

Archaeal diversity in the seawater of Changjiang River estuary reveals its adaptability to bottom seawaters*

Yan HUANG, Wu QU, Yingping FAN, Jianxin WANG**

Marine Microorganism Ecological & Application Lab., Zhejiang Ocean University, Zhoushan 316022, China

Received Feb. 8, 2021; accepted in principle Apr. 14, 2021; accepted for publication Jul. 16, 2021 © Chinese Society for Oceanology and Limnology, Science Press and Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract Archaea regulate the biogeochemical processes of ocean systems. The Changjiang (Yangtze) River estuary (CRE) is a complex and dynamic area strongly affected by seawaters and ocean currents. In this study, the planktonic archaeal communities in the surface and bottom seawaters of the CRE were investigated. Significant differences in the archaeal community composition were found between the surface and bottom seawaters (P<0.001). Marine Group II (MG-II) was dominant in the surface layers, and Nitrosopumilales was enriched in the bottom layers. Marine Group III (MG-III) was more abundant in the bottom layers than in the surface ones (P<0.001). These results were completely different from previous findings in the CRE seawater. Instead of dissolved oxygen (DO), temperature and salinity were the most vital environmental variations in the distribution of the archaeal communities. According to the predicted metabolic pathways, the following functional subcategories were enriched in the hypoxic condition: replication and repair, membrane transport, glycan biosynthesis and metabolism, amino acid metabolism, metabolism of cofactors and vitamins, and xenobiotics biodegradation and metabolism (P < 0.001), which indicated the strong adaptability of archaea to the harsh environment in the bottom seawater. These findings expand the understanding on archaeal structure and functions in the surface and bottom seawaters, including the hypoxic zones in the CRE, and may contribute to further works of the archaeal community in the CRE.

Keyword: archaeal communities; adaptability; predicted metabolism; hypoxic zone; Changjiang River estuary

1 INTRODUCTION

In marine environments, microorganisms account for nearly 90% of biomass and 98% of ocean primary productivity and are the key players in biogeochemical processes (Caron, 2005; Sogin et al., 2006). Planktonic bacteria and archaea are important ocean microbial communities with different evolutionary histories. Initial researches revealed that bacteria are distributed globally, and archaea prefer extreme environments, such as psychrophilic and hypersaline environments, deep-sea hydrothermal vents and hot springs (Ochsenreiter et al., 2002; Miroshnichenko, 2004; Cavicchioli, 2006; Mirete et al., 2011). However, the applications of molecular technologies in microbial ecology have completely changed our understanding of archaea in marine ecosystems (Schleper et al., 2005). Analysis of 16S rRNA gene sequences from numerous environmental samples showed that archaea are widespread and play important roles in the global carbon and nitrogen cycle. It was also found that Euryarchaeota and Thaumarchaeota dominate most marine environments (DeLong, 1992; Karner et al., 2001; Francis et al., 2005; Wuchter et al., 2006; Kubo et al., 2012; Vila-Costa et al., 2013). The archaeal strains were difficult to be isolated or purified. So far, the marine archaea strains that could be purely cultured were from the

^{*} Supported by the National Key R&D Program of China (No. 2019YFD0901305), the Science and Technology Program of Zhoushan (No. 2019C21011), the Research Project of Ecological Environment Protection and Restoration of Yangtze River in Zhoushan (No. SZGXZS2020068), the National Natural Science Foundation of China (Nos. 31270160, J1310037), the Natural Science Foundation of Zhejiang Province, China (No. LY12C03003), the Zhejiang Public Welfare Technology Application Research Project (No. 2016C33084), and the Province Key Research and Development Program of Zhejiang (No. 2021C02047)

^{**} Corresponding author: jxwang@zjou.edu.cn

phylum Thaumarchaeota, which has made great progress in the physiology, biochemistry, and niche specification (Ingalls et al., 2006; Martens-Habbena et al., 2009; Qin et al., 2017). Most Euryarchaeota have not been cultured; however, their lifestyle was mainly obtained based on metagenome-assembled genomes (Iverson et al., 2012; Deschamps et al., 2014; Martin-Cuadrado et al., 2015). The phylogeny of Euryarchaeota is diverse, including physiologically distinct groups, such as strict anaerobic methanogens, extreme halophiles, and extreme acidophiles (Lewalter and Müller, 2006). The clade Marine Group II (MG-II), which affiliated to Euryarchaeota, is globally distributed in the seas and open ocean (Massana et al., 2000). The identification of proteorhodopsins in genomes derived from the surface water layer indicated that MG-II is a taxon of photoheterotrophs (Frigaard et al., 2006; Pereira et al., 2019). Some subclades can degrade the algalose or protein in the surface layers, and some lack proteorhodopsins in the mesopelagic zones (Tully, 2019). These metabolic characteristics make MG-II significant contributors to the global oceanic carbon cycle. Deep ocean environment lacks sunlight and is one of the few ecosystems on the earth that is mainly driven chemolithoautotrophy by rather than photosynthesis (Yakimov et al., 2011). Thaumarchaeota were predominant and regarded as chemolithoautotrophs that use nitrification as a major energy acquiring mechanism in the dark primary production process (Könneke et al., 2005, 2014; Yakimov et al., 2011; Stahl and de la Torre, 2012). Zhong et al. (2020)assembled several thaumarchaeotal genomes, which obtained from the Mariana Trench. Through comparative genomics, and Zhong et al. (2020) predicted unexpected genes involved in bioenergetics, which may be of great help to the success under extreme conditions. Zhong et al. (2020) also found that the clade has genetic potential to import a far higher range of organic compounds than their shallower water counterparts. Michoud et al. (2021) studies the composition of prokaryotic communities in the Red Sea deep halocline found that Thaumarchaeota play an important role in adapting to the changing conditions of the chemocline.

The Changjiang (Yangtze) River estuary (CRE), a transitional zone between the land and open ocean, is a complex and dynamic region suffering from the strong influence of seawaters and ocean currents all year round. In summer, seasonal hypoxia often occurs

in the bottom layers of the CRE, which may be caused by Taiwan Warm Current, and the high concentrations of particle organic carbon and nitrogen (Li et al., 2002). In addition, it is common in some estuaries, such as the Zhujiang (Pearl) River estuary (Liu et al., 2014; Orita et al., 2015). Hypoxia may be caused by water stratification and the decomposition of deposited organic matter from rivers mediated by microorganisms (Diaz and Rosenberg, 2008; Lohrenz et al., 2008; Grenz et al., 2010).

Estuaries have always been hotspots for biogeochemical cycling. Many high-throughput sequencing studies were conducted on the distribution of microbial communities in estuaries (Liu et al., 2014, 2015, 2020; Ye et al., 2016; Wu et al., 2019). However, only a few works have explored the distribution of archaeal community structure in the CRE. Zeng et al. (2007) found the main groups in the seawater of the CRE were Marine Group I (MG-I), which was affiliated to the phylum Thaumarchaeota, and MG-II. Moreover, MG-I dominated the surface layers. Dang et al. (2008) proposed that ammoniaoxidizing archaea (AOA) are unique in the seawater of the CRE mixing zone, and its community distribution is crucially related to salinity and sediment sorting coefficient. Liu et al. (2011) found similar results to Zeng et al. (2007) by denaturing gradient gel electrophoresis technology and proposed that MG-II is the abundant group in the bottom seawater and salinity seriously affects the archaeal community composition. He et al. (2016) found that AOA were substantially related to nitrite in the CRE sediments.

Although research progress has been achieved regarding archaeal distributions in the ocean, the adaptive mechanism of archaea communities to the corresponding environments in the CRE remains unclear. In this study, the structures and adaptability of archaeal community in surface and bottom water layers of the CRE were determined through 16S rRNA gene analysis and metagenomic functional prediction. The results may lay the foundation for the functional researches of archaea in estuaries.

2 MATERIAL AND METHOD

2.1 Sample collection and physicochemical measurements of seawater

During a summer cruise in July 2016, 24 seawater samples were collected from 12 stations in the CRE (Fig.1). Seawater from the surface (1.9–2.1-m depth; Sample C3S

C4S

C5S

D3S

D4S

D5S

E3S

E4S

E5S

F3S

F4S

F5S

C3B

C4B

C5B

D3B

D4B

D5B

E3B

E4B

E5B

F3B

F4B

F5B

Depth (m)

1.942

1.986

1.955

1.957

1.962

1.943

1.975

1.948

2.033

1.898

1.991

1.995

41.828

59.669

50.725

27.731

46.821

50.979

26.983

42.962

54.540

35.762

57.476

51.706

DO

2 2 5 9

1.682

1.975

1.877

2.539

2.208

2.601

2.132

2.249

1.972

in the Changjiang River estuary								
O (mg/L)	COD (mg/L)	pН	NO ₃ (mg/L)	NO ₂ (mg/L)	PO ₄ ³⁻ (mg/L)	NH ₄ (mg/L)	Salinity	Temperature (°C)
5.294	0.578	8.180	45.120	0.409	0.339	1.548	18.769	25.072
9.894	2.429	8.690	45.559	0.413	0.593	0.469	13.788	26.667
6.040	0.634	8.200	39.357	0.409	0.339	1.066	23.226	23.994
4.216	0.610	7.930	57.163	0.464	1.186	0.811	11.544	25.972
5.692	0.663	8.170	38.757	0.399	0.339	0.998	20.442	24.500
5.062	0.634	8.130	35.968	0.473	0.254	0.847	20.390	24.702
4.274	0.803	7.930	50.010	0.340	1.016	0.472	18.130	24.546
4.155	0.788	7.940	50.274	0.500	1.186	0.350	21.703	23.890
7.974	2.025	8.460	63.872	0.496	0.508	1.011	19.570	25.664
4.367	0.606	7.930	30.615	0.340	0.678	0.626	26.353	23.626
7.578	0.894	8.280	24.529	0.455	0.169	0.602	24.013	25.791
4.881	0.840	8.200	10.155	0.184	0.169	0.416	26.273	26.941
1.805	0.335	7.770	14.554	0.312	1.016	0.210	33.927	20.164
2.045	0.343	7.780	15.642	0.280	1.355	0.293	34.054	20.041

1.186

1.186

1.016

0.762

1.101

1.186

0.762

1.016

1.186

1.270

0

0.214

0.162

0.159

0.165

0.464

0.176

0.334

0.187

0.177

34.080

33.153

33.930

34.179

33.004

34.151

34.229

34.139

34.364

34.421

20.436

20.013

20.039

20.602

20.382

20.255

20.316

20.171

19.878

20.194

Table 1 Environmental and physicochemical factors of the seawater from two layers (surface and bottom) of the 24 samples in the 12 sites in

0.270 The actual measured value of the NH⁺₄ concentration in C5B was -0.041, which replaced by 0.

0.367

0.372

0.264

0.359

0.263

0.403

0.315

0.320

0.156

7.740

7.730

7.780

7.540

7.820

7.820

7.850

7.810

7.810

7.830

13.945

19.169

16.401

15.441

20.615

10.779

15.846

16.411

17.637

16.241

0.280

0.243

0.161

0.202

0.441

0.303

0.211

0.156

0.142

0.133

S) and bottom layers (26-60-m depth; B) was collected at each site (Table 1) by using Niskin bottles mounted on an SBE32 CTD (Sea-Bird Electronics). Each 2 L of seawater sample was pre-filtered through a 3-µm pore size filter (Millipore Corporation, Billerica, MA, USA) with a gentle vacuum pressure <33.3 kPa and then re-filtered with a 0.22-µm filter to collect free-living microbial cells. The 24 filters with the pore size of 0.22 µm were temporarily stored at -20 °C on board and then transferred to -80 °C in the laboratory until DNA extraction. The 24 samples were divided into two groups: surface and bottom. Each sample was named with the location name, followed by the abbreviation of the layer. For example, the C3S sample means sampling from the surface seawater in the C3 site.

Seawater physicochemical parameters, including depth, salinity, and temperature, were measured by CTD in situ. Other parameters, such as the concentration of nitrate (NO_3) , nitrite (NO_2) ,



Fig.1 A map showing the study areas in the Changjiang **River estuary**

Red dots: the sampling sites.

ammonium (NH_4^+) , chemical oxygen demand (COD), phosphate (PO_4^{3-}) , and dissolved oxygen (DO), were measured following the specifications for marine monitoring prior to chemical parameter analysis (Wu et al., 2019).

2.2 DNA extraction, PCR, and sequencing

The DNA of the 24 samples was extracted with a FastDNA spin kit for soil (MP Biomedicals, USA) in accordance with the specification. The DNA extracts were quantified using a NanoDrop 2000 (Thermo Fisher Scientific, USA) and then sent to Biozeron Company (Shanghai, China) for 16S rRNA gene amplification (V4-V5 region) using dual-indexed pairs archaeal barcoded primer 524F (5'-TGYCAGCCGCCGCGGTAA-3') and 958R (5'-YCCGGCGTTGAVTCCAATT-3'). Prior to PCR, the 12-bp barcode sequences were linked to the primers during primer synthesis. Each DNA sample was amplified as an undiluted template and at a 1:10 template dilution to reduce the potential effects of PCR bias. The 16S amplicons were pooled together and their quality was verified by Bioanalyzer (Agilent Technologies). Following dilution, the library pool was quantified by quantitative-PCR. Finally, the pooled samples were run on the Illumina HiSeq platform. Raw sequence files are available at the NCBI Sequence Read Archive under accession SUB6193127.

2.3 Sequence quality control and amplicon sequence variant (ASV) assignment

The raw reads FASTQ files were processed and analyzed using the QIIME 2 bioinformatics program (Bolyen et al., 2019). Plugin "giime tools import" was used to import the raw FASTQ data by a manifest file including the samples' IDs and absolute pathways. Barcodes and primers were removed by the plugins "qiime cutadpt demux-paired" and "qiime cutadapt trim-paired", respectively. Plugin "giime dada2 denoise-paired" was used to perform quality filtering, denosing and chimera removal to cluster the sequences into ASVs (i.e., the representative sequences). After the base with average quality lower than 20 were removed, the average length of reads was 455 bp. A total of 780 721 sequences were obtained after denosing and chimera removal. Finally, plugin "qiime feature-classifier classify-sklearn" was used to assign taxonomy with Silva 132 database (16S rRNA gene; 97% similarity level) trained by the primers used in this study (Quast et al., 2012).

Nitrosopumilales and Marine Group III (MG-III) were the focus of analysis. These orders were aligned against the GenBank database in National Center for Biotechnology Information (NCBI, http://www.ncbi. nlm.nih.gov) data and the top-hit sequence was

downloaded for each ASV. The cloned and ASV sequences of Nitrosopumilales and MG-III were used to construct the phylogenetic trees, respectively by using the neighbor-joining (NJ) methods and MEGA-X with Tanura-Nei model (Kumar et al., 2018). The tree topologies were checked by 1 000 bootstrap.

2.4 Statistical analysis

For diversity analysis, the ASVs table was used to calculate the alpha and beta diversities in R v4.0 (R Core Team, 2013). Package picante was applied to calculate the diversity and richness indexes (Shannon, Chao1, and Simpson) (Lee and Chao, 1994; Kembel et al., 2010). Alpha diversity indexes were analyzed with the function "ggsignif" in the "ggplot2" package to compare the diversities and richness of archaeal communities in the two groups (surface and bottom seawater layers) (Wickham and Chang, 2009). Beta diversity was explored with the Bray-Curtis dissimilarity index by the function "vegdist" of the "vegan" package (Bray and Curtis, 1957; Oksanen et al., 2010). Complete linkage hierarchical clustering based on Bray-Curtis dissimilarity using the normalized ASVs matrix was performed using the package "vegan" (Oksanen et al., 2010). Non-metric multidimensional scaling (NMDS) analysis was also conducted with normalized ASVs using the "vegan" and "ggplot2" packages (Wickham and Chang, 2009). Co-occurrence networks were constructed for archaeal communities in all the samples, and ASVs affiliated with Nitrosopumilales, MG-II, and MG-III were included in analysis. The correspondences between the ASVs and the groups were formed the edges, while selected ASVs served as network nodes. The edge and the node files were formed in R (R Core Team, 2013), and the network topology were shaped in Cytoscape (Baryshnikova et al., 2016).

Redundancy analysis (RDA) combined with 999 Monte Carlo permutation was employed with "vegan" package in R to analyze the influence of environmental factors on archaeal communities (Oksanen et al., 2010). Hellinger was used to standardize the ASV data and the dissimilarity in environmental factors was log-transformed. A heatmap was plotted to visualize the Spearman correlations between the environmental factors and ASVs of Nitrosopumilales with "pheatmap" package in R (Wickham and Chang, 2009). Principal component analysis (PCA) was performed with "vegan" package in R based on the relative abundance of different individual pathways to analyze the relationship among archaeal communities and environmental factors (Oksanen et al., 2010). Hellinger was also used to standardize the relative abundance of pathways.

2.5 Prediction of functionality of archaeal communities

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) was used to predict the functional potential of prokaryotic community based on marker genes, particularly 16S rRNA genes (Ye and Doak, 2009). ASV sequences were used to clustered into a reference tree to predict functions, which were pre-calculated for genes in databases including Kyoto Encyclopedia of Genes and Genomes (KEGG) and Clusters of Orthologous Groups of proteins (COG). Script "picrust2 pipeline.py" was used to predict the functions of the archaeal ASVs and received the predictions for enzyme commission (EC) number metagenome, KEGG orthology (KO) metagenome, and pathway abundance. Script "pathway pipline. py" was employed to collapse the predictions at the level of the individual pathways. Linear discriminant analysis effect size (LEfSe) was used to test the data for statistical significance, and biological consistency was applied to identify differentially abundant pathways between the surface and bottom seawaters (Segata et al., 2011). Cladogram plot was presented hierarchically with category, subcategory, and individual pathways. Linear discriminant analysis (LDA) scores were calculated, and the barplots for the relative abundance of individual pathways were displayed. PCA biplot was constructed based on relative abundance of individual pathways to relationship among understand the archaeal communities and environmental factors.

3 RESULT

3.1 Environmental factors in seawaters

The physicochemical parameters of 24 samples from 12 sites are summarized in Table 1. Most environmental factors changed vertically in the 12 sites (Supplementary Fig.S1). Temperature, pH, and the concentrations of COD, NO₂⁻, NO₃⁻, NH₄⁺, and DO in the surface layers were significantly higher than those in the bottom ones (P<0.01), whereas salinity and the concentration of PO₄⁻ in the surface layers were significantly lower than those in the bottom ones (P<0.001). In this study, the DO concentration in the surface water layers was greater than 4 mg/L, whereas that in the bottom layers was less than 2.6 mg/L. In accordance with the definitions (Li et al., 2011), the surface layers were called oxic ones, and the bottom layers were called hypoxic zones.

3.2 Diversity of archaeal community and community comparison

The 24 samples yielded 782 095 high quality archaeal sequences ranging from 23 734 to 38 164 (Supplementary Table S1). A total of 121 ASVs were generated from the 24 samples at a 97% similarity level. Normalized Shannon and Chao1 indexes were used to represent the diversity and richness, respectively. The Shannon index of archaea ranged from 1.62 to 3.83, and its Chao1 index ranged from 24 to 48 (Supplementary Table S1). The Shannon and Chao1 indexes in the bottom layers were significantly higher than those in the surface layers (P<0.05, Wilcoxon test, Supplementary Fig.S2), indicating that the bottom layers had higher archaeal diversity and richness than the surface ones.

Beta diversity was used to explore the transition of archaeal diversity in different environments. Hierarchical clustering dendrogram showed the separation of archaeal community composition from the surface layers to the bottom layers (Fig.2a). NMDS analysis also separated the samples from the depth by the first axis (Fig.2b). Analysis of similarity (ANOSIM) tested the dissimilarity of all the samples based on the Bray-Curtis distance and also confirmed the significant difference in the archaeal community composition between the depth groups (R=0.992 6, P=0.001).

3.3 Taxonomic assignment

All sequences were classified into two main phyla, Euryarchaeota and Thaumarchaeota (99.8%-100% of the total population), with a few belonging to Crenarchaeota, Diapherotrites, Hydrothermarchaeota, and Nanoarchaeaeota. The two major phyla displayed different distribution patterns in the two layers of the CRE (Fig.3a). Euryarchaeota ($P \le 0.001$) accounted for the most abundant proportion of archaea in the surface samples, and Thaumarchaeota (P < 0.001) was the most enriched in the bottom zones. The archaeal communities at the class level mainly belonged to two main classes: Thermoplasmata and Nitrososphaeria, which are affiliated to Euryarchaeota and Thaumarchaeota, respectively (Supplementary Table S2). Some rare taxa mostly existed in the bottom



Fig.2 Bray-Curtis dissimilarity based dendrogram showing the clustering of samples using the archaeal amplicon sequence variants (ASVs) (a); non-metric multidimensional scaling (NMDS) results showing the relationship among all the samples based on the archaeal community composition using ASVs (stress=0.035) (b)

Hierarchical clustering was calculated with complete algorithms in (a). Samples are colored according to the depth (purple for surface seawater samples and dark green for bottom seawater ones).

layers. For example, Bathyarchaeia was found in C4B, D5B, F3B, and F4B, and Woesearchaeia was observed in C5B and D5B. At a fine taxonomic resolution, MG-II and Nitrosopumilales were prevalent across all samples. MG-II was relatively abundant in the surface layers, and Nitrosopumilales was more predominant in the bottom ones. In all samples, the most abundant order in E5S was MG-II (93.97%), and in C3B was Nitrosopumilales (74.87%). Notably, MG-III (P<0.001), which is affiliated to Euryarchaeota, was highly abundant in the bottom layers. C4B contained relatively high percentages of MG-III (9.14%), followed by F5S (4.95%).

The co-occurrence network also showed the distribution of the ASVs of MG-II, MG-III, and Nitrosopumilales in the two layers (Supplementary Fig.S3). Most ASVs of MG-II were distributed in both layers, while Nitrosopumilales and MG-III were mainly distributed in the bottom seawater.

3.4 Correlation between archaeal communities and environmental variations

From all samples, 40 ASVs of MG-II, 4 ASVs of MG-III, and 25 ASVs of Nitrosopumilales were extracted for reducing complexity and eliminating rare taxa. RDA was used to analyze the variation of archaeal communities as a function of environmental



Fig.3 Archaeal community compositions at different taxonomic levels in each seawater sample

a. relative abundance of the dominant archaeal phyla in the 24 samples; b. relative abundance of the dominant archaeal orders in the 24 samples. In (b) "others" referred to the orders with a total relative abundance of less than 0.3%.

factors including salinity, temperature, DO, NO₃, NO₂, NH₄, COD, PO₄³⁻, and pH. RDA1 and RDA2 explained 80.81% of the total variations (Fig.4). On the horizontal axis (RDA1, 74.3% of constrained variability), the most influential constraining variable was temperature (biplot score=-0.97) followed by DO (biplot score=-0.93) and salinity (biplot score=-0.89). The sites were distinctly separated into two groups (surface and bottom layers, P < 0.001) by RDA1. F5S relatively deviated from the 95% confidence interval of the surface groups, and the bottom groups gathered closely. On the horizontal axis (RDA2, 8.03% of constrained variability), the most influential constraining variable was NO3 (biplot score=0.50), followed by PO_4^{3-} (biplot score=0.44). Function "envfit" was used to measure each factor onto an ordination. Salinity and temperature were significantly related to the archaeal community (Supplementary Table S3, P<0.001). In addition, the ASVs of MG-II and Nitrosopumilales was separated by RDA1 (P < 0.05), indicating that most ASVs of the two groups prefer different biotopes.

No.3

A heatmap of Nitrosopumilales (25 ASVs) and the environmental factors was constructed to determine the relationship between Nitrosopumilales and environmental factors (Supplementary Fig.S4). The ASVs were sorted according to the relative abundance. Except for ASV24, ASV8, and ASV55, the top 20 ASVs were positively related to PO_4^{3} , depth, and salinity and negatively related to other factors. Except for ASV24, most of the top 10 ASVs were correlated with all the environmental factors (|R|>0.40) and displayed coordinated relationships with each factor.

3.5 Phylogenetical diversity of Nitrosopumilales and MG-III

Compared with archaeal communities in the surface water, Nitrosopumilales and MG-III were more abundant in the bottom layers. The NJ trees of the ASVs affiliated to Nitrosopumilales and MG-III, respectively. The Nitrosopumilales phylogenetic tree identified two major clusters (Fig.5a). Most ASVs were relatively abundant in the bottom layers, and others were abundant in the surface layers. For example, ASV24, ASV55, ASV36, and ASV61 were more abundant in the surface layers than in the bottoms and were clustered in another branch. In addition, most sequences of genetically close species downloaded from the NCBI nt database were derived from deep seawater, sediments, or the hypoxic zones, such as the northeast Pacific Ocean and the northern Gulf of Mexico.

The MG-III phylogenetic tree also identified two clusters (Fig.5b). The sequences in one cluster were mainly recovered from the deep seas, and those in the other cluster were from the surface seawater or hypoxic areas. ASV50, which was assigned into the second cluster, occurred in both layers.

3.6 Predicted metabolic potentials

PICRUSt2 analysis detected 78 individual pathways. LEfSe was used to reveal the significant differences of the metabolic functionality between the two water layers (Fig.6, Supplementary Table S3). In the functional categories, the surface layers were enriched with genetic information processing

1057



Redundancy analysis (RDA) results showing the ordination diagram of the archaeal community composition (ASVs) associated with environmental factors. Environmental factors are indicated as arrows. Different shapes represented different seawater layers (surface layer samples are represented by triangles and bottom layer samples by squares). The ASVs affiliated to the three orders of Marine Group II (MG-III), Marine Group III (MG-III), and Nitrosopumilales were marked in light blue, orange, and red, respectively. Box plots showing the overall distribution of RDA1 and RDA2 scores within the three orders (**:

P<0.05, ns: no significance; Wilcoxon rank-sum test). DO: dissolved oxygen; COD: chemical oxygen demand.

 $(P \le 0.001)$, and the bottom layers were enriched with environmental information processing (P<0.001), cellular processes (P<0.001), and organismal systems (P<0.001). Several functional subcategories, including "cell growth and death", "folding, sorting and degradation", "translation; amino acid metabolism", "carbohydrate metabolism", "metabolism of terpenoids and polyketides" and "nucleotide metabolism" were significantly higher (P < 0.001) in the surface layers compared to the bottom ones. Subcategories, such as "cell motility," "membrane transport," "replication and repair," "biosynthesis of other secondary metabolites," "energy metabolism; glycan biosynthesis and metabolism," "lipid metabolism; metabolism of cofactors and vitamins,"

"metabolism of other amino acids," and "xenobiotics biodegradation and metabolism" were enriched in the bottom layers (P < 0.001). The boxplot showed the significant enrichment in the bottom layers (Supplementary Fig.S5, *P*<0.001). Functional subcategories, such as replication and repair, amino acid metabolism, carbohydrate metabolism and metabolism of cofactors and vitamins, were abundant in the bottom layers. Individual pathways, such as chemotaxis, basal transcription factors, sulfur metabolism, biotin metabolism, and biosynthesis of vancomycin group antibiotics, were significantly enriched in the bottom layers (P<0.001). The PCA plot constructed based on relative abundance of different metabolisms presented the clustering of the



Fig.5 Phylogenetic tree based on the 16S rRNA gene from the ASVs of Nitrosopumilales (a) and Marine Group III (b) detected in our study

Support values, with 1 000 replicates for neighbor-joining (NJ) analyses, are shown in the order of NJ at nodes (values lower than 50% are not shown). The ASVs of the present study are shown in boldface. The scale bar indicates 0.01 nucleotide substitutions per site. The circle size indicates the relative abundance of sequences in each ASV at different seawater layers. S: surface; B: bottom.



a. overall exhibition of three-level pathways by cladogram. The abbreviations represent KEGG pathway Level-2, and each circle represents the KEGG pathway; b. LDA scores (LDA>2, P<0.05) of the enriched KEGG pathway level-2 enriched in each group.

samples. All surface and bottom samples formed two distinct clusters by the first axis (Supplementary Fig. S6, P=0.001). Similar clustering was observed in the RDA and NMDS analysis (Figs.2 & 4) which was conducted because of the archaeal community composition. A similar trend was obtained with different statistical analyses based on community composition and predicted community function, indicating that different archaeal communities reflect the community function. In addition, the environmental factors driving the predicted metabolic pathways were similar to those actuating the taxonomic composition. Temperature and salinity were also the main important chemical factors that explained most of the variations (P<0.01, $R^2>0.8$; Supplementary Tables S4 & S5).

4 DISCUSSION

4.1 Comparison of archaeal communities between surface and bottom water layers

In general, the ocean is characteristically stratified as reflected in the vertical distributions of zooplankton or microbial communities (Banse, 1964; DeLong et al., 2006). 16S rRNA gene sequencing was performed on 12 seawater samples from the surface layers (DO \geq 3 mg/L) and 12 seawater samples from the bottom layers (DO<3 mg/L) to reveal the features of the archaeal community structure in the CRE. The environmental factors were measured in situ. The two layers showed significantly different environmental factors and archaeal communities (P<0.01; Fig.2,

Supplementary Figs.S1 & S2). The archaeal richness and diversity were higher in the bottom water $(P \le 0.01)$ than the surface layers, suggesting the presence of complex archaeal communities in deep layers. Similar results of the microbial community were also found in the saltwater of the Zhujiang estuary and in the same sites of our study (Liu et al., 2014, 2015; Wu et al., 2019). However, the bottom layers in this work were under hypoxia, which can vary the composition of microbial loop (Naqvi et al., 2000). The archaeal community is less abundant in the hypoxic seawater, which is relative to oxic layers (He et al., 2016). Considering that the samples were obtained from two different depths, relabeling them as hypoxic and hypoxic groups based solely on the DO values was inappropriate. RDA analysis showed that salinity and temperature were the most important environmental factors to the archaeal community. Therefore, we speculated that DO value was not the most important factor for the vertical distribution of the archaeal community in the CRE. Liu et al. (2011) also found that salinity has a remarkable effect on archaeal community composition. In the present work, although the surface and bottom samples were distinctly divided into two branches during clustering (Fig.2a), the archaeal community compositions in the surface samples were not uniform (Fig.2b) due to some environmental factors (Supplementary Fig.S1). The quartile deviations of NO₃, DO, salinity, and NH₄⁺ were long in the surface group, indicating that the values of the measured factors were largely different within the surface samples (Supplementary Fig.S1). In F5S, salinity was the highest in the surface group, and the concentrations of NO₂ and NO₃ were the lowest among all surface samples. RDA analysis also presented that the surface sample sites were relatively dispersive (Fig.4).

In line with previous studies in the CRE and the Zhujiang River estuary (Liu et al., 2014, 2020), MG-II was predominant in the surface seawater, and Thaumarchaeota and MG-III prevailed in deep layers (López-García et al., 2001; DeLong et al., 2006; Zhang et al., 2015). Surprisingly, these results were completely different from previous findings in the CRE. Zeng et al. (2007) and Liu et al. (2011) proposed that MG-I is dominant in the surface seawater, and MG-II is abundant in the bottom layers. Massana et al. suggested that Euryarchaeota might be more limited in the temperate zone (Massana et al., 2000) and dominated in the middle and deeper seawater (Massana et al., 1997). Some studies also found the similar phenomenon (DeLong et al., 1994). A research found that the MG-II were dominant in planktonic archaea throughout the water column in the northeastern South China Sea (Liu et al., 2017) possibly due to strong vertical mixing (Tian et al., 2009; Jiao et al., 2014). Besides, Orsi et al. (2015) also proposed that MG-II preferred to attach to particles. Considering that the CRE is often strongly influenced by various ocean currents and water masses in summer, we suggested that the previous results might also be related to the current fluctuation or the preference of archaea for particles. Unfortunately, these speculations have not been confirmed and further verification. MG-II contributes more to archaeal assemblages from the surface than from deeper waters (López-García et al., 2001; Herndl et al., 2005; DeLong et al., 2006; Tseng et al., 2015). Similar to our results, Liu et al. (2009) found that MG-II showed decreased diversity with the increase of depth in the Gulf of Mexico, and Liu et al. (2014) has reported that MG-II were more predominant in the surface saltwater sites of Zhujiang River estuary. In phylogenetic analysis based on the 16S rRNA genes (Massana et al., 2000; Martin-Cuadrado et al., 2008; Belmar et al., 2011), MG-II was classified into two major clusters: MG-IIA, which was more common in the surface seawaters (Frigaard et al., 2006), and MG-IIB, which is abundant in deep water (Hugoni et al., 2013). The distribution of MG-II (ASVs) in the RDA plot also displayed different preferences of environmental factors, and most were negatively related to salinity and positively related to DO and temperature (Fig.4). The wide distribution of MG-II indicated its extensive adaption to diverse marine habitats (Zhang et al., 2015).

MG-III, another dominant family of Euryarchaeota, presented relatively higher abundance in the bottom layers. A few studies confirmed that MG-III is mainly retrieved from deeper seawater (Galand et al., 2009; Tseng et al., 2015; Tarn et al., 2016). Similar to MG-II, no strain of MG-III has been cultured, which explains its unclear ecological and physiological characteristics. The phylogenetic structure of MG-III showed that most referenced sequences were mainly from the deep sea or hypoxic zones (Fig.5b). ASV50 occurred in both groups, and the clustered sequences, JX281472 (unpublished) and JF715301, were also from the surface water lavers in the eastern South Pacific Ocean seawater (Belmar et al., 2011). Six novel MG-III genome sequence bins were recovered in the photic zone (Haro-Moreno et al., 2017), and a

small amount of MG-III was found in surface Arctic waters and the South China Sea (Galand et al., 2009; Li et al., 2021). Notably, F5S possessed the highest amount of MG-III among all the surface samples and detached from the surface group in the RDA plot (Figs.3 & 4). Among the environmental factors, salinity in F5S was the highest among the surface samples, and the concentrations of NO₃ and NO₂ in F5S were extremely lower than in other surface samples. F5S processed similar environmental status to the bottom layers, thus explaining why MG-III is relatively abundant in the site. Metagenomics analysis revealed that the DNA fragments of MG-III genes resembled those in ammonia-oxidizing archaea (AOA), indicating that some members of MG-III may oxidize ammonia (Martín-Cuadrado et al., 2007). In general, we speculated that environmental factors affect the archaeal community components.

Thaumarchaeota had a niche preference for the hypoxic bottom layers, and this finding is in agreement with several works (Massana et al., 2000; Molina et al., 2010; Beman et al., 2012; Tolar et al., 2013). Thaumarchaeota, initially known as mesophilic Crenarchaeota, is a new phylum (Brochier-Armanet et al., 2008). Most of the studies proposed that marine Thaumarchaeota are chemolithoautotrophs, and all cultured representatives of Thaumarchaeaota are AOA (Pester et al., 2011; Hatzenpichler, 2012). Nitrosopumilales, which also belong to AOA, were the most abundant order of Thaumarchaeota in the previous study (Könneke et al., 2005). AOA and AOB play important roles in the ocean nitrogen cycle. Moreover, AOA dominates in many environments, indicating that AOA play more important role in ammonia oxidation (Albers et al., 2004; Shen et al., 2008; Kembel et al., 2010; Jin et al., 2011; R Core Team, 2013; Liu et al., 2017). For the correlations between each ASV of Nitrosopumilales and the environmental factors (Supplementary Fig.S4), most ASVs were positively related with depth and salinity, and negatively related with temperature, DO, pH, and the concentration of NO_3^- , NO_2^- , and PO_4^{3-} . Environmental factors, such as salinity, temperature, nitrate concentration, and total nitrogen, are the driving forces affecting the diversity of AOA communities (Cao et al., 2011; Yu et al., 2016). A small amount of Nitrosopumilales also existed in the surface layers (Fig.3b). As shown in the phylogenetic tree (Fig.5a), several ASVs, such as ASV24, ASV55, and ASV61, were relatively abundant in the surface layers and clearly clustered in the upper topologic structure. Some referenced sequences were also found from the surface waters. ASV24 (Candidatus Nitrosopumilus) was one of the top ASVs of Nitrosopumilales in the relative abundance. The clustered sequences FJ355404 and JQ226368 were detected from the surface water in Industrial Canal (Amaral-Zettler et al., 2008) and 10-m seawater in the Northern Pacific Ocean oxygen minimum zone, respectively. ASV36 (Candidatus Nitrosoarchaeum) and ASV61 (Candidatus Nitrosotenuis) were also closely clustered with KY356879 and KY356871, respectively, which were obtained from the nitrateand radionuclide-contaminated groundwater. Hence, several relatively abundant ASVs of Nitrosopumilales in surface layers, which have strong adaptability, may be injected from terrestrial freshwater rivers and even industrial swages to the coastal surface seawater (Amaral-Zettler et al., 2008; Ren et al., 2019). Furthermore, some researches show that Candidatus Nitrosotenuis uzonensis and Nitrosopumilus sp. are associated with low-salinity and freshwater environments (Mosier and Francis, 2008; Restrepo-Ortiz et al., 2014; Alves et al., 2018). Most of the ASVs of Nitrosopumilales in this study exhibited high similarity to the uncultured archaeal sequences from other environments, such as the Northeast Pacific Ocean, the northern Gulf of Mexico, Atlantic Ocean, and South China Slope. Therefore, these ASVs did not represent the special groups in the CRE and constantly adapt to different environments worldwide.

4.2 Metabolic prediction of archaeal community in the hypoxic layers

Identifying the archaeal communities responding to hypoxia is vital to understand the structure and function of estuarine ecosystems. Here, PICRUSt2 was applied to predict the archaeal metabolic function in the CRE. Comparison of the metabolic pathways in different water layers revealed many relative abundant pathways were shown in the hypoxic bottom layers.

The replication and repair of genetic information processing were enriched in the bottom layers (P<0.001; Supplementary Fig.S5). The rugged environment condition in the bottom layers, such as low pH, high salinity, and hypoxia, acted as the DNAdamaging agents to accelerate DNA damage (Zatopek et al., 2018). Owing to the toxic nature of DNA lesions, organisms have evolved several DNA replication and repair pathways. Base excision repair, homologous recombination, mismatch repair, and nucleotide excision repair were abundant in the bottom layers (P<0.001; Supplementary Fig.S5). Therefore, under environmental pressure, archaea may need powerful DNA replication and repair compounds to ensure genomic fidelity (Shin et al., 2014).

The membrane transport and signal transduction of environmental information processing were relatively abundant in the bottom layers (*P*<0.001; especially Supplementary Fig.S5), adenosinetriphosphate (ATP) binding cassette (ABC) transporters pathways. Different from most bacteria, archaea prefer primary ATP-driven uptake systems for carbon and energy sources (Konings et al., 2002). The high affinity of the binding proteins may enable them to survive in habitats with low carbon sources such as sugars and peptides (Albers et al., 2004). Konings et al. (2002) suggested that archaeal ABC sugar transporters are equipped with an exceptionally high affinity for the subnanomolar substrate, which is beneficial for archaea to compete with others in harsh environments and thereby maximize their ability to absorb nutrients. Here, the starch and sucrose metabolism and the degradation of some amino acids were relatively vigorous in the bottom hypoxic layers (P<0.001) possibly because the archaea consume these substrates for energy through ABC transporters.

Some subcategories pathways affiliated to metabolism enriched in the bottom layers, including amino acid metabolism, carbohydrate and lipid metabolism, metabolism of cofactors and vitamins, and energy metabolism. Arginine and methionine are sulfur-containing amino acids. In hypoxic environments, some organic sulfides, such as methanethiol and dimethyl sulfide, can be derived from the degradation of sulfur-containing amino acids (Kadota and Ishida, 1972; Lohrenz et al., 2008). Sulfur metabolism was vigorous in the bottom layers (P<0.001, Supplementary Fig.S5), which was consistent with other studies (Liu et al., 2014; Wang et al., 2016; Wu et al., 2019). In addition to being the basic structure of protein synthesis, amino acids are also precursors for the production of secondary metabolites. The synthesis of streptomycin and vancomycin was also vigorous in the bottom layer. Vancomycin is a class of glycopeptide antibiotics that effectively inhibit the biosynthesis of bacterial peptidoglycans (Khelaifia and Drancourt, 2012), and streptomycin is a class of amino sugar antibiotics, that prevent bacterial ribosomes or their reaction substrates (Weisblum and Davies, 1968). These antibiotics may be protective agents that archaea could use to maintain

their growth in complex environments. Some sponge archaeal communities could produce tetracycline with protective properties against potential sponge pathogenic microorganisms and competitors (Polónia et al., 2014). In this work, glycan biosynthesis was enriched in the bottom layers. The cell surface of prokaryotes is particularly rich in glycans, which are glycosylated with the proteins and play fundamental roles in cell physiology (Doyle, 2002). In archaea, N-linked glycosylation is the most common and mainly occurrs on S-layer proteins (Mescher and Strominger, 1976; Kärcher et al., 1993; Schäffer and Messner, 2001) and flagellins (Voisin et al., 2005; Kelly et al., 2009). The S-layers also have two noteworthy functions. First, they can minimize unspecific binding and are important for nutrient absorption in harsh environments (Pohlschroder et al., 2018). Second, they can also reduce flow resistance to promote archaea-driven movement due to their structural characteristics (Sleytr et al., 2014). These functions are necessary for the archaea that live in harsh environments to maintain their own growth and division. The current results also showed that protein export and some amino acids synthesis pathways were vigorous and could satisfy the S-layer synthesis.

Xenobiotics biodegradation and metabolism, one of the functional subcategories, were abundant in seawater (Jain et al., 2005). In the bottom layers, the degradation pathways of aminobenzoate benzoate, chlorocyclohexane, and chlorobenzeneare were enriched (P<0.001) (Battersby, 1990). These organic compounds are widely used in many industries (paints, textiles, wood, and chemistry) and agriculture (herbicides and pesticides) and have been considered as important environmental contaminants. The CRE is adjacent to the Changjiang River Delta area with developed industry and agriculture, which is highly affected by human activities. The above organic compounds might flow into the aquatic environment mainly through agricultural and industrial runoff, and gradually sink to the bottom (Embrandiri et al., 2016). These findings indicated that archaeal communities could be regarded as xenobiotic biodegradants for environmental remediation. The archaea community could also obtain carbon, nitrogen, and energy to adapt the harsh niches (Polónia et al., 2018; Wang et al., 2018).

5 CONCLUSION

The archaeal communities in the surface and bottom layers of the CRE were detected on July 2016.

Distinct archaeal community structure was observed between the two layers. At the taxonomic levels, MG-II dominated in the surface layers, whereas Nitrosopumilales was enriched in the bottom water. However, the results were completely different from previous studies in the CRE. Salinity and temperature were the most important environmental factors affecting the archaeal community. In addition, metabolic prediction revealed that the functional subcategories such as replication and repair, membrane transport, and xenobiotics biodegradation and others, indicating that archaea have a strong ability to protect themselves against harsh environmental conditions. These findings expend the understanding of the archaeal structure and functions in different water layers, even in the hypoxic zones in the CRE, and may contribute to further works of the archaeal community in the CRE.

6 DATA AVAILABILITY STATEMENT

The raw sequence datasets in this study are available in GenBank under BioProject PRJNA632685 (BioSample accession Nos.: SAMN16378058– SAMN16378081) (https://www.ncbi.nlm.nih.gov/ bioproject?LinkName=biosample_bioproject&from_ uid=16378061)

7 AUTHORS' CONTRIBUTION

Yan HUANG processed the experimental data, performed the analysis, and wrote the manuscript. Wu QU analyzed the data. Yingping FAN carried out the part of experiments. Jianxin WANG supervised the project from experimental design to submission of the manuscript. All authors agreed and approved the manuscript to be published.

8 ACKNOWLEDGMENT

We are grateful to all the staff who assisted in the field sampling.

References

- Albers S V, Koning S M, Konings W N, Driessen A J M. 2004. Insights into ABC transport in archaea. *Journal of Bioenergetics and Biomembranes*, **36**(1): 5-15, https:// doi.org/10.1023/B:JOBB.0000019593.84933.e6.
- Alves R J E, Minh B Q, Urich T, Haeseler A V, Schleper C. 2018. Unifying the global phylogeny and environmental distribution of ammonia-oxidising archaea based on *amoA* genes. *Nature Communications*, 9(1): 1517, https:// doi.org/10.1038/s41467-018-03861-1.

- Amaral-Zettler L A, Rocca J D, Lamontagne M G, Dennett M R, Gast R J. 2008. Changes in microbial community structure in the wake of Hurricanes Katrina and Rita. *Environmental Science & Technology*, **42**(24): 9072-9078, https://doi.org/10.1021/es801904z.
- Banse K. 1964. On the vertical distribution of zooplankton in the sea. *Progress in Oceanography*, 2: 53-125, https://doi. org/10.1016/0079-6611(64)90003-5.
- Baryshnikova A. 2016. Exploratory analysis of biological networks through visualization, clustering, and functional annotation in cytoscape. Cold Spring Harbor Protocols, 6, https://doi.org/10.1101/pdb.prot077644.
- Battersby N S. 1990. A review of biodegradation kinetics in the aquatic environment. *Chemosphere*, **21**(10-11): 1243-1284, https://doi.org/10.1016/0045-6535(90)90144-I.
- Belmar L, Molina V, Ulloa O. 2011. Abundance and phylogenetic identity of archaeoplankton in the permanent oxygen minimum zone of the eastern tropical South Pacific. *FEMS Microbiology Ecology*, **78**(2): 314-326, https://doi.org/10.1111/j.1574-6941.2011.01159.x.
- Beman J M, Popp B N, Alford S E. 2012. Quantification of ammonia oxidation rates and ammonia-oxidizing archaea and bacteria at high resolution in the Gulf of California and eastern tropical North Pacific Ocean. *Limnology and Oceanography*, **57**(3): 711-726, https://doi.org/10.4319/ lo.2012.57.3.0711.
- Bolyen E, Rideout J R, Dillon M R, Bokulich N A, Abnet C C, Al-Ghalith G A, Alexander H, Alm E J, Arumugam M, Asnicar F, Bai Y, Bisanz J E, Bittinger K, Brejnrod A, Brislawn C J, Brown C T, Callahan B J, Caraballo-Rodríguez A M, Chase J, Cope E K, Da Silva R, Diener C, Dorrestein P C, Douglas G M, Durall D M, Duvallet C, Edwardson C F, Ernst M, Estaki M, Fouquier J, Gauglitz J M, Gibbons S M, Gibson D L, Gonzalez A, Gorlick K, Guo J R, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley G A, Janssen S, Jarmusch A K, Jiang L J, Kaehler B D, Kang K B, Keefe C R, Keim P, Kelley S T, Knights D, Koester I, Kosciolek T, Kreps J, Langille M G I, Lee J, Ley R, Liu Y X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin B D, McDonald D, McIver L J, Melnik A V, Metcalf J L, Morgan S C, Morton J T, Naimey A T, Navas-Molina J A, Nothias L F, Orchanian S B, Pearson T, Peoples S L, Petras D, Preuss M L, Pruesse E, Rasmussen L B, Rivers A, Robeson M S II, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song S J, Spear J R, Swafford A D, Thompson L R, Torres P J, Trinh P, Tripathi A, Turnbaugh P J, Ul-Hasan S, van der Hooft J J J, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y H, Wang M X, Warren J, Weber K C, Williamson C H D, Willis A D, Xu Z Z, Zaneveld J R, Zhang Y L, Zhu Q Y, Knight R, Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37(8): 852-857, https://doi.org/10.1038/ s41587-019-0209-9.
- Bray J R, Curtis J T. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, 27(4): 325-349, https://doi.org/10.2307/1942268.

- Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P. 2008. Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Reviews Microbiology*, 6(3): 245-252, https://doi.org/10.1038/ nrmicro1852.
- Cao H L, Hong Y G, Li M, Gu J D. 2011. Diversity and abundance of ammonia-oxidizing prokaryotes in sediments from the coastal Pearl River estuary to the South China Sea. *Antonie Van Leeuwenhoek*, **100**(4): 545-556, https://doi.org/10.1007/s10482-011-9610-1.
- Caron D A. 2005. Marine microbial ecology in a molecular world: what does the future hold? *Scientia Marina*, 69(S1): 97-110.
- Cavicchioli R. 2006. Cold-adapted archaea. Nature Reviews Microbiology, 4(5): 331-343. https://doi.org/10.1038/ nrmicro1390
- Dang H Y, Zhang X X, Sun J, Li T G, Zhang Z N, Yang G P. 2008. Diversity and spatial distribution of sediment ammonia-oxidizing crenarchaeota in response to estuarine and environmental gradients in the Changjiang Estuary and East China Sea. *Microbiology*, **154**(7): 2084-2095, https://doi.org/10.1099/mic.0.2007/013581-0.
- DeLong E F, Preston C M, Mincer T, Rich V, Hallam S J, Frigaard N U, Martinez A, Sullivan M B, Edwards R, Brito B R, Chisholm S W, Karl D M. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science*, **311**(5760): 496-503, https://doi. org/10.1126/science.1120250.
- DeLong E F, Wu K Y, Prézelin B B, Jovine R V M. 1994. High abundance of archaea in Antarctic marine picoplankton. *Nature*, **371**(6499): 695-697, https://doi.org/10.1038/ 371695a0.
- DeLong E F. 1992. Archaea in coastal marine environments. Proceedings of the National Academy of Sciences, 89(12): 5685-5689, https://doi.org/10.1073/pnas.89.12.5685.
- Deschamps P, Zivanovic Y, Moreira D, Rodriguez-Valera F, López-García P. 2014. Pangenome evidence for extensive interdomain horizontal transfer affecting lineage core and shell genes in uncultured planktonic thaumarchaeota and euryarchaeota. *Genome Biology and Evolution*, 6(7): 1549-1563, https://doi.org/10.1093/gbe/evu127.
- Diaz R J, Rosenberg R. 2008. Spreading dead zones and consequences for marine ecosystems. *Science*, **321**(5891): 926-929, https://doi.org/10.1126/science.1156401.
- Doyle R J. 2002. Glycomicrobiology. Springer, Boston, MA, https://doi.org/10.1007/b113570.
- Embrandiri A, Kiyasudeen S K, Rupani P F, Ibrahim M H. 2016. Environmental xenobiotics and its effects on natural ecosystem. *In*: Singh A, Prasad S, Singh R eds. Plant Responses to Xenobiotics. Springer, Singapore. p.1-18, https://doi.org/10.1007/978-981-10-2860-1_1.
- Francis C A, Roberts K J, Beman J M, Santoro A E, Oakley B B. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the* United States of America, **102**(41): 14683-14688, https:// doi.org/10.1073/pnas.0506625102.

- Frigaard N U, Martinez A, Mincer T J, Delong E F. 2006. Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. *Nature*, 439(7078): 847-850, https://doi.org/10.1038/nature04435.
- Galand P E, Casamayor E O, Kirchman D L, Potvin M, Lovejoy C. 2009. Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. *The ISME Journal*, 3(7): 860-869, https://doi. org/10.1038/ismej.2009.23.
- Grenz C, Denis L, Pringault O, Fichez R. 2010. Spatial and seasonal variability of sediment oxygen consumption and nutrient fluxes at the sediment water interface in a subtropical lagoon (New Caledonia). *Marine Pollution Bulletin*, **61**(7-12): 399-412, https://doi.org/10.1016/j. marpolbul.2010.06.014.
- Haro-Moreno J M, Rodriguez-Valera F, López-García P, Moreira D, Martin-Cuadrado A B. 2017. New insights into marine group III Euryarchaeota, from dark to light. *The ISME Journal*, **11**(5): 1102-1117, https://doi. org/10.1038/ismej.2016.188.
- Hatzenpichler R. 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. *Applied* and Environmental Microbiology, 78(21): 7501-7510, https://doi.org/10.1128/AEM.01960-12.
- He H, Zhen Y, Mi T Z, Yu Z G. 2016. Community composition and abundance of ammonia-oxidizing archaea in sediments from the Changjiang estuary and its adjacent area in the East China Sea. *Geomicrobiology Journal*, 33(5): 416-425, https://doi.org/10.1080/01490451.2014.9 86695.
- Herndl G J, Reinthaler T, Teira E, Van Aken H, Veth C, Pernthaler A, Pernthaler J. 2005. Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. *Applied* and *Environmental Microbiology*, **71**(5): 2303-2309, https://doi.org/10.1128/AEM.71.5.2303-2309.2005.
- Hugoni M, Taib N, Debroas D, Domaizon I, Dufournel I J, Bronner G, Salter I, Agogué H, Mary I, Galand P E. 2013. Structure of the rare archaeal biosphere and seasonal dynamics of active ecotypes in surface coastal waters. *Proceedings of the National Academy of Sciences of the* United States of America, **110**(15): 6004-6009, https:// doi.org/10.1073/pnas.1216863110.
- Ingalls A E, Shah S R, Hansman R L, Aluwihare L I, Santos G M, Druffel E R M, Pearson A. 2006. Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proceedings of the National Academy* of Sciences of the United States of America, **103**(17): 6442-6447, https://doi.org/10.1073/pnas.0510157103.
- Iverson V, Morris R M, Frazar C D, Berthiaume C T, Morales R L, Armbrust E V. 2012. Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. *Science*, **335**(6068): 587-590, https://doi. org/10.1126/science.1212665.
- Jain R K, Kapur M, Labana S, Lal B, Sarma P M, Bhattacharya D, Thakur I S. 2005. Microbial diversity: application of micro-organisms for the biodegradation of xenobiotics. *Current Science*, 89(1): 101-112.

- Jiao N, Luo T, Zhang R, Yan W, Lin Y, Johnson Z I, Tian J, Yuan D, Yang Q, Zheng Q, Sun J, Hu D, Wang P. 2014. Presence of *Prochlorococcus* in the aphotic waters of the western Pacific Ocean. *Biogeosciences*, **11**(8): 2391-2400, https://doi.org/10.5194/bg-11-2391-2014.
- Jin T, Zhang T, Ye L, Lee O O, Wong Y H, Qian P Y. 2011. Diversity and quantity of ammonia-oxidizing Archaea and Bacteria in sediment of the Pearl River Estuary, China. *Applied Microbiology and Biotechnology*, **90**(3): 1137-1145, https://doi.org/10.1007/s00253-011-3107-8.
- Kadota H, Ishida Y. 1972. Production of volatile sulfur compounds by microorganisms. *Annual Review of Microbiology*, **26**(1): 127-138, https://doi.org/10.1146/ annurev.mi.26.100172.001015.
- Kärcher U, Schröder H, Haslinger E, Allmaier G, Schreiner R, Wieland F, Haselbeck A, König H. 1993. Primary structure of the heterosaccharide of the surface glycoprotein of *Methanothermus fervidus. Journal of Biological Chemistry*, **268**(36): 26821-26826, https://doi. org/10.1016/S0021-9258(19)74185-4.
- Karner M B, DeLong E F, Karl D M. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature*, 409(6819): 507-510, https://doi.org/10.1038/35054051.
- Kelly J, Logan S M, Jarrell K F, VanDyke D J, Vinogradov E. 2009. A novel N-linked flagellar glycan from *Methanococcus maripaludis*. *Carbohydrate Research*, **344**(5): 648-653, https://doi.org/10.1016/j.carres.2009.01.006.
- Kembel S W, Cowan P D, Helmus M R, Cornwell W K, Morlon H, Ackerly D D, Blomberg S P, Webb C O. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**(11): 1463-1464, https://doi. org/10.1093/bioinformatics/btq166.
- Khelaifia S, Drancourt M. 2012. Susceptibility of archaea to antimicrobial agents: applications to clinical microbiology. *Clinical Microbiology and Infection*, **18**(9): 841-848, https://doi.org/10.1111/j.1469-0691.2012.03913.x.
- Konings W N, Albers S V, Koning S, Driessen A J M. 2002. The cell membrane plays a crucial role in survival of bacteria and archaea in extreme environments. *Antonie Van Leeuwenhoek*, **81**(1-4): 61-72, https://doi. org/10.1023/A:1020573408652.
- Könneke M, Bernhard A E, de la Torre J R, Walker C B, Waterbury J B, Stahl D A. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature*, 437(7058): 543-546, https://doi.org/10.1038/nature03911.
- Könneke M, Schubert D M, Brown P C, Hügler M, Standfest S, Schwander T, von Borzyskowski L S, Erb T J, Stahl D A, Berg I A. 2014. Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO₂ fixation. *Proceedings of the National Academy of Sciences of the United States of America*, **111**(22): 8239-8244, https://doi. org/10.1073/pnas.1402028111.
- Kubo K, Lloyd K G, Biddle J F, Amann R, Teske A, Knittel K. 2012. Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. *The ISME Journal*, 6(10): 1949-1965, https:// doi.org/10.1038/ismej.2012.37.

- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**(6): 1547-1549, https://doi.org/10.1093/molbev/ msy096.
- Lee S M, Chao A. 1994. Estimating population size via sample coverage for closed capture-recapture models. *Biometrics*, 50(1): 88-97, https://doi.org/10.2307/2533199.
- Lewalter K, Müller V. 2006. Bioenergetics of archaea: ancient energy conserving mechanisms developed in the early history of life. *Biochimica et Biophysica Acta (BBA) -Bioenergetics*, **1757**(5-6): 437-445, https://doi. org/10.1016/j.bbabio.2006.04.027.
- Li D J, Zhang J, Huang D J, Wu Y, Liang J. 2002. Oxygen depletion off the Changjiang (Yangtze River) estuary. *Science in China Series D: Earth Sciences*, 45(12): 1137-1146, https://doi.org/10.1360/02yd9110.
- Li J T, Gu L Y, Bai S J, Wang J, Su L, Wei B B, Zhang L, Fang J S. 2021. Characterization of particle-associated and free-living bacterial and archaeal communities along the water columns of the South China Sea. *Biogeosciences*, 18: 113-133, https://doi.org/10.5194/bg-18-113-2021.
- Li X G, Yu Z M, Song X X, Cao X H, Yuan Y Q. 2011. The seasonal characteristics of dissolved oxygen distribution and hypoxia in the Changjiang Estuary. *Journal of Coastal Research*, 27(6A): 52-62, https://doi.org/10.2112/ JCOASTRES-D-11-00013.1.
- Liu B, Ye G B, Wang F P, Bell R, Noakes J, Short T, Zhang C L. 2009. Community structure of Archaea in the water column above gas hydrates in the Gulf of Mexico. *Geomicrobiology Journal*, **26**(6): 363-369, https://doi. org/10.1080/01490450902929290.
- Liu H D, Zhang C L, Yang C Y, Chen S Z, Cao Z W, Zhang Z W, Tian J W. 2017. Marine Group II dominates planktonic Archaea in water column of the Northeastern South China Sea. *Frontiers in Microbiology*, 8: 1098, https://doi. org/10.3389/fmicb.2017.01098.
- Liu J W, Fu B B, Yang H M, Zhao M X, He B Y, Zhang X H. 2015. Phylogenetic shifts of bacterioplankton community composition along the Pearl Estuary: the potential impact of hypoxia and nutrients. *Frontiers in Microbiology*, 6: 64, https://doi.org/10.3389/fmicb.2015.00064.
- Liu J W, Yu S L, Zhao M X, He B Y, Zhang X H. 2014. Shifts in archaeaplankton community structure along ecological gradients of Pearl Estuary. *FEMS Microbiology Ecology*, **90**(2): 424-435, https://doi.org/10.1111/1574-6941.12404.
- Liu J W, Zhu S Q, Liu X Y, Yao P, Ge T T, Zhang X H. 2020. Spatiotemporal dynamics of the archaeal community in coastal sediments: assembly process and co-occurrence relationship. *The ISME Journal*, **14**(6): 1463-1478, https://doi.org/10.1038/s41396-020-0621-7.
- Liu M, Xiao T, Wu Y, Zhou F, Zhang W C. 2011. Temporal distribution of the archaeal community in the Changjiang Estuary hypoxia area and the adjacent East China Sea as determined by denaturing gradient gel electrophoresis and multivariate analysis. *Canadian Journal of Microbiology*, 57(6): 504-513, https://doi.org/10.1139/w11-037.

- Lohrenz S E, Redalje D G, Cai W J, Acker J, Dagg M. 2008. A retrospective analysis of nutrients and phytoplankton productivity in the Mississippi River plume. *Continental Shelf Research*, **28**(12): 1466-1475, https://doi.org/10. 1016/j.csr.2007.06.019.
- López-García P, López-López A, Moreira D, Rodríguez-Valera F. 2001. Diversity of free-living prokaryotes from a deepsea site at the Antarctic Polar Front. *FEMS Microbiology Ecology*, **36**(2-3): 193-202, https://doi.org/10.1016/ S0168-6496(01)00133-7.
- Martens-Habbena W, Berube P M, Urakawa H, de la Torre J R, Stahl D A. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature*, **461**(7266): 976-979, https://doi.org/10.1038/ nature08465.
- Martin-Cuadrado A B, Garcia-Heredia I, Moltó A G, López-Úbeda R, Kimes N, López-García P, Moreira D, Rodriguez-Valera F. 2015. A new class of marine Euryarchaeota group II from the Mediterranean deep chlorophyll maximum. *The ISME Journal*, 9(7): 1619-1634, https://doi.org/10.1038/ismej.2014.249.
- Martín-Cuadrado A B, López-García P, Alba J C, Moreira D, Monticelli L, Strittmatter A, Gottschalk G, Rodríguez-Valera F. 2007. Metagenomics of the deep Mediterranean, a warm bathypelagic habitat. *PLoS One*, 2(9): e914, https://doi.org/10.1371/journal.pone.0000914.
- Martin-Cuadrado A B, Rodriguez-Valera F, Moreira D, Alba J C, Ivars-Martínez E, Henn M R, Talla E, López-García P. 2008. Hindsight in the relative abundance, metabolic potential and genome dynamics of uncultivated marine archaea from comparative metagenomic analyses of bathypelagic plankton of different oceanic regions. *The ISME Journal*, 2(8): 865-886, https://doi.org/10.1038/ ismej.2008.40.
- Massana R, DeLong E F, Pedrós-Alió C. 2000. A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. *Applied and Environmental Microbiology*, **66**(5): 1777-1787, https://doi.org/0.1128/AEM.66.5.1777-1787.2000.
- Massana R, Murray A E, Preston C M, DeLong E F. 1997. Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Applied and Environmental Microbiology*, **63**(1): 50-56, https://doi.org/10.1128/aem.63.1.50-56.1997.
- Mescher M F, Strominger J L. 1976. Purification and characterization of a prokaryotic glycoprotein from the cell envelope of Halobacterium salinarium. *Journal of Biological Chemistry*, 251(7): 2005-2014, https://doi. org/10.1016/S0021-9258(17)33647-5.
- Michoud G, Ngugi D K, Barozzi A, Merlino G, Calleja M L, Delgado-Huertas A, Morán X A G, Daffonchio D. 2021. Fine-scale metabolic discontinuity in a stratified prokaryote microbiome of a Red Sea deep halocline. *The ISME Journal*, **15**: 2351-2365, https://doi.org/10.1038/ s41396-021-00931-z.
- Mirete S, de Figueras C G, González-Pastor J E. 2011. Diversity of Archaea in Icelandic hot springs based on

16S rRNA and chaperonin genes. *FEMS Microbiology Ecology*, **77**(1): 165-175, https://doi.org/10.1111/j.1574-6941.2011.01095.x.

- Miroshnichenko M L. 2004. Thermophilic microbial communities of deep-sea hydrothermal vents. *Microbiology*, **73**(1): 1-13, https://doi.org/10.1023/B: MICI.0000016360.21607.83.
- Molina V, Belmar L, Ulloa O. 2010. High diversity of ammonia-oxidizing archaea in permanent and seasonal oxygen-deficient waters of the eastern South Pacific. *Environmental Microbiology*, **12**(9): 2450-2465, https:// doi.org/10.1111/j.1462-2920.2010.02218.x.
- Mosier A C, Francis C A. 2008. Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. *Environmental Microbiology*, **10**(11): 3002-3016, https://doi.org/10.1111/ j.1462-2920.2008.01764.x.
- Naqvi S W A, Jayakumar D A, Narvekar P V, Naik H, Sarma V V S S, D'Souza W, Joseph S, George M D. 2000. Increased marine production of N₂O due to intensifying anoxia on the Indian continental shelf. *Nature*, **408**(6810): 346-349, https://doi.org/10.1038/35042551.
- Ochsenreiter T, Pfeifer F, Schleper C. 2002. Diversity of Archaea in hypersaline environments characterized by molecular-phylogenetic and cultivation studies. *Extremophiles*, **6**(4): 267-274, https://doi.org/10.1007/ s00792-001-0253-4.
- Oksanen J, Blanchet F G, Kindt R, Legendre P, O'Hara R B, Simpson G L, Solymos P, Henry M and Stevens W H. 2010. Vegan: community ecology package. R package, version 1.17-4. http://cran.R-project.org.
- Orita R, Umehara A, Komorita T, Choi J W, Montani S, Komatsu T, Tsutsumi H. 2015. Contribution of the development of the stratification of water to the expansion of Dead Zone: a sedimentological approach. *Estuarine*, *Coastal and Shelf Science*, **164**: 204-213, https://doi. org/10.1016/j.ecss.2015.07.028.
- Orsi W D, Smith J M, Wilcox H M, Swalwell J E, Carini P, Worden A Z and Santoro A E. 2015. Ecophysiology of uncultivated marine euryarchaea is linked to particulate organic matter. *The ISME Journal*, **9**(8): 1747-1763, https://doi.org/10.1038/ismej.2014.260.
- Pereira O, Hochart C, Auguet J C, Debroas D, Galand P E. 2019. Genomic ecology of Marine Group II, the most common marine planktonic archaea across the surface ocean. *MicrobiologyOpen*, 8(9): e00852, https://doi. org/10.1002/mbo3.852.
- Pester M, Schleper C, Wagner M. 2011. The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. *Current Opinion in Microbiology*, 14(3): 300-306, https:// doi.org/10.1016/j.mib.2011.04.007.
- Pohlschroder M, Pfeiffer F, Schulze S, Halim M F A. 2018. Archaeal cell surface biogenesis. *FEMS Microbiology Reviews*, 42(5): 694-717, https://doi.org/10.1093/femsre/fuy027.
- Polónia A R M, Cleary D F R, da Costa Coelho F J R, Becking L E, De Voogd N J, Toha A H A, Gomes N C M. 2018.

Compositional analysis of archaeal communities in high and low microbial abundance sponges in the Misool coral reef system, Indonesia. *Marine Biology Research*, **14**(6): 537-550, https://doi.org/10.1080/17451000.2018.149897 7.

- Polónia A R M, Cleary D F R, Duarte L N, de Voogd N J, Gomes N C M. 2014. Composition of Archaea in seawater, sediment, and sponges in the Kepulauan Seribu reef system, Indonesia. *Microbial Ecology*, 67(3): 553-567, https://doi.org/10.1007/s00248-013-0365-2.
- Qin W, Heal K R, Ramdasi R, Kobelt J N, Martens-Habbena W, Bertagnolli A D, Amin S A, Walker C B, Urakawa H, Könneke M, Devol A H, Moffett J W, Armbrust E V, Jensen G J, Ingalls A E, Stahl D A. 2017. Nitrosopumilus maritimus gen. nov., sp. nov., Nitrosopumilus cobalaminigenes sp. nov., Nitrosopumilus oxyclinae sp. nov., and Nitrosopumilus ureiphilus sp. nov., four marine ammonia-oxidizing archaea of the phylum Thaumarchaeota. International Journal of Systematic and Evolutionary Microbiology, 67(12): 5067-5079, https:// doi.org/10.1099/ijsem.0.002416.
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner F O. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, **41**(D1): D590-D596, https://doi.org/10.1093/nar/gks1219.
- R Core Team. 2013. R: A language and environment for statistical computing. Vienna, Austria.
- Ren M L, Feng X Y, Huang Y J, Wang H, Hu Z, Clingenpeel S, Swan B K, Fonseca M M, Posada D, Stepanauskas R, Hollibaugh J T, Foster P G, Woyke T, Luo H W. 2019. Phylogenomics suggests oxygen availability as a driving force in Thaumarchaeota evolution. *The ISME Journal*, 13(9): 2150-2161, https://doi.org/10.1038/s41396-019-0418-8.
- Restrepo-Ortiz C X, Auguet J C, Casamayor E O. 2014. Targeting spatiotemporal dynamics of planktonic SAGMGC-1 and segregation of ammonia-oxidizing Thaumarchaeota ecotypes by newly designed primers and quantitative polymerase chain reaction. *Environmental Microbiology*, **16**(3): 689-700, https://doi.org/10.1111/ 1462-2920.12191.
- Schäffer C, Messner P. 2001. Glycobiology of surface layer proteins. *Biochimie*, **83**(7): 591-599, https://doi.org/10. 1016/S0300-9084(01)01299-8.
- Schleper C, Jurgens G, Jonuscheit M. 2005. Genomic studies of uncultivated archaea. *Nature Reviews Microbiology*, 3(6): 479-488, https://doi.org/10.1038/nrmicro1159.
- Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett W S, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. *Genome Biology*, **12**(6): 1-18, https://doi.org/10.1186/gb-2011-12-6-r60.
- Shen J P, Zhang L M, Zhu Y G, Zhang J B, He J Z. 2008. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environmental Microbiology*, **10**(6): 1601-1611, https://doi.org/10.1111/j.1462-2920.2008.01578.x.

- Shin D S, Pratt A J, Tainer J A. 2014. Archaeal genome guardians give insights into eukaryotic DNA replication and damage response proteins. *Archaea*, 2014: 206735, https://doi.org/10.1155/2014/206735.
- Sleytr U B, Schuster B, Egelseer E M, Pum D. 2014. S-layers: principles and applications. *FEMS Microbiology Reviews*, 38(5): 823-864, https://doi.org/10.1111/1574-6976.12063.
- Sogin M L, Morrison H G, Huber J A, Welch D M, Huse S M, Neal P R, Arrieta J M, Herndl G J. 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the United States of America*, **103**(32): 12115-12120, https://doi.org/10.1073/pnas.0605127103.
- Stahl D A, de la Torre J R. 2012. Physiology and diversity of ammonia-oxidizing Archaea. Annual Review of Microbiology, 66(1): 83-101, https://doi.org/10.1146/ annurev-micro-092611-150128.
- Tarn J, Peoples L M, Hardy K, Cameron J, Bartlett D H. 2016. Identification of free-living and particle-associated microbial communities present in hadal regions of the Mariana Trench. *Frontiers in Microbiology*, 7: 665, https://doi.org/10.3389/fmicb.2016.00665.
- Tian J W, Yang Q X, Zhao W. 2009. Enhanced diapycnal mixing in the South China Sea. *Journal of Physical Oceanography*, **39**(12): 3191-3203, https://doi.org/10. 1175/2009JPO3899.1.
- Tolar B B, King G M, Hollibaugh J T. 2013. An analysis of Thaumarchaeota populations from the Northern Gulf of Mexico. *Frontiers in Microbiology*, 4: 72, https://doi. org/10.3389/fmicb.2013.00072.
- Tseng C H, Chiang P W, Lai H C, Shiah F K, Hsu T C, Chen Y L, Wen L S, Tseng C M, Shieh W Y, Saeed I, Halgamuge S, Tang S L. 2015. Prokaryotic assemblages and metagenomes in pelagic zones of the South China Sea. BMC Genomics, 16(1): 219, https://doi.org/10.1186/s12864-015-1434-3.
- Tully B J. 2019. Metabolic diversity within the globally abundant Marine Group II Euryarchaea offers insight into ecological patterns. *Nature Communications*, 10: 271. https://doi.org/10.1038/s41467-018-07840-4.
- Vila-Costa M, Barberan A, Auguet J C, Sharma S, Moran M A, Casamayor E O. 2013. Bacterial and archaeal community structure in the surface microlayer of high mountain lakes examined under two atmospheric aerosol loading scenarios. *FEMS Microbiology Ecology*, 84(2): 387-397, https://doi.org/10.1111/1574-6941.12068.
- Voisin S, Houliston R S, Kelly J, Brisson J R, Watson D, Bardy S L, Jarrell K F, Logan S M. 2005. Identification and characterization of the unique N-linked glycan common to the flagellins and S-layer glycoprotein of *Methanococcus voltae. Journal of Biological Chemistry*, 280(17): 16586-16593, https://doi.org/10.1074/jbc.M500329200.
- Wang K, Ye X S, Zhang H J, Chen H P, Zhang D M, Liu L. 2016. Regional variations in the diversity and predicted metabolic potential of benthic prokaryotes in coastal northern Zhejiang, East China Sea. *Scientific Reports*, 6: 38709, https://doi.org/10.1038/srep38709.

- Wang L, Zhang J, Li H L, Yang H, Peng C, Peng Z S, Lu L. 2018. Shift in the microbial community composition of surface water and sediment along an urban river. *Science* of the Total Environment, 627: 600-612, https://doi. org/10.1016/j.scitotenv.2018.01.203.
- Weisblum B, Davies J. 1968. Antibiotic inhibitors of the bacterial ribosome. *Bacteriological Reviews*, **32**(4 Pt 2): 493-528.
- Wickham H, Chang W. 2009. ggplot2: an implementation of the Grammar of Graphics. R package version 0.8.3.
- Wu D M, Dai Q P, Liu X Z, Fan Y P, Wang J X. 2019. Comparison of bacterial community structure and potential functions in hypoxic and non-hypoxic zones of the Changjiang Estuary. *PLoS One*, **14**(6): e0217431, https://doi.org/10.1371/journal.pone.0217431.
- Wuchter C, Abbas B, Coolen M J L, Herfort L, van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl G J, Middelburg J J, Schouten S, Damsté J S S. 2006. Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, **103**(33): 12317-12322, https://doi.org/10.1073/pnas.0600756103.
- Yakimov M M, Cono V L, Smedile F, Deluca T H, Juárez S, Ciordia S, Fernández M, Albar J P, Ferrer M, Golyshin P N, Giuliano L. 2011. Contribution of crenarchaeal autotrophic ammonia oxidizers to the dark primary production in Tyrrhenian deep waters (Central Mediterranean Sea). *The ISME Journal*, 5(6): 945-961, https://doi.org/10.1038/ismej.2010.197.
- Ye Q, Wu Y, Zhu Z Y, Wang X N, Li Z Q, Zhang J. 2016. Bacterial diversity in the surface sediments of the hypoxic zone near the Changjiang Estuary and in the East China Sea. *MicrobiologyOpen*, 5(2): 323-339, https://doi.

org/10.1002/mbo3.330.

- Ye Y Z, Doak T G. 2009. A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. *PLoS Computational Biology*, 5(8): e1000465, https://doi.org/10.1371/journal.pcbi.1000465.
- Yu S L, Yao P, Liu J W, Zhao B, Zhang G L, Zhao M X, Yu Z G, Zhang X H. 2016. Diversity, abundance, and niche differentiation of ammonia-oxidizing prokaryotes in mud deposits of the eastern China marginal seas. *Frontiers in Microbiology*, 7: 137, https://doi.org/10.3389/ fmicb.2016.00137.
- Zatopek K M, Gardner A F, Kelman Z. 2018. Archaeal DNA replication and repair: new genetic, biophysical and molecular tools for discovering and characterizing enzymes, pathways and mechanisms. *FEMS Microbiology Reviews*, 42(4): 477-488, https://doi.org/10.1093/femsre/ fuy017.
- Zeng Y H, Li H Y, Jiao N Z. 2007. Phylogenetic diversity of planktonic archaea in the estuarine region of East China Sea. *Microbiological Research*, **162**(1): 26-36, https://doi. org/10.1016/j.micres.2006.03.007.
- Zhang C L, Xie W, Martin-Cuadrado A B, Rodriguez-Valera F. 2015. Marine Group II Archaea, potentially important players in the global ocean carbon cycle. *Frontiers in Microbiology*, 6: 1108, https://doi.org/10.3389/fmicb. 2015.01108.
- Zhong H H, Lehtovirta-Morley L, Liu J W, Zheng Y F, Lin H Y, Song D L, Todd J D, Tian J W, Zhang X H. 2020. Novel insights into the Thaumarchaeota in the deepest oceans: their metabolism and potential adaptation mechanisms. *Microbiome*, 8(1): 78, https://doi.org/10.1186/s40168-020-00849-2.

Electronic supplementary material

Supplementary material (Supplementary Tables S1–S5 and Figs.S1–S6) is available in the online version of this article at https://doi.org/10.1007/s00343-021-1035-8.