, and averaged values for the three traditional saline ranges, i.e., <5% (saline), 5-15% (hypersaline), and >15% (highly hypersaline), respectively.

Fig 3. Relative abundances and OTU richness for the four most abundant groups (Woesearchaeota, Pacearchaeota, Nanohaloarchaeota, Halobacteria) along the salinity gradient. Correlations shown for the three traditional saline ranges, i.e., <5% (saline), 5-15% (hypersaline), and >15% (highly hypersaline), respectively. Spearman's rho correlation and p value are shown when significant; *ns* = non-significant.

Fig 4. NMDS representation based on the genetic distances among OTUs in the three main salinity ranges (<5% saline, 5-15% hypersaline, and >15% highly hypersaline). Each OTU from the same lineage is connected in the two-dimensional space through the centroid. Point sizes show relative abundances.

Fig 5. Community ecology descriptors for the main phyla of the Archaea assemblage in Monegros saline lakes. Nucleotidic diversity related to both lagoon occurrence (**A**) and environmental amplitude (**B**). Spearman's rho correlation shown when significant. Evenness (Pielou's index) based on abundance distribution related to group OTUs richness (**C**). "Colonization and extinction" turnover rates (day^{-1}) based on the absence/presence of the local communities in consecutive sampling times (**D**). Point sizes show OTU richness.

SUPPLEMENTAL MATERIAL

Table S1: Detailed information on sampling date, sampling site, water height (cm), salinity (% w/v), conductivity (mS/cm), water temperature (°C), pH, redox conditions (mV), dissolved oxygen (mg/l) and Chl a, for each sample in Monegros.

Table S2: Accession numbers for the most abundant OTUs sequences uploaded to Genbank. Additional accession numbers for Halobacteria and Nanohaloarchaeota are shown in Fig S1.

Fig S1. ARB maximum parsimony tree provide by default with short 16S rRNA gene sequences added using the tool “add species to existed tree / ARB-parsimony tool interactive”. The most abundant OTUs (>0.01%) of Halobacteria and Nanohaloarchaeota from the most hypersaline samples (>35% salinity) are included. Genbank accessions numbers are shown.

Table 1. Range of environmental parameters measured

Lagoon/pond	Salinity (%, w/v)	Conductivity (S/m)	Temperature (°C)	pH	DO (mg/l)	Redox (mV)	Chl <i>a</i> (µg/l)
Guallar	2.8-23	26.2-197.6	2-26.5	7.6-8.4	8.8-12.8	145.5-350	0.9-10
Pinol	1.2-8.4	14.4-103	5-25.1	8-8.8	8.9-13.3	187.1-315	0.9-27.9
Muerte	2-32	20.3-207.4	7.5-32	7.3-8.61	7.4-12.8	224-312	0.9-5.2
Camaron	1.2-15.4	14.2-164.7	2.3-29.5	8.0-9.0	8.8-14.1	220-305	0.5-46
Pez	1.1-13.8	8.5-165.5	2-31	7.8-8.42	8.4-13.1	200-320	1.1-6.7
Rollico	0.8-5.8	5.6-77.6	8.7-21.5	8-8.8	9.1-12.8	205-345	0.7-13.7
Rebollon	1-2.4	5.1-27.2	7.5-14.8	8.1-9.1	10.4-14.6	225-340	1-27.1
Pito	1.2-28	12-94.1	1.5-34.5	7.5-8.3	7.5-12.7	240-335	1.1-6.6
Salineta	6.9-40	66.3-167.9	7.4-34	7.7-9.2	7.8-15.7	188.6-315	2.4-237.4
Playa	2.1-21	26.3-194.5	4-3	8-8.8	7.3-12.4	144-312	0.4-29.6
Amarga Alta	0.9-1.7	3.6-15.6	3-15	7.9-8.4	10.9-12.5	248-326	0.5-6.8
Amarga Baja	0.9-1.8	4.3-15.6	1.5-11.2	7.9-9.2	10.5-13.1	242-339	0.7-11.4
Salobral	2.9-3.8	31.3-47.1	5.9-30.3	8.8-9.5	8.1-14.2	122.6-341	1.7-15.5
Balsa Molinos	0.1-0.3	0.7-0.9	8-17.4	8.3-9.1	9.8-12.1	195-240	3-9.7

Table 2. OTU richness, occurrence and relative abundances for the archaeal lineages in Monegros

Archaeal lineage	OTUs	Occurrence (%)	Max. abundance (%)*
DPANN: Woesearchaeota	402	123 (96,09%)	3,34
DPANN: Pacearchaeota	376	112 (87,5%)	4,98
EURY: Halobacteria	105	105 (82,03%)	22,08
DPANN: Nanohaloarchaeota	62	63 (49,22%)	23,94
EURY: Thermoplasmata	19	30 (23,44%)	2,99
TACK: Thaumarchaeota	8	44 (34,38%)	0,23
Archaea unclass.	6	14 (10,94%)	0,27
DPANN: Aenigmarchaeota	5	24 (18,75%)	0,35
EURY: Unclass.	2	5 (3,91%)	0,01
Asgard: Lokiarchaeota	1	5 (3,91%)	0,04
EURY: Methanomicrobia	1	3 (2,34%)	0,04
EURY: WSA2	1	2 (1,56%)	0,02
EURY: Archaeoglobi	1	3 (2,34%)	0,01
EURY: Miscellaneous Group (MEG)	1	1 (0,78%)	0,01

* Relative abundances in the whole prokaryotic amplicon mixture

Archaea percentage on the pool of 16S

$\rho = 0.68$
 $p \text{ value} < 0.001$

Salinity % (w/v)

DPANN: Nanohaloarchaeota

DPANN: Pacearchaeota

EURY: Halobacteria

DPANN: Woesearchaeota

● — Relative abundance of the group on the sample ● — OTU richness of the group on the sample * p value < 0.05 ** p value < 0.01

■ **DPANN: Aenigmarchaeota** ■ **DPANN: Nanohaloarchaeota** ■ **DPANN: Pacearchaeota** ■ **DPANN: Woesearchaeota** ■ **EURY: Halobacteria** ■ **EURY: Thermoplasmata** ■ **TACK: Thaumarchaeota**

A **B** **C** **D**

Table S1.

Sample code	Lagoon	Sampling date (dd/mo/yr)	Water height (cm)	Salinity (% w/v)	Conductivity (mS/cm)	Temperature (°C)	pH	Redox (mV)	Dissolved oxygen (mg/l)	Chl <i>a</i> (µg/l)
MON_009	Amarga Alta	13/11/12	10	1	4.7	8.6	7.9	326	11.8	0.5
MON_022		5/12/12	5	1.2	8.1	7.8	7.9	290	11.3	0.6
MON_035		10/1/13	5	1.7	15.6	10	8.0	285	11.1	3.0
MON_055		21/3/13	2	1	10.4	15	8.4	265	12.5	0.6
MON_098		28/11/13	20	0.9	3.6	3.0	8.1	320	12.1	3.4
MON_111		19/12/13	5	0.9	5.3	7.5	8.2	275	10.9	6.8
MON_124		23/1/14	8	1	8.7	8.5	8.0	248	11.8	0.9
MON_148		23/12/14	25	1	6.95	6.3	8.25	299	11.1	1.2
MON_008	Amarga Baja	13/11/12	5	0.9	4.6	6.3	8.2	339	12	1.2
MON_021		5/12/12	2	1.2	7.8	5.9	7.9	242	11.9	0.7
MON_034		10/1/13	3	1.8	15.6	9.5	8.0	256	11.6	0.8
MON_054		21/3/13	2	1.5	13.3	11.2	8.6	258	11.8	0.9
MON_097		28/11/13	4	0.9	4.3	1.5	9.2	280	11.7	5.5
MON_110		19/12/13	3	0.9	6.4	7.9	8.3	270	10.5	11.4
MON_123		23/1/14	2	1.1	10.3	8.0	8.0	244	11.6	1.4
MON_147		23/12/14	3	0.9	5.95	7.2	8.34	296	13.1	1.9
MON_011	Camarón	13/11/12	40	1.2	14.2	11.7	8.3	305	12.2	0.5
MON_030		9/1/13	40	2.5	27	7.0	8.3	250	12.6	6.0
MON_046		23/2/13	30	3.1	34.3	2.3	8.0	288	12.4	1.8
MON_050		20/3/13	20	3.2	39.8	15.3	8.4	288	10.3	5.7
MON_063		16/4/13	10	4.3	68	29.5	9.0	220	12.3	4.2
MON_071		26/5/13	5	9.8	117.3	22.5	8.2	220	8.8	3.9
MON_093		27/11/13	15	2	22.7	10	8.3	270	10.9	9.5
MON_106		17/12/13	5	3.1	38.5	9.1	8.6	235	14.1	1.0
MON_119	Guallar	22/1/14	3	4.6	59.2	11.4	8.3	270	10.8	4.3
MON_129		14/3/14	1	15.4	164.7	10	8.0	242	9.7	46
MON_139		22/12/14	10	4.3	51.3	8.5	8.26	262	11.6	2.1
MON_014		4/12/12	10	4	40	10.5	8.1	350	10.6	3.7
MON_027		9/1/13	8	6	65.3	4.5	8.4	225	12.8	0.9
MON_040		22/2/13	10	10.5	118.9	9.4	8.2	244	10.6	1.6
MON_047		20/3/13	10	6.8	80.6	13.6	8.2	245	10.3	5.9
MON_060		16/4/13	3	16.9	166.4	23.3	7.9	270	8.8	2.9
MON_069	Molinos	26/5/13	0.5	23	197.6	26.5	7.6	270	8.8	10
MON_090		27/11/13	10	2.8	26.2	2.0	8.1	250	12.1	1.1
MON_116		22/1/14	6	5.8	67.4	8.5	8.4	265	11.6	1.7
MON_136		22/12/14	10	3.95	42	7.9	8.35	145	10.65	2.5
MON_077		26/5/13	NA	0.1	0.9	17.4	8.3	195	9.8	3.0
MON_128		22/1/14	40	0.3	0.7	8.0	9.1	240	12.1	9.7
MON_017	Muerte	4/12/12	2	3.4	37.8	12	8.1	312	10.3	5.2
MON_029		9/1/13	5	5.8	65.6	7.8	8.2	260	12.3	0.9
MON_062		16/4/13	1	32	207.4	32	7.3	290	7.4	2.1
MON_092		27/11/13	5	2	20.3	7.5	8.3	270	11.2	1.4
MON_118		22/1/14	3	5.9	71.1	9.7	8.3	275	11.3	2.9
MON_138		22/12/14	6	4	44.8	8.4	8.61	224	12.8	1.5
MON_020	Pez	4/12/12	4	2.1	21.7	8.1	8.0	287	11.1	4.2
MON_033		9/1/13	3	3.3	38	8.3	8.2	280	12.7	3.6
MON_045		23/2/13	10	2.3	26.6	2.0	8.0	285	12.5	2.1
MON_053		20/3/13	5	4	50.1	14	8.3	303	10.1	6.6
MON_064		16/4/13	2	13.8	165.5	31	7.8	260	8.4	6.4
MON_073		27/5/13	2	6	79.1	21.5	8.1	200	9.1	6.7
MON_096		27/11/13	2	1.1	8.5	5.2	8.0	320	11.6	1.1
MON_122		23/1/14	6	2.9	35	11.5	8.0	300	10.5	4.3
MON_142	Piñol	22/12/14	5	1.6	17.68	8.1	8.4	279	13.1	3.2
MON_002		12/11/12	13	1.2	16	13.5	8.6	315	11.3	5.4
MON_015		4/12/12	10	2	21.6	12	8.3	305	10.6	8.4
MON_028		9/1/13	10	4	32.5	5.5	8.7	206	13.3	1.4
MON_048		20/3/13	8	4	49.6	15	8.6	275	10.5	9.6
MON_061		16/4/13	5	7.8	102.2	24.9	8.8	235	10.6	6.3
MON_070		26/5/13	1	8.4	103	25.1	8.6	220	8.9	27.9
MON_091		27/11/13	8	1.6	14.4	5.0	8.0	245	11.8	0.9
MON_117	Pito	22/1/14	10	4.1	49	9.0	8.3	272	11.8	5.4
MON_137		22/12/14	8	2.9	31.4	8.1	8.39	187	11.9	2.3
MON_010		13/11/12	15	1.5	18.3	13.9	7.8	330	11.7	6.2
MON_023		5/12/12	4	2.3	25	9.2	8.0	302	10.7	2.3
MON_036		10/1/13	10	3	36.5	12	8.0	295	10.5	4.2
MON_044		23/2/13	10	4.8	53.4	1.5	7.9	293	12.7	3.8
MON_056		21/3/13	5	3.8	45.4	13.9	8.0	240	11	6.1
MON_065		17/4/13	3	7	94.1	22.1	8.1	325	9.7	6.6
MON_078		12/6/13	0.2	28	NA	34.5	7.5	260	7.5	1.8
MON_099		28/11/13	10	1.2	12	3.5	8.0	295	12.6	1.1
MON_112		19/12/13	5	1.7	20	6.7	8.3	275	11.6	1.4
MON_125		23/1/14	8	2.8	34.5	11.2	8.0	304	10.8	2.7
MON_146		23/12/14	15	2.8	30.7	6.1	8.2	335	11.7	1.5

MON_012	Playa	13/11/12	10	2.1	26.3	14.4	8.8	306	12	1.2
MON_024		5/12/12	15	3	31	8.5	8.2	303	11.5	0.4
MON_037		10/1/13	30	4.5	49.7	11.3	8.4	270	11.6	0.5
MON_041		22/2/13	40	5.9	67	11	8.5	230	11.3	3.3
MON_057		21/3/13	30	5.8	67.5	16.3	8.6	250	12.4	5.9
MON_066		17/4/13	40	10.1	124.4	23.5	8.1	312	9.4	3.8
MON_074		26/5/13	40	10.6	117.6	17.5	8.7	225	9.2	14
MON_079		12/6/13	25	14.8	169.3	27.9	8.1	225	8.8	11.7
MON_083		8/7/13	30	16.2	168.1	30	8.2	230	7.3	4.0
MON_100		28/11/13	40	5	58.1	4.0	8.0	280	11.7	0.8
MON_113	Rebollón	18/12/13	25	6	78.3	8.5	8.4	144	12	8.0
MON_126		22/1/14	30	6.6	86.4	10.8	8.6	285	11.5	6.0
MON_130		14/3/14	25	11.5	134.9	10	8.4	215	9.9	29.6
MON_133		30/4/14	20	21	194.5	25	8.0	215	10.1	23.6
MON_145		23/12/14	40	4.9	55.6	6.0	8.7	295	11.8	5.2
MON_004		12/11/12	5	1	7.8	14.3	8.2	340	10.4	1.3
MON_019		4/12/12	5	1.8	15.7	9.1	8.1	280	10.9	1.3
MON_032		9/1/13	3	2.4	27.2	9.5	8.8	245	14.6	1.0
MON_052		20/3/13	5	2.2	26.2	14.8	9.1	258	10.5	13.2
MON_095		27/11/13	15	1	5.1	7.5	8.1	305	11.6	1.8
MON_108	Rollico	18/12/13	3	1.1	12.4	10	9.1	242	12.6	27.1
MON_141		22/12/14	3	1.5	14.57	8.9	8.8	225	14.3	1.6
MON_005		12/11/12	10	1.1	11.6	12.5	8.6	345	10.5	1.7
MON_018		4/12/12	10	2	20.4	10	8.1	318	10.3	6.7
MON_031		9/1/13	5	3.3	37.8	9.5	8.4	238	12.2	0.7
MON_051		20/3/13	5	3.2	40.3	15.4	8.2	245	10	13.7
MON_072		27/5/13	2	5.8	77.6	21.5	8.1	205	9.1	2.8
MON_094		27/11/13	5	0.8	5.6	9.0	8.1	305	11	1.9
MON_107		18/12/13	5	1	13.3	11.3	8.8	230	11.5	2.8
MON_120		23/1/14	6	2.1	28.7	11.3	8.0	290	10.6	10.6
MON_140	Salineta	22/12/14	4	1.8	18.36	8.7	8.28	261	12.8	1.0
MON_013		13/11/12	13	6.9	66.3	13.1	9.2	278	10.6	79.8
MON_025		5/12/12	25	7.8	77.1	8.2	9.0	286	12.1	237.4
MON_038		10/1/13	50	10	94.3	12	8.7	275	11.8	71.3
MON_042		22/2/13	30	15	128.8	9.4	8.4	246	10.4	35.8
MON_058		21/3/13	30	11	107.5	16.1	8.4	270	10.1	40.9
MON_067		17/4/13	15	24	156.8	28	8.1	188	9.0	23.8
MON_075		26/5/13	5	23	152	19.1	8.2	235	12.9	85.7
MON_080		12/6/13	5	35	150.3	34	7.9	220	9.5	54.3
MON_084		8/7/13	1	38	142	32.5	7.9	230	7.9	5.6
MON_088	Salobral	9/10/13	1	40	143.3	18.8	7.7	265	8.9	2.4
MON_101		28/11/13	10	7.5	67.5	7.4	NA	315	11.6	3.7
MON_114		18/12/13	10	9	85.6	11	8.4	245	15.7	54.7
MON_127		23/1/14	15	11.2	105.2	12.2	8.8	278	11.1	201.5
MON_131		14/3/14	10	24.2	167.9	10	8.0	235	10.7	95.5
MON_134		30/4/14	1	37	129.3	24	7.8	235	7.8	87.3
MON_007		13/11/12	20	3.3	31.7	5.9	9.0	341	9.4	2.9
MON_026		5/12/12	30	3.2	31.3	8.0	8.8	275	13.5	1.7
MON_039		10/1/13	40	3.2	32	11	8.9	280	13.8	2.9
MON_043		22/2/13	30	3.7	35.6	10.1	8.9	210	11.5	3.8
MON_059	MON_085	21/3/13	50	3	31.3	16	8.9	230	10.8	15.5
MON_068		17/4/13	40	2.9	34.3	25.8	9.3	170	8.2	4.1
MON_076		26/5/13	40	3.4	36.2	20	9.5	180	14.2	4.4
MON_081		12/6/13	40	3	42.4	30.3	9.1	190	11.5	2.8
MON_085		8/7/13	30	3.8	47.1	29.6	8.8	122	8.1	5.4

Table S2. GenBank accession numbers for the most abundant OTUs for Aenigmarchaeota
Pacearchaeota, Thermoplasmata, and Woesearchaeota

Archaeal lineage	OTU id	Genbank accession number
Aenigmarchaeota	OTU_714	MH024548
	OTU_314	MH024528
	OTU_390	MH024534
	OTU_435	MH024536
Pacearchaeota	OTU_671	MH024546
	OTU_721	MH024549
	OTU_4358	MH024585
	OTU_9144	MH024587
Thermoplasmata	OTU_274	MH024525
	OTU_316	MH024526
	OTU_772	MH024547
Woesearchaeota	OTU_146	MH024522
	OTU_171	MH024524
	OTU_305	MH024530
	OTU_388	MH024533
	OTU_377	MH024535
	OTU_413	MH024537
	OTU_463	MH024538
	OTU_559	MH024541
	OTU_530	MH024543
	OTU_549	MH024544
	OTU_561	MH024545
	OTU_872	MH024551
	OTU_810	MH024552

Fig S1. GenBank accession numbers for the most abundant OTUs of Halobacteria and Nanohaloarchaeota found in Monegros