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Studies of sediments, dry mud and water samples from lake elmenteita reveal the presence of thaumarchaeota and euryarchaeota

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Abstract

So as to provide new insight into the diversity of Archaea in dry mud, sediment and water from Lake Elmenteita in Kenya, an investigation using a culture-independent approach was conducted after extraction of total genomic DNA from the environmental samples using suitable extraction methods. Small insert clone libraries were constructed by amplifying 16S rRNA genes using archaea-specific primers, followed by cloning from which 94 non-chimeric sequences were obtained and a total of 34 operational taxonomic units (OTUs) were identified. The OTUs were grouped into *Thaumarchaeota* (6%) and *Euryarchaeota* (94%). Approximately 90% of the clones were related with genes from uncultured Archaea, compared to the 10% that showed affiliation with genes from previously cultured Archaea. The similarity of the sequenced clones to type strains was consistently lower than the similarity to uncultured members of Archaea. Clones from water and sediment were dominated by sequences from Euryarchaea, while clones from the dry mud samples showed affiliations to both *Euryarchaeota* and *Thaumarchaeota*, with the predominant phylum being *Euryarchaeota*. This is the first study reporting the presence of representatives of *Thaumarachaeota* from Lake Elmenteita and adds sequences from Lake Elmenteita to the developing database of 16S rRNA clone libraries obtained from environmental sources.

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Introduction

Archaea are found in a wide range of habitats such as soils, oceans, lakes, marshland and plankton, among others. Ever since 16S rRNA sequence comparisons were first used to identify Archaea in bacterioplankton samples from the Pacific Ocean (Fuhrman *et al*., 1992), the diversity of Archaea in both extreme and moderate environments has been established by different molecular phylogenetic studies (Bintrim *et al*., 1997, Burggraf *et al*., 1997, Callieri *et al*., 2009, Hu *et al*., 2011). Dawson *et al*., (2006), have postulated that there are four main phyla of Archaea, namely *Euryarchaeota*, *Crenarchaeota*, *Korarchaeota* and *Nanoarchaeota*. Another phylum, *Thaumarchaeota*, was suggested after the genome of *Cenarchaeum symbiosum* was sequenced and found to differ significantly from other members of the hyperthermophilic phylum *Crenarchaeota*. Initially, they were referred to as mesophilic Crenarchaeota and later renamed to *Thaumarchaeota*, based on the genome sequence of *Cenarchaeum symbiosum* strain A, which was discovered in marine environment (Brochier-Armanet *et al*., 2008). Various studies have shown that *Thaumarchaeota* can grow at temperatures of up to 74°C (Hatzenpichler *et al*., 2008; de la Torre *et al*., 2008). To date, there are only two well-characterized *Thaumarchaeota* (Müller *et al*., 2010). Preston *et al*., 1996 were the first to describe the psychrophilic archaeon, *C. symbiosum*, while Konneke *et al*., 2005 isolated and characterized the ammonia-oxidizing marine archaeon, candidatus *Nitrosopumilus maritimus*. Therefore, identification of *Thaumarchaeota*

Various studies that have been conducted in Kenyan soda lakes, using either traditional cultural methods or modern molecular techniques or both, have revealed diverse groups of halophilic bacteria and archaea (Tindall *et al*., 1984; Duckworth *et al*., 1996, Jones *et al*., 1998, Grant *et al*., 1999, Baumgarten *et al*., 2003, Rees *et al*., 2004, Mwirichia *et al*., 2010). Work done on Lake Elementaita by Mwirichia *et al*., 2010, using Archaea-specific primers and 16S rDNA cloning, indicate the presence of the members of the domain Archaea mainly belonging to *Halobacteriaceae*, *Methanomicrobiales* and *Methanosarcinales*. Within the soda lake ecosystem, hot springs may be found, and these have been shown to be predominated by hyperthermophilic bacteria such as *Thermotoga* and *Aquifex*, and by hyperthermophilic archaea such as the *Nanoarchaea*. Amylases, lipases and proteases within these hyperthermophiles have been targeted for various biotechnological applications such as the detergent and starch production industries. Lake Elmenteita contains one such seasonal hot spring (Kekopey hot springs) that may habor such hyperthermophilic Archaea that may be of biotechnological importance. To our knowledge, previous studies on Lake Elmenteita have not been able to detect any *Thaumarchaeota*. Thus there is need for further studies on this ecosystem.

However, despite the various studies conducted, the actual diversity of Kenyan soda lakes including Lake Elmenteita is still unknown since most microorganisms in these environments are difficult to culture and isolate. Thus, there is need to use culture-independent techniques to capture the diversity both cultured and not-yet-cultured microorganisms from the samples. Through the use of metagenomics, scientists are able to access complete genes and pathways without any prior knowledge of sequence information of the target gene, enabling the discovery of new and previously unknown genes and products (Handelsman, 2004). The present study aimed to assess the diversity of Archaea in water, sediment (mud) and dry mud samples from Lake Elmenteita using metagenomic techniques. To be able to capture any Archaea that may have been missed during previous studies, a different set of primers (A109f and A915r) and a different DNA extraction method were used compared to those used in a previous study by Mwirichia *et al*., (2010) who used arc8f and arc1492r as primers. In this study we report the presence of representatives of *Thaumarachaeota* from Lake Elmenteita. The sequences obtained in this study

adds to the developing database of 16S rRNA clone libraries obtained from environmental sources.

Materials and methods

Study site

Lake Elmenteita is a shallow lake located 20 km to the south-east of Nakuru town and 120 km northwest of Nairobi in Kenya (0°27'S, 36°15'E) at 1776 meter above sea level (Melack 1988, Mwirichia *et al*., 2010). The average area of the lake fluctuates between 15 and 22 km² while its average depth is 0.7 meters. Important feeders for the lake include Mereroni River which is the main source of water for Lake Elmenteita (Sorokin *et al*., 2011, Bennun & Njoroge, 1999). Other feeders are Chamuka and Mbaruk rivers, Kariandusi Springs, and Kekopey hot springs, (Bennun & Njoroge, 1999). The area around the lake experiences unpredictable rainfall which seldom exceeds 600 mm annually and the lake can completely dry out during some seasons to form concentrated salt pans (Mwirichia *et al*., 2010). The water temperatures ranges between 30°C to 40°C and high pH above 9 is also recorded (Mwaura, 1999, Mwirichia *et al*., 2010). The area is full of volcanic rocks while the soil type is silt clay.

Sample collection

Sampling was done on 28th August 2010. The sampling sites were distributed in a manner that helped to capture the lake's spatial variability. Replicate water samples were collected using sterile 250 ml bottles. The pH and temperature were recorded on site. The samples were transported to the laboratory immediately, in a cooler (4 °C), for filtration. They were filtered first through a 0.45 µm Nucleopore filters (GF/F, Whatman) and then a 0.22 µm pore size filters (Type GS; Millipore) using a vacuum pump. The filter papers were packed in sterile aluminium foil and transported under dry ice to DSMZ, Braunschweig, then stored in -80°C in the laboratory until further analysis. Dry mud (soil) were collected on the shores of the lake while sediment samples (100 g) were collected at the same point as the water samples by scooping from between 10-20 cm depth using a sterile shovel. The samples were labelled and put in pre-sterilized falcon tubes, then in sterile Ziploc bags, before being transported immediately in a cool box on ice to the laboratory in JKUAT, Nairobi. Once at the laboratory, the samples were stored at 4°C. The samples were transported under dry ice to DSMZ laboratories for further analysis. On arrival at DSMZ the samples were lyophilized, ground into a fine powder with sterile mortar and pestle and then stored at -80°C until processing.

DNA extraction and purification

High molecular weight environmental DNA was extracted from sediment and soil (dry mud) samples from Lake Elmenteita using the freeze-thaw DNA extraction method, modified from the protocol used by Zhou *et al*., (1996). The DNA was purified using the Phenol: Chloroform: Isoamylalcohol (25: 24: 1) method (Sambrook & Russel, 2001). To further purify the DNA, pulsed field gel electrophoresis (PFGE) was performed and the DNA of the appropriate size was extracted from the gel using the PeqGold gel extraction kit (PeqLab Biotechnologie GmbH, Erlangen, Germany). Extraction of genomic DNA from water samples was done using the genomic Nucleospin kit for soil samples (Machenery, Germany). The method was modified from that used to extract soil, to suit and optimize the extraction of genomic DNA from water samples. The total genomic DNA was later used as a template in PCR reactions to amplify 16S rRNA.

PCR amplification and 16S rRNA clone library construction

The following oligonucleotide primers were used for PCR amplification to generate PCR fragments of approximately 800bp: forward primer (A109F) (Großkopf *et al*., 1998) and reverse primer (A915R), (DeLong, 1992, Chin *et al*., 1999). The PCR mixture (50µl) contained 1 µl of template DNA (100 ng/µl), 1 µl of forward and reverse primers (10 mM each), 10 µl of 10X PCR buffer, 1.25 µl of deoxyribonucleotide triphosphates (10 mM each), 0.3 µl Taq DNA

polymerase (3 units/ml) and 1 µl of 10× bovine serum album (BSA). PCR cycle was modified from that used by Ochsenreiter *et al.*, 2002 as follows: Initial denaturation at 94 °C for 5 min was followed by 32 cycles of 45 s at 94 °C, 1 min at 52 °C and 2 min at 72 °C, followed by a final extension of 10 min at 72 °C. PCR products were purified using the QIAquick PCR Purification kit (Qiagen, Hilden). The purified PCR fragments were ligated into pGEM-T plasmid vector (Promega, Madison, WI) and transformed into *E. coli* DH10B competent cells (Sambrook & Russel, 2001) The presence of correct inserts was checked by performing PCR using the M13 primers (Promega, Madison, WI) which flank the cloning sites on the vector. Plasmid DNA was prepared from the positive clones according to the plasmid Miniprep Kit I (PEQLAB Biotechnologies GmbH), to obtain high copy/number plasmids.

Sequencing and Phylogenetic Analysis

Cycle sequencing of the inserts was done using the Applied Biosystems ABI prism 3730 automated DNA sequencer (Applied Biosystems, USA). Sequencing was performed using the Archaea-specific primers (A109F/915R). The generated sequence data was edited manually to remove low quality regions, typically at the beginning and end of the sequences (Neufeld *et al.*, 2004). The sequences were also checked for chimeric artifacts using Mallard (Ashelford *et al.*, 2006) and the CHIMERA_CHECK program (Maidak *et al.*, 2001), of the Ribosomal Database Project (RDP) II database (Cole *et al.*, 2003, Neufeld *et al.*, 2004). The cloned sequences were clustered into operational taxonomic units (OTUs) at 97% identity threshold using the CAP3 program (Huang and Madan, 1999). The taxonomic affiliation of the representative clones (one from each OTU) was determined using the Basic Local Alignment Tool (BLAST) of the National Center for Biotechnology Information (NCBI) database (Altschul *et al.*, 1990). The representative sequences of each OTU and selected closest similar references were aligned using multiple sequence alignments in ClustalW program. Construction of phylogenetic trees was performed using Maximum Likelihood analyses as determined using the Jukes Cantor method in Mega 6 (Jukes & Cantor, 1969, Tamura *et al.*, 2013). Computation of a majority rule consensus tree was made out of 1000 bootstrap tree replicates (Felsenstein, 1985).

Nucleotide sequence accession numbers

Nucleotide sequences generated in this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers KC753234 - KC753327.

Results

Physico-chemical characteristics of sampling sites

The sampling area was characterized by volcanic rock soil type to silty clay soil type. The sediment samples (obtained from the bottom of the lake) were silty clay. In sites 1, 3, and 4 temperatures ranged between 26.3°C and 27.9°C while the pH ranged from 8.3 to 8.4. In the hot spring (site 2), the temperature and pH were 56.7°C and 8.2 respectively. The temperature of the Kekopey' hot springs (site 2), found on the southern end of the lake, was slightly lower than expected, due to rain the previous day and morning of sampling day.

PCR, clone library construction and sequencing

In order to determine the archaeal diversity in Lake Elmenteita, four small insert clone libraries containing ~800 bp PCR fragments were successfully constructed from dry mud (DM1 and DM2), water (WA1) and sediment (SE1) samples using primers A109F and A915R. This resulted in a total of 1440 plasmid clones which were selected for PCR and sequencing. The average insert sizes of plasmids for PCR were estimated to be ~800 bp after restriction enzyme analysis of 20 randomly picked small insert clones using BamHI enzyme (Data not shown). 117 sequences were obtained, and after quality checking, 94 sequences remained for further analysis.

Phylogenetic analysis of archaeal clone library

To be able to get an accurate representation of the archaeal clones from Lake Elmenteita, sequences of type strains of Archaea from environmental sources

were obtained from Genbank and included in the phylogenetic analysis. Sequences of uncultivated members of Archaea were also included in the analysis. The sequences were classified according to sample type as well as according to phyla. Selected clones from a previous study on samples from Lake Elmenteita were included for comparison purposes (Mwrichia *et al*., 2010).

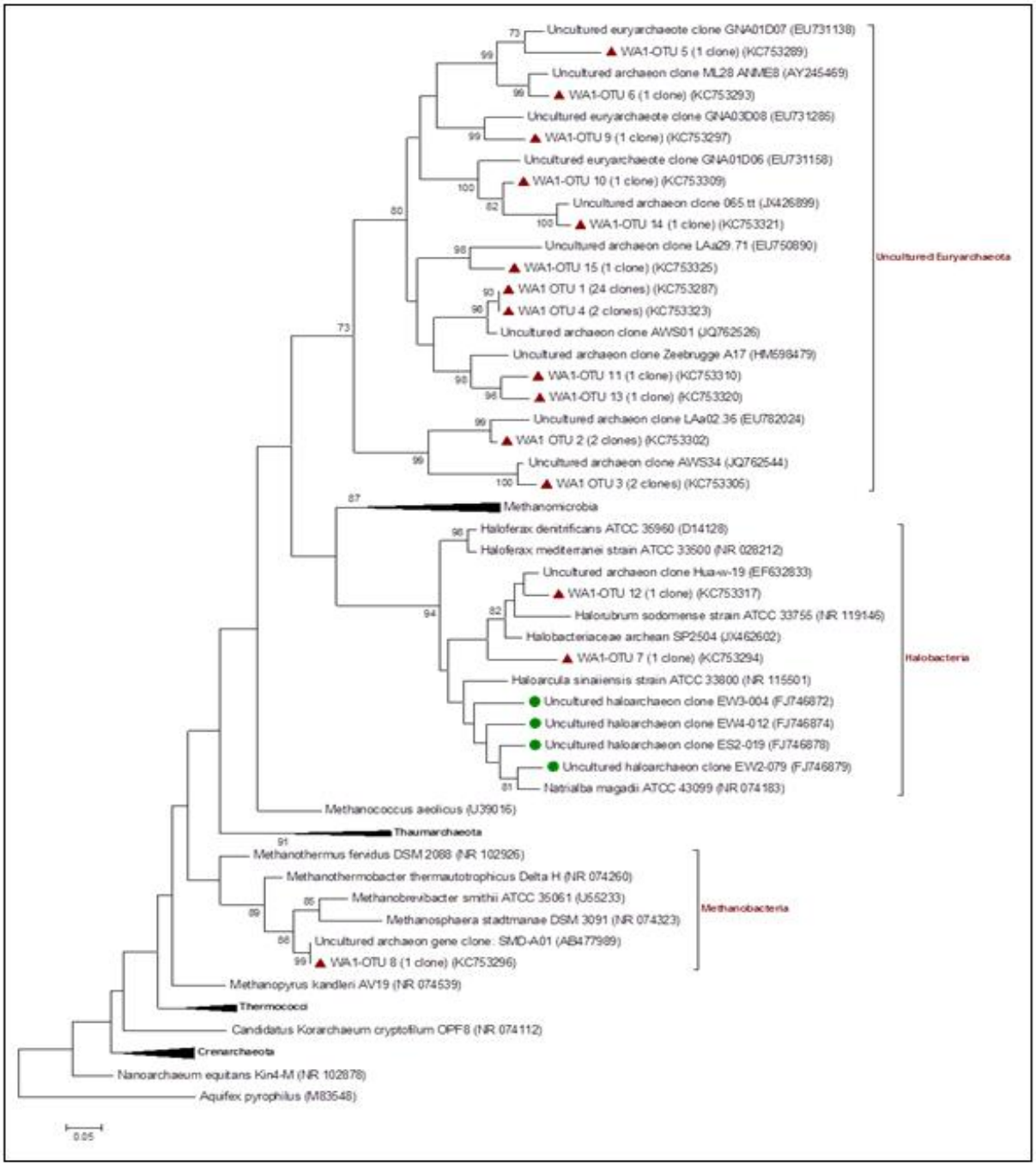

Fig. 1. 16S rRNA sequence-based phylogenetic tree of water samples from Lake Elmenteita. The number of clones within each OTU is shown and the gene accesion number of one representative clone type within each OTU is shown at the end in brackets. Bootstrap values are reported as percentages of 1000 bootstrap replications. The scale bar represents the substitutions per nucleotide position. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates are collapsed. *Aquifex pyrophilus* (M83548) is used as the outgroup.

Maximum Likelihood phylogenetic trees of the cloned 16S rRNA genes and their closest cultivated and uncultivated relatives from water samples (Fig. 1), dry mud samples (Fig. 2) and sediment samples (Fig. 3) were generated. Percentage relationships were described as per the calculations from the Maximum Likelihood analyses. The four libraries revealed a total of 34 different OTUs distributed within the *Euryarchaeota* and *Thaumarchaeota* phyla. There was a clear separation between the two phyla with majority (94%) of the cloned sequences showing affinity to *Euryarchaeota* and 6% related to *Thaumarchaeota*. None of clones clustered with members of either *Korarchaeota* or *Nanoarchaeota*. This may be due to the choice of primers used for amplification, which may not have been able to capture the genes of *Nanoarchaeota* and *Korarchaeota* from samples from Lake Elmeteita. The 41 sequences from water (WA) sample clone library were distributed into 15 OTUs, making it to have the highest phylotype richness as compared to the dry mud (DM) and sediment (SE) clone libraries, each having 11 OTUs and 8 OTUs respectively.

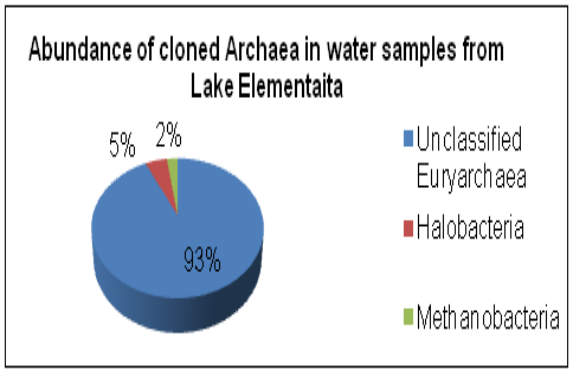

Fig. 2. Abundance of Archaea in water samples from Lake Elmenteita.

Archaeal diversity in water Samples

The phylogenetic tree analyses revealed close relatedness of all OTUs from water samples to the phylum *Euryarchaeota*. The dominant OTU from the WA clone library was OTU 1 comprising of 24 clones out of the total 41 clones. The 24 clones from OTU 1 and 2 clones from OTU 4 showed high similarity to clone AWS01 (Accession JQ762526) that was retrieved from Lake Awong Co on the Tibetan Plateau (Liu *et al.*, 2013). These uncultured euryarchaeal clones from water samples were found to be relatively similar to one another and clustered with clones from other aquatic environments. OTUs 7 and 12 clustered with sequences belonging to uncultured members of the *Halobacteriales*, OTU 7 showed 82% similarity to an uncultured halobacterial clone SP2504 (Accession JX462602) retrieved from Abijata Soda Ash Factory Pond System in Ethiopia, while OTU 12 had 54% relatedness to an uncultured archaeal clone Hua-w-19 (Accession EF632833), retrieved from aquatic environments of the high altitude Andean Altiplano in northern Chile. These clones also clustered together with clones (FJ746872, FJ746874, FJ746878 and FJ746879, retrieved from Lake Elmenteita by Mwirichia *et al.*, 2010. None of the clones clustered closely with type strains belonging to *Halobacteriales*. OTU 8 clustered with sequences belonging to members of *Methanomicrobiales*, a group of Archaea known to utilize H_2 and CO_2 for growth (Anderson *et al.*, 2009). It had 99% similarity to an uncultured archaeon clone (Accession AB477989) that was previously retrieved from a geothermal groundwater sample in Japan (Kimura *et al.*, 2010). 10 OTUs (OTU 1, 4, 5, 6, 9, 10, 11, 13, 14 and 15) clustered together with other uncultured members of the *Euryarchaeota* with percentage sequence similarities ranging between 73% and 100% as (Fig. 1). OTU 2 and 3 branched away from the rest of the OTUs but clustered closely with uncultured members of the *Euryarchaeota*. As shown in Fig. 2, 93% of the clones from water samples belonged to uncultured members of *Euryarchaeota*, while 5% belonged to Halobacteria and 2% to Methanogenic archaea.

Archaeal diversity in dry mud samples

4 OTUs (OTU 1, 2, 3, and 4) from the dry mud (DM1) clone library, consisting of 24 clones belong to the *Euryarchaeota* phylum within the *Halobacateriales* order. They clustered consistently with clones retrieved from various soda lake environments (Liu *et al.*, 2013, Antony *et al.*, 2012, Mwirichia *et al.*, 2010). OTU 9 had 60% similarity to the uncultured

methanomicrobiales archaeon clone ES5-50 (Accession FJ746884) from Lake Elmenteita, retrieved from brackish sediment (Hirschler-Réa *et al.*, 2012). OTU 10 clustered at 100% similarity together with an uncultured archaeon clone ArchSMTZ 12G (FR695321) that was retrieved from sediment samples from Aarhus Bay, Denmark (Webster *et al.*, 2011), suggesting relations to methanogens. The rest 5 OTUs (OTU 5, 6, 7, 8 and 11) of the DM2 clone library clustered together with uncultured clones of *Thaumarchaeota* as shown in fig. 3. None of the *Thaumarchaeota* clones from dry mud samples occurred at site 1 and they were all concentrated at site 2 (hot spring). The 5 OTUs and their related sequences were closely related to the ammonia-oxidizing Candidatus *Nitrosphaera gargensis* Ga9.2, (Spang *et al.*, 2012). Based on the phylogenetic inference, the thaumarchaeal sequences were mostly associated with uncultured 16S rRNA clones from various soil environments (Zhalnina *et al.*, 2014, Pester *et al.*, 2011, Nishizawa *et al.*, 2008, Quaiser *et al.*, 2002). The largest percentage (57%) of the clones belonged to halobacteria and 41% belonged to uncultured members of *Thaumarchaeota*. Only 2% were halobacteria (Fig. 4).

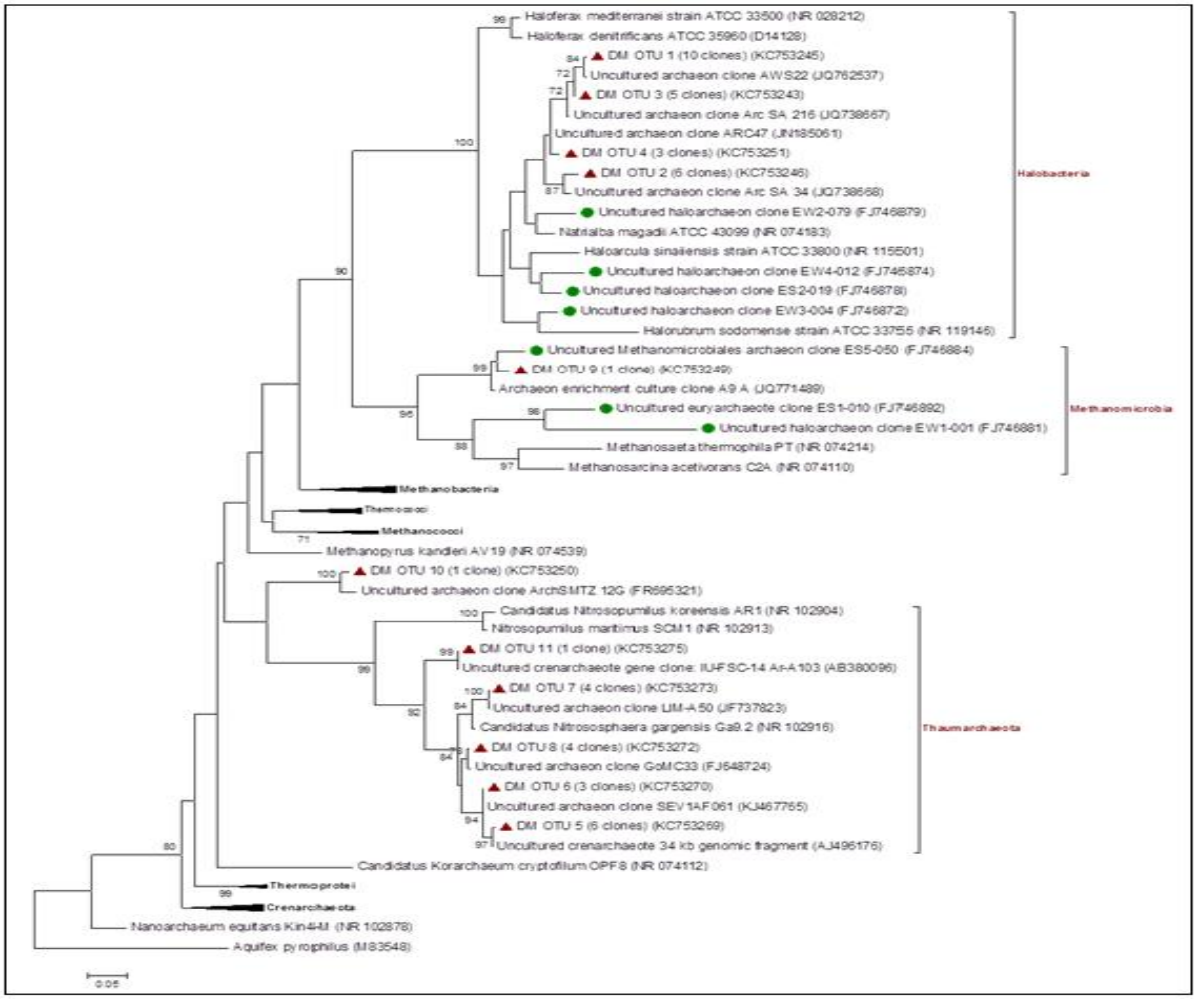

Fig. 3. 16S rRNA sequence-based phylogenetic tree of dry mud samples from Lake Elmenteita. The number of clones within each OTU is shown and the gene accesion number of one representative clone type within each OTU is shown at the end in brackets. Bootstrap values are reported as percentages of 1000 bootstrap replications. The scale bar represents the substitutions per nucleotide position. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates are collapsed. *Aquifex pyrophilus* (M83548) is used as the outgroup.

Archaeal Diversity in Wet Sediment Samples

8 OTUs were observed in the wet sediment (SE) clone library and as shown in Fig. 3 below, only OTU 2 containing 1 clone clustered together with members of the *Thaumarchaeota*, with a similarity of 100% to an uncultured archaeon clone Arch_J3_32 (KC925982) retrieved from sediment samples of Jiulongjiang and Minjiang, Fujian province of China. The rest of the OTUs were clustered with members of *Euryarchaeota*. Sediment OTU 1 and 4 comprised of clones affiliated with uncultured members of *Halobacteriales* from various lake environments as well as from Lake Elmenteita, while OTU 6 clustered with members of the order *Methanobacteriales*, showing 74% similarity to an uncultured archaeon clone (AB477989) from a geothermal groundwater sample in Japan (Kimura *et al.*, 2010). The *Methanomicrobia* were represented by OTUs 3 5, 7 and 8. OTU 3 clustered at 100% similarity with an uncultured clone Arc30 (JN185052) from soda lake sediments (Antony *et al.*, 2012). OTU 5 was closely related at 100% similarity to the uncultured archaeon clone AWS24 (JQ762539) retrieved from Lake Awong Co on the Tibetan Plateau (Liu *et al.*, 2013). On the other hand OTU 7 showed 99% similarity to the uncultured archaeon clone Hua6-s87 (EU481591) retrieved from sediment samples from a saline wetland in northern Chile (Dorador *et al.*, 2010) and OTU 8 clustered at 89% similarity with clone EW1-001 (FJ746881) retrieved from Lake Elmenteita (Mwirichia *et al.*, 2010). The sediment clones showed a more proportionally distributed representation of the various members of Archaea as compared to the other clones from water and dry mud samples (Fig. 6).

Discussion

Archaeal diversity in three types of sampling material (dry mud cake, sediment and water samples) from Lake Elmenteita (a soda lake environment) was investigated in this study. The primer pairs used were specific for Archaea and generated amplicons for *Euryarchaeota* as well as for *Thaumarchaeota*. The sequences obtained from the water, sediment and dry mud samples showed similarity to both cultivated and uncultivated Archaea from similar environments. *Euryarchaeota* were found to be the dominant archaeal group in all the samples. The halophiles and methanogens represented the major Euryarchaeal groups detected.

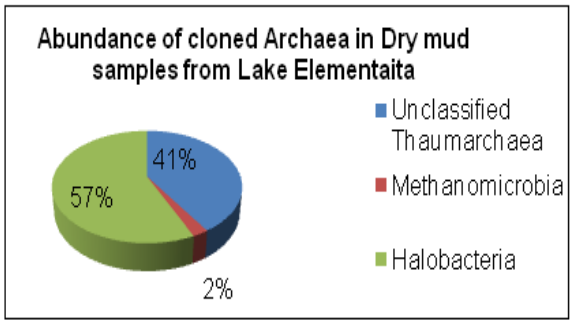

Fig. 4. Abundance of Archaea in dry mud samples from Lake Elmenteita.

The detection of genes related to methanogenic Archaea in the environmental samples indicates a predominant tolerance and utilization of H_2/CO_2 and the suitability of these environments for the survival of the retrieved methanogenic Archaea. Methanogens play a major role in the global carbon cycle (Etheridge *et al.*, 1998) and they have been shown to be associated with low temperature anoxic marine sediments as well as with lake water clones (Kim *et al.*, 2005). The group of unclassified *Euryarchaeota* from water samples (Fig. 1) may be indicative of a new group of *Euryarchaea* that might be playing unknown important roles in the biogeochemical cycles in saline lakes Elmenteita. However, more extensive research is needed to confirm this theory.

Besides high pH, soda lakes such as Lake Elmenteita have high levels of sodium carbonate and the optimal functioning of the biogeochemical cycle in these lakes is attributed to the utilization of the sulfur cycle. In particular, it is thought that the haloarchaeal organisms take part in the reductive and oxidative dissimilatory conversion of inorganic sulfur compounds and this could explain the predominance of chemolithoautotrophs and haloalkalophiles in soda lakes (Sorokin *et al.*, 2011). This theory ties in well with the observation of high numbers of haloarchaeal strains. The results are also in agreement with various

other studies which have indicated that ecological differentiation affect the diversity of Archaea in habitats (Teske *et al*., 2002, Sørensen *et al*., 2004, Clementino *et al*., 2001). Previous studies on Lake Elmenteita by Rees *et al*., (2004) and Mwirichia *et al*., (2010) had already shown a significant diversity of halobacteria, and the results in this study are in agreement as indicated by the predominant presence of *Halobacteriales*-related sequences in all the clone libraries (57% in dry mud samples, 34% in sediment samples and 5% in water samples). This indicates a predominant tolerance to NaCl. So far, various novel archaeal sequences have been reported in a wide range of habitats such as soda lakes (Jones *et al*., 1998, Grant *et al*., 1999), forest soils (Bintrim *et al*., 1997), open ocean waters (Fuhrman *et al*., 1992), and coastal waters (Massana *et al*., 1997), sediments (Vetriani *et al*., 1998) and hydrothermal vents (Takai & Horikoshi, 1999). Halophilic Archaea have been shown to be the dominant group of organisms in soda lakes (Yildiz *et al*., 2012, Mwirichia *et al*., 2010, Ochsenreiter *et al*., 2002).

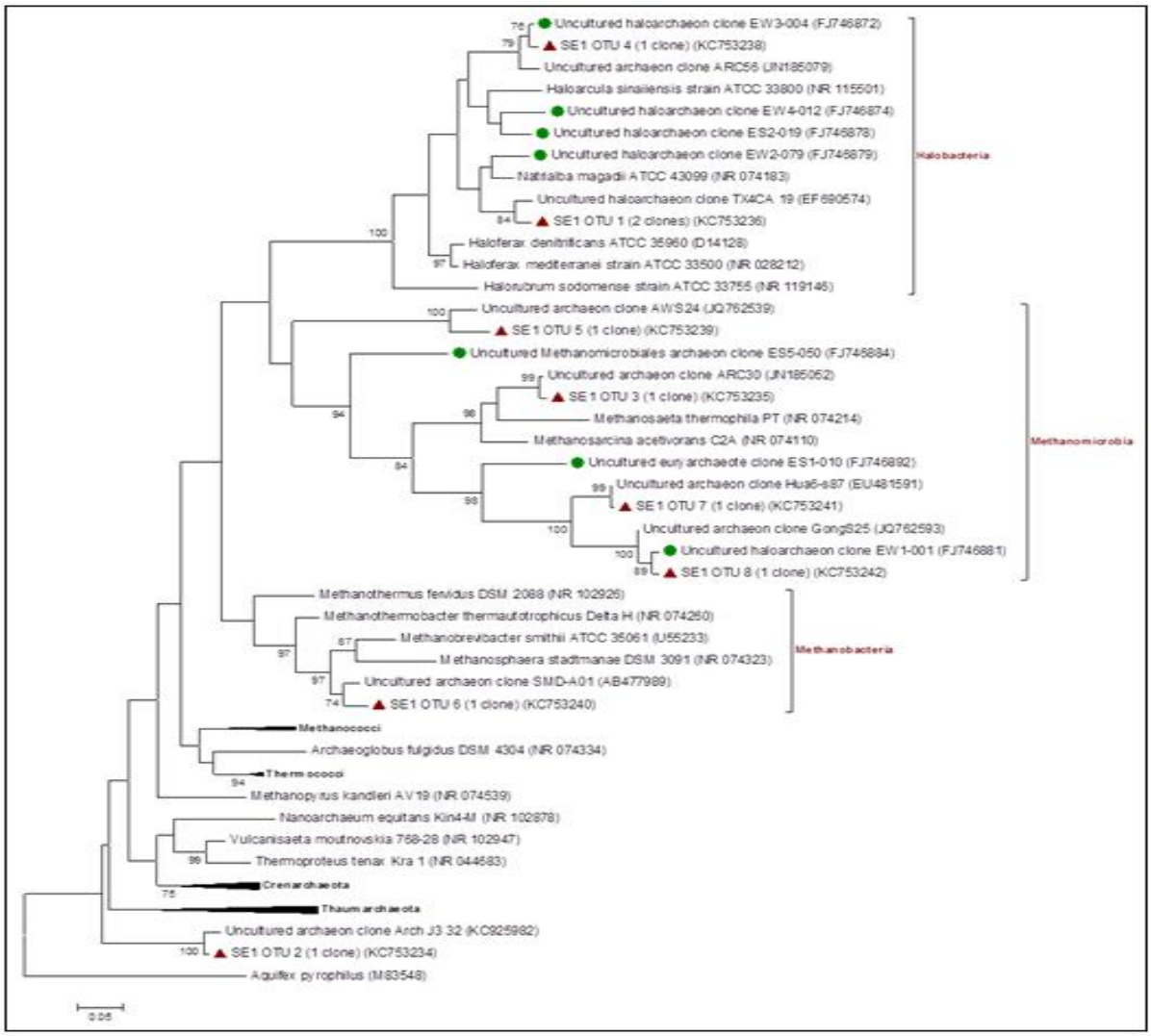

Fig. 5. 16S rRNA sequence-based phylogenetic tree of wet sediment samples from Lake Elmenteita. The number of clones within each OTU is shown and the gene accession number of one representative clone type within each OTU is shown at the end in brackets. Bootstrap values are reported as percentages of 1000 bootstrap replications. The scale bar represents the substitutions per nucleotide position. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates are collapsed. *Aquifex pyrophilus* (M83548) is used as the outgroup.

The culture independent technique used in this study also enabled the recovery of *Thaumarchaeota* 16S rRNA sequences within the soda lake environment of Lake Elmenteita. All but one (SE1 OTU 2) of the *Thaumarchaeota* clones were identified from the DM clone library (near the hot spring) with a water temperature of 56.7°C. On the other hand, the DM clone library from site 1 was predominated by the uncultured Euryarchaea, most of which belonged to the haloarchaea. The temperature at site 1 was much lower (26.3°C) than that at site 2 (hot spring). This observation supports the hypothesis of sequence clustering prevalent in several works and which presupposes that phylotypes always cluster based on their habitats (Pouliot *et al.*, 2009, Beman & Francis, 2006, Francis *et al.*, 2005). According to Kim *et al.*, 2005, if a sequence is similar to that of a group of cultivated organisms with common properties, then the environmental organism represented only by the sequence should also be expected to exhibit those properties. The close relation of the 18 sequenced clones to ammonia-oxidizing Candidatus *Nitrosphaera gargensis* Ga9.2 suggests that they belong to ammonia-oxidizing *Thaumarchaeota*.

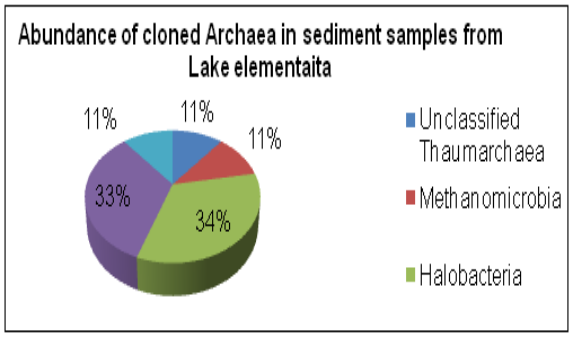

Fig. 6. Abundance of Archaea in sediment (mud) samples from Lake Elmenteita.

The elevated temperature at the sampling site may be a contributing factor to the expression of Thaumarchaeal genes, resulting in the clear separation of genes obtained from the hot spring site, and the rest of the genes. This concurs with other studies that have suggested that elevated temperatures favor the survival and growth of *Thaumarchaeota* (Marusenko *et al.*, 2013, Kim *et al.*, 2012, de la Torre *et al.*, 2008). However, various studies have also shown that the ubiquity of *Thaumarchaeota* in many habitats is due to their ability to survive at even lower temperatures (lower than 74°C), in swamps, sediments, mud, and soil among other habitats (Swan *et al.*, 2014, Hatzenpichler 2012, and Müller *et al.*, 2010). This study revealed 18 sequenced cloned DNA fragments of *Thaumarchaeota* that were isolated in dry mud from site 2 where the temperature was 56.7°C.

The detection of Archaea in the WA, DM and SE clone libraries from Lake Elmenteita adds a significant amount of sequences to the ever growing database of archaeal 16S rRNA clone libraries from soda lakes. The results from this study indicate that apart from the *Euryarchaeota* and other phyla that were captured from previous and present studies, *Thaumarchaeota* are also a part of the microbial diversity of Lake Elmenteita. The results also indicate that majority of the sequences were uniquely similar to sequences from diverse environments and were not restricted to sequences from soda lakes. This is an indication of the ecological differentiation exhibited by environmental Archaea and their adaptation to various physiological environments as shown by various environmental studies. The phylogenetic results indicated that temperature and pH play an important role in archaeal diversity; especially in hot springs. Most of the clones identified with uncultured clones from various environments and had lower sequence identity to known type/reference species. Although sequenced data does not necessarily represent the actual diversity of microorganisms in the environment, they however give an indication of the expected diversity. As expected, the data clearly shows that Lake Elmenteita harbors as yet unexplored Archaea and that they may be possible new archaeal lineages. The findings enhance our understanding of the complexity of the soda lake environments and ecological importance of Archaea in Lake Elmenteita. That detection of various groups of Archaea in Lake Elmenteita is dependent on various factors such as type of primers used and PCR conditions used to amplify the DNA fragments.

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