Hoshino and Inagaki
Progress in Earth and Planetary Science (2024) 11:19
https://doi.org/10.1186/s40645-024-00621-2

Progress in Earth and Planetary Science

RESEARCH ARTICLE

Open Access

Distribution of eukaryotic environmental DNA in global subseafloor sediments

Tatsuhiko Hoshino[1,2*] and Fumio Inagaki[2,3]

Abstract

The analysis of eukaryotic environmental DNA (eDNA) in sediment has the potential for understanding past ecosystems, even for taxa lacking skeletons or preserved only as a part of necromass. Despite the paleoenvironmental and ecological importance of eukaryotic eDNA in marine sediment, the duration of remaining eDNA and the species of eDNA present in marine sediment has not been well investigated. Here, we analyzed eDNA extracted from 299 sediment samples down to 678 m below the seafloor at 40 geologically distinct sites. The results showed that eukaryotic eDNA was amplified from more than 80% of the sediments with a depositional age of less than 100,000 years. The eDNA was well conserved in anoxic sediments than in oxic sediments, with PCR success rates of 48% and 18%, respectively. The eukaryotic communities include non-benthic organisms such as marine plankton, including diatoms, dinoflagellates, and coccolithophores. A freshwater diatom genus *Aulacoseira* was detected in the Baltic Sea sediments from the last glacial lacustrine environment. These results provide new insights into the global-scale distribution of the past eukaryotic eDNA preserved in marine sediment.

Keywords Environmental DNA, Eukaryote, Marine sediment, Past environment

1 Introduction

Environmental DNA remaining in the environment, including sediment, over time scales of hundreds or thousands of years is referred to as "ancient eDNA (aeDNA)" (Taberlet et al. 2018), "fossil DNA" or "paleomes" (Inagaki et al. 2005; Coolen et al. 2013). Paleoenvironmental studies utilizing those eDNA have been mainly progressed for localized settings; however, the spatiotemporal distribution of eukaryotic eDNA in deep marine sediments that capture a global historical record has not been thoroughly investigated. Knowledge gained from the comprehensive analysis of eukaryotic eDNA in deep marine sediments with a more diverse range of sedimentological, geochemical, and paleontological characteristics would provide a valuable benchmark for establishing sedimentary eukaryotic eDNA as a genetic proxy for past ecosystems.

The relatively well-preserved eDNA has been extracted and sequenced, successfully, including Holocene and Pleistocene plant and animal DNA from Siberian permafrost (Willerslev et al. 2003) and the diatomaceous DNA from the Holocene sediment of the Antarctic Lake (Coolen et al. 2004). DNA of fish and other aquatic organisms has also been recovered from surface sediments outside of permafrost and polar regions, which are cold and thus favorable for DNA preservation, being studied as "sedimentary ancient DNA (*seda*DNA)" for understanding past ecosystems (Turner et al. 2015; Kuwae et al. 2020; Ogata et al. 2021). In addition, it has been often pointed out that the adsorption of DNA to the inorganic matter (e.g., sediment particles) might facilitate the long-term preservation of DNA under anaerobic

*Correspondence:
Tatsuhiko Hoshino
hoshinot@jamstec.go.jp
[1] Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Kochi Institute for Core Sample Research, Kochi, Japan
[2] JAMSTEC, Advanced Institute for Marine Ecosystem Change (WPI-AIMEC), Yokohama, Japan
[3] Department of Earth Sciences, Graduate School of Science, Tohoku University, Sendai, Japan

sedimentary conditions (Corinaldesi et al. 2011; Pedersen et al. 2015).

Microfossils of plankton (diatoms, coccolithophorids, foraminifera, etc.) in sediment have been widely used for geological dating and paleoenvironmental reconstruction. Nevertheless, restoration of past ecosystems based on non-fossilized organisms has not been thoroughly explored, and technical difficulties remain due to their decomposable nature. For example, analysis of eukaryotic eDNA in Black Sea sediments revealed that the same virus–host relationship had continued to occur in phytoplanktonic populations for centuries (Coolen 2011). In addition, those marine planktonic populations over about 11,400 years have responded to the gradual increase in salinity after the latest marine reconstruction (Coolen et al. 2013). In the northeastern Arabian Sea, the depth and age profile of sedimentary eDNA and paleoceanographic proxies synchronously demonstrated that the protist community has responded to the variability of the oxygen minimum zone over the last 43,000 years (More et al. 2018). Recently, metagenomic analysis of eukaryotic eDNA in sediments from the Scotia Sea has shown changes in diatom species composition corresponding to glacial–interglacial cycles over the past few hundred thousand years (Armbrecht et al. 2022). However, these studies have focused on one specific site in each study, and sufficient information on the distribution of eDNA and its persistence in marine sediments of different geological settings has not yet been obtained.

In this study, we evaluated the presence of eukaryotic eDNA and its taxonomic composition by PCR amplification of 18S rRNA genes in 299 eDNA assemblages from global subseafloor sediments, including those from depths down to 678 m below the seafloor (mbsf) and dates to over 10 million years before present. The consistent methodology used in this study for DNA extraction, library preparation, and sequencing allowed for unbiased comparisons and analysis across all samples. This broad analysis of eDNA recovered from marine sediments around the world has confirmed that eukaryotic eDNA of past organisms is long preserved globally in oxic and anoxic sediment after burial from the surface world and is potentially useful for understanding ancient ecosystems that include non-fossilized organisms.

2 Methods

2.1 Sediment samples and DNA

The present study used 299 subseafloor sediment samples collected from depths of up to 678 mbsf at 40 sites over the past 20 years (Fig. 1, Additional file 1: Table S1). The all DNA samples used in this study were already extracted in an earlier study and stored at − 80 °C (Hoshino and Inagaki 2019; Wörmer et al. 2019; Hoshino et al. 2020) except for sediments of KR0805 from which

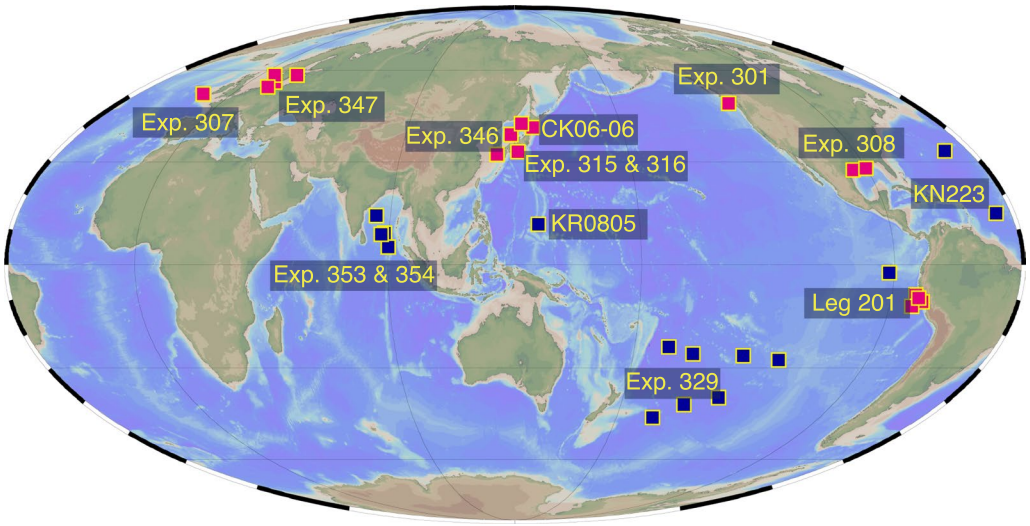

Fig. 1 A site location map. Red and dark blue squares indicate margin and open ocean sites, respectively. Sediment samples were collected at different depths from the surface to 678 mbsf during 14 scientific cruises from 40 sites. A total of 299 sediment samples were used. Leg 201: Ocean Drilling Program Leg 201 Peru Deep Biosphere, Exp. 301: Integrated Ocean Drilling Program (IODP) Expedition 301 Juan de Fuca Hydrogeology, Exp. 307: IODP Expedition 307 Modern Carbonate Mounds: Porcupine Drilling, Exp. 308: IODP Expedition 308 Gulf of Mexico Hydrogeology, Exp. 315 & 316: IODP Expeditions 315 and 316 NanTroSEIZE, Exp. 346: IODP Expedition 346 Asian Monsoon, Exp. 347: IODP Expedition 347 Baltic Sea Paleoenvironment, Exp. 353: International Ocean Discovery Program (IODP) Expedition 353 Indian Monsoon Rainfall, Exp. 354: IODP Expedition 354 Bengal Fan, CK06-06: D/V *Chikyu* Shakedown Cruise off Shimokita Peninsula, KN223: R/V *Knorr* Cruise 223 in North Atlantic, KR0805: R/V Kairei KR08-05 Cruise. This figure is adapted from Hoshino and Inagaki 2019

DNA was extracted in this study. To briefly describe the DNA extraction method, sediment samples were frozen immediately after the core sample delivery onboard and stored at − 80 °C until DNA extraction. To avoid external contamination as much as possible, all DNA extractions were performed from 5 g of the frozen sediments in a clean room of the Kochi Institute for Core Sample Research, JAMSTEC, using a Dneasy PowerMax Soil Kit (QIAGEN). The sedimentation age of each sediment sample was described in the previous study (Hoshino et al. 2020, Additional file 1: Table S1). Sedimentological characteristics by smear slide observations in the previous study were also used as part of the data analysis in this study. In brief, the sediments were smeared on slide glasses. These smear slides were then examined using a transmitted-light, petrographic microscope. The relative proportions of biogenic, mineralogic, and authigenic components were determined.

2.2 PCR amplification and library preparation

By using the primer set Euk_1391f-EukBr (Stoeck et al. 2010), a fragment of the eukaryotic 18S rRNA gene was amplified by PCR. The PCR was performed using MigtyAmp DNA polymerase (Takara Bio) for 40 cycles of (98 °C for 10 s, 58 °C for 15 s, and 68 °C for 15 s) after initial denaturation at 98 °C for 2 min. The PCR products obtained were purified by agarose gel electrophoresis, followed by index PCR for sequencing. After measuring the concentration of the products from the index PCR, equal amounts of the PCR products from each sample were mixed to prepare a sequence library. Sequencing was performed by MiSeq using the MiSeq Reagent Kit v3 (600 cycles; Illumina), according to the manufacturer's instructions. The resulting sequences were quality filtered, merged, and clustered into ZOTUs (referred to as sequence variants: ASVs in this study) using Usearch (64-bit version; www.drive5.com/usearch/) and phylogenetically identified by Mothur (Schloss et al. 2009) using the SILVA 138 SSU database (Quast et al. 2012; https://www.arb-silva.de/). Analysis of the obtained data was performed using vegan, and plotting was performed using the ggplot2 package in R.

3 Results

3.1 Amplification of 18S rRNA gene

Amplification of the V9 region of the eukaryotic 18S rRNA gene from 299 sediment samples was attempted, and 124 samples yielded visible bands on agarose gels after PCR. The success rate of PCR amplification varied from site to site (Fig. 2A): For example, in sediment samples obtained from depths shallower than 100 mbsf, successful PCR amplifications were observed in 21 out of 23 (91%) sediment samples from Expedition 308 in the Gulf of Mexico, and in all 25 sediment samples from Expedition 347 in the Baltic Sea. However, PCR amplifications were observed in only 3 out of 39 sediment samples (7%) from Expeditions 315 and 316 in the Nankai Trough forearc basin off the Kii Peninsula. No amplification was observed from all ten sediment samples cored during the D/V Chikyu Shakedown Expedition CK06-06 off the Shimokita Peninsula and collected in KR0805 at Mariana Trench. The deepest sediment sample from which amplicons were obtained was from Expedition 308 in the Gulf of Mexico, at Site U1324 392.2 mbsf, with a depositional age of ~ 94,000 years, while the oldest sediment with amplification was from Expedition 353 in the Bay of Bengal, at Site U1443, 310.7 mbsf, with an age of ~ 65 Ma.

The subseafloor sedimentary biosphere is generally anoxic in eutrophic marginal areas and oxic in oligotrophic open ocean gyres. It was estimated that oxygen reaches the basement rock in up to 37% of global marine sediments (D'Hondt et al. 2015). In the oxic oligotrophic ocean, amplification products were obtained from only 12 of 66 sediment samples (18.2%), whereas 111 of 233 samples (47.6%) were obtained from anoxic sediment samples (Fig. 2B). The higher PCR success rate in anaerobic sediments suggests a higher input of eDNA at the time of sedimentation and/or a more stable condition for DNA than in aerobic sediments.

PCR success rates were 52%, 44%, and 16% on average for 0–10 mbsf, 10–100 mbsf, and depths below 100 m, respectively. This trend suggests that PCR products were more readily obtained from shallower and younger sediments than deeper and older sediments, most likely due to the fragmentation and decomposition of the eukaryotic eDNA during burial processes (Fig. 2C). The PCR success rate for sediments deposited after 100,000 years ago including all the Exp.347 sediments (Andrén et al. 2015) was approximately 80%, which is higher than for the older sediment samples (Fig. 2D). The PCR success rate declined sharply as the sediment age exceeded 100,000 years: 27% from 100,000 to 1 million years, 11% from 1 to 10 million years, and 7.4% from > 10 million years. This trend strongly indicates that increase in depth and burial time has a critical impact on eukaryotic eDNA preservation in marine sediment.

3.2 Sequencing and eukaryotic community composition

After quality filtering, a total of 10.1 million 18S rRNA gene sequences were obtained. The average number of sequences per sample was 5.73×10^4. The obtained sequences were clustered into amplicon sequence variants (ASVs), resulting in 20,658 ASVs, of which 12,918 ASVs were classified as eukaryotes. For further analysis, only the sequences of those eukaryotic ASVs were used, and the number of sequences in each sample

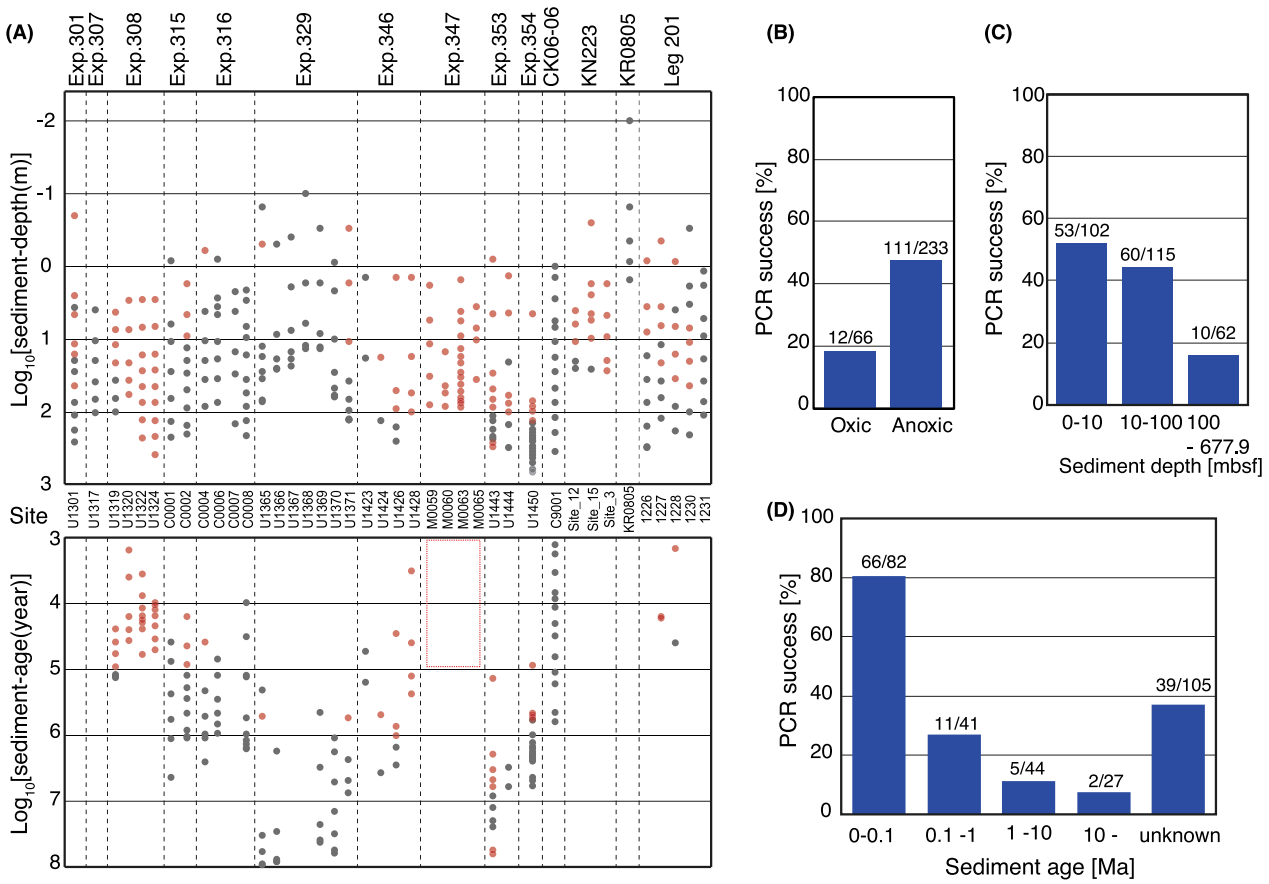

Fig. 2 PCR amplification of 18S rRNA genes from eDNA extracted from 299 marine sediment samples. **A** Success or failure of PCR amplification of 18S rRNA gene in every sample. Red and gray circles indicate PCR success and failure, respectively. Only the plots for which sediment ages are available are shown in the lower panel. The red dashed squares indicate successful amplification from all Expedition 347 sediment samples known to be younger than 105 years. **B** The summary of PCR success rates in the oxic and anoxic sediments. **C** The summary of PCR success rates in the three different sediment depth classes, 0–10, 10–100, and 100–677.9 mbsf. **D** The PCR success rates in the four different sediment age classes, 0–0.1, 0.1–1, 1–10, and > 10 million years ago

used for phylogenetic and beta-diversity analyses was randomly rarefied to 5,000 sequences. Phylogenetic classification was performed using the ARB silva v138 database as a reference. However, many of the sequences could not be classified even at the phylum level, which was either due to the lack of references in the database or to short sequence length (approximately 130 bases; see "Eukaryotic_unclassified" in Fig. 3). Excluding those unclassified sequences, Phragmoplastophyta was the most predominantly detected eukaryotic clade, followed by Ascomycota, Dinoflagellata, Vertebrata, and Basidiomycota. In the sediments in Baltic Sea collected during Expedition 347, Diatomea was the most predominant eukaryotic clade at Site M0059, whereas sequences affiliated with the phylum Dinoflagellata were more predominantly detected at Sites M0063 and M0065 than at any other sites. Three sites of Expedition 353, U1443, U1444, and U1450 in the Bay of Bengal were also distinguished by the predominance of Phragmoplastophyta and Vertebrata. The beta-diversity of eukaryotic community compositions within the recoverable pool of eDNA indicated that the communities differed significantly between marginal and open ocean sediments (PERMANOVA, $p = 0.001$, Fig. 4). The samples from the Baltic Sea (Expedition 347) constituted an independent cluster, representing different eukaryotic communities between the shallower and deeper sediment horizons. The samples from the Bay of Bengal (Expeditions 353 and 354) derived from greater than 50 m-deep beneath the open ocean were clustered in the upper right-hand corner (Fig. 4). These global-scale data set suggests that the distribution of eukaryotic eDNA is spatiotemporally vast in the marine sedimentary environment

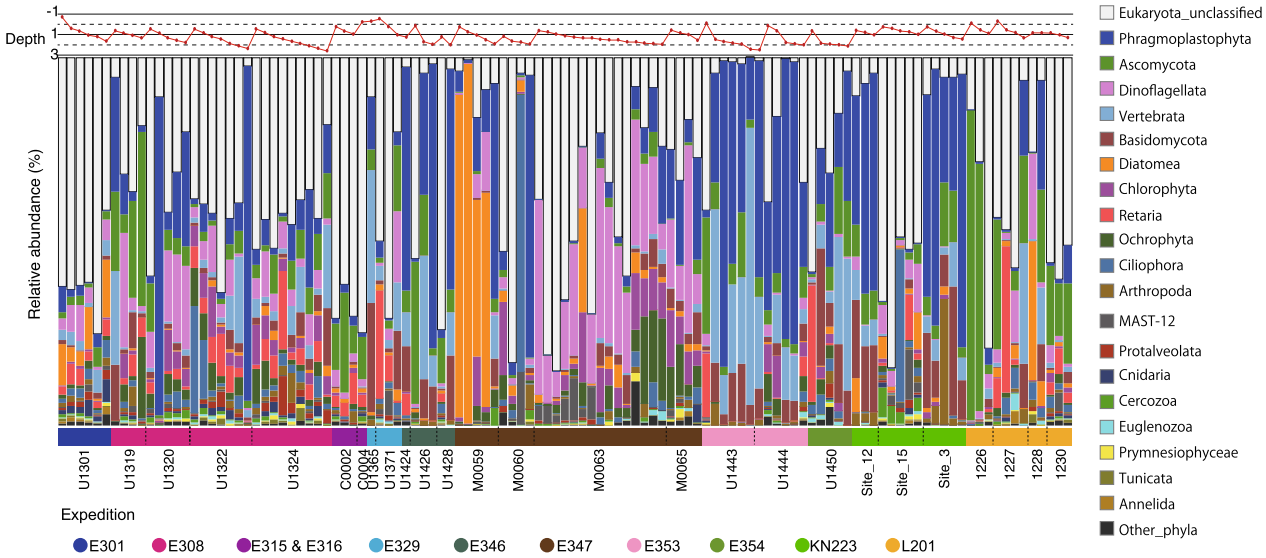

Fig. 3 Phylum-level taxonomic composition of eukaryotic communities in subseafloor sediment. The upper red line chart shows sediment depth on a logarithmic (log10) scale. The colored bar below the bar chart indicates expeditions. Classification is based on the SILVA 138 SSU database (https://www.arb-silva.de/)

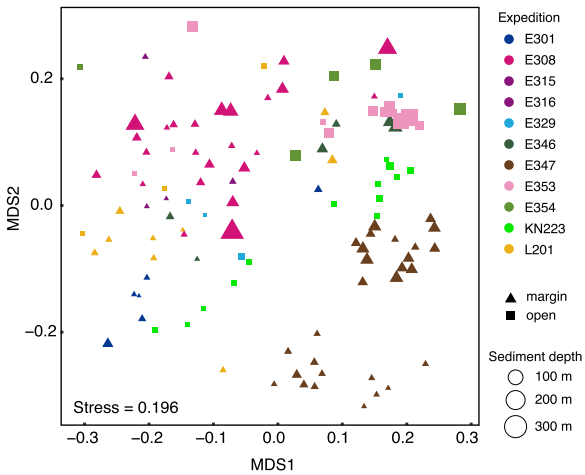

Fig. 4 NMDS ordination plots for the sedimentary eukaryotic communities. The Jaccard index was derived from the rarefied amplicon sequence variants composition. Triangles and squares indicate marginal and open ocean sediments, respectively

and depends on the geological and paleoenvironmental history (Orsi and Inagaki 2023).

3.3 Correlation of eukaryotic eDNA with microfossils

Microfossils of marine plankton are generally preserved in sediment (e.g., diatom spicules). Therefore, their stratigraphy (occurrence and disappearance) has historically been employed as an index fossil record for dating and paleoenvironmental reconstructions. We determined whether there is a correlation between the occurrence of microfossils in those sediments and the results of eDNA analysis. Smear slide analysis results from the previous studies were used to determine the occurrence of three typical microfossils, Diatomea, Prymnesiophyceae, and Retaria, in each of the sediment samples. We then examined whether there were differences in the relative abundance of eDNA of organisms corresponding to the microfossils in the sediments with and without the microfossils (Fig. 5). Among these, foraminiferal microfossils were observed on smear slides in several sediment samples; however, they were not detected by 18S rRNA gene sequencing probably because the primer set does not amplify those sequences. For Diatomea, the median relative abundance of sequences in samples where smear slides did not contain Diatomea microfossils was 4.95×10^{-3} and that of samples with microfossils was 1.25×10^{-2}, showing a significant difference in the Welch two-sample t test ($p = 0.0159$, Fig. 5A). On the other hand, for the nannoplankton Prymnesiophyceae, there was no significant difference ($p = 0.198$, Fig. 5B), with 7.90×10^{-4} and 1.66×10^{-4} for the fossil-undetected and -detected smear slides, respectively.

3.4 Correlation between paleoclimate and eukaryotic eDNA data

To test whether eukaryotic eDNA in marine sediment can be used as a proxy for paleoenvironmental changes, we focused on diatom eDNA in sediment samples from the Baltic Sea collected during Expedition 347. Expedition 347 explored a sedimentary sequence from the last

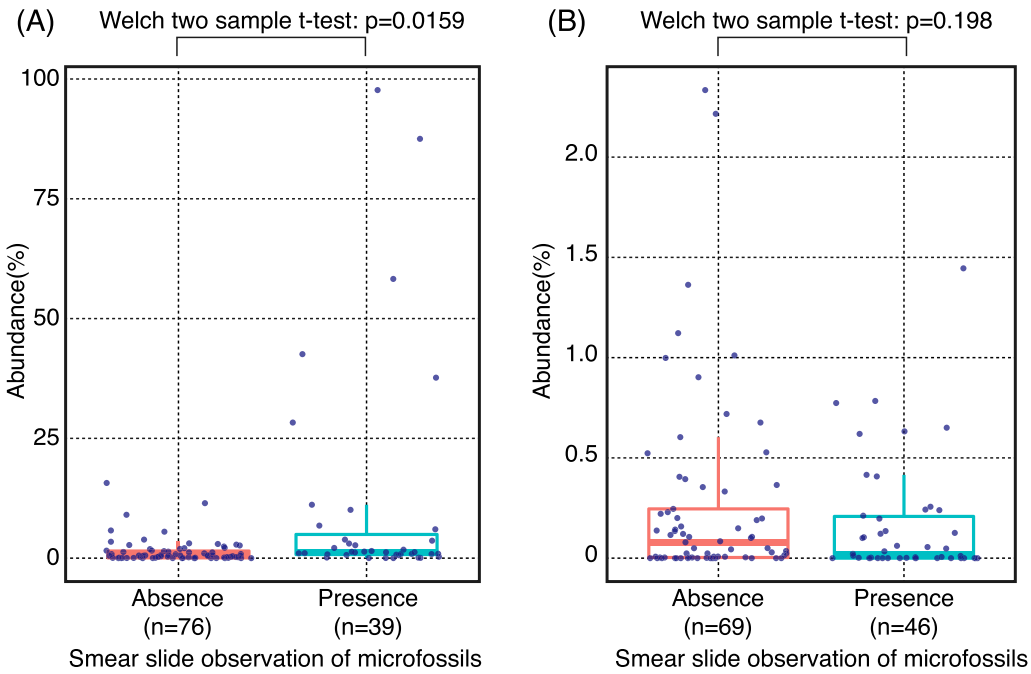

Fig. 5 Correlation between the presence or absence of microfossils in sediment samples (using the results of smear slide analysis in Ref. 3) and the relative proportion of environmental DNA. The panels A and B indicate the results for Diatomea and Prymnesiophyceae, respectively

glacial and interglacial periods, and thus the obtained sediment samples are distinguishable between freshwater and seawater depositional environments according to the depth, age, and sedimentological characteristics (Andrén et al. 2015).

Of the top 20 ASVs classified in Diatomea, 14 ASVs were identified at the genus level. These were marine genera Chaetoceros, Skeletonema, Thalassiosira, Eucampia, Attheya, and Cylindrotheca, and freshwater genera Aulacoseira, Stephanodiscus. We focused on the frequency of occurrence of ASVs in freshwater and marine sediments, focusing on Aulacoseira, which has a high abundance. Aulacoseira was detected in only seven sediments, six of which were from the Baltic Sea, out of a total of 114 sediments used for ASV analysis (Fig. 6). Among the Baltic Sea sites, *Aulacoseira* was detected at Site M0063, located in Landsort Deep, and Site M0065, located in the Borhholm Basin, while it was not detected at Sites M0059 and M0060, located in the Little Belt and southeast of Anholt at the bay entrance. At Site M0063, *Aulacoseria* were frequently detected in deep sediments, 5.6%, 2.0%, and 1.0% of 5,000 sequences at 64.2 m, 70.1 m, and 87.8 m, respectively. Four sediment samples greater than 64.2 m at Site M0063 are identified as freshwater deposits in the Glacio-lacustrine with the detection of *Aulocoseira*. On the other hand, the 17.5 m sediment with only 0.02% (1/5000sequences) of *Aulascoseira* detected is a Holocene marine sediment, so the detected sequence probably is distal origin. At Site M0065, 1.6% *Aulacoseira* was detected in the sediments from 10.3 m in the lacustrine depositional environment, while they were not detected in the upper marine sediments.

In contrast, no *Aulacoseira* were detected in the 29.2 and 33.9 m sediments of Site M0063, which are classified as Ancylus Lake sediments, or in the 36.4 m deep sediment of M0065, which is a glacial lacustrine depositional environment. The absence of *Aulacoseira* may be due to environmental factors other than salinity, but further research is needed to understand the diatom community at the time. These data indicate that *Aulacoseira* eDNA can serve as a proxy for a freshwater ecosystem in the Baltic Sea during the last glacial period.

4 Discussion

In this study, we showed the distribution of eukaryotic eDNA in marine sediments by analyzing eukaryotic eDNA in sediments from a wide range of sites around the world. With greater sediment depth or age, lower PCR success was observed, as seen at Sites U1301, C0002, U1450, U1371, and 1226 (Fig. 2). The lower PCR success rate could be due to the gradual degradation of eukaryotic eDNA over time. Deeper and older subseafloor sediments are typically unfavorable habitats for active growth and/or long-term survival of most eukaryotes due to energy and space limitations, with some exceptions,

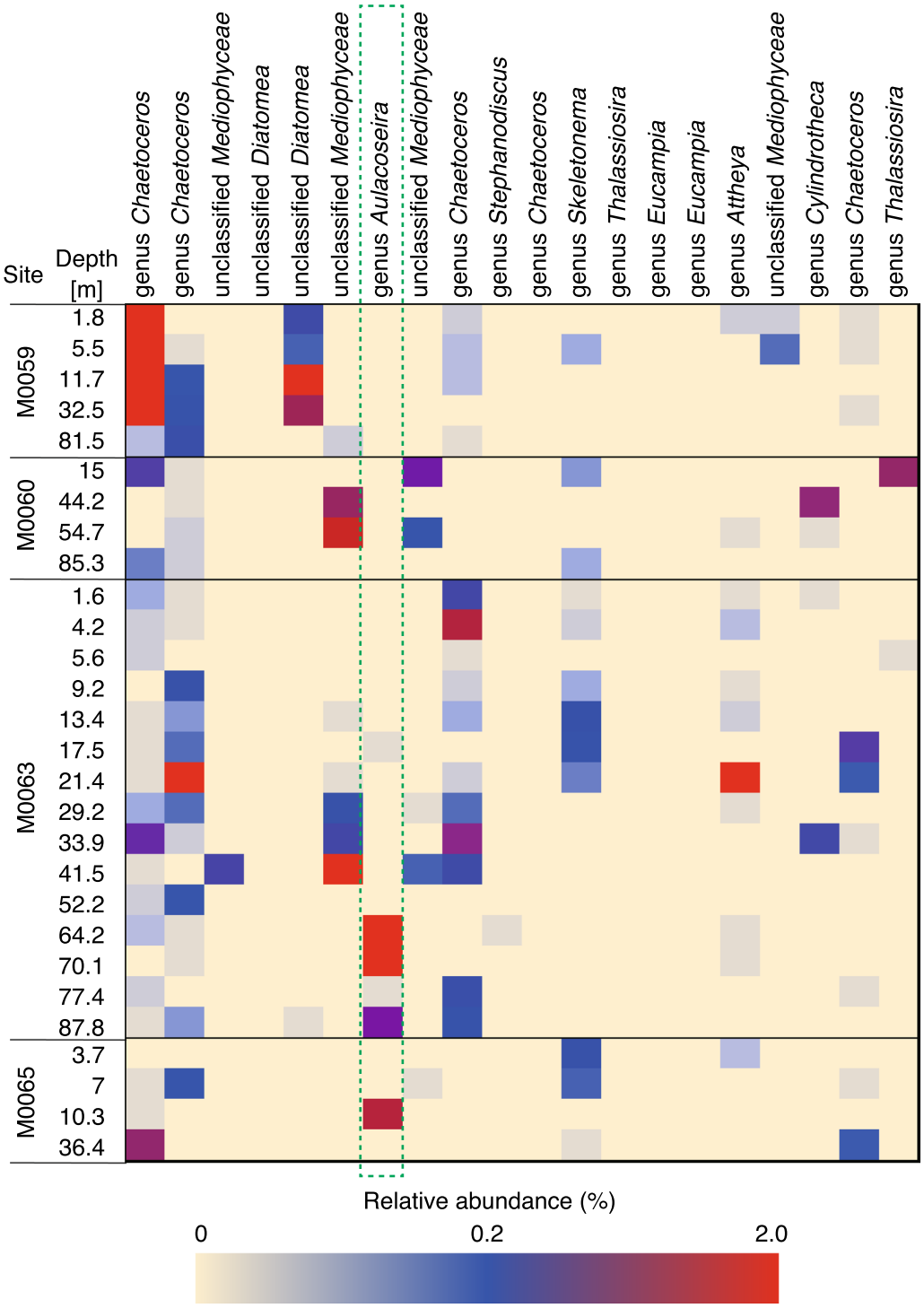

Fig. 6 Relative abundance of top 20 ASVs belonging to phylum Diatomea in sediments of the Baltic Sea, IODP Expedition 347. Genus *Aulacoseira*, enclosed by green dashed line, is known as freshwater diatom

including spore-forming organisms. This is one of the reasons why the depth-dependent decrease in amplifiable 18S rRNA genes was consistently observed at all sites investigated in this study. A previous study of Moa bones (flightless birds endemic to New Zealand) suggested that the half-life for 242-base mitochondrial DNA

(mtDNA) is estimated to be 521 years, and that the average strand length of mtDNA is 88 bp after 10,000 years at 5 °C, a temperature close to that of many shallow marine sediments (Allentoft et al. 2012). Given this estimate and the fact that the size of the PCR amplicons obtained in this study was about 140–170 bp, it was somewhat unexpected that the PCR success rate was as high as approximately 80% for sediments younger than 100,000 years. DNA degradation rate is influenced by physical, chemical, and biological factors of the surrounding environment; for example, minerals can stabilize eDNA in soil where a substantial amount of eDNA can accumulate over time (Kirkpatrick et al. 2016; Morrissey et al. 2015). Similarly, the absorption of eukaryotic eDNA to mineral particles or skeletal remains in marine sediments may also help to stabilize those eDNA from spontaneous decomposition. And thus, subseafloor sediment may therefore be an environment in which eDNA is relatively stable against degradation. Fungi and some other eukaryotes, including diatoms and dinoflagellates, are known to form spores in their life cycle that permit long-term survival (Ribeiro et al. 2011), and the intracellular DNA is likely more protected within such dehydrated periplasmic structures. Alternatively, it has been reported that marine phytoplankton including diatoms, dinoflagellates, and haptophytes have other resting stages than spores, either with minimal activity or as dormant cells (Ellegaard and Ribeiro 2018). If these organisms are present, they might be preferentially detected as their DNA is more easily extracted than that of spores.

Our research has shown that fragmented DNA remaining in subseafloor sediments can also be amplified by PCR and sequenced to identify species. It should be noted, however, that the community composition obtained by PCR amplification may be biased because of the trace amount of target DNAs in the sediments. A comparative study of sedimentary 16S rRNA gene amplicon libraries with shotgun metagenomic data suggested that a shotgun metagenomic approach may help obtain more quantitative data on the buried eukaryotic community in sediment (Ziesemer et al. 2016). In fact, metagenomic analysis without DNA amplification has been applied to shallow sediments with ages up to 6000 years from the Arabian Sea, demonstrating that the abundance of the chlorophyll biosynthesis gene of diatoms can be used as a proxy for past photosynthetic plankton abundance (Giosan et al. 2018). However, eDNA is more fragmented in older sediments, thus hampering metagenomic analysis. In recent years, DNA enrichment of specific taxa by hybridization capture has been applied to eDNA analysis (Armbrecht et al. 2021), which may be useful for metagenomic analysis of such samples with extensive DNA fragmentation.

Correlations between the presence/absence of Diatomeae and Prymnesiophyceae microfossils and the relative abundance of eDNA were found to be correlated for the former, but not for the latter. These algal skeletal fossils and eDNA represent differences in the mechanisms of persistence in sediments. The coccolithophyte skeleton is composed of carbonate, which does not persist at depths greater than the carbonate compensation depth, whereas the diatom skeleton is composed of silicate, which is more stable. Therefore, it is possible that eDNA would persist even if the carbonate skeletons were dissolved. On the other hand, in the present study, Prymnesiophyceae eDNA was rarely detected (around 0–0.01%) in sediments where nanofossil predominated in smear analysis (Additional file 1: Table), e.g., sediments from Site 3 of KN224 in Fig. 3. The relationship between the abundance of microfossils and eDNA in sediments and the differences in their respective physicochemical and biochemical degradation processes remains largely open to research.

While diatom eDNA was detected in the Baltic Sea sediments, including those from glacial sediments, dinoflagellate DNA was also abundant in other inner bays (Fig. 3, sites M0063 and M0065). The Baltic Sea is a region of high primary production, resulting in a greater frequency of these primary producers. In addition, the ratio of dinoflagellates to diatoms is reported to be high in the inner Baltic Sea and low near the outlet at present (Klais et al. 2011). This may be consistent with the high abundance of diatom environmental DNA detected in the sediments of M0059 (located in the bays of Kiel and Mecklenburg), which is the outlet to the Baltic Sea. However, as the proportion of diatoms is known to vary with environmental change, it is debatable whether diatoms were the dominant species at the time of deposition at the M0059 site.

In this study, a high relative abundance of eukaryotic eDNA belonging to the phylum Phragmoplastophyta, most of which consist of terrestrial organisms, was observed in samples from multiple sites and depths/ages (Fig. 3). It may be of particular interest to see the predominance of Phragmoplastophyta-related sequences dominated at sites U1443 and U1444 in the Bay of Bengal. The Bengal Fan is the largest estuarine fan on Earth and its massive terrigenous influx likely leads to the wide dispersal of Phyragmoplastophyta from land to sea. Nonetheless, one could argue that the eukaryotic eDNA of terrestrial plants is difficult to distinguish from the modern DNA contaminants introduced into amplified DNA products during sampling and laboratory analysis. Our negative control experiments where DNA extraction blanks were applied to the same PCR procedure used for the sediment samples resulted in no-visible amplification

on agarose gel electrophoresis. However, it would be very hard to eliminate this possibility at any stage of the analyses completely. For example, it is physically impossible to achieve no contamination from the seawater that is used as a drilling fluid during the drilling process (Lever et al. 2006). In addition, onboard sampling was not be conducted in a clean environment at that time, although sediment samples of DNA analysis were taken from the near center of the core. Therefore, cross-validation with other data such as lipid biomarkers and geochemical proxies is necessary for interpreting eukaryotic eDNA data, as discussed in earlier studies (Armbrecht et al. 2019 and papers cited therein). Also, focusing only on organisms that are typically not present in the laboratory environment could minimize the complications of data interpretation (More et al. 2018; Lejzerowicz et al. 2013). For example, the application of PCR targeting phytoplankton, fish and seaweeds, rather than universal primer sets as used in this study, is promising for obtaining data that will be useful for understanding past ecosystems.

5 Conclusions

This study demonstrated that eukaryotic eDNA has long been globally preserved in subseafloor sediment for as long as 100,000 years. Comparisons with paleoenvironmental and microfossil records support the notion that eukaryotic eDNA in the deep sedimentary biosphere includes taxa from the past surface biosphere and thus is a useful proxy for gaining a deeper understanding of paleoenvironments and ecosystem's coevolution at the DNA level.

Abbreviations

eDNA	Environmental DNA
ASVs	Amplicon sequence variants
mbsf	Meters below sea floor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40645-024-00621-2.

Additional file 1: Metadata of the sediment samples.

Acknowledgements

The samples and/or data used in this study were provided by the Ocean Drilling Program (ODP), the Integrated Ocean Drilling Program, and the International Ocean Discovery Program (IODP). The authors thank all the crew, drilling team members, and shipboard scientists and technical staff for core sampling during ODP Leg 201; IODP Expeditions 301, 307, 308, 315, 316, 329, 346, 347, 353, and 354; D/V *Chikyu* shakedown cruise CK06-06; R/V *Kairei* Cruise KR08-05; and R/V *Knorr* Cruise KN223. We thank R. R. Adhikari, Y. Morono, T. Nunoura, G.-I. Uramoto, and N. Xiao for their assistance in sediment core sampling as a shipboard scientist during the IODP expedition. We are also grateful for the technical assistance provided by S. Hashimoto and T. Terada at the Kochi Institute for Core Sample Research, JAMSTEC. This work was supported by World Premier International Research Center Initiative (WPI), MEXT, Japan.

Author contributions

Both authors designed the study. T.H. performed PCR, sequencing, and data analysis. Both authors wrote and approved the final version of the manuscript. TH and FI helped in conceptualization. TH contributed to methodology. TH and FI assisted in investigation. TH helped in writing—original draft.

Funding

Japan Society for the Promotion of Science Grant-in-Aid for Science Research 22H00429 (FI, TH) and 21K19876 and 22H01347 (TH).

Availability of data and materials

The DNA sequence datasets supporting the conclusions of this article are deposited in DDBJ/EMBL/GenBank under the accession number PRJDB14385.

Declarations

Competing interests

Authors declare that they have no competing interests.

Received: 5 December 2023 Accepted: 22 March 2024
Published: 7 April 2024

References

Allentoft ME, Collins M, Harker D, Haile J, Oskam CL, Hale ML, Campos PF, Samaniego JA, Gilbert MTP, Willerslev E, Zhang G, Scofield RP, Holdaway RN, Bunce M (2012) The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. Proc R Soc B 279:4724–4733

Andrén T, Jørgensen BB, Cotterill C, the Expedition 347 Scientists (2015) Proc IODP, 347: College Station, TX, Integrated ocean drilling program

Armbrecht LH, Coolen MJL, Lejzerowicz F, George SC, Negandhi K, Suzuki Y, Young J, Foster NR, Armand LK, Cooper A, Ostrowski M, Focardi A, Stat M, Moreau JW, Weyrich LS (2019) Ancient DNA from marine sediments: precautions and considerations for seafloor coring, sample handling and data generation. Earth-Sci Rev 196:102887

Armbrecht L, Hallegraeff G, Bolch CJS, Woodward C, Cooper A (2021) Hybridisation capture allows DNA damage analysis of ancient marine eukaryotes. Sci Rep 11:3220

Armbrecht L, Weber ME, Raymo ME, Peck VL, Williams T, Warnock J, Kato Y, Hernández-Almeida I, Hoem F, Reilly B, Hemming S, Bailey I, Martos YM, Gutjahr M, Percuoco V, Allen C, Brachfeld S, Cardillo FG, Du Z, Fauth G, Fogwill C, Garcia M, Glüder A, Guitard M, Hwang JH, Iizuka M, Kenlee B, O'Connell S, Pérez LF, Ronge TA et al (2022) Ancient marine sediment DNA reveals diatom transition in Antarctica. Nat Commun 13:5787

Coolen MJL (2011) 7000 years of *Emiliania huxleyi* viruses in the Black Sea. Science 333:451–452

Coolen MJ, Muyzer G, Rijpstra WI, Schouten S, Volkman JK, Damsté JS (2004) Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake. Earth Plan Sci Lett 223(1–2):225–239

Coolen MJL, Orsi WD, Balkema C, Quince C, Harris K, Sylva SP, Filipova-Marinova M, Giosan L (2013) Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene. Proc Natl Acad Sci USA 110:8609–8614

Corinaldesi C, Barucca M, Luna GM, Dell'Anno A (2011) Preservation, origin and genetic imprint of extracellular DNA in permanently anoxic deep-sea sediments. Mol Ecol 20:642–654

D'Hondt S, Inagaki F, Zarikian CA, Abrams LJ, Dubois N, Engelhardt T, Evans H, Ferdelman T, Gribsholt B, Harris RN, Hoppie BW, Hyun JH, Kallmeyer J, Kim J, Lynch JE, McKinley CC, Mitsunobu S, Morono Y, Murray RW, Pockalny R, Sauvage J, Shimono T, Shiraishi F, Smith DC, Smith-Duque CE, Spivack AJ, Steinsbu BO, Suzuki Y, Szpak M, Toffin L et al (2015) Presence of oxygen and aerobic communities from sea floor to basement in deep-sea sediments. Nature Geosci 8:299–304

Ellegaard M, Ribeiro S (2018) The long-term persistence of phytoplankton resting stages in aquatic 'seed banks': persistence of phytoplankton resting stages. Biol Rev 93:166–183

Giosan L, Orsi WD, Coolen M, Wuchter C, Dunlea AG, Thirumalai K, Munoz SE, Clift PD, Donnelly JP, Galy V, Fuller DQ (2018) Neoglacial climate anomalies and the Harappan metamorphosis. Clim past 13:1669–1686
Hoshino T, Inagaki F (2019) Abundance and distribution of Archaea in the subseafloor sedimentary biosphere. ISME J 13:227–231
Hoshino T, Doi H, Uramoto GI, Wörmer L, Adhikari RR, Xiao N, Morono Y, D'Hondt S, Hinrichs KU, Inagaki F (2020) Global diversity of microbial communities in marine sediment. Proc Natl Acad Sci 117:27587–27597
Inagaki F, Okada H, Nealson TAI, KH, (2005) Microbial survival: the paleome: a sedimentary genetic record of past microbial communities. Astrobiolgy 5:141–153
Kirkpatrick JB, Walsh EA, D'Hondt S (2016) Fossil DNA persistence and decay in marine sediment over hundred-thousand-year to million-year time scales. Geology 44:615–618
Klais R, Tamminen T, Kremp A, Spilling K, Olli K (2011) Decadal-scale changes of dinoflagellates and diatoms in the anomalous Baltic Sea spring bloom. PLoS ONE 6:e21567
Kuwae M, Tamai H, Sakata MK, Minamoto T, Suzuki Y (2020) Sedimentary DNA tracks decadal-centennial changes in fish abundance. Commun Biol 3:1–12
Lejzerowicz F, Esling P, Majewski W, Szczuciński W, Decelle J, Obadia C, Arbizu PM, Pawlowski J (2013) Ancient DNA complements microfossil record in deep-sea subsurface sediments. Biol Lett 9:20130283
Lever MA, Alperin M, Engelen B, Inagaki F, Nakagawa S, Steinsbu BO, Teske A (2006) Trends in basalt and sediment core contamination during IODP expedition 301. Geomicrobiol J 23:517–530
More KD, Orsi WD, Galy V, Giosan L, He L, Grice K, Coolen MJL (2018) A 43 Kyr record of protist communities and their response to oxygen minimum zone variability in the Northeastern Arabian Sea. Earth Planet Sci Let 496:248–256
Morrissey EM, McHugh TA, Preteska L, Hayer M, Dijkstra P, Hungate BA, Schwartz E (2015) Dynamics of extracellular DNA decomposition and bacterial community composition in soil. Soil Biol Biochem 86:42–49
Ogata M, Masuda R, Harino H, Sakata MK, Hatakeyama M, Yokoyama K, Yamashita Y, Minamoto T (2021) Environmental DNA preserved in marine sediment for detecting jellyfish blooms after a tsunami. Sci Rep 11:16830
Orsi WD, Inagaki F (2023) Decoding geobiological evolution from microbiomes. Sci Adv 9:eadg5448
Pedersen MW, Overballe-Petersen S, Ermini L, Sarkissian CD, Haile J, Hellstrom M, Spens J, Thomsen PF, Bohmann K, Cappellini E, Schnell IB, Wales NA, Carøe C, Campos PF, Schmidt AM, Gilbert MT, Hansen AJ, Orlando L, Willerslev E (2015) Ancient and modern environmental DNA. Philos Trans R Soc Lond B Biol Sci 370:20130383
Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596
Ribeiro S, Berge T, Lundholm N, Andersen TJ, Abrantes F, Ellegaard M (2011) Phytoplankton growth after a century of dormancy illuminates past resilience to catastrophic darkness. Nat Commun 2:311
Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
Stoeck T, Bass D, Nebel M, Christen R, Jones MD, Breiner HW, Richards TA (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol Suppl 1:21–31
Taberlet P, Bonin A, Zinger L, Coissac E (2018) Environmental DNA: for biodiversity research and monitoring. Oxford University Press, Oxford
Turner CR, Uy KL, Everhart RC (2015) Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol Conserv 183:93–102
Willerslev E, Hansen AJ, Binladen J, Brand TB, Gilbert MT, Shapiro B, Bunce M, Wiuf C, Gilichinsky DA, Cooper A (2003) Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science 300:791–795
Wörmer L, Hoshino T, Bowles MW, Viehweger B, Adhikari RR, Xiao N, Uramoto GI, Könneke M, Lazar CS, Morono Y, Inagaki F (2019) Microbial dormancy in the marine subsurface: global endospore abundance and response to burial. Sci Adv 5(2):eaav1024
Ziesemer KA, Mann AE, Sankaranarayanan K, Schroeder H, Ozga AT, Brandt BW, Zaura E, Waters-Rist A, Hoogland M, Salazar-García DC, Aldenderfer M, Speller C, Hendy J, Weston DA, MacDonald SJ, Thomas GH, Collins MJ, Lewis CM, Hofman C, Warinner C (2016) Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification. Sci Rep 13:16498

Publisher's Note