# Diversity and biogeography of eukaryotic microorganism in the deep-sea sediment of the Eastern Indian Ocean

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## Abstract

**Marine eukaryotic microbe plays an important role in many biogeochemical processes, such as carbon, nitrogen and primary and secondary productivity in marine ecosystem. Despite being prevalent, the diversity and spatial distribution of eukaryotic microbe in the deep-sea sediment of the Eastern Indian Ocean (EIO) remain rarely studied. Here, the phylogenetic diversity and community structure of eukaryotic microorganism community in relation to environmental factors in the deep-sea sediment of the EIO were investigated by the high throughout sequencing of 18S rRNA genes technique. In general, Fungi were the most abundant phylum in all samples, followed by Rhizaria, Chloroplastida and so on. Saccharomycetes, Unclassified _c_Ascomycota, Exobasidiomycetes, Magnoliophyta and Silicofilosea dominated the eukaryotic microorganism at the order level. UniFrac PCoA (principal coordinate analysis) and UPGMA (unweighted pair group method with arithmetic mean) analyses revealed that the benthic eukaryotic microbial communities varied with geographic distance. TOC, TP and sediment texture could significantly shape the community structure and maintain the diversity of benthic eukaryotic microorganisms in the deep-sea sediments of EIO. These results provide a foundation for interpreting future studies on the adaptive mechanism of eukaryotic microbe under deep-sea conditions.**

## Keywords

**Eukaryotic microorganism, Illumina MiSeq sequencing, the Eastern Indian Ocean (EIO), 18S rRNA gene, deep-sea sediment.**

## 1. Introduction

Eukaryotic microbe is the common and numerous organisms in the world. It is widely distributed on the planet, including soil, water and harsh conditions, even extreme heat, cold, pressed and saline environment [1-8]. Marine eukaryotic microbe communities play a decisive role in the marine ecosystem. They can drive a wide range of many biogeochemical processes, such as carbon, nitrogen and primary and secondary productivity, makes them indispensable to any marine ecosystem [3, 4, 9, 10]. Marine eukaryotic microorganism influences the nutrient composition and energy flow in both sediments and water column through food webs and mediating chemical transformations [9, 11]. Since its indisputable importance in marine ecosystem, the composition and distribution of eukaryotic microorganism has been widely studied in different ocean [1, 3, 4, 5, 10, 12, 13].

As the third largest ocean, the Indian Ocean approximately covers 20% of the water on the Earth's surface. The Eastern Indian Ocean (EIO) is a part of the Indo-Pacific warm pool and strongly influenced by monsoon, however, as for the biological research on the Eastern Indian Ocean is much fewer [14]. Compared to the other oceans, the Indian Ocean remains one of the most undersampled and least understood basin of the world oceans with respect to its physical and biogeochemical dynamics [15], especially the research on eukaryotic microbe in the deep-sea sediment of the EIO.

The deep-sea was identified the least explored regions on the earth, which environments is known as an extreme environment (high pressure, absence of sunlight and generally low temperature or occasionally extremely high, >400 °C near hydrothermal vents) [16].

In the present study, sediment samples of the EIO were collected, and then Illumina MiSeq sequencing of 18S rRNA genes technique was introduced to investigate the diversity and spatial distribution of eukaryotic microorganism, and how the environmental factors to shape the communities of eukaryotic microorganism in the deep-sea surface sediments of the EIO.

## 2. Materials and methods

### 2.1 Sample collection

The study area lies in the EIO (6°S-6°N, 80°E-88°E). Three sediment samples were selected in water depths ranging from 3898 to 5172 m during a multidisciplinary cruise carried out in the EIO by R/V Shiyan 1 from March 8 to May 27, 2015 (Fig. 1). The surface sediment samples (0-2 cm) were collected in triplicate and stored at -20 °C in sterile polyethylene bags and then transferred to the laboratory and stored at -80 °C until DNA extraction and sediment property analyses.

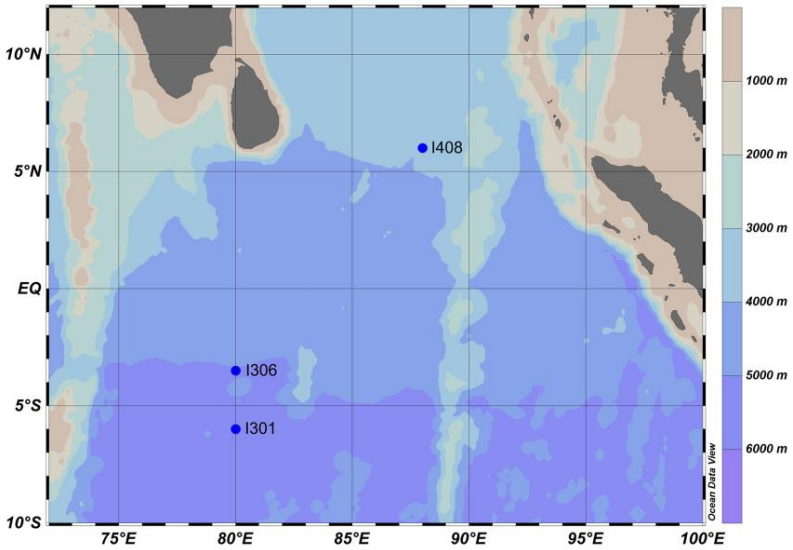


Fig. 1 Location of sampling stations

### 2.2 Environmental parameter measurements

The in situ seawater depth of each sampling site was recorded by the CTD (Seabird, NE). The grain size of the sediment was analyzed using a Mastersizer 2000 particle size analyzer (Malvern, England), and the particle was divided into three size fraction: >63, 4-63 and <4 μm [17]. Sediments were freeze-dried and sieved (2 mm) for further analyses. The total nitrogen (TN) and total phosphorus (TP) were analyzed according to Yuan et al [18]. Total organic carbon (TOC) content was measured with a Shimadzu TOC-V CSH/CSN and a SSM-5000A solid sample module (Shimazdu, Japan).

### 2.3 Total community DNA extraction and Illumina MiSeq sequencing

Total DNA was extracted directly from 1.0 g of the sample using FastDNA® spin kit (MP bio, Santa Ana, USA) following the manufacturer's protocol. And all DNA samples met the criteria: 260 nm/280 nm > 1.70, and 260 nm/230 nm > 1.8. The entire V7-V8 region of 18S rRNA gene was selected for targeting amplicons (forward primer, CGWTAACGAACGAG; reverse primer, AICCA TTCAATCGG). Next generation sequencing library preparations and Illumina MiSeq sequencing were conducted at GENEWIZ, Inc. (Suzhou, China). All sequences obtained from this study were deposited in NCBI sequence read archive (SRA) with the accession numbers PRJNA492346.

### 2.4 Statistical analysis

The software package QIIME 1.9.1 was used for 18S rRNA gene data analysis [19]. The forward and reverse reads were from the original DNA fragments were merged by using FLASH and assigned to

sample based on barcode and truncated by cutting off the barcode and primer sequence [20]. Sequences were grouped into operational taxonomic units (OTUs) using the clustering program VSEARCH 1.9.6 at 97% sequence identity. Rarefaction analysis was conducted using the original detected OTUs. The taxonomic assignment was performed by the RDP classifier at confidence threshold of 0.8 [21].

Shannon index, Simpson index, Chao1 index and Good's coverage of the three sediment samples were determined as described previously [22]. Weighted and unweighted UniFrac, Bray-Curtis and principal coordinate analysis (PCoA) were calculated by QIIME. Unweighted pair group method with arithmetic mean (UPGMA) clustering was conducted by unweighted and weighted UniFrac based on the protocol published previously [19].

Redundancy analysis (RDA) was executed in CANOCO 4.5 for Windows (Microcomputer Power, Ithaca, USA) to determine the correlations between the community composition of eukaryotic microorganism and environmental parameters [23]. Pearson correlation coefficient analysis was evaluated to relate the environmental variables and the diversity and richness indices of the 18S rRNA gene sequences.

## 3. Results

### 3.1 Environmental parameters of study area

The basic environmental variables at each sampling site were summarized in Table 1. The water depth is from 3898 m to 5172 m. The content of TOC, TN, TP and clay mainly decreased as the depth increase. Compared to other sampling sites, the depth of sampling site was deeper at site I301 and lower TOC, TN, TP and present of clay were detected at the same site, while the shallowest and highest TOC and TN were observed at site I408.

Table 1 Environmental characteristics of sediments obtained from each study site

| Site | Depth (m) | TOC (g/kg) | TN (mg/kg) | TP (mg/kg) | Sand (%) | Silt (%) | Clay (%) |
|---|---|---|---|---|---|---|---|
| I301 | 5172 | 1.67 | 12.78 | 0.18 | 2.98 | 89.75 | 7.27 |
| I306 | 4989 | 4.44 | 26.83 | 0.32 | 9.76 | 60.67 | 29.57 |
| I408 | 3898 | 34.82 | 38.47 | 0.21 | 3.21 | 52.15 | 44.64 |

### 3.2 Diversity analysis of eukaryotic microorganisms

After quality filtering the raw reads, a total of 560690 18S rRNA gene sequences (with an average of 186897) were obtained. The numbers of different eukaryotic OTUs at the 97% similarity level ranged from 458 to 991 per sample with an average of 745 OTUs (Table 2). The rarefaction curves obtained with the OTUs number nearly reached saturation level for all the samples, which demonstrated bacterial communities were well covered and the coverage was above 99.9% (Table 2). OTUs diversity index (Shannon and Simpson) and richness estimators (ACE and Chao1) were introduced to evaluate the sequences of this study. These four indices at site I306 were relatively higher than other sites.

Table 2 Sequencing information and diversity index analyses of 18S rRNA gene in the sediments of the EIO

| Site | Valid sequence No. | OTUs | Shannon | Simpson | Chao1 | ACE | Coverage |
|---|---|---|---|---|---|---|---|
| I301 | 136611 | 458 | 3.92 | 0.84 | 642 | 631 | 99.9% |
| I306 | 171919 | 991 | 4.97 | 0.92 | 1031 | 1066 | 99.9% |
| I408 | 252160 | 786 | 3.56 | 0.76 | 810 | 831 | 100% |

Venn diagram was introduced to evaluate the number and identity of the shared OTUs between soil samples of surface sediments from the EIO (Fig. 2). All three eukaryotic microorganisms shared 158 (10.7%) of 1472 OTUs (34.5%, 15.9% and 20.1% of the total OTUs identified at sites I301, I306 and I408, respectively)(Fig. 2). The characteristic sequence of site I301 was less than other two sites (79).

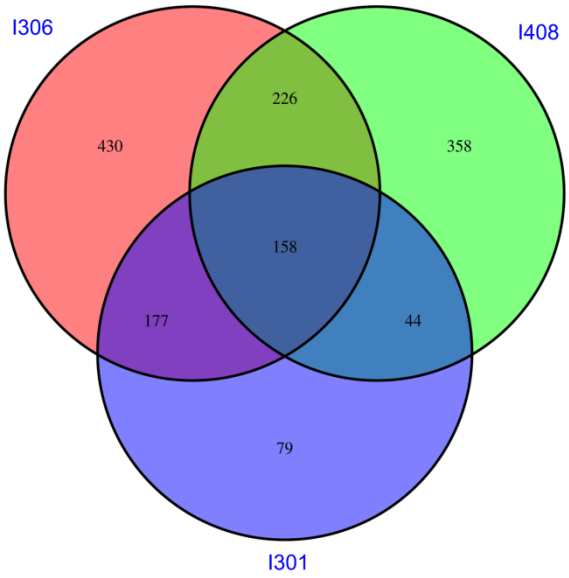


Fig. 2 Venn diagram showing the shared OTUs of eukaryotic microorganisms

### 3.3 Spatial variation in community structure of eukaryotic microorganisms

Relative abundance (RA) of the total 10 phyla of eukaryotic microorganisms from each sample accounted for more than 96.6% of the whole amplicons (Fig. 3A). Fungi were the most abundant phylum in all samples, accounting for 71.1 to 82.4 % of the total valid reads in all samples, with an average relative abundance of 78.5 %. Rhizaria was the second most abundant phylum at site I301 (12.6 %), while Chloroplastida was the second most abundant phylum at sites I306 and I408 (17.3 % and 15.6 %, respectively).

At the order level (Fig. 3B), RA of the top 20 orders from each sample accounted for more than 94.2 % of the total amplicons. Saccharomycetes was the most abundant order at site I408 (48.7 %), while Unclassified_c_Ascomycota was the most abundant order at sites I301 and I306 (36.5 % and 20.2 %, respectively). Saccharomycetes, Unclassified_c_Ascomycota, Exobasidiomycetes, Magnoliophyta and Silicofilosea dominated the eukaryotic microorganism at the order level.

### 3.4 Community analyses of the eukaryotic microorganism assemblages

The weighted UniFrac PCoA was shown in Fig. 4A. The first principal coordinate (P1) explained 58.24 % of the total community structure variation among all samples. Obviously, the sample from three sites, which located in the three different quadrants, could be separated from each other. Similar classification was revealed in the hierarchical clustering analysis (UPGMA) (Fig. 4B). Samples from sites I301 and I306 clustered together in a subgroup, while sample of site I408 was distinguished separately in the different group.

### 3.5 Environmental parameters explaining spatial variability of the eukaryotic microorganism community

Redundancy analysis (RDA) was performed to discern possible linkages between environmental factors and eukaryotic microorganism distribution (Fig. 5). Each environmental variable in the RDA biplot was represented by an arrow and the length of the individual arrow indicated how much variance was explained by that variable. The RDA analysis based on the eukaryotic microorganism community composition was in accord with the PCoA (Fig. 4A). The three sites located in the three different quadrants.

Eigenvalues (indicating strength of the model) for the first two multivariate axes were 0.78 and 0.22, respectively. The first canonical axis was positively correlated with TN, TOC and clay, but negatively correlated with the other environmental variables. The second canonical axis was positively correlated with all the factors except silt and TOC. The eukaryotic microorganism distribution at site I306 was positively correlated with TP and sand, while that at site I301 influenced by slit. The

concentrations of TN, TOC and clay positively influenced on the eukaryotic microorganism composition at site I408.

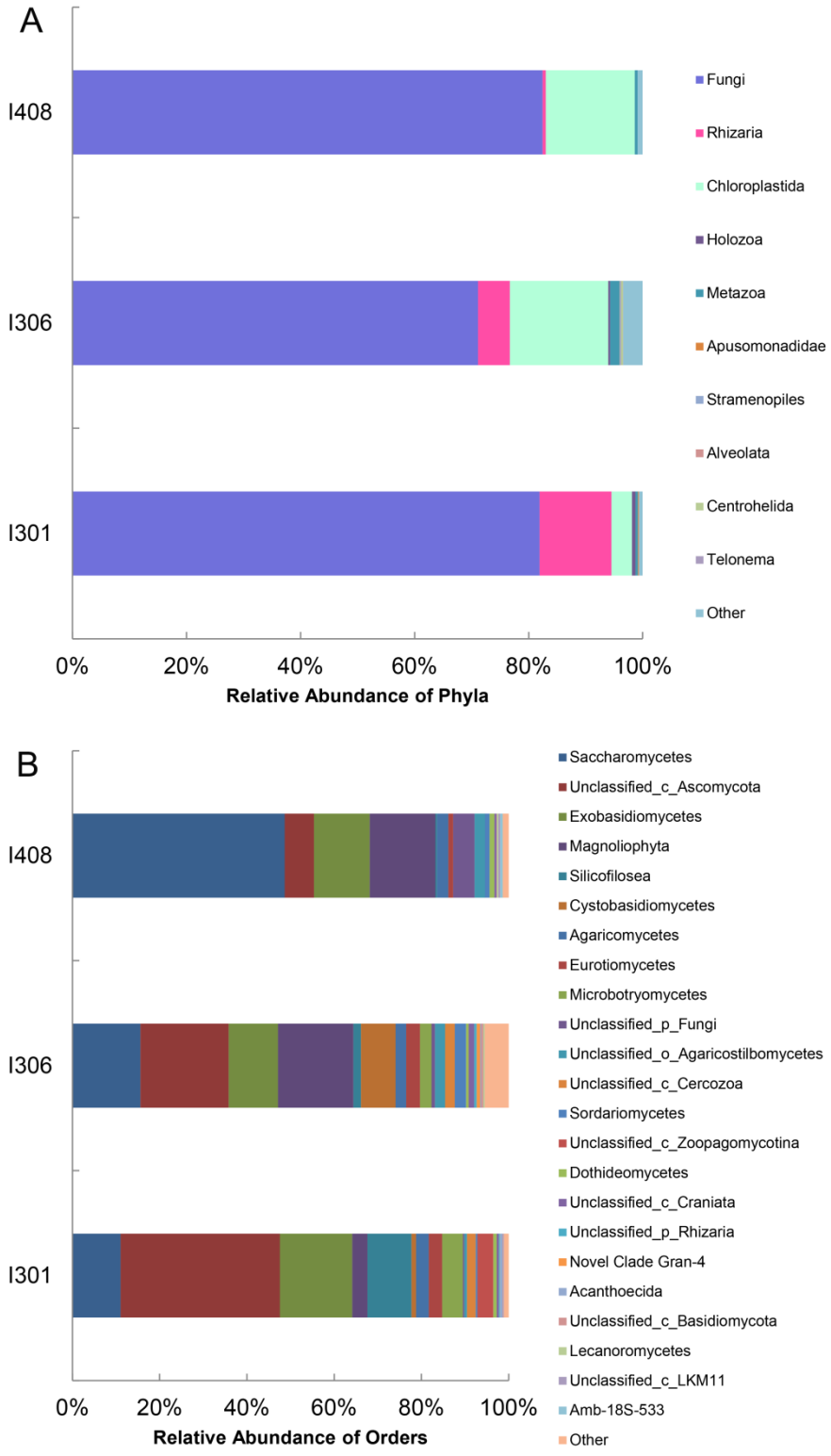


Fig. 3 Compositions of eukaryotic microorganism at the phylum (A) and order (B) levels

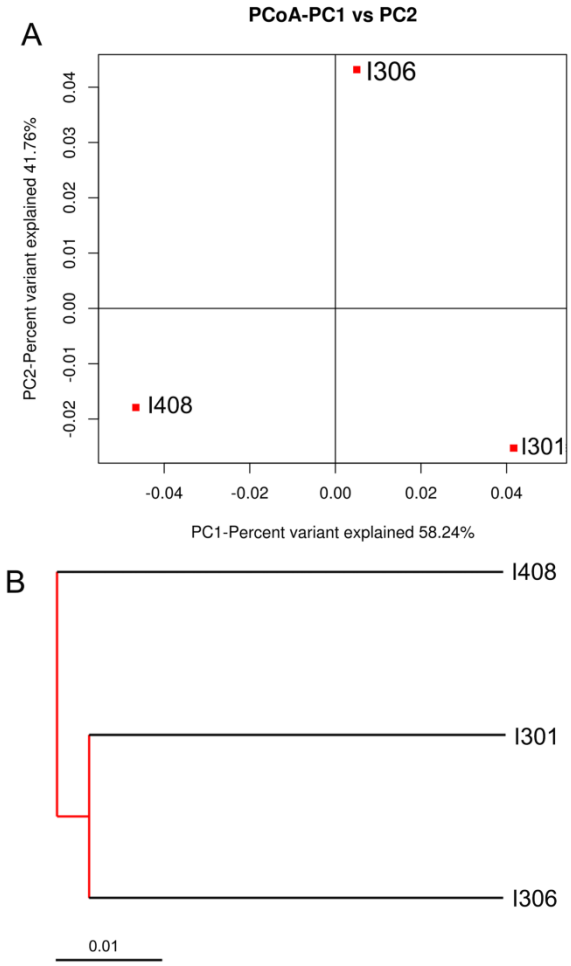


Fig. 4 Weighted UniFrac principal coordinates analysis (PCoA) (A) hierarchical clustering analysis (UPGMA) (B) of eukaryotic microorganism communities from deep-sea sediments

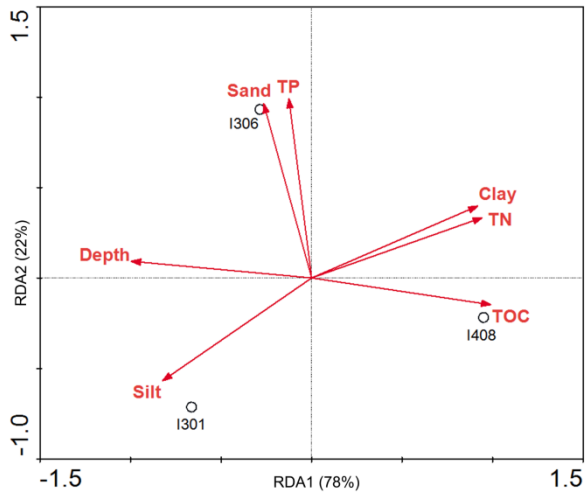


Fig. 5 Redundancy analysis (RDA) ordination plot of environmental parameters and the eukaryotic microorganism communities

## 4. Discussion

### 4.1 Diversity of eukaryotic microbial communities

In this study, the Illumina MiSeq sequencing technology was introduced to reveal the relatively rich diversity of eukaryotic microorganism community in the deep-sea surface sediments of the EIO.

Comparison of the community structure based on OTU percentages indicated a divergent distribution pattern of individual eukaryotic taxa.

In the deep sea, bathypelagic environment spatially and temporally maintain constant through long timescales, with consequence for genetic material and slow selection accumulation [24]. Hence, the deep-sea sediments with the relatively high diversity may act as a 'seed-bank' of eukaryotic microorganisms.

Fungi were the most abundant phylum in all samples (RA >70%), but Rhizaria (site I301) or Chloroplastida (sites I306 and I408) was the second most abundant phylum at the different sites (Fig. 3A). Fungi occupy a position of prominence in the eukaryotes among the water and sediment of different oceans, such as the Atlantic Ocean [10], the Mariana Trench and hydrothermal vents [25, 16], the South China Sea [5], the Pacific Ocean [26]. Although the part played by fungi in deep-sea environments remains unclear, fungi are one of the most ecologically successful eukaryotic lineages and occupy the terrestrial, aquatic and marine niches, even such harsh environment in the deep-sea sediments. Our work revealed that 13 of the top 20 orders clustered in fungi (Fig. 3B), including the three highest relative abundance orders, Saccharomycetes, Unclassified_c_Ascomycota and Exobasidiomycetes. Within these 13 orders, four groups cannot cluster in the exact order lineage belonging to fungi phylum, which will be needed further investigation.

### 4.2 Influencing environmental factors for eukaryotic microbial community

Eukaryotic microbial communities could be largely influenced by ambient environmental factors, such as oxygen availability, temperature, total organic carbon and nitrogen and phosphate [3, 27, 28, 29]. Jiang et al demonstrated temperature, salinity, phosphorus and silicate indicated to have a significant impact on the community structure of eukaryotic ultraplankton of the nSCS [3]. Based on the RDA (Fig. 5), this study indicated that TOC, TP and sediment texture could significantly shape the community structure and maintain the diversity of benthic eukaryotic microorganisms in the deep-sea sediments of EIO. Previous studies have reported that TOC, TP and TN influence microbial community through changing nutrients availability (Verma et al. 2017). Similarly, TOC and TP may affect the benthic eukaryotic microorganisms by the same way. Different sediment textures take on particle surface area supplying microbial attachment and growth with the surface area available. Moreover, sediment texture can influence oxygen holding capacity and affect physicochemical processes in the sediment and then vary the structure of the benthic microbial community [30, 31].

In the present study, UniFrac PCoA and UPGMA analyses shown that the benthic eukaryotic microbial communities varied with geographic distance (Fig. 4). Previous studies have reported that deep-sea microbial biogeography depend on the ability of microorganisms to disperse [32, 33]. Therefore, the dispersal ability gets weaker with the geographic distance increase, and then tends to variation benthic eukaryotic microbial communities in the EIO.

## 5. Conclusions

In this study, 18S rRNA gene was introduced as a biomarker to investigate the diversity and biogeography of eukaryotic microorganism in the deep-sea sediment of the Indian Ocean. The presented results showed that Fungi were predominant at the phylum level, Saccharomycetes, Unclassified_c_Ascomycota, Exobasidiomycetes, Magnoliophyta and Silicofilosea dominated the eukaryotic microorganism at the order level. A geographic separation was present in the composition of eukaryotic microbial communities. TOC, TP and sediment texture drove the eukaryotic microbial community structure. This knowledge contributes to reveal the adaptive mechanism of eukaryotic microorganism for living in the deep-sea environment.

## Acknowledgements

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