INSIGHTS INTO THE DISTRIBUTION AND ECOLOGICAL ROLE OF MEMBERS OF THE ARCHEAL PHYLUM BATHYARCHAEOTA. FROM DE GLOBAL TO THE LOCAL SCALE

Mireia Fillol Homs

Universitat de Girona

Doctoral Thesis

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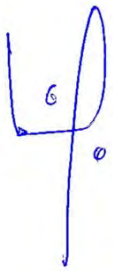

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The present thesis contain one annex containing the supplementary material from the chapters at the end of the document

This thesis is submitted in fulfilment of the requirements to obtain the doctoral degree from the Universitat de Girona

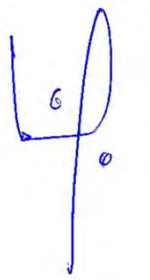
Universitat de Girona

Hereby, Dr. Carles Borrego Moré, Associate Professor of Microbiology at the University of Girona,

C E R T I F I E S

That the doctoral thesis entitled "**Insights into the distribution and ecological role of members of the archaeal Phylum Bathyarchaeota. From the global to the local scale**", that *Mireia Fillol Homs* has submitted to obtain the doctoral degree from the Universitat de Girona has been completed under my supervision, and meets all the requirements to opt for the *International Doctor* mention.

In witness whereof and for such purposes as may arise, I signed this certificate in Girona, on 25th January 2017



Dr. Carles Borrego Moré
Thesis supervisor

"In science it often happens that scientists say, 'You know that's a really good argument; my position is mistaken,' and then they would actually change their minds (...) I cannot recall the last time something like that happened in politics or religion."

Carl Sagan

Dedicatòria

Una Tesi és un treball d'equip. I si bé podriem dir que som un grup petit, a la pràctica som un equip molt gran. De totes i cadascuna de les persones amb qui he tractat al llarg d'aquests anys n'he après alguna cosa important i per tant, a totes els pertoca un trosset d'aquesta tesi.

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intensos. I també a la Mercè de qui he après que sempre et pots organitzar millor i a la Itziar pels consells i les converses, perquè els primers mesos de la reincorporació vau ser vitals per poder seguir el ritme. Una dedicatòria especial per l'Imma i l'Olga amb qui vam fer el camí de la UdG a l'ICRA juntes i amb qui hem compartit infinitat de moments, també us pertoca una bona porció de tesi.

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List of publications

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Chapter 1

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IF_{2015} = 3.500; Quartile 2 in category Microbiology (38/123)

List of abbreviations

Abbreviation	Description
16S rRNA	small subunit ribosomal ribonucleic acid
AICc	Akaikes' information criterion corrected
ANME	anaerobic methanotroph
ANOSIM	analisys of similarity
AOA	ammonia-oxidizing archaea
ARMAN	archaeal Richmond Mine acidophilic nanoorganisms
ASR	ancestral state reconstruction
CARD-FISH	catalyzed reported deposition-fluorescence in situ hybridization
cDNA	complementary DNA
CL	core lipids
dbRDA	distance-based redundancy analysis
DGGE	denaturing gradient gel electrophoresis
DHVEG	deep hydrothermal vent euryarchaeota group
DIC	dissolved inorganic carbon
DistLM	distance based linear model permutation test
DOC	dissolved organic carbon
DPANN	Diapherotrites-Parvarchaeota-Aenigmarchaeota-Nanoarchaeota-Nanohaloarchaeota archaeal superphylum
DSAG	Deep Sea Archaeal Group
DSEG	Deep Sea Euryarchaeota Group
GDGT	Glycerol Dialkyl Glycerol Tetraether

HGT	horizontal gene transfer
HWCGI	Hot Water Crenarchaeota Group I
ILs	indicator lineages
IndVal	indicator value
IPL	intact polar lipids
iTOL	interactive Tree of Life
MANOVA	multivariate analysis of variance
MBG-B	Marine Benthic Group B
MBG-D	Marine Benthic Group D
MCG	Miscellaneous Crenarchaeotic Group
MEG	Miscellaneous Euryarchaeotic Group
MG-I	Marine Group I
ML	maximum likelihood
MRT	multivariate regression tree
NCBI	National Center for Biotechnology Information
NJ	Neighbour-Joining
NMDS	non-metric multidimensional scaling
ORF	open reading frame
OTU	operational taxonomic unit
PCoA	principal coordinate analysis
PCR	polimerase chain reaction
PD	phylogenetic diversity
qPCR	quantitative polimerase chain reaction

RAxML	randomized axelerated maximum likelihood
RNA	ribonucleic acid
SAD	species abundance distribution
SAGMEG	South African Gold Mine Euryarchaeota Group
SCG	Soil Crenarchaeotic Group
SES	standarized effect size
SIMPER	similarity percentages
SIP	stable isotope probing
SMTZ	sulphate-methane transition zone
TACK	archaeal superphylum Thaumarchaeota-Aigarchaeota-Crenarchaeota-Korarchaeota
TMCG	Terrestrial Miscellaneous Crenarchaeotic Group
TMEG	Terrestrial Miscellaneous Euryarchaeotal Group
TOC	total organic carbon
TP	total phosphorous

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Summary

The Phylum Bathyarchaeota is currently one of the most studied uncultured archaeal phyla. Bathyarchaeota are phylogenetically diverse, globally ubiquitous and especially abundant in marine subsurface sediments. Despite their global significance, their ecological role is still unknown due to the lack of cultivated representatives and the paucity of available sequenced genomes. Besides, current distribution patterns provide few clues about their metabolic capabilities and physiological requirements.

The current work tackled the problem by combining different phylogenetic and molecular techniques to shed light on the main environmental drivers that affect the distribution patterns of Bathyarchaeota at a global and regional scale. Our results showed that the phylum Bathyarchaeota went across various saline–freshwater transitions during its evolution that caused diversification events that resulted in subgroups specifically adapted to marine and saline habitats. Besides, our approach provided a robust ecological framework in which Bathyarchaeota appear as a core generalist group in the sediment realm, sharing their key role with other uncultured archaeal lineages such as the Thermoplasmata. At a regional scale, a molecular survey carried out in different stratified lakes of the Banyoles Karstic System provided evidences that Bathyarchaeota are a common component of archaeal assemblages thriving in both euxinic water compartments and sediments. Multivariate analyses identified sulfide and dissolved organic carbon as main environmental variables that explain the distribution of Bathyarchaeota subgroups between the planktonic and sedimentary habitats. In the latter, Bathyarchaeota were particularly prevalent in biofilms developed on leaf litter settled in sediments of Lake Cisó, that provided a natural enrichment where to better characterize their habitat segregation, phylogenetic diversity and membrane lipid composition. The application of complementary molecular tools such as massive sequencing of 16S rRNA gene and lipidomics revealed that biofilms were almost solely composed of subgroup Bathyarchaeota-6 and possessed a characteristic, and hitherto unknown, profile of isomeric isoprenoid tetraether lipids GDGT-1, -2 and -3.

Altogether, our study recognised Bathyarchaeota as key players in marine and freshwater sediments at both global and regional scales. We also identified the main environmental drivers behind their group diversification and current subgroup segregation, and stablished the co-occurrence and putative synergy between members of the Phylum Bathyarchaeota and the class Thermoplasmata. Besides, our work provides new molecular tools to better address ecological studies focused on the Bathyarchaeota and make an important step forward to finally find a specific lipid biomarker for this widespread and diverse archaeal lineage.

Resum

El Fílum Bathyarchaeota és un dels llinatges d'arqueus no cultivats més estudiat degut a la seva diversitat filogenètica i ubiqüitat, especialment en sediments marins on són particularment abundants. Tot i així, la manca de representants cultivats i els pocs genomes seqüenciats del que es disposa limita el coneixement sobre el seu paper en l'ecosistema, els factors ambientals que afecten la seva distribució, les seves capacitats metabòliques i requeriments fisiològics.

El treball que aquí es presenta combina tècniques moleculars i filogenètiques per conèixer quins factors ambientals són els que afecten la distribució dels Bathyarchaeota a escala global i regional. Els resultats mostren que el Fílum Bathyarchaeota ha patit, al llarg de l'evolució, diverses transicions entre ambients salins i d'aigua dolça que han resultat en una diversificació i adaptació dels diferents subgrups a cadascun d'aquests ambients. Les aproximacions moleculars que hem desenvolupat defineixen un marc ecològic robust en el que Bathyarchaeota apareix com a un grup central, generalista en termes de recursos i que desenvolupa, juntament amb altres llinatges d'arqueus no cultivats com ara els Thermoplasmata, un paper clau en els sediments. A escala regional, hem aplicat un anàlisi molecular en diversos llacs estratificats del Sistema Càrstic de Banyoles que ha permès determinar que els Bathyarchaeota són membres estables de la comunitat d'arqueus en ambients euxínics tant de la columna d'aigua com del sediment. Les anàlisi multivariants han identificat que el sulfhídric i el carboni orgànic dissolt són les principals variables ambientals que expliquen la segregació dels subgrups de batiarqueus entre nínxols planktònics i sedimentaris. Concretament en ambients bentònics, els Bathyarchaeota són particularment abundants en biofilms desenvolupats sobre fulles en descomposició acumulades al sediment del llac Cisó, representant un vertader "enriquiment natural" d'aquests arqueus. Estudis de lipidòmica i seqüenciació massiva del gen del 16S rRNA han mostrat que en aquests biofilms la comunitat d'arqueus està composada gairebé en la seva totalitat per Bathyarchaeota del subgrup–6 i mostren un perfil d'isòmers dels lípids isoprenoides de membrana (GDGT–1, –2 i –3) únic i fins ara desconegut.

En conjunt, el nostre estudi identifica als Bathyarchaeota com a grup clau en sediments marins i lacustres tant a escala global com regional. També identifica els principals factors ambientals que han afectat la seva diversificació durant l'evolució així com en la segregació que actualment mostren en ambients lacustres. També es presenten evidències de la co-ocurrència freqüent entre membres del fílum Bathyarchaeota i la classe *Thermoplasmata* en sediments, fet que suggereix una possible relació sintrófica entre aquests dos llinatges. El treball aporta, a més, eines moleculars que permetran estudiar els Bathyarchaeota amb més detall i avenços clau per la identificació de biomarcadors lipídics específics per aquest grup d'arqueus tan ubic i divers.

Resumen

El Fílum Bathyarchaeota es uno de los linajes de Archaea no cultivadas más estudiados debido a su diversidad filogenética y ubicuidad, especialmente en sedimentos marinos donde son particularmente abundantes. Sin embargo, el bajo número de genomas secuenciados así como la falta de representantes cultivados limita el conocimiento sobre su papel en el ecosistema, los factores que afectan a su distribución, sus capacidades metabólicas y requerimientos fisiológicos.

Este trabajo combina técnicas moleculares y filogenéticas para identificar los factores ambientales que controlan sus patrones de distribución a escala global y regional. Los resultados muestran que el Fílum Bathyarchaeota sufrió, a lo largo de la evolución, varias transiciones entre ambientes marinos y de agua dulce que resultó en una diversificación y adaptación de los distintos subgrupos a cada uno de estos ambientes. Las aproximaciones moleculares desarrolladas definen un marco ecológico robusto en el que Bathyarchaeota aparece cómo un grupo central, generalista en términos de recursos y que desarrolla un papel clave en los sedimentos junto con otros linajes de archaea cómo las Thermoplasmata. A escala regional hemos realizado un análisis molecular en diversos lagos estratificados del Sistema Cárstico de Banyoles que ha permitido determinar que las Bathyarchaeota son miembros estables de la comunidad de archaea de ambientes euxínicos, tanto en columna de agua cómo en el sedimento. Mediante análisis multivariante se identificó el sulfhídrico y el carbono orgánico disuelto cómo las variables ambientales que mejor explican la segregación de los diferentes subgrupos entre el plancton y el sedimento. Concretamente en los ambientes bentónicos, las Bathyarchaeota son particularmente abundantes en biofilms desarrollados sobre hojarasca en descomposición acumulada en el sedimento de la laguna del Cisó, constituyendo un verdadero "enriquecimiento natural" de estas archaea. Análisis de lipidómica y de secuenciación masiva del gen 16S rRNA han mostrado que en estos biofilms la comunidad de archaea está dominada prácticamente en su totalidad por Bathyarchaeota del subgrupo-6 y mostrando un perfil de isómeros de lípidos isoprenoides de membrana (GDGT–1, –2 y –3) único y hasta ahora desconocido.

En conjunto, nuestro estudio identifica las Bathyarchaeota como un grupo clave en los sedimentos marinos y lacustres tanto a escala global cómo regional. También identifica los principales factores ambientales que han afectado su diversificación durante la evolución así como la segregación que actualmente muestran en ambientes lacustres. También se presentan evidencias de la co-ocurrencia y posible relación sintrófica entre los miembros del fílum Bathyarchaeota y la clase *Thermoplasmata*. El trabajo aporta, además, herramientas moleculares que facilitarán el estudio de las Bathyarchaeota con más detalle y avances clave para identificar un biomarcador lipídico específico para este grupo de archaea tan diverso y ubicuo.

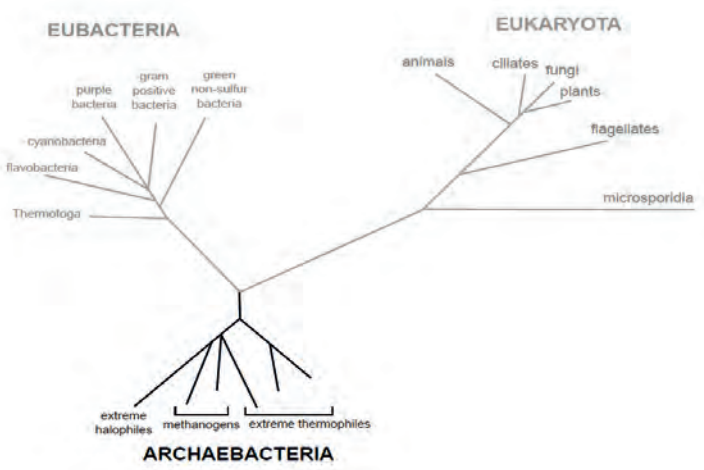
1.Introduction

1.1. Domain Archaea

'Advances in molecular phylogenetics, macromolecular sequencing techniques, and emerging genomic technologies, (…), are changing the playing field dramatically for microbial biologists'
(DeLong and Pace, 2001)

1.1.1. From Woese to Woesearchaeota

Our view of the phylogenetic diversity and ecological distribution of Archaea (Woese and Fox, 1977) has suffered drastic changes in the last decades (Pace, 1997). The large majority of these prokaryotes remain uncultured and our knowledge relies on cultivation-independent techniques based on conserved marker genes (Rinke *et al.*, 2013). The use of the 16S rRNA gene as biomarker for their occurrence and abundance has expanded our view of the distribution and ecology of Archaea. Sequences affiliated to Domain *Archaea* have been retrieved from the most disparate environments (soils (Bintrim *et al.*, 1997; Walsh *et al.*, 2005; Bates *et al.*, 2011; Tripathi *et al.*, 2013), terrestrial hot springs (Barns *et al.*, 1996), marine environments (DeLong, 1992; Fuhrman *et al.*, 1992; Massana *et al.*, 1997; Karner *et al.*, 2001), marine hydrothermal vents (Teske *et al.*, 2002), deep subsurface (Biddle *et al.*, 2006, 2008; Teske and Sørensen, 2008), freshwater ecosystems (Stein *et al.*, 2002; Llirós *et al.*, 2008; 2010; 2011; Lin *et al.*, 2012; Yergeau *et al.*, 2012; Berdjeb *et al.*, 2013; Bricheux *et al.*, 2013; Silveira *et al.*, 2013; Vila-Costa *et al.*, 2013), volcanic ash layers (Inagaki *et al.*, 2006) among others) thus enlarging its phylogenetic tree (Figure 1.1A) and establishing the ubiquity of the Archaea on a global scale (DeLong and Pace 2001, Schleper *et al.*, 2005; Chaban *et al.*, 2006; Auguet and Casamayor, 2008; Llirós *et al.*, 2008; Teske and Sørensen, 2008; Casamayor and Borrego, 2009; Auguet *et al.*, 2010). Recently, advances in genomic reconstruction from metagenomic sequence data have yielded valuable insights into the metabolic capabilities and ecological roles of many novel lineages (Hallam *et al.*, 2004; Konneke *et al.*, 2005; Elkins *et al.*, 2008; Walker *et al.*, 2010; Lloyd *et al.*, 2013; He *et al.*, 2016; Vanwonterghem *et al.*, 2016). Moreover, methodological and computational advances in molecular techniques at single cell level have accelerated the identification and genomic characterization of high-rank archaeal lineages. More new phyla or candidate phyla have been described in the last 6 years than in the past 30 years. (Woese *et al.*, 1990; Barns *et al.*, 1994; Huber *et al.*, 2002; Brochier-Armanet *et al.*, 2008; Baker *et al.*, 2010; Nunoura *et al.*, 2010; Narasingarao *et al.*, 2012; Rinke *et al.*, 2013; Kozubal *et al.*, 2013; Meng *et al.*, 2014; Spang *et al.*, 2015; Castelle *et al.*, 2015; Seitz *et al.*, 2016; Baker *et al.*, 2016).



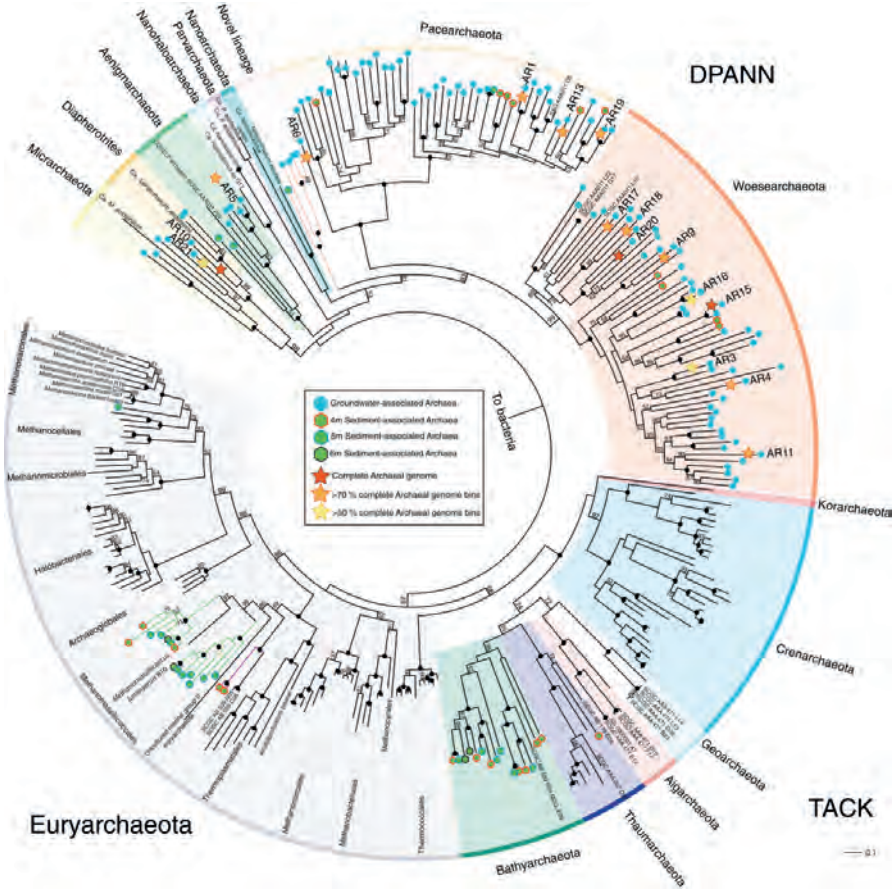


Figure 1.1. Archaeal phylogenetic tree based on (A) 16S rRNA gene sequences alignment (extracted from Woese, 1987) and (B) and archaeal genomic tree based on the alignment of 15 concatenated ribosomal proteins (extracted from Castelle *et al.*, 2015).

1.1.2. The current archaeal tree

Our view of the phylogeny and diversity of Archaea is rapidly evolving. The most recent and comprehensive phylogenomic-based assessment of the domain *Archaea* (Rinke *et al*., 2013) proposed three superphyla encompassing different archaeal phyla, namely: superphyla TACK and DPANN, and the phylum Euryarchaeota. Table 1.1 summarizes all new phyla according to Castelle *et al* 2015 (Figure 1.1B). As more genomes and improved phylogenetic inference methods will be applied, the currently proposed lineages delineations and their evolutionary relationships will be re-evaluated (Rinke *et al*., 2013)

1.1.2.1 Phylum Euryarchaeota

Phylum Euryarchaeota was first described as one of the two main lineages of the domain Archaea (Woese *et al*., 1990). Initially, all methanogenic archaea affiliated to the Euryarchaeota and were classified into seven orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanocellales*, *Methanopyrales and Methanomasiliicoccales*. Phylum Euryarchaeota also comprises halophiles (*Halobacteriales*), sulfate-reducing species (*Archaeoglobales*), and two orders that include (hyper)thermophiles, the *Thermoplasmatales* (also comprising mesophilic lineages) and the *Thermococcales*.

1.1.2.2. Superphylum TACK

Superphylum TACK (Guy and Ettema, 2011) included the Thaumarchaeota, the Aigarchaeota, the Crenarchaeota and the Korarchaeota. New lineages were later also included within TACK, namely, the Phylum Bathyarchaeota and the Phylum Geoarchaeota (Castelle *et al*., 2015).

Phyla Crenarchaeota and Korarchaeota branched together. The Crenarchaeota was initially described from cultured representatives as a group encompassing (hyper)thermophilic and thermoacidophilic representatives, mainly related with the sulfur cycle (Woese *et al*., 1990). Phylum Korarchaeota was postulated on the basis of environmental 16S rRNA gene surveys and phylogenies from enrichments of the hyperthermophilic *Candidatus* 'Korarchaeum cryptophilum' (Barns *et al*., 1996; Elkins *et al*., 2008). The Phylum Thaumarchaeota was proposed after sequencing the genome of *Candidatus* 'Cenarchaeum symbiosum', a symbiont of the marine sponge *Axinella Mexicana* (Preston *et al*., 1996), and demonstrated that mesophilic archaea were widespread in temperate environments (Brochier-Armanet *et al*., 2008). Thaumarchaeota comprises all known ammonia-oxidizing Archaea (AOA). The discovery of Thaumarchaeota has resulted in major contributions to our understanding of global C and N cycling in the open ocean (Ingalls *et al*., 2006; Kozubal *et al*., 2013). Interestingly, Thaumarchaeota possess a specific and unique membrane lipid, the crenarchaeol, which is consistent with the segregation of the phylum in the archaeal phylogenetic tree (Schouten *et al*., 2013; Pester *et al*, 2011). Phylum Aigarchaeota arises from the former lineage *Hot Water Crenarchaeotic*

Group I (HWCGI) (Nunoura *et al*., 2005). Metagenomic analyses allowed the almost complete genome sequence of the uncultivated *Ca.* Caldiarchaeum subterraneum (Nunoura *et al*., 2010). Most recently, Phylum Geoarchaeota was described from 16S rRNA gene sequences retrieved from thermophilic habitats (Kozubal *et al*., 2013). Finally, the formerly known *Miscellaneous Crenarchaeotic Group* (MCG) was proposed as the new Phylum Bathyarchaeota after phylogenetic reconstructions that placed members of this group in a deep branching position together with the Thaumarchaeota and the Aigarchaeota (Meng *et al*., 2014). A detailed description of the origin and characteristics of the Bathyarchaeota is provided in Section 1.2 of this introductory chapter.

After the comprehensive work of Rinke *et al* (2013) and Castelle *et al* (2015), several new candidate phyla have been described. Thorarchaeota and Lokiarchaeota (formerly *Deep Sea Archaeal Group* (DSAG)/*Marine Benthic Group* B (MBG-B)) are two new proposed phyla described from sequences retrieved from marine and estuarine sediments, respectively (Spang *et al*., 2015; Seitz *et al*., 2016). They both branch deeply within the TACK superphylum and provide insights into the ancestral relationships between the archaea and the eukaryotes. The separation between the Euryarchaeota and the 'TACK' group represents the primary split among the known archaeal species. Petitjean *et al*. (2014) proposed to call this new superphylum Proteoarchaeota, as a reference to the Greek god of the sea Proteus, able to display many different forms.

1.1.2.3. Superphylum DPANN

The DPANN superphylum initially encompassed Phyla Diapherotrites (Rinke *et al*., 2013), Parvarchaeota (Baker *et al*., 2010), Aenigmarchaeota (Rinke *et al*., 2013), Nanohaloarchaeota (Narasingarao *et al*., 2012), and the Nanoarchaeota (Waters *et al*., 2003; Podar *et al*., 2013). Later, Castelle *et al*. (2015) included Phyla Micrarchaeota (formerly *Archaeal Richmond Mine acidophilic nanoorganisms group* 2 (ARMAN-2, Baker *et al*., 2006)), Pacearchaeota and Woesearchaeota (both included in the formerly known *Deep Hydrothermal Vent Euryarchaeotal Group* 6 (DHVE-6) (Takai and Horikoshi, 1999)). Originally, most of these new phyla classified within the Euryarchaeota (Table 1.1). The precise position of superphylum DPANN into the archaeal tree is still a matter of debate in the specialized literature (Petitjean *et al*., 2014; Williams and Embley *et al*., 2014). For instance, Petitjean *et al*., (2014) described an archaeal phylogenetic tree in which members of the DPANN (Nanoarchaeota, Parvarchaeota and Nanohaloarchaeota) were classes included into the Phylum Euryarchaeota. In the current work, we have used the classification proposed by Rinke *et al*. (2013) and further refined by Castelle *et al*. (2015). According to these authors, the DPANN superphylum is a monophyletic clade in which the small cell and genomic size are unifying features (Rinke *et al*., 2013). Notwithstanding this, DPANN superphylum represents an intriguing collection of phyla with disparate physiological requirements and environmental distribution (Youssef *et al*., 2014), ranging from strict symbiotic and thermophilic species within the Nanoarchaeota (Waters *et al*., 2003; Podar *et al*., 2013), to the acidophilic candidate phylum Parvarchaeota (formerly ARMAN-4 and

ARMAN-5 (Baker *et al*., 2006)) and to the non-extremophilic candidate phyla Aenigmarchaeota (Rinke *et al*., 2013) and Diapherotrites (formerly known as pMC2A384 (Takai and Horikoshi, 1999)). However, many other characteristics seem to be common to all members of this phylum, probably as a consequence of their streamlined, reduced-size genomes. For instance, they may not have the ability to synthesize the glycerol-1-phosphate (G1P) backbone of archaeal lipids and so they rely on host cells or cell debris for the synthesis of their lipids (Villanueva *et al*., 2016).

Table 1.1. Current archaeal phylogeny

Superphylum	Phylum/Candidate Phylum	Former clasification	Candidatus-type species cultured or enriched	Technique	References
	Euryarchaeota	Euryarchaeota	Yes, many	16S rRNA gene sequence comparision	[1]
TACK[a]*	Crenarchaeota	Crenarchaeota	Yes, many	16S rRNA gene sequence comparision	[1]
	Korarchaeota	Korarchaeota	Yes, *Korarchaeum cryptofilum*	Genome sequencing from enrichment	[2]
	Thaumarchaeota	Group I (non-thermophilic Crenarchaeota group)	Yes, *Cenarchaeum symbiosum*	Genome sequencing from enrichment	[3]
	Aigarchaeota	HWCG* (Crenarchaeota)	None	Metagenomics	[4]
	Geoarchaeota (NAG1*)	Crenarchaeota	None	Metagenomics	[5]
	Bathyarchaeota	MCG* (Crenarchaeota)	None	Metagenomics	[6]
	Thorarchaeota		None	Metagenomics	[7]
	Lokiarchaeota		None	Metagenomics	[8]

Guy *et al*., 2011 [b]Rinke *et al*., 2013

*Abreviations: SCG: Single Cell Genomics; NAG1: New Arcaheal Group 1; HWCG: Hot Water Crenarchaeota Group; ARMAN: Archaeal Richmond Mine acidophilic nanoorganisms; MEG: Miscellaneous Euryarchaeotal Group; DSEG: Deep Sea Euryarchaeotal Group; DHVEG-6: Deep Hydrothermal Vent Euryarchaeotal Group 6; MCG: Miscellaneous Crenarchaeotic Group; SAGMEG: South African Gold Mine Euryarchaeota Group;TACK: acronym for Thaumarchaeota-Aigarchaeota-Crenarchaeota-Korarchaeota; DPANN: acronym for Diapherotrites-Parvarchaeota-Aenigmarchaeota-Nanoarchaeota-Nanohaloarchaeota.

[1]: Woese *et al* 1990, [2]: Barns *et al*., 1994, [3]: Brochier-Armanet *et al*., 2008, [4]: Nunoura *et al*., 2010, [5]: Kozubal *et al*., 2013, [6]: Meng *et al*., 2014, [7]: Seitz *et al*., 2016, [8]: Spang *et al*., 2015, [9]: Huber *et al*., 2002, [10]: Baker *et al*., 2010, [11]: Rinke *et al*., 2013, [12]: Narasingarao *et al*., 2012, [13]: Castelle *et al*., 2015

Superphylum	Phylum/Candidate Phylum	Former clasification	Candidatus-type species cultured or enriched	Technique	Reference
DPANN[b]	Nanoarchaeota	Nanoarchaeota	Yes, *Nanoarchaeum equitans*	Genome sequencing from enrichment	[9]
	Micrarchaeota	ARMAN2*	None	Metagenomics	[10]
	Diapherotrites	MEG* (Euryarchaeota)	None	SCG*	[11]
	Nanohaloarchaeota	Euryarchaeota	None	Metagenomics	[12]
	Aenigmarchaeota	DSEG* (Euryarchaeota)	None	SCG	[11]
	Parvarchaeota	ARMAN5*	None	SCG	[11]
	Pacearchaeota	DHVEG-6* (Euryarchaeota)	None	Metagenomics	[13]
	Woesearchaeota	DHVEG-6 (Euryarchaeota)	None	Metagenomics	[13]

Table 1.1. Current archaeal phylogeny *(continued)*

[a]Guy et al., 2011 [b]Rinke et al., 2013

*Abreviations: SCG: Single Cell Genomics; NAG1: New Arcaheal Group 1; HWCG: Hot Water Crenarchaeota Group; ARMAN: Archaeal Richmond Mine acidophilic nanoorganisms; MEG: Miscellaneous Euryarchaeotal Group; DSEG: Deep Sea Euryarchaeotal Group; DHVEG-6: Deep Hydrothermal Vent Euryarchaeotal Group 6; MCG: Miscellaneous Crenarchaeotic Group; SAGMEG: South African Gold Mine Euryarchaeota Group;TACK: acronym for Thaumarchaeota-Aigarchaeota-Crenarchaeota-Korarchaeota; DPANN: acronym for Diapherotrites-Parvarchaeota-Aenigmarchaeota-Nanoarchaeota-Nanohaloarchaeota.

[1]: Woese et al 1990, [2]: Barns et al., 1994, [3]: Brochier-Armanet et al., 2008, [4]: Nunoura et al., 2010, [5]: Kozubal et al., 2013, [6]: Meng et al., 2014, [7]: Seitz et al., 2016, [8]: Spang et al., 2015, [9]: Huber et al., 2002, [10]: Baker et al., 2010, [11]: Rinke et al., 2013, [12]: Narasingarao et al., 2012, [13]: Castelle et al., 2015

1.2. Phylum Bathyarchaeota

(*Miscellaneous Crenarchaeotic Group*)

"At present, no other deep subsurface archaeal lineage has such a diversified habitat range (...) and the high intragroup phylogenetic depth of the MCG Archaea (...)."
(Teske and Sørensen, 2008)

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1.2.1. From "*Miscellaneous*" to Bathyarchaeota

The Phylum Bathyarchaeota is one of the most interesting uncultured archaeal lineages considering its ubiquity, abundance, phylogenetic diversity and apparent metabolic versatility. Members of the Phylum Bathyarchaeota were described for the first time as a monophyletic clade by DeLong (1998) on the first comprehensive study of the non-extremophilic archaea. DeLong described the Group I of the Phylum Crenarchaeota containing all the marine and terrestrial non-thermophilic archaeal 16S rRNA sequences. DeLong divided this Group I into three subclusters, I.1 (currently the Phylum Thaumarchaeota), I.2 (currently the subgroup 15 within the Bathyarchaeota) and I.3 (currently the Phylum Bathyarchaeota). Later on, those groups were refined and relabelled as I.1 (formerly C1), I.2 (formerly C3) and I.3 (formerly C2) (DeLong and Pace, 2001) Concurrently, similar sequences of the group C2 were found in deep terrestrial subsurface in South African goldmines clustering with other sequences from diverse terrestrial habitats, and thus the group was labelled as 'Terrestrial Miscellaneous Crenarchaeotic Group' (TMCG) (Takai *et al*., 2001). Later on, groups C2 and the TMCG were classified into a single lineage, finally named Miscellaneous Crenarchaeotic Group (Inagaki *et al*., 2003). Recently, this group was proposed as a new Phylum "Bathyarchaeota" from the greek "*bathys*" meaning "deep" as reminder of its deep branching within the archaeal tree (Meng *et al*., 2014). The Phylum Bathyarchaeota was described as a sister lineage of the Phylum Aigarchaeota and phylogenetically close to the Phylum Thaumarchaeota (Nunoura *et al*., 2011, Rinke *et al*., 2014).

Sequences of the Phylum Bathyarchaeota have similarities as low as 76% between the most distant members (Fry *et al*., 2008; Kubo *et al*., 2012). This large intragroup phylogenetic diversity compelled researchers to identify smaller, phylogenetically coherent subgroups and to identify potential biogeographical trends and ecological constraints to understand their distribution.

1.2.2. Sorting out the "miscellany"

So far, three different studies tried to sort out the Bathyarchaeota lineage into smaller and more manageable subgroups. Sørensen and Teske (2006), tried to discern which archaeal groups from marine sediments were active and to establish which groups dominated the active community on different subsurface layers. They realized that the MCG dominated many different layers, indicating a flexible ecophysiology of the group and di-

vided them into subclusters (MCG-1 to MCG-4), linking them to specific habitats that could be used as indicator for putative metabolisms. Later, Jiang *et al.* (2011), established that members of the Bathyarchaeota dominated the community of estuarine sediments of the Pearl River Estuary and classified bathyarchaeotal sequences into seven subgroups (A, B, C, D, E, F and G) increasing the coverage of the previous classification (Figure 1.2). In 2012, Kubo and co-workers developed the most complete classification identifying 18 phylogenetic subgroups (from MCG-1 to MCG-17 with a later subdivision of MCG-5 into MCG-5a and MCG-5b). This new classification expanded the previous one by Jiang and coworkers and included several new clusters that were not originally included within the Bathyarchaeota. For instance, the previously group named pMARA-4 (Nercessian *et al.*, 2005) was renamed as MCG-16, the group C3 described by Inagaki *et al.* (2006) was renamed as MCG-15, and the MBG-C was relabelled as MCG-8 (Figure 1.2). This classification was later expanded to 21 subgroups (See Chapter 1).

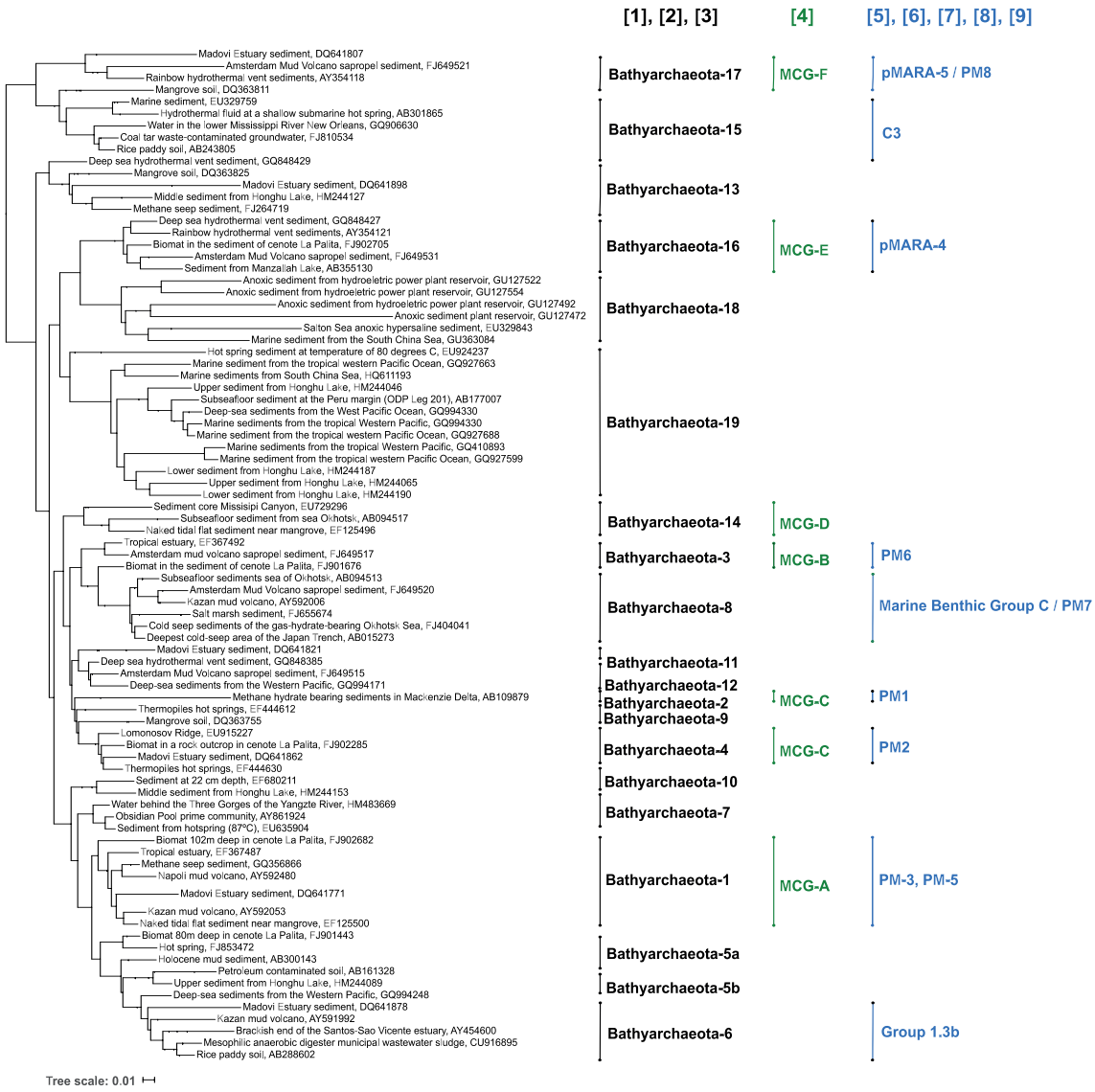

Figure 1.2. Neighbour-joining phylogenetic tree of the Phylum Bathyarchaeotabased on 16S rRNA gene sequences alignment. Columns on the right list different classifications of the sub-groups. [1] Sørensen and Teske, 2006; [2] Kubo *et al.*, 2012; [3] Fillol *et al.*, 2016; [4] Jiang *et al.*, 2011; [5] Nercessian *et al.*, 2005; [6] Webster *et al.*, 2010; [7] Inagaki *et al.*, 2006; [8] Vetriani *et al.*, 1999; [9] Ochsenreiter *et al.*, 2003.

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1.2.3. Distribution, crossing borders.

The difficulty to categorize the wide habitat range of members of the Bathyarchaeota was reflected on the label 'miscellaneous' from its former name (the *Miscellaneous Crenarchaeotic Group*). Indeed, the Bathyarchaeota have a cosmopolitan distribution in natural ecosystems and appears to have no well-defined boundaries in terms of salinity, temperature or trophic status. They are particularly abundant and widespread in marine environments, from the oligotrophic deep subsurface sediments (Biddle *et al*., 2006, 2008; Teske, 2006; Fry *et al*., 2008; Durbin and Teske 2012; Kubo *et al*., 2012; Lloyd *et al*., 2013; Lazar *et al*., 2014; Evans *et al*., 2015; He *et al*., 2016) to the organic rich surficial marine sediments (Vetriani *et al*., 1999). They are occurring in basement fluids of buried sediments (Jungbluth *et al*., 2012), cold seep sediments populated by tubeworms (Lazar *et al*., 2010) or the buried coral fossils (Hoshino *et al*., 2011). Members of the Bathyarchaeota tolerate a wide range of salinities since they have been identified in hypersaline sediments (Lazar *et al*., 2011), brackish lakes (Hershberger *et al*., 1996), estuarine sediments (Jiang *et al*., 2011; Kubo *et al*., 2012; Meng *et al*., 2014), freshwater lakes (Jiang *et al*., 2008; Llirós *et al*., 2008, 2011, 2014; Buckles and Villanueva *et al*, 2013; Fillol *et al*., 2015; Fan and Xing, 2016), stream sediments (Porat *et al*., 2010) and groundwater (Chen *et al*., 2009). Regarding their temperature tolerance, they have been identified in samples from marine hydrothermal vents (Sørensen *et al*., 2004) and terrestrial hot springs (Barns *et al*., 1996; Teske *et al*., 2002; Wemheuer *et al*., 2013) but also from the Artic permafrost (Shcherbakova *et al*., 2016). They also have been identified in terrestrial environments as deep-subsurface paleosols (Chandler *et al*., 1998), forest soils (Tupinambá *et al*., 2016) and the deep terrestrial subsurface (Takai *et al*., 2001). The Bathyarchaeota are also present in other environments such as mud volcanoes (Oni *et al*., 2015) and salt marsh sediments (Seyler *et al*., 2014). Sequences affiliated to the Bathyarchaeota have consistently been retrieved from oil contaminated soils (Kasai *et al*., 2005), landfill leachates (Huang *et al*., 2003), anaerobic wastewater reactors (Collins *et al*., 2005; Kuroda et al., 2015), human-perturbed water bodies (Hu *et al*., 2016), termite guts (Friederich *et al*., 2001), the surface of sugar beet plants (Shi *et al*., 2015) and fermented sea-food (Roh *et al*., 2010).

1.2.4. Putative metabolisms

Our understanding of the metabolic capabilities and potential biogeochemical roles of the Phylum Bathyarchaeota is still limited. The vast phylogenetic diversity and the widespread distribution suggest a wide metabolic versatility, but the lack of cultured representatives and the paucity of sequenced genomes still impede a better understanding of their metabolic versatility and ecological roles (He *et al*., 2016).

Firsts approaches on the metabolism of the Phylum Bathyarchaeota derived from subsurface marine sediments where members of the Phylum Bathyarchaeota were dominant (Biddle *et al*., 2006; Webster *et al*., 2006). Results on isotope labelling revealed that these archaea were able to feed on buried organic carbon (Biddle *et al*., 2006), and hence, they were identified as heterotrophic. These results were later supported by results obtained

from estuarine sediments (Kubo *et al*., 2012). The lack of correlation between their abundance and any geochemical gradient indicated that they might obtain carbon and energy from substrates that vary little along sediment depth and between habitats. Accordingly, potential metabolisms included the fermentative degradation of different types of organic matter buried into sediments were chronic energy stress is the common situation. Thus, the Bathyarchaeota were likely to access substrates that are physically or chemically recalcitrant (Biddle *et al*., 2006; Lipp *et al*., 2008; Takano *et al*., 2010; Webster *et al*., 2010; Llirós *et al*., 2011; Kubo *et al*., 2012). Same conclusions were reached after Stable Isotope Probing (SIP) experiments using salt march sediment samples (Seyler *et al*., 2014). Results suggested that members of the subgroups Bathyarchaeota-8 and Bathyarchaeota-6 were able to assimilate a wide variety of organic substrates, including recalcitrant biopolymers (Seyler *et al*., 2014). Moreover, Meng *et al*. (2014) sequenced one fosmid related with the Bathyarchaeota-8 encoding genes involved in protocatechuate degradation, a key step on the catabolism of aromatic compounds of the ß-ketoadipate pathway (Stanier and Ornston, 1973).

Despite evidences for the heterotrophy of the Bathyarchaeota, their widespread distribution linked to the great phylogenetic diversity (21 subgroups, Fillol *et al*., 2016) suggested a great versatility in their physiological capabilities. Indeed, recent metagenomic surveys revealed distinct carbon substrate preferences and ecological niches among bathyarchaeotal subgroups (Lazar *et al*., 2014). The genomic information of one member of the subgroup Bathyarchaeota-6 suggested their ability of degrading extracellular plant-derived mono- and polysaccharides (Lazar *et al*., 2016). Recently, shotgun metagenomics have revealed that members of the subgroup Bathyarchaeota-16 may carry out acetogenesis, degrading complex organic carbon and producing acetate (He *et al*., 2016). In contrast, Bathyarchaeota-15 seems to bypass those substrates and focus on diagenetically processed low-molecular-weight carbon substrates and proteins (Lazar *et al*., 2016). Similar results were obtained by single-cell genome sequencing of one member of the Bathyarchaeota-15 from a marine sediment, where authors found genomic evidences for exogenous protein degradation (Lloyd *et al*., 2013). Finally, a study based on genome-centric metagenomics applied on water samples from coal-bed methane wells revealed the potential capacity to carry out methylotrophic methanogenesis using a wide range of methylated compounds (Evans *et al*., 2015).

1.3. Archaeal membrane lipids.

Where biogeochemistry meets microbial ecology.

'The pathways for the biosynthesis of the lipid components are those shared by most microorganisms and demonstrate a close relationship; however, an independent line of descent is indicated by the formation of the isopranyl glycerol ether lipids'
(Tornabene *et al.*, 1980)

Microbial ecologists have been relying on genetic biomarkers for the study of microbial communities in the environment, mainly by the amplification of the 16S rRNA gene sequences. However, in environmental sciences, membrane lipids are valuable biomarkers that can provide information on the composition of ancient and/or modern prokaryotic ecosystems. Even though 16S rRNA-based molecular techniques enable description of a natural community on a higher phylogenetic resolution, the potential biases associated to the PCR-dependent techniques may result in unreliable results about microbial diversity. On the other hand, lipid-based techniques provide complementary information about *in situ* communities because they are directly measured and provide information on the relative importance of distinct prokaryotic groups (Sturt *et al.*, 2004; Schouten *et al.*, 2013; Buckles and Villanueva *et al.*, 2013).

1.3.1. Specific archaeal membrane lipids, the Glycerol Dialkyl Glycerol Tetraethers (GDGT)

The composition of membrane lipids is one of the most remarkable traits distinguishing the three domains of life, Archaea, Bacteria, and Eukarya (Woese and Fox, 1977; Villanueva *et al.*, 2016). Figure 3 compiles the main characteristics distinguishing archaeal lipids from those typically found in Bacteria and Eukarya. Briefly, archaeal isoprenoid hydrocarbon chains are covalently linked forming a bipolar lipid monolayer instead of the polar bilayer composed of linear fatty acid chains in Bacteria and Eukarya. In Archaea, the isoprenoid hydrocarbon chains are linked to the glycerol moiety by ether linkages instead of the esther-linkages as it occurs in Bacteria and Eukarya. Finally, they differ in the stereochemistry of glycerol phosphate backbones, Archaea have sn-glycerol-1-phosphate as opposed of the glycerol-3-phosphate backbones from Bacteria and Eukarya (Figure 1.3). Besides, isoprenoid hydrocarbon chains may contain cyclopentyl and cyclohexyl moieties (Figure 1.4).

After cell death, intact polar GDGTs rapidly lose their polar head groups via enzymatic hydrolysis (Harvey *et al.*, 1986; White *et al.*, 1979). Thus, the presence of Intact Polar Lipids (IPL) can be indicative of living (or recently living) cells whereas the core lipids (CL), which have great stability and are able to persist for long periods of time, may indicate the occurrence of a given taxa in the past (Hopmans *et al.*, 2000; Sturt *et al.*, 2004).

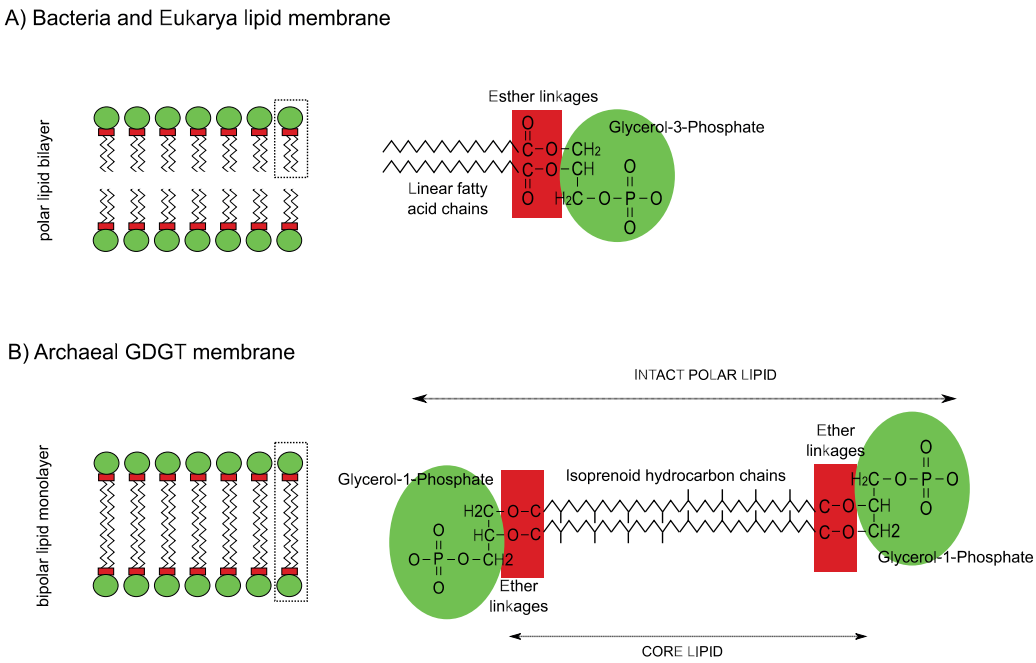

Figure 1.3. Membrane lipids from (A) Bacteria and Eukarya and (B) Archaea.

1.3.2. Lipids as biomarkers for archaeal diversity

In microbial ecology, composition of GDGTs has traditionally been used to identify the main carbon assimilation pathway through calculation of stable isotopic ratios (Biddle *et al.*, 2006) and also have been applied as a complementary tool for tracking the presence of certain archaeal groups (Leininger *et al.*, 2006; Wuchter *et al.*, 2006; Buckles and Villanueva *et al.*, 2013). The use of GDGTs as specific biomarkers for archaeal lineages is however, limited. Most data on archaeal GDGTs derives from enrichments or pure cultures but the paucity in the number of archaeal isolates hinders a comprehensive understanding of the true diversity and occurrence of GDGTs across the Archaea (Schouten *et al.*, 2013). Moreover, the apparent low diversity of GDGTs (Figure 1.4) compared with the large phylogeny of the domain Archaea makes difficult to infer direct link between a given membrane lipid and its archaeal source. So, although isoprenoid GDGTs are specific for Archaea only a few are specific for classes within the Archaea. Although indirect and not conclusive, some inferences can be draw by linking data from genetic analysis and lipid analysis (Sturt *et al.*, 2004; Buckles and Villanueva *et al.*, 2013) and by metagenomic analysis that provide complete or near-complete genome sequencing and provides clues on the kind of membrane lipids they may be producing (Villanueva *et al.*, 2016).

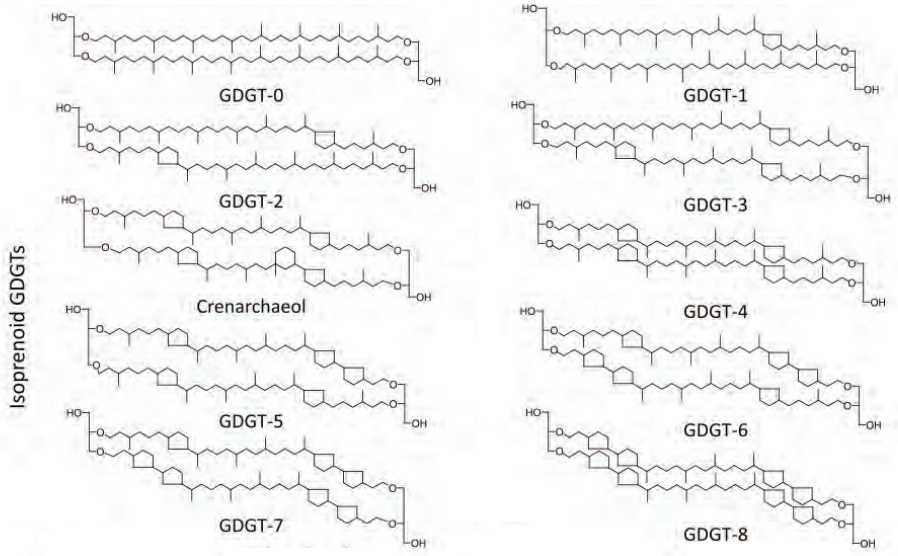

Figure 1.4. Structures of isoprenoid GDGTs containing different cyclopenthane moieties. Extracted from Schouten *et al.*, 2013.

In this regard, a study focused on the identification of genes encoding for enzymes involved in membrane lipid biosynthesis in archaeal genomes revealed that members of the Superphylum DPANN, some groups within the Phylum Euryarchaeota and the Phylum Lokiarchaeota (Superphylum TACK) do not have the ability to synthesize their own GDGTs (Villanueva *et al.*, 2016). On the other hand, cell membranes of members of the class *Halobacteria* (Phylum Euryarchaeota) are composed by archaeol-based lipids instead of GDGTs (Rossel *et al.*, 2011). According to these results, GDGTs can be related with the Superphylum TACK (being the Phylum Lokiarchaeota the exception to this rule) and with non-halophilic Euryarchaeota. Within the cultivated euryarchaeons available to date, GDGT-0 is the most common GDGT. Using carbon isotopic analysis, GDGT-0 has specifically been related to methanogens since its backbone is depleted in ^{13}C (Pancost *et al.*, 2000; Schouten *et al.*, 2001). GDGT-0 is also common on cultivated (hyper-)thermophilic crenarchaeota. In clear contrast, GDGTs containing 1-4 cyclopentane moieties are widespread in cultured Crenarchaeota and Thaumarchaeota but nearly absent in Euryarchaeota being the thermophilic members of the order *Thermoplasmatales* the unique euryarchaeons possessing this type of GDGTs. The occurrence of GDGT-0 and GDGTs with 1–4 cyclopentane moieties in cultivated Archaea suggests that they might be also common in uncultivated representatives (*e.g.* mesophilic Archaea). A recent study linking

GDGT membrane lipid distributions with gene abundances of ammonia-oxidizing Thaumarchaeota and uncultured crenarchaeotal groups pointed to an origin of GDGT-0 from uncultured members of the Superphylum TACK (Buckles and Villanueva *et al.*, 2013). In contrast, GDGTs with more than four cyclopentane moieties (GDGTs 5–8) have not been identified in temperate environments yet but only in high-temperature habitats suggesting that they are restricted to hyperthermophilic archaea (Ward *et al.*, 1985; Pearson *et al.*, 2004, 2008; Schouten *et al.*, 2007b; Zhang *et al* 2006, 2008; Kaul *et al.*, 2011b; Burgess *et al.*, 2012). Finally, crenarchaeol, which contain one cyclohexyl moiety and four cyclopentyl moieties (Figure 1.4), is specific for ammonia-oxidising archaea (*i.e.* Thaumarchaeota) (Sinninghe Damsté et al., 2002b) and it has been extensively used as a tracer for the presence and abundance of archaeal nitrifiers in both terrestrial and aquatic habitats (Leininger *et al.*, 2006; Wuchter *et al.*, 2006b; Pitcher *et al.*, 2011b).

2.Objectives and outline of the Thesis

This PhD Thesis is focused on the study of the uncultured archaeal Phylum Bathyarchaeota. We aim to examine their ecological significance within archaeal communities and to assess the main environmental parameters driving their distribution. To address these issues we stablished three main objectives:

1. To assess the ecological preferences of subgroups of the Phylum Bathyarchaeota at a global scale.

2. To design new molecular tools for the identification and quantification of members of the Phylum Bathyarchaeota.

2.1. Genomic biomarkers: to design a new primer pair for the identification and quantification of members of the Phylum Bathyarchaeota (qPCR).

2.2 Lipidic biomarkers: to identify specific isoprenoidal membrane lipid of the Phylum Bathyarchaeota.

3. To assess the abundance and diversity of the Phylum Bathyarchaeota in freshwater environments.

To assess the ecological preferences of bathyarchaeotal subgroups we developed a global study of the phylum Bathyarchaeota using publicly available archaeal 16S rRNA gene sequences from globally distributed studies (**Chapter 1**). We examined the ecological significance of the Phylum Bathyarchaeota within archaeal communities in both freshwater and marine sediments and investigated the hypothesis of a relationship between diversification and habitat during the evolution of the lineage and the transition between marine and freshwater sediments. In addition, we used network analysis in an attempt to unveil potential syntrophic and/or mutualistic interactions in which Phylum Bathyarchaeota would be involved and, by association, in the potential metabolisms they might harbor (**Objective 1**).

From the global study, we detected a powerful bias on the published studies involving the Phylum Bathyarchaeota towards the saline environment. To overcome this limitation we developed a study at a local scale at the Banyoles Karstic System (**Chapter 2**). Here we studied the composition of archaeal communities and the abundance and diversity of Phylum Bathyarchaeota by massive parallel sequencing and quantitative PCR (qPCR) (**Objective 3**) using a newly designed primer set that provides deep phylogenetic resolution of the Phylum Bathyarchaeota (**Objective 2.1**).

Finally, in **Chapter 3** we compared the archaeal community composition of different niches at the sediment surface of a karstic lake (Lake Cisó) using gene-based (*i.e.* 16S rRNA gene) and lipid-based (*i.e.* isoprenoid IPL-GDGT) surveys with the aim of determining niche preferences of the Bathyarchaeota as well as their potential lipid biomarkers (**Objectives 2.2** and **3**).

3.Results and discussion

Chapter 1

Insights in the ecology and evolutionary history of the *Miscellaneous Crenarchaeotic Group* lineage

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ABSTRACT

Members of the archaeal *Miscellaneous Crenarchaeotic Group* (MCG) are among the most successful microorganisms on the planet. During its evolutionary diversification, this very diverse group has managed to cross the saline–freshwater boundary, one of the most important evolutionary barriers structuring microbial communities. However, the current understanding on the ecological significance of MCG in freshwater habitats is scarce and the evolutionary relationships between freshwater and saline MCG remains poorly known. Here, we carried out molecular phylogenies using publicly available 16S rRNA gene sequences from various geographic locations to investigate the distribution of MCG in freshwater and saline sediments and to evaluate the implications of saline–freshwater transitions during the diversification events. Our approach provided a robust ecological framework in which MCG archaea appeared as a core generalist group in the sediment realm. However, the analysis of the complex intragroup phylogeny of the 21 subgroups currently forming the MCG lineage revealed that distinct evolutionary MCG subgroups have arisen in marine and freshwater sediments suggesting the occurrence of adaptive evolution specific to each habitat. The ancestral state reconstruction analysis indicated that this segregation was mainly due to the occurrence of a few saline–freshwater transition events during the MCG diversification. In addition, a network analysis showed that both saline and freshwater MCG recurrently co-occur with archaea of the class *Thermoplasmata* in sediment ecosystems, suggesting a potentially relevant trophic connection between the two clades.

Chapter 2

Diversity of *Miscellaneous Crenarchaeotic Group* archaea in freshwater karstic lakes and their segregation between planktonic and sediment habitats

Fillol, Mireia, Sànchez-Melsió, Alexandre, Guich, Frederic, Borrego, Carles. "Diversity of Miscellaneous Crenarchaeotic Group archaea in freshwater karstic lakes and their segregation between planktonic and sediment habitats". *FEMS microbiology ecology,* Vol. 91, 4, (1 April 2015): fiv020

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ABSTRACT

The *Miscellaneous Crenarchaeotic Group* (MCG) is an archaeal lineage whose members are widespread and abundant in marine sediments. MCG archaea have also been consistently found in stratified euxinic lakes. In this work, we have studied archaeal communities in three karstic lakes to reveal potential habitat segregation of MCG subgroups between planktonic and sediment compartments. In the studied lakes, archaeal assemblages were strikingly similar to those of the marine subsurface with predominance of uncultured *Halobacteria* in the plankton and *Thermoplasmata* and MCG in anoxic, organic-rich sediments. Multivariate analyses identified sulphide and dissolved organic carbon as predictor variables of archaeal community composition. Quantification of MCG using a newly designed qPCR primer pair that improves coverage for MCG subgroups prevalent in the studied lakes revealed conspicuous populations in both the plankton and the sediment. Subgroups MCG-5a and -5b appear as planktonic specialists thriving in euxinic bottom waters, while subgroup MCG-6 emerges as a generalist group able to cope with varying reducing conditions. Besides, comparison of DNA- and cDNA-based pyrotag libraries revealed that rare subgroups in DNA libraries, i.e. MCG-15, were prevalent in cDNA-based datasets, suggesting that euxinic, organic-rich sediments of karstic lakes provide optimal niches for the activity of some specialized MCG subgroups.

KEYWORDS
anoxic sediments, euxinia, karstic lakes, Miscellaneous Crenarchaeotic Group, sulphidic redoxclines, uncultured archaea

Issue Section: Research article

Chapter 3

Leaf litter biofilms in euxinic sediments as a new niche for Bathyarchaeota

Leaf litter biofilms in euxinic sediments as a new niche for Bathyarchaeota

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RUNNING TITLE: *New niche for the Bathyarchaeota*

KEYWORDS: Bathyarchaeota, Miscellaneous Crenarchaeotic Group (MCG), Leaf litter, euxinia, anoxic sediments, Biofilms, Lake Cisó, archaeal lipids, biomarker, glycerol dibiphytanyl glycerol tetraether (GDGT)

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Summary

Bathyarchaeota (also known as Miscellaneous Crenarchaeotic Group, MCG) is currently one of the most studied uncultured archaeal lineages due to its ubiquity and phylogenetic diversity. Here, we investigated the distribution of Bathyarchaeota in different niches (i.e. bottom water, surface sediment, biofilm from leaf litter, and biofilm from plant debris) in the bottom of an anoxic karstic lake receiving a high contribution of terrestrial organic matter depositions by combining 16S rRNA gene amplicon sequencing and archaeal lipid composition analysis by high performance liquid chromatography tandem mass spectrometry. We found a clear dominance (85% of total archaeal reads) of Bathyarchaeota group-6 in biofilms growing on leaf litter and a positive co-occurrence of this group with isomeric forms of the archaeal lipids tetraether glycerol dibiphytanyl glycerol tetraether (GDGT) with one and two cyclopentane moieties (GDGT-1 and GDGT-2), which points to these membrane lipids as potential biomarkers of this Bathyarchaeota group. The preference of the Bathyarchaeota-6 for benthic growth on the surface of decomposing leaves agrees well with available genomic data that indicates that this group has the potential capacity to degrade recalcitrant organic matter.

4.General discussion

4.1. Phylogenetic diversity

The Phylum Bathyarchaeota has been described as one of the most phylogenetically diverse lineages of archaea (Teske and Sorensen, 2008; Kubo *et al.,* 2012; Fillol *et al*., 2016, among others). In the first comprehensive study of 16S rRNA gene sequences from Bathyarchaeota (Kubo *et al*., 2012), the minimum sequence identity between the most distant sequences was close to 76%, which is similar to the range used to define prokaryotic phyla (between 76.4% to 80.4%, according to Yarza *et al*., 2010). Such large phylogenetic diversity hinders the proper design of new molecular tools targeting the 16S rRNA gene for the whole Phylum (see Chapter 2). Despite the primer pair designed in the course of this work has a better coverage than that already available (Kubo *et al*., 2012), it would be of great interest to design molecular tools (*e.g.* qPCR primers or FISH probes) that specifically target the different subgroups within the bathyarchaea. However, the intragroup classification of the Bathyarchaeota is still not well resolved and large discrepancies still exist among phylogenies inferred by different authors that pose major constraints to the design of subgroup-specific primers of probes. One of those discrepancies appears when trying to define the taxonomical category of each subgroup. Based uniquely on the percentage of identity of 16S rRNA gene sequences, the minimum cutoff of sequence similarity for 16S rRNA gene ranges from 85.2% to 90.2% to define Families (Yarza *et al*., 2010). Assuming this cutoff, some subgroups (*e.g.* Bathy-1, -3, -5b, -6, -8, -9 and -16) would be considered as true families (see Table 4.1). Other subgroups showed lower sequence similarity and, accordingly, they would be classified as Orders or Classes (*e.g.* Bathy-13, -15 and -17) whereas some others (*e.g.* Bathy-5a) might be considered as Genus (94.9±0.4% of sequence identity, Yarza *et al*., 2010). As illustrated in Table 4.1, some subgroups (Bathy-2, -4, -7, -10, -11, -12 and -14) are currently on the boundary between different taxonomical categories and their classification would differ according to the phylogenetic inference used. More problematic are those differences that modify the subgroup composition. For instance, our analysis splitted Bathy-5b into 5b and 5bb (see Figure 3 in Chapter 1) when two of the Bathy-5b reference sequences branched separately from the rest. The phylogenetic tree published by Lazar and co-workers (2014) is even more ambiguous since subgroups Bathy-7/Bathy-17 and Bathy-5/Bathy-8 grouped together and authors proposed their clustering into single subgroups (i.e. Bathy-7/17 and Bathy-5/8). Information regarding these 'new' subgroups is scarce in the specialized literature but, in our opinion, this combined clustering is probably an artifact derived from the use of sequences from a single source (*i.e.* White Oak River (WOR) estuary (North Carolina, USA)). According to our phylogenetical analysis, not only subgroups Bathy-7 and Bathy-17 but also Bathy-5 and Bathy-8 are distantly related (see Figure 3 in Chapter 1). Moreover, they probably evolved separately during the early-stages of the Bathyarchaeota diversification. While Subgroups Bathy-17 and Bathy-8 are true specialists from saline environments, Bathy-7 and Bathy-5 (including Bathy-5a, -5b and -5bb) seem to be adapted to freshwater (see Figure 6 in Chapter 1). Accordingly, the grouping of Bathy-5 and Bathy-8 into a single subgroup is surprising considering that the former has

been identified as an indicator lineage for saline environments and the latter for freshwater environments (see Figure 5 in Chapter 1, Fillol *et al.*, 2016). This apparent contradiction is probably a consequence of the use of phylogenetic inferences based exclusively on the 16S rRNA gene. New approaches to resolve evolutionary relationships among microbial lineages currently rely on genome-based phylogenetic analysis using concatenated alignments of multiple universally distributed single copy marker genes (Rinke *et al.*, 2013). To resolve the problems exposed above and to properly delineate the intragroup phylogeny of the Bathyarchaeota it is then mandatory to use genome-centric approaches instead of focusing in a single gene (i.e. 16S rRNA).

Table 4.1. Minimum intragroup similarity of bathyarchaeotal subgroups based on the % similarity of the 16S rRNA gene.

Subgroup	*minimum intragroup similarity (%)*		Taxonomic level[a]
	Kubo *et al.* (2012)	Fillol *et al.* (2015)	
1	88	89	F[b]/F[c]
2	94	89	G/F
3	89	91	F/F
4	91	94	F/G
5a	94	93	G/G
5b	91	91	F/F
5bb	n.c.	n.c.	n.d.
6	90	89	F/F
7	92	96	F/G
8	87	88	F/F
9	87	88	F/F
10	84	91	O-C/F
11	87	93	F/G
12	92	96	F/G
13	85	85	O-C/O-C
14	84	91	O-C/F
15	82	83	O-C/O-C
16	87	92	F/F
17	84	82	O-C/O-C
18	n.c.	n.c.	n.d.
19	n.c.	n.c.	n.d.
20	n.c.	n.c.	n.d.
18*	n.c.	n.c.	n.d.
19*	n.c.	n.c.	n.d.
5/8	n.c.	n.c.	n.d.
7/17	n.c.	n.c.	n.d.
All	76	n.d.	n.d.

[a] Taxonomic level considering the minimum similarity according to Yarza *et al.*, 2010.
O-C = Order-Class; F = Family; G = Genus.
[b] based on 16S rRNA gene sequence similarities published in Kubo *et al.*, 2012
[c] based on 16S rRNA gene sequence similarities published in Fillol *et al.*, 2015
n.d.: no data available.
n.c. subgroups not considered.
*: Subgroups described by Fillol and co-workers (2016) are not the same than subgroups -18 and -19 described by Lazar *et al.* (2014).

Actually, the need of a refined intragroup classification for the Bathyarchaeota goes beyond the mere phylogenetic framework and extends into physiology and ecology. Once confirmed their habitat diversification the next step is to unveil which are the main environmental factors that drive their habitat distribution. At this moment, the physicochemical conditions and the availability of energy and carbon sources in the habitats where Bathyarchaeota usually thrive are very diverse thus creating confusion about their physiological requirements and ecological constraints. In their pioneering study, Kubo and co-workers (2012) did not find any biogeographical trend for the habitat preferences of the distinct Bathyarchaeota subgroups and they merely described that all subgroups encompass sequences from disparate environments (*e.g.* soil, freshwater and marine sediments). The global analysis carried out in the present work (Chapter 1) unveiled clear niche preferences for the different subgroups, specially in relation to salinity. In our opinion, this work is a first step forward to a better comprehension of the main environmental drivers that affect the distribution of the different Bathyarchaeota subgroups.

4.2. Ecological role

The paucity of available sequenced genomes for members of the Bathyarchaeota currently impedes a comprehensive understanding of their metabolic capabilities and, consequently, of their ecological roles (He *et al*., 2016). At this point, there are only twelve partial or near-complete published genomes from which 8 classify into 5 described subgroups and the remaining four are still unclassified (Table 4.2). The available genomic data suggest that most Bathyarchaeota are anaerobic heterotrophs that preferentially fed on carbohydrates and proteins (Lloyd *et al*. 2013; Meng *et al.,* 2014; Lazar *et al*., 2016; He *et al*., 2016) (Table 4.2). For instance, genome fragments obtained by techniques such as single-cell sequencing or short-read genome assembly provide evidences that members of the subgroup Bathyarchaeota-6 possess genes related to the degradation of plant-derived mono- and polysaccharides (Lazar *et al*., 2016). In turn, genes related to the degradation of lignin-derived aromatic compounds have been identified in genome fragments from Bathyarchaeota-8 (Meng *et al*., 2014). In contrast, genomes from Bathyarchaeota-15 encode the enzymatic equipment necessary for the degradation of persistent components of microbial cell walls (*i.e.* peptidoglycan) (Lloyd *et al*., 2013).

Table 4.2. Metabolic traits and potential carbon sources predicted from available genomic data for members of the Phylum *Bathyarchaeota*.

Subgroup	Methodological approach[a]	Fosmid or genomic bins	% genome coverage	Isolation source	Probable C sources	Putative main metabolism	Metabolic alternatives	Reference
1	MG	SG8-32-3	51	estuarine sediment	Plant-derived polymeric carbohydrates, CO_2, other	Fermentation	Acetogenesis (auto- or heterotrophic)	[1]
6	FL	37F10	-	estuarine sediment	–	–	–	[2]
	MG	AD8-1	96	estuarine sediment	Plant-derived polymeric carbohydrates, CO_2, other	Fermentation	Acetogenesis (auto- or heterotrophic)	[1]
8	FL	75G8	-	estuarine sediment	Aromatic compounds	Fermentation	–	[2]
	FL	26B6	-	mangrove sediment	–	–	–	[2]
15	SCG	MCG_E09	41	organic-rich marine	Detrital proteins	Fermentation	–	[3]
	MG	DG-45	73	estuarine sediment	Detrital proteins, lmw* carbon substrates, CO_2	Fermentation	Acetogenesis (auto- or heterotrophic)	[1]
	MG	B23	60	marine sediment	Detrital proteins, cellulose, aromatic compounds, CO_2	Acetogenesis (auto- or heterotrophic)	?	[4]
16	MG	B26-1	74	marine sediment	Detrital proteins, cellulose, aromatic compounds, CO_2	Acetogenesis (auto- or heterotrophic)	?	[4]
	MG	B26-2	77	marine sediment	Detrital proteins, cellulose, aromatic compounds, CO_2	Acetogenesis (auto- or heterotrophic)	?	[4]

[a]: MG: Metagenomics; FL: Fosmid clone Library; SCG: Single Cell Genomics

**: Sulfate-Methane Transition Zone

[1] Lazar *et al.* (2016); [2] Meng *et al.* (2014); [3] Lloyd *et al.* (2013); [4] He *et al.* (2016); [5] Evans *et al.* (2015)

Table 4.2. Metabolic traits and potential carbon sources predicted from available genomic data for members of the Phylum Bathyarchaeota.(continued)

Subgroup	Methodological approacha	Fosmid or genomic bins code	% genome coverage	Isolation source	Probable C sources	Putative metabolism	Alternative metabolic	Reference
7/17	MG	SMTZ-80	62	estuarine sediment	Detrital proteins	Fermentation	Acetogenesis (auto- or	[1]
Unclassified	MG	BA1	92	deep anoxic	Glucose, oligopeptides,	Methanogenesis	Wood-Ljungdahl pathway,	[5]
	MG	BA2	94	deep anoxic	Fatty acid, methylated C1-compounds	Methanogenesis	Fermentation	[5]
	MG	B24	94	marine sediment	Detrital proteins, cellulose, aromatic	Acetogenesis (auto- or	Wood-Ljungdahl pathway	[4]
	MG	B25	70	marine sediment	Detrital proteins, cellulose, aromatic	Acetogenesis (auto- or	Wood-Ljungdahl pathway	[4]

[a]: MG: Metagenomics; FL: Fosmid clone Library; SCG: Single Cell Genomics

**: Sulfate-Methane Transition Zone

[1] Lazar *et al.* (2016); [2] Meng *et al.* (2014); [3] Lloyd *et al.* (2013); [4] He *et al.* (2016); [5] Evans *et al.* (2015)

Considering that plant-derived aromatic compounds and peptidoglycan are among the most persistent sedimentary detrital matter in marine sediments, which comprise the largest organic carbon sink on Earth (Hedges *et al.*, 1995), and assuming that Bathyarchaeota are widespread and abundant in the marine subsurface (Lloyd *et al.*, 2013), their impact on mineralization of buried organic carbon is probably greater than expected (Biddle *et al.*, 2006; Lloyd *et al.*, 2013; Seyler *et al.*, 2014; He *et al.*, 2016). This hypothesis agrees with the identification of Bathyarchaeota as 'keystone species' in the sedimentary realm as revealed from network analysis (see Chapter 1). In fact, their potential role as versatile degraders in sediments may stimulate the growth of other microbial groups by providing labile organic compounds to fuel heterotrophic metabolisms such as acetoclastic methanogenesis (He *et al.*, 2016). Interestingly, other uncultured archaea from sediments may also share this role as key stones species in sediment archaeal communities. Our network analysis identified a high number of interconnections between OTUs affiliated to Phylum Bathyarchaeota and class *Thermoplasmata*. These 'in silico' results agree with results obtained in Chapter 2 where OTUs affiliated to both lineages co-occur together in sediments from karstic lakes. Besides, recent survey carried out in 21 inland water bodies in the Iberian Peninsula also indicate that the co-distribution of Bathyarchaeota and Thermoplasmata are the rule rather than the exception (Compte *et al.*, submited). Despite co-occurrence is not directly an indicator of synergistic relationships and could be an artefact caused by a niche overlap (Barberan *et al.*, 2012), the recurrent association between Bathyarchaeota and Thermoplasmata is compelling and points towards a potential syntrophy between both groups. According to the putative metabolisms associated to both lineages (Lloyd *et al.*, 2013), members of the Bathyarchaeota and *Thermoplasmata* seem to perform similar ecological functions. For instance, members of the *Marine Benthic Group D* (class *Thermoplasmata*) may be involved in the degradation of detrital proteins both in marine and inland sediments (Lloyd *et al.*, 2013) similarly to some bathyarchaeons. In clear contrast, members of the *Methanomassiliicoccales* (Iino *et al.*, 2013) are true methylotrophic methanogens obtaining energy and carbon from methanol and methylated amines (Paul *et al.*, 2012; Dridi *et al.*, 2012; Iino *et al.*, 2013; Borrel *et al.*, 2014) and, therefore, could be synergistically related to Bathyarchaeota by consuming C_1-compounds derived from the degradation of complex organic matter. On the other hand, analysis of near-completed genomes of members of the Bathyarchaeota revealed the presence of divergent homologs of the genes necessary to perform methylotrophic methanogenesis from a wide range of methylated compounds (Evans *et al.*, 2015). Thus, archaea from both lineages seem to possess not only the ability to degrade complex organic matter but also to carry out methanogenesis from simple organic compounds, which makes synthrophy a reasonable hypothesis for their interaction. Besides, recent metagenomic approaches found genes involved in heterotrophic acetate production in members of the Bathyarchaeota-16 (He *et al.*, 2016) and also in the reductive acetyl-CoA pathway (the autotrophic direction for the Wood- Ljungdahl pathway (Wood, 1991)) in four nearly-completed genomes of Bathyarchaeota-1, -6, -7/17 and -15) inferring that these archaea are organoheterotrophic and/or autotrophic

acetogens (Lazar *et al.*, 2016). The occurrence of acetogenic and methanogenic bathyarchaeons, two of the most ancient biochemical reactions (He *et al.*, 2016), expands the range of metabolisms within this phylum and raises interesting questions regarding their contribution to global carbon cycle, global warming and the evolution of early life. Finally, we may also highlight that most sequenced genomes available so far were originally obtained from marine samples (10 out of 12 genomes were obtained from marine or estuarine sediments and the remaining 2 from an anoxic aquifer) thus pointing to a clear bias towards marine environments. This 'marine bias' is probably a consequence of a preferential strategy towards sampling marine sediments (Philips *et al.*, 2009) due to the higher impact that marine microbes may have on global geochemical cycles (Fry *et al.* 2008) in comparison to the presumed lower influence of microbiota from inland sediments (Figure 4.1).

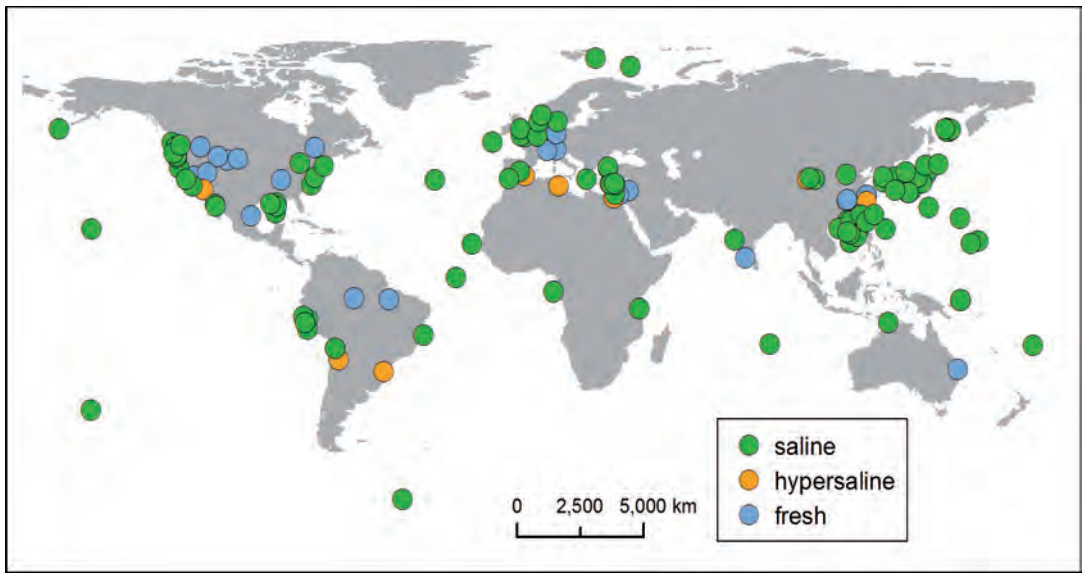

Figure 4.1. Global distribution of the available archaeal 16S rRNA gene sequence libraries from the GenBank NCBI-nr database (before January 2014) and the salinity (see dot color legend) associated to the environment at the sampling point.

In this regard, is it plausible to assume a similar role on C cycle for lacustrine bathyarchaeons? For instance, can we assume that members of Bathy-6 and Bathy-15 in Lake Cisó (Chapters 2 and 3) are metabolically similar to their marine and estuarine counterparts? Unfortunately, these questions can only be answered if genomes from these freshwater relatives could be sequenced and/or members of these groups can be isolated in pure cultures and properly characterized. Combination of genome-based approaches using state-of-the-art molecular techniques and cultivation-based methodologies is then mandatory to get a deeper insight on the ecophysiology of the Phylum Bathyarchaeota.

4.3. Cultivation in the -omics era

Microbial ecologists mostly agree that cultivation techniques are inadequate for describing naturally occurring microbial assemblages (DeLong and Pace, 2001) but they are useful to properly interpret molecular data into their ecological context (Rothschild, 2006; Nichols, 2007). Cultivation-independent approaches have facilitated not only the exploration of the unculturable majority of microorganisms (Pace, 1997; Morales and Holben, 2011) but also have permitted the precise delineation of their evolutionary relationships. One limitation of gene-based approaches is they reliance on the existence of curated sequence databases to compare against (Abulencia *et al.*, 2006). The proper interpretation of environmental molecular data largely depends on the information (*i.e.* genes, transcripts, proteins) obtained from pure cultures grown under different treatment conditions. Even for microbial species from which their complete genomes are sequenced, around 40% of encoded proteins remain functionally unassigned (Nichols *et al.*, 2007). Taking into account the rapid development of sequences technologies, the number of assembled genomes of uncultured microbes will probably multiply in the near future thus providing scaffolds to compare against. Despite these advances, the role that a gene is playing and how the environmental conditions modulate its expression can not be directly inferred from its nucleotide sequence. Besides, interactions between microbial cells or populations are difficult, if not impossible, to infer from genomic data although their pivotal role in ecosystem functioning. Most of the studies centred on the analysis of bathyarchaeotal genomes highlight the necessity to put extra efforts in trying to enrich and isolate any member of the Bathyarchaeota. Up to date, however, no members of this lineage have been brought into culture. In a recent study, Gagen an coworkers tried to enrich members of the Bathyarchaeota using sediments from the Oak River Estuary, where they accounted for the 100% of the archaeal community (Gagen *et al.*, 2013). Authors amended estuarine sediments with a large variety of organic substrates (*e.g.* acetate, glucose, amino acids, methanol, protocatechuate, pyruvate, glycerol and TCA cycle intermediates) and also used dissolved organic matter from the source sediment and different gas mixtures (H_2:CO_2, N_2:CO_2, and aerobic conditions). Despite they observed a 1-log increment in gene copies of bathyarchaeotal 16S rRNA after three weeks of incubation, any of the assayed substrates yielded significant differences when compared to control conditions. These negative results were at the same time accompanied by a reduction in the abundance of Bathyarchaeota in subsequent subcultures, thus supporting the idea that unknown compounds from the original sediment or accompanying microbes were critical for their growth and survival. Even though authors proved that growth of Bathyarchaeota was possible and amenable under laboratory conditions, the adverse results clearly demonstrated that more innovative strategies should be implemented to overcome their low culturability.

Enrichment and isolation were not primary issues of the current PhD workplan but results obtained in Chapter 3 encouraged us to use leaf litter from Lake Cisó as starting

material to enrich Bathyarchaeota. Our experimental design was aimed to detect ^{13}C incorporation into archaeal membrane lipids considering that Bathyarchaeota were prevalent in the biofilms growing in the surface of degraded leaves (≈80–90% of total archaeal reads). Biofilms were incubated for two weeks in filtered lake water supplemented with ^{13}C-labeled acetate, aminoacids from algal origin and 4-OH-benzoate to assess if Bathyarchaeota were able to incorporate any of the labeled substrates into their membrane lipids. Unfortunately, we did not detect ^{13}C incorporation into archaeal GDGTs mainly because extracts did not reach the detection limit of the system (GC-MS (Agilent 6800 GC coupled to a ThermoFisher Delta V isotope ratio monitoring mass spectrometer). Future enrichments would probably need longer incubation time and a higher concentration of the labeled substrates to ensure the minimum concentration of the analyte to allow its proper quantification. Despite these drawbacks, we obtained encouraging results regarding the putative lipid composition of Bathyarchaeota by identifying new isomers of the GDGT that may be related with members of the Bathyarchaeota-6 (see Chapter 3 for details). The biofilm under study is, however, a very promising material to use in future experiments using either stable isotope probing, metagenomics, single cell genomics or NanoSIMS (Nanoscale Secondary Ion Mass Spectometry) procedures. Alternatively, artificial substrates such as aluminum or PVC could be alternatives to natural biofilms since they are easy to handle and process under controlled conditions (Zhang *et al*., 2016).

5.Conclusions

I. Bathyarchaeota is a core generalist lineage in sediments. Their members are widespread and abundant in both marine and freshwater sediments and they can tolerate a large spectrum of environmental conditions and use a broad range of resources. Thus, the Phylum Bathyarchaeota can be considered an indicator lineage for sedimentary habitats.

II. The evolutionary history of the Bathyarchaeota shows few environmental transition events (that is, switch from a freshwater ancestor to a saline descendant and vice versa), which influenced the phylogenetic diversification of distinct subgroups. Salinity explains most of the phylogenetic variation within the phylum and thus distinct evolutionary subgroups occurred in marine and freshwater sediments.

III. Bathyarchaeota subgroups –1 and –8 are indicator lineages for marine sediments whereas subgroups –11 and –5b are indicator lineages for freshwater sediments.

IV. Bathyarchaeota systematically co-occurred often with members of the class Thermoplasmata suggesting a putative syntrophic interaction between these uncultured archaea. Both groups might be considered 'keystone species' in the sediment realm by performing major biogeochemical processes such as the degradation of recalcitrant organic matter.

V. Archaeal communities in stratified karstic lakes are mainly composed of uncultured groups within the classes Halobacteria and Thermoplasmata and phylum Bathyarchaeota, sharing striking similarities with those inhabiting the marine subsurface.

VI. The distribution and diversity of archaeal communities in stratified karstic lakes are mainly driven by the concentration of sulphide and dissolved organic carbon. This observation agrees with the putative heterotrophic metabolism assumed for most of the identified archaeal groups.

VII. Bathyarchaeota subgroups –5a and –5b are adapted to the planktonic habitat, especially in euxinic water layers where inorganic and organic reduced compounds accumulate. In turn, subgroup 6 appears to be a generalist subgroup, adapted to both the planktonic and the sediment habitat and able to thrive in a wide range of sulfidic conditions.

VIII. Bathyarchaeota subgroup–6 dominates archaeal communities in biofilms growing on the surface of plant debris in the anoxic, sulfide-rich sediment of the karstic Lake Cisó.

IX. Lipidomic analysis revealed a singular and hitherto unknown isomeric GDGT profile in euxinic niches from Lake Cisó (plankton, sediment and biofilms). Besides, isomers of isoprenoid tetraether lipids GDGT–1, –2 and –3 showed strong correlation with Bathyarchaeota subgroup–6, thus suggesting that these isomeric forms might be a specific lipid biomarker for this lineage.

X. Biofilms grown on leaf litter settled in Lake Cisó sediment are 'natural enrichments' of Bathyarchaeota subgroup–6. Thus, they may be regarded as useful samples to address experimentation aimed to answer questions regarding substrate preferences, growth requirements and cultivability of lacustrine Bathyarchaeota.

6.References

Abulencia C.B., Wyborski D.L., Garcia J.A., Podar M., Chen W., Chang S.H., Chang H.W., Watson D., Brodie E.L., Hazen T.C., Keller M. (2006). Environmental whole-genome amplification to access microbial populations in contaminated sediments. *Appl. Environ. Microbiol.* 72:3291–3301.

Auguet J-C., Barberan A. & Casamayor E.O. (2010) Global ecological patterns in uncultured Archaea. *ISME J.* 4: 182–190.

Auguet J-C. & Casamayor E.O. (2008) A hotspot for cold crenarchaeota in the neuston of high mountain lakes. *Environ. Microbiol.* 4: 1080–1086.

Baker B.J., Tyson G.W., Webb R. I., Flanagan J., Hugenholtz P., Allen E.E., Banfield J.F. (2006). Lineages of Acidophilic archaea revealed by community genomic analysis. *Science* 314:1933-1935.

Baker BJ Comolli, L.R., Dick, G.J., Hauser, L.J., Hyatt, D., Dill, B.D., Land, M.L., Verberkmoes, N.C., Hettich, R.L., Banfield, J.F. (2010) Enigmatic, ultrasmall, uncultivated Archaea. *Proc. Natl. Acad. Sci. USA*. 107: 8806–8811.

Baker B.J., Saw J.H., Lind A.E., Lazar C.S., Hinrichs K., Teske A.P. & Ettema T.J.G. (2016) Genomic inference of the metabolism of cosmopolitan subsurface Archaea, Hadesarchaea. *Nat. Microbiol.* 1: 16002.

Barberan A., Bates S.T., Casamayor E.O. & Fierer N. (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* 6: 343–351.

Barns S.M., Delwiche C.F., Palmer J.D. & Pace N.R. (1996) Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proc. Natl. Acad. Sci. USA*. 93: 9188–9193.

Barns S.M., Fundyga R.E., Jeffries M.W. & Pace N.R. (1994) Remarkable archaeal diversity detected in a Yellowstone-National-Park hot-spring environment. *Proc. Natl. Acad. Sci. USA*. 91: 1609–1613.

Bates S.T., Berg-Lyons D., Caporaso J.G., Walters W.A., Knight R. & Fierer N. (2011) Examining the global distribution of dominant archaeal populations in soil. *ISME J.* 5171: 908–917.

Berdjeb L., Pollet T., Chardon C. & Jacquet S. (2013) Spatio-temporal changes in the structure of archaeal communities in two deep freshwater lakes. *FEMS Microbiol. Ecol.* 86: 215–230.

Biddle J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T. J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H., Hinrichs, Kai-Uwe U (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl. Acad. Sci. USA* 103: 3846–3851.

Biddle J.F., Fitz-Gibbon S., Schuster S.C., Brenchley J.E. & House C.H. (2008) Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proc. Natl. Acad. Sci. USA* 105: 10583–10588.

Bintrim S.B., Donohue T.J., Handelsman J., Roberts G.P. & Goodman R.M. (1997) Molecular phylogeny of Archaea from soil. *Proc. Natl. Acad. Sci. USA* 94: 277–282.

Borrel G., O'Toole P.W., Harris H.M.B., Peyret P., Brugère J-F. & Gribaldo S. (2013) Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of Methanogenesis. *Genome Biol. Evol.* 5: 1769–1780.

Borrel G., Parisot, N., Harris, Hugh M.B., Peyretaillade, E., Gaci, N., Tottey, W., Bardot, O., Raymann, K., Gribaldo, S., Peyret, P., O'Toole, P.W. & Brugère, J-F. (2014) Comparative genomics highlights the unique biology of Methanomassiliicoccales, a Thermoplasmatales-related seventh order of methanogenic archaea that encodes pyrrolysine. *BMC Genomics* 15: 1–24.

Bricheux G., Morin L., Le Moal G., Coffe G., Balestrino D., Charbonnel N., Bohatier J. & Forestier C. (2013) Pyrosequencing assessment of prokaryotic and eukaryotic diversity in biofilm communities from a French river. *Microbiology open* 2: 402–414.

Brochier-Armanet C., Boussau B., Gribaldo S. & Forterre P. (2008) Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nat. Rev. Microbiol.* 6: 245–252.

Buckles L.K., Villanueva L., Weijers J.W.H., Verschuren D. & Damsté J.S.S. (2013) Linking isoprenoidal GDGT membrane lipid distributions with gene abundances of ammonia-oxidizing Thaumarchaeota and uncultured crenarchaeotal groups in the water column of a tropical lake (Lake Challa, East Africa). *Environ. Microbiol.* 15: 2445–2462.

Burgess E.A., Unrine J.M., Mills G.L., Romanek C.S. & Wiegel J. (2012) Comparative geochemical and microbiological characterization of two Thermal pools in the Uzon Caldera, Kamchatka, Russia. *Microb. Ecol.* 63: 471–489.

Casamayor EO & Borrego CM (2009) Archaea in inland waters. In: Enclyclopedia of Inland Waters. Likens G (Ed.), Elsevier Academic Press, Oxford, pp 167–181.

Castelle C.J., Wrighton, K.C., Thomas, B.C., Hug, L.A., Brown, C.T., Wilkins, M.J., Frischkorn, K.R., Tringe, S.G., Singh, A., Markillie, L.M., Taylor, R. C., Williams, K.H., Banfield, J.F. (2015) Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. *Curr. Biol.* 25: 690–701.

Chaban B., Ng, S.Y.M. & Jarrell, K.F. (2006) Archaeal habitats — from the extreme to the ordinary. *Can. J. Microbiol.* 52: 73–116.

Chandler D.P., Brockman F.J., Bailey T.J. & Fredrickson J.K. (1998) Phylogenetic diversity of archaea and bacteria in a deep subsurface paleosol. *Microb. Ecol.* 36: 37–50.

Chen Y., Wu L., Boden R., Hillebrand A., Kumaresan D., Moussard H., Baciu M., Lu Y. & Colin M.J. (2009) Life without light: microbial diversity and evidence of sulfur- and ammonium-based chemolithotrophy in Movile Cave. *ISME J.* 3: 1093–1104.

Collins G., Mahony T., McHugh S., Gieseke A., de Beer D. & Flaherty V. (2005) Distribution, dynamics and in situ ecophysiology of Crenarchaeota in anaerobic wastewater treatment granular biofilms. *Water Sci. Technol.* 52: 233-239.

DeLong E.F. (1992) Archaea in coastal marine environments. *Proc. Natl. Acad. Sci. U. S. A.* 89: 5685–5689.

DeLong E (1998) Archaeal means and extremes. *Science* 280: 542–543.

DeLong E.F. & Pace N.R. (2001) Environmental diversity of bacteria and archaea. *Syst. Biol.* 50: 470–478.

Dridi B., Fardeau M.L., Ollivier B., Raoult D. & Drancourt M. (2012) Methanomassiliicoccus luminyensis gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 62: 1902–1907.

Durbin A.M. & Teske A. (2012) Archaea in organic-lean and organic-rich marine subsurface sediments: an environmental gradient reflected in distinct phylogenetic lineages. *Front. Microbiol.* 3: 168.

Elkins J.G., Podar, M., Graham, D.E., Makarova, K.S., Wolf, Y.. Randau, L., Hedlund, B.P., Brochier-Armanet, C., Kunin, V., Anderson, I., Lapidus, A., Goltsman, E., Barry, K., Koonin, E.V., Hugenholtz, P., Kyrpides, N., Wanner, G.. Richardson, P.. Keller, M., Stetter, K.O. (2008) A korarchaeal genome reveals insights into the evolution of the Archaea. *Proc. Natl. Acad. Sci. U. S. A.* 105: 8102–8107.

Evans P.N., Parks D.H., Chadwick G.L., Robbins S.J., Orphan V.J., Golding S.D. & Tyson G.W. (2015) Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science* 350(6259):434-8

Fan X. & Xing P. (2016) Differences in the Composition of Archaeal Communities in Sediments from Contrasting Zones of Lake Taihu. *Front. Microbiol.* 7: 1–11.

Fillol M., Sànchez-Melsió À., Gich F. & Borrego C.M. (2015) Diversity of Miscellaneous Crenarchaeotic Group archaea in freshwater karstic lakes and their segregation between planktonic and sediment habitats. *FEMS Microbiol. Ecol.* 91: fiv020.

Fillol M., Auguet J-C., Casamayor E.O. & Borrego C.M. (2016) Insights in the ecology and evolutionary history of the Miscellaneous Crenarchaeotic Group lineage. *ISME J.* 10(3):665–677.

Friedrich M.W., Schmitt-Wagner D., Lueders T. & Andreas B. (2001) Axial differences in community structure of Crenarchaeota and Euryarchaeota in the highly compartmentalized gut of the soil-feeding termite *Cubitermes orthognathus*. *Appl. Environ. Microbiol.* 67: 4880–4890.

Fry J.C., Parkes R.J., Cragg B.A., Weightman A.J. & Webster G. (2008) Prokaryotic biodiversity and activity in the deep subseafloor biosphere. *FEMS Microbiol. Ecol.* 66: 181–196.

Fuhrman J.A., McCallum K. & Davis A.A. (1992) Novel major archaebacterial group from marine plankton. *Nature* 356: 148–149

Gagen E.J., Huber H., Meador T., Hinrichs K-U. & Thomm M. (2013) Novel cultivation-based approach to understanding the miscellaneous crenarchaeotic group (MCG) archaea from sedimentary ecosystems. *Appl. Environ. Microbiol.* 79: 6400–6406.

Guy L. & Ettema T.J.G. (2011) The archaeal "TACK" superphylum and the origin of eukaryotes. Trends Microbiol. 19: 580–587.

Hallam S.J., Putnam N., Preston C.M., Detter J.C., Rokhsar D., Richardson P.M. & DeLong E.F. (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science* (80) 305: 1457-1462.

Harvey H.R., Fallon R.D. & Patton J.S. (1986) The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. *Geochim. Cosmochim. Acta* 50: 795–804.

He Y., Li M., Perumal V., Feng X., Fang J., Xie J., Sievert S.M. & Wang F. (2016) Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. *Nat. Microbiol.* 16035.

Hedges J.I. & Keil R.G. (1995). Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem*. 49, 81–115.

Hershberger K.L., Barns S.M., Reysenbach A-L., Dawson S.C. & Pace N.R. (1996) Wide diversity of Crenarchaeota. *Nature* 384.

Hopmans, E.C., Schouten, S., Pancost, R.D., van der Meer, M.T., and Sinninghe Damsté, J.S. (2000) Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun Mass Spectrom* 14: 585–589.

Hoshino T., Morono Y., Terada T., Imachi H., Ferdelman T.G. & Inagaki F. (2011) Comparative study of subseafloor microbial community structures in deeply buried coral fossils and sediment matrices from the challenger mound in the porcupine seabight. *Front. Microbiol.* 2: 231.

Hu A., Wang H., Li J., Liu J., Chen N. & Yu C.P. (2016) Archaeal community in a human-disturbed watershed in southeast China: diversity, distribution, and responses to environmental changes. *Appl. Microbiol. Biotechnol.* 1–14.

Huang L.N., Chen Y.Q., Zhou H., Luo S., Lan C.Y. & Qu L.H. (2003) Characterization of methanogenic Archaea in the leachate of a closed municipal solid waste landfill. *FEMS Microbiol. Ecol.* 46: 171–177.

Huber H., Hohn M.J., Rachel R., Fuchs T., Wimmer V.C. & Stetter K.O. (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* 417: 63–67.

Iino T., Tamaki H., Tamazawa S., Ueno Y., Ohkuma M., Suzuki K., Igarashi Y. & Haruta S. (2013) Candidatus Methanogranum caenicola: a novel methanogen from the anaerobic digested sludge, and proposal of Methanomassiliicoccaceae fam. nov. and Methanomassiliicoccales ord. nov., for a methanogenic lineage of the Class *Thermoplasmata*. *Microbes Environ.* 28: 244–250.

Inagaki F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F.S., Nealson, K.H., Horikoshi, K., D'Hondt, S., Jørgensen, B.B. (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. U. S. A.* 103: 2815–2820.

Inagaki F., Suzuki M., Takai K., Oida H., Sakamoto T., Aoki K., Nealson K.H. & Horikoshi K. (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl. Environ. Microbiol.* 69(12):7224.

Ingalls A.E., Shah S.R., Hansman R.L., Aluwihare L.I., Santos G.M., Druffel E.R.M. & Pearson A. (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proc. Natl. Acad. Sci. U. S. A.* 103: 6442–6447.

Jiang H., Dong H., Yu B., Ye Q., Shen J., Rowe H. & Zhang C. (2008) Dominance of putative marine benthic Archaea in Qinghai Lake, north-western China. *Environ. Microbiol.* 10: 2355–2367.

Jiang L., Zheng Y., Chen J., Xiao X. & Wang F. (2011) Stratification of Archaeal communities in shallow sediments of the Pearl River Estuary, Southern China. *Antonie Van Leeuwenhoek* 99: 739–751.

Jungbluth S.P., Grote J., Lin H-T., Cowen J.P. & Rappé M.S. (2012) Microbial diversity within basement fluids of the sediment-buried Juan de Fuca Ridge flank. *ISME J.* 161–172.

Karner M.B., Delong E.F. & Karl D.M. (2001) Archaeal dominance in the mesopelagic zone of thePacific Ocean. *Nature* 507–510.

Kasai Y., Takahata Y., Hoaki T. & Watanabe K. (2005) Physiological and molecular characterization of a microbial community established in unsaturated, petroleum-contaminated soil. *Environ. Microbiol.* 7: 806–818.

Kaur G., Mountain B.W., Hopmans E.C. & Pancost R.D. (2011) Relationship between lipid distribution and geochemical environment within Champagne Pool, Waiotapu, New Zealand. *Org. Geochem.* 42: 1203–1215.

Könneke M., Bernhard A.E., de la Torre J.R., Walker C.B., Waterbury J.B. & Stahl D. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543–546.

Kozubal M.A., Romine M., Jennings R.M., Jay Z.J., Tringe S.G., Rusch D.B., Beam J.P., McCue L.A. & Inskeep W.P. (2013) Geoarchaeota: a new candidate phylum in the Archaea from high-temperature acidic iron mats in Yellowstone National Park. *ISME J.* 7: 622–634.

Kubo K., Lloyd K.G., F Biddle J., Amann R., Teske A. & Knittel K. (2012) Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *ISME J.* 6: 1949–1965.

Kuroda K., Hatamoto M., Nakahara N., Abe K., Takahashi M., Araki N. & Yamaguchi T. (2015) Community composition of known and uncultured archaeal lineages in anaerobic or anoxic wastewater treatment sludge. *Microb. Ecol.* 69: 586–596.

Lazar C.S., Baker B.J., Seitz K., Hyde A.S., Dick G.J., Hinrichs K.U. & Teske A.P. (2016) Genomic evidence for distinct carbon substrate preferences and ecological niches of Bathyarchaeota in estuarine sediments. *Environ. Microbiol.* 18: 1200–1211.

Lazar C.S., Biddle J.F., Meador T.B., Blair N., Hinrichs K-U. & Teske A.P. (2014) Environmental controls on intragroup diversity of the uncultured benthic archaea of the Miscellaneous Crenarchaeotal Group lineage naturally enriched in anoxic sediments of the White Oak River Estuary (North Carolina, USA). *Environ. Microbiol.* 17(7): 2228-2238

Lazar C.S., Dinasquet J., Pignet P., Prieur D. & Toffin L. (2010) Active archaeal communities at cold seep sediments populated by Siboglinidae tubeworms from the Storegga Slide. *Microb. Ecol.* 60: 516–527.

Lazar C.S., L'haridon S., Pignet P. & Toffin L. (2011) Archaeal populations in hypersaline sediments underlying orange microbial mats in the Napoli mud volcano. *Appl. Environ. Microbiol.* 77: 3120–3131.

Leininger S., Urich T., Schloter M., Schwark L., Qi J., Nicol G.W., Prosser J.I., Schuster S.C. & Schleper C. (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442: 806–809.

Lin X., McKinley J., Resch C.T., Kaluzny R., Lauber C.L., Fredrickson J., Knight R. & Konopka A. (2012) Spatial and temporal dynamics of the microbial community in the Hanford unconfined aquifer. *ISME J.* 6: 1665–1676.

Lipp J.S., Morono Y., Inagaki F. & Hinrichs K-UU. (2008) Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature* 454: 991–994.

Llirós M., Casamayor E.O. & Borrego C. (2008) High archaeal richness in the water column of a freshwater sulfurous karstic lake along an interannual study. *FEMS Microbiol. Ecol.* 66: 331–342

Lliros M., Gich F., Plasencia A., Auguet J-C., Darchambeau F., Casamayor E.O., Descy J-P. & Carles Borrego (2010) Vertical distribution of ammonia-oxidizing crenarchaeota and methanogens in the epipelagic waters of Lake Kivu (Rwanda-Democratic Republic of the Congo). *Appl. Environ. Microbiol.* 76: 6853–6863.

Llirós M Alonso-Saez, L., Gich, F., Plasencia, A., Auguet, O., Casamayor, E.O. &

Borrego, C.M. (2011) Active bacteria and archaea cells fixing bicarbonate in the dark along the water column of a stratified eutrophic lagoon. *FEMS Microbiol. Ecol.* 77: 370–384

Llirós M., Trias R., Borrego C. & Bañeras L. (2014) Specific archaeal communities are selected on the root surfaces of *Ruppia* spp. and *Phragmites australis*. *Wetlands* 34: 403–411.

Lloyd K.G., Schreiber, L., Petersen, D.G., Kjeldsen, K.U., Lever, M., Steen, A.D., Stepanauskas, R., Richter, M., Kleindienst, S., Lenk, S., Schramm, A. & Jørgensen, B.B. (2013) Predominant archaea in marine sediments degrade detrital proteins. *Nature* 496: 215-218

Massana R., Murray A.E., Preston C.M. & DeLong E.F. (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Appl Env. Microbiol* 63: 50–56.

Meng J., Xu J., Qin D., He Y., Xiao X. & Wang F. (2014) Genetic and functional properties of uncultivated MCG archaea assessed by metagenome and gene expression analyses. *ISME J.* 8: 650–659.

Morales S.E. & Holben W.E. (2011) Linking bacterial identities and ecosystem processes: Can "omic" analyses be more than the sum of their parts? *FEMS Microbiol. Ecol.* 75: 2–16.

Narasingarao P., Podell S., Ugalde J., Brochier-Armanet C., Emerson J.B., Brocks J.J., Heidelberg K.B., Banfield J.F. & Allen E.E. (2012) De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities. *ISME J.* 6: 81–93.

Nercessian O., Fouquet Y., Pierre C., Prieur D. & Jeanthon C. (2005) Diversity of Bacteria and Archaea associated with a carbonate-rich metalliferous sediment sample from the Rainbow vent field on the Mid-Atlantic Ridge. *Environ. Microbiol.* 7: 698–714.

Nichols D. (2007) Cultivation gives context to the microbial ecologist. *FEMS Microbiol. Ecol.* 60: 351–357.

Nunoura T., Oida, H., Nakaseama, M., Kosaka, A., Ohkubo, S.B., Kikuchi, T., Kazama, H., Hosoi-Tanabe, S., Nakamura, K-I., Kinoshita, M., Hirayama, H., Inagaki, F., Tsunogai, U., Ishibashi, J.-I. & Takai, K. (2010) Archaeal diversity and distribution along thermal and geochemical gradients in hydrothermal sediments at the Yonaguni Knoll IV hydrothermal field in the Southern Okinawa trough. *Appl. Environ. Microbiol.* 76: 1198–1211.

Nunoura T., Hirayama, H., Takami, H., Oida, H., Nishi, S., Shimamura, S., Suzuki, Y., Inagaki, F., Takai, K., Nealson, K.H. & Horikoshi, K. (2005) Genetic and functional properties of uncultivated thermophilic crenarchaeotes from a subsurface gold mine as revealed by analysis of genome fragments. *Environ. Microbiol.* 7: 1967–1984.

Nunoura T., Takaki, Y., Kakuta, J., Nishi, S., Sugahara, J., Kazama, H., Chee, G-J., Hattori, M., Kanai, A., Atomi, H., Takai, K. & Takami, H. (2011) Insights into the evolution of Archaea and eukaryotic protein modifier systems revealed by the genome of a novel archaeal group. *Nucleic Acids Res.* 39: 3204–3223.

Ochsenreiter T., Selezi D., Quaiser A., Bonch-Osmolovskaya L., Schleper C. (2003) Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. *Environ. Microbiol.* 5(9): 787–797.

Oni O.E., Schmidt F., Miyatake T., Kasten S., Witt M., Hinrichs K.U. & Friedrich M.W. (2015) Microbial communities and organic matter composition in surface and subsurface sediments of the Helgoland mud area, North Sea. *Front. Microbiol.* 6: 1–16.

Pace N.R. (1997) A molecular view of microbial diversity and the biosphere. *Science* 276: 734–740.

Pancost R.D., J, Sinninghe Damsté J.S., Lint S. De, Maarel M.J.E.C. Van Der & Gottschal J.C. (2000) Biomarker evidence for widespread anaerobic methane oxidation in mediterranean sediments by a consortium of methaogenic Archaea and Bacteria. *Appl. Environ. Microbiol.* 66: 1126–1132.

Paul K., Nonoh J.O., Mikulski L. & Brune A. (2012) "Methanoplasmatales," Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. *Appl. Environ. Microbiol.* 78: 8245–8253.

Pearson A., Pi, Y., Zhao, W., Li, W., Li, Y., Inskeep, W., Perevalova, A., Romanek, C., Li, S. & Zhang, C.L. (2008) Factors controlling the distribution of archaeal tetraethers in terrestrial hot springs. *Appl. Environ. Microbiol.* 74: 3523–3532.

Pearson A., Huang Z., Ingalls A.E., Romanek C.S., Wiegel J., Freeman K.H., Smittenberg R.H. & Zhang C.L. (2004) Nonmarine crenarchaeol in Nevada hot springs. *Appl. Environ. Microbiol.* 70: 5229–5237.

Pester M., Schleper C. & Wagner M. (2011) The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. *Curr. Opin. Microbiol.* 14: 300–306.

Petitjean C., Deschamps P., López-García P. & Moreira D. (2014) Rooting the Domain Archaea by Phylogenomic Analysis Supports the Foundation of the New Kingdom Proteoarchaeota. *Genome Biol.* Evol.

Hugenholtz P. and Kyrpides N. A changing of the guard: Genomics update. (2009). *Environ. Microbiol*. 11(3):551-553.

Pitcher A., Hopmans E.C., Mosier A.C., Park S.J., Rhee S.K., Francis C.A., Schouten S. & Sinninghe Damsté J.S. (2011a) Core and intact polar glycerol dibiphytanyl glycerol tetraether lipids of ammonia-oxidizing Archaea enriched from marine and estuarine sediments. *Appl. Environ. Microbiol*. 77: 3468–3477.

Podar M., Makarova K.S., Graham D.E., Wolf Y.I., Koonin E.V. & Reysenbach A-L. (2013) Insights into archaeal evolution and symbiosis from the genomes of a nanoarchaeon and its inferred crenarchaeal host from Obsidian Pool, Yellowstone National Park. *Biol. Direct* 8: 9.

Porat I., Vishnivetskaya, T.A., Mosher, J.J., Brandt, Craig C., Yang, Zamin K., Brooks, Scott C., Liang, L., Drake, M.M.,Podar, M., Brown, S.D. & Palumbo, A.V. (2010) Characterization of archaeal community in contaminated and uncontaminated surface stream sediments. *Microb. Ecol.* 60: 784–795.

Preston C.M., Wu K.Y., Molinski T.F., DeLong E.F. (1996) A psychrophilic crenarchaeon inhabits a marine sponge: Cenarchaeum symbiosum gen. nov., sp. nov. *Proc Natl Acad Sci USA* 93:6241–6246.

Rinke C Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J.-F., Darling, A., Malfatti, S., Swan, B.K.,Gies, E., Dodsworth, J., Hedlund, B.P., Tsiamis, G., Sievert, S.M., Liu, W-T., Eisen, J., Hallam, S.J., Kyrpides, N.C., Stepanauskas, R., Rubin, E.M., Hugenholtz, P. & Woyke, T. (2013) Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 499: 431–437.

Roh S.W., Kim K-H., Nam Y-D., Chang H-W., Park E-J. & Bae J-W. (2010) Investigation of archaeal and bacterial diversity in fermented seafood using barcoded pyrosequencing. *ISME J.* 4: 1–16.

Rossel P.E., Elvert M., Ramette A., Boetius A. & Hinrichs K.U. (2011) Factors controlling the distribution of anaerobic methanotrophic communities in marine environments: Evidence from intact polar membrane lipids. *Geochim. Cosmochim. Acta* 75: 164–184.

Rothschild, L. (2006) A microbiologist explodes the myth of the unculturables. *Nature* 443: 249

Schleper C., Jurgens G. & Jonuscheit M. (2005) Genomic studies of uncultivated archaea. Nat. Rev. Microbiol. 3: 479–488.

Schouten S., Huguet C., Hopmans E. C., Kienhuis M. and Sinninghe Damste´ J. S. (2007a) Analytical methodology for TEX86 paleothermometry by high-performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. *Anal. Chem*. 79: 2940-2944.

Schouten S., Hopmans E.C. & Sinninghe Damsté J.S. (2013) The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. *Org. Geochem*. 54: 19–61.

Schouten S., Van Der Meer M.T.J., Hopmans E.C., Rijpstra W.I.C., Reysenbach A.L., Ward D.M. & Damsté J.S.S. (2007) Archaeal and bacterial glycerol dialkyl glycerol tetraether lipids in hot springs of Yellowstone National Park. *Appl. Environ. Microbiol.* 73: 6181–6191.

Schouten S., Rijpstra W.I., Kok M., Hopmans E., Summons R., Volkman J. & Sinninghe Damsté J.S. (2001) Molecular organic tracers of biogeochemical processes in a saline meromictic lake (Ace Lake). *Geochim. Cosmochim. Acta* 65: 1629–1640.

Seitz K.W., Lazar C.S., Hinrichs K-U., Teske A.P. & Baker B.J. (2016) Genomic reconstruction of a novel, deeply branched sediment archaeal phylum with pathways for acetogenesis and sulfur reduction. *ISME J.* 1–10.

Seyler L.M., McGuinness L.M. & Kerkhof L.J. (2014) Crenarchaeal heterotrophy in salt marsh sediments. *ISME J.* 8: 1534–1543.

Shcherbakova V., Yoshimura Y., Ryzhmanova Y., Taguchi Y., Segawa T., Oshurkova V. & Rivkina E. (2016) Archaeal communities of Arctic methane-containing permafrost. *FEMS Microbiol. Ecol.* dx.doi.org/10.1093/femsec/fiw135

Shi Y.W., TaPa, M.S., Li, C., Yang, H.M., Zhang, T., Gao, Y., Sun, J., Zeng, J., Lin, Q. Cao, & Z.H., (2015) Diversity and space-time dynamics of endophytic archaea from sugar beet in the north slope of Tianshan Mountain revealed by 454 pyrosequencing and T-RFLP. *World J. Microbiol. Biotechnol.* 31: 1031–1039.

Silveira C.B., Cardoso A.M., Coutinho F.H., Lima J.L., Pinto L.H., Albano R.M., Clementino M.M., Martins O.B. & Vieira R.P. (2013) Tropical Aquatic Archaea Show Environment-Specific Community Composition. *PLoS One* 8: 1–12.

Sinninghe Damsté J.S., Schouten S., Hopmans E., van Duin A. & Geenevasen J. (2002a) Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. *J. Lipid Res.* 43: 1641–1651.

Sørensen K.B., Lauer A. & Teske A. (2004) Archaeal phylotypes in a metal-rich and low-activity deep subsurface sediment of the Peru Basin , ODP Leg 201 , Site 1231. *Geobiology* 2: 151–161.

Sørensen K.B. & Teske A. (2006) Stratified communities of active Archaea in deep marine subsurface sediments. Appl. Environ. Microbiol. 72: 4596–4603.

Spang A., Saw, J.H., Jørgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E., van Eijk, R., Schleper, C., Guy, L. & Ettema, T.J.G. (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521: 173–179.

Stainer R.Y. & Ornston L.N. (1973) The β-Ketoadipate Pathway. *Adv. Microb. Physiol.* 9: 89–151.

Stein L.Y., Jones G., Alexander B., Elmund K., Wright-Jones C. & Nealson K.H. (2002) Intriguing microbial diversity associated with metal-rich particles from a freshwater reservoir. *FEMS Microbiol. Ecol.* 42: 431–440.

Sturt H.F., Summons R.E., Smith K., Elvert M. & Hinrichs K-U. (2004) Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry--new biomarkers for biogeochemistry and microbial ecology. *Rapid Commun. Mass Spectrom.* 18: 617–628.

Takai K. & Horikoshi K. (1999) Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics* 152: 1285–1297.

Takai K., Moser D.P., DeFlaun M., Onstott T.C. & Fredrickson J.K. (2001) Archaeal diversity in waters from deep South African gold mines. *Appl. Environ. Microbiol.* 67: 5750–5760.

Takai K. & Sako Y. (1999) A molecular view of archaeal diversity in marine and terrestrial hot water environments. *FEMS Microbiol. Ecol.* 28: 177–188.

Takano Y., Chikaraishi Y., Ogawa N.O., Nomaki H., Morono Y., Inagaki F., Kitazato H., Hinrichs K-U. & Ohkouchi N. (2010) Sedimentary membrane lipids recycled by deep-sea benthic archaea. *Nat. Geosci* 3: 858–861.

Teske A., Hinrichs K.U., Edgcomb V., De Vera Gomez A., Kysela D., Sylva S.P., Sogin M.L. & Jannasch H.W. (2002) Microbial diversity of hydrothermal sediments in the Guaymas Basin: Evidence for anaerobic methanotrophic communities. *Appl. Environ. Microbiol.* 68: 1994–2007.

Teske A. & Sørensen K.B. (2008) Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME J.* 2: 3–18.

Teske A.P. (2006) Microbial community composition in deep marine subsurface sediments of ODP Leg 201: sequencing surveys and cultivations. *Proceedings of the Ocean Drilling Program, Scientific Results* 201: 1–19.

Tornabene T.G., Holzer G. & Peterson S.L. (1980) Lipid profile of the halophilic alga, Dunaliella salina. *Biochem. Biophys. Res. Commun.* 96: 1349–1356.

Tripathi B.M., Kim M., Lai-Hoe A., Shukor N.A.A., Rahim R.A., Go R. & Adams J.M. (2013) PH dominates variation in tropical soil archaeal diversity and community structure. *FEMS Microbiol. Ecol.* 86: 303–311.

Tupinambá D.D., Cantão M.E., Yonara O., Costa A., Bergmann J.C., Kruger R.H., Kyaw C.M., Barreto C.C. & Quirino B.F. (2016) Archaeal community changes associated with cultivation of Amazon forest soil with oil palm. *Archaea* 2016: 0–8.

Vanwonterghem I., Evans P.N., Parks D.H., Jensen P.D., Woodcroft B.J., Hugenholtz P. & Tyson G.W. (2016) Methylotrophic methanogenesis discovered in the novel archaeal phylum Verstraetearchaeota. *Nature Microb.* 1–9.

Vetriani C., Jannasch H.W., Gregor B.J.M.A.C., Stahl D.A. & Reysenbach A. (1999) Population Structure and Phylogenetic Characterization of Marine Benthic Archaea in Deep-Sea Sediments. *Appl. Environ. Microbiol.* 65: 4375–4384.

Vila-Costa M., Barberan A., Auguet J.C., Sharma S., Moran M.A. & Casamayor E.O. (2013) Bacterial and archaeal community structure in the surface microlayer of high mountain lakes examined under two atmospheric aerosol loading scenarios. *FEMS Microbiol. Ecol.* 84: 387–397.

Villanueva L., Schouten S. & Damsté J.S.S. (2016) Phylogenomic analysis of lipid biosynthetic genes of Archaea shed light on the "lipid divide." *Environ. Microbiol.* 1–34.

Walker C.B. de la Torre, J.R., Klotz, M.G., Urakawa, H., Pinel, N., Arp, D.J., Brochier-Armanet, C., Chain, P.S.G., Chan, P.P., Gollabgir, A., Hemp, J., Hügler, M., Karr, E.A., Könneke, M., Shin, M., Lawton, T.J., Lowe, T., Martens-Habbena, W., Sayavedra-Soto, L.A., Lang, D., Sievert, S.M., Rosenzweig, A.C., Manning, G. & Stahl, D.A. (2010) Nitrosopumilus maritimus genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. *Proc. Natl. Acad. Sci. U. S. A.* 107: 8818–8823.

Walsh D.A., Papke R.T. & Doolittle W.F. (2005) Archaeal diversity along a soil salinity gradient prone to disturbance. *Environ. Microbiol.* 7: 1655–1666.

Ward D.M., Brassell S.C. & Eglinton G. (1985) Archaebacterial lipids in hot-spring microbial mats. Nature 318: 656–659.

Waters E., Hohn, M.J., Ahel, I., Graham, D.E., Adams, M.D., Barnstead, M., Beeson, K.Y., Bibbs, L., Bolanos, R., Keller, M., Kretz, K., Lin, X., Mathur, E., Ni, J., Podar, M., Richardson, T., Sutton, G.G., Simon, M., Soll, D., Stetter, K.O., Short, J.M. & Noordewier, M. (2003) The genome of Nanoarchaeum equitans: insights into early archaeal evolution and derived parasitism. *Proc. Natl. Acad. Sci. U. S. A.* 100: 12984–12988.

Webster G., Parkes, J.R., Cragg, B.A., Newberry, C.J., Weightman, A.J., Fry, J.C. (2006) Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. *FEMS Microbiol. Ecol.* 58: 65–85.

Webster G., Rinna J., Roussel E.G., Fry J.C., Weightman A.J. & Parkes R.J. (2010) Prokaryotic functional diversity in different biogeochemical depth zones in tidal sediments of the Severn Estuary, UK, revealed by stable-isotope probing. *FEMS Microbiol. Ecol.* 72: 179–197.

Wemheuer B., Taube R., Akyol P., Wemheuer F. & Daniel R. (2013) Microbial diversity and biochemical potential encoded by thermal spring metagenomes derived from the Kamchatka peninsula. *Archaea* 2013 ID 136714.

White D.C., Davis W.M., Nickels J.S., King J.D. & Bobbie R.J. (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia* 40: 51–62.

Williams T.A. & Embley T.M. (2014) Archaeal "dark matter" and the origin of eukaryotes. *Genome Biol. Evol.* 6: 474–481.

Woese C.R. & Fox G.E. (1977) Phylogenetic structure of the prokaryotic domain : The primary kingdoms. *Proc. Natl. Acad. Sci. U. S. A.* 74: 5088–5090.

Woese C.R. (1987) Bacterial evolution. *Microbiol. Rev.* 2: 221-271.

Woese C.R., Kandler O. & Wheelis M.L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci. U. S. A.* 87: 4576–4579.

Wood (1991). Life with CO or CO_2 and H_2 as a source of carbon and energy. *FASEB J* 5:156-63

Wuchter C., Parkes, J.R., Cragg, B.A., Newberry, C.J., Weightman, A.J. & Fry, J.C. (2006a) Archaeal nitrification in the ocean. *Proc. Natl. Acad. Sci. U. S. A.* 103: 12317–12322.

Yarza P., Ludwig W., Euzéby J., Amann R., Schleifer K.H., Glöckner F.O. & Rosselló-Móra R. (2010) Update of the all-species living tree project based on 16S and 23S rRNA sequence analyses. *Syst. Appl. Microbiol.* 33: 291–299.

Yergeau E., Lawrence J.R., Sanschagrin S., Waiser M.J., Korber D.R. & Greer C.W. (2012) Next-generation sequencing of microbial communities in the athabasca river and its tributaries in relation to oil sands mining activities. *Appl. Environ. Microbiol.* 78: 7626–7637.

Youssef N.H., Rinke C., Stepanauskas R., Farag I., Woyke T. & Elshahed M.S. (2014) Insights into the metabolism, lifestyle and putative evolutionary history of the novel archaeal phylum "Diapherotrites". *ISME J.* 9: 1–14.

Zhang C.L., Pearson A., Li Y.L., Mills G. & Wiegel J. (2006) Thermophilic temperature optimum for crenarchaeol synthesis and its implication for archaeal evolution. *Appl. Environ. Microbiol.* 72: 4419–4422.

Zhang W., Ding W., Yang B., Tian R., Gu S., Luo H. & Qian P-Y. (2016) Genomic and transcriptomic evidence for carbohydrate consumption among microorganisms in a cold seep brine pool. *Front. Microbiol.* 7: 1825

ANNEX

Supplementary information Chapter 1

SUPPLEMENTARY FIGURE S1

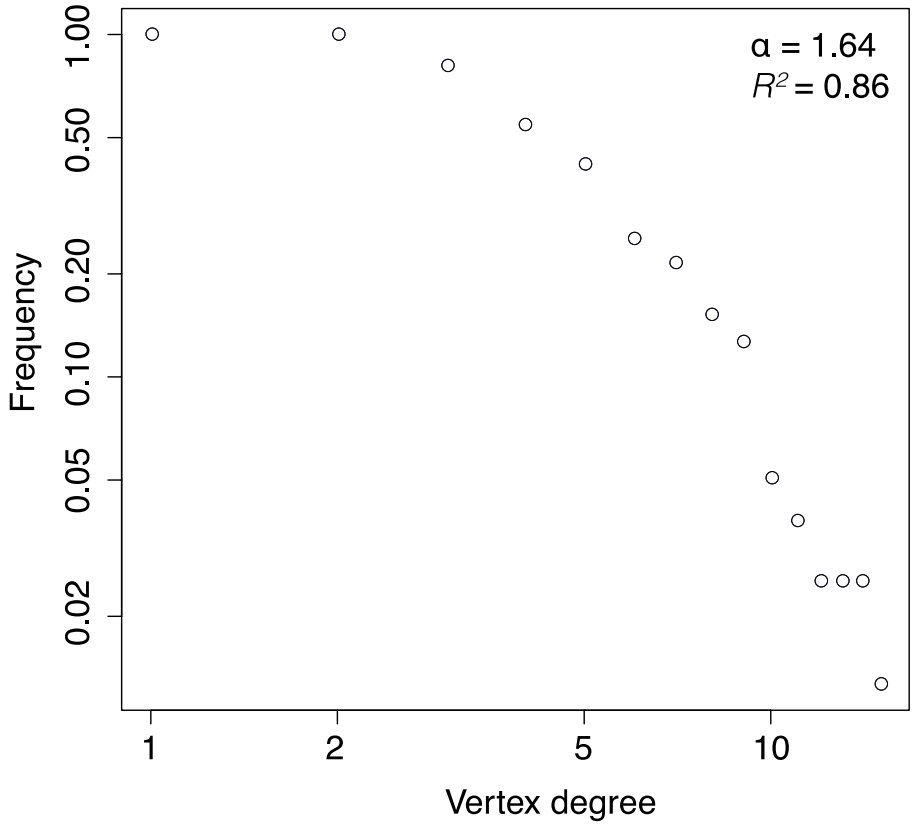

Figure S1: Cumulative degree distribution of the archaeal sediment network. The connectivity distribution is adjusted to a power law distribution expressed as $P(k) \approx k^{-\alpha}$, where P is the number of nodes with k degrees, k is connectivity/degrees and α is a constant.

Table S1. Summary of the 207 archaeal libraries included in the analysis and the environmental matrix associated.

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
Freshwater	Alpine Lake Joeri XIII, Switzerland	1	17	10	<10	1	1	1	unpublished
	Qinghai Province Cold springs, China	1	16	10	<10	1	1	1	unpublished
	Lake Kinneret, Israel	1	35	12	<10	1	2	0	Schwarz *et al.*, 2007
	Shrimp pond sediment, India	1	25	16	10	1	2	0	unpublished
	Lake Geneva, Switzerland	1	36	20	13	1	2	0	Haller *et al*., 2011
	Amazonia Lakes	1	87	55	27	1	2	0	unpublished
	Lake Grosse Fuchskuhle,	1	72	53	39	1	2	0	Chan *et al*., 2002
	Kentucky lake reservoir, USA	1	83	61	<10	1	2	0	unpublished
	Lake Suwa, Japan	1	70	27	<10	1	2	0	Sakai *et al*., 2009
	Salar de Huasco (H1), Chile	1	23	22	14	1	2	0	Dorador *et al*., 2007
	Contaminated aquifer sediment, Wyoming, USA	1	11	10	<10	1	2	0	Callaghan *et al*., 2010
	Middle sediment from Honghu Lake, China	2	246	164	90	1	2	0	unpublished
	Andean Altiplano	1	95	74	29	1	2	0	unpublished

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Drinking water reservoir	1	341	199	119	1	2	0	unpublished
	Tamaulipas, cenote La Palita, Mexico	1	150	100	41	1	2	0	unpublished
	Lake Coeur d'Alene, Idaho, USA	1	111	28	<10	1	2	0	Rastogi *et al*., 2009
	Wind Cave, South Dakota, USA	1	22	20	<10	1	2	0	Chelius *et al*., 2010
	Tucurui hydroeletric power plant reservoir, Brazil	1	82	122	58	1	2	0	unpublished
	Lake Manzallah, Egypt	1	25	21	<10	1	2	0	Elsaied *et al*., 2008
	Lake Taihu, China	1	23	19	12	1	2	0	Ye *et al*., 2009
	Lake Wivenhoe, Australia	1	78	58	26	1	2	0	unpublished
	Xiangjiang River, China	1	36	29	<10	1	2	0	unpublished
	Kanto Plain, Japan	1	15	11	10	1	2	0	Takeuchi *et al*., 2011
	Middle Island sinkhole, Lake Huron, USA	1	18	16	13	1	2	0	Nold *et al*., 2010
	Western Ghats, India	2	74	71	39	1	2	0	unpublished
	Lake Taihu, China	5	236	172	101	1	2	1	unpublished
	Honghu Lake, China	1	101	62	32	1	2	1	unpublished
	River Leine, Germany	1	58	21	<10	1	2	1	unpublished
	Hotsprings Ambite Island, Papua Nova Guinea	1	19	17	13	1	3	0	unpublished

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Little Hot Creek (80ºC), California, USA	1	21	18	15	1	3	0	unpublished
	Hot spring Yellowstone National Park, USA	1	13	12	<10	1	3	0	de la Torre *et al*., 2008
	Lake Taihu, China	2	122	100	45	1	3	1	unpublished
	Great Basin, Mud Hot Springs, Nevada, USA	2	169	27	23	1	3	1	Costa *et al*., 2009
Saline	Arctic Circle, Svalbard, Norway	1	17	17	15	2	1	0	Park *et al*., 2011
	Weddell Sea, Antarctica	1	31	10	<10	2	1	0	Brandt *et al*., 2007
	Warm pool, West Pacific	1	19	16	<10	2	2	0	Wang *et al*., 2005
	Warm pool, West Pacific	1	33	16	<10	2	2	0	unpublished
	Deep subsurface groundwater, Japan	1	27	25	21	2	2	0	Shimizu *et al*., 2006
	Aquifers, Japan	1	39	13	<10	2	2	0	unpublished
	Cold Seep Dongsha Area of South China Sea, China	1	62	56	40	2	2	0	unpublished
	Nankai Trough, China	1	20	17	<10	2	2	0	Arakawa *et al*., 2006
	Cold-seep sediments in Japan Trench	1	22	14	10	2	2	0	unpublished
	Eel River basin; USA	1	125	72	34	2	2	0	Beal *et al*., 2009
	methane seep sediment	1	125	49	25	2	2	0	unpublished

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Qinghai Lake, China	1	26	18	<10	2	2	0	Dong *et al.*, 2006
	Urania brine lake, Eastern Mediterranean	1	33	12	<10	2	2	0	Heijs *et al.*, 2008
	Brackish lake, Hampshire, UK	1	18	17	12	2	2	0	Banning *et al.*, 2005
	Qinghai Lake, China	1	74	43	15	2	2	0	Jiang *et al.*, 2008
	Gulf of Mexico	1	497	146	69	2	2	0	unpublished
	Pearl River Estuary, China	3	43	43	30	2	2	0	Jiang *et al.*, 2011
	Yellow River Delta, China	1	113	44	22	2	2	0	unpublished
	Hainan island, China	1	128	69	31	2	2	0	unpublished
	Tirez lagoon, Spain	1	33	20	12	2	2	0	Montoya *et al.*, 2013
	South China Sea	1	53	47	25	2	2	0	unpublished
	South China Sea	1	64	64	36	2	2	0	unpublished
	Lake Erie, Michigan, USA	1	59	28	<10	2	2	0	Chaudhary *et al.*, 2009
	Severn estuary, Portishead, UK	1	19	16	11	2	2	0	Webster *et al.*, 2010
	Gabon continental margin, Atlantic Ocean	1	16	11	<10	2	2	0	Nadalig *et al.*, 2009
	Sandy carbonate sediment, Hawai	1	14	14	10	2	2	0	Sorensen *et al.*, 2007

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Nankai Trough, Japan	1	11	11	<10	2	2	0	Miyazaki *et al.*, 2009
	Holocene sediment, Japan	1	23	21	12	2	2	0	unpublished
	Peru Margin (ODP Leg 201), Peru	1	31	19	12	2	2	0	Parkes *et al.*, 2005/ Webster *et al.*, 2006
	Aarhus Bay site M5 SMTZ, Denmark	2	25	24	10	2	2	0	Webster *et al.*, 2011
	Marine sediments, Korea	1	60	36	19	2	2	0	unpublished
	Hydrate Ridge Oregon/Northern Cascadia Margin/ Indian Ocean	1	19	19	12	2	2	0	Briggs *et al.*, 2011
	Hydrate Ridge Oregon/Southern Cascadia Margin	1	62	26	<10	2	2	0	Knittel *et al.*, 2005/Lösekann *et al.*, 2007
	SMTZ continental margin sediments, Santa Barbara, California, USA	1	52	44	21	2	2	0	Harrison *et al.*, 2009
	Skan Bay, Alaska, USA	1	140	79	32	2	2	0	Kendall *et al.*, 2007
	Hydrate Ridge, Oregon, USA	1	23	21	<10	2	2	0	Kendall *et al.*, 2009
	South Pacific Gyre	1	15	12	11	2	2	0	unpublished
	South China Sea, China	1	128	105	63	2	2	0	Wang *et al.*, 2010

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	South China Sea, China	1	67	65	35	2	2	0	unpublished
	East Pacific Rise	1	30	28	16	2	2	0	Li *et al.*, 2008
	Monterey Canyon, USA	1	17	12	<10	2	2	0	Goffredi *et al.*, 2008
	Chefren mud volcano	2	113	41	29	2	2	0	Omoregie *et al.*, 2008
	Pang Chau, Victoria Harbour	2	155	122	62	2	2	0	unpublished
	Mangrove Soil, China	1	40	40	29	2	2	0	unpublished
	White Oak River estuary, USA	6	171	170	112	2	2	0	Kubo *et al.*, 2012
	Peru margin (ODP Leg 201 Site 1229), Peru	2	22	21	15	2	2	0	Biddle *et al.*, 2006/ Sorensen and Teske, 2006
	Peru margin (ODP Leg 201 Site 1227), Peru	1	48	42	18	2	2	0	Sørensen and Teske, 2006
	Marine sediments, Brazil	1	17	17	11	2	2	0	Clementinoetal., 2007
	Central Pacific Ocean	1	36	23	<10	2	2	0	unpublished
	Kazan mud volcano in the deep Eastern Mediterranean sea (22-34 cm)	3	145	45	16	2	2	0	Heijs *et al.*, 2007
	Nankai Trough (ODP Leg 190), Japan	1	18	11	<10	2	2	0	Newberry *et al.*, 2004

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Rainbow vent field on the Mid-Atlantic Ridge	1	16	10	<10	2	2	0	Nercessian *et al*., 2005
	Mississippi canyon, USA	1	47	46	22	2	2	0	Lloyd *et al*., 2010
	Qiongdongnan basin, South China Sea, China	2	59	57	43	2	2	0	unpublished
	Colne River estuary, UK	1	178	12	<10	2	2	0	unpublished
	Orikasa River estuary, Japan	1	52	50	35	2	2	0	Kaku *et al*., 2005
	West Pacific ocean, China	3	720	415	165	2	2	0	unpublished
	Cascadia Margin, Oregon, USA	1	29	24	17	2	2	0	unpublished
	Okhotsk Sea, China	1	124	56	21	2	2	0	unpublished
	Gulf of Mexico (IODP site 1319, 1320, 1324)	3	70	65	43	2	2	0	Nunoura *et al*., 2009
	Amsterdam Mud Volcano, Mediterranean Sea	1	21	21	14	2	2	0	unpublished
	Marmara Sea, Turkey	1	135	123	121	2	2	0	Kolukirik *et al*., 2011
	Cascadia Margin (ODP Leg 201, 204), Peru	3	118	99	60	2	2	0	Inagaki *et al*., 2006
	Tokyo Bay, Japan	1	20	13	10	2	2	0	unpublished
	Okhotsk sea, China	1	49	48	24	2	2	0	Inagaki *et al*., 2003
	Virginia, Chesapeake Bay, Virginia, USA	2	161	22	<10	2	2	0	Breuker *et al*., 2011

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Joetsu, Japan Sea, methane seep sediment	1	14	13	<10	2	2	0	Yanagawa *et al.*, 2011
	Tainan Ridge, Taiwan	1	60	38	21	2	2	0	unpublished
	Shimokita Peninsula, Japan	1	54	43	16	2	2	0	Imachi *et al.*, 2011
	Zeebrugge sea port, Belgium	1	88	55	34	2	2	0	Siegert *et al.*, 2011
	Nankai Trough, Japan	1	41	39	26	2	2	0	unpublished
	Ogasawara Trench, Japan	1	16	12	<10	2	2	0	Nunoura *et al.*, 2013
	Northern South China Sea	1	54	52	35	2	2	0	unpublished
	North Sea	1	11	10	<10	2	2	0	unpublished
	Gulf of Mexico, Mexico	2	95	45	21	2	2	0	Orcutt *et al.*, 2010
	North Sea, Norway	1	28	11	<10	2	2	0	Wegener, *et al.*, 2008
	Altiplano, Salar de Huasco (H1), Chile	1	12	11	<10	2	2	0	Dorador *et al.*, 2010
	Yung-An Ridge, Taiwan	1	82	30	17	2	2	0	unpublished
	Ulleung Basin, South Corea	1	126	67	34	2	2	0	unpublished
	Xiamen, China	1	24	24	11	2	2	0	unpublished
	Western Pacific, China	1	199	136	61	2	2	0	Zhang *et al.*, 2010
	Pacific Ocean, China	2	37	25	<10	2	2	0	Liao *et al.*, 2011
	Barents Sea	1	52	33	<10	2	2	0	unpublished

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Guaymas Basin, Gulf of California, Mexico	1	19	12	<10	2	2	0	Kellermann *et al*., 2012
	Northern slope of South China sea	1	33	19	<10	2	2	0	unpublished
	Okhotsk Sea, China	1	64	62	34	2	2	0	unpublished
	Surface sediment in South China Sea	2	88	85	56	2	2	0	unpublished
	Porcupine Seabight, Atlantic Ocean	1	345	39	21	2	2	0	unpublished
	Salt marsh sediment, USA	1	59	19	<10	2	2	0	unpublished
	Mediterranean Sea	1	54	21	<10	2	2	1	Heijs *et al*., 2008
	Takuyo daigo seamount, Japan	1	21	10	<10	2	2	1	Nitahara *et al*., 2011
	East Sea, Korea	1	75	25	10	2	2	1	Park *et al*., 2008
	Okhotsk Sea, China	1	63	35	12	2	2	1	Dang *Et al*., 2009
	Timor Sea, Australia	1	49	36	18	2	2	1	Wasmund *et al*., 2009
	South China sea, China	2	43	40	30	2	2	1	Li *et al*., 2008
	Kagnhwa Island, Dongmak, South Korea	1	167	92	46	2	2	1	Kim *et al*., 2005
	Western Pacific, China	1	119	93	53	2	2	1	unpublished
	Hokkaido, Shiribeshi Trough, Japan	1	20	20	18	2	2	1	unpublished

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	South Pacific Gyre	1	92	31	<10	2	2	1	Durbin and Teske, 2010
	Sagami Bay, Japan	1	224	74	31	2	2	1	unpublished
	Mid-Atlantic Ridge, Atlantic Ocean	2	121	22	<10	2	2	1	Schauer *et al*., 2011
	Hydrate mounds, Gulf of Mexico	1	46	22	13	2	2	1	Mills *et al*., 2003
	Sea of Marmara	1	62	29	20	2	2	1	Quaiser *et al*., 2011
	Okhotsk Sea, China	1	111	58	24	2	2	1	Dang *et al*., 2010
	Mtoni creek, Tanzania	1	14	13	10	2	2	1	Lyimo *et al*., 2009
	Pacific Ocean	1	30	12	<10	2	2	1	Liao *et al*., 2011
	Central west coast, India	1	179	74	34	2	2	1	Singh *et al*., 2010
	Salt Marsh, Long Island Sound, New England	1	191	49	17	2	2	1	Nelson *et al*., 2009
	Pacific Artic Ocean	3	245	37	<10	2	2	1	unpublished
	Napoli/Amsterdam mud volcano, Eastern Mediterranean	3	108	53	13	2	3	0	unpublished
	Southern Okinawa trough, Japan	2	70	69	54	2	3	0	Nunoura *et al*., 2010
	East Lau Basin, Pacific Ocean	4	91	84	37	2	3	0	unpublished

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Guaymas Basin, Gulf of California, Mexico	1	26	20	15	2	3	0	Dhillon *et al.*, 2005
	Amsterdam mud volcano, Mediterranean Sea	1	55	25	15	2	3	0	Pachiadaki *et al.*, 2011
	Mud volcano, Gulf of Cadiz, Spain	1	61	17	12	2	3	0	unpublished
	Juan de Fuca, Pacific Ocean, Mexico	1	49	35	24	2	3	0	unpublished
	Napoli/Amsterdam mud volcano, Eastern Mediterranean Sea	2	79	34	<10	2	3	1	unpublished
	Ambitle Island, Tutum Bay, Papua New Guinea	1	13	10	<10	2	3	1	unpublished
Hypersaline	Hypersaline lakes, Wadi An Natrun, Egypt	3	81	75	36	3	2	0	Mesbah *et al.*, 2007
	Leachate sediment, China	1	59	37	17	3	2	0	Liu *et al.*, 2011
	Altiplano, Salar de Aguas Calientes, Chile	1	13	12	<10	3	2	0	unpublished
	Salton Sea, California, USA	1	103	103	78	3	2	0	Swan *et al.*, 2010
	Hypersaline sediments, Gulf of Mexico	1	31	23	10	3	2	0	Lloyd *et al.*, 2006
	Altiplano, Salar de Huasco (H6), Chile	1	32	28	17	3	2	0	Dorador *et al.*, 2010

Environment	Site location	Clone Libraries	Seqs	OTU 97%	OTU 90%[4]	Sal[1]	Temp[2]	Ox[3]	Reference[5]
	Multipond solar saltern, Tunisia	1	103	55	27	3	2	0	Baati *et al*., 2010
	Hypersaline pond, Spain	1	24	20	<10	3	2	0	unpublished
	Lake Chaka, China	1	37	27	19	3	2	1	Jiang *et al*., 2006
	Rambla Salada, Murcia, Spain	1	29	14	10	3	2	1	Luque *et al*., 2012
	Lagoa Vermelha, Brazil	1	22	15	13	3	2	1	unpublished

[1]Sal= Salinity: 1= Freshwater (<0.1%), 2=Saline (0.1-3.5%), 3=Hypersaline (>3.5%)

[2]Temp= Temperature: 1=Psicrophile (<10ºC), 2= Mesophile (10-50ºC), 3= Thermophile (>50ºC)

[3]Ox=Oxygen: 0= anoxic, 1=oxic

[4]Studies with less than 10 representative sequences were discarded from the analysis.

[5]unpublished indicates direct submissions to GenBank without any published paper related.

References for Supplementary Table S1

Arakawa S, Sato T, Yoshida Y, Usami R, Kato C. (2006). Comparison of the microbial diversity in cold-seep sediments from different depths in the Nankai Trough. *J Gen Appl Microbiol* **52**:47–54.

Baati H, Guermazi S, Gharsallah N, Sghir A, Ammar E. (2010). Novel prokaryotic diversity in sediments of Tunisian multipond solar saltern. *Res Microbiol* **161**:573–582.

Banning N, Brock F, Fry JC, Parkes RJ, Hornibrook ERC, Weightman AJ. (2005). Investigation of the methanogen population structure and activity in a brackish lake sediment. **7**:947–960.

Beal EJ, House CH, Orphan VJ. (2009). Manganese- and iron-dependent marine methane oxidation. *Science* **325**:184–187.

Biddle JF, Lipp JS, Lever M a, Lloyd KG, Sørensen KB, Anderson R, *et al.* (2006). Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc Natl Acad Sci U S A* **103**:3846–3851.

Brandt A, Gooday AJ, Brandão SN, Brix S, Brökeland W, Cedhagen T, Choudhury M, Cornelius N, Danis B, Mesel ID, Diaz RJ, Gillan DC, Ebbe B, Howe JA, Janussen D, Kaiser S, Linse K, Malyutina M, Pawlowski J, Raupach M, Vanreusel A (2007). First insights into the biodiversity and biogeography of the Southern Ocean deep sea. *Nature* **447**:307-311.

Breuker A, Köweker G, Blazejak A, Schippers A. (2011). The deep biosphere in terrestrial sediments in the chesapeake bay area, Virginia, USA. *Front Microbiol* **2**:156.

Callaghan A V, Davidova I a, Savage-Ashlock K, Parisi V a, Gieg LM, Suflita JM, *et al.* (2010). Diversity of benzyl- and alkylsuccinate synthase genes in hydrocarbon-impacted environments and enrichment cultures. *Environ Sci Technol* **44**:7287–7294.

Chan OC, Wolf M, Hepperle D, Casper P. (2002). Methanogenic archaeal community in the sediment of an arti ¢ cially partitioned acidic bog lake. *FEMS Microbiol Ecol* **42**:119–129.

Chaudhary A, Haack S. (2009). Bacterial and archaeal phylogenetic diversity of a cold sulfur-rich spring on the shoreline of Lake Erie, Michigan. *App Environ Microbiol* **75**:5025–5036.

Chelius MK, Moore JC. (2004). Molecular Phylogenetic Analysis of Archaea and Bacteria in Wind Cave, South Dakota. *Geomicrobiol J* **21**:123–134.

Clementino MM, Fernandes CC, Vieira RP, Cardoso a M, Polycarpo CR, Martins OB. (2007). Archaeal diversity in naturally occurring and impacted environments from a tropical region. *J Appl Microbiol* **103**:141–151.

Costa KC, Navarro JB, Shock EL, Zhang CL, Soukup D, Hedlund BP. (2009). Microbiology and geochemistry of great boiling and mud hot springs in the United States Great Basin. *Extremophiles* **13**:447–459.

Dang H, Luan X, Zhao J, Li J. (2009). Diverse and novel nifH and nifH-like gene sequences in the deep-sea methane seep sediments of the Okhotsk Sea. *Appl Environ Microbiol* **75**:2238–2245.

Dang H, Luan X-W, Chen R, Zhang X, Guo L, Klotz MG. (2010). Diversity, abundance and distribution of amoA-encoding archaea in deep-sea methane seep sediments of the Okhotsk Sea. *FEMS Microbiol Ecol* **72**:370–385.

Dhillon A, Lever M, Lloyd K. (2005). Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (mcrA) genes in hydrothermal sediments of the Guaymas Basin. *Appl Environ Microbiol* **71**:4592–4601.

Dong H, Zhang G, Jiang H, Yu B, Chapman LR, Lucas CR, *et al.* (2006). Microbial diversity in sediments of saline Qinghai Lake, China: linking geochemical controls to microbial ecology. *Microb Ecol* **51**:65–82.

Dorador C, Vila I, Remonsellez F, Imhoff JF, Witzel K-P. (2010). Unique clusters of Archaea in Salar de Huasco, an athalassohaline evaporitic basin of the Chilean Altiplano. *FEMS Microbiol Ecol* **73**:291–302.

Durbin AM, Teske A. (2010). Sediment-associated microdiversity within the Marine Group I Crenarchaeota. *Environ Microbiol Rep* **2**:693–703.

Elsaied HE, Maruyama A (2008). Biodiversity of archaea in Manzala Lake, Egypt, based on 16S rRNA gene. *Egypt J Genet Cytol* **37**:57-72.

Goffredi SK, Wilpiszeski R, Lee R, Orphan VJ. (2008). Temporal evolution of methane cycling and phylogenetic diversity of archaea in sediments from a deep-sea whale-fall in Monterey Canyon, California. *ISME J* **2**:204–220.

Haller L, Tonolla M, Zopfi J, Peduzzi R, Wildi W, Poté J (2011). Composition of bacterial and archaeal communities in freshwater sediments with different contamination levels (Lake Geneva, Switzerland). *Water Res* **45**:1213-1228.

Heijs SK, Haese RR, van der Wielen PWJJ, Forney LJ, van Elsas JD. (2007). Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean cold seep. *Microb Ecol* **53**:384–398.

Heijs SK, Laverman AM, Forney LJ, Hardoim PR, van Elsas JD. (2008). Comparison of deep-sea sediment microbial communities in the Eastern Mediterranean. *FEMS Microbiol Ecol* **64**:362–377.

Imachi H, Aoi K, Tasumi E, Saito Y, Yamanaka Y, Saito Y, *et al.* (2011). Cultivation of methanogenic community from subseafloor sediments using a continuous-flow bioreactor. *ISME J* **5**:1913–1925.

Inagaki F, Nunoura T, Nakagawa S, Teske A, Lever M, Lauer A, *et al.* (2006). Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc Natl Acad Sci USA* **103**:2815–2820.

Inagaki F, Suzuki M, Takai K. (2003). Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl Environ Microbiol* **69**:7224–7235.

Jiang H, Dong H, Yu B, Ye Q, Shen J, Rowe H, *et al.* (2008). Dominance of putative marine benthic Archaea in Qinghai Lake, north-western China. *Environ Microbiol* **10**:2355–2367.

Jiang L, Zheng Y, Chen J, Xiao X, Wang F. (2011). Stratification of Archaeal communities in shallow sediments of the Pearl River Estuary, Southern China. *Antonie Van Leeuwenhoek* **99**:739–751.

Kaku N, Ueki A, Ueki K, Watanabe K. (2005). Methanogenesis as an important terminal electron accepting process in estuarine sediment at the mouth of Orikasa River. *Microbes Environ* **20**:41–52.

Kellermann MY, Wegener G, Elvert M, Yoshinaga MY, Lin Y-S, Holler T, *et al.* (2012). Autotrophy as a predominant mode of carbon fixation in anaerobic methane-oxidizing microbial communities. *Proc Natl Acad Sci USA* **109**:19321–19326.

Kendall MM, Wardlaw GD, Tang CF, Bonin AS, Liu Y, Valentine DL. (2007). Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of Methanogenium boonei sp. nov. *Appl Environ Microbiol* **73**:407–414.

Kim BS, Oh HM, Kang H, Chun J (2005). Archaeal diversity in tidal flat sediment as revealed by 16S rDNA analysis. *J Microbiol* **43**:144-151.

Knittel K, Lo T, Boetius A, Kort R, Amann R. (2005). Diversity and Distribution of Methanotrophic Archaea at Cold Seeps. *Appl Environ Microbiol* **71**:467–479.

Kolukirik M, Ince O, Cetecioglu Z, Celikkol S, Ince BK. (2011). Spatial and temporal changes in microbial diversity of the Marmara Sea sediments. *Mar Pollut Bull* **62**:2384–2394.

Kubo K, Lloyd KG, F Biddle J, Amann R, Teske A, Knittel K. (2012). Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *ISME J* **6**:1949–1965.

De la Torre JR, Walker CB, Ingalls AE, Könneke M, Stahl D a. (2008). Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. *Environ Microbiol* **10**:810–818.

Li Y, Li F, Zhang X, Qin S, Zeng Z, Dang H, *et al.* (2008). Vertical distribution of bacterial and archaeal communities along discrete layers of a deep-sea cold sediment sample at the East Pacific Rise (approximately 13 degrees N). *Extremophiles* **12**:573–585.

Liao L, Xu X-W, Jiang X-W, Wang C-S, Zhang D-S, Ni J-Y, *et al.* (2011). Microbial diversity in deep-sea sediment from the cobalt-rich crust deposit region in the Pacific Ocean. *FEMS Microbiol Ecol* **78**:565–585.

Lloyd KG, Lapham L, Teske A. (2006). An anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Appl Environ Microbiol* **72**:7218–7230.

Lloyd KG, Macgregor BJ, Teske A. (2010). Quantitative PCR methods for RNA and DNA in marine sediments: maximizing yield while overcoming inhibition. *FEMS Microbiol Ecol* **72**:143–151.

Luque R, González-Domenech CM, Llamas I, Quesada E, Béjar V (2012). Diversity of culturable halophilic archaea isolated from Rambla Salada, Murcia (Spain). *Extremophiles* **16**:205-213.

Lyimo TJ, Pol A, Jetten MSM, den Camp HJMO. (2009). Diversity of methanogenic archaea in a mangrove sediment and isolation of a new Methanococcoides strain. *FEMS Microbiol Lett* **291**:247–253.

Mesbah NM, Abou-El-Ela SH, Wiegel J. (2007). Novel and unexpected prokaryotic diversity in water and sediments of the alkaline, hypersaline lakes of the Wadi An Natrun, Egypt. *Microb Ecol* **54**:598–617.

Mills HJ, Hodges C, Wilson K, Macdonald IR, Sobecky P a. (2003). Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. *FEMS Microbiol Ecol* **46**:39–52.

Miyazaki J, Higa R, Toki T, Ashi J, Tsunogai U, Nunoura T, *et al.* (2009). Molecular characterization of potential nitrogen fixation by anaerobic methane-oxidizing archaea in the methane seep sediments at the number 8 Kumano Knoll in the Kumano Basin, offshore of Japan. *Appl Environ Microbiol* **75**:7153–7162.

Montoya L, Vizioli C, Rodríguez N, Rastoll MJ, Amils R, Marin I. (2013). Microbial community composition of Tirez lagoon (Spain), a highly sulfated athalassohaline environment. *Aquat Biosyst* **9**:19.

Nadalig T, Roussel E, Delage E, Olu K, Duperron S, Caprais J, Boetius A, Sibuet M, Cambon-Bonavita MA (2009). Diversity and distribution of methane-oxidizing microbial communities associated with different faunal assemblages in a giant pockmark of the Gabon continental margin. *Deep-sea Res II, Top Stud Oceanogr* **56**: 2248-2258.

Nelson K a, Moin NS, Bernhard AE. (2009). Archaeal diversity and the prevalence of Crenarchaeota in salt marsh sediments. *Appl Environ Microbiol* **75**:4211–4215.

Nercessian O, Fouquet Y, Pierre C, Prieur D, Jeanthon C (2005). Diversity of Bacteria and Archaea associated with a carbonate-rich metalliferous sediment sample from the Rainbow vent field on the Mid-Atlantic Ridge. *Environ Microbiol.* 7(5):698-714.

Newberry CJ, Webster G, Cragg BA, Parkes RJ, Weightman AJ, Fry JC (2004). Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. *Environ Microbiol* **6**:274-287.

Nold SC, Zajack HA, Biddanda BA (2010) Eukaryal and Archaeal Diversity in a Submerged Sinkhole Ecosystem Influenced by Sulfur-Rich, Hypoxic Groundwater. *Journal of Great Lakes Research* **36**:366-375.

Nunoura T, Soffientino B, Blazejak A, Kakuta J, Oida H, Schippers A, *et al.* (2009). Subseafloor microbial communities associated with rapid turbidite deposition in the Gulf of Mexico continental slope (IODP Expedition 308). *FEMS Microbiol Ecol* **69**:410–424.

Nunoura T, Oida H, Nakaseama M, Kosaka A, Ohkubo SB, Kikuchi T, *et al.* (2010). Archaeal diversity and distribution along thermal and geochemical gradients in hydrothermal sediments at the Yonaguni Knoll IV hydrothermal field in the Southern Okinawa trough. *Appl Environ Microbiol* **76**:1198–1211.

Nunoura T, Nishizawa M, Kikuchi T, Tsubouchi T, Hirai M, Koide O, Miyazaki J, Hirayama H, Koba K, Takai K (2013). Molecular biological and isotopic biogeochemical prognoses of the nitrification-driven dynamic microbial nitrogen cycle in hadopelagic sediments. *Environ Microbiol* **15**:3087-3107.

Orcutt BN, Joye SB, Kleindienst S, Knittel K, Ramette A, Reitz A, *et al.* (2010). Impact of natural oil and higher hydrocarbons on microbial diversity, distribution, and activity in Gulf of Mexico cold-seep sediments. *Deep Sea Res Part II Top Stud Oceanogr* **57**:2008–2021.

Pachiadaki MG, Kallionaki A, Dählmann A, De Lange GJ, Kormas KA. (2011). Diversity and spatial distribution of prokaryotic communities along a sediment vertical profile of a deep-sea mud volcano. *Microb Ecol* **62**:655–668.

Park SJ, Park BJ, Rhee SK (2008). Comparative analysis of archaeal 16S rRNA and *amo A* genes to estimate the abundance and diversity of ammonia-oxidizing archaea in marine sediments. *Extremophiles* **12**:605-610.

Park SJ, Park BJ, Jung MY, Kim SJ, Chae JC, Roh Y, Forwick M, Yoon HI, Rhee SK (2011). Influence of Deglaciation on Microbial Communities in Marine Sediments off the Coast of Svalbard, Arctic Circle. *Microbial Ecology* **62**:537-548.

Parkes RJ, Webster G, Cragg BA, Weightman AJ, Newberry CJ, Ferdelman TG, *et al.* (2005). Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* **436**:390–394.

Quaiser A, Zivanovic Y, Moreira D, López-García P. (2011). Comparative metagenomics of bathypelagic plankton and bottom sediment from the Sea of Marmara. *ISME J* **5**:285–304.

Rastogi G, Sani RK, Peyton BM, Moberly JG, Ginn TR. (2009). Molecular studies on the microbial diversity associated with mining-impacted Coeur d'Alene River sediments. *Microb Ecol* **58**:129–139.

Sakai S, Imachi H, Sekiguchi Y, Tseng I-C, Ohashi A, Harada H, *et al.* (2009). Cultivation of methanogens under low-hydrogen conditions by using the coculture method. *Appl Environ Microbiol* **75**:4892–4896.

Schauer R, Røy H, Augustin N, Gennerich H-H, Peters M, Wenzhoefer F, *et al.* (2011). Bacterial sulfur cycling shapes microbial communities in surface sediments of an ultramafic hydrothermal vent field. *Environ Microbiol* **13**:2633–2648.

Schubert CJ, Vazquez F, Lösekann-Behrens T, Knittel K, Tonolla M, Boetius A. (2011). Evidence for anaerobic oxidation of methane in sediments of a freshwater system (Lago di Cadagno). *FEMS Microbiol Ecol* **76**:26–38.

Schwarz JIK, Eckert W, Conrad R. (2007). Community structure of Archaea and Bacteria in a profundal lake sediment Lake Kinneret (Israel). *Syst Appl Microbiol* **30**:239–254.

Shimizu S, Akiyama M, Ishijima Y, Hama K, Kunimaru T, Naganuma T (2006). Molecular characterization of microbial communities in fault-bordered aquifers in the Miocene formation of northernmost Japan. *Geobiology* **4**:203-213.

Siegert M, Cichocka D, Herrmann S, Gründger F, Feisthauer S, Richnow H-H, *et al.* (2011). Accelerated methanogenesis from aliphatic and aromatic hydrocarbons under iron- and sulfate-reducing conditions. *FEMS Microbiol Lett* **315**:6–16.

Singh SK, Verma P, Ramaiah N, Chandrashekar A a, Shouche YS. (2010). Phylogenetic diversity of archaeal 16S rRNA and ammonia monooxygenase genes from tropical estuarine sediments on the central west coast of India. *Res Microbiol* **161**:177–186.

Sørensen KB, Teske A. (2006). Stratified communities of active Archaea in deep marine subsurface sediments. *Appl Environ Microbiol* **72**:4596–4603.

Swan BK, Ehrhardt CJ, Reifel KM, Moreno LI, Valentine DL. (2010). Archaeal and bacterial communities respond differently to environmental gradients in anoxic sediments of a California hypersaline lake, the Salton Sea. *Appl Environ Microbiol* **76**:757–768.

Takeuchi M, Yoshioka H, Seo Y, Tanabe S, Tamaki H, Kamagata Y, Takahashi HA, Igari S, Mayumi D, Sakata S (2011). A distinct freshwater-adapted subgroup of ANME-1 dominates active archaeal communities in terrestrial subsurfaces in Japan. *Environmental Microbiology* **13**:3206-3218.

Vick TJ, Dodsworth J a, Costa KC, Shock EL, Hedlund BP. (2010). Microbiology and geochemistry of Little Hot Creek, a hot spring environment in the Long Valley Caldera. *Geobiology* **8**:140–154.

Wang P, Xiao X, Wang F. (2005). Phylogenetic analysis of Archaea in the deep-sea sediments of west Pacific Warm Pool. *Extremophiles* **9**:209–217.

Wang P, Li T, Hu A, Wei Y, Guo W, Jiao N, *et al.* (2010). Community structure of archaea from deep-sea sediments of the South China Sea. *Microb Ecol* **60**:796–806.

Wasmund K, Kurtböke DI, Burns K a, Bourne DG. (2009). Microbial diversity in sediments associated with a shallow methane seep in the tropical Timor Sea of Australia reveals a novel aerobic methanotroph diversity. *FEMS Microbiol Ecol* **68**:142–151.

Webster G, Parkes RJ, Cragg B a, Newberry CJ, Weightman AJ, Fry JC. (2006). Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. *FEMS Microbiol Ecol* **58**:65–85.

Webster G, Rinna J, Roussel EG, Fry JC, Weightman AJ, Parkes RJ. (2010). Prokaryotic functional diversity in different biogeochemical depth zones in tidal sediments of the Severn Estuary, UK, revealed by stable-isotope probing. *FEMS Microbiol Ecol* **72**:179–197.

Wegener G, Shovitri M, Knittel K, Niemann H, Hovland M, Boetius A (2008). Biogeochemical processes and microbial diversity of the Gullfaks and Tommeliten methane seeps (Northern North Sea). *Biogeosci Discuss* **5**:1127-1144

Yanagawa K, Sunamura M, Lever MA, Morono Y, Hiruta A, Ishizaki O, *et al.* (2011). Niche Separation of Methanotrophic Archaea (ANME-1 and -2) in Methane-Seep Sediments of the Eastern Japan Sea Offshore Joetsu. *Geomicrobiol J* **28**:118–129.

Ye G, Wang S, Jiang L, Xiao X, Wang F, Noakes J, *et al.* (2009). Distribution and Diversity of Bacteria and Archaea in Marine Sediments Affected by Gas Hydrates at Mississippi Canyon in the Gulf of Mexico. *Geomicrobiol J* **26**:370–381.

Ze XL, Duncan SH, Louis P, Flint HJ (2012). Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *ISME J* **6:**1535-1543.

Zhang W, Saren G, Li T, Yu X, Zhang L. (2010). Diversity and community structure of archaea in deep subsurface sediments from the tropical Western pacific. *Curr Microbiol* **60**:439–445.

Supplementary information Chapter 2

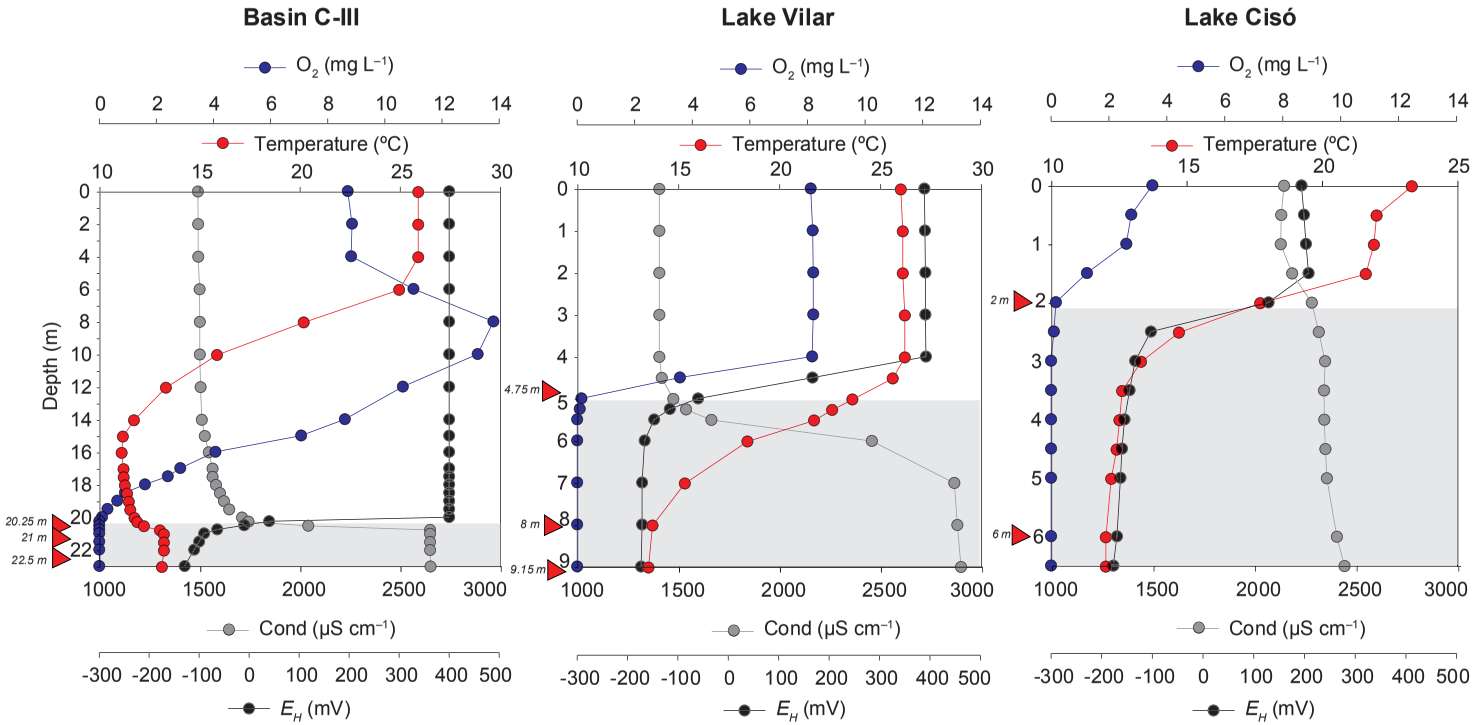

Figure S1. Physicochemical depth profiles for temperature, dissolved oxygen, conductivity and RedOx potential in the three studied lakes ate the day of sampling. The shaded area indicates the anoxic water compartment. Red arrowheads indicate sampling depths.

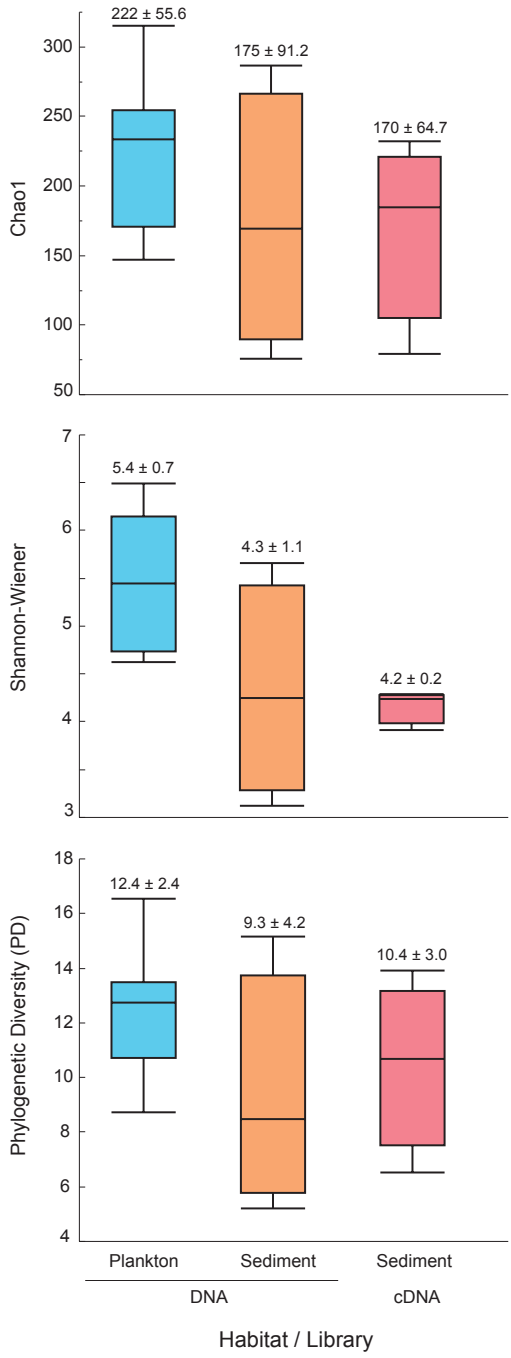

Figure S2. Box-plots of alpha-diversity indicators calculated from archaeal SSU rRNA pyrotag sample libraries. Samples were grouped according to habitat (planktonic *vs.* sediment) and library (DNA *vs.* cDNA) for a better comparison. Alpha-diversity indicators were calculated in QIIME v10.8 (see Supporting Information).

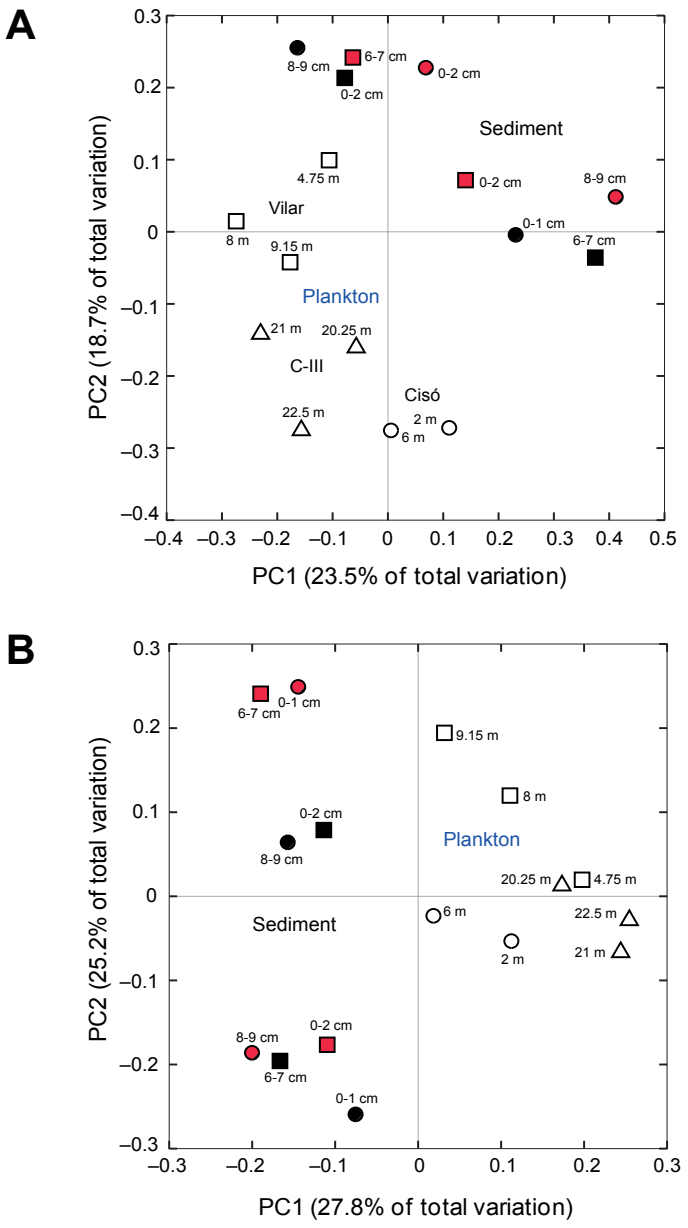

Figure S3. PCoA ordination of samples according to unweighted (**A**) and weighted (**B**) UniFrac distance matrices. Analysis was performed in QIIME v10.8 (see Supporting Information). Basin C-III (triangles), Lake Cisó (circles), Lake Vilar (squares). Planktonic and sediment samples are indicated by open and filled symbols, respectively. Sample labels indicate sampling depth.

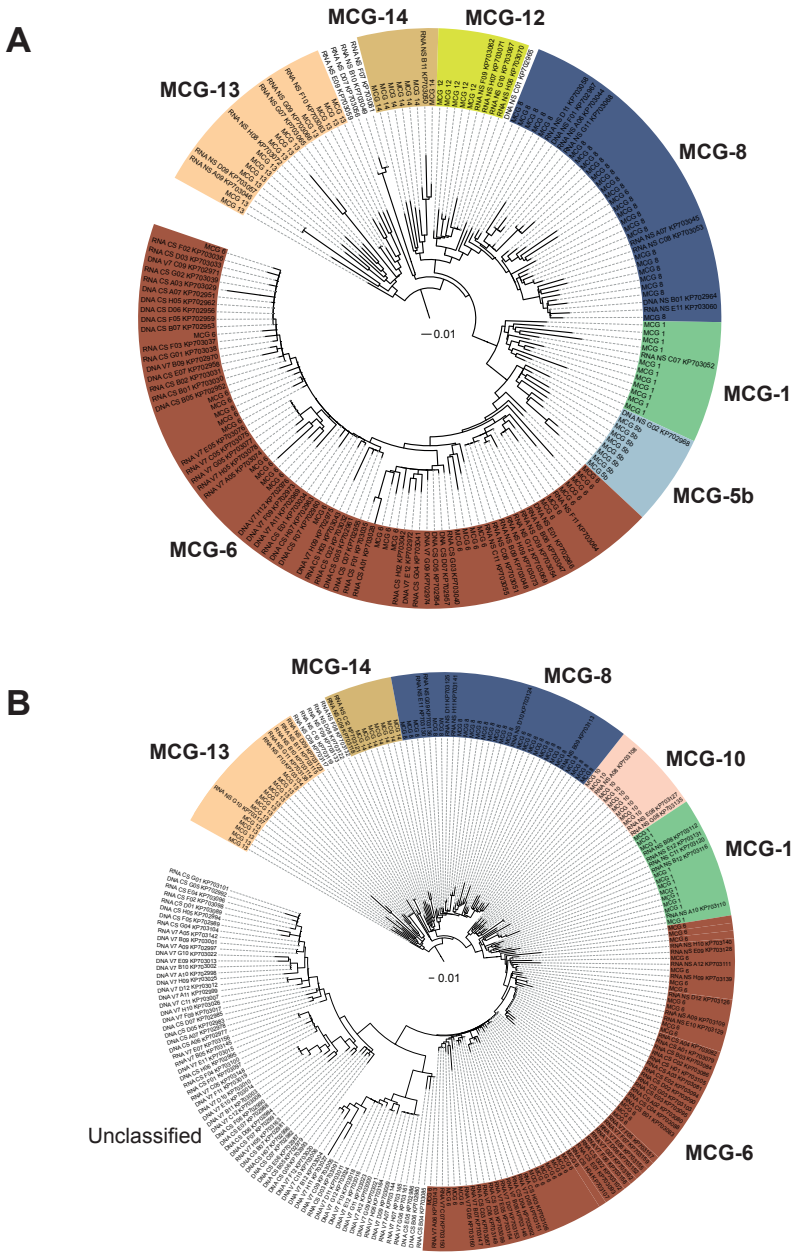

Figure S4. Neighbour-Joining (NJ) phylogenetic tree based on MCG SSU rRNA gene sequences showing the affiliation of clones obtained from qPCR amplicons using (**A**) new primer pair MCG-242dF/MCG-678R and (**B**) primer pair MCG-528F/MCG-732R (Kubo et al., 2012). Representative sequences for each MCG subgroup are used as phylogenetic anchors and labelled according to subgroup numbering (Kubo et al., 2012). Uncoloured leaves indicate unassigned sequences. NJ tree was constructed in ARB (Ludwig et al., 2004) and edited in the Interactive Tree of Life (iTOL) tool (Letunic and Bork, 2011). C: Lake Cisó; V: Lake Vilar; NS: North Sea.

Supplementary Table S1. Alignment of the designed forward primer sequence against a representative sequence of each of the described MCG subgroups. The degenerate forward primer contains two degeneracies (**D**: A, G or T) at the second and ninth positions (highlighted in red).

MCG	MCG-242F sequence (5'–3')																	
Subgroup*	T	**D**	A	C	C	G	G	T	**D**	C	G	G	G	C	C	G	T	G
MCG-1	·	G/A	·	·	·	·	·	·	A/G	·	·	·	·	·	·	·	·	·
MCG-2	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-3	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-4	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	A	·	·
MCG-5a	·	A	·	·	·	·	·	·	A/T	·	·	·	·	·	·	·	·	·
MCG-5b	·	A/G	·	·	·	·	·	·	A/T/G	·	·	·	·	·	·	·	·	·
MCG-6	·	A/G/T	·	·	·	·	·	·	A/G	·	·	·	·	·	·	C	·	·
MCG-7	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-8	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-9	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-10	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-11	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-12	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
MCG-13	·	A	·	·	·	·	·	·	A/T	·	·	·	·	·	·	·	·	·
MCG-14	·	A/G	·	·	·	·	·	·	A/T	·	·	·	·	·	·	A/C/G	·	·
MCG-15	·	G	·	·	·	·	·	·	-	·	·	·	·	·	·	A/G	·	·
MCG-16	·	A/G	·	·	·	·	·	·	A	·	·	·	·	·	T/C	·	C/T	·
MCG-17	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	C	·	·

* Subgroup classification according to Kubo et al. (2012)

References:

Kubo K, Lloyd KG, Biddle J, Amann R, Teske A, Knittel K & Biddle JF (2012) Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *ISME J* 6: 1949–1965.

Supplementary Table S2. Primers and conditions used for qPCR analyses.

Primer	Sequence (5'–3')	Target	Reference	Gradient[†]	R^2	Efficiency (%)
1048F	GTGSTGCAYGGYTGTCGTCA	Univ. Bacteria	[1]	3' at 95ºC 15" at 95ºC 60" at 60ºC	0.99[a] / 0.99[b]	99[a] / 102[b]
1194R	ACGTCRTCCMCACCTTCCTC	Univ. Bacteria				
806F	ATTAGATACCCSBGTAGTCC	Univ. Archaea	[2]	3' at 95ºC 20" at 95ºC 60" at 60ºC	0.99[a] / 0.99[b]	98[a] / 98[b]
915R	AGGAATTGGCGGGGGAGCAC	Univ. Archaea	[3]			
242dF	TDACCGGTDCGGGCCGTG	MCG	This study	3' at 95ºC 15" at 95ºC 60" at 68ºC	0.99[a] / 0.99[b]	81[a] / 99[b]
678R	CACCGTCGGGCGCGTTCT	MCG	This study			

[†] The program consists in 35 cycles of amplification.

[a]: planktonic samples; [b]: sediment samples

References

[1] Maeda H, Fujimoto C, Haruki Y & Maeda T (2003) Quantitative real-time PCR using TaqMan and SYBR Green for *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *tetQ* gene and total bacteria. *FEMS Immunol Med Microbiol* 39: 81–86.

[2] Takai K & Horikoshi K (2000) Rapid Detection and Quantification of Members of the Archaeal Community by Quantitative PCR Using Fluorogenic Probes. *Appl Environ Microbiol* 66: 5066–5072.

[3] Stahl DA and Amann R (1991) Development and application of nucleic acid probes, p. 205–248. In: Stackebrandt E and Goodfellow M (Eds.), *Nucleic acid techniques in bacterial systematics*. John Wiley & 553 Sons Ltd., Chichester, United Kingdom

Supplementary Table S3. Nutrient concentrations measured at selected depths of the water column of the three studied lakes at the day of sampling. TP: Total phosphorous; DOC: Dissolved Organic Carbon; DIC: Dissolved Inorganic Carbon. LoQ: Limit of Quantification[†]; n.a. not available.

Depth (m)	NO_3^- (mg L^{-1})	NO_2^- (mg L^{-1})	NH_4^+ (mg L^{-1})	H_2S (mg L^{-1})	SO_4^{2-} (mg L^{-1})	TP (mg L^{-1})	DOC (mg L^{-1})	DIC (mg L^{-1})
Basin C-III								
2	<LoQ	<LoQ	0.452	<LoQ	191.04	0.013	2.997	19.18
8	0.034	<LoQ	0.538	<LoQ	188.16	0.065	3.377	*n.a.*
17	0.091	<LoQ	0.663	<LoQ	185.28	0.016	1.696	*n.a.*
19.75	0.054	<LoQ	0.932	0.001	204.2	0.013	1.768	*n.a.*
20.25	<LoQ	<LoQ	1.010	0.004	217.1	0.018	4.210	*n.a.*
21	<LoQ	<LoQ	1.450	5.064	315.0	0.014	2.008	*n.a.*
22.5	<LoQ	<LoQ	2.383	12.47	414.6	0.032	6.490	46.5
Lake Vilar								
2	n.a.	n.a.	<LoQ	<LoQ	158.9	0.050	4.760	35.50
4.75	0.031	<LoQ	0.357	0.020	154.0	0.100	4.710	*n.a.*
5	<LoQ	<LoQ	0.586	0.051	157.8	0.070	4.410	*n.a.*
5.25	<LoQ	<LoQ	0.337	1.637	146.9	0.080	4.610	*n.a.*
6	<LoQ	<LoQ	3.612	17.17	242.7	0.070	4.350	*n.a.*
8	<LoQ	<LoQ	9.995	23.48	395.3	0.740	2.230	*n.a.*
9.15	<LoQ	<LoQ	11.15	28.81	383.4	0.730	2.360	97.01
Lake Cisó								
2	<LoQ	<LoQ	1.336	0.010	306.3	0.100	5.424	*n.a.*
4	<LoQ	0.054	6.533	38.39	303.0	0.490	2.711	*n.a.*
6	<LoQ	0.054	9.842	54.00	287.3	0.820	3.106	113.9

[†]Limits of quantification for cation and anion ionic chromatography: NH4+ = 0.010 mg N-NH_4 L^{-1}; NO_3^- = 0.002 mg N-NO_3 L^{-1}; NO_2^- = 0.003 mg N-NO_2 L^{-1}; SO_4^{2-}= 0.017 mg S-SO_4 L^{-1}; for spectrophotometry: PO_4^{3-}= 0.003 mg P-PO4 L^{-1}; for catalytic oxidation (DOC/DIC) = 0.05 mg C L^{-1}.

Supplementary Table S4. Nutrient concentrations measured at different layers of sediment corers collected in lakes Cisó and Vilar at the day of sampling. DOC: Dissolved Organic Carbon; DIC: Dissolved Inorganic Carbon. LoQ: Limit of Quantification[†]; n.a. not available.

Depth (cm)	NO_3^- (mg L^{-1})	NO_2^- (mg L^{-1})	NH_4^+ (mg L^{-1})	SO_4^{2-} (mg L^{-1})	PO_4^{2-} (mg L^{-1})	DOC (mg L^{-1})	DIC (mg L^{-1})
Lake Vilar							
0	<LoQ	0.142	14.02	233.1	0.064	29.00	128.0
1	<LoQ	<LoQ	23.16	87.86	0.016	54.50	165.7
2	<LoQ	<LoQ	27.13	36.87	0.261	121.2	197.1
3	<LoQ	<LoQ	30.53	20.90	0.189	113.1	199.7
4	<LoQ	<LoQ	32.80	19.50	0.775	108.0	183.4
5	<LoQ	<LoQ	33.61	20.06	1.051	121.5	197.5
6	<LoQ	<LoQ	36.68	20.13	0.953	108.9	217.8
7	<LoQ	<LoQ	37.53	54.93	0.814	155.5	203.1
8	<LoQ	<LoQ	40.77	46.37	0.700	124.8	205.0
9	<LoQ	0.213	31.96	82.94	0.670	90.26	176.9
10	<LoQ	<LoQ	23.32	75.85	0.039	53.11	170.5
11	<LoQ	<LoQ	32.28	13.07	0.621	96.65	202.9
12	<LoQ	<LoQ	36.95	1.153	1.345	96.75	215.2
13	<LoQ	<LoQ	49.36	1.285	0.928	123.7	225.6
14	<LoQ	<LoQ	58.80	0.818	1.637	90.11	242.6
15	0.078	<LoQ	61.19	1.870	2.601	102.0	243.5
Lake Cisó							
0	<LoQ	<LoQ	1.671	274.9	0.218	70.12	53.61
1	<LoQ	<LoQ	1.933	286.6	0.136	102.0	51.10
2	<LoQ	<LoQ	2.162	273.2	0.043	878.5	50.89
3	0.076	<LoQ	2.365	272.0	0.054	296.1	49.72
4	<LoQ	<LoQ	2.053	267.4	0.049	170.3	53.32
5	0.085	<LoQ	2.054	263.2	0.039	138.2	55.78
6	<LoQ	<LoQ	1.790	260.3	0.050	111.9	53.67
7	<LoQ	<LoQ	1.835	254.6	0.057	86.40	54.54
8	<LoQ	<LoQ	1.593	244.9	0.043	70.30	55.90
9	<LoQ	<LoQ	1.488	239.6	0.050	165.4	55.89
10	<LoQ	<LoQ	1.390	245.6	0.044	66.30	50.37
11	<LoQ	<LoQ	1.453	242.7	0.024	75.15	51.64
12	0.405	<LoQ	1.558	241.7	0.060	121.7	55.30
13	0.192	<LoQ	1.991	233.0	0.008	145.1	46.64

[†]Limits of quantification for cation and anion ionic chromatography: NH4+ = 0.010 mg N-NH_4 L^{-1}; NO_3^- = 0.002 mg N-NO_3 L^{-1}; NO_2^- = 0.003 mg N-NO_2 L^{-1}; SO_4^{2-}= 0.017 mg S-SO_4 L^{-1}; for spectrophotometry: PO_4^{3-}= 0.003 mg P-PO4 L^{-1}; for catalytic oxidation (DOC/DIC) = 0.05 mg C L^{-1}.

Supplementary Table S5. R statistic and *p*-values obtained after analyses of similarity (ANOSIM) carried out by comparing the composition of archaeal communities (at different taxonomic levels) between samples grouped according to different factors, namely: Habitat (Planktonic *vs.* Benthic), System (basin C-III, Lake Vilar, Lake Cisó), Library (DNA *vs.* cDNA). The number of permutations was 9,999 using a significance level of 5% for the statistic. Significative values are shown in bold type. Analysis was done in PRIMER-6 statistical package (PRIMER-E, Plymouth Marine Laboratory, UK).

Taxonomic level	Factor	R	*p-value*
Archaeal classes	Habitat	0.558	**0.0002**
	System	0.016	0.3980
	Library	0.267	**0.0390**
MCG subgroups	Habitat	0.181	0.0530
	System	0.204	**0.0460**
	Library	0.408	**0.0070**
Halobacteria clades	Habitat	0.008	0.3530
	System	–0.131	0.9120
	Library	0.366	**0.0140**
Thermoplasmata clades	Habitat	0.451	**0.0009**
	System	–0.044	0.5850
	Library	0.090	0.2090

Supplementary Table S6. Average relative abundance and contribution percentage of each archaeal taxon to Bray-Curtis dissimilarity (SIMPER analysis) between sample groups. Only significant comparisons are shown (see Table S3). SIMPER was run in PRIMER 6 statistical package with the PERMANOVA+ add-on (PRIMER-E, Plymouth Marine Laboratory, UK). Cumulative contribution cutoff was set to 80%.

Comparison	Taxa[a]	Avrg. abund. in 1st group (%)	Avrg. abund. in 2nd group (%)	Contrib. (%)	Cum. Contrib. (%)
Archaeal classes					
Plankton *vs.* Sediment (avrg. diss.= 39.5)	*Halobacteria*	44.1	3.24	28.9	28.9
	Methanococci	4.77	8.76	13.6	42.4
	Methanobacteria	3.88	3.61	13.5	55.9
	Thermoplasmata	16.2	28.1	19.7	68.6
	MCG	16.0	29.0	17.8	79.6
	MBG-B	3.31	1.42	9.50	88.5
DNA *vs.* cDNA (avrg. diss.= 37.9)	*Halobacteria*	24.2	4.53	20.9	20.9
	Methanococci	5.06	3.48	16.4	37.3
	Methanobacteria	3.88	2.22	15.5	52.8
	Thermoplasmata	23.0	18.3	12.8	65.6
	MCG	19.6	30.4	12.0	77.6
	MBG-B	3.24	0.38	8.59	86.2
MCG subgroups					
Vilar *vs.* C-III (avrg. diss.= 51.6)	*MCG-6*	43.7	8.76	21.0	21.0
	MCG-5a	0.83	24.3	20.8	41.8
	MCG-5b	6.10	34.9	19.6	61.4
	MCG-15	9.80	4.75	14.6	76.0
	MCG-11	9.00	6.55	10.6	86.2
Cisó *vs.* C-III (avrg. diss.= 65.0)	*MCG-6*	64.4	8.76	24.1	24.1
	MCG-5b	0.91	34.9	23.2	47.3
	MCG-5a	0.08	24.3	20.9	68.2
	MCG-15	11.0	4.75	11.9	80.1
Vilar *vs.* Cisó (avrg. diss.= 42.0)	*MCG-15*	9.80	11.0	21.3	21.3
	MCG-11	9.00	1.99	20.7	42.0
	MCG-6	43.7	62.4	20.3	62.3
	MCG-5b	6.10	0.91	15.3	77.6
	MCG-11	0.94	0.20	6.96	84.5
DNA *vs.* cDNA (avrg. diss.= 54.6)	*MCG-15*	2.62	52.4	30.8	30.8
	MCG-6	48.7	22.2	19.3	50.1
	MCG-5b	9.48	0.58	14.0	64.2
	MCG-11	5.71	1.45	13.8	77.9
	MCG-5a	3.46	0.02	9.11	87.0

Supplementary Table S6. *(continued)*

Comparison	Taxa[a]	Avrg. abund. in 1st group (%)	Avrg. abund. in 2nd group (%)	Contrib. (%)	Cum. Contrib. (%)
Halobacteria clades					
DNA *vs.* cDNA (avrg. diss.= 25.5)	*DHVEG-6*	42.5	18.0	31.6	31.6
	DSEG	6.05	21.9	23.3	55.0
	MEG	31.8	38.6	19.0	74.0
	Other *Halobacteria*	8.29	6.35	13.5	87.5
Thermoplasmata clades					
Plankton *vs.* Sediment (avrg. diss.= 50.1)	*ASC21*	9.12	57.3	23.9	23.9
	MBG-D and DHVEG-1	50.0	9.86	21.8	45.7
	SAGMEG	5.29	0.29	8.69	54.4
	AMOS-1A-4113	8.18	4.04	8.09	62.5
	DHVEG-2	3.46	0.09	7.43	69.9
	Other *Thermoplasmata*	3.10	0.55	5.14	75.1
	Marine Group-III	0.30	1.00	4.48	79.6
	ANTO_06-05	0.19	1.12	4.29	83.9

Supplementary Table S7. Results of the distance-based multivariate linear model (DistLM, McArdle & Anderson, 2001) on the relative composition (square root transformation of relative abundances at class level) of archaeal communities and environmental variables (log transformed and standardized). Variables included in the model were ammonia, sulphate, sulfide, dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) concentrations. The proportion of variance explained by environmental variables in stepwise sequential tests (9,999 permutations) using the AICc selection criterion (Aikake, 2006) is showed first. Only sulfide and DOC concentrations resulted in significant probabilities, explaining ≈ 56% of total variance. *Prop.* = Proportion of variance explained by each single variable, Cumul. P. = Cumulative proportion of variance explained by multiple variables; res. def: residual degrees of freedom.

Sequential tests for stepwise model

Variable	AICc	SS (trace)	Pseudo-F	*p-value*	Cumul. P.	res. df.
+Sulfide	74.54	2393.2	6.240	0.001	0.384	10
+DOC	74.34	1055.1	3.415	0.033	0.554	9

Best solution

AICc	R^2	RSS	Nº Var.	Selections
74.345	0.554	2780.4	2	Sulfide, DOC

Percentage of variation explained by individual axes

	% explained variation out of fitted model		% explained variation out of total variation	
Axis	Individual	Cumulative	Individual	Cumulative
1	75.16	75.16	41.61	41.91
2	24.84	100	13.75	55.36

References

Akaike H (1978) A new look at the Bayes procedure. *Biometrika*, 65: 53–59.

McArdle BH & Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, 82: 290–297.

Supplementary Table S8. Quantification of Bacteria, Archaea and MCG by qPCR along depth in the two sediment corers collected from lakes Vilar and Cisó. Sediment layers analyzed by pyrosequencing are indicated by grey shading. qPCR conditions and primers used are compiled in Table S8.

	16S rRNA gene copies (g sediment)$^{-1}$		
Sediment layer	Bacteria	Archaea	MCG
Lake Vilar			
0–2 cm	3.04×10^{10}	8.24×10^{9}	1.80×10^{8}
2–3 cm	3.75×10^{10}	11.1×10^{10}	2.34×10^{8}
3–4 cm	2.20×10^{10}	7.37×10^{9}	2.24×10^{8}
4–5 cm	5.34×10^{10}	1.86×10^{10}	4.80×10^{8}
5–6 cm	3.91×10^{10}	1.56×10^{10}	3.90×10^{8}
6–7 cm	5.28×10^{10}	2.13×10^{10}	4.10×10^{8}
7–8 cm	4.65×10^{10}	1.98×10^{10}	4.03×10^{8}
8–9 cm	7.57×10^{10}	3.21×10^{10}	5.38×10^{8}
9–10 cm	3.10×10^{10}	1.13×10^{10}	2.94×10^{8}
10–11 cm	5.76×10^{10}	1.99×10^{10}	4.02×10^{8}
11–12 cm	6.43×10^{10}	2.42×10^{10}	4.74×10^{8}
12–13 cm	8.92×10^{10}	2.95×10^{10}	5.21×10^{8}
13–14 cm	5.40×10^{10}	1.71×10^{10}	3.79×10^{8}
14–15 cm	4.83×10^{10}	1.72×10^{10}	4.36×10^{8}
15–16 cm	1.58×10^{10}	6.50×10^{9}	3.66×10^{8}
Lake Cisó			
0–1 cm	1.18×10^{10}	2.77×10^{9}	1.06×10^{8}
1–2 cm	1.81×10^{10}	3.71×10^{9}	1.60×10^{8}
2–3 cm	7.21×10^{9}	1.38×10^{9}	6.18×10^{7}
3–4 cm	9.55×10^{9}	3.37×10^{9}	1.20×10^{8}
4–5 cm	9.62×10^{9}	1.64×10^{9}	9.87×10^{7}
5–6 cm	6.93×10^{9}	1.55×10^{9}	8.25×10^{7}
6–7 cm	6.58×10^{9}	1.49×10^{9}	7.76×10^{7}
7–8 cm	5.11×10^{9}	1.38×10^{9}	6.93×10^{7}
8–9 cm	6.86×10^{9}	1.66×10^{9}	8.51×10^{7}
9–10 cm	1.47×10^{10}	4.72×10^{9}	1.67×10^{8}
10–11 cm	1.18×10^{10}	2.70×10^{9}	1.37×10^{8}
11–12 cm	9.23×10^{9}	1.60×10^{9}	8.99×10^{7}
12–13 cm	9.53×10^{9}	2.95×10^{9}	1.44×10^{8}

Supplementary information Chapter 3

Supplementary Information

DNA extraction and high-throughput sequencing

DNA from all type of samples was extracted using FastDNA™ Spin Kit for Soil (MP Biomedicals, Irvine, CA) following manufacturer instructions. The concentration of DNA extracts was measured using QUBIT2.0 Fluorometer (Invitrogen Molecular probes Inc., Oslo, Norway). DNA extracts were analyzed through tag-encoded FLX-Titanium amplicon pyrosequencing at the Research and Testing Laboratory (Lubbock, TX, USA). Briefly, genomic DNA from planktonic, biofilm and sedimentary communities was used as a template in PCR reactions using archaeal primers PARCH340f (Øvreås *et al*., 1997) and ARCH958R (DeLong, 1992) targeting the V3–V5 hyper-variable regions of the archaeal 16S rRNA gene complemented with 454-adapters and sample-specific barcodes. Raw sequence dataset was pre-processed at RTL facilities to reduce noise and sequencing artifacts as previously described (Dowd *et al*., 2008). Demultiplexing, quality filtration, sample barcodes, sequence quality assessments, chimera detection and downstream phylogenetic analyses were conducted in QIIME (Caporaso *et al*., 2010). Taxonomy was assigned to each OTU (cutoff 97%) based on blast and the SILVA database (Altschul *et al*., 1990; Quast *et al*., 2013). Pyrosequencing data of this study have been deposited in the NCBI database under accession number **PRJNA350216**.

Lipid analyses

For the analysis of biofilm samples (BLL and BPD), freeze-dried material was cut into small pieces and homogenized using a mortar and a pestle. The same procedure was carried out with filters from bottom water samples (BW). Freeze-dried surface sediment (SS) was homogenized using a glass mortar and pestle. Lipids from sediment, biofilms and filters were extracted by a modified Bligh-Dyer extraction method (Pitcher *et al*., 2009). Briefly, lipids were extracted from sediments, leaves and filters ultrasonically three times in a mixture of methanol (MeOH):dichloromethane (DCM):phosphate buffer (2:1:0.8, v/v/v) and the supernatant was carefully removed following each extraction after centrifugation for 2 min at 3,000 rpm. The solvent ratio was then adjusted to 1:1:0.9 (MeOH:DCM:phosphate buffer, v/v/v) by addition of DCM and phosphate buffer to initiate phase separation. Polar fraction was removed and aqueous fraction was washed three times with DCM. DCM fractions were combined and rotary evaporated to near-dryness and passed through pre-extracted cotton wool and collected into a pre-weighed vial. Fifty per cent of the Bligh Dyer extract was separated into core lipid (CL) and intact polar lipid (IPL) GDGT fractions by silica gel column separation. CL fraction was eluted with five column volumes of hexane:ethyl acetate (1:1, v:v) and IPL fraction was eluted with five column volumes of MeOH. Fractions were collected into pre-weighed vials. An aliquot (10%) of the IPL fraction was split to determine the carry-over of CL-GDGT into the IPL fraction by measuring directly via high-performance liquid chromatography-atmospheric pressure chemical ionization/mass spectrometry (HPLC-APCI/MS). The

other 90% was acid hydrolysed under refluxing in 1.5N HCl/MeOH for 2 h, to cleave the polar head groups from IPLs to obtain IPL-derived CL-GDGTs. After reflux extracts were cooled down and pH adjusted to 4–5 by adding 2N KOH/MeOH (1:1, v/v), followed by addition of water and extraction of the aqueous phase with DCM, three times. Remaining water in the IPL-derived CL-GDGT was removed through anhydrous NA_2SO_4 columns and the extract was dried under nitrogen. GDGTs contained in the IPLs were quantified after hydrolysis of the IPL fraction and will be referred to as IPL-derived GDGTs. Fractions were analysed by a high performance liquid chromatography (HPLC)– mass spectrometry (MS) method with improved chromatographic separation as described by Hopmans *et al.* (2016). Selected ion monitoring (SIM) detection of $[M+H]^+$ ions of GDGTs was used, in combination with HP Chemstation software, for the quantification of GDGTs. A standard mixture of crenarchaeol:C_{46} GDGT was run regularly to check for differences in ionisation efficiencies, which were compensated for if present.

References from Supplementary Information

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data. Nature methods 7 (5): 335-336.

DeLong, E.F. (1992) Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA. 89: 5685-5689.

Dowd, S.E., Callaway, T.R., Wolcott, R.D., Sun, Y., McKeehan, T., Hagevoort, R.G., Edrington, T.S. (2008) Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC microbiology 8: 125.

Hopmans EC, Schouten S, Sinninghe Damsté JS. (2016). The effect of improved chromatography on GDGT-based palaeoproxies. Org Geochem 93:1–6.

Øvreås, L., Forney L, Daae FL, Torsvik V. (1997). Distribution of bacterioplankton in meromictic Lake Saelen- vannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Appl Environ Microbiol 63: 3367–3373.

Quast, C., Pruesse, E., Yilmaz, Pelin., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nuc. Acid. Res. 41:590-596.

Pitcher, A., Schouten, S. and Damsté, J.S.S. (2009) In situ production of Crenarchaeol in two California hot springs. Appl. Environ. Microbiol. 75, 4443–4451.

Supplementary Figures and Tables

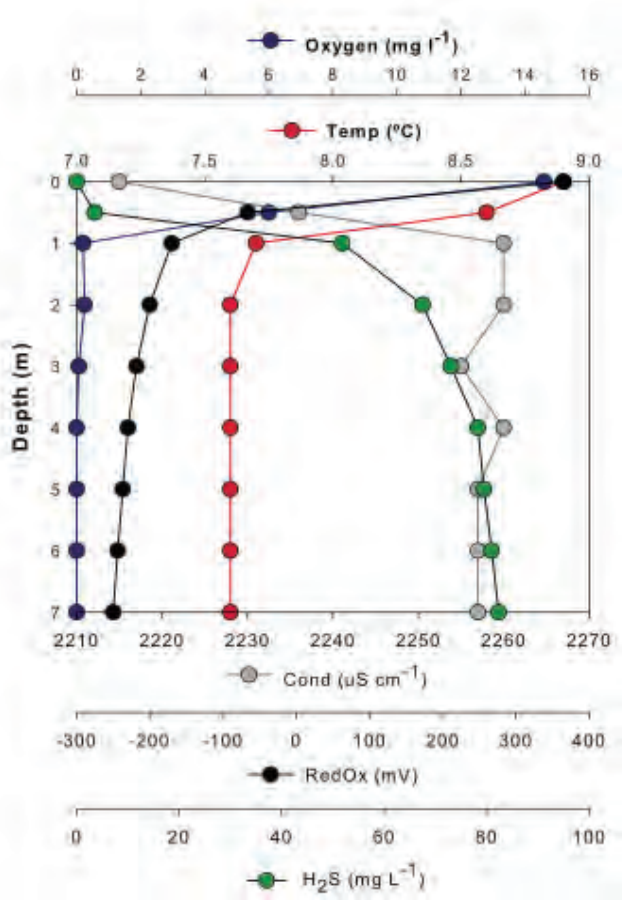

Figure S1. Physicochemical profile of the water column of Lake Cisó at the day of sampling

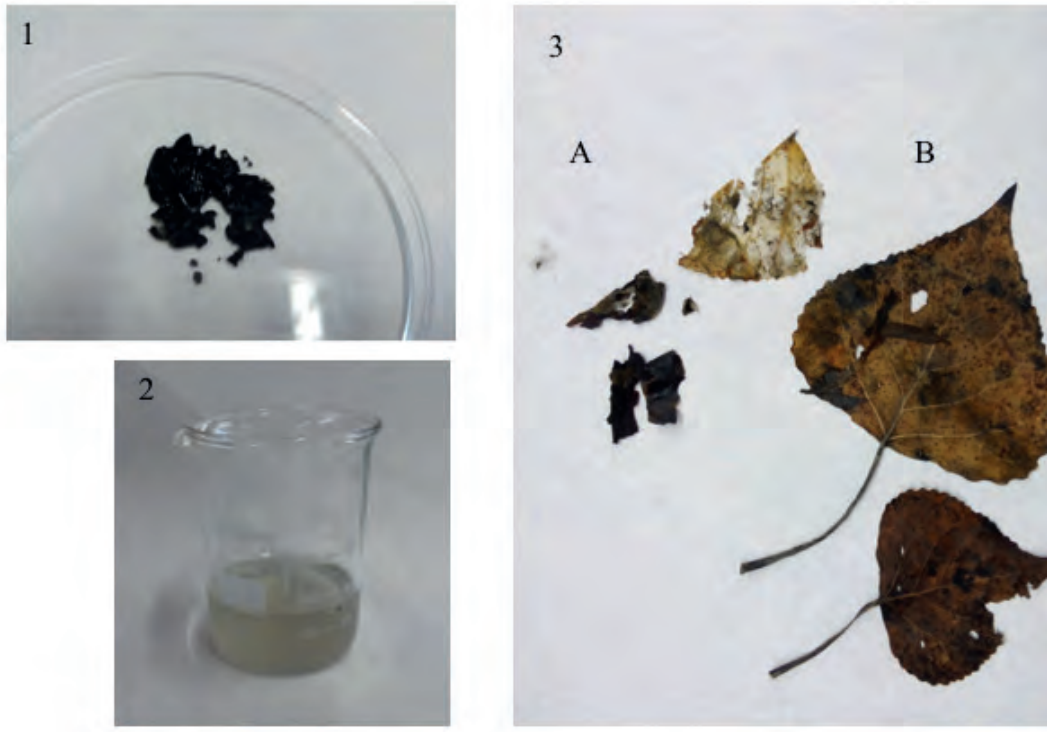

Figure S2. Picture of the samples from Lake Cisó: sediment (1), bottom water (2), plant debris (3.A) and leaf litter (3.B).

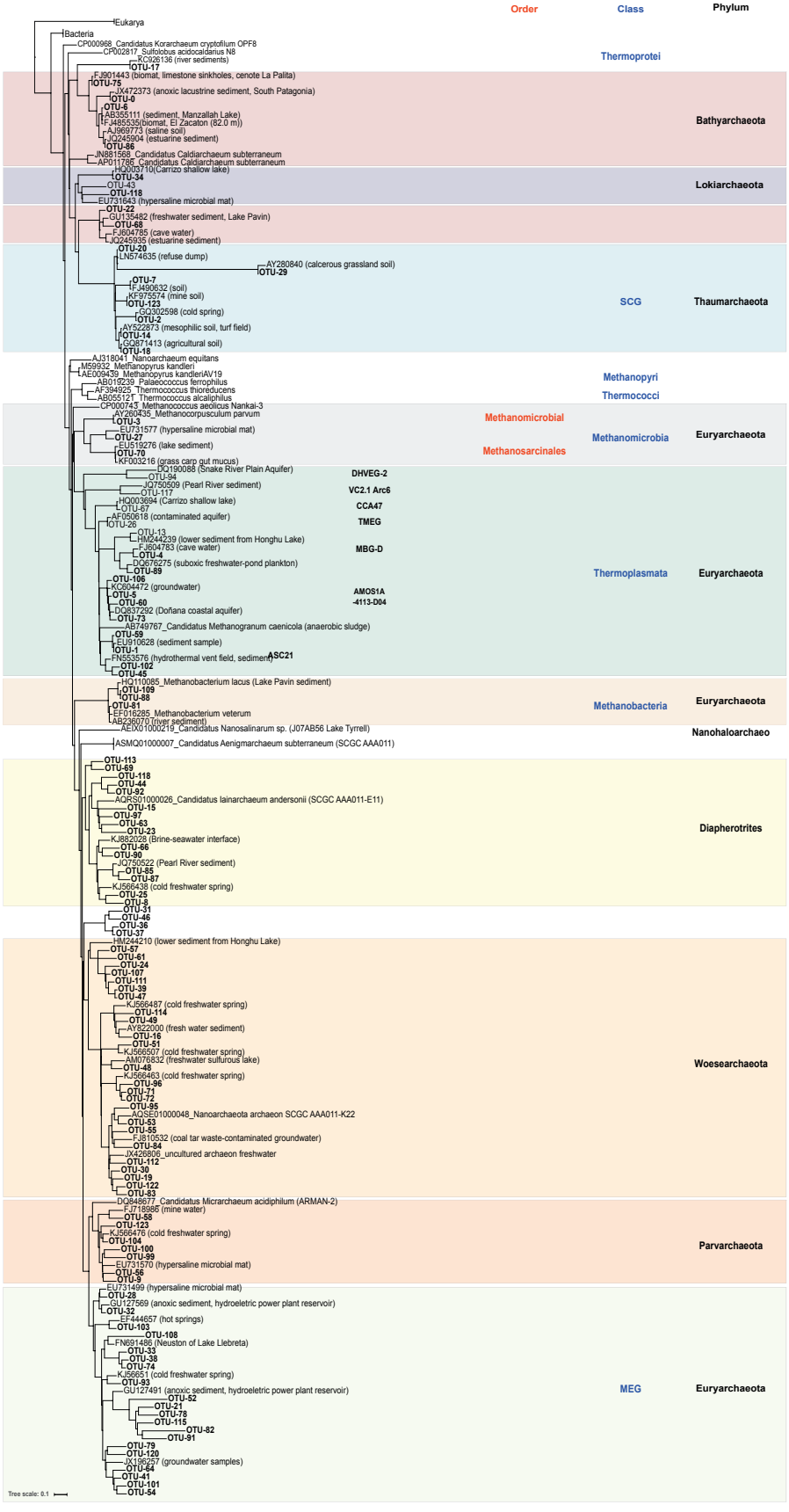

Figure S3. Neighbor-Joining phylogenetic tree of the 16S rRNA gene reads from the tag-encoded FLX-Titanium amplicon pyrosequencing (Lubbock, TX, USA) using ARB (Ludwig et al., 2004) loaded with the 123 Silva NR SSU Reference database (http://www.arb-silva.de/) (Quast et al., 2013). Sequences were added using the ARB Parsimony tool. OTUs were delineated at 97% similarity. Coloured shadows refer to colouring in Figure 1

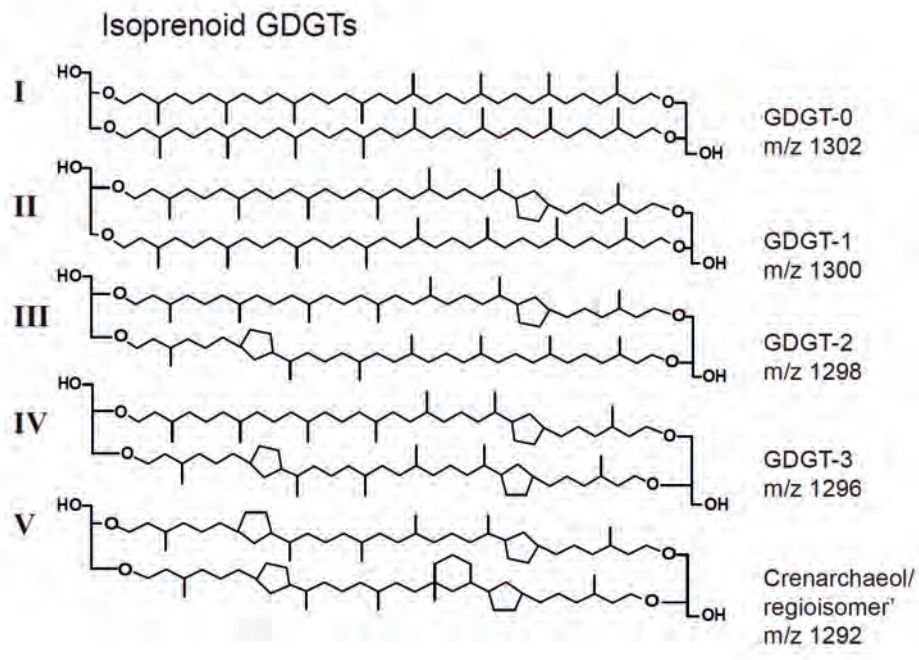

Figure S4. Isoprenoid glycerol dialkyl tetraether (GDGT) structures and their mass-to-charge (m/z) ratios

Supplementary Table S1. Abundance of IPL-derived GDGTs (nanograms per liter or per gram of dry weight) in bottom water (BW), sediment surface (SS), biofilm from plant debris (BPD) and biofilm from leaf litter (BLL) from Lake Cisó. Σ = sum. nd = not detected.

GDGT	BLL	BPD	BW	SS
0	402.0	837.4	606.7	713.0
1d	0.00	0.00	0.00	0.00
1c	0.00	5.41	0.00	0.52
1b	3.80	6.66	4.83	6.70
1a	180.5	283.1	193.3	249.9
1	9.18	0.00	3.44	10.6
2e	6.95	12.6	7.47	5.46
2d	3.04	5.20	2.37	0.00
2c	52.9	92.0	19.2	27.8
2b	58.2	0.00	28.1	22.7
2a	5.88	66.9	4.11	9.84
2	12.3	6.76	4.74	11.2
3f	1.40	0.00	0.00	0.39
3e	5.33	6.06	0.00	0.60
3d	3.55	0.00	1.56	1.30
3c	0.59	0.00	0.76	0.79
3b	3.42	2.10	1.60	4.08
3a	2.88	0.00	0.56	1.11
3	2.43	0.00	1.66	4.45
4c	0.00	0.00	0.00	0.00
4b	0.00	0.00	0.30	0.80
4a	0.00	0.00	0.22	0.58
4	2.33	0.00	1.80	4.37
cren-b	0.00	0.00	0.00	0.00
cren	44.7	9.73	22.4	67.2
cren-a	1.60	0.00	0.59	1.15
cren'	7.38	1.54	5.14	11.7
average	810.4	1335.5	910.9	1156.4

[Microorganisms] may not build cities or have interesting social lives, but they will be here when the Sun explodes. This is their planet, and we are on it only because they allow us to be.

Bill Bryson
A short story of nearly everything

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