ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 4, pp. 570–576. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © A.Y. Merkel, V.A. Korneeva, I.Yu. Tarnovetskii, A.L. Bryukhanov, V.K. Chasovnikov, E.A. Taranov, S.V. Toshchakov, N.V. Pimenov, 2015, published in Mikrobiologiya, 2015, Vol. 84, No. 4, pp. 476–484.

EXPERIMENTAL ARTICLES

Structure of the Archaeal Community in the Black Sea Photic Zone

A. Y. Merkel[a], V. A. Korneeva[a, b], I. Yu. Tarnovetskii[a, b], A. L. Bryukhanov[a, b], V. K. Chasovnikov[c], E. A. Taranov[a], S. V. Toshchakov[d], and N. V. Pimenov[a, 1]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia*
[b] *Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia*
[c] *Shirshov Institute of Oceanology, Southern Branch, Gelendzhik, Russia*
[d] *Immanuel Kant Baltic Federal University, Kaliningrad, Russia*

Received December 1, 2014

Abstract—Qualitative and quantitative analysis of the structure of the archaeal community of the photic zone of the Black Sea water column was carried out. Real-time PCR revealed 2×10^4 archaeal cells/mL (4.2% of the total cell number) at a 15-m depth. The structure of archaeal communities in the subsurface water column was investigated using the sequencing by synthesis technology (Illumina/Solexa) of the 16S rRNA genes. The Marine Group II phylogenetic cluster belonging to the phylum *Euryarchaeota* was the most numerous archaeal group ($1.2–1.7 \times 10^4$ cells/mL). The Marine Group I phylogenetic cluster (phylum *Thaumarchaeota*) was the second most numerous group (40% of the free-living archaea or 7.7×10^3 cells/mL). Sequences of the *'Nitrosopumilus'* cluster were revealed among Marine Group I sequences due to high homology (over 90%). A group of archaea belonging to the Deep-sea Hydrothermal Vent Euryarchaeotic Group 6 (DHVEG-6) (phylum *Euryarchaeota*) was also detected. The 16S rRNA gene sequences belonging to this cluster were revealed only in the suspension fraction. High homology level (over 90%) suggested classification of most DHVEG-6 sequences within the *'Parvarchaeum'* cluster. In spite of a noticeable methane peak detected at 15-m depth, no sequences of methanogens were found.

Keywords: structure of archaeal communities, real-time PCR, 16S rRNA gene, photic zone, water column, Black Sea

DOI: 10.1134/S0026261715040128

The Black Sea is known as the world's largest meromictic water body, with high concentrations of dissolved sulfide, methane, and CO_2 in its anoxic horizons.

Due to considerable differences in the mineral and gas composition of the Black Sea surface and deep water layers, specific microbial communities develop at different depths, which are responsible for the major biogeochemical processes of turnover of the biogenic elements. The redox zone, where the upper, oxygen-containing water contacts with anoxic deep water, has traditionally been of special interest to microbiologists. This zone is highly heterogeneous in its geochemical parameters and consists of relatively thin layers with the maximal content of chemical components; their composition and content depend on the ambient redox conditions [1].

A series of works using radioisotope, isotope geochemical, direct microbiological, and molecular genetic approaches revealed the presence of obligately aerobic lithotrophic and methanotrophic bacteria in the upper part of the redox zone, which are responsible for chemosynthesis and methane oxidation. Apart from the classical aerobes, the lower part of the redox zone was found to contain facultative and obligate anaerobes involved in anaerobic oxidation of sulfur compounds, methane, and ammonium, as well as in decomposition of organic matter [2–5].

The first data on archaeal vertical distribution in the water column were obtained over a decade ago [2] by fluorescent in situ hybridization (FISH) with the universal archaeal probe ARCH915. The authors detected archaea not only in the deep, sulfide-rich layers, but also in the oxygen-containing surface layer and in the cold intermediate layer. Detection of archaea (up to 10×10^3 cells/mL) in the oxic horizons, as well as the presence of a methane peak at the depth of 20–40 m, were attributed to methane release from zooplankton intestines and to formation of anaerobic microniches in the pellets and other large particles of suspended organic matter (SOM) [6].

Numerous recent findings indicate that archaea, as well as bacteria, are almost ubiquitous inhabitants of the water column of marine and freshwater habitats, constituting a considerable portion of the picoplankton [7–9].

According to Lin et al., group I *Crenarchaeota* predominated among the Black Sea archaea both within

[1] Corresponding author; e-mail: npimenov@mail.ru

the redox zone and in deep water horizons [10]. The works [11, 12] presenting evidence of the role of archaea in ammonium oxidation are the only attempts at elucidating the ecological and physiological role of archaea in the Black Sea water column. The markers of pelagic marine *Crenarchaeota*—both molecular (the 16S rRNA gene and the *amoA* archaeal gene) and lipid (crenarchaeol)—were revealed in the upper layers of the redox zone, where oxygen concentrations do not exceed 1 μM. Activity of ammonium-oxidizing archaea at the interface between the oxidized and reduced water may result in formation of nitrite, which is used by anaerobic planctomycetes in the anammox reaction [12, 13].

In spite of considerable methodological progress in investigation of the archaeal communities, the works on their phylogenetic diversity and geochemical role in the picoplankton of the Black Sea water column are scarce. Almost no data are available concerning archaeal taxonomic and functional diversity in the upper oxic (photic) Black Sea water. The goal of the present work was to apply molecular genetic techniques to investigation of archaeal distribution in the photic zone of the deep-water area of the Black Sea.

MATERIALS AND METHODS

Subjects of research. Water from the oxic zone (15-m depth) was collected in July 2014 from on board the *Ashamba* fishing boat, Shirshov Institute of Oceanology, Russian Academy of Sciences, at the station (44°491 N, 37°873 Å) in the Gelendzhik area within the continental shelf of the Russian sector of the Black Sea (depth 1200 m). The samples were collected using 5-L bathometers of a Rosette complex equipped with a hydrophysical probe (Sea-Bird Electronics, United States) with temperature, salinity, and pressure sensors. For general description of the water profile, samples of the photic zone (3, 10, 15, 20, 25, and 30 m) and the cold intermediate layer (50, 110, and 130 m) were collected from the oxic zone (0–130 m). Water samples were dispensed into glass vials for determination of dissolved O_2, nitrates, nitrites, ammonium, and methane. The hydrochemical parameters were determined according to the standard procedures used in the Laboratory of Chemistry, Southern Branch, Shirshov Institute of Oceanology RAS. Methane was determined in the Winogradsky Institute of Microbiology RAS on a Crystal gas chromatograph (Russia) equipped with a flame ionization detector.

Microbiological analyses. To determine total cell numbers, the samples were filtered through GTBP 2500 membranes (25 mm diameter, 0.2 μm pore size, Millipore, United States) with a nitrocellulose layer (25 mm diameter, 0.45 μm pore size) and stained with DAPI. The cells were enumerated under an Axio Imager D1 epifluorescence microscope (Carl Zeiss, Germany) using the Axio Vision software package.

Suspended matter was separated using GF/C glass fiber filters (Whatman, United Kingdom), which retain the particles larger than 1.2 μm, with subsequent filtration through 0.22 μm membrane filters (Millipore, United States). For each sample, ~5 L of seawater was filtered. The GF/C filters retaining the objects larger than 1.2 μm concentrated mainly large particles, which could contain the suspension-associated cells, while smaller particles (mostly free-living microorganisms) were retained by the membrane filters. The filters were then fixed in a solution with 0.15 M NaCl and 0.1 M disodium EDTA (pH 8.0).

Molecular genetic techniques and bioinformation analysis of the data. DNA was isolated as described in [14] with some modifications. A FastPrep® 24 homogenizer (MP Biomedicals, United States) was used for cell desintegration. The DNA preparations were assessed using a DropSense-96® spectrophotometer (Trinean, Belgium). PCR was carried out according to the standard protocol [15]. The Univ515F [16]–Arch915R [17] primers were used for amplification of the fragments of archaeal 16S rRNA genes. The *mcrA* gene fragments were amplified using the mlas–mcrA-rev primers [18]. For sequencing, the amplicons were separated by electrophoresis in agarose gel, excised, and purified using the Cleanup Standard kit (Evrogen, Russia). The multiplexed amplicon libraries for sequencing on a MiSeq system (Illumina, United States) were prepared using the NEBNext® kit for fragment libraries (New England BioLabs, United States). Sequencing was carried out using the reagent kit providing for reading 300 nucleotides from each end of the amplicon. Primary treatment of the readings (filtration and demultiplexing) was carried out using CLC Genomics Workbench 7.5 software (Qiagen, United States). The resultant data were processed using the SILVAngs online service (https://www.arb-silva.de/ngs/). To estimate occurrence of the members of uncultured groups, all their 16S rRNA gene sequences from the Silva database (https://www.arb-silva.de/) were used for comparison. The distribution of the sequences according to the sources of their isolation was determined using the GetIsolationSources software package developed by the authors. This open-source free program is available at https://github.com/allista/GetIsolationSource.

Real-time PCR was carried out as described in [19] using the SYBR Green I® intercalating dye. The total number of prokaryotic 16S rRNA genes was determined using the primer pair Uni515F–806R [20]. The calibration curve was built using genomic DNA of *Melioribacter roseus* [21]. The number of archaeal 16S rRNA genes was assessed using the primer pair Uni515F–Arch915R. The calibration curve was built using genomic DNA of *Thermococcus sibiricus* [22]. For all calibration curves, the correlation coefficient was at least 0.99, and the reaction efficiency was at

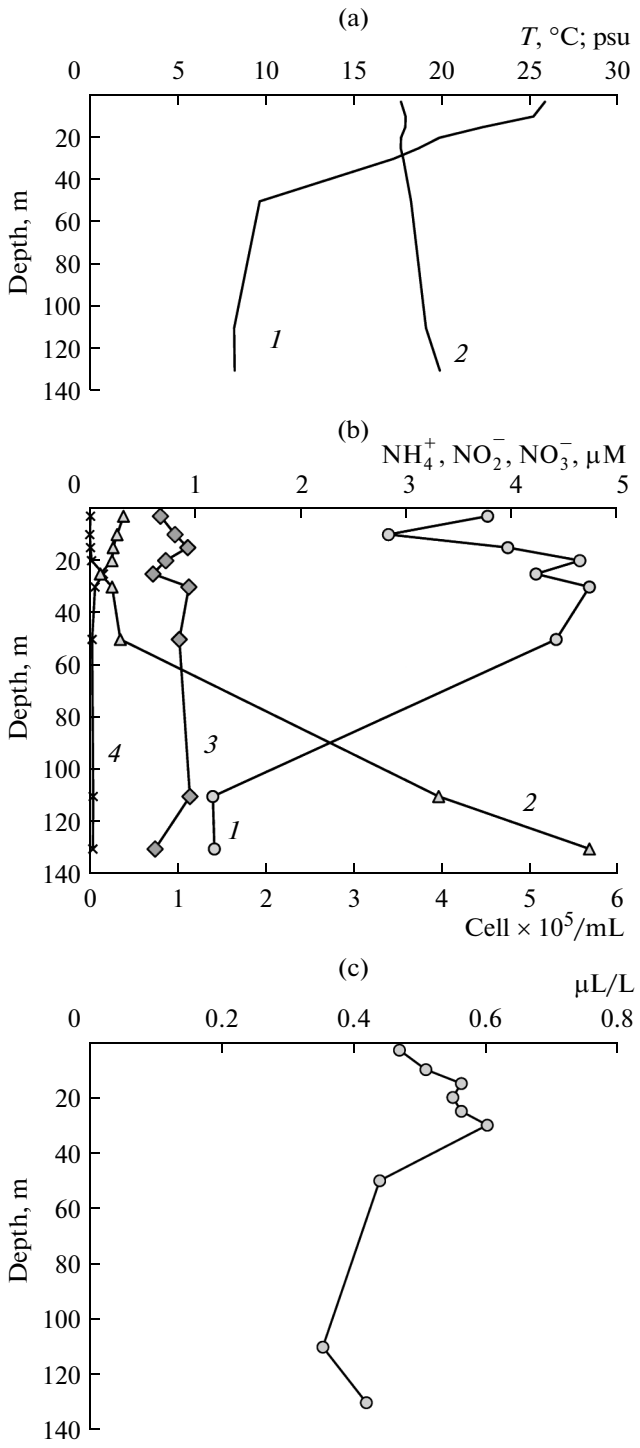

Fig. 1. Characteristics of the Black Sea water column (July 2014); (a) temperature (*1*) and salinity, psu (*2*); (b) microbial cell number, 10^5/mL (*1*), concentrations of NO_3^-, NH_4^+, and NO_2^-, μM (*2, 3*, and *4*, respectively); (c) methane profile, μL/L.

least 70%. Real-time PCR was carried out on a StepOnePlus® Real-Time PCR System (Life Technologies, United States) using the qPCRmix-HS SYBR PCR mixture (Evrogen, Russia). All real-time PCR measurements of the concentrations of the target DNA molecules were carried out in three replicates; the standard deviation did not exceed 15%.

RESULTS AND DISCUSSION

Hydrochemical parameters and total microbial abundance. The hydrochemical parameters determined in mid-July 2014 were within the range of the typical summer hydrophysical structure, but with an uncharacteristic local increase in salinity of the subsurface layer. The seasonal thermocline was at the 10–50-m depth, and the temperature in the horizons of the cold intermediate layer was ~ 8°C. The halocline was located in the 110–140-m horizon (Fig. 1a). Sulfide was first detected at the depth of 170 m. The distribution of inorganic nitrogen species was characterized by a typical increase in ammonium concentration in deep layers (from the oxic–anoxic interface) and by elevated nitrate concentrations within the 100–130 m depth range (Fig. 1). Elevated nitrite concentration was detected in subsurface water (25 m); the lowest concentrations of nitrate and ammonium were found in the same horizon (Fig. 1b). Such distribution of mineral nitrogen species indicates active oxidation–reduction processes in this horizon. In the oxidized water column, the highest number of microorganisms (up to 5.7×10^5 cells/mL) was observed within the thermocline (15–50 m). The subsurface methane maximum was located at 10–25 m (Fig. 1c). The 15-m horizon was chosen for detailed investigation of the structure of the archaeal community based on the data of physicochemical probing and the profiles on the concentrations of methane and mineral nitrogen species.

Molecular genetic analysis of archaeal phylogenetic diversity. A total of 309 sequences of archaeal 16S rRNA genes were collected: 165 and 144 sequences for the samples filtered through the membrane filter and the GF/C filter, respectively. For each sample, the set of sequences was analyzed using the SILVAngs online service. As a result, qualitative and quantitative assessment of the composition of the archaeal population in the photic zone of the Black Sea water column was obtained (Fig. 2). While the results of analysis of the ratio of the number sequences obtained to the number of operational taxonomic units (OTU) at the 98% similarity level indicated the obtained number of sequences to be insufficient for complete description of archaeal phylotypes from the photic zone of the Black Sea, the graphs constructed based on this ratio exhibited near-saturation (Fig. 3).

Quantitative ratios of archaeal groups in the microbial community of the Black Sea photic zone. Real-time PCR with the primers amplifying the 16S rRNA gene fragments of most archaea was used to assess the relative abundance of archaea in the Black Sea photic zone. These results were compared to those obtained by real-time PCR with the universal primers amplify-

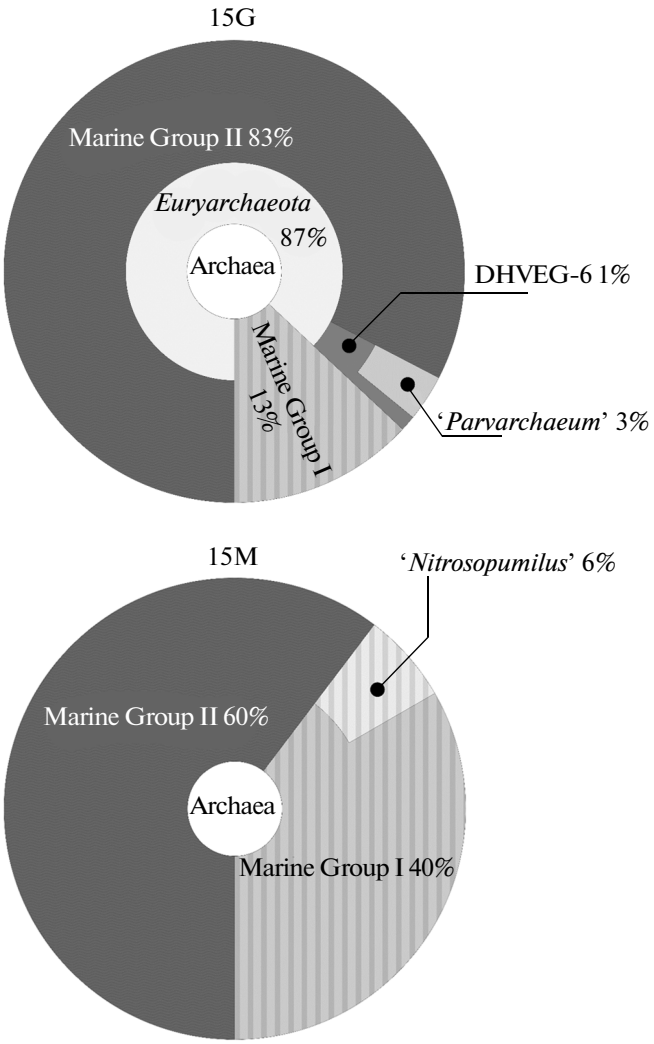

Fig. 2. Relative abundance of archaeal groups in the libraries of archaeal 16S rRNA genes. DNA samples were obtained by precipitation on a GF/C with 1 μm pore diameter (suspension-associated archaea, 15G) or by filtration through a membrane filter with 0.22 μm pore diameter (free-living cells, 15M).

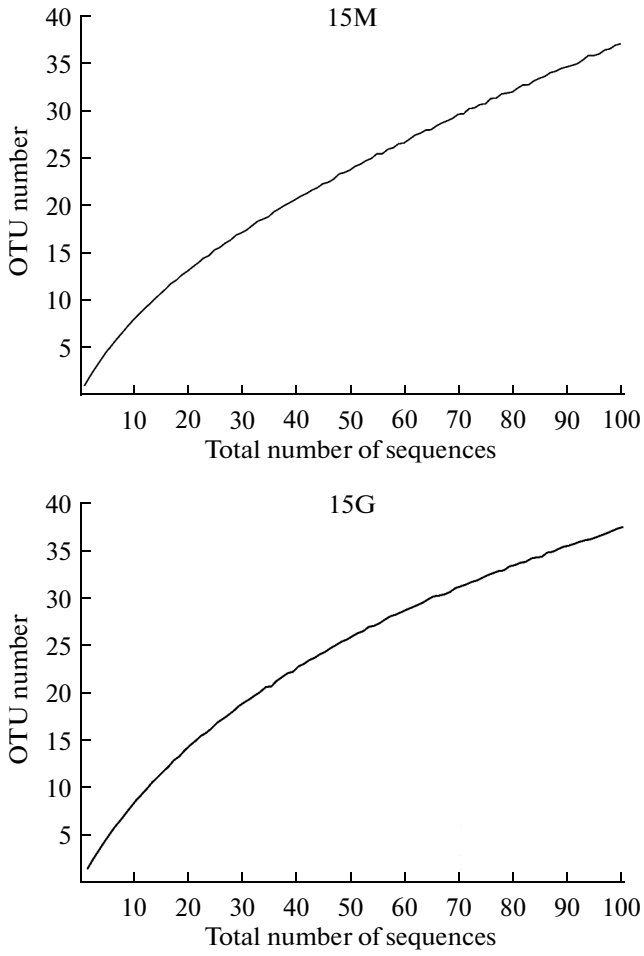

Fig. 3. Ratio between the total number of archaeal 16S rRNA gene sequences and the number of operational taxonomic units (OTU) clustered at 98% homology. The samples were obtained by precipitation on a GF/C with 1 μm pore diameter (suspension-associated archaea, 15G) or by filtration through a membrane filter with 0.22 μm pore diameter (free-living cells, 15M).

ing the 16S rRNA gene fragments of most prokaryotes. Archaea were found to constitute 4.2% of the total microbial population of the studied Black Sea water horizon. Based on the results of total counts of DAPI-stained cells, ~2×10^4 cells/mL may be accepted as the absolute number of archaeal cells in the photic zone of the Black Sea (14 m).

Analysis of the 16S rRNA gene sequences revealed several major archaeal groups in the community of the Black Sea surface waters.

Marine Group II. The Marine Group II phylogenetic cluster (phylum *Euryarchaeota*) was the most numerous group of archaea in the Black Sea photic zone. In the DNA samples collected from the membrane filter and from GF/C filter, the organisms of this group constituted 60 and 83% of archaea, respectively. Thus, abundance of Marine Group II in the photic zone of the studied Black Sea area was (1.2–1.7) × 10^4 cells/mL.

The Marine Group II phylogenetic cluster belongs to the order *Thermoplasmatales* and comprises the sequences isolated mainly from the upper layers of marine and oceanic water [23, 24]. This group was originally detected by molecular techniques based on the 16S rRNA gene sequencing [25, 26]. This phylogenetic cluster was previously considered to be restricted to marine ecosystems [24]. Our analysis of over 7000 partial sequences of the 16S rRNA gene belonging to this cluster indicates that, while the overwhelming majority of these sequences was indeed retrieved from the oceanic water column, some phylotypes have been detected in soil ecosystems [27, 28].

High-throughput sequencing technologies made it possible to sequence the untangled genome of one member of this group of uncultured microorganisms [29]. It was determined to be a motile, photoheterotrophic microorganism adapted to the degradation of proteins and lipids. It is still unclear whether other members of this group also possess photoheterotrophic metabolism and whether this is the property common to the entire cluster.

Marine Group I. The second most numerous archaeal group in the photic zone of the studied Black Sea area belonged to the Marine Group I phylogenetic cluster of uncultured microorganisms (phylum *Thaumarchaeota*), which is one of the major phylogenetic groups of marine archaea [30]. This group was originally detected by molecular techniques based on the 16S rRNA gene sequencing [25, 26]. Numerous works revealed Marine Group I to constitute up to 40% of the total prokaryotic biomass in aphotic seawater horizons [31, 32]. In the present work, the highest number of the sequences belonging to this group was found in the DNA fraction from the membrane filter. This may indicate Marine Group I archaea, which constituted up to 40% of the total archaeal population (7.7×10^3 cells/mL), are free-living organisms.

Several members of Marine Group I have been cultivated: '*Nitrosoarchaeum,*' '*Nitrosopumilus,*' '*Crenarchaeum.*' Among the sequences belonging to Marine Group I, the fraction obtained from the membrane filter contained the sequences identified as members of the genus '*Nitrosopumilus*' based on their high homology (over 90%). '*Nitrosopumilus maritimus*' the first cultured member of this genus, is a mesophilic nitrifying microorganism capable of autotrophic growth. Organic compounds, even in minor amounts, inhibit growth of this microorganism. This may be the reason why the sequences identified as belonging to the '*Nitrosopumilus*' were detected only in the fraction collected from the membrane filter, i.e., they were not associated with suspended organic matter. Abundance of this archaeal group in the sea is probably limited by ammonium concentration and by the presence of organic matter [32]. '*Nitrosopumilus maritimus*' was the first cultured member of the *Archaea* domain capable of aerobic ammonium oxidation. Moreover, the ability of these microorganisms to fix inorganic carbon via the 3-hydroxypropionate/4-hydroxybutyrate pathway [33] may indicate their considerable contribution to chemoautotrophic primary production in various layers of marine and oceanic water [34]. Similar to '*Nitrosopumilus,*' '*Nitrosoarchaeum*' is capable of aerobic ammonium oxidation. This may be an indication of wide occurrence of this feature among members of the Marine Group I phylogenetic cluster. Some works, however, presented the data indicating heterotrophic metabolism of the members of this cluster [35, 36].

Deep-sea Hydrothermal Vent Euryarchaeotic Group 6 (DHVEG-6). The Deep-sea Hydrothermal Vent Euryarchaeotic Group 6 (DHVEG-6) phylogenetic cluster of uncultured microorganisms was another archaeal group detected in the photic zone of the studied Black Sea area. The 16S rRNA gene sequences belonging to this cluster were revealed only in the DNA fraction associated with suspended matter. Members of this cluster were among the minor components of the archaeal population (4%, or less than 800 cells/mL). The DHVEG-6 cluster belongs to the order *Halobacteriales* (phylum *Euryarchaeota*). While this cluster was originally identified as a separate phylogenetic group in the study of archaeal diversity in deep-sea hydrothermal ecosystems [37], it was subsequently detected in various environments. Our analysis of over 4500 16S rRNA gene sequences belonging to this cluster revealed that the microorganisms of this phylogenetic group inhabit a wide range of ecotopes: microbial mats, marine and freshwater environments, various soils, hypersaline and thermal environments, etc.

Due to high homology levels (over 90%), most of the DHVEG-6 sequences were assigned to the '*Parvarchaeum*' suncluster. The names *Candidatus* "Parvarchaeum acidiphilum" and *Candidatus* "Parvarchaeum acidophilus" were proposed for two microorganisms, for which partial genomes were assembled during metagenomic analysis of chemoautotrophic microbial epibioses formed in mines under conditions of high acidity and high concentrations of metal ions [38]. These microorganisms are characterized by their cell size (0.009 to 0.04 μm^3) [39], which is close to the theoretical limit for free-living organisms. Microscopy of the Black Sea water samples revealed relatively high occurrence of ultra-small cells. Their metabolism, however, remains unknown.

Thus, our results make it possible to suggest that the distribution of physiologically active *Euryarchaeota* in the Black Sea water column is not restricted to the oxic–anoxic interface, the zone with low oxygen concentrations where thaumarchaea were reported to oxidize ammonium aerobically to nitrite, while anammox bacteria used nitrite for anaerobic ammonium oxidation [12, 13]. Our work also demonstrated that in surface water *Thaumarchaeota* was among the most widespread phylogenetic groups of archaea. High abundance of the sequences belonging to the Marine Group II phylogenetic cluster (phylum *Euryarchaeota*), for which the only assembled complete genome indicates photoheterotrophic metabolism, is of considerable interest. In spite of a significantly increased methane concentration in subsurface water, the sequences typical of methanogenic *Euryarchaeota* were not detected at 15-m depth. At this depth methane formation may result from the recently described process of production of methylphosphonic acid by nitrifying *Thaumarchaeota* with its subsequent transformation to methane mediated by a typical marine alphaproteobacterium of the order *Pelagibacterales* [40].

ACKNOWLEDGMENTS

The authors are grateful to Prof. M.V. Flint (Shirshov Institute of Oceanology) for his help in organizing the sea expedition and to the researchers of the Winogradsky Institute of Microbiology: Dr. I.I. Rusanov (for measuring methane content in the water samples), Dr. T.A. Kanapatskii (for enumeration of microorganisms by epifluorescence microscopy), and E.E. Zakharova (for help in filtration of the samples).

The work was supported by the Russian Foundation for Basic Research, project no. 13-04-00033-a.

REFERENCES

1. Yakushev, E.V., Podymov, O.I., and Chasovnikov, V.R., Seasonal changes in the hydrochemical structure of the Black Sea redox zone, *Oceanography*, 2005, vol. 18, pp. 48–55.
2. Pimenov, N.V., Rusanov, I.I., Yusupov, S.K., Fridrich, J., Lein, A.Yu., Wehrli, B., and Ivanov, M.V., Microbial processes at the aerobic–anaerobic interface in the deep-water zone of the Black Sea, *Microbiology* (Moscow), 2000, vol. 69, pp. 436–448.
3. Vetriani, C., Tran, H.V., and Kerkhof, L.J., Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the Black Sea, *Appl. Environ. Microbiol.*, 2003, vol. 69, pp. 6481–6488.
4. Durisch-Kaiser, E., Klauser, L., Wehrli, B., and Schubert, C., Evidence of intense archaeal and bacterial methanotrophic activity in the Black Sea water column, *Appl. Environ. Microbiol.*, 2005, vol. 71, pp. 8099–8106.
5. Pimenov, N. and Neretin, L., Composition and activities of microbial communities involved in carbon, sulfur, nitrogen and manganese cycling in the oxic/anoxic interface of the Black Sea, in *Past and Present Water Column Anoxia,* Neretin, L., Ed., New York: Springer, NATO Sci. Ser., 2006, pp. 501–522.
6. Rusanov, I.I., Yusupov, S.K., Savvichev, A.S., Pimenov, N.V., Lein, A.Yu., and Ivanov, M.V., Microbial production of methane in the aerobic water layer of the Black Sea, *Doklady Biol. Sci.*, 2004, vol. 399, no. 4, pp. 493–495.
7. DeLong, E.F., Wu, K.Y., Prezelin, B.B., and Jovine, R.V.M., High abundance of archaea in Arctic marine picoplankton, *Nature*, 1994, vol. 371, pp. 695–697.
8. Karner, M.B., DeLong, E.F., and Karl, D.M., Archaeal dominance in the mesopelagic zone of the Pacific Ocean, *Nature*, 2001, vol. 409, no. 6819, pp. 507–510.
9. Auguet, J.C. and Casamayor, E.O., A hotspot for cold crenarchaeota in the neuston of high mountain lakes, *Environ. Microbiol.*, 2008, vol. 10, no. 4, pp. 1080–1086.
10. Lin, X., Wakeham, S.G., Putnam, I.F., Astor, Y.M., Scranton, M.I., Chistoserdov, A.Y., and Taylor, G.T., Comparison of vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and Black Sea by use of fluorescence in situ hybridization, *Appl. Environ. Microbiol.*, 2006, vol. 72, no. 4, pp. 2679–2690.
11. Lam, P., Jensen, M.M., Lavik, G., McGinnis, D.F., Müller, B., Schubert, C.J., Amann, R., Thamdrup, B., and Kuypers, M.M., Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, vol. 104, pp. 7104–7109.
12. Coolen, M.J.L., Abbas, B., van Bleijswijk, J., Hopmans, E.C., Kuypers, M.M.M., Wakeham, S.G., Jaap, S., and Damsté, J.S., Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids, *Environ. Microbiol.*, 2007, vol. 9, pp. 1001–1016.
13. Kuypers, M.M.M., Sliekers, A.O., Lavik, G., Schmid, M., Jorgensen, B.B., Kuenen, J.G., Damste, J.S.S., Strous, M., and Jetten, M.S., Anaerobic ammonium oxidation by anammox bacteria in the Black Sea, *Nature*, 2003, vol. 422, pp. 608–611.
14. Tsai, Y.L. and Olson, B.H., Rapid method for direct extraction of DNA from soil and sediments, *Appl. Environ. Microbiol.*, 1991, vol. 57, pp. 1070–1074.
15. Kramer, M.F. and Coen, D.M., Enzymatic amplification of DNA by PCR: standard procedures and optimization, *Curr. Protoc. Immunol.*, 2001, vol. 10.20, pp. 10.20.1–10.20.10.
16. Kublanov, I.V., Perevalova, A.A., Slobodkina, G.B., Lebedinsky, A.V., Bidzhieva, S.K., Kolganova, T.V., Kaliberda, E.N., Rumsh, L.D., Haertlé, T., and Bonch-Osmolovskaya, E.A., Biodiversity of thermophilic prokaryotes with hydrolytic activities in hot springs of Uzon Caldera, Kamchatka (Russia), *Appl. Environ. Microbiol.,* 2009, vol. 75, pp. 286–291.
17. Stahl, D.A. and Amann, R., Development and application of nucleic acid probes in bacterial systematic, in *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M., Eds., Chichester: Wiley, 1991, pp. 205–248.
18. Steinberg, L.M. and Regan, J.M., Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge, *Appl. Environ. Microbiol.*, 2008, vol. 74, pp. 6663–6671.
19. Kubista, M., Andrade, J.M., Bengtsson, M., Forootan, A., Jonák, J., Lind, K., Sindelka, R., Sjöback, R., Sjögreen, B., Strömbom, L., Ståhlberg, A., and Zoric, N., The real-time polymerase chain reaction, *Mol. Aspects Med.*, 2006, vol. 27, pp. 95–125.
20. Walters, W.A., Caporaso, J.G., Lauber, C.L., Berg-Lyons, D., Fierer, N., and Knight, R., PrimerProspector: *de novo* design and taxonomic analysis of barcoded PCR primers, *Bioinformatics*, 2011, vol. 27, pp. 2–4.
21. Podosokorskaya, O.A., Kadnikov, V.V., Gavrilov, S.N., Mardanov, A.V., Merkel, A.Y., Karnachuk, O.V., Ravin, N.V., Bonch-Osmolovskaya, E.A., and Kublanov, I.V., Characterization of *Melioribacter roseus* gen. nov., sp. nov., a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class *Ignavibacteria*, and a proposal of a novel bacterial phylum *Ignavibacteriae, Environ. Microbiol.*, 2013, vol. 15, pp. 1759–1771.
22. Miroshnichenko, M.L., Hippe, H., Stackebrandt, E., Kostrikina, N.A., Chernyh, N.A., Jeanthon, C., Nazina,T.N., Belyaev, S.S., and Bonch-Osmolovskaya, E.A., Isolation and characterization of

Thermococcus sibiricus sp. nov. from a Western Siberia high-temperature oil reservoir, *Extremophiles*, 2001, vol. 5, pp. 85–91.

23. Massana, R., Murray, A.E., Preston, C.M., and DeLong, E.F., Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel, *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 50–56.
24. Moreira, D., Rodríguez-Valera, F., and López-García, P., Analysis of a genome fragment of a deep-sea uncultivated Group II *Euryarchaeota* containing 16S rDNA, a spectinomycin-like operon and several energy metabolism genes, *Environ. Microbiol.*, 2004, vol. 6, pp. 959–969.
25. DeLong, E.F., Archaea in coastal marine environment, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, vol. 89, pp. 5685–5689.
26. Fuhrman, J.A., McCallum, K., and Davis, A.A., Novel major archaebacterial group from marine plankton, *Nature*, 1992, vol. 356, pp. 148–149.
27. Nishizawa, T., Komatsuzaki, M., Kaneko, N., and Ohta, H., Archaeal diversity of upland rice field soils assessed by the terminal restriction fragment length polymorphism method combined with real time quantitative-PCR and a clone library analysis, *Microbes Environ.*, 2008, vol. 23, pp. 237–243.
28. Midgley, D.J., Saleeba, J.A., Stewart, M.I., and McGee, P.A., Novel soil lineages of Archaea are present in semi-arid soils of eastern Australia, *Can. J. Microbiol.*, 2007, vol. 53, pp. 129–138.
29. Iverson, V., Morris, R.M., Frazar, C.D., Berthiaume, C.T., Morales, R.L., and Armbrust, E.V., Untangling genomes from metagenomes: revealing an uncultured class of marine *Euryarchaeota, Science*, 2012, vol. 335, pp. 587–590.
30. Hu, A., Jiao, N., Zhang, R., and Yang, Z., Niche partitioning of Marine Group I *Crenarchaeota* in the euphotic and upper mesopelagic zones of the East China Sea, *Appl. Environ. Microbiol.*, 2011, vol. 77, pp. 7469–7478.
31. Schattenhofer, M., Fuchs, B.M., Amann, R., Zubkov, M.V., Tarran, G.A., and Pernthaler, J., Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean, *Environ. Microbiol.*, 2009, vol. 11, pp. 2078–2093.
32. Könneke, M., Bernhard,A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., and Stahl, D.A., Isolation of an autotrophic ammonia-oxidizing marine archaeon, *Nature*, 2005, vol. 437, pp. 543–546.
33. Berg, I.A., Kockelkorn, D., Buckel, W., and Fuchs, G., A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea, *Science*, 2007, vol. 318, pp. 1782–1786.
34. Swan, B.K., Chaffin, M.D., Martinez-Garcia, M., Morrison, H.G., Field, E.K., Poulton, N.J., Masland, E.D., Harris C.C., Sczyrba, A., Chain, P.S., Koren S., Woyke T., and Stepanauskas R., Genomic and metabolic diversity of Marine Group I *Thaumarchaeota* in the mesopelagic of two subtropical gyres, *PLoS One*, 2014, vol. 9(4):e95380.
35. Ouverney, C.C. and Fuhrman, J.A., Marine planktonic archaea take up amino acids, *Appl. Environ. Microbiol.*, 2000, vol. 66, pp. 4829–4833.
36. Hansman, R.L., Griffin, S., Watson, J.T., Druffel, E.R., Ingalls, A.E., Pearson, A., Aluwihare, L.I., The radiocarbon signature of microorganisms in the mesopelagic ocean, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, vol. 106, pp. 6513–6518.
37. Takai, K. and Horikoshi, K., Genetic diversity of archaea in deep-sea hydrothermal vent environments, *Genetics*, 1999, vol. 152, pp. 1285–1297.
38. Baker, B.J., Comolli, L.R., Dick, G.J., Hauser, L.J., Hyatt, D., Dill, B.D., Land, M.L., Verberkmoes, N.C., Hettich, R.L., and Banfield, J.F., Enigmatic, ultrasmall, uncultivated Archaea, *Proc. Natl. Acad. Sci. U. S. A.* 2010, vol. 107, pp. 8806–8811.
39. Comolli, L.R., Baker, B.J., Downing, K.H., Siegerist, C.E., and Banfield, J.F., Three-dimensional analysis of the structure and ecology of a novel, ultrasmall archaeon, *ISME J.*, 2009, vol. 3, pp. 159–167.
40. Carini, P., White, A.E., Campbell, E.O., and Giovannoni, S.J., Methane production by phosphate-starved SAR11 chemoheterotrophic marine bacteria, *Nat. Commun.*, 2014, vol. 7, p. 4346.

Translated by P. Sigalevich