BIODIVERSITY OF ARCHAEA IN MANZALA LAKE IN EGYPT BASED ON 16S rRNA GENE

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One of the primary challenges in modern microbial molecular genetic is effectively and accurately assessing total microbial diversity, particularly with regard to detection of unculturable and fastidious archaeal species existing in low abundance (i.e., minority populations), in the natural aquatic environment. The domain archaea represents a third line of evolutionary descent and separates from bacteria and eukarya. Initial studies seemed to limit archaea to various extreme environments. These included habitats at the extreme limits that allow life on earth, in terms of temperature, pH, salinity, and anaerobiosis, which are the homes to hyper thermophiles, extreme acidophiles, extreme halophiles, and methanogens, respectively (Chaban *et al*., 2006). Typical environments, from which pure cultures of archaeal species have been isolated, included hot springs, hydrothermal vents, solfataras, salt lakes, soda lakes, sewage digesters, and the rumen (Cytryn *et al*., 2000; Huber *et al*., 2002; Mesbah *et al*., 2007).

A common thread described in numerous published studies and textbooks regarding archaeal community diversities is that in most environments, only 0.1 to 1.0% of archaea detected by direct microscopic enumeration can be recovered on even the most general of laboratory media (Tailliez *et al*., 2002). As a result, microbial ecologists generally have the opinion that the vast majority of archaeal diversity remains uncharacterized due to this gap between culturable and direct estimates of archaeal biomass and diversity. In order to bridge this gap, numbers of molecular approaches have been developed for studying microbial communities, often based on analysis of nucleic acids directly extracted from environmental samples (Olsen *et al*., 1986). Within the past two decades, the use of molecular techniques, including polymerase chain reaction (PCR)-based amplification of ribosomal RNA genes, has allowed a culture-independent assessment of archaeal diversity (Madsen, 2000).

Ribosomal RNA (rRNA) genome is the most conserved (least variable) genome in all individuals of one species (Woese and Fox, 1977). For this reason, genes that encode the rRNA, especially 16S rRNA gene in prokaryotes, have been sequenced to identify an organism' taxonomical group, calculate related groups and estimate rates of species divergence (Olsen *et al*., 1986). By the

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analysis of the 16S rRNA gene, it was possible to discover the domain archaea and separate them from the domain bacteria (Woese and Fox, 1977). Based on the 16S rRNA, archaea have been classified into four phyla, which are Euryarchaeota, Crenarchaeota, Korarchaeota and Nanoarchaeota (DeLong, 1992; Fuhrman *et al*., 1992; Huber *et al*., 2002). Euryarchaeota is a major group of archaea. They include the extremely anaerobic methanogens, which produce methane, the halophiles, which survive in extreme concentrations of salt, and some extremely thermophilic aerobes and anaerobes (Barns *et al*., 1996; Bapteste *et al*., 2005). Crenarchaeotes are thought to be extremophiles (e.g., thermophilic and psychrophilic organisms). Recent studies have identified them as the most abundant archaea in the marine environment (Madigan and Martinko, 2005). The Korarchaeotes were known only from direct 16S rRNA gene analysis of samples from high temperature hydrothermal environments (Takai and Horikoshi, 1999). Recently, Nanoarchaeotes were discovered as tiny hyperthermophilic symbionts (Huber *et al*., 2002). The direct analysis of the 16S rRNA gene from environmental samples uncovered a diverse of unclassified archaea in both extreme and ordinary aquatic environments and could expand our knowledge to unlimited archaeal diversity (Pace, 1997; Gribaldo and Armanet, 2006).

Egyptian lakes show high variations in physicochemical characteristics due to natural and anthropogenic water sources that feed these lakes and make them as big reservoirs for highly diverse microbial communities. The studies regarding the diversity of microbial communities in Egyptian lakes were very few and restricted only on culture and isolation of specific types of bacterioplanktons, while ignoring the others in the sample (El-Naggar *et al*., 1998; Abdel-Monem, 2001; Abdel-Karim *et al*., 2006; Sabae, 2006). These traditional methodologies could not identify the actual composition of microbial community in the sample. In addition, studies on the diversity of archaea have never been done in the majority of Egyptian lakes.

Manzala is one of the most Egyptian lakes affected by anthropogenic pollutants. Bashtir and Genka, sampling sites of this study, are the most two sites of Manzala Lake suffering from high input of pollutants come from discharging of sewage drain, Bahr El-Baqar, and agricultural waste drain, Bahr Hados, respectively. This high organic pollution leads to accumulation of methane, hydrogen sulfide and other chemical hazards (Abbassy *et al*., 2003). Since microbial flora, especially archaea, are considered as bio-indicators for ecological processes in extreme aquatic environments, especially those constructed by heavy anthropogenic pollution, it is very important to investigate the actual composition of archaeal communities living in Bashtir and Genka sites. This work is a complement to a previous work done for molecular genetic analysis of bacterial diversities from the same lake

sites (Elsaied, 2007). This well helps to design a complete concept to understand the microbial role that may control ecological processes within studied lake sites. In this study, the archaeal 16S rRNA gene was applied, for the first time, to analyze the archaeal composition within water and sediment at Bashtir and Genka sites of Manzala Lake. This is an efficient molecular method for identification of even unculturable and minor archaeal species that can not be detected by normal culture methodology. Bulk microbial DNA was extracted directly from the samples. The archaeal 16S rRNA gene was amplified by PCR. Molecular statistical analyses, based on gene diversity and Libshuff analyses of clone libraries, were used to compare archaeal community structures between the two sites. Phylogenetic tree, based on 16S rRNA gene sequences, was constructed to show the phylogenetic position of each recovered archaeal phylotype.

MATERIALS AND METHODS

Sampling and preparation for DNA extractions

Surface water and sediment samples were collected from Bashtir, N31 11.938′; E32 12.419′, and Genka, N31 08.815′; E32 05.429′, located at the eastern part of Manzala Lake, Egypt, as presented in a previous work (Elsaied, 2007). Approximately, 10 L of surface water, from each site, were filtered on cylindrical 0.2 µm filter membrane units (type Sterivex-GS, Millipore Corp., USA), 500 ml per each filter unit. Filters were washed with 10 ml sterile SET buffer (20% sucrose, 50 mM EDTA, 50 mM Tris-HCl, pH 7.6). The inlet and outlet of the filters were capped, and the filters were stored at -30°C until processed for DNA extraction. Sediments were collected by a clean sterile Ekman grab. At each site, about 100 g were taken from the middle part of the collected sediment, put inside a clean sterile propylene tube and covered with Tris-EDTA buffer, with high concentration of EDTA (100 mM), to chelate Mg^{++} and other nuclease co-enzymes. The sediment samples were stored at -30°C for DNA extraction.

DNA extraction and PCR amplification of archaeal 16S rRNA gene

In case of water samples, bulk microbial DNA was extracted essentially within the Sterivex-GS filters housing according to the method of Somerville *et al*. (1989) and modification of Elsaied and Naganuma (2001). For each site, the extracted DNAs from filters were combined to obtain DNA of the 10 L filtrated water. DNA was extracted from 100 g of sediment, from each sampling site, using the method of Porteuous *et al.* (1994).

PCR amplifications of the archaeal 16S rRNA gene, from the purified genomic DNAs, were carried out using the primer sets Arch-21F (5′-TTCCGGTTG ATCCYGCCGGA-3′) and Arch-958R (5′-YCCGGCGTTGAMTCCAATT-3′) (DeLong and Pace, 2001). PCR was performed with an initial denaturation

step of 9 min at 96°C. The standard reaction continued with 30 cycles of 1 min at 95°C, 40 sec. at the desirable annealing temperature and 1 min extension at 72°C. The 30 thermal cycles were followed by a final extension of 10 min at 72°C to allow 3′-A overhangs for the amplified PCR product to facilitate TA-cloning.

Construction of clone libraries and sequence analyses

The products of triplicate PCR reactions for 16S rRNA gene amplification were combined and cloned into TOP10 *Escherichia coli* using a TOPO TA-cloning kit according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, California, USA). White transformed clones were grown overnight in Luria-Bertani broth prior to plasmid extraction with a Qiagen plasmid purification kit (Qiagen, USA). Inserts in the plasmids were sequenced using vector primer T7 and an ABI model 377 sequencer (Applied Biosystems, Foster City, USA). Clone sequences were analyzed by FASTA screening to determine their similarity to known sequences in the DNA database (http:// ddbj.nig.ac.jp). The recovered sequences were aligned using CLUSTAL W software (DDBJ, http://clustalw.ddbj.nig.ac.jp). The sequences that had > 97% nucleotide identity within each clone library were grouped into a phylotype (Godon *et al*., 1997).

Statistical analyses and construction of the phylogenetic tree

The diversity of the resulted phylotypes was analyzed by two statistical methods. (1) Gene diversity within each clone library was estimated using the freeware program Arlequin (Schneider *et al*., 2000), where phylotype pairwise nucleotide differences were measured to extrapolate similarities or differences within the constructed clone libraries. (2) The computer program LIBSHUFF (Singleton *et al*., 2001) was used to estimate homologous and heterologous coverage of clone libraries as a function of evolutionary distance for pairwise reciprocal comparisons (library A compared with library B and *vice versa*). Differences in coverage were considered significant at P values of < 0.05.

The phylogenetic analyses of the resulted phylotypes and corresponded sequences from the databases were performed by applying the neighbour-joining algorithm and drawing the trees using the MEGA 3.1 software (http:// www.megasoftware.net/). The branching patterns of the constructed phylogenetic tree were confirmed by reconstruction of the phylogenies using two other methods of analysis, namely maximum-parsimony and maximum-likelihood, contained within the PHYLIP package (http:// evolution.gs.washington.edu/phylip.html).

Nucleotide sequence accession numbers and nomenclature of phylotypes

The archaeal 16S rRNA gene sequences resulting from this study were deposited in the DNA international database (http://www.ddbj.nig.ac.jp/Welcome-e.html) under the accession no. AB355090-AB355095 for Bashtir water phylotypes;

AB355096-AB355105 for Genka water phylotypes; AB355106-AB355121 for Bashtir sediment phylotypes; AB355122-AB355130 for Genka sediment phylotypes.

The direct recovery of archaeal 16S rRNA genes from environmental samples (Ex. water and sediment) means that the source archaeon can not be identified. Consequently, we have adopted a nomenclature whereby each archaeal phylotype had the descriptor of the names of sampling site followed by sample type and the number of the recovered phylotype, for example, Bashtir-water-1, Genka-sediment-1 etc.

RESULTS AND DISCUSSION

Efficiency of PCR amplification of archaeal 16S rRNA gene

All water and sediment samples showed positive amplification of archaeal 16S rRNA, an implication for existence of archaea in both collected waters and sediments. Archaeal sequences are often recovered from extreme environments (Rothschild and Mancinelli, 2001). Occurrence of unculturable archaea, based on rRNA gene analysis, in ordinary habitats such as Lake Surface waters has been documented (Chaban *et al*., 2006). The G+C nucleotide content value percentages of the amplified fragments, from both water and sediment samples, were high, showing a range from 52.59 to 59.32% compared with 16S rRNA fragments, amplified by same primers, from other habitats (Spring *et al*., 2000). This indicated that the initial denaturation step (96°C, 9 min) of PCR was essential to denature the current archaeal DNA duplexes. The PCR amplified fragments from each sample had sizes ranging from 912 to 917 bp for Bashtir water; from 911 to 940 bp for Genka water; from 914 to 947 bp for Bashtir sediment and from 915 to 945 for Genka sediment. These variations in amplicon size within each sample indicated the possibility of bias in PCR amplification was minimized. This is because the PCR was tested on the bulk DNA extracted from each sample by A) Increasing the annealing temperature 2°C every 10 PCR cycles to give annealing temperatures ranging from 56 to 60°C, and B) Doing PCR using number of cycles ranged from 26 to 30 cycles, and the amplicons were combined for cloning. These conditions gave the flexibility for the primers to anneal with various 16S rRNA gene templates with different G+C nucleotide contents (Suzuki and Giovannoni, 1996). Hence, the PCR could screen as much as possible of the actual composition of archaeal 16S rRNA gene variants in the samples, as presented in this work.

Diversity of archaeal 16S rRNA gene based on molecular statistical analysis

One hundred clones from each clone library were screened directly by direct sequencing to obtain an equilibrium distribution for the diversity of archaeal 16S rRNA gene in the studied samples. Analysis of two hundred clones, representing water clone libraries, produced 6 and 10 archaeal phylotypes for

Bashtir and Genka, respectively (Table 1). On the other hand, the sequence screening of the two hundred clones from sediment clone libraries showed the occurrence of 16 and 9 phylotypes in Bashtir and Genka, respectively. These observations may indicate that the diversity of archaea in Bashtir sediment is more than in water (Table 1). Although the numbers of recovered phylotypes from water and sediment samples of Genka were relatively close to each others (10 and 9 phylotypes), the gene diversity value in water (0.207) was relatively double than that in sediment (0.13). This was due to the number of variable nucleotide positions recorded from paired-reciprocal comparisons between water phylotypes (195.34) was higher than that between sediment phylotypes (120.79) of the Genka site (Table 1). In the term of comparison of water samples between the two sites, Genka water showed high intra-gene diversity (0.207) more than Bashtir water (0.137). This highly archaeal variation in Genka water may be correlated with the intensive human activities, like illegal fish culture enclosures, which constitute water nutrient sources, beside those come from Bahr Hados drain, for diverse microbial communities in that area (Martone *et al*., 2005). Archaeal species, belonging to methanogens, have been discovered as predominant species in some fish aquaculture areas (Lai *et al*., 1999; Lai and Chen, 2001).

The results from LIBSHUFF analyses confirmed that Bashtir sediment community was more diverse than that of Genka sediment. Paired reciprocal library comparisons showed that the Bashtir sediment clone library differed significantly (P = 0.008) from that of Genka sediment, but Genka sediment clone library did not differ from that of Bashtir (P = 0.902) (Table 2). One explanation for this pattern is that the diversity of Bashtir sediment archaeal community encompassed and described some species in the Genka sediment community, while Genka sediment community accounted for only a portion of the Bashtir sediment diversity.

Phylogenetic characterization of the recovered archaeal phylotypes

All the recovered phylotypes located in 8 phylogenetic clusters (from A to H) (Fig. 1). Most of these clusters belonged to the phyla Euryarchaeota and Crenarchaeota. Neither korarchaeota nor Nanoarchaeota-like phylotypes could be detected in the studied samples.

1- Distinction between water and sediment phylotypes

Within the phylogenetic clusters A, B, C, E, and H, the recovered water phylotypes formed monophyletic and paraphyletic clades with the recovered sediment phylotypes (Fig. 1). In the cluster A, the phylotype Bashtir-sediment-10 formed paraphyletic clade with the water phylotypes Genka-water-3 and 9 and both showing nucleotide identity average 93.5%. The phylotypes Bashtir-

sediment-16 formed monophyletic and paraphyletic clades with the water phylotypes Genka-water-2 and 8, respectively in the cluster B. Within this cluster, the nucleotide identity between Bashtir-sediment-16 and Genka-water-2, the most closely related phylotypes, was 96%. The phylotypes Bashtir-sediment-1, in the cluster C, was clearly distinct from Bashtir-water-2 and Genka-water-1, and each of the three phylotypes formed independent monophyletic clade. The phylotypes Genka-water-10 and Bashtir-sediment-2, in the cluster E, showed nucleotide identity 99% and may represent two strains of one species from two different locations. In the cluster H, the phylotype Genka-water-4 formed monophyletic clade with anaerobic unculturable archaeon (Chin *et al*., 1999) and clearly distinct from current sediment phylotypes located within the same cluster. These results indicated that the amplified sequences of the 16S rRNA gene derived from water or sediment, at the same site, independently, rather than from sediment contaminated with water. The nucleotide identities between water phylotypes and sediment phylotypes, rooted with each others, at the corresponding studied clusters, except cluster E, were less than 97%, the limit of consideration of one species (Godon *et al*., 1997). This may indicate that the archaeal species derived from surface waters were distinct from those derived from sediments, but have the same ancestors. Diversity of archaeal species, belonging to one ancestor, was a kind of microbial phylogenetic succession for adaptation to habitat changes (Yannarell and Triplett, 2005).

2- The recovered phylotypes formed unique phylogenetic clusters

The presented water and sediment phylotypes formed unique phylogenetic lineages and largely expanded the diversity within the domain archaea (Fig. 1). Some of these phylotypes affiliated with previously recorded unculturable archaea, forming clusters, which can be considered as unclassified archaeal clusters (Fig. 1). Clusters A and B located in the branch of the phylum Euryarchaeota. The cluster A was predominated by phylotypes recovered from water and phylogenetically rooted with unculturable Euryarchaeotes from sulfide-rich environments (Skirnisdottir *et al*., 2000). The cluster B haboured phylotypes from both sediment and water, forming monophyletic and paraphyletic clades, affiliating with unculturable Euryarchaeote from salty Solar lake, Sinai, Egypt (Cytryn *et al*., 2000). This observation may implicate the biogeographic distribution of this unculturable Euryarchaeote in both marine and freshwater Egyptian inland lakes.

The phylogenetic fingerprints of archaeal populations in the studied sites were represented by the clusters E and F. These two clusters had a unique phylogenetic position between the phyla Nanoarchaeota and Crenarchaeota. The phylotype Genka- water-5 constituted the cluster F and represented the fingerprint

phylotype of Genka water archaeal population. The phylotype members of the cluster E showed nucleotide identity averages 73.5% and 75% with the species *Nanoarchaeum equitans* and *Thermofilum pendens*, the representatives of Nanoarchaeota and Crenarchaeota, respectively. Also, the nucleotide identity values recorded between the phylotype Genka-water-5 and cluster E phylotypes, *Nanoarchaeum equitans* and *Thermofilum pendens* were < 75%, the 16S rRNA gene similarity value for differentiation of archaeal phyla (Gribaldo and Armanet, 2006). These observations suggested that the clusters E and F may represent two new phyla within the domain archaea. Filing up the archaeal evolution in Egyptian lakes, by adding presently uncultivated species from Manzala Lake, is a complement to other previous studies (Cytryn *et al*., 2000; Mesbah *et al*., 2007).

3- Occurrence of methanogen-like phylotypes in water, while crenarchaeota-like phylotypes are abundant in sediments

One of the features of the current phylogenetic tree (Fig. 1) was that the water phylotypes that located within the branch of Euryarchaeota and belonged to known Euryarchaeotes clustered only with methanogenic species, as presented in clusters C and D. This observation suggested that occurrence of methanogens in the water samples is common. Methanogens are frequently retrieved from anoxic lake sediments (Spring *et al*., 2000), implicating the anoxic feature of the water beside sediment at the studied sites. Within the cluster C, the phylotype Genka-sediment-6, characterizing the archaeal population in Genka sediment, formed a strong clade with the *Methanosaeta harundinacea* (Ma *et al*., 2006), represented by the high bootstrap value. On the other hand, the water phylotypes Bashtir-water-1 and 5; and Genka- water-7 were rooted with *Methanospirillum hungatei* and *Methanocorpusculum labreanum*, forming the cluster D. Occurrence of *M. harundinacea* in sediment and *M. hungatei* in water may be an indicator for the existence of acetate, an essential substrate for the growth of *M. harundinacea*, in Genka sediment, and hydrogen, the main electron donor for production of methane by *M. hungatei*, in the waters from both the studied sites (Leibo *et al*., 2006; Ma *et al*., 2006). On the other hand, only, *Methanosarcinals*-like phylotypes have been detected in lakes of Wadi Al-Natrun, while Solar Lake was characterized by occurrence of the genera *Methanobacterium* and *Methanococcus* (Cytryn *et al*., 2000; Mesbah *et al*., 2007). In contrast with Manzala Lake, both Wadi Al-Natrun and Solar lakes are hypersaline lakes. So, the genera *Methanosaeta*, *Methanospirillum*, *Methanothrix* and *Methanocorpusculum* may characterize the methanogenic archaeal community in freshwater Manzala Lake. Generally, methanogens are frequently more abundant in hyper-eutrophic freshwater lakes than marine ones (Whitby *et al*., 2004).

Both the clusters G and H harboured the majority of phylotypes recovered from sediment samples and belonged to the phylum Crenarchaeota, suggesting the dominance of Crenarchaeotes in the studied sediments. In contrast, Crenarchaeotes have not been recorded at previously studied Egyptian Solar and Wadi An Natrun lakes (Cytryn *et al*., 2000; Mesbah *et al*., 2007), a phenomenon characterizing only Manzala sediment. The phylotypes Bashtir-sediment-7 and Genka-sediment-2 and 9 showed phylogenetic affiliation with *Thermofilum pendens* and *Staphylothermus achaiicus*, hyperthermophilic Crenarchaeotes (Kjems *et al*., 1990; Arab *et al*., 2000). Occurrence of these two thermophilic Crenarchaeotes in moderately temperate aquatic environment, like Manzala Lake, implicates the global distribution of these Crenarcaheotes. Recently, Crenarchaeotes have been recorded widely in various temperate marine environments (Chaban *et al*., 2006).

Cluster H had a unique location at the phylogenetic branch of Crenaracheotes. All the members of this cluster were unculturable Crenarchaeotes, including the presented sediment phylotypes. The nucleotide identity averages between the members of this cluster and known recorded Crenarchaeotes *Thermofilum pendens* and *Staphylothermus achaiicus*, located in this branch, were around 80% and 79%, suggesting that cluster H represented a new class of Crenarchaeota, characterizing Manzala sediments.

Correlation between archaeal diversity and environmental characteristics as implicated from 16S rRNA gene analysis

The presented molecular study showed the phylogenetic similarity between archaeal populations in Bashtir and Genka water (Table 2 and Fig. 1). On the other hand, Bashtir sediment is characterized by highly diverse archaeal community, which included phylotypes similar to that of Genka, located mainly in the cluster H (Fig. 1). Genka was the ancient dischargeable site of Bahr El-Baqar drain. Hence, Genka may have a sediment archaeal flora similar to that of Bashtir, the new dischargeable site of Bahr El-Baqar. In comparisons with bacterial diversity from the same locations (Elsaied, 2007), the presented archaeal diversity is lower than that of bacteria and more restricted to few species, mainly methanogens and two species of Crenarchaeotes, beside unculturable species with unknown ecological role. This low diversity of archaea, compared to bacteria, has been also recorded in the lake of Wadi An Natrun (Mesbah *et al*., 2007). Accumulation of methanogen-like phylotypes in the water samples may be correlated with the high input load of sewage untreated acidic-water that contains high concentration of reducing hydrogen and acetates, essential substrates for growth of methanogens and production of methane (El-Naggar *et al*.,

1998). In addition, abundance of extremophilic Crenarchaeota-like phylotypes in sediment samples (Fig. 1 cluster H) implicated that Bashtir and Genka of Manzala have been becomes extreme environments and the rate of aerobic life in these habitats is almost non-existent. This is the second biological evidence beside the previous one (Elsaied, 2007) to pay attention for the heavy anthropogenic pollution attacking these habitats. More molecular genetic studies are required to monitor the microbial communities in other areas of Manzala Lake and other Egyptian lakes in order to make a concrete concept on the actual composition of microbial flora, as efficient bioindicators for natural and anthropogenic activities, in Egyptian lake environments.

SUMMARY

A first molecular genetic study was done on the diversity of archaeal communities in two sites, Bashtir and Genka, of the Manzala Lake, Egypt, based on culture-independent 16S rRNA gene analysis. Bulk microbial DNAs were extracted from surface water and sediment samples collected from both the two studied sites. The archaeal 16S rRNA gene was positively amplified from the DNAs of all samples by PCR. The amplicons were cloned and analyzed directly by sequencing followed by statistical and phylogenetic analyses. The results recorded 6, 10, 16 and 9 archaeal phylotypes in Bashtir water, Genka water, Bashtir sediment and Genka sediment, respectively. Based on Libshuff pairwise clone library comparisons, the 16S rRNA gene phylotypes from Bashtir sediment encompassed the phylotypes of Genka sediment, while the phylotypes of Genka sediment accounted only a minor portion of those of Bashtir sediment. The recovered phylotypes from water samples showed phylogenetic differentiation from those from sediment samples, an observation implicating the phylogenetic distinction between water and sediment archaeal communities. Several recovered phylotypes from water and sediment samples located in the phylogenetic branch of the phylum Euryarchaeota. On the other hand, the phylum Crenarchaeota characterized, mainly, the phylotypes recovered from sediment samples. In the term of affiliation to species, several phylotypes, mainly from water samples, closely related to known Euryarchaeotic methanogenic species *Methanothrix soehngenii*, *Methanospirillum hungatei* and *Methanocorpusculum labreanum*, implying uncommon occurrence of methanogens in surface waters. The Crenarchaeota-like phylotypes affiliated to the thermophilic species *Thermofilum pendens* and *Staphylothermus achaiicus*, implicating the global distribution of those species in even moderately temperate habitats like that of Manzala. Most of the recovered phylotypes belonged to unculturable archaeal species. Several recovered phylotypes from water and sediment samples grouped into unique phylogenetical clusters, suggesting new archaeal phyla and classes characterizing

the sampling sites. The success of this study was identification of the total composition of archaeal communities in the studied sites and the discovery of novel archaeal species, that never recorded by traditional techniques

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Table (1): Gene diversities and average pair wise nucleotide position differences θ (π) between phylotypes within each clone library (intra-population diversity).

Sample	Clone library	No. phylotypes	θ(π)	Gene diversity
Water	Bashtir	6	125.78 (54.39)	0.14 (0.07)
	Genka	10	195.34 (84.31)	0.21 (0.09)
Sediment	Bashtir	16	177.05 (76.4)	0.20 (0.09)
	Genka	9	120.79 (52.3)	0.13 (0.06)

Both θ(π) and gene diversity are expressed as means (± standard deviation) for each library.

Table (2): LIBSHUFF comparison of clone libraries

Sample	Clone library	Cov_{hom} (%)	Cov_{het} (%)	*P*
Water	Bashtir	83.0	84.0	0.900
	Genka	74.0	69.0	0.440
Sediment	Bashtir	87.3	76.4	0.008
	Genka	92.0	93.0	0.902

Homologous (Cov_{hom}) and heterologous (Cov_{het}) coverage percentages of libraries are given. Probability values (*P*) for the significance of differences between homologous and heterologous coverage in reciprocal comparisons as a function of evolutionary distance are also given.

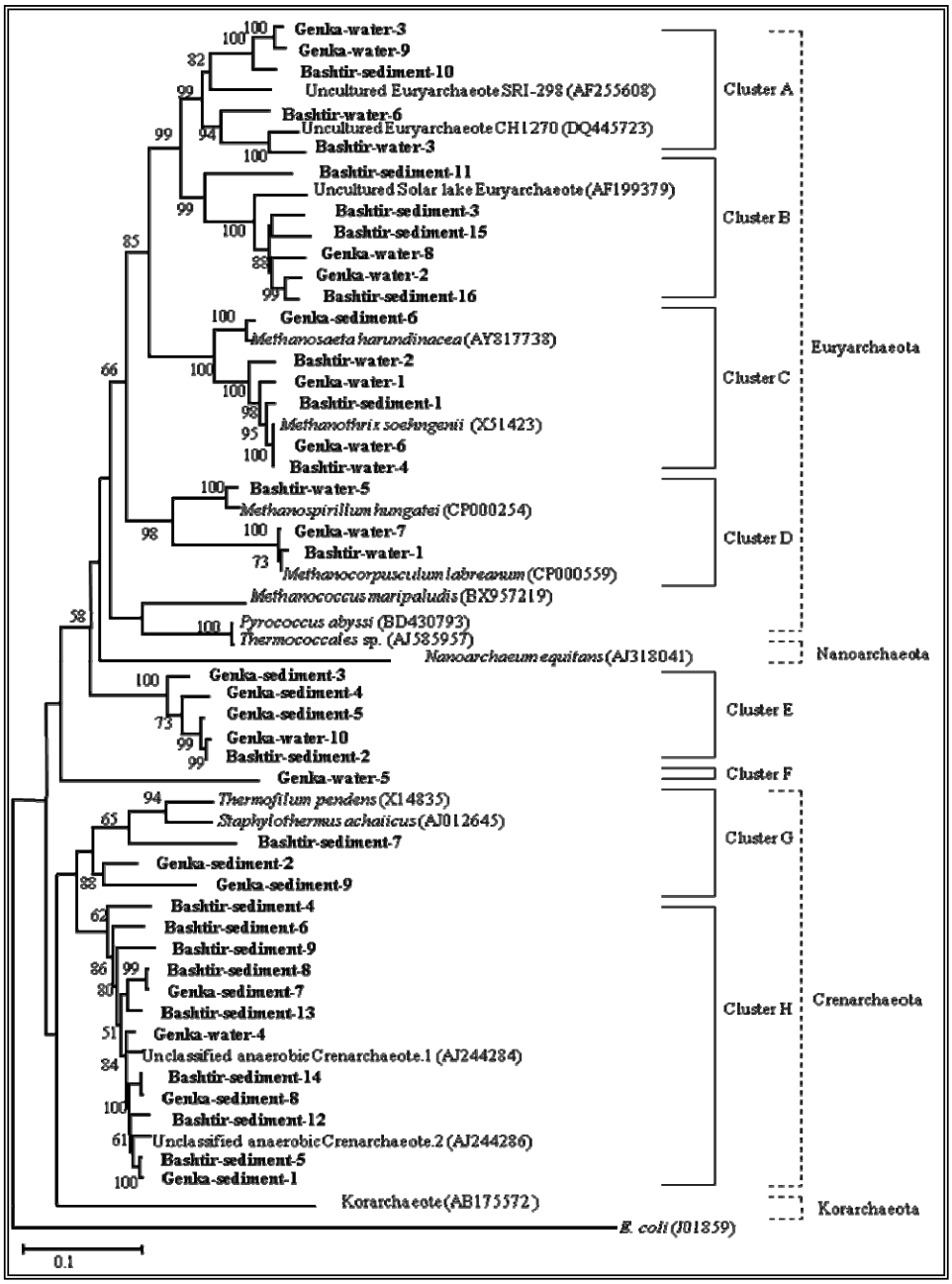

Fig. (1): Phylogenetic tree based on 16S rRNA gene nucleotide sequences. The tree shows the phylogenetic positions of the recovered water and sediment phylotypes, marked with bold, among archaeal species sequences, from different phyla, collected from DNA databases. An out-group sequence from the bacterium *Escherichia coli* was used to define the archaeal groups. Bootstrap values were calculated by neighbour-joining algorithm of > 50% and indicated at the roots of each cluster. Accession numbers of the sequences retrieved from DNA databases were shown between brackets. The bar represents 0.1 changes per nucleotide.