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Phylogenetic diversity of sediment bacteria from the deep Northeastern Pacific Ocean: a comparison with the deep Eastern Mediterranean Sea

Ioanna Kouridaki,[1,2] Paraskevi N. Polymenakou,[2]* Anastasios Tselepides,[2,3] Manolis Mandalakis,[2] Kenneth L. Smith, Jr.[4]

[1]Department of Chemistry, University of Crete, Heraklion, Crete, Greece. [2]Institute of Marine Biology and Genetics, Hellenic Centre for Marine Research, Heraklion, Crete, Greece. [3]Department of Maritime Studies, University of Piraeus, Piraeus, Greece. [4]Monterey Bay Aquarium Research Institute, Moss Landing, California, USA

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Summary. The variability of bacterial community composition and diversity was studied by comparative analysis of five 16S rRNA gene clone libraries from deep-sea sediments (water column depth: 4000 m) of the Northeastern Pacific Ocean and Eastern Mediterranean Sea. This is the first comparison of the bacterial communities living in these deep-sea ecosystems. The estimated chlorophyll *a*, organic carbon, and C/N ratio provided evidence of significant differences in the trophic state of the sediments between the Northeastern Pacific Ocean and the much warmer Eastern Mediterranean Sea. A diverse range of 16S rRNA gene phylotypes was found in the sediments of both regions. These were represented by 11 different taxonomic groups, with Gammaproteobacteria predominating in the Northeastern Pacific Ocean sediments and Acidobacteria in the Eastern Mediterranean microbial community. In addition, several 16S rRNA gene phylotypes only distantly related to any of the previously identified sequences (non-affiliated rRNA genes) represented a significant fraction of the total sequences. The potential diversity at the two sites differs but remains largely unexplored and remains of continuing scientific interest. [**Int Microbiol** 2010; 13(3):143-150]

Keywords: marine bacterial diversity · deep-sea sediments · 16S rRNA genes · Northeastern Pacific Ocean · Eastern Mediterranean Sea

Introduction

Deep-sea sediments (water depth ≥1000 m) cover about 95% of the total oceanic bottom and 67% of the Earth's surface, thus representing one of the planet's largest ecosystems [46]. For years, the deep-sea floor was considered to be a very stable and biologically inert environment due to its high hydrostatic pressure and the absence of sunlight. However, recent technological developments and monitoring methods have shown that deep-sea ecosystems constitute a dynamic environment linked to upper water column processes through the influx of organic matter [18,37,43]. Moreover, it has also become clear that the microbial processes occurring along the deep-sea floor are essential in sustaining oceanic primary and secondary production. Host microbial cells account for most of the benthic biomass, with an enormous number of undiscovered species ($0.3–8.3 \times 10^6$) estimated to be living in the deep-sea floor [11].

***Corresponding author:** P.N. Polymenakou
Institute of Marine Biology and Genetics
Hellenic Centre for Marine Research
Gournes Pediados, P.O. Box 2214
GR-71003 Heraklion, Crete, Greece
Tel. +30-2810337855. Fax +30-2810337822
Email: polymen@her.hcmr.gr

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Despite the vast contribution of microbial biomass to the total benthic biomass of the deep ocean floor and its indispensable role in biogeochemical cycling, the cultivated strains constitute only a small fraction of the total biodiversity [1,44]. Molecular methods targeting 16S rRNA genes have revealed that only a very small fraction of the diversity of the microbial world is known, as most of it is hidden in the pool of yet uncultured bacteria [1,16]. Similarly, with the exception of a few functionally defined bacterial groups (methanogens, methanotrophs, sulfate-reducing bacteria), our knowledge regarding the ecology and physiology of sediment bacteria is fragmentary due to the lack of extensive biogeographical studies of sediment bacterial populations [3].

The goal of the present study was therefore to shed light on the nature of these populations by using molecular-based methods to analyze the microbial components in the deep-sea sediments (water-column depth: 4000 m) of two contrasting environments, the oligotrophic, warm Eastern Mediterranean Sea and the eutrophic, cold Northeastern (NE) Pacific Ocean off the Californian coast. The deep eastern basin of the Mediterranean Sea is one of the most oligotrophic regions in the world [17,19], characterized by a complex hydrographic and geomorphologic regime [26]. Despite its depth (average: 2000 m), its deep water mass temperature is at least 13.5ºC. Hence, the Eastern Mediterranean Sea constitutes a model for a deep, relatively warm, and highly oligotrophic bathypelagic habitat [36]. By contrast, in the NE Pacific Ocean, surface nutrient levels, chlorophyll concentrations, and biological productivity are high [38], comprising a eutrophic but relatively cold environment.

In the following, we present a comparative phylogenetic analysis of the microbial community composition and biodiversity dwelling in the aforementioned environments, by means of 16S rRNA gene clone libraries. If the trophic state or/and the temperature play a key role in microbial community composition, then the eutrophic and cold environment of the NE Pacific Ocean should support bacterial assemblages different from those in the oligotrophic and warm Mediterranean Sea. To our knowledge, this is the first comparison of bacterial communities living in drastically different deep-sea ecosystems.

Materials and methods

Sampling sites and environmental variables. Four sampling sites at the NE Pacific Ocean (station M, *Alvin* samples) and one at the Eastern Mediterranean Sea (station KM3, South Ionian Sea; Fig. 1 and Table 1) were selected, each at a water column depth of approximately 4000 m. Undisturbed Pacific sediment samples were collected by means of the research submersible *Alvin* (Woods Hole Oceanographic Institution, Woods Hole, MA, USA) in August 2006 (Fig. 1A). Piston cores manipulated by the arms of the submersible allowed the collection of undisturbed sediment samples from four microenvironments in the NE Pacific Ocean (station M). The sediment core Alvin24 was collected on a bioturbation mound, whereas the core Alvin25, used as a control, was collected away from the observed mound. Similarly, core Alvin28 was collected near a detrital kelp holdfast, whereas core Alvin29 was used as a control. A Bowers and Connelly multiple-corer (8 cores, internal diameter 9.0 cm) was used to collect cores with an undisturbed sediment-water interface and with overlying bottom water from the South Ionian Sea in May 2007, on board the R/V Aegaeo of the Hellenic Centre for Marine Research (Fig. 1B). Three independent deployments were made in the South Ionian sampling site (station KM3). Mixed surface sediment samples (0–1 cm) from each of the sampling sites (one Ionian and four

Table 1. Geochemical characteristics of the deep-sea sediments collected from the Northeastern Pacific Ocean (station M) and Eastern Mediterranean Sea

	KM3	Alvin24	Alvin25	Alvin28	Alvin29
Station character	Deep-sea basin	Away from bioturbation mound	On bioturbation mound	Near a detrital kelp holdfast	Away from detrital kelp holdfast
Depth (m)	4015	4100	4100	4100	4100
Temperature (°C)	14.00	1.48	1.48	1.48	1.48
Chl *a* (μg/g)	0.12	0.94	0.97	0.90	0.83
CPE (μg/g)	1.07	7.75	7.38	7.46	7.04
OC (%)	0.82	1.53	1.69	1.66	1.53
C/N	10.45	9.11	6.60	6.10	6.87
Sequenced clones	105	73	65	87	81
OTUs	82	55	43	64	60
Coverage (%)	43.44	26.31	54.68	44.79	43.51
Chao-1	188.78	209.08	78.64	142.89	137.88

Chl *a:* chlorophyll *a*; CPE: chloroplastic pigment equivalents; OC (%): organic carbon content; C/N: organic carbon to nitrogen ratio.

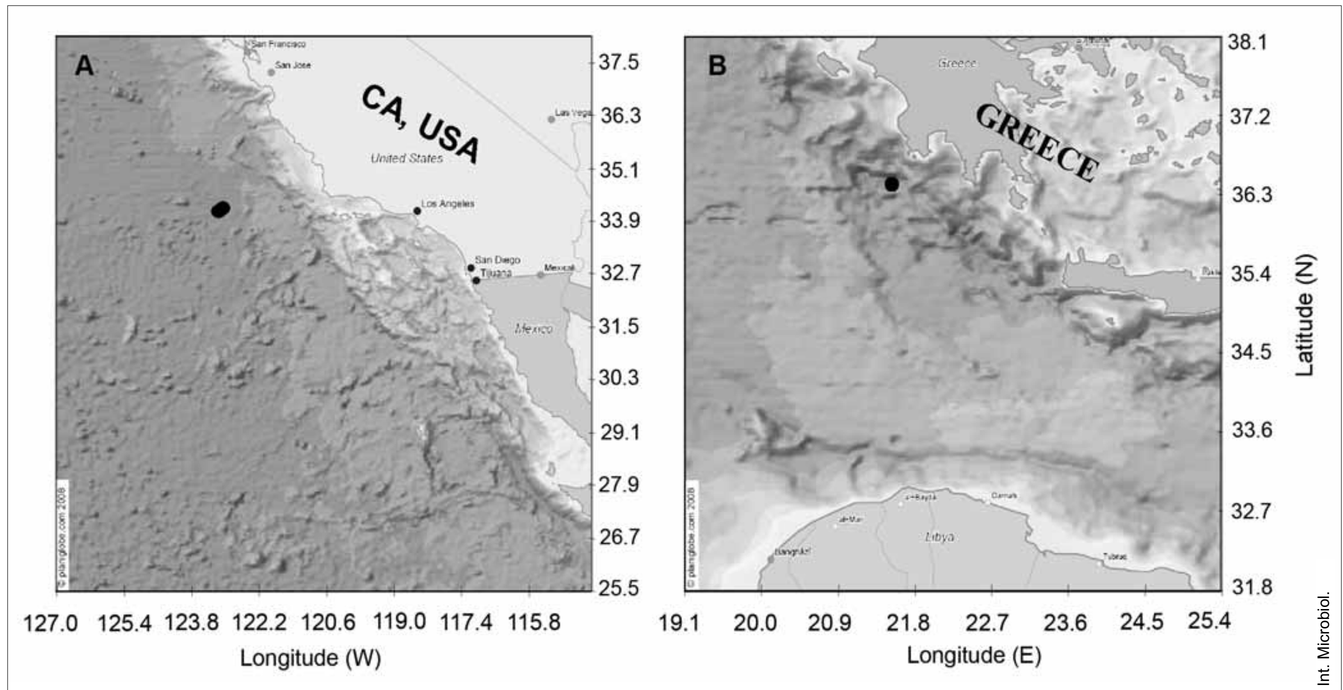

Fig. 1. Map showing the positions of the sampling sites in (**A**) the Northeastern Pacific Ocean (station M) and (**B**) the Eastern Mediterranean Sea (station KM3).

Pacific oxic sediments) were stored frozen in sterile vials for subsequent analysis in the laboratory. Chloroplastic pigments (chlorophyll *a* and phaeopigments) and total organic carbon and nitrogen concentrations were estimated using a Turner TD-700 fluorometer [23] and a Perkin Elmer CHN 2400 analyzer [13], respectively. Chloroplastic pigment equivalents (CPE) are defined as the sum of chlorophyll *a* and phaeopigments.

16S rRNA gene clone library. Five 16S rRNA gene clone libraries were constructed from the surface sediments in order to study benthic bacterial diversity and community composition. Total sedimentary DNA (approximately 0.5 g) was extracted directly from the deep-sea samples using the FastDNA-SPIN Kit for Soil (Q-Biogene, Carlsbad, CA, USA) following the manufacturer's instructions. The concentration of DNA in the extracts was measured using the spectrophotometer ND-1000 (NanoDrop, Wilmington, DE, USA). Bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) with the universal bacterial primers 27f (modified to match also Planctomycetales, 5′-AGRGTTTGATCMTGGCTCAG-3′ [45]) and 1492r (5′-GGYTACCTTGTTACGACTT-3′ [20]). For each sample, eight replicate PCRs of 20 µl were amplified in a Perkin-Elmer cycler, with an initial denaturation at 94°C for 3 min followed by 30 cycles of 1 min at 94°C, 1 min annealing at 55°C, 2 min primer extension at 72°C, and a final extension at 72°C for 7 min. In the case of KM3 samples, 16S rRNA gene amplification was possible at a PCR annealing temperature of 50°C. Each PCR tube contained 1–4 ng of target DNA, PCR buffer (10 mM Tris-HCl, pH 9, 50 mM KCl, 0.1% Triton X-100, and 2 mM $MgCl_2$), 100 nM of each primer, 200 µM of each dNTP, and 0.25 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). All PCRs were then pooled and precipitated in a centrifugal vacuum evaporator (SpeedVac; Heraeus Instruments, Hanau, Germany) followed by gel purification using the Quiaquick PCR purification kit (Quiagen, Valencia, CA, USA). For each sample, 10 ng of PCR product was cloned into the pCR 4-TOPO vector and transformed into chemically competent cells of *E. coli* One shot TOP10 cells using the TOPO TA cloning kit (version M), as recommended by the manufacturer (Invitrogen). The bacterial clones were collected and transferred to 96 deep-well plates and incubated for 24 h at 37°C in Luria-Bertani (LB) medium containing 25 mg kanamycin/ml. Aliquots of the individual clones were either kept at –80°C in 7% dimethyl sulfoxide or processed for plasmid DNA extraction using 800-µl Unifilter microplates (Whatman). The extracts were further used for sequencing.

Phylogenetic analysis, species richness and statistical analysis. A total of 472 clones were successfully sequenced with either the vector primer M13f-20 (5′-GTAAAACGACGGCCAG-3′) or the vector primer M13r (5′-CAGGAAACAGCTATGAC-3′, Invitrogen) on an ABI 3700 96-capillary sequencer (Applied Biosystems) using the BigDye terminator kit (v.3.1, Applied Biosystems). This procedure generated high-quality reads of 450–780 bases. Three sequences were identified as likely chimeric products according to Chimera Check, included in Ribosomal Database Project II (Michigan State University, East Lansing, MI, USA), and were omitted from the phylogenetic analysis. The remaining sequences were compared to GenBank entries using BLAST (Basic Local Alignment Search Tool, National Center for Biotechnology Information, Bethesda, MD, USA) in order to obtain preliminary phylogenetic affiliations of the clones. Additional sequences excluded from further analysis were 55 clones related to eukaryotic organelles and clone vector sequences, and three clones not having any relation to the GenBank reference clones. The sequences of the remaining 411 clones were imported to the ARB software (version 2.5b, Technical University of Munich, Germany [24]) and aligned using the integrated alignment tool and the fast alignment option followed by manual alignment of the sequences to closely related sequences in the ARB database. Similarity matrices among the clone sequences were calculated to identify operational taxonomic units (OTUs, minimum sequence similarity of 98%), which were further used to estimate species richness (Chao-1) using the web-based rarefaction calculator software [http://www2.biology.ualberta.ca./jbrzusto/rarefact.php] [5]. Finally, 304 OTUs were identified and subsequently used to construct phylogenetic trees by applying the maximum parsimony method. The robustness of tree topologies was confirmed by maximum parsimony analysis [24] with 100 bootstrap replica-

tions; values <50 were removed. All 304 partial 16S rRNA gene sequences generated in the present study were deposited in GenBank under accession numbers FJ197343–FJ197646. Novel clusters of uncultured sediment bacteria were defined as monophyletic groups of 16S rRNA gene sequences with a minimum sequence similarity of 98%. Additional criteria were that the cluster must contain sequences from a minimum of two libraries. Multivariable analysis using Euclidean distance, as integrated in the PRIMER 6.1.5 software (Plymouth Marine Laboratory, UK), was carried out to compare bacterial community composition among the environmental samples, at the level of large taxonomic groups.

Analysis of clone library significance testing. To determine the significance of differences between two clone libraries (e.g., X and Y), differences (ΔC) between homologous $C_X(D)$ and heterologous $C_{XY}(D)$ coverage curves were calculated using the statistical tool LIBSHUFF [http://libshuff.mib.uga.edu/] [39]. Genetic distance matrices among the clone libraries were calculated in the ARB software and further imported to the LIBSHUFF program (perl environment). If the clone libraries are similar, then the coverage curves $C_X(D)$ and $C_{XY}(D)$ should also be similar. For each pairwise comparison, if the lower of the two critical P-values (P) returned by LIBSHUFF was ≤ 0.025, then the two libraries were considered significantly different in community composition, with a confidence of 95% ($P = 0.05$).

Results

Geochemical sediment characteristics. Chloroplastic pigments, organic carbon, and carbon-to-nitrogen ratio (C/N) are useful indicators of the amount of food available to sediment dwellers. According to the results of the chemical analysis (Table 1), the Alvin samples contained greater quantities of chlorophyll *a* (0.83–0.97 μg/g), chloroplastic pigment equivalents (7.04–7.75 μg/g), and organic carbon content (1.53–1.69%) than the Eastern Mediterranean sediments, where the corresponding values were only 0.12 μg/g, 1.07 μg/g, and 0.82% respectively. Amongst the Alvin samples, slightly lower concentrations of organic carbon were determined for the control sampling sites. As for the ratio C/N, the KM3 sediment had the highest value (10.45), paralleled only by the Alvin24 sample (9.11). In the rest of the Alvin samples, the C/N ratios were lower, ranging from 6.1 to 6.87.

Diversity of bacterial phylotypes. Species richness was estimated by means of rarefaction analysis, with the rarefaction curve constructed (Fig. 2) such that it illustrated the bacterial diversity of the KM3 and Alvin deep-sea microenvironments. Among the 411 clones that were compared, 82 different OTUs were identified among the 105 screened clones from the KM3 clone library, whereas among the *Alvin* samples 55 of 73, 43 of 65, 64 of 87, and 60 of 81, from the Alvin24, Alvin25, Alvin28, and Alvin29 clone libraries, respectively, were identified. Apparently, all of the 16S rRNA gene libraries were highly diverse, while their curves diverged only marginally from the reference curve.

Taxonomic groups and phylogenetic analysis. Our comparative phylogenetic analysis among the five clone libraries was based on 304 unique 16S rRNA gene phylotypes. Phylogenetic analysis (Fig. 3 and Fig. S1) led to the classification of the majority of sequences (252 phylotypes) in eleven widespread taxonomic groups: Acidobacteria, Actinobacteria, Planctomycetes, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Nitrospirae, Chloroflexi, Verrucomicrobia and Bacteroidetes. From the remaining 52 phylotypes, five were classified as cluster1 and cluster2, one phylotype was affiliated with the candidate division OP11, but the majority (40 phylotypes) did not fall into any known taxonomic group.

Regarding the NE Pacific Ocean microenvironments (222 phylotypes), the differences occurring at the level of large taxonomic groups were small. In general, Gammaproteobacteria constituted the dominant class (23.3%), followed by Deltaproteobacteria (13.6%) and Actinobacteria (12.1%). Additionally retrieved bacterial phylotypes were those affiliated with the Alphaproteobacteria, Betaproteobacteria, Acidobacteria, Bacteroidetes, Verrucomicrobia, Nitrospirae, Chloroflexi, cluster2, candidate division OP11, and a significant percentage of phylotypes not affiliated with any known taxonomic group (10.3%).

The KM3 clone library (82 phylotypes) included bacterial phylotypes sorted in 11 phylogenetic groups: Acidobacteria, Actinobacteria, Planctomycetes, Deltaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria,

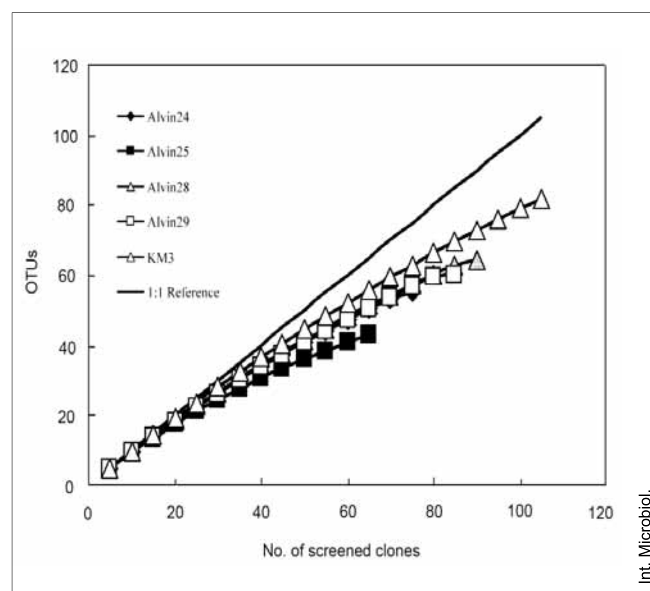

Fig. 2. Rarefaction analysis of 16S rRNA gene sequence heterogeneity in clone libraries from the sediment samples. Total numbers of screened clones are plotted against unique operational taxonomic units (OTUs).

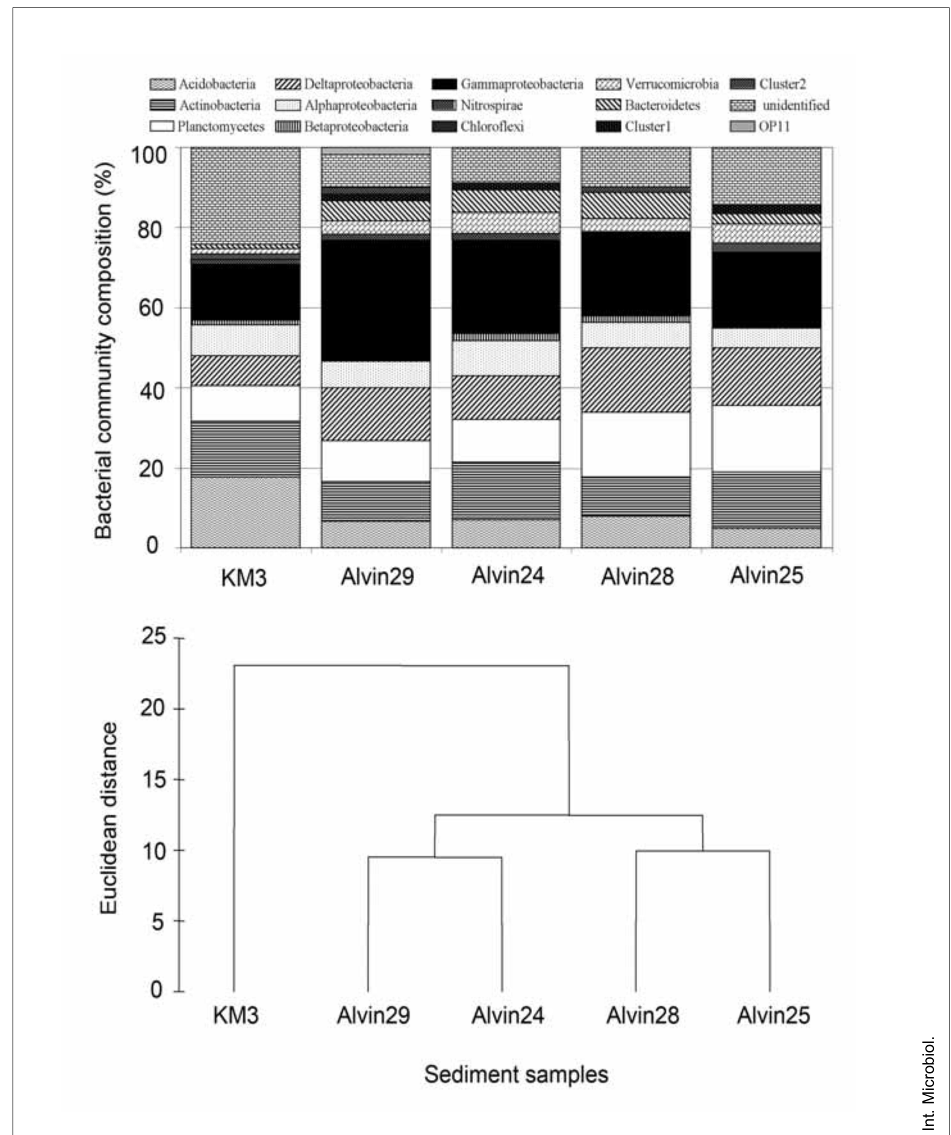

Fig. 3. (**A**) Column chart denoting the bacterial community composition of the Northeastern Pacific Ocean and Eastern Mediterranean sediments. (**B**) Cluster analysis of bacterial community composition at the level of large taxonomic groups from the different surface sediment samples. Scale indicates Euclidean distance.

Nitrospirae, Chloroflexi, Verrucomicrobia, and Bacteroidetes. Note that the 16S rRNA gene sequences not affiliated with any known taxonomic group were the most abundant (24.1%). Acidobacteria was the most prevalent known phylum (17.7%) followed by Gammaproteobacteria and Actinobacteria (17.7% and 13.9%, respectively).

Hierarchical cluster analysis based on the similarity matrices for bacterial community composition at the phylum/class level revealed differences between the Alvin and KM3 clone libraries. Specifically, although Acidobacteria and Gammaproteobacteria were the most prevalent phylogenetic groups in the sediments of both areas, the KM3 clone library had the highest percentage of 16S rRNA gene sequences not clustered into any known taxonomic group (Fig. 3 and Fig. S1).

Clone library significance testing. To determine the significance of differences between the clone libraries based on sequence similarity, LIBSHUFF analysis was applied (Fig. S2). A comparison of all libraries revealed that bacterial community composition differed significantly only between sediments Alvin25 and Alvin28 ($P = 0.002$, Fig. S2). By contrast, there were no noticeable discrepancies regarding bacterial community composition between the Mediterranean sediment KM3 and the Pacific sediments Alvin24 ($P = 0.439$), Alvin25 ($P = 0.038$), Alvin28 ($P = 0.256$), and Alvin29 ($P = 0.161$, Fig. S2). The coverage curves for pairs of clone libraries showed differences only at high levels of genetic distance ($D < 0.2$, Fig. S2); these differences were more obvious for the significantly different sediments (Alvin25 and Alvin28, Fig. S2A).

Discussion

Deep-sea sediments have been characterized as the final depository for the accumulation of autochthonous and allochthonous organic matter (e.g., [7,12]), with the levels of chlorophyll *a* and CPE widely used in assessing the contribution of phytodetritus to the sediments. Based on these levels, areas with a variable food input or trophic state can be defined. For example, in deep-sea oligotrophic ecosystems, chlorophyll *a* concentrations are < 0.06 μg/g in the central equatorial Pacific [40] and average 0.15 μg/g in the Indian sector of the Southern Ocean and a maximum of 0.15 μg/g at the permanently open-ocean zone [35]. At NE Pacific Ocean sampling sites, chlorophyll *a* values were higher (0.83–0.94 μg/g, Table 1) than in the aforementioned deep-sea ecosystems and were comparable to the values typical of mesotrophic environments. The sampled area of the NE Pacific Ocean lies below the California current, whose surface water chlorophyll concentrations show large seasonal variations. However, previous measurements from the same area were not indicative of regular seasonal fluctuations in sediment chlorophyll *a* and phaeopigments between 1992 and 1996 [6]; rather, the values were comparable to those of the present study. In Eastern Mediterranean sediments, the chlorophyll *a* value (0.12 μg/g, Table 1) was in accordance with previous measurements from the South Ionian Sea sediments (0.05 μg/g, water depth: 2790 m; [27,28]), typical for oligotrophic environments. The organic carbon content of NE Pacific sediments, as determined in this study, was approximately 1.53–1.69%, comparable to values reported in an 8-year time-series study (for the period 1990–1998) in the same area (ca. 1.5–2.0% [42]). At station KM3, however, the organic carbon content was much lower (0.82%) than in NE Pacific sediments. Our data confirm the organic carbon values previously documented for the Eastern Mediterranean Sea sediments, which ranged from 0.37% to 1.63% [27,31,33].

Overall, because of the negligible photosynthetic rates, the availability of deep-sea organic matter regulates benthic productivity and biomass [18]. Previous time-series monitoring at the NE Pacific (station M) sampling site revealed also a coupling between the maximum flux of particulate matter entering the benthic boundary layer and the presence of detrital aggregates on the sea floor. In addition, seasonal inputs of phytodetritus appear to be an important food source for epibenthic fauna at station M [2,41]. Polymenakou et al. [27] found that both organic carbon and chlorophyll *a* are linked to bacterial community composition in Eastern Mediterranean Sea sediments at depths of 30–2860 m. In the present study, all 16S rRNA gene clone libraries proved to be highly diverse. Several 16S rRNA gene phylotypes were affiliated with bacterial sequences from a wide variety of environments, such as terrestrial, anoxic habitats, and mud volcanoes. This high diversity in deep-sea sediments has also been identified in the Arctic Ocean [34], Antarctica [3], and the Eastern Mediterranean [28]. In fact, Bowman and McGuaig [3] reported that the sedimentary biodiversity of the oxic and the anoxic layers is comparable to that of terrestrial ecosystems.

Bacterial community composition at station M resembled that of other regions in the Pacific Ocean. In a similar research effort conducted in deep-sea sediments near Japan (water column depths: 2339–4031 m), Li et al. [21] retrieved 16S rRNA gene phylotypes that clustered into the Alpha-, Beta-, Gamma-, Delta-, and Epsilonproteobacteria, Bacteroidetes, and Actinobacteria. Furthermore, Gammaproteobacteria were the dominant taxonomic group in a 37-clone cosmid library constructed from deep-sea sediments (depth: 5274 m) retrieved from the Pacific Nodule Province [47]. Alpha- and Deltaproteobacteria, Actinobacteria, and Firmicutes were the other groups retrieved. According to Li et al. [21], Alpha- and Gammaproteobacteria as well as Bacteroidetes are common in deep-sea sediments. In fact, Gammaproteobacteria seem to be the predominant bacterial group, as it prevailed over other taxa identified in several deep-sea investigations (e.g., [3,21,28]).

Deltaproteobacteria was one of the most abundant taxonomic groups defined in NE Pacific sediments. Due to its common role in regulation of the sulfur cycle, Epsilonproteobacteria were also expected; nonetheless, affiliated phylotypes were not identified, perhaps due to the fact that they inhabit shallow coastal marine waters and deep hydrothermal vents [22]. The finding that several phylotypes from the NE Pacific sampling sites were closely related to 16S rRNA gene sequences previously found in the Eastern Mediterranean Sea (i.e., phylotypes from the Thermaikos Gulf, South Ionian Sea, and Cretan Sea [28]) is intriguing.

Based on the phylogenetic analysis of the KM3 16S rRNA gene clone library, this bacterial community was apparently dominated by Acidobacteria, Actinobacteria, and Gammaproteobacteria. Despite the absence of certain phylogenetic groups, the results of the analysis were in accordance with those of a previous study of deep-sea sediments in the Ionian Sea [28]. Specifically, the 17 16S rRNA gene phylotypes from the KM3 library had high sequence similarity (98–99%) to phylotypes obtained from various other sampling sites around the Mediterranean Sea [28]. The dominance of Acidobacteria in the KM3 clone library is consistent with previous investigations in the Eastern Mediterranean Sea. This phylum has been reported to be the prevalent one in sedimentary bacterial communities of the South Ionian Sea

[28] and in deep-sea sediments from both the submarine Samaria gorge and the Paximades Channel in the southern Cretan margin [29]. In general, representatives of Acidobacteria predominate in a variety of soils, hot springs, and sediments [10].

Actinobacteria and Gammaproteobacteria were the next most abundant phylogenetic taxa in the KM3 clone library. However, a relatively limited number of actinobacterial phylotypes have been found in Eastern Mediterranean sediments [28]. Actinobacteria form a major or dominant component of the bacterial populations both at bathyal and abyssal depths [4]. The 16S rRNA gene sequences not affiliated with any known bacterial clone comprised the most abundant KM3 group, as their abundance (24.05%) was higher than that of Acidobacteria. Surprisingly, 16S rRNA gene phylotypes affiliated with the Firmicutes were not detected in the KM3 sediments, even though this phylum is quite widespread in the Eastern Mediterranean Sea [28,29] and especially in the air [30].

The NE Pacific and Eastern Mediterranean ecosystems have different trophic states and temperature conditions but the same depth. Nonetheless, LIBSHUFF analysis, which is based on sequence similarity, did not show substantial differences in bacterial community composition among the sampling sites. By contrast, cluster analysis, which is based on the level of large taxonomic groups, identified differences in the presence or absence of certain minor phyla and in the percentages of the phylotypes assigned to each phylogenetic group. In fact, several Mediterranean and Pacific phylotypes appeared to be affiliated with each other in the phylogenetic trees. Note that all sampling sites were located at the same latitude. Generally, most organisms show latitudinal gradients in species diversity, with their species richness usually increasing from the poles towards the equator [15,25]. Although this pattern is poorly understood, the few suggested mechanisms are based on species-area relationships, productivity levels, and the evolutionary history of the ecosystem [15]. Finlay [9] suggested that free-living microbial species (e.g., protists) do not show biogeographies; instead, their ubiquity-biogeography transition can be explained by species size, which ranges from 1 to 10 mm. However, Pommier et al. [32], by analyzing the bacterioplankton communities from coastal waters at nine world-wide distributed locations, demonstrated that these tiny communities share many of the biogeographical and macroecological features of macroscopic organisms. In particular, the investigated marine bacterioplankton community had a high degree of endemism and followed a latitudinal gradient of species richness. Although biogeographical patterns and the ubiquitous distribution of microbial taxa, as well as the occurrence of endemic bacterial communities, have been widely discussed, most studies have been restricted to free-living bacterioplankton communities and microbial eukaryotes (e.g., [9,32]). This reflects the fact that planktonic communities are well mixed, as there are few barriers to microbial dispersal [8]. Sediments, on the other hand, are unlikely to support the ubiquitous dispersal of microorganisms relative to the water column. Nonetheless, although there is little evidence to support ubiquitous dispersal in sediments, habitat specificity of different types of bacteria has been suggested. Recent studies have shown that water column depth is one of the factors controlling microbial community composition in sediments [14,27] regardless of the trophic state of the ecosystem. Our results confirm this model, since similar bacterial communities were observed between widely dispersed habitats of different trophic state and temperature conditions.

The present study has provided only a small fraction of the information that can be obtained by studying benthic communities. This limitation is due to the low sequence capacity of the clone library analysis using Sanger-based technology. State-of-the-art pyrosequencing technology, which is able to generate thousands of sequences from each sampling site, would provide greater insight into the magnitude of the biodiversity within these highly diverse environments. However, it is not only the high diversity but also the significant percentage of non-affiliated bacterial phylotypes that define these microenvironments as microbial hotspots.

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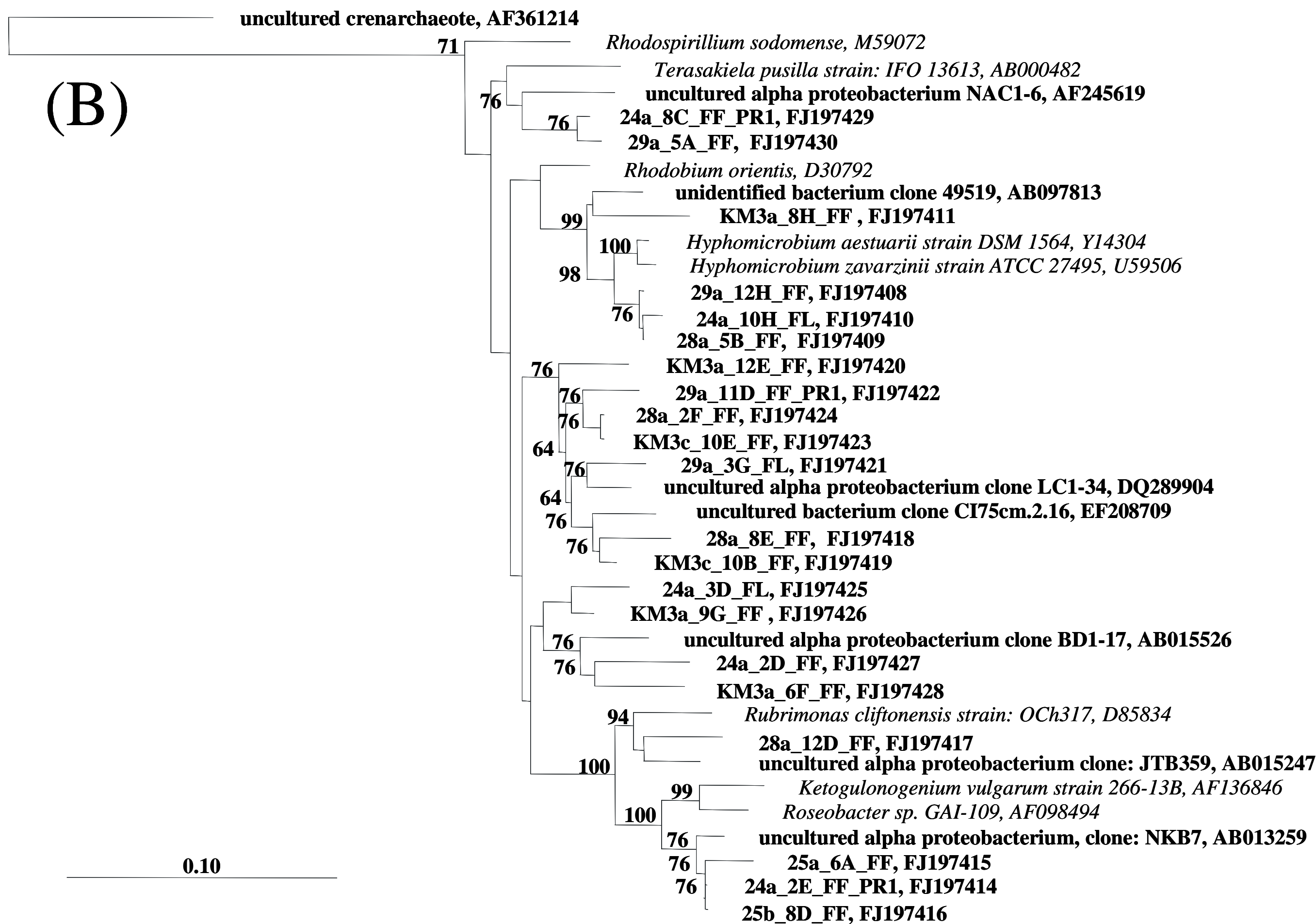
Supplementary : Figure S1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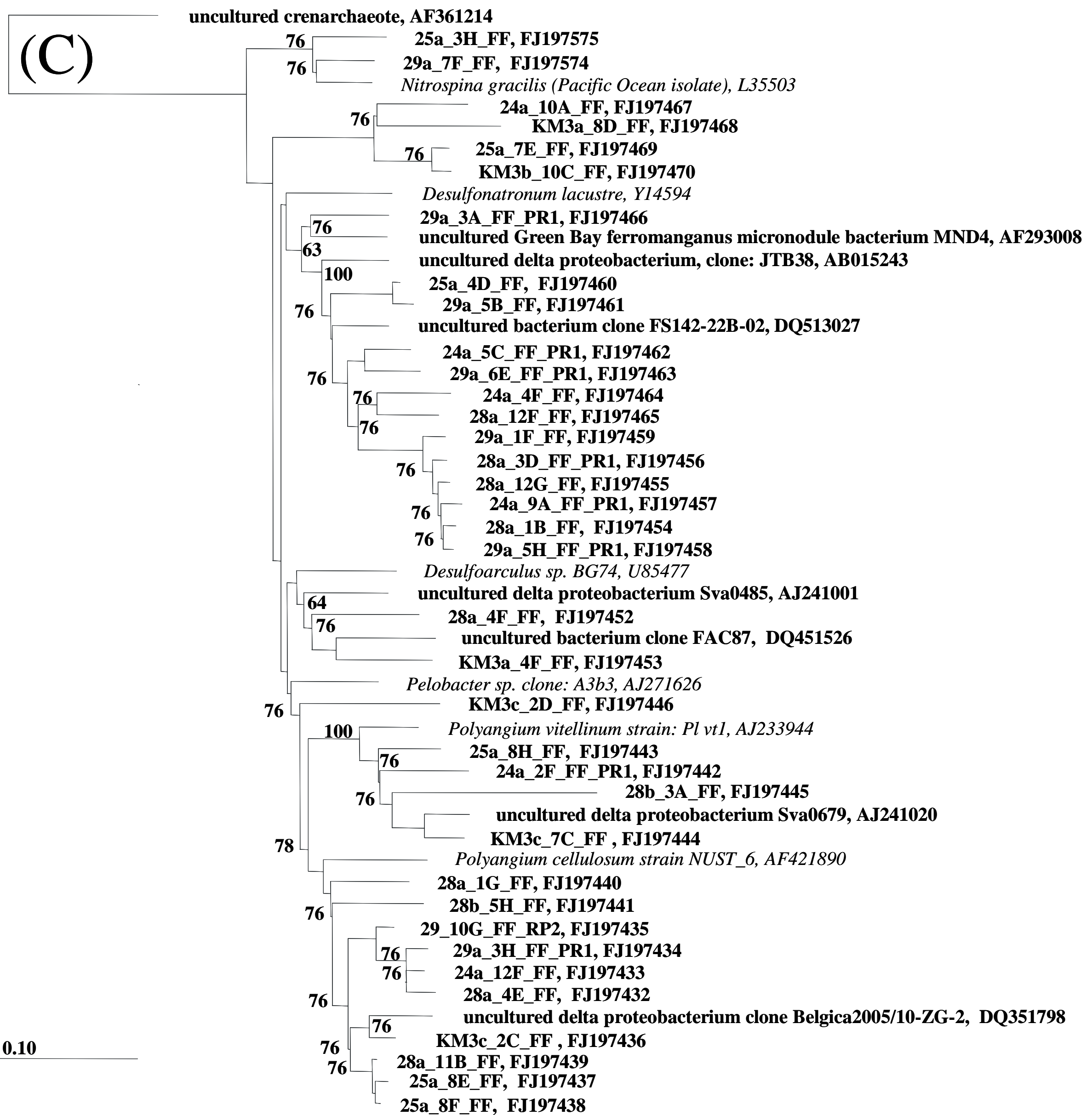
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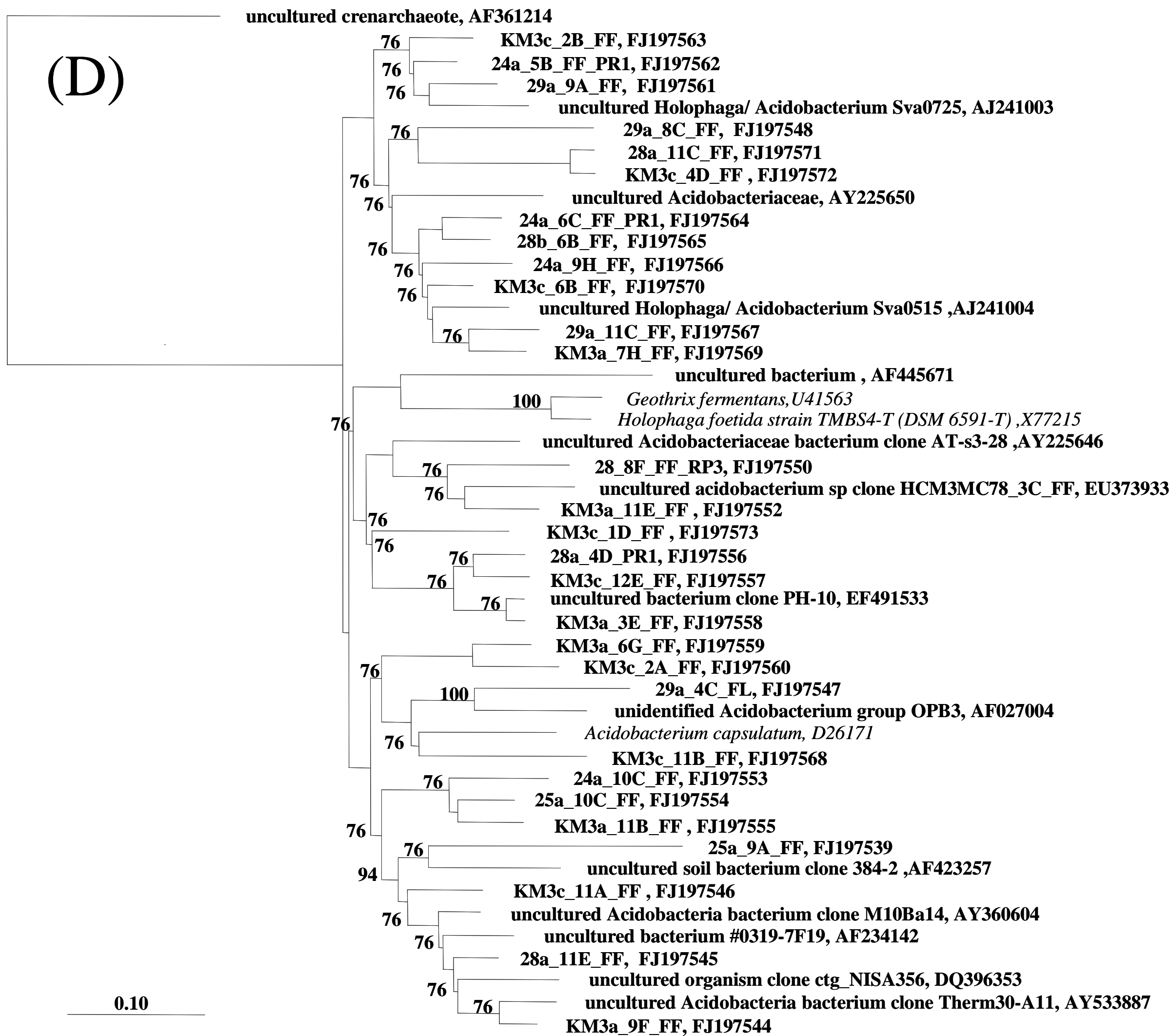
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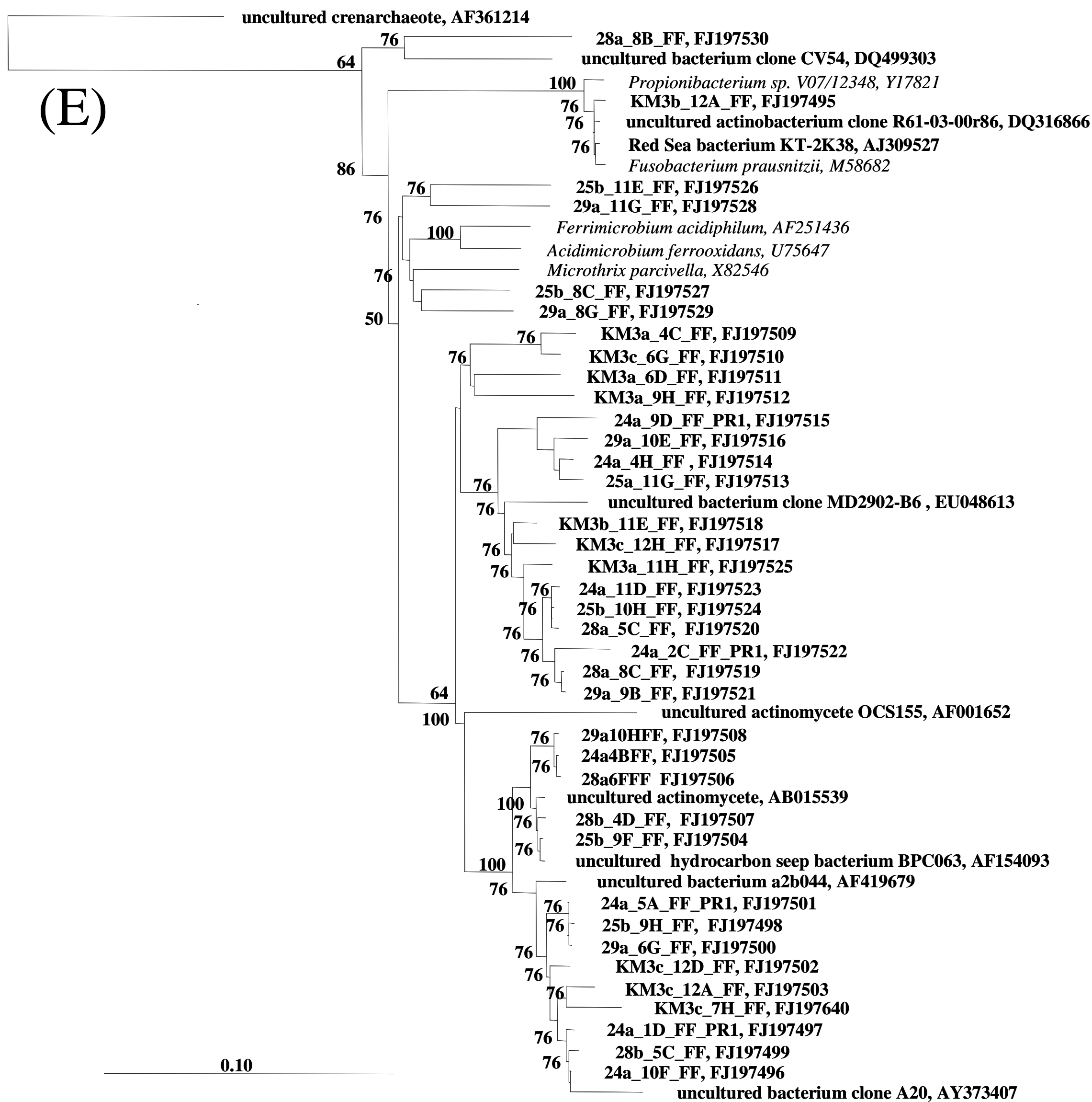
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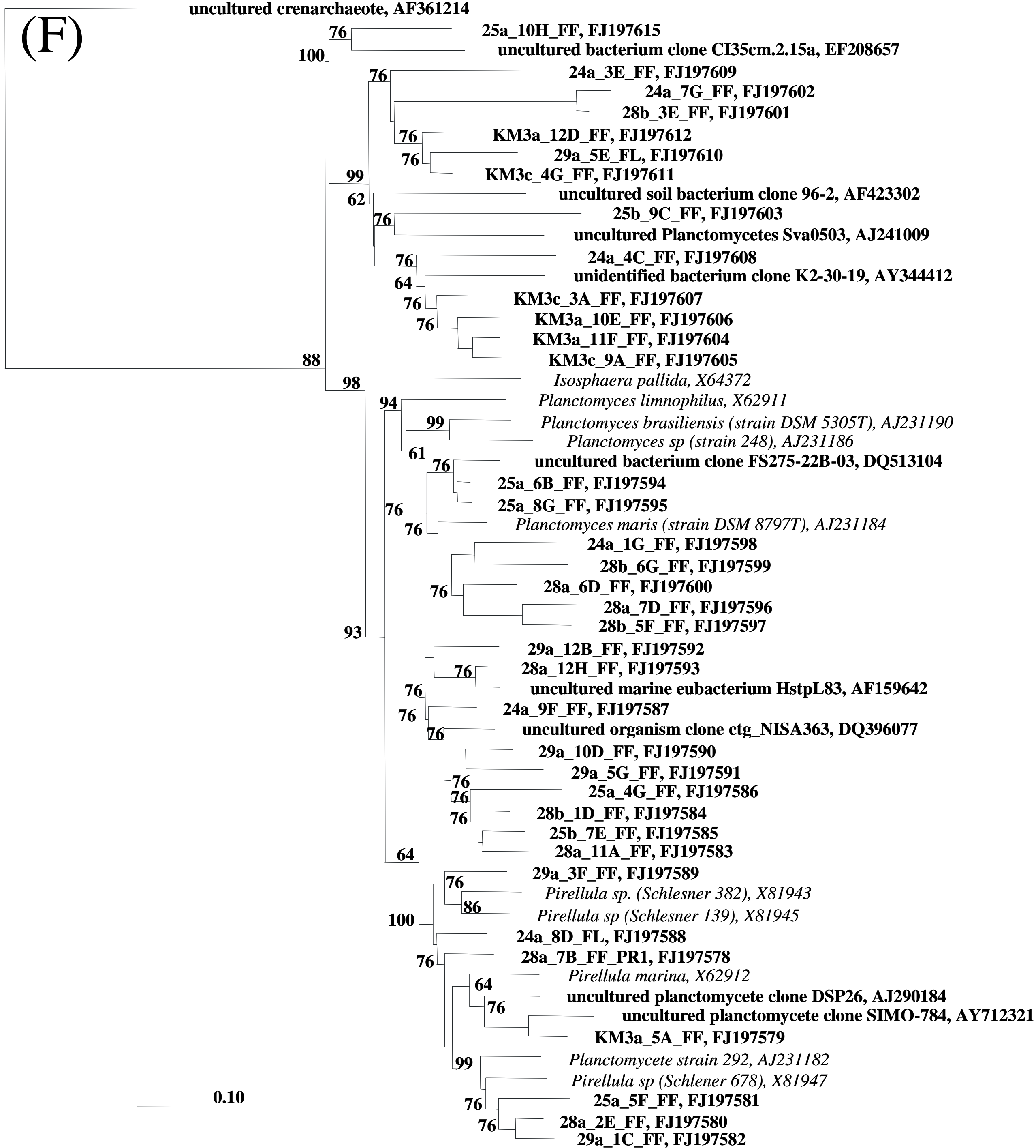
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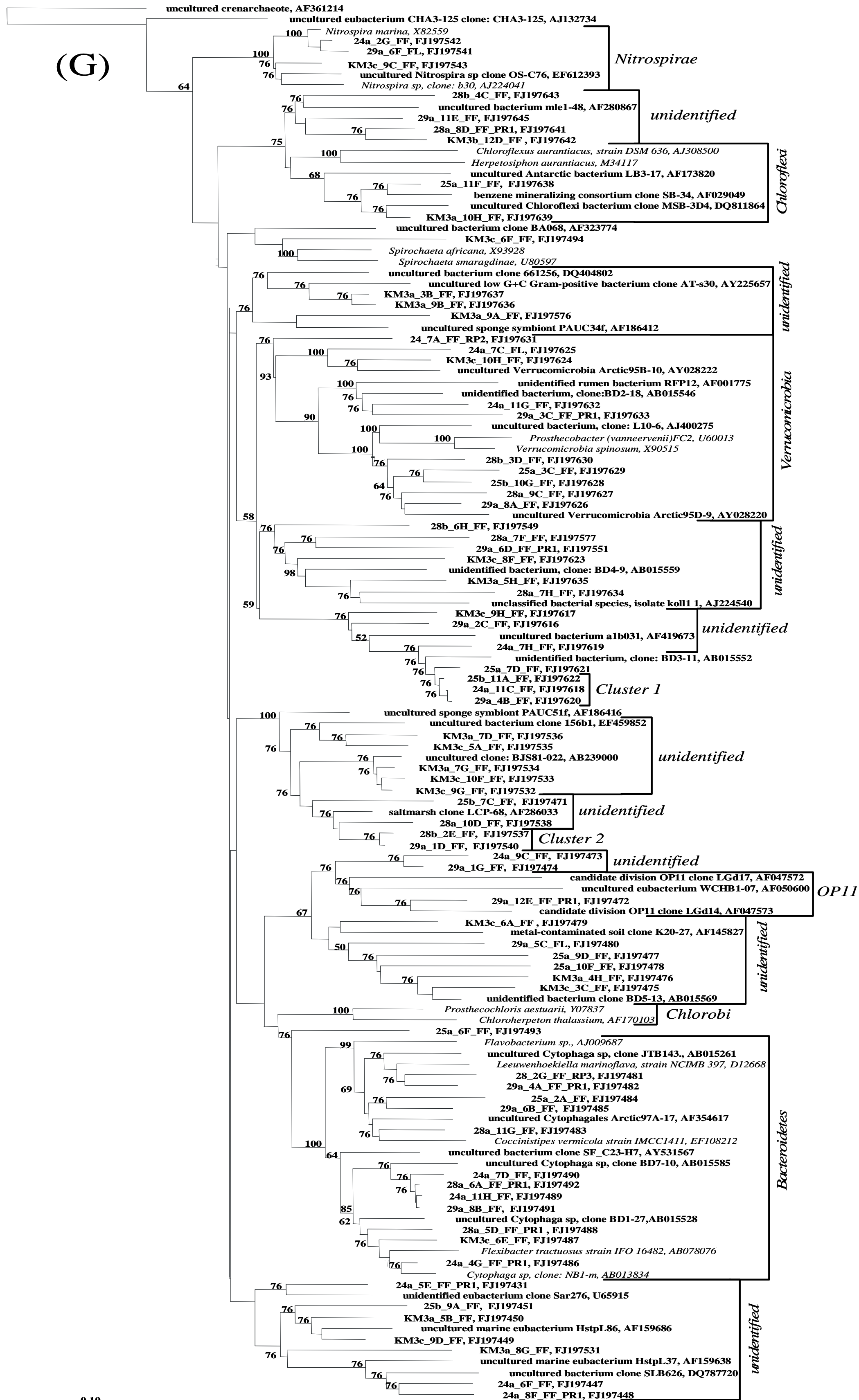

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Spirochaeta smaragdinae, U80597
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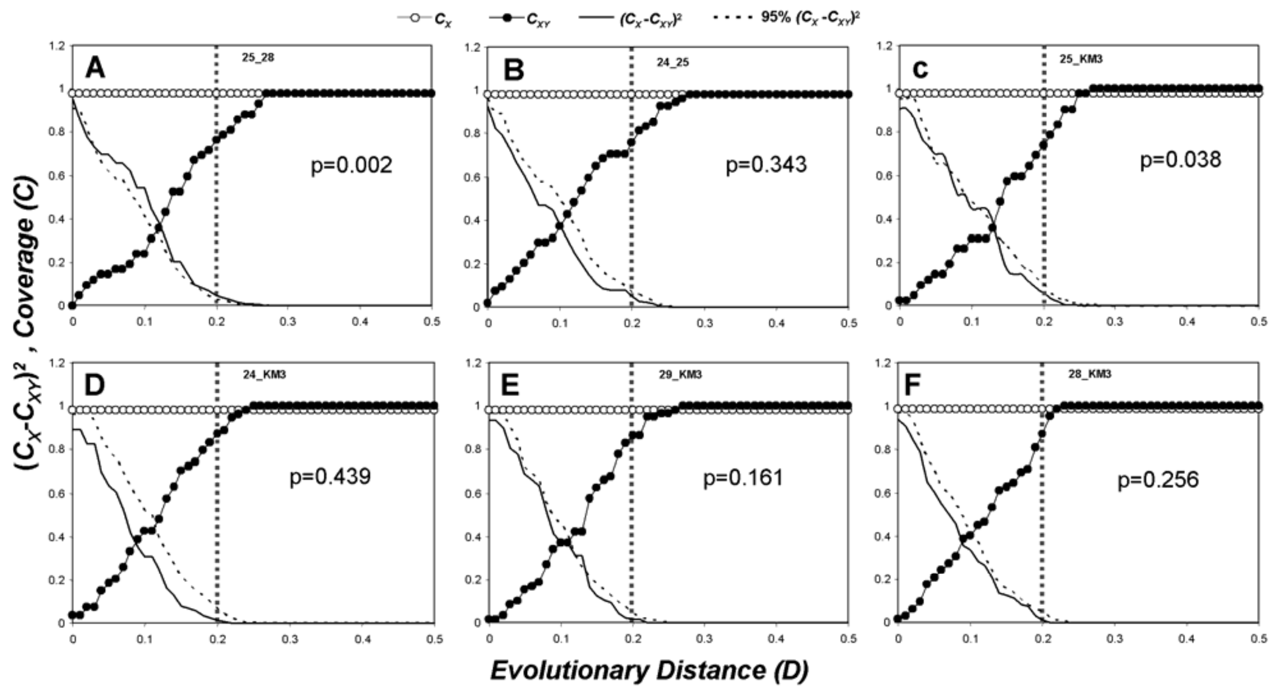
KM3a_5B_FF, FJ197450
uncultured marine eubacterium HstpL86, AF159686
KM3c_9D_FF, FJ197449
KM3a_8G_FF, FJ197531
uncultured marine eubacterium HstpL37, AF159638
uncultured bacterium clone SLB626, DQ787720
24a_6F_FF, FJ197447
24a_8F_FF_PR1, FJ197448
unidentified
0.10

Supplementary : Figure S2



Supplementary material

Fig S1. Maximum parsimony (MP) 16S rRNA gene trees showing positions of phylotypes affiliated with (A) Betagammaproteobacteria, (B) Alphaproteobacteria, (C) Deltaproteobacteria, (D) Acidobacteria, (E) Actinobacteria, (F) Planctomycetes, (G) Nitrospirae, Chloroflexi, Verrucomicrobia, Bacteroidetes, Clusters 1, and 2, candidate division OP11, and unidentified clones from the sediment samples. The closest matching entries in GenBank were also included in the tree. The trees are a summary of 100 multiple bootstrapped replicates with MP method and the bootstrap values, determined as percentages of the 100 trees inferred by MP method, are given for branches with greater than 50% support. The scale bar indicates 10% nucleotide change per 16S rRNA gene sequence positions. Sequences from cultured representatives are indicated in italics.

Fig S2. Results of selected LIBSHUFF (Singleton et al., 2001) comparisons of clones from (A) Alvin25 (*X*) to Alvin28 (*Y*), (B) Alvin24 (*X*) to Alvin25 (*Y*), (C) Alvin25 (*X*) to KM3 (*Y*), (D) Alvin24 (*X*) to KM3 (*Y*), (E) Alvin29 (*X*) to KM3 (*Y*) and (F) Alvin28 (*X*) to KM3 (*Y*) clone libraries. Homologous (*CX*) and heterologous (*CXY*) coverage curves for 16S rRNA gene clones are presented. Solid lines indicate $(CX - CXY)2$ for the original samples at each value of genetic distance (*D*) and broken lines indicate the 950th value (or $p = 0.05$) of $(CX - CXY)2$ for the randomized samples.