

16S rRNA-based bacterial diversity in the organic-rich sediments underlying oxygen deficient waters of eastern Arabian Sea

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Abstract

The eastern Arabian Sea has a unique and permanent oxygen minimum zone (OMZ) that extends along the western continental margin of India. The sediment below this region is rich in organic matter. This study describes the bacterial community structure and diversity in OMZ sediments of the eastern Arabian Sea (AS) through 16S rRNA clone library analysis. Phylogenetic analysis of the sequences demonstrated that phylum *Proteobacteria* (52%), followed by *Planctomycetes* (12.7%), *Chloroflexi* and an unidentified bacterial group (8.8% each) were represented in the library. *Deltaproteobacteria* was the dominant class (62.5%) in phylum *Proteobacteria* with clones falling in orders *Syntrophobacterales* and *Desulfovibrionales*. Few minor phylogenetic groups, corresponding to *Spirochetes*, *Firmicutes*, *Acidobacteria* and *Verrucomicrobia* were found. Unidentified candidate groups falling in OP11, OP8 and OP3 were represented by 0.9%, 2.9% and 3.9%, respectively and two clusters of the cloned sequences in this study showed very low identity to known sequences. This is the first report that discusses the phylogenetic groups in the OMZ sediments of eastern Arabian Sea individually and compares it with available data from marine hypoxic locales and water mass. LIBSHUFF statistics revealed high richness of the bacterial community of the Arabian Sea OMZ (AS-OMZ) compared to the other regions.

Keywords: bacterial diversity; cultivation-independent; oxygen minimum zone; sediments, eastern Arabian Sea

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Introduction

Oxygen minimum zones (OMZ) are unique habitats with pronounced oxygen deficiency and are permanent features in the most productive regions of all oceans where dissolved oxygen concentrations at intermediate depths are as low as 0.5 ml L^{-1} (Kamykowski and Zentera 1990; Stramma et al. 2008). The seabed below the OMZ is a region of “large organic food falls” due to the deposition of particulate organic matter from the productive waters above, harbouring enormous and unique microbial biomass. Studying the diversity and distribution of natural microbial communities of marine sediments has been a long-standing challenge in microbial ecology. Over the last decade, the understanding of microbial diversity and dynamics in marine sediments has significantly increased due to the development of culture-independent methods which circumvented the associated culture biases (Pace et al. 1986; Giovannoni et al. 1990; Amann et al. 1995; Fuhrman and Davis 1997). Despite the two decades of advent of molecular techniques, studies have focussed mainly on functional gene diversity of anoxic and OMZ sediments (Schaefer et al. 2007; Schippers and Neretin 2006; Liu et al. 2003 a, b). Thus very little has been studied on the bacterial diversity of OMZ.

Indian Ocean covers ca 59% of the total global OMZ area, inclusive of Arabian Sea and Bay of Bengal (Helly and Levin 2004). The AS-OMZ extends from 150-1500 m water depth and impinges on the continental slope (Wyrski 1971; von Stackelberg 1972; Codispoti et al. 2005). Most of the bacteriological studies in the eastern Arabian Sea were limited to pelagic region (OMZ and non-OMZ) dealing with abundance (Ramaiah et al. 1996; Ducklow et al. 2001), taxonomic diversity (Riemann et al. 1999, Fuchs et al. 2005) and functional gene diversity (Woebken et al. 2008; Ward et al. 2009). However, very little is known about the diversity and phylogenetic affiliation of bacteria in the underlying sediments of the AS-OMZ despite its contribution to the biogeochemical cycles (Boetius et al. 2000, Hedges et al. 2001). For better understanding of the ecosystem and the processes, it is imperative to carry out an exhaustive accounting of the contributing bacterial populations and their diversity. It is difficult to isolate and cultivate bacteria representative of natural communities and this limitation is even more pronounced in extreme and unique environments (Boivin-Jahns et al. 1996). Though, diversity relies on the use of culture-independent methods, these techniques have biases (Farrelly et al. 1995; Acinas et al. 2004; Morasch et al. 2001; Chandler et al. 1997) which may influence the results. In spite of these caveats, among the various culture-independent tools 16S rDNA is, nevertheless, a widely employed technique to describe the composition of complex microbial community and to gain a descriptive overview of possible difference among communities (Yakimov et al 2005). The present study provides a detailed 16S

rDNA clone library analysis of the AS-OMZ sediment. This being the first study it has contributed to our fragmented knowledge on the biogeographical studies of sediment bacterial populations.

Methods

Collection of sediments

Sediment samples were collected from off Goa region of AS-OMZ, onboard FORV Sagar Sampada (Cruise # 254) from three different locations. Samples were handled aseptically and preserved in liquid nitrogen until DNA extraction.

DNA extraction from sediments

DNA was extracted using modified method of Zhou et al. (1996). The crude DNA extract from different OMZ locations were purified using the UltraClean Soil DNA kit (MoBio Laboratories, USA), according to manufacturer's instructions except that the bead-beating step was avoided (Sorensen et al. 2004). The purified DNA was estimated using Nanodrop spectrophotometer (NanoDrop Technologies, Inc, USA) and pooled.

Clone library construction

The purified DNA was subjected to PCR for the amplification of 16S rRNA genes using the primers 27F: AGAGTTTGTCTGGCTCA and 1090R: GCTCGTTGCGGGACTTAACC that amplify 1099 bp fragment of 16S rDNA (Amann et al., 1995). Amplification was carried out in a 50 μ l reaction mixture containing 50 ng of purified DNA (ie., 5 μ l from a 1:5 diluted genomic DNA), 3 mM concentration of MgCl₂, 2.5 mM of each dNTP, 10 pM of each primer and 3U/ μ l of high fidelity Taq polymerase in 1X of Taq buffer (Bangalore Genei, India). The PCR conditions followed were: initial denaturation at 94°C for 5 minutes, 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 minute and elongation at 72°C for 2 minutes and final extension at 72°C for 10 minutes. The PCR amplification was performed in a Thermal cycler (Eppendorf, Germany). The 16S rRNA gene amplicons were separated on 1.5% (w/v) agarose gel electrophoresed in Tris acetic acid-EDTA (TAE) buffer stained with 0.5 μ g/ml final concentration of ethidium bromide (BioRad, USA). The gels were further visualized using the gel documentation system (Eastman Kodak Company, USA). The amplified PCR products were cloned using TOPO TA cloning kit (Invitrogen, USA) designed for high cloning efficiency of long PCR products following manufacturer's instructions (Zhou and Gomez-Sanchez, 2000). A total of 180 clones were obtained out of which 132 were positive. Recombinant plasmids were extracted using QIA-prep Spin Miniprep kit (Qiagen,

USA). The plasmids were screened for the 16S rDNA inserts by PCR using the primers M13F: GTAAAACGACGGCCAG and M13R: CAGGAAACAGCTATGAC which are specific for the vector. Sequencing was carried out by a commercial company (BioServe Biotechnologies Pvt. Ltd., India).

Phylogenetic analysis

A total of 115 sequences obtained from the clone library were assembled using DNA Baser sequence assembly software Version 2 (DNA Baser, USA) and analyzed for putative chimeric sequences using Chimera_Check software of Ribosomal Database Project (Cole et al. 2003) and Pintail Software (Ashelford et al. 2005). Similarities among sequences were calculated from the phylogenetic tree and sequences showing $\geq 97\%$ sequence similarity were grouped into the same OTU (Stackebrandt and Goebel 1994). As the percentage of identical sequences was negligible (ie no two sequences showed 100% similarity) all the sequences were taken for the phylogenetic analysis. A total of 103 different sequences were aligned using multiple sequence alignment tools Clustal X version 1.83 (Thompson et al. 1997). A PHYLIP tree was generated by neighbour-joining method and edited using MEGA (Kumar et al. 2004). Bootstrap analysis was carried out using 1000 iterations.

Diversity analysis

The clone library of AS-OMZ was compared with those of other anoxic sediments from Gulf of Mexico, North Sea and South China Sea (28, 60 and 87 sequences, respectively) to understand the similarity between the libraries. Selected 16S rRNA gene sequences of the above libraries were retrieved from the Genbank database (NCBI) (Accession numbers: EU886379–EU886464; FM179819–FM179914; AM745141–AM746064). DOTUR software was used to assign sequences to operational taxonomic units (OTU), perform rarefaction analysis and calculation of diversity indices (Schloss and Handelsman 2005). Sequences with distance values of 0.03 ($\geq 97\%$ sequence similarity) corresponding to the species level were grouped into a single operational taxonomic unit (Stackebrandt and Goebel 1994). Rarefaction curves with 95% confidence intervals were constructed by plotting the number of OTUs observed against the number of sequences sampled in each of the clone libraries. The diversity indices included the Shannon-Wiener diversity index, Simpson diversity index, non-parametric richness estimators such as Chao1 richness estimate and the abundance-based richness estimator (ACE).

LIBSHUFF analysis was performed to compare the four clone libraries for homologous C_X (D) and heterologous C_{XY} (D) coverage using the software weblibshuff (<http://libshuff.mib.uga.edu>) (Singleton et al. 2001). For DOTUR and Libshuff analysis, all the sequences were aligned (multiple sequence alignment) using Clustal X in PHYLIP format, followed by the generation of distance matrices in the DNADIST program of PHYLIP (version 3.68), which were further used as the input files in the respective programs. The partial 16S rRNA gene environmental clone sequences were deposited in the Genbank database under accession numbers EU071379-EU071386, EU445347-EU445355 and FJ268480-FJ268573.

Results

Phylogenetic Analysis

The abundance of each taxonomic group is given in Figure 1. The clones were

—————→ **Figure 1**

distributed among 14 phyla. Majority of the clones (52%) belonged to *Proteobacteria* followed by *Planctomycetes* (12.7%) and *Chloroflexi* (8.8%). *Firmicutes*, *Bacteroidetes* and *Verrucomicrobia* formed 0.9% each of the total sequences. *Acidobacteria* and *Spirochetes* were 3.9% each. *Nitrospira* was represented by 2.9%. Interestingly, there were three uncultured candidate divisions OP3, OP8 and OP11 accounting for 3.9%, 2.9% and 0.9% of the sequences, respectively.

The phylogenetic affiliations of all the clones are represented in Figure 2a and are compared to the sequences from the other non-OMZ marine sediments such as mangroves, hydrothermal vents, shelf sediments etc.

—————→ **Figure 2a**

The dominant phylum, *Proteobacteria* was represented by *Deltaproteobacteria* (62.5%), *Gammaproteobacteria* (25%), *Alphaproteobacteria* (8%) and *Betaproteobacteria* (4%). The flagged groups in Figure 2a such as *Deltaproteobacteria*, *Planctomycetes* and *Chloroflexi* were the dominant ones and their phylogenetic affiliation are shown separately. However, the other classes of *Proteobacteria* are elaborately represented in supplementary material of the paper (Figure S1, S2 and S3 - See Electronic Supplementary Material for this paper). In OMZ sediments class *Alphaproteobacteria* were represented by a few sequences and they showed affiliation to both cultured and uncultured clades (Figure S1). One of the sequences showed similarity to the Order *Rhizobiales*. The *Betaproteobacteria* clustered with culturable taxa and showed high similarity to

Burkholderia cepacia (Figure S2). In the case of *Gammaproteobacteria*, majority showed similarity to *Thiorhodospira sibirica*, while the rest were affiliated to *Vibrionales* (Figure S3). However, fifteen clones could not be assigned to any of known phyla and are placed under two clusters.

Among the *Deltaproteobacteria*, 73% showed similarity to the sulphate reducing bacteria falling in the orders *Syntrophobacterales* and *Desulfovibrionales* while 26% did not show any similarity to any cultivated representatives of the class (Figure 2b).

—————→ **Figure 2b**

None of the sequences were similar to the SAR clusters that are found in the OMZ waters. Interestingly, representatives of orders *Desulfurellales*, *Bdellovibrionales*, *Nannocystinae*, *Desulfobacterales*, *Myxococcales* and *Desulfromonales* were not obtained.

Phylum *Planctomycetes* formed dominant group (12.7%) after *Proteobacteria*, suggesting its importance in the OMZ (Figure 2c). In this Phylum 92% of the clones did not belong to the cultured representative and showed similarity to the uncultured

—————→ **Figure 2c**

representatives from other areas like sediments of mangrove, Black Sea and shelf sediments except a clone which showed a close resemblance to reported uncultured *Planctomycetes* from the OMZ waters of Eastern Pacific Ocean. The sequences of the present study did not show any resemblance to the Candidatus *Scalindua* cluster which was present in the AS-OMZ waters.

The phylum *Chloroflexi* the third dominant group and were mostly affiliated to *Anaerolinea thermophila* (Figure 2d). Sequences showed close similarity to the uncultured representatives of the organic rich sediments of mangrove and continental slopes.

—————→ **Figure 2d**

Bacterial diversity and richness

To understand the bacterial diversity and richness of the AS-OMZ sediment clone library, comparisons were made with suboxic sediment clone libraries from South China Sea, North Sea and Gulf of Mexico. The DOTUR analysis showed that AS-OMZ sediment clone library had the highest number of OTUs (90) at an evolutionary distance of ≥ 0.03 (species level), followed by South China Sea sediment library with 74 unique OTUs and the lowest number was recorded in Gulf of Mexico sediment with only 23 OTUs (Table 1).

————→ **Table 1**

Rarefaction curve showed that the AS-OMZ sediment library showed close to plateau (data not shown). The Shannon-Wiener diversity index and Chao 1 richness estimator were the highest for the AS-OMZ sediment library with values 4.4 and 484.6 respectively (Table 1). The distribution of Delta C as a function of evolutionary distance (D) showed that delta C was maximum at 0.2 genetic distances in all the combinations using LIBSHUFF analysis. The bacterial community composition differed significantly among the four clone libraries ($p=0.001$) (Figure 3)

————→ **Figure 3**

Discussion

Phylogenetic analysis

Sedimentary microorganisms are poorly understood as majority are uncultivated and most are phylogenetically distinct from those in terrestrial environments (D'Hondt et al. 2002). Bacteriological studies of OMZ are quite recent and most of them are pertaining to water column. In the water, unusually diverse bacterial groups, which are mostly exclusive to the region, have been reported (Fuchs et al. 2005; Stevens and Ulloa 2008). Schippers et al. (2006) has attempted to quantify the microbial community in near-surface and deeply buried marine sediments from the Peru continental margin. Despite their obvious ecological importance, microbial communities thriving in the sediments below the OMZ are hardly studied with the exceptions of certain functional gene-based studies (Liu et al. 2003 a,b; Schaefer et al. 2007). Since, being the first in eastern Arabian Sea, the phylogenetic groups of our study are discussed individually and are also compared with available published data from other non-OMZ sediments and OMZ water.

The phylogenetic analysis of the clones derived from OMZ sediments of AS showed a wide representation of various taxonomic groups as the eastern Arabian Sea sediments underlying the OMZ consist of laminated mud, rich in organic matter (Paropkari et al. 1993), which forms an excellent habitat for the microorganisms to flourish. Gupta (2000) in his review on the phylogeny of prokaryotes states that phylum *Proteobacteria* is of biological significance as it is the most dominant and diverse group of the microbial assemblage. Occurrence of classes of *Proteobacteria* in sediments has been reported from water or/and sediment with the dominance of *Proteobacteria* in certain regions (Yakimov et al. 2002; Knittel et al. 2003). *Alphaproteobacteria* are encountered in the marine habitats especially in the pelagic zone frequently (Hugenholtz et al. 1998). Occurrence of *Gammaproteobacteria* has been reported among culturable and non-culturable bacteria of pelagic waters (Lopez Garcia et al. 2001, Fuhrman and Davis 1997) and sediments (Hugenholtz et al. 1998;

Cho and Giovannoni 2004). In the OMZ sediment, *Proteobacteria* was the most prevalent phylum (52%) represented by both cultivable and non-culturable classes viz. *Alpha*, *Beta*, *Gamma* and *Delta*-*proteobacteria*. The *Deltaproteobacteria*, which include the anaerobic sulphate reducing bacteria, were the most abundant class (62.5%), suggesting the prevalence of sulphate reduction in this region. *Syntrophobacterales* and *Desulfovibrionales* were the predominant groups. However, 26% of the sequences did not show any resemblance to cultured representatives, but were closely related to clones detected at cold seeps, mud volcano and harbour sediments (Fang et al. 2006; Losekann et al. 2007; Zhang et al. 2008). Sulphate concentrations vary from 39.8 to 12.7 mM in the pore waters of the eastern Arabian Sea, indicating sulphate reduction (Karisiddaiah et al. 2006). The presence in this zone of sulphate reducing bacterial communities indicates the probable biological agents responsible for this process. Similar functional diversity of sulfate-reducing bacteria of the eastern Pacific continental margin (Liu et al. 2003b) and microbial diversity of sulfate-reducing and methanogenic subsurface sediments of the Benguela Upwelling System (Schaefer et al. 2007) have been reported. Occurrence of high sulphate reducers has been reported in other marine sediments (Gray and Herwig 1996; Ravenschlag et al. 1999; Bowman and McCaiug 2003, Bisset et al. 2007). Dominance of this group was not only restricted to Mid-Atlantic Ridge (Lopez Garcia et al. 2003) but was also reported in Tokyo and Sagami Bays (Urakawa et al. 1999 and 2000). *Planctomycetes*, though sparsely studied, the members of this phylum are significant because of their abundance in marine environment (Glockner et al. 2003) and their role in anaerobic ammonium oxidation (anammox) (Allredge 2000; Strous et al. 1999; Kuypers et al. 2005; Thamdrup et al. 2006). A few clones seem to group with the anammox lineages suggesting they are especially abundant in surface sediment. This monophyletic and deeply branching bacterial phylum has also been reported in permanently anoxic basins such as Black Sea, Golfo Dulce (Kuypers et al. 2003; Dalsgaard et al. 2003), OMZ waters (Hamersley et al. 2007; Woebken et al. 2008) and other marine sediments (Musat et al. 2006). Members of phylum *Chloroflexi*, exhibits unusual metabolic diversity like anoxygenic photosynthesis, organotrophy and reduction of chlorinated hydrocarbons (Jackson et al. 1973; Maymo-Gatell et al. 1995). This phylum was represented by only Family *Anaerolineae* in the AS OMZ. Various other groups such as *Firmicutes*, *Bacterioidetes*, *Acidobacteria*, *Nitrospira*, *Spirochetes* and *Verrucomicrobia* reported from marine realms were represented as a minority in the AS OMZ. These are reported from marine sediments and water and their tight association with particles is thought to be helping them for the degradation of recalcitrant compounds (Ravenschlag et al. 1999; Polymenakou et al. 2005; Martin-Cuadrado et al. 2007; Quaiser et al. 2008). Among them *Bacterioidetes* group is known to be taking part in organic matter degradation and have been isolated from wide range of habitats (Chang et al. 2003). *Verrucomicrobia* is known to include both aerobic

and anaerobic heterotrophs (Hedlund et al. 1997; Chin et al. 2001). Hence occurrence of the anaerobic members of this group in the sub-oxic sediments in the present study is not unusual. Candidate divisions which involve groups that have no cultivated representatives in environment are assigned as OP series (Hugenholtz et al. 1998). In the OMZ sediments, Candidate divisions were spread in OP3, OP8 and OP11. Uncultured groups I and II which were not assigned to any taxonomic group formed a major part of the library. The OP11 candidate divisions are widespread and phylogenetically diverse (Harris et al. 2004) and have been detected primarily in the anaerobic ecosystems (Barns et al. 1994; Ludwig et al. 1997; Dojka et al. 2000).

Though sediments may seem unlikely to support ubiquitous dispersal of microorganisms compared to water, some bacterial taxa have shown widespread distribution in marine sediments. This may be due to environmental selection. Though phylum *Acidobacteria* with a few culturable representatives is well represented in soils, they are only rarely encountered in marine habitats (DeLong et al. 2006; Quaiser et al. 2008). Five phylogenetic groups (*Proteobacteria*, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia* and Candidate division OP 11) present in the OMZ water column of the AS (Fuchs et al. 2005) were also found in the sediments. Though all the four classes of *Proteobacteria* (*Alpha*, *Beta*, *Gamma* and *Delta*) were present in AS OMZ water and sediments, *Alphaproteobacteria* were found to be under represented in OMZ sediments compared to that in AS waters (Fuchs et al. 2005). Similar assemblages may be due to transportation of pelagic population to the seabed via constant detritus rain, followed by the genetic exchange between pelagic and benthic population. Similarly, certain groups which were present in the AS pelagic waters such as *Cytophaga*, *Nitrospina*, *Actinobacteria* and *Cyanobacteria* were absent in the sediment (Fuchs et al. 2005). Interestingly, though SAR clades are common in the pelagic zone (Fuchs et al. 2005), it was not detected in the sediment. *Betaproteobacteria* include ammonia oxidizers such as *Nitrosospira*-like clusters, which have been well documented in the OMZ waters off north Chile and marine sediments (Molina et al. 2007; Freitag and Prosser 2004), were not recorded in the AS-OMZ sediment. Thus few groups were cosmopolitan and rest were exclusive. Coolen et al. (2002) opined that the relative abundance of organic matter in the AS sediment may alter the microbial community. The presence of *Chloroflexi* related SAR 202 cluster, which forms a monophyletic subgroup in the phylum, has been reported in the oxic (Morris et al. 2004) and the oxygen minimum zone waters (Stevens and Ulloa 2008), but did not occur in our study. Also phylum *Bacteroidetes* was represented by less than 2% in the OMZ sediments whereas in the waters their relative abundance was 2-10% (Kirchman 2002; Abell and Bowman 2005; DeLong et al. 2006). The characteristic of this group is that only one predominant family occurs in an environment. In the sediment, we also observed the presence of only *Flavobacteriaceae* family (Order-Flavobacteriales) which have also

been reported in the hydrothermal sediments (Lopez-Garcia et al. 2003). Thus phylogenetic analysis of AS-OMZ suggest that bacterial diversity was unexpectedly high compared to other hypoxic locales and quite distinct from the water mass.

Statistical analysis

Comparative analysis of diversity and paired reciprocal comparisons using LIBSHUFF statistics between the AS-OMZ sediment library and libraries of Gulf of Mexico (Orcutt et al. 2007), North Sea (Wegener et al. 2008) and South China Sea (Dai et al. 2002), showed high number of OTUs which differed significantly. This is further supported by the higher values of the diversity indices and richness. However, ACE was 484.6 at a distance of ≥ 0.03 lesser than reported value of 757 for South China Sea sediment clone library (Dai et al. 2002). The difference between these hypoxic regions may be due the selective pressure that shape diversity within communities (Dunbar et al. 1999). The OMZ sediments represented a greater diversity of bacteria which may be due to the accumulation of less recalcitrant organic matter. The diversity analysis of OMZ clone library of the AS showed that the diversity indices and estimates were high, though the coverage was 22%. Since the homologous coverage of our library with respect to evolutionary distance was high at $D < 0.2$, it suggests that the library contained the representatives of the original community. In the present study, the coverage was comparatively higher than the three areas and hence it is reasonable to suggest that the data were representative of the *in situ* bacterial community inspite of the drawbacks of clone library method (Sogin et al. 2006). Though the biogeochemical implications of the phylogenetic diversity observed in marine microbial communities are not yet well understood, the bacterial species composition is significant in controlling the rates and patterns of organic matter hydrolysis (Riemann et al. 1999). This study is the first information on the bacterial diversity in the OMZ sediments which provides the baseline for further studies on the microbial groups along with functional probes for a better understanding of the OMZ ecosystem.

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LEGENDS TO TABLE AND FIGURES

Table 1: Diversity indices of the four 16S rDNA clone libraries.

Figure 1: Abundance of phylogenetic groups in the AS-OMZ sediments

Figure 2a. Phylogenetic tree of 16S rRNA gene sequences of AS-OMZ sediments and their closest marine sediment sample entries in the GenBank. Sequences with prefix 'PL' are results of the present library. Groups flagged represent the three most dominant groups. Tree topology was inferred by neighbour-joining method, using 1000 bootstrap iterations. *Methanothermobacter wolfeii* is the outgroup. Scale bar shows 2% sequence divergence. Numbers in parenthesis represent the percentage of abundance of classes *Alpha*, *Beta*, *Gamma* and *Delta-proteobacteria* in the phylum *Proteobacteria*.

Figure 2b. Phylogenetic tree of 16S rRNA gene sequences of AS-OMZ sediments showing affiliation within the *Deltaproteobacteria*. Flagged group represent orders of the class *Deltaproteobacteria*.

Figure 2c. Phylogenetic tree of 16S rRNA gene sequences of AS-OMZ sediments showing affiliation within the Phylum *Planctomycetes*.

Figure 2d. Phylogenetic tree of 16S rRNA gene sequences of AS-OMZ sediments showing affiliation within the Phylum *Chloroflexi*.

Figure 3. Comparisons of AS-OMZ sediment clone library with A) South China Sea B) Gulf of Mexico C) North Sea. Homologous (red) and heterologous (green) coverage curves are depicted. Blue line indicates the value of $(C_X - C_{XY})^2$ for the original samples at each value of evolutionary distance (D).

Fig.1. Abundance

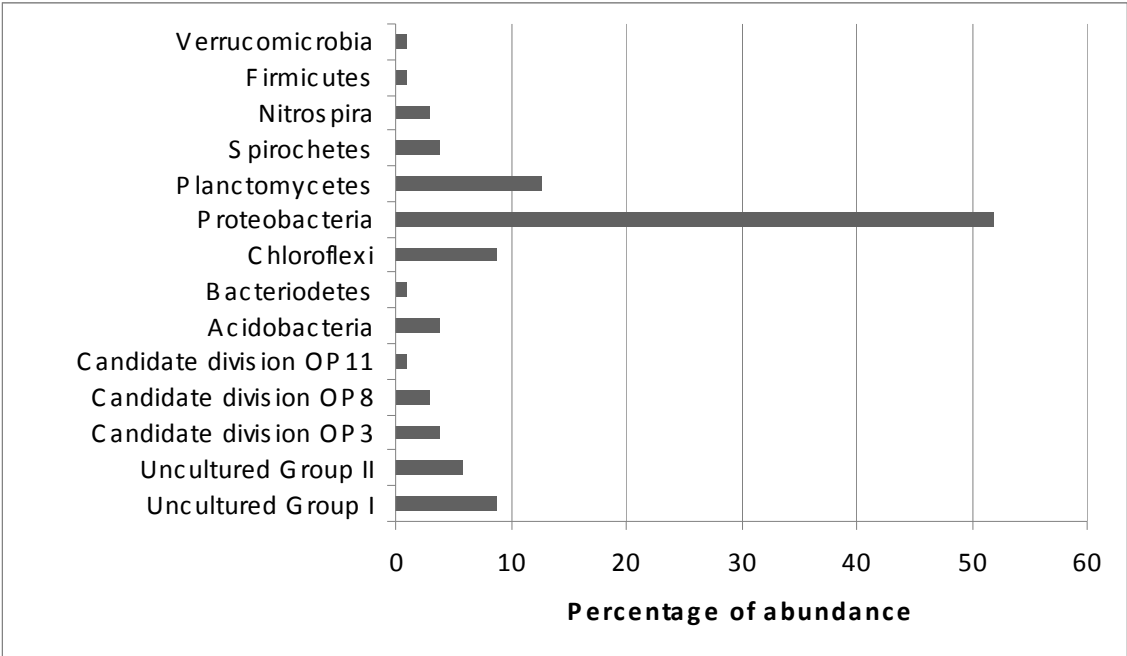


Fig.2a. Phylogenetic tree of 16S rRNA gene sequences of OMZ sediments.

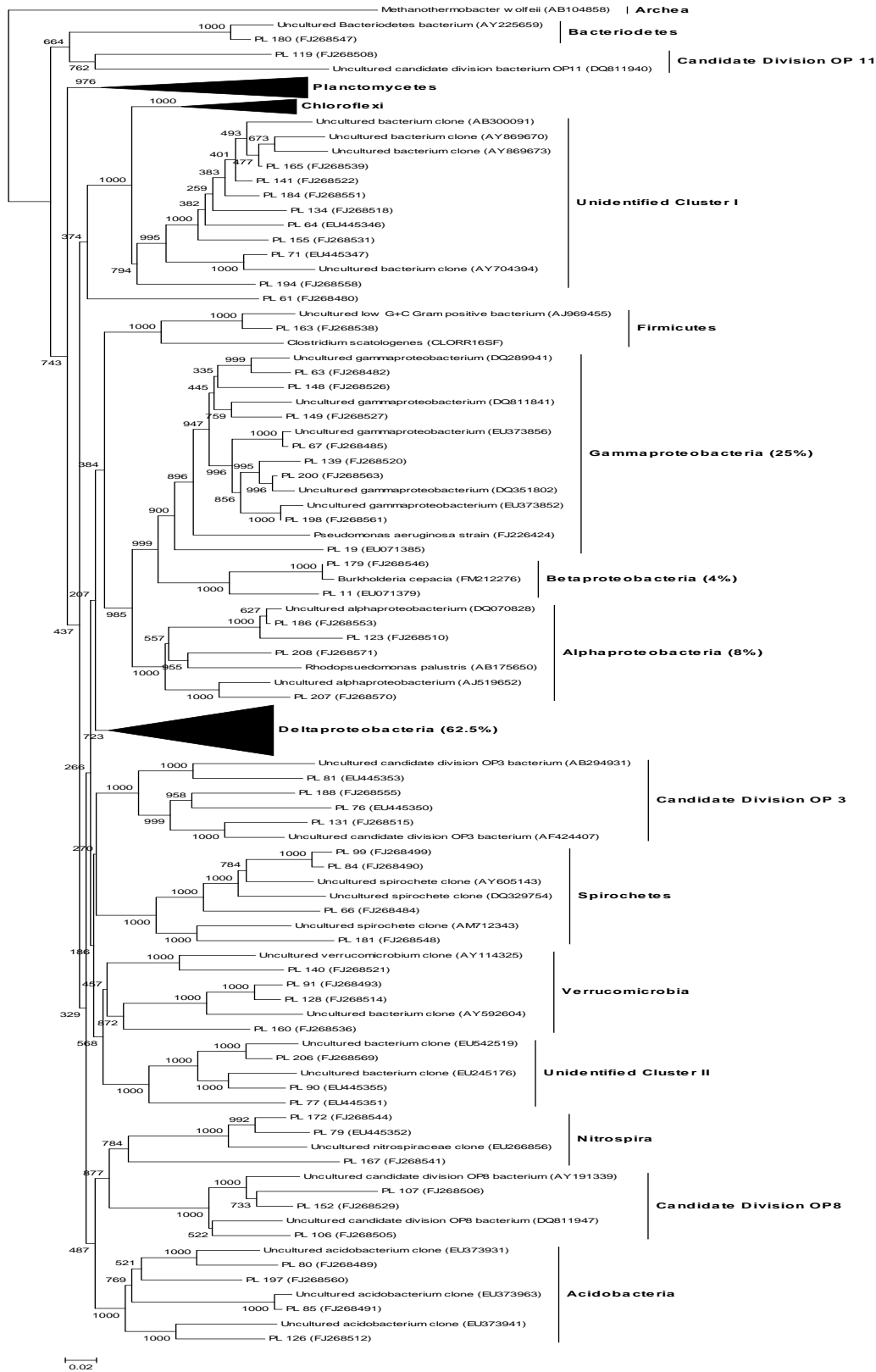


Fig.2b. Phylogenetic tree of *Deltaproteobacteria*

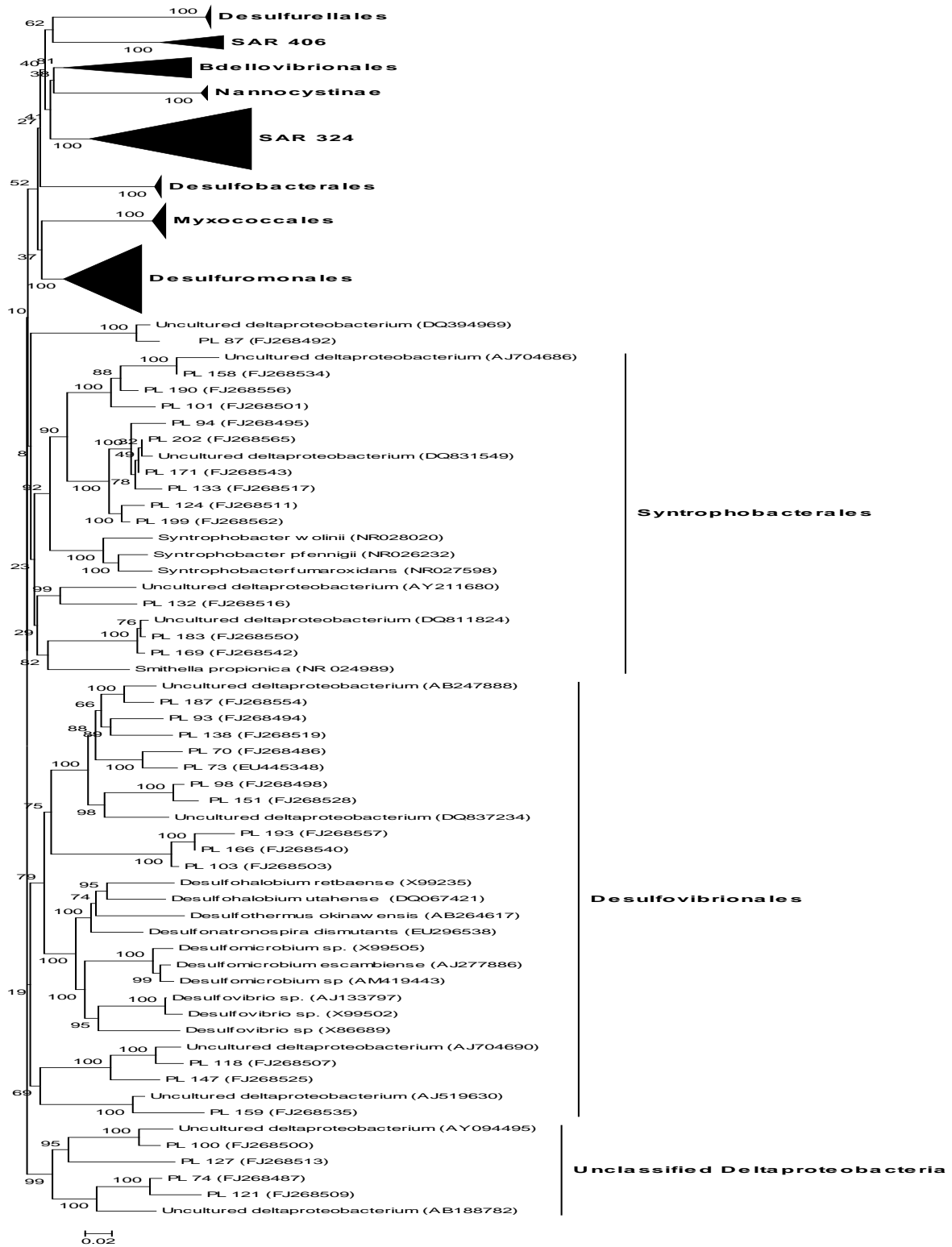


Fig.2c. Phylogenetic tree of *Planctomycetes*

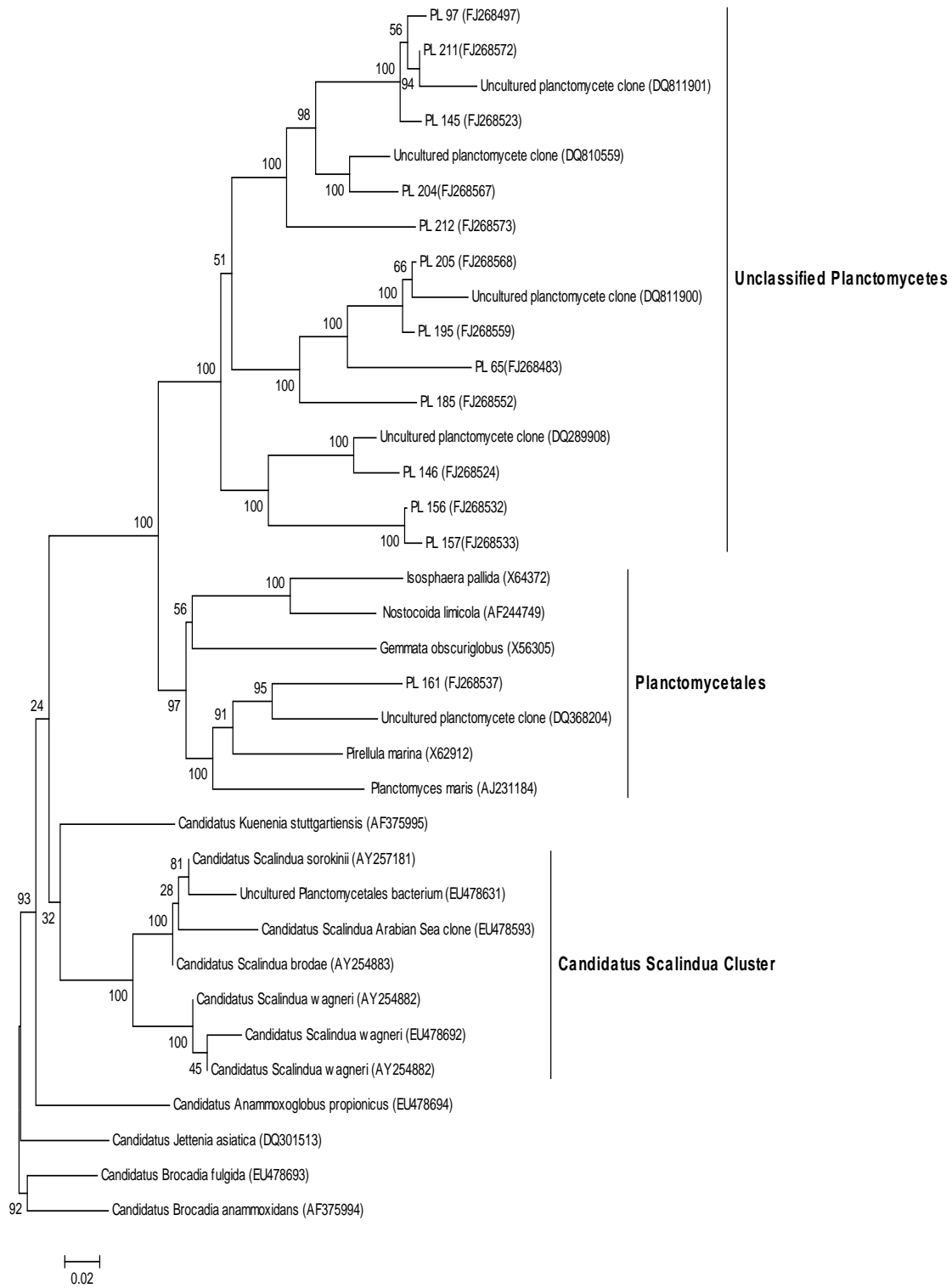


Fig.2d. Phylogenetic tree of Phylum *Chloroflexi*

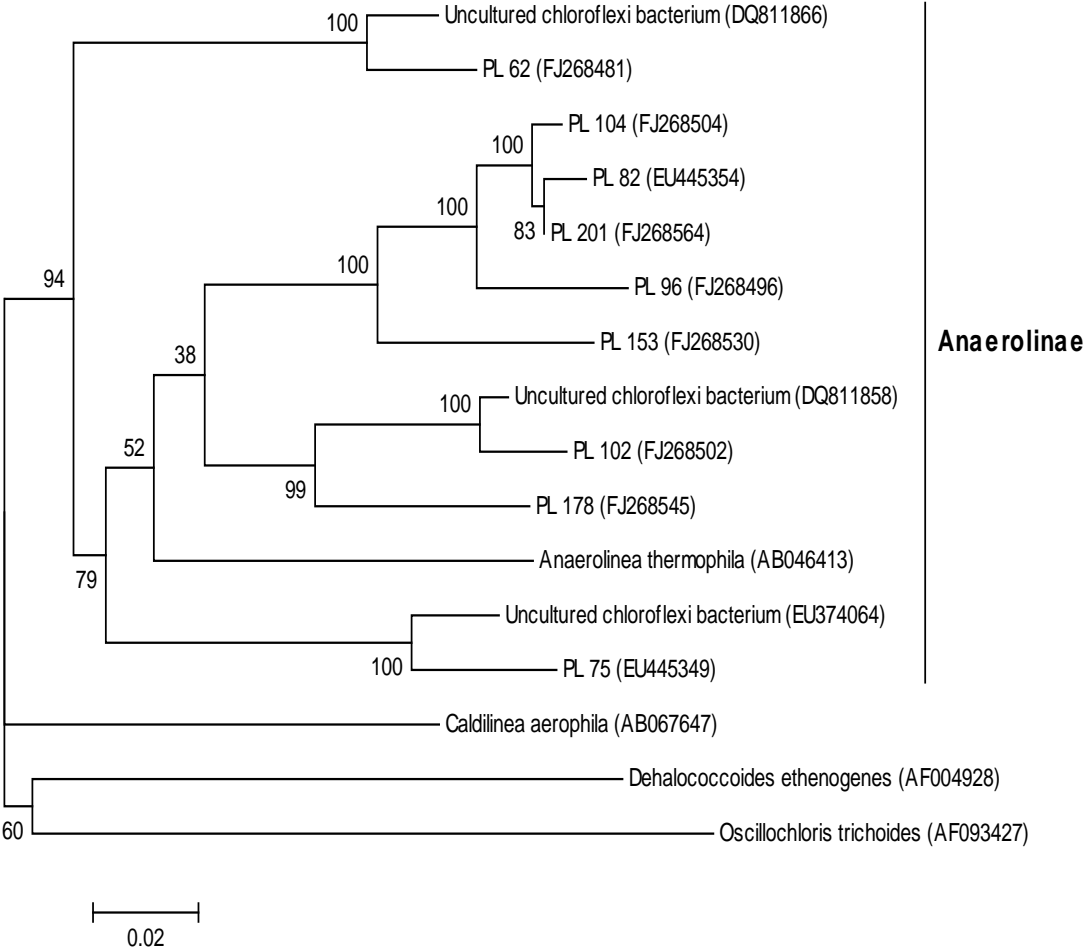
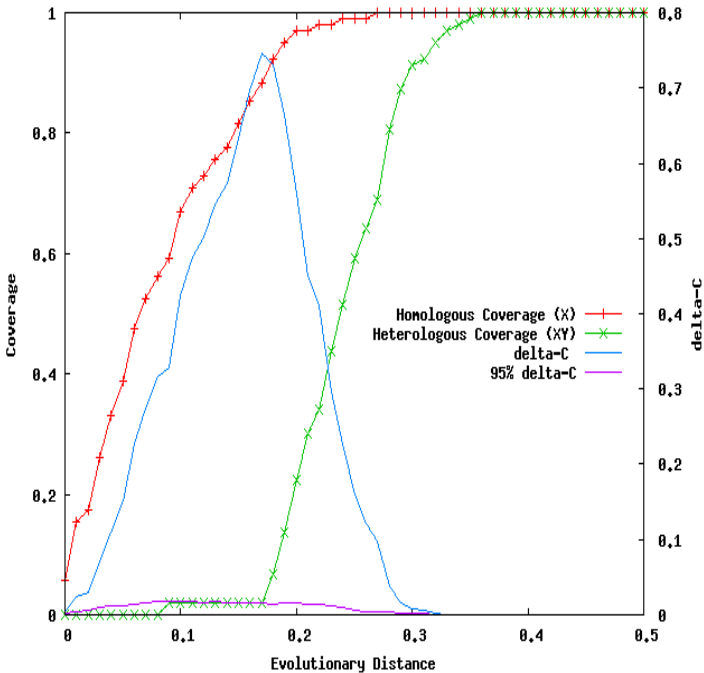
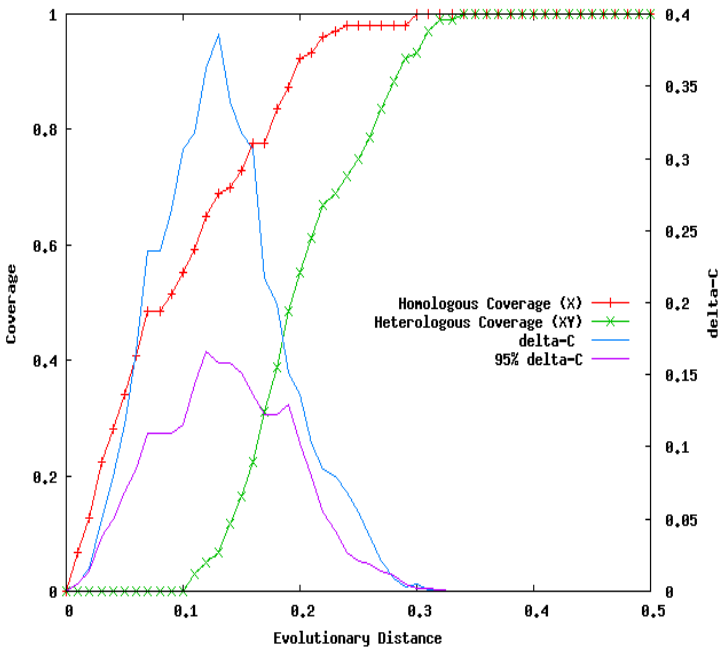


Fig.3. Comparisons of OMZ sediment clone library

A



B



C

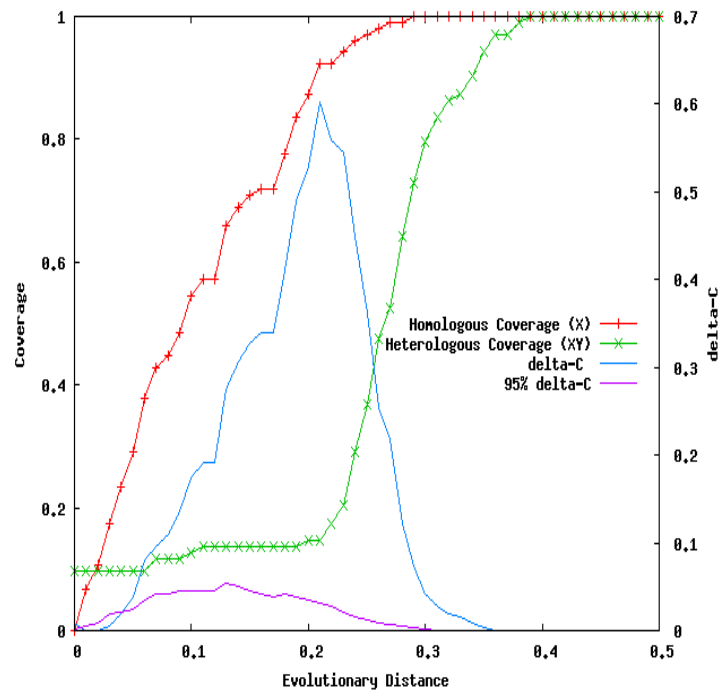


Table 1: Comparison of diversity indices

Sediment	No. of unique OTUs	Abundance-based coverage estimator (ACE)	Chao 1 richness estimate	Shannon -Wiener Index (H)	Simpson Index (1/D)
Arabian Sea OMZ	90	484.6	485	4.4	0.003
North Sea	47	139.7	121	3.7	0.009
South China Sea	74	757	442.5	4.1	0.007
Gulf of Mexico	23	114.8	118	3.04	0.018

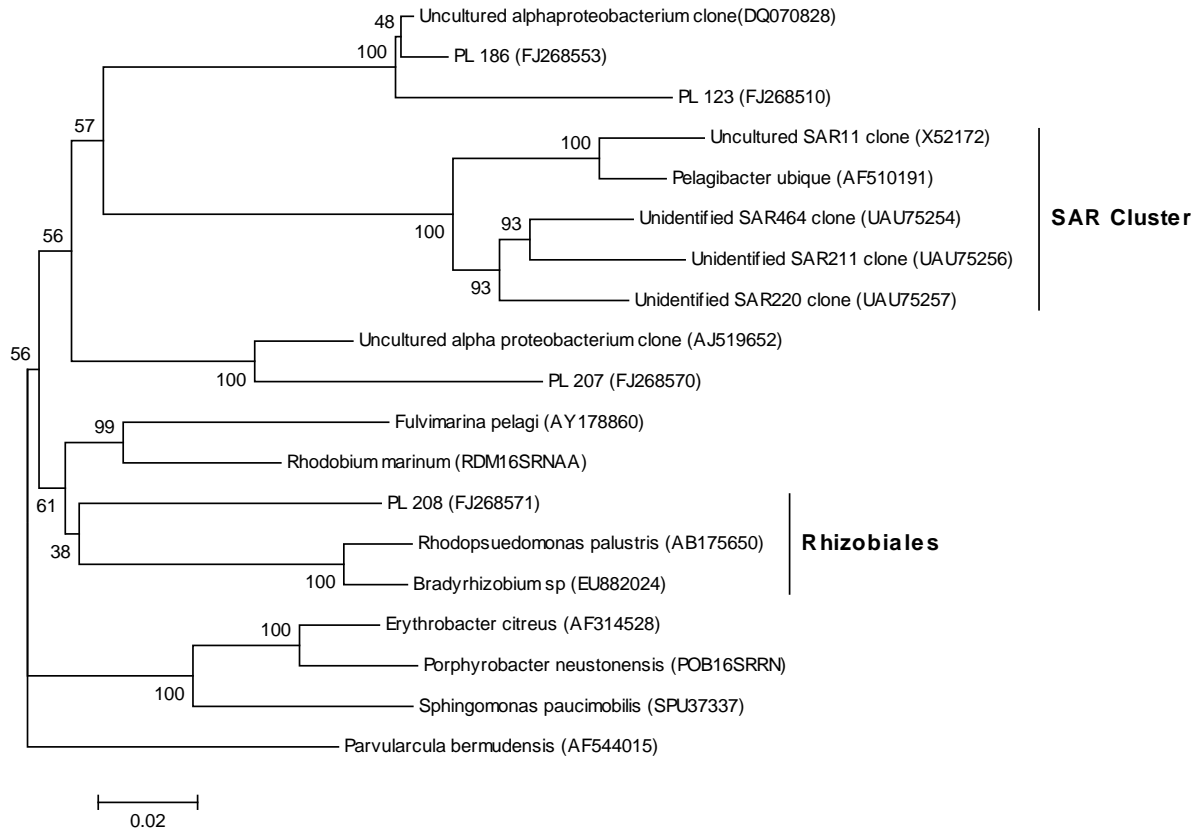


Fig. S1 Phylogenetic tree of 16S rRNA gene sequences of AS-OMZ sediments showing affiliation within the *Alphaproteobacteria*.

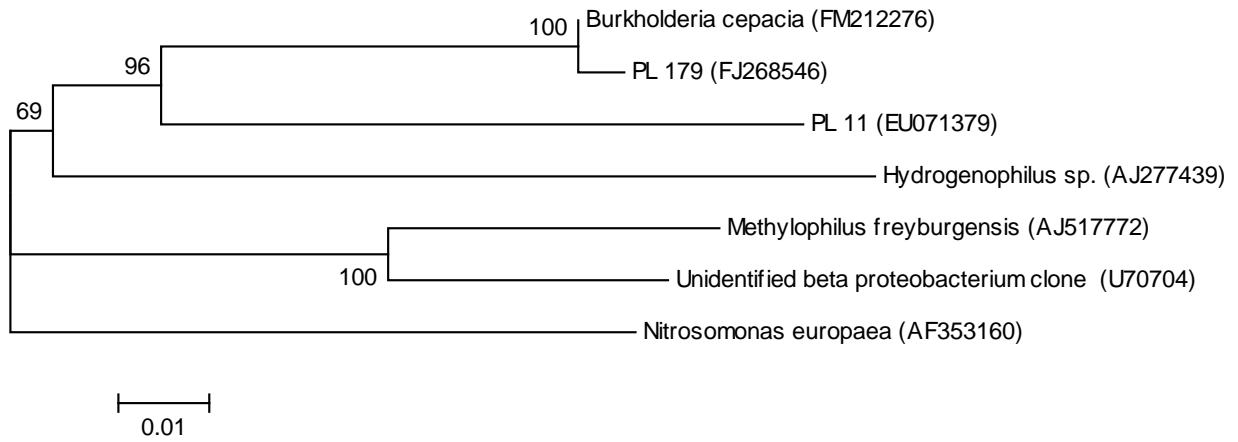


Fig. S2 Phylogenetic tree of 16S rRNA gene sequences of AS-OMZ sediments showing affiliation within the *Betaproteobacteria*.

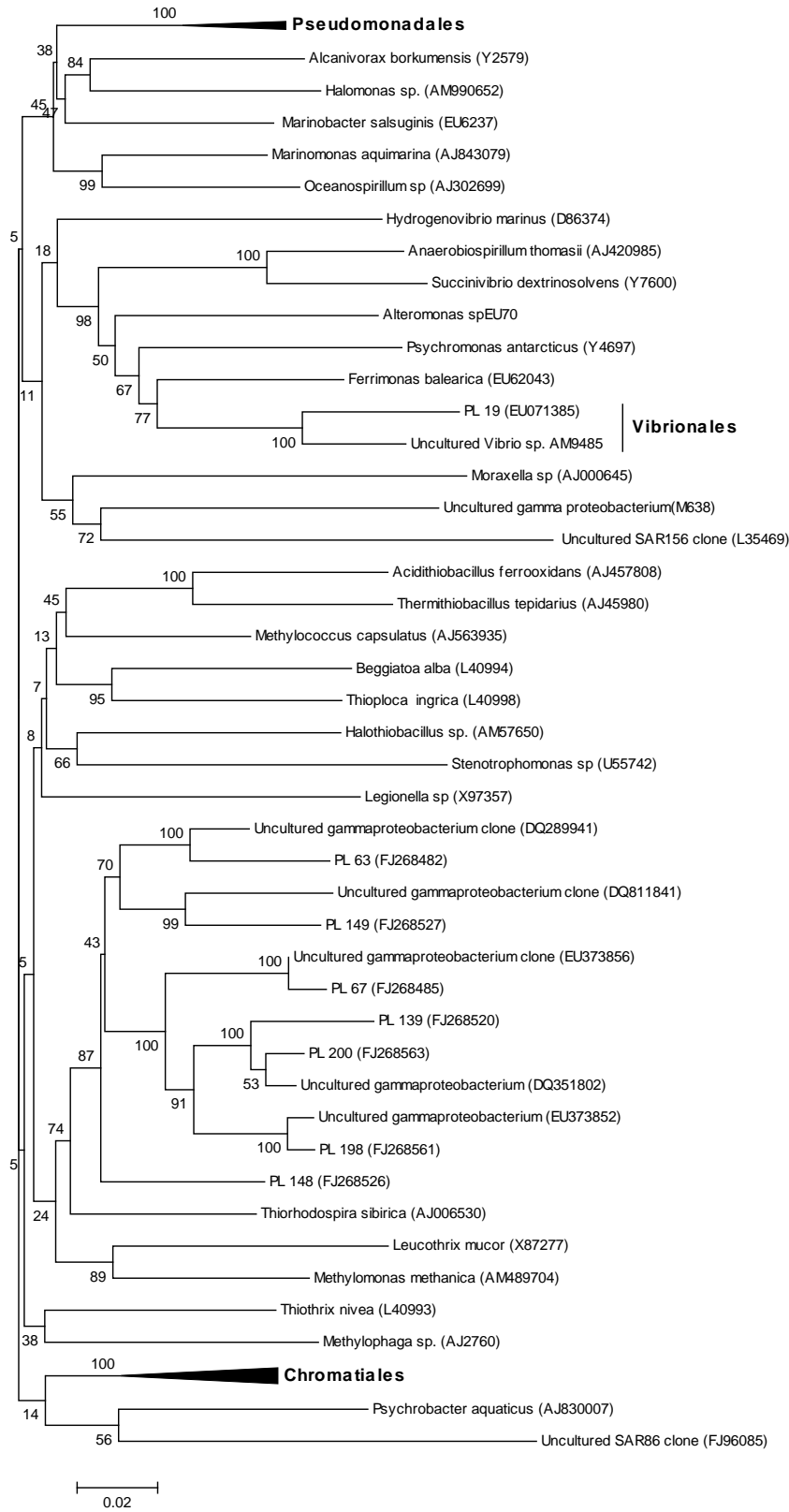


Fig S3: Phylogenetic tree of 16S rRNA gene sequences of AS-OMZ sediments showing affiliation within the *Gammaproteobacteria*.