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Archaeal diversity and abundance within different layers of summer sea-ice and seawater from Prydz Bay, Antarctica

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Abstract Fluorescence in-situ hybridization (FISH) and 16S rRNA gene clone library analyses were used to determine the abundance and diversity of archaea in Prydz Bay, Antarctica. Correlation analysis was also performed to assess links between physicochemical parameters and archaeal abundance and diversity within the sea-ice. Samples of sea-ice and seawater were collected during the 26th Chinese National Antarctic Research Expedition. The results of FISH showed that archaea were relatively abundant within the top layer of the sea-ice, and correlation analysis suggested that the concentration of NH_4^+ might be one of the main factors underlying this distribution pattern. However, using 16S rRNA gene libraries, archaea were not detected in the top and middle layers of the sea-ice. All archaeal clones obtained from the bottom layer of the sea-ice were grouped into the Marine Group I *Crenarchaeota* while the archaeal clones from seawater were assigned to Marine Group I *Crenarchaeota*, Marine Group II *Euryarchaeota*, and Marine Group III *Euryarchaeota*. Overall, the findings of this study showed that the diversity of archaea in the sea-ice in Prydz Bay was low.

Keywords **Antarctica, summer sea-ice, archaea, diversity, abundance**

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1 Introduction

Research has shown that archaea are widespread and abundant in many different environments[1-3]. The domain Archaea consists of five distinct phyla, *Crenarchaeota*, *Euryarchaeota*, *Nanoarchaeota*, *Korarchaeota*, and *Thaumarchaeota*[4-7]. Archaea were first identified in Antarctic ecosystems in 1988[8], and to date archaea have been found in marine waters, frozen lakes, and other Antarctic marine environments[9-10]. Previous studies in Antarctic waters have found high archaeal abundance with archaea accounting for 34% of prokaryotes[11-12]. In Antarctic marine environments, Group I *Crenarchaeota* and Group II *Euryarchaeota* were predominant, and Group I was generally more abundant than Group II[13].

Archaea make up a sizable proportion of the biomass in Antarctic frazil ice[9]. However, archaea have not been identified in sea-ice until recently, although they are abundant in Arctic pelagic communities. Brown and Bowman[14] and Brinkmeyer and Knittel[15] found that archaea were absent from Arctic summer sea-ice and sea-ice near Antarctica. Junge et al.[16] investigated the abundance of archaea using fluorescence in-situ hybridization (FISH) and found that they accounted for 0–3.4% of the total cells in Arctic winter sea-ice. Collins et al.[17] showed that archaeal sequences were present in both Arctic winter sea-ice and seawater clone libraries, and the majority (91%) of sequences from the archaeal libraries belonged to Marine Group I[17]. Cowie et al.[10] found a low abundance of archaea in Antarctic sea-ice with archaea making up just 6.6% of the prokaryotic community. The majority (90.8%) of the sequences were

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clustered within Marine Group I[10].

The main aim of this study was to quantify archaeal abundance using FISH and to describe archaeal diversity using 16S rRNA gene molecular methods. In addition, correlation analysis was carried out to investigate the relationship between physicochemical parameters and archaeal diversity and abundance within the sea-ice. To our knowledge, this is the first study to investigate the abundance and diversity of archaea in different sea-ice layers. Therefore, this study provides important new information on archaea in Antarctic sea-ice ecosystems and forms a theoretical basis for future research.

2 Materials and methods

2.1 Sample collection and DNA extraction

Sea-ice samples (Table 1) were collected from Prydz Bay, Antarctica, during the 26th Chinese National Antarctic Research Expedition (26th CHINARE; November 2008 to May 2009). Samples were collected using the method described by Li et al.[18] Sea-ice samples were kept frozen during transportation and were stored at −20°C until analysis. The fluorometric method was used to measure chlorophyll *a* (Chl *a*) concentrations. Total organic carbon (TOC) and total organic nitrogen (TON) were measured by researchers at Tongji University in Shanghai. The concentrations of nitrate–nitrogen (NO_3^-), ammonium–nitrogen, phosphate–phosphorus, and silica were measured using a continuous flow nutrient analyzer (Skalar San++, Skalar UK (Ltd.), York, UK). The DNA extraction was carried out following the method described by Li et al.[18]

2.2 Fluorescence *in-situ* hybridization (FISH)

The abundance of archaea was determined using FISH with probe ARCH915-Cy3 (5'-GTG CTC CCC CGC CAA TTC CT-3'). Hybridization was carried out following the method described by Junge et al.[16] A Nikon 80i fluorescence microscope was used to visualize samples under excitation and emission filters of 360 and 420 nm, respectively, for DAPI-labeled cells (blue), and under excitation and emission filters of 550 and 570 nm, respectively, for Cy3-labeled cells (red). For each sample, cells in 20 randomly selected microscopic fields were counted and the concentration was then determined as follows: cells·cm^{-3} = (the mean number of cells per field/the volume of the sample trapped on the filter) × (the area of the filter/the area of the field).

Table 1 Ice core collection stations and sea-ice layers

Station	Date (YYYY-MM-DD)	Longitude	Latitude	Length of ice core /cm	Layers /cm
I01	2009-12-09	76°26′589″E	69°17′500″S	167	Top: 0–20 Middle: 76–96 Bottom: 161–167
I02	2009-12-09	76°26′756″E	69°17′572″S	168	Top: 0–20 Middle: 75–94 Bottom: 158–168
I03	2009-12-09	76°26′750″E	69°17′564″S	168	Top: 0–20 Middle: 74–94 Bottom: 158–168
I04	2009-12-14	76°27′368″E	69°19′774″S	183	Top: 0–20 Middle: 83–103 Bottom: 172–183
I05	2009-12-14	76°27′308″E	69°19′774″S	191	Top: 0–20 Middle: 80–100 Bottom: 181–191

2.3 Phylogenetic analysis

Samples from station I02 were selected for the analysis of diversity. The 16S rRNA gene was amplified with the primer pair Arch-21F, and Arch-958R[19]. Clone library construction, screening, and processing followed the methods described by DeLong[19]. Briefly, the PCR products were purified with a gel extraction kit (Watson, Shanghai, China) according to the manufacturer's instructions. Cloning was conducted with the pGEM-T Vector (Promega, Madison, WI, USA) following the manufacturer's instructions. Libraries were screened for the 0.8 kb 16S rDNA insert by PCR with M13 primers. The restriction endonuclease Alu I was used for amplified ribosomal DNA restriction analysis (ARDRA). The sequences that had different ARDRA patterns were sequenced on an ABI PRISM 3730 sequencer.

Clustal X1.8, BioEdit Sequence Alignment Editor (version 5.0.9), and MEGA (version 4) software packages were used for the analysis of all nucleotide sequences. The 16S rRNA gene sequences obtained in this study

were submitted to the GenBank database (accession nos. JQ753077–JQ753094).

2.4 Estimation of operational taxonomic unit richness

To estimate richness and to ensure rigorous comparison among the communities, sequences were placed into operational taxonomic units (OTUs) at a level of sequence similarity of ⩾ 97%. All OTU richness and sample coverage calculations were performed with the program EstimateS (version 8.0). The OTU richness was calculated for each sample using the non-parametric estimator Chao 1[20]. Extrapolation using best-fit regression analysis was performed to calculate the point at which 95% confidence intervals (CIs) did not overlap[21].

2.5 Correlation analysis

Correlation analysis was performed to investigate the relationship between physicochemical parameters and archaeal diversity and abundance within the sea-ice. The analysis was performed using SPSS Statistics 17 software package.

3 Results

3.1 Physicochemical parameters of the sea-ice

Physical and chemical characteristics of the sea-ice samples are shown in Table 2. The highest concentrations of Chl *a*, TOC, TON, ammonium–nitrogen, phosphate, nitrate–nitrogen, and silicate were found within the bottom layer of the sea-ice.

3.2 Total cell numbers and archaeal abundance

The fluorescent stain 4,6-diamidino-2-phenylindole 2HCl (DAPI) for DNA was used to determine the abundance of total bacteria in sea-ice samples melted in the dark at 4℃ (Figure 1a). The total number of bacterial cells in the sea-ice samples was 1.97×10^5–1.8×10^6 cells·cm^{-3}. The mean numbers of bacterial cells in the top, middle, and bottom layers of the sea-ice were $(8.84\pm0.66)\times10^5$, $(2.78\pm1.02)\times10^5$, and $(9.26\pm4.4)\times10^5$ cells·cm^{-3}, respectively. The results of FISH showed that the total number of archaeal cells in the sea-ice was 5.1×10^4–2.5×10^5 cells·cm^{-3} (Figure 1b), and the mean numbers of archaeal cells in the top, middle, and bottom layers were $(2.5\pm2.2)\times10^5$, $(1.05\pm0.97)\times10^5$, and $(5.12\pm1.17)\times10^4$ cells·cm^{-3}, respectively. The total number of bacterial cells in seawater samples was 1.5×10^4–6.8×10^4 cells·cm^{-3} and the mean abundance of archaea in the seawater was $(3.4\pm2.9)\times10^4$ cells·cm^{-3}.

Table 2 Physical and chemical characteristics of sea-ice

Station	Section	T_m/℃	Salinity/‰	TOC /(mg·L^{-1})	TON /(μg·mL^{-1})	Chl *a* /(μg·L^{-1})	PO_4^{3-} /(μg·L^{-1})	SiO_4^{3-} /(μg·L^{-1})	NH_4^+ /(μg·L^{-1})	$(NO_3^- + NO_2^-)$ /(μg·L^{-1})
I01	I01T	−2.7	7.1	2.213	0.5013	0.264	20.34	234.88	255.55	11.55
	I01M	−2.0	4.1	1.434	0.4729	0.150	47.72	105.46	61.60	10.42
	I01B	−1.45	5.3	7.989	2.117	8.142	395.88	293.35	489.67	704.67
I02	I02T	−2.3	7.1	1.509	0.471	3.513	177.41	78.58	217.95	15.05
	I02M	−1.9	4.2	1.033	0.253	0.117	14.73	130.25	ND	10.53
	I02B	−1.6	6.8	7.074	1.692	7.499	289.76	384.11	73.79	513.33
I03	I03T	−2.4	7.4	1.332	0.294	0.892	38.49	79.57	ND	9.87
	I03M	−2.5	3.7	0.781	0.167	0.103	13.64	114.13	40.20	8.87
	I03B	−1.0	6.1	8.452	1.824	7.095	353.78	397.46	ND	376.73
I04	I04T	−1.5	4.6	0.953	0.322	0.311	12.03	14.17	103.23	3.33
	I04M	−1.9	3.8	1.442	0.229	7.466	6.78	83.95	120.93	9.59
	I04B	−1.1	7.9	11.4	3.078	8.885	603.55	823.18	387.89	622.58
I05	I05T	−1.8	3.0	1.162	0.279	0.845	166.54	120.44	394.34	7.56
	I05M	−2.3	4.5	0.923	0.302	1.405	20.18	124.93	274.61	6.81
	I05B	−1.7	7.6	10.99	2.592	5.473	180.60	322.77	884.83	14.89

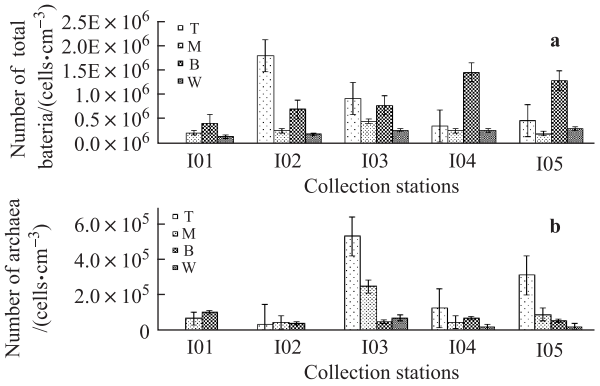

Figure 1 The abundance of total bacteria and archaea in sea-ice and seawater samples. T, M, and B represent the top, middle, and bottom layers of sea-ice, respectively, and W represents seawater.

3.3 Correlation analysis

Alu I enzyme pattern analysis was used in this study. Four phylotypes were found in the 16S rRNA gene clone analysis of samples of the bottom layer of the sea-ice and nine phylotypes were detected in the seawater samples. Correlation analysis was performed to investigate the relationship between the number of phylotypes and the physicochemical parameters of the sea-ice and the seawater. The results presented in Table 3 show that archaeal diversity was significantly positively correlated with salinity (r=0.965, P=0.035) and the concentration of silicate (r=0.996, P=0.004). It was therefore suggested that salinity and the concentration of silicate could play an important role in determining the distribution of archaeal cells in sea-ice. At sites I02 and I03, there was a significant negative correlation between the abundance of archaea and the concentration of ammonium–nitrogen (r=−1.000), but there was no obvious correlation between the abundance and other physicochemical parameters.

3.4 Analysis of archaeal 16S rRNA gene clone libraries

In total, 144 positive archaeal 16S rRNA gene clones from the top, middle, and bottom layers of sea-ice from station I02 and from seawater were PCR screened with M13 primers. After sequencing and chimera checking, a total of 37 clones of 16S rRNA gene sequences from the four archaeal clone libraries (12 from the bottom layer and 25 from seawater) were obtained for further analysis. The majority of sequences were similar to 16S rRNA gene sequences in GenBank with similarities ranging from 90%–100%. Sequences having the same type after ARDRA were assigned to the same phylotype. Four and nine phylotypes were defined within the bottom layer of the sea-ice and the seawater libraries, respectively. The phylogenetic relationships among clones are displayed in Figure 2. All the clones from bottom layer libraries were affiliated with Marine Group I and most of the clones from the libraries were similar to environmental sequences recovered from Arctic or Antarctic sea-ice and seawater. Most clones from the seawater libraries were affiliated with Marine Group I and Marine Group III; only one clone was affiliated with Marine Group II. Six sequences detected in sea-ice and six sequences detected in seawater samples were associated with clones from Arctic winter sea-ice samples (99%)[17] and all were affiliated with Marine Group I. The archaeal sequence I02W106 affiliated with Marine Group II was associated with the clones from Arctic sea-ice samples (99%). Marine Group III, as a unique representative phylotype in the library, was associated with the clone SHZW623 from seawater.

3.5 Estimation of OTU richness of clone libraries

As the clone libraries were constructed concurrently, the archaeal diversity of Antarctic sea-ice and seawater clone libraries underwent comparative analysis to extrapolate species richness. Plotting the cumulative number of OTUs estimated against the sampling effort generates species richness curves (Figure 3). The highest estimated number of species was present in the seawater samples, which contained seven OTUs (95% CI, 7 to 37), whereas the bottom sea-ice samples yielded three OTUs (95% CI, 2 to 6). The 95% CIs for the bottom sea-ice and seawater sample communities overlapped, therefore there was no significant difference in species richness among the samples (P>0.05). The Shannon's index of diversity for bottom sea-ice (I02B) and seawater (I02W) samples was 0.72 and 1.34, respectively.

Table 3 Correlation analysis between physicochemical parameters and archaeal diversity and abundance within sea-ice

		T_m	Salinity	TOC	TON	Chl *a*	PO_4^{3-}	SiO_4^{3-}	NH_4^+	$NO_3^- + NO_2^-$
Diversity I02	Correlation	0.684	0.965*	−0.043	0.092	−0.201	−0.268	0.996**	0.851	0.436
	Significance	0.316	0.035	0.957	0.908	0.799	0.732	0.004	0.352	0.564
FISH I02	Correlation	0.705	−0.822	0.105	0.035	−0.297	−0.438	0.328	−1.00**	0.167
	Significance	0.502	0.385	0.933	0.978	0.808	0.712	0.787	.	0.893
I03	Correlation	−0.762	−0.420	−0.537	−0.656	−0.531	−0.588	−0.649	−1.000**	−0.850
	Significance	0.238	0.580	0.463	0.344	0.469	0.412	0.351	.	0.150
I04	Correlation	0.276	−0.059	−0.281	−0.213	−0.907	−0.234	−0.316	−0.295	−0.250
	Significance	0.822	0.963	0.818	0.863	0.277	0.850	0.795	0.810	0.839
I05	Correlation	0.240	−0.827	−0.588	−0.611	−0.689	0.313	−0.620	−0.447	−0.535
	Significance	0.846	0.380	0.600	0.581	0.516	0.797	0.574	0.705	0.640

Notes: **significant correlation at the 0.01 level (double sided); *significant correlation at the 0.05 level (double sided).

Figure 2 Phylogenetic tree of archaeal 16S rRNA gene sequences obtained from the bottom sea-ice layer at station I02 and seawater under ice clone libraries. The trees were constructed using Jukes-Cantor distances and the neighbor-joining method. Bootstrap support values >55% (of 100 replicates) are shown. The numbers are the numbers of closely related sequences with similarity >99% in the same library and the numbers in parentheses are the accession numbers of sequences. The scale bar indicates the estimated number of base changes per nucleotide sequence position. I02B and I02W represent the bottom layer of sea-ice and the seawater samples, respectively.

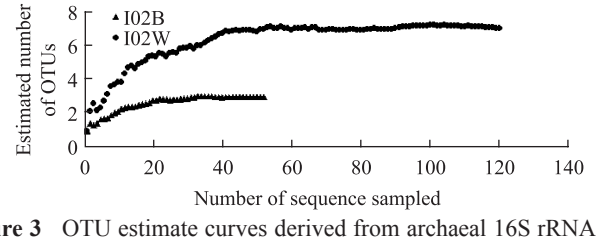

Figure 3 OTU estimate curves derived from archaeal 16S rRNA gene clone libraries data. I02B and I02W represent the bottom layer of sea-ice and the seawater samples, respectively.

4 Discussion

Our results showed that the mean abundance of archaea in sea-ice was 5.1×10^4–2.5×10^5 cells·cm^{-3}, making up 6.6% of the total picoplankton abundance in the Antarctic sea-ice samples. Results of the present study were consistent with those of Cowie et al.[10], who also found that archaea were present in low abundance and accounted for 6.6% of the

prokaryotic community in Antarctic sea-ice. The abundance of archaea was also low in Arctic sea-ice samples and Brinkmeyer and Knittel[15] showed that 93%–96% of DAPI-stained cells hybridized with the general bacterial probe, and archaea were below the detection limit of FISH in Arctic and Antarctic pack-ice communities. Junge et al.[16] determined that archaeal cells made up 0–3.4% of total cells in Arctic winter sea-ice. In our study, the mean abundance of archaea was lowest in the bottom layer of the sea-ice, and archaeal abundance showed a significant negative correlation with the concentration of ammonium–nitrogen, suggesting that the concentration of ammonium–nitrogen might be identified as a major driver of this distribution pattern.

Church et al.[13] reported that the abundance of archaea increased significantly with the depth of seawater, averaged 2.1×10^4 cells·cm^{-3}, and accounted for 9%–39% of the total picoplankton abundance in Antarctic Peninsula water. These findings are consistent with our results; the mean abundance of archaea in our seawater sample was $(3.4\pm2.9)\times10^4$ cells·cm^{-3}, making up 14.7% of the total picoplankton abundance. Kirchman et al.[22] found that *Crenarchaeota* increased from about 10% of prokaryotes in surface waters of the western Arctic waters to as much as 40% in samples from depths of 100–200 m. Kirchman et al.[22] also found that the abundance of *Crenarchaeota* was correlated with ammonium–nitrogen concentrations.

The present study provides characterization of archaeal diversity in sea-ice and seawater collected from Antarctica, based on 16S rRNA gene clone libraries. Overall, a total of 37 archaeal 16S rRNA gene clones from the bottom layer of the sea-ice and seawater samples revealed low phylogenetic diversity. All sequences derived from the bottom layer of the sea-ice fell into one archaeal phylogenetic lineage, Marine Group I. Collins et al.[17] found that Marine Group I *Crenarchaeota* was the dominant archaeal phylotype in Arctic sea-ice and seawater libraries, and they also detected the rarer Marine Group II *Euryarchaeota*. Cowie et al.[10] obtained similar results with the majority (90.8%) of sequences clustered within Marine Group I. Archaeal abundance measured using quantitative PCR in Antarctic sea-ice samples was found to be low, accounting for just 6.6% of the prokaryotic community[10]. We did not detect Marine Group II in our Antarctic sea-ice samples.

Marine Group I and Marine Group III were the predominant groups in our Antarctic seawater samples and our results suggested that Group II populations are not numerically abundant in Antarctic plankton communities. Our results were in agreement with previous studies. Galand et al.[23] reported an archaeal community dominated by Group III *Euryarchaeota* and demonstrated that Group III *Euryarchaeota* were more abundant in deep-water masses, representing the second most abundant tags after Group I *Crenarchaeota* in the deep Atlantic layer of the central Arctic Ocean. Group III *Euryarchaeota* were first identified in the Northeast Pacific[24] and were later detected in different marine environments. However, Group III is rarely represented in marine ecosystems, in which Marine Group I *Crenarchaeota* and Group II *Euryarchaeota* are the most abundant groups. Recently, metagenomic analysis revealed the presence of genes that resembled those found in ammonia-oxidizing bacteria in Group III DNA fragments, suggesting that at least some members of Group III *Euryarchaeota* may oxidize ammonia[23]. If this finding is confirmed it suggests that the group could play an important role in the nitrogen cycle of Antarctica.

The objectives of this study were to quantify archaeal abundance and to investigate the distribution of archaea in Antarctic sea-ice and seawater. Our data showed that the mean abundance of archaea was higher in the top and middle layers of sea-ice. However, we did not detect archaea in the top and middle layer sea-ice samples on 16S rRNA gene analysis, suggesting that the general archaeal primer may underestimate total archaeal copy numbers. The diversity of archaea in sea-ice was low and our findings were consistent with previous results.

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