

## THE MICROFUNGAL COMMUNITIES IN DEEP-SEA SEDIMENTS FROM THE EQUATORIAL ATLANTIC

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Microfungi of deep-sea sediments, and especially those several meters below the water–sediment interface, are poorly studied. In this work, for the first time, microfungal communities isolated by cultivation from deep-sea sediments of the eastern part of the Equatorial Atlantic (the Romanche and Chain Fracture Zones) were investigated. Fungi were isolated from sediments sampled at each of 12 stations from horizons 1.0–4.7 m below the sediment–water interface. To study microscopic fungi, one sediment horizon was isolated from each core. The fungal abundances were within the range of 0.0–3300.0 CFU g<sup>-1</sup> sediment dry weight. A total of 19 fungal taxa from the phyla *Ascomycota* (18) and *Basidiomycota* (1) were identified, and *Mycelia sterilia* 1 strain was also isolated. Seven fungal species were encountered only once. In this case, the maximum similarity of species composition, in terms of the Bray – Curtis coefficient, was 57.14% (horizons 1.0 and 3.6 m, four common species). A comparison of the taxonomic structures of fungal communities from the study area was made with those from sediments of the Indian and Pacific Oceans and other areas of the Atlantic. The fungal communities from sediments in the study area were compared with those from the Indian and Pacific Oceans and other areas of the Atlantic. From the literature data and present study results, a list of fungal species with 180 names was compiled. The fungi belonged to 97 genera, 57 families, 32 orders and 13 classes of the phyla *Ascomycota*, *Basidiomycota*, and *Mucoromycota*. The diversity of fungal communities was assessed using indicators of taxonomic richness (number of taxa from different ranks), proportions (genera/families, species/families, species/genera), Average Taxonomic Distinctness index (AvTD,  $\Delta^+$ ) and Variation in Taxonomic Distinctness index (VarTD,  $\Lambda^+$ ). Four and twelve fungal classes were identified in sediments in the Eastern Equatorial Atlantic and the Indian Ocean, respectively. The species/genera proportions in the communities varied from 1.33 (Indian Ocean) to 3.8 (other areas of the Atlantic Ocean). For the fungal communities of the Eastern Equatorial Atlantic, the AvTD index value was minimal ( $\Delta^+ = 50.19$ ), the VarTD index was maximal ( $\Lambda^+ = 945.38$ ), and they were beyond the 95% confidence interval. This was due to the small number of the fungal classes and vertical and horizontal unevenness of species distribution along taxonomic branches, which was manifested in the dominance of species of the family *Aspergillaceae* (78.9% of the species in the class *Saccharomycetes* and *Eurotiomycetes*), only two species belonging to the classes *Sordariomycetes* and one species belonging to the class *Microbotryomycetes* (phylum *Basidiomycota*). Consequently, statistically significant differences were found between the taxonomic structures of the fungal communities of the Eastern Equatorial Atlantic and the other regions of the World Ocean, which are due to the insufficient amount of data obtained on the species composition of fungi in the sediments of this area. The study did not reveal any pattern in the change in the number of fungal species and their abundance in relation to the water characteristics (temperature, pH, and salinity), horizon depth in the sediment core, sediment type, or sampling station location in the Romanche and Chain Fracture Zones.

**Keywords:** abiotic factors, *Aspergillaceae*, Average Taxonomic Distinctness index, Distinctness index, subsurface sediment horizons, marine fungi

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## INTRODUCTION

The largest ecosystem on Earth is the deep waters of the World Ocean and its floor, but these biotopes have not been sufficiently studied in terms of organic matter content, chemical composition, and biotic components. Living or viable-in-culture microorganisms (bacteria, archaea and fungi) have been detected in ocean sediment layers that lie several hundred meters below the water–sediment interface. However, there is very little information about the general diversity and growth of microorganisms in this biotope (Rédou et al., 2015; Rojas-Jimenez et al., 2020; Florio Furno et al., 2022). The results of studying sediments to a depth of 1922 m using molecular and cultural methods simulating *in situ* conditions made it possible to revise the previously established maximum depths from 159 to 1740 m for the habitat of eukaryotic microorganisms and from 518 to 1922 m for that of prokaryotes (Ciobanu et al., 2014).

Cultivation allows isolating only a small number of species; however, the merit of the method is the possibility of further dealing with pure cultures of microorganisms to estimate their physiological effects and to extract and study biologically active compounds from them. Bacteria and fungi isolated from deep-sea sediments are known to produce extracellular hydrolases and antibacterial metabolites that are active against human clinical pathogens (Padmanaban et al., 2019; Zhou et al., 2021). Such species as: *Acremonium fusidioides* (Nicot) W. Gams, *Penicillium allii-sativi* Frisvad, Houbraken et Samson, *P. chrysogenum* Thom, *P. palitans* Westling, *P. solitum* Westling, and *Pseudogymnoascus verrucosus* A.V. Rice et Currah, were isolated from sediments in the Antarctic region. Some isolates of these species exhibited antifungal, trypanocidal, leishmanicidal, antimalarial, nematocidal or herbicidal activities (Ogaki et al., 2020).

In deep-sea ecosystems, fungi are involved in biogeochemical cycles and food chains and enter into diverse interactions (biotrophy, parasitism or symbiotrophy) with other organisms (Marchese et al., 2021). It is known that endolithic fungi (i.e. those living inside rocks) destroy the calcareous structures of hydrobiots: mollusk shells, barnacles, foraminifera, corals, and the burrow linings of marine wood borers (Kopytina, Bocharova, 2022).

Studies have been carried out on marine fungi from deep-sea sediments of the Mariana (Nagano et al., 2010) and Atacama Trenches (Edgcomb et al., 2011; Wang et al., 2019), Mid-Indian Basin (Singh et al., 2012), South China Sea (Zhang et al., 2013), Tropical Eastern Pacific (Rojas-Jimenez et al., 2020), Atlantic Ocean (Marchese et al., 2021; Zhou et al., 2021), and Black Sea (Zaitsev, Polikarpov, 2008; Sergeeva, Kopytina, 2014). Mainly

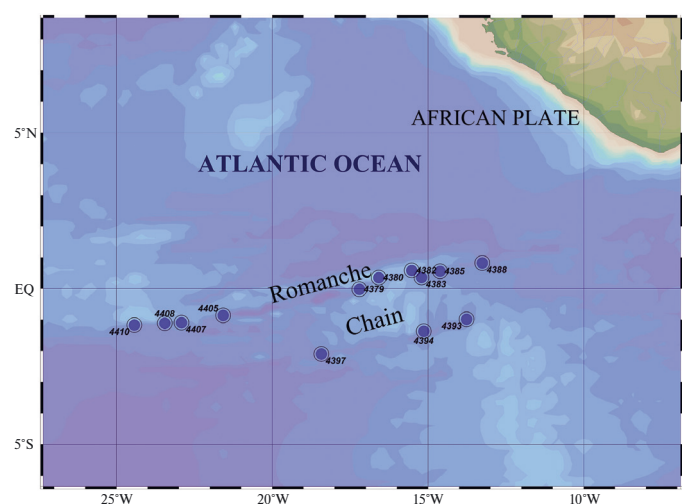
cosmopolitan fungi have been identified at different horizons in sediments. This raises the question on the origin of the fungi and their ability to adapt to the deep-sea conditions (Damare et al., 2006; Rédou et al., 2015; Wang et al., 2019, Zvereva, Borzykh, 2022). Molecular studies discover operational taxonomic units (OTU) of uncultivated fungi for which there are no data in the GenBank database (Xu et al., 2014; Marchese et al., 2021).

Studies on the fungal species diversity in the deep pelagic and benthic zones of the World Ocean are important for understanding the number of fungal species on the planet and their ecophysiological role in extreme environmental conditions (Marchese et al., 2021).

The purposes of the present study are: 1) to assess the taxonomic diversity of fungi and the structural features of their communities in sediments up to the horizons 4.7 m below the water-sediment interface in the Eastern Equatorial Atlantic; and 2) to assess the influence of abiotic factors – characteristics of water above the sediments (temperature, salinity, and pH), horizon level in the sediment core, and sediment type – on the structure of fungal communities.

## MATERIALS AND METHODS

**Sediments and water sampling.** RV “Akademik Ioffe” cruise 63 (Shirshov Institute of Oceanology of the Russian Academy of Sciences, RAS) took place from 29.09.2022 to 08.12.2022 in the eastern part of the Equatorial Atlantic in the area of the Romanche and Chain Fracture Zones (Fig. 1). Teams of geologists and lithologists from the Shirshov Institute of Oceanology of RAS collected sediment cores at 12 stations using a marine



**Fig. 1.** Map of stations at which sediment cores were sampled with water characteristics in the 63th cruise RV “Akademik Ioffe”.

geological coring tube with a diameter of 110 mm. To study microscopic fungi, one sediment horizon was isolated from each core.

Water temperature, salinity and pH in the near-sediment layer (at a distance of about 7 m from the ocean floor) were measured using a CTD-probe *Sea-Bird 19plus* and a pH-meter *HANNA instruments HI98128 PHep5* with a precision of 0.01°C and 0.01 PSU for the thermohaline characteristics and 0.01 for pH values.

**Fungal isolation.** Fungi were isolated on Czapek medium (LLC Research and Development Center “Biokompas-S”, Uglich, Russia) and Sabouraud agar (Obolensk, Serpukhov, Moscow Region, Russia). 1 mL of sediment suspension (dilution 1 : 10) was added into a sterile Petri dish and the dish was filled with molten medium cooled to approximately 45°C. Three replicates were prepared for each medium (72 dishes in total). A 3% chloramphenicol (antibiotic) solution in ethanol was added to the media in a proportion of 1 mL per 1 L of media to suppress bacterial growth. The media were prepared using sterile artificial seawater, which was obtained by dissolving 34 g of sea salt (“Marbelle”, Taganrog, Russia) in 1 liter of distilled water. The Petri dishes with the material were incubated in a thermostat at 18°C for one month.

Two sediment samples of equal weights were taken. One of them was used to prepare a suspension, and the other one was dried in an oven to constant weight at a temperature of 105°C. The number  $N$  of colony-forming units (CFU) of micromycetes was calculated per 1 g of dry sediment using the formula:  $N = a \cdot b \cdot m \cdot g^{-1}$ , [1], where  $a$  is the average number of colonies in Petri dishes,  $b$  is the sediment dilution factor,  $m$  is the wet sediment weight, and  $g$  is the dry sediment weight (Bilay, 1982).

To examine fungal communities, we used an ADF U300 light microscope equipped with a camera ADF Pro 08 (China). Microfungi were identified by morphological and cultural characteristics using the works (Bilay, Koval, 1988; De Hoog et al., 2000; Klich, 2002; Refai et al., 2014; Visagie et al., 2014) and others. Valid names and taxonomic affiliation of fungi were taken from the international electronic database Index Fungorum (2024).

Permanent preparations of isolated fungi are in the personal collection of N.I. Kopytina at the Papanin Institute of Biology of Inland Waters of RAS.

**Data processing.** Data processing was carried out using MS Excel and the statistical software package PRIMER® 5.2.8. The input of the PRIMER® software is a sample  $\times$  taxon matrix supplemented with the corresponding grouping factors. The similarity of the structure of fungal communities across stations/horizons were assessed using the Bray – Curtis similarity coefficient (the Similarity analysis) in two variations: species composition similarity was assessed by the presence/absence of species, and similarity

of the quantitative structure of communities was evaluated from the number and abundance of species.

The systematic features of a fungal community are described using two taxonomic indices: Average Taxonomic Distinctness index (AvTD,  $\Delta^+$ ) and Variation in Taxonomic Distinctness index (VarTD,  $\Lambda^+$ ). These indices are calculated based on the presence/absence of species. To calculate index values, two matrices are used: Sample data (main matrix) and Aggregation data with the systematic position of each species according to the hierarchy of C. Linnaeus (species, genus, family, etc.). The TAXDTEST function plots index graphs and the arrangement of symbols demonstrates the proximity of the taxonomic structure of the complexes under consideration.

Average Taxonomic Distinctness index ( $\Delta^+$ ) shows the vertical breadth of the distribution of taxa at the previous and next levels of the taxonomic tree. The  $\Delta^+$  index is the mean length of links between species in the taxonomic tree. When two species belong to the same genus, one link leads to the common node representing the genus. If species belong to different genera of the same family, two different link levels are involved (species – genus and genus – family), and the index is calculated using the formula:  $\Delta^+ = [\sum_{i < j} \omega_{ij}] / [S(S-1)/2]$  [2], where  $S$  is the number of species in the sample and  $\omega_{ij}$  is a measure of taxonomic difference given by the length of the path that connects species  $i$  and  $j$  in the hierarchical classification.

Variation in Taxonomic Distinctness index (VarTD,  $\Lambda^+$ ) shows the degree of horizontal evenness in the distribution of the number of lower taxa in the branches of high levels. It is calculated using the formula:  $\Lambda^+ = [\sum_{i < j} (\omega_{ij} - \Delta^+)^2] / [S(S-1)/2]$  [3], with the same notations as in Eq. [2]. The more representatives from polyspecific branches in the community, the lower the indicators of hierarchical evenness of the taxonomic structure and the lower the values of the indices  $\Delta^+$  and  $\Lambda^+$  (Clarke et al., 2014).

Systematic features of the fungal communities of the Eastern Equatorial Atlantic are considered in comparison with similar communities from other areas of the World Ocean. A list of fungal species in sediments of the Indian Ocean (cores at 0.3, 0.4 and 4.7 m) (Raghukumar, Raghukumar, 1998; Raghukumar et al., 2010; Damare et al., 2006; Zhang et al., 2014; Xu et al., 2018), the Pacific (0.0–3.58, 4, 12, 21, 25, 34, 37, 137, 403, 765, 1478 and 1884 m) (Xu et al., 2014; Rédou et al., 2015; Keeler et al., 2021), and the Atlantic (surficial horizons in the areas of Porcupine Bank, the Whittard Canyon, and the South Atlantic Ocean) (Marchese et al., 2021; Zhou et al., 2021) is compiled. This list includes fungal species that were isolated by culture or identified by genetic analysis with an accuracy of at least 98%. If only the fungal genus was reported, then the genus name is supplemented with the designations: sp. 1, sp. 2. The list consists of 180 fungal names and includes also the



**Table 1.** Characteristics of sampling stations, sediment samples and water above sediments

Station N	Coordinates	Ocean depth, m	T, °C	S, PSU	pH	Sediment horizon, m	Sediment type
4379	00°01.218' S; 17°11.983' W	5493	1.96	34.86	7.00	3.00	sandy clay
4380	00°21.151' N; 16°34.747' W	5602	1.91	34.85	7.02	1.00	sandy silt
4382	00°34.846' N; 15°31.27' W	4814	2.20	34.84	6.94	3.60	silty sand
4383	00°21.293' N; 15°12.424' W	4165	1.19	34.77	6.89	2.68	clayey sand
4385	00°33.135' N; 14°36.729' W	4838	1.35	34.79	6.83	4.50	clayey sand
4393	00°59.275' S; 13°45.671' W	4688	1.28	34.78	6.78	2.95	clayey sand
4394	01°22.236' S; 15°08' W	5535	1.23	34.76	6.78	2.60	sandy silt
4397	02°05.9' S; 18°25.4' W	5189	0.82	34.72	6.69	3.75	clay
4405	00°51.47' S; 21°34.5' W	5964	1.16	34.77	7.02	3.00	clayey sand
4407	01°05.66' S; 22°54.587' W	4824	1.28	34.76	6.31	3.10	sandy silt
4408	01°06.794' S; 23°26.982' W	4860	1.24	34.76	6.82	2.19	sandy silt
4410	01°10.356' S; 24°25.5' W	5415	1.13	34.76	6.74	4.70	silty sand

taxa found in this study. Using the list of species, the taxonomic richness (number of taxa of different ranks) and proportions (genera/families, species/families, species/genera) in the communities of the compared areas are determined and the graphs of the  $\Delta^+$  and  $\Lambda^+$  indices are plotted. This analysis is shown in Discussion.

The outcome of the BIOENV analysis (Biota and/or Environment matching) is the highest possible values of Spearman's rank correlation coefficients ( $\rho_{\max}$ ). The values of the coefficients show the combination of environmental parameters that most closely match variations in the distribution of numbers and species composition of organisms by comparing biotic (i.e. related to number of species and number of organisms) and abiotic (i.e. related to physicochemical parameters of the environment) similarity matrices. The software uses Z-standardization of environmental parameters with different measurement units. The Shannon diversity index  $H'$  used in the study indicates the complexity of the fungal community structure and is calculated based on the abundance (or biomass) and number of species as follows:  $H' = \sum_{i=1}^R \rho_i \log_e \rho_i$ , [4], where  $\rho_i$  is the proportion of the  $i$ -th species in the total count (or total biomass) and  $R$  is the number of species. In this study, the Shannon index based on abundance was calculated using the function DIVERSE (Clarke et al., 2014).

The similarity in the quantitative structure of the fungal communities was analyzed using MDS ordination (ordination of samples by Multi-Dimensional Scaling), whose outcome representation contains the intervals among stations in multidimensional space and is projected to 3D plots (MDS function).

Ecological analysis of fungal community includes: species composition, number of species, number of colony-forming units (CFU g<sup>-1</sup> dry sediment), and frequency of

species occurrence. The frequency of occurrence was calculated using the number of stations (12) taken as 100%.

## RESULTS

### Characteristics of sediment samples and water

Station coordinates and characteristics of the sediment samples and water layer above sediments are presented in Table 1. The sampled sediments were sandy, clayey or silty odorless sediments, light gray or dark gray in color at natural moisture content. The physicochemical characteristics of water at the sampling stations were close.

### Fungal communities

In the deep-sea sediments from the Romanche and Chain Fracture Zones, 2543 colonies of fungi belonging to 18 species of the phylum *Ascomycota* and one species of the phylum *Basidiomycota* were identified, and one unidentified taxon *Mycelia sterilia* 1 was detected. The species composition of the fungal complexes was dominated by representatives of the class *Eurotiomycetes*, family *Aspergillaceae*: six species from the genus *Aspergillus*, seven ones from the genus *Penicillium*, one species from the genus *Emericella*, and one from the genus *Talaromyces* (Fig. 2, Table 2). The maximum frequency of occurrence (50%) among stations was noted for the yeast *Metschnikowia* sp. 1.

Representatives of obligate marine fungi were not found in the sediments, and all species belonged to terrigenous cosmopolitan fungi. Low abundance (in CFU g<sup>-1</sup>) was noted in all samples, and growth of micromycetes was recorded in 33 Petri dishes (45.8%). From the sediments sampled at Station N 4393 (sampling horizon 2.95 m), no fungi were isolated.

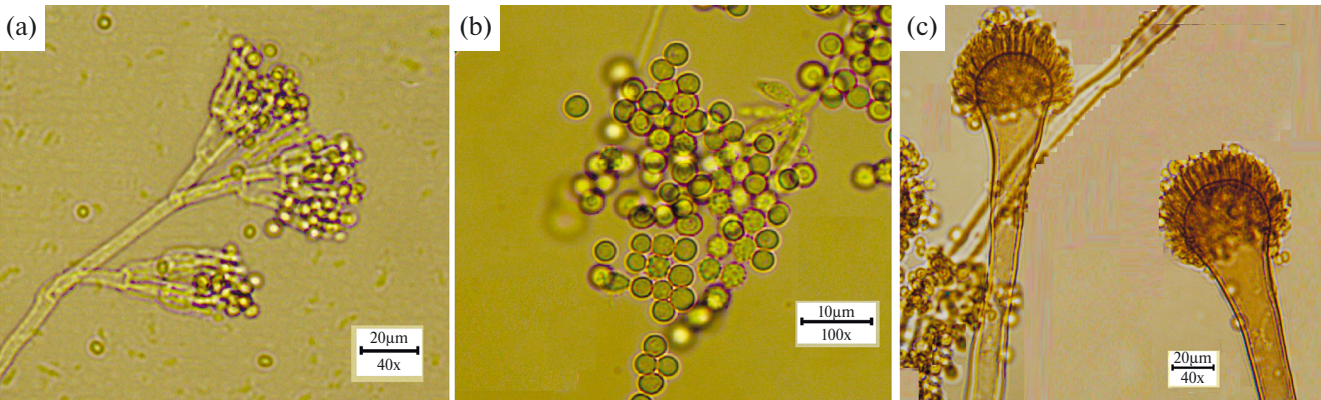


Fig. 2. Main fungal species isolated: (a) *Penicillium citrinum*; (b) *Microascus brevicaulis*; (c) *Aspergillus fumigatus*.

Table 2. Taxonomic composition, abundance (CFU g<sup>-1</sup> dry sediment) and at different horizons (stations) in deep-sea sediments from the eastern part of the Equatorial Atlantic

Sediment horizon, m	3.0	1.0	3.6	2.68	4.5	2.6	3.75	3.0	3.1	2.19	4.7
Station #	4379	4380	4382	4383	4385	4394	4397	4405	4407	4408	4410
CFU g <sup>-1</sup> dry sediment											
Ascomycota											
Eurotiomycetes											
* <i>Aspergillus aculeatus</i> Iizuka	0	0	0	0	0	60	0	0	0	0	0
* <i>A. carneus</i> Blochwitz	0	0	0	70	0	0	0	0	0	0	0
<i>A. conicus</i> Blochwitz	0	200	0	0	0	0	0	0	0	0	0
* <i>A. fumigatus</i> Fresen.	0	100	0	70	0	60	30	0	0	0	0
* <i>A. terreus</i> Thom	0	0	0	0	0	0	30	0	0	50	0
* <i>Emericella usta</i> (Bainier) Pitt et A.D. Hocking	0	0	0	0	0	60	0	0	0	0	0
* <i>A. sydowii</i> (Bainier et Sartory) Thom & Church	0	0	0	0	100	0	0	0	0	50	0
<i>Penicillium aurantiogriseum</i> Dierckx	0	0	0	150	0	0	0	0	1600	0	40
* <i>P. brevicompactum</i> Dierckx	0	0	0	70	0	0	0	0	30	0	0
* <i>P. chrysogenum</i> Thom	100	100	150	0	100	0	0	0	0	0	500
* <i>P. citrinum</i> Thom	100	0	0	0	0	0	0	0	0	0	0
<i>P. expansum</i> Link	0	0	0	0	100	0	0	0	0	0	0
<i>P. paradoxum</i> (Fennell et Raper) Samson, Houbraken, Visagie et Frisvad	0	100	0	0	0	0	40	0	0	50	0
<i>P. purpurogenum</i> Stoll	0	0	0	0	0	0	0	0	0	0	500
<i>Talaromyces rugulosus</i> (Thom) Samson, N. Yilmaz, Frisvad et Seifert	0	0	30	70	150	0	0	0	0	0	0
Sordariomycetes											
<i>Sarocladium kiliense</i> (Grütz) Summerb.	0	0	30	0	0	0	0	90	70	0	0
* <i>Microascus brevicaulis</i> S.P. Abbott	0	100	30	150	0	0	0	0	0	50	0
Saccharomycetes											
<i>Metschnikowia</i> sp. 1	635	200	185	0	1300	0	0	0	1600	504	0
Basidiomycota											
Microbotryomycetes											
<i>Rhodotorula</i> sp. 1	0	500	300	0	0	0	0	0	0	0	0

End of Table 2

Sediment horizon, m	3.0	1.0	3.6	2.68	4.5	2.6	3.75	3.0	3.1	2.19	4.7
Station #	4379	4380	4382	4383	4385	4394	4397	4405	4407	4408	4410
Unidentified taxon											
Mycelia sterilia 1	220	0	30	0	0	0	0	30	0	0	40
Total abundance and indicators of fungal species diversity											
Total abundance, CFU g <sup>-1</sup> dry sediment	1055	1300	755	580	1750	180	100	120	3300	704	1080
Number of taxa	4	7	7	6	5	3	3	2	4	5	4
Shannon Index, $H'(\log_e)$	1.079	1.733	1.545	1.720	0.922	1.099	1.089	0.562	0.826	0.991	0.957

Note. \*Terrestrial fungal species with proven ability to live in the marine environment, according to molecular data (Jones et al., 2015).

**Table 3.** The highest values of Spearman's rank correlation coefficients ( $\rho_{\max}$ ) for combinations of different numbers of variables influencing the structure of the fungal communities

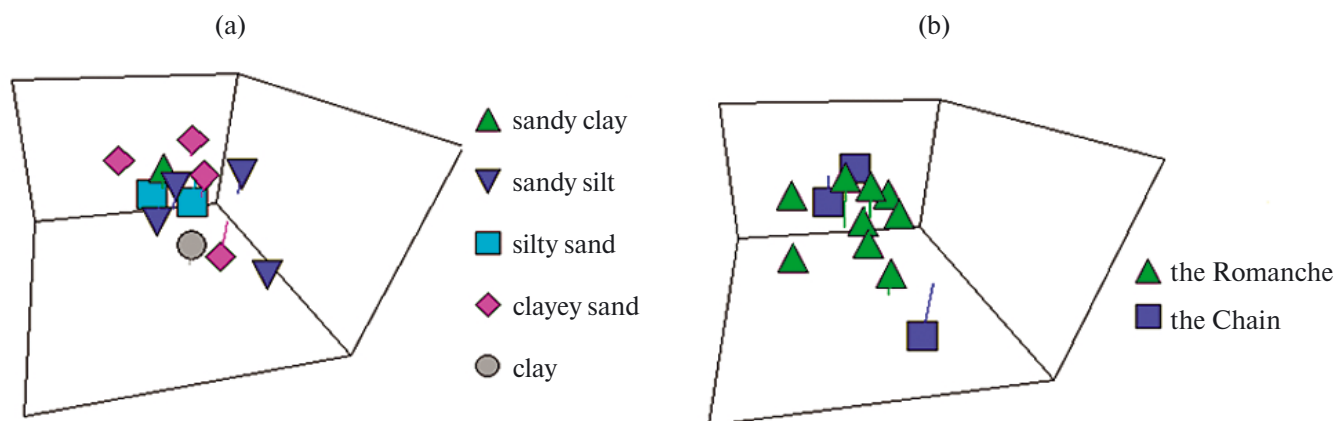
Max. Spearman's coefficient ( $\rho_{\max}$ )	Combination of factors (water characteristics)
0.103	Temperature, salinity
0.090	Temperature
0.090	Temperature, salinity, pH
0.088	Ocean depth, temperature, salinity
0.082	Ocean depth, temperature
0.081	pH
0.079	Temperature, pH
0.077	Ocean depth, temperature, pH
0.074	Salinity
0.063	Ocean depth, temperature, salinity, pH

Seven fungal species were encountered only once. In this case, the maximum similarity of species composition, in terms of the Bray-Curtis coefficient, was 57.14% (Stations NN 4380 and 4382, horizons 1.0 and 3.6 m, four common

species), and the maximum similarity of the quantitative structure was 59.59% at these stations (with the abundances of 1300 and 755 CFU g<sup>-1</sup> dry sediment). The biodiversity across horizons (stations) was also very low as seen in the Shannon Index values.

As a result of comparing matrices of the biotic and abiotic factors (ocean depth, sediment horizon, water temperature, pH and salinity) using BIOENV analysis, maximum Spearman correlation coefficients were obtained, which ranged from 0.063 to 0.103. The values of the coefficients were not statistically significant; therefore, the environmental parameters under consideration did not significantly affect the structure of the fungal communities (Table 3). We did not reveal any pattern of changes in the number of species or fungal abundance with the sediment horizon (at the ocean depth differences up to 1799 m and at the sediment horizon differences up to 3.7 m).

No differences were detected among fungal communities from sediments of different types and from the Romanche and Chain Fracture Zones, as shown in the graphs of ordination of samples by Multi-Dimensional Scaling [MDS (3D)] (Fig. 3).



**Fig. 3.** MDS (3D) similarity of fungal communities: a – in sediments of different types; b – in sediments of the Romanche and Chain Fracture Zones (according to the Bray-Curtis similarity coefficient).

DISCUSSION

Molecular and cultural mycological studies of sub-surface horizons in deep-sea sediments of the World Ocean show that species of the phylum *Ascomycota* represent 43.0–80.0%, *Basidiomycota* 3–20%, *Mucoromycota* 0.55–1.5%, *Chytridiomycota* 0.8%, and those with unidentified sequences account for 2.4–64.0% of the species composition (Xu et al., 2018; Rojas-Jimenez et al., 2020; Florio Furno et al., 2022).

Species of the phylum *Ascomycota* dominated the sediments in the Indian, Pacific, and Atlantic Oceans, representing from 76.4% (Indian Ocean) to 89.5% (Atlantic Ocean) of the species composition. In the present study, fungal taxa from the phylum *Ascomycota* accounted for 92.5% of the species composition and abundance. Using the species lists, indicators of taxonomic richness and diversity of fungal communities were calculated, and graphs of the taxonomic indices Delta<sup>+</sup> and Lambda<sup>+</sup> were plotted for each region in question (Table 4, Fig. 3, a, b).

The highest similarity of species composition was in the fungal communities of PO and IO, 20.6 (the common species were: *Acremonium obclavatum* W. Gams, *Aspergillus terreus*, *Aureobasidium pullulans* (de Bary et Löwenthal) G. Arnaud, *Candida parapsilosis* (Ashford)

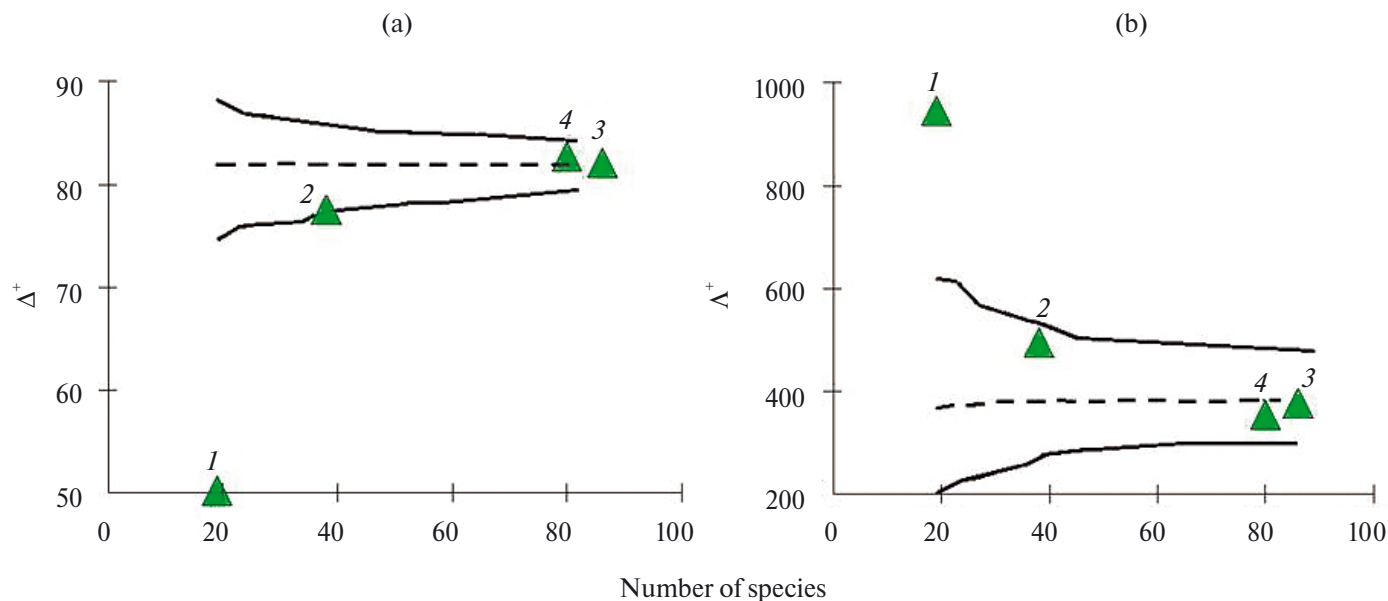
Langeron et Talice, *Cladosporium sphaerospermum* Penz., *Cutaneotrichosporon curvatum* (Diddens et Lodder) Yurkov, Xin Zhan Liu, F.Y. Bai, M. Groenew. et Boekhout, *C. mucoides* (E. Guého et M.T. Sm.) Xin Zhan Liu, F.Y. Bai, M. Groenew. et Boekhout, *Emericella sydowii*, *Fusarium oxysporum* Schltdl., *F. solani* (Mart.) Sacc., *Meyerozyma guilliermondii* (Wick.) Kurtzman et M. Suzuki, *Penicillium chrysogenum*, *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison, *Sarocladium kiliense*, and *Starmerella etchellsii* (Lodder et Kreger-van Rij) C.A. Rosa et Lachance, and the common genus was *Exophiala*). The similarity of the fungal communities in the EEA sediments was 10.5 with those in sediments of the other AO areas (*Aspergillus conicus*, *Penicillium brevicompactum*, *P. chrysogenum*); 11.4 with those in the IO sediments (*Aspergillus terreus*, *Emericella sydowii*, *E. usta*, *Penicillium chrysogenum*, *Sarocladium kiliense*); and 16.3 with those in the PO sediments (*Aspergillus fumigatus*, *A. terreus*, *Emericella sydowii*, *Penicillium brevicompactum*, *P. chrysogenum*, *Sarocladium kiliense*, yeasts of the genera *Metschnikowia* and *Rhodotorula*). In the EEA sediments, nine fungal species are noted that were encountered in other regions. Common to the compared communities were *Penicillium chrysogenum* and yeasts of the genus *Rhodotorula*.

**Table 4.** Indicators of taxonomic richness and diversity of fungal communities in deep-sea sediments of the Eastern Equatorial Atlantic, other Atlantic areas, Indian and Pacific oceans

Ocean	Number of taxa					Proportions		
	species	genera	families	orders	classes	G/F	S/F	S/G
<i>Ascomycota</i>								
Eastern Equatorial Atlantic (EEA)	18	6	4	4	3	1.50	4.50	3.00
Atlantic (AO)	34	15	12	11	5	1.25	2.83	2.27
Indian (IO)	68	37	23	12	6	1.61	2.96	1.84
Pacific (PO)	63	39	24	15	6	1.63	2.63	1.62
<i>Basidiomycota</i>								
Eastern Equatorial Atlantic (EEA)	1	1	1	1	1	1.00	1.00	1.00
Atlantic (AO)	4	4	3	3	3	1.33	1.33	1.00
Indian (IO)	18	15	10	8	6	1.50	1.80	1.20
Pacific (PO)	16	12	7	7	3	1.71	2.29	1.33
<i>Mucoromycota</i>								
Eastern Equatorial Atlantic (EEA)	0	0	0	0	0	0	0	0
Atlantic (AO)	0	0	0	0	0	0	0	0
Indian (IO)	0	0	0	0	0	0	0	0
Pacific (PO)	1	1	1	1	1	1.00	1.00	1.00

Note. G/F – genera/families; S/F – species/families; S/G – species/genera.





**Fig. 4.** Average Taxonomic Distinctness index ( $\Delta^+$ ) (a) and Variation in Taxonomic Distinctness index ( $\Lambda^+$ ) (b) based on a list of fungal species in subsurface horizons of deep-sea sediments: 1 – Eastern equatorial Atlantic; 2 – Atlantic Ocean, other areas; 3 – Indian Ocean; 4 – Pacific Ocean. Solid lines demarcate the 95% probability funnels. The dotted line runs through the center of the 95% probability funnel – average calculated index value.

The smallest value of the Average Taxonomic Distinctness index  $\Delta^+ = 50.19$  was recorded for the EEA communities. It was beyond the confidence interval ( $\approx 73$ – $88$ ), and its symbol on the chart (Fig. 4, a) is below the probability funnel. This indicates the uneven distribution of the species across higher taxonomic ranks and statistically significant difference from the taxonomic structure of the other fungal communities under consideration. The species in this area belong to four classes (out of 13). The class *Eurotiomycetes* is dominated by representatives of the family *Aspergillaceae*, 78.9% of the species composition; this proportion is 18.6% in the Indian Ocean, 20.3% in the Pacific, and 23.7% in the other areas of the Atlantic. The symbol of the communities from the other areas of AO (eight classes) is on the lower border of the confidence funnel ( $\Delta^+ = 77.55$ ). The indices of the communities from the Indian and Pacific Oceans (10–12 classes) are the closest to the calculated mean value ( $\Delta^+ = 83.22$ – $82.66$ ). Representatives of the classes *Dothideomycetes*, *Eurotiomycetes*, *Sordariomycetes*, *Agaricomycetes*, *Leotiomycetes*, *Saccharomycetes*, *Microbotryomycetes* were noted in sediments of AO, IO and PO. In the eastern equatorial Atlantic, fungi from the classes *Dothideomycetes*, *Eurotiomycetes*, *Sordariomycetes*, and *Microbotryomycetes* were recorded.

The highest value of in the index  $\Lambda^+ = 945.38$  is found for the communities from the EEA sediment samples, and it is also beyond the confidence interval ( $\approx 220$ – $670$ ). Its symbol is above the confidence funnel on the graph (Fig. 4, b), confirming a high degree of horizontal unevenness in the species distribution along

taxonomic branches and a small number of higher taxonomic ranks. Species of the family *Aspergillaceae* were dominant, and there were one-two species from the classes *Sordariomycetes* and *Saccharomycetes* and one species from the phylum *Basidiomycota* (class *Microbotryomycetes*). The index values for the other regions ( $\Lambda^+ = 364.18$ – $495.22$ ) are within the confidence funnel.

The taxonomic richness and diversity of fungal communities in the oceans (Table 4) and the values of the genera/families, species/families and species/genera proportions reflect the average distribution of genera at two relatively low taxonomic levels, but do not reveal the distribution of species in them and, even more so, in the higher taxonomic ranks in specific regions. At the same time, the kingdom Fungi includes a great number of classes and, accordingly, taxa of lower levels. The graphs of the  $\Delta^+$  and  $\Lambda^+$  indices clearly show the similarities/differences in the structure of the communities under consideration, with the distribution of species in the high ranks taken into account. Thus, these graphs are useful when analyzing large numbers of species and regions, and data on taxonomic richness and diversity are helpful in justifying the values of the indices.

In the EEA sediments, fungi of the classes *Eurotiomycetes*, *Sordariomycetes*, *Saccharomycetes*, and *Microbotryomycetes* were detected, which fact is consistent with results obtained for the other regions of the World Ocean (Raghukumar, Raghukumar, 1998; Raghukumar et al., 2010; Damare et al., 2006; Zhang et al., 2014; Xu et al., 2014, 2018; Rédou



et al., 2015; Wang et al., 2019; Rojas-Jimenez et al., 2020; Keeler et al., 2021). In our study, common fungal species of the genera *Alternaria*, *Cladosporium*, *Phoma* and *Fusarium* were not found, which make up from 11.4% (Pacific Ocean) to 31.6% (other areas of the Atlantic) of the species composition. Apparently, this is due to the small amount of data and insufficient research on fungi in sediments of the Eastern Equatorial Atlantic.

It is known that the structure of fungal communities in sediments is not dependent on such factors as hydrostatic pressure, water salinity, presence of oxygen, and geographical location of site (Rojas-Jimenez et al., 2020; Marchese et al., 2021). This is confirmed also by the results of the present research. Further studies on fungi in sediment layers are needed to make more comprehensive and substantiated conclusions about the influence of environmental conditions on these organisms.

## CONCLUSION

For the first time, fungal communities from sediment samples collected in the Eastern Equatorial Atlantic from ocean depths of 4165–5964 m at horizons 1.0–4.7 m below the water–sediment interface have been characterized. In similar studies, names of fungal taxa no lower than at the genus level are typically indicated, and frequently, they are for higher taxonomic ranks up to phyla. Therefore, it is difficult to compare the obtained results with the literature data. Based on the literature data, a list of species in sediments from the Indian, Pacific and Atlantic oceans, including the Equatorial Atlantic, has been compiled. This has allowed us to identify common features of the structure of the fungal communities: 1) species from the phylum *Ascomycota* account for 92.5% of the species composition and abundance; 2) there is a high incidence of yeasts (50.0% for *Saccharomycetes*); 3) a yeast of the genus *Rhodotorula* (*Basidiomycota*) has been isolated. The features of the fungal communities from the Eastern Equatorial Atlantic are: 1) identified species are predominantly from the family *Aspergillaceae* (78.9%); 2) small number of fungal classes (four only) have been identified; 3) the widespread genera *Alternaria*, *Cladosporium*, *Phoma*, and *Fusarium* are lacking. The values for the Average Taxonomic Distinctness index (Delta+) and Variation in Taxonomic Distinctness index (Lambda+) calculated for the fungal communities from EEA samples were beyond the 95% confidence interval. Consequently, the taxonomic structure of these communities is significantly different from those of the other areas of the World Ocean in question, which is clearly displayed in the index graphs and is confirmed by the data on taxonomic richness and diversity. It has been found in the present study that the structure of the fungal communities from the Eastern Equatorial Atlantic was not influenced by the ocean depth at the sampling stations or physicochemical characteristics

(temperature, salinity and pH) of water above the sediments. Also, no differences in the fungal communities from different types of sediments and between the Romanche and Chain Fracture Zones have been detected.

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## Сообщества микроскопических грибов в глубоководных осадках экваториальной Атлантики

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Микобиота глубоководных отложений, особенно тех, которые находятся на глубине нескольких метров ниже границы раздела “вода — осадок”, изучена слабо. В этой работе впервые были исследованы комплексы микроскопических грибов, выделенных методом культивирования из глубоководных отложений восточной части экваториальной Атлантики (зоны разломов Романш и Чейн). Грибы выделены из осадков, отобранных на каждой из 12 станций с горизонтов на глубине 1.0–4.7 м ниже границы раздела донные “отложения — вода”. Для изучения микроскопических грибов из каждого керна был выделен один горизонт донных отложений. Численность грибов изменялась в пределах 0.0–3300.0 КОЕ на 1 г сухого веса осадка. В общей сложности было идентифицировано 19 таксонов грибов из отделов *Ascomycota* (18) и *Basidiomycota* (1) и выделен неопознанный вид *Mycelia sterilia* 1. Семь видов грибов были обнаружены по одному разу. Максимальное сходство видового состава по коэффициенту Брэя — Кертиса составило 57.14% (горизонты 1.0 и 3.6 м, четыре общих вида). Было проведено сравнение таксономических структур комплексов грибов из района исследования с комплексами из осадков Индийского и Тихого океанов и других районов Атлантики. На основе литературных данных и результатов настоящего исследования был составлен список видов грибов из 180 наименований. Грибы принадлежали к 97 родам, 57 семействам, 32 отрядам и 13 классам отделов *Ascomycota*, *Basidiomycota* и *Mucoromycota*. Разнообразие грибных сообществ оценивалось с использованием показателей таксономического богатства (количество таксонов разных рангов), пропорций (род/семейство, вид/семейство, вид/род), среднего индекса таксономической отличительности ( $AvTD$ ,  $\Delta^+$ ) и вариации индекса таксономической отличительности ( $VarTD$ ,  $\Lambda^+$ ). В донных отложениях восточной части экваториальной Атлантики и Индийского океана были обнаружены четыре и двенадцать классов грибов соответственно. Соотношение видов и родов в сообществах варьировало от 1.33 (Индийский океан) до 3.8 (другие районы Атлантического океана). Для комплексов грибов восточной экваториальной Атлантики значение индекса  $AvTD$  было минимальным ( $\Delta^+ = 50.19$ ), индекса  $VarTD$  — максимальным ( $\Lambda^+ = 945.38$ ), и они находились за пределами 95% доверительного интервала. Это связано с малочисленностью классов грибов, а также с вертикальной и горизонтальной неравномерностью распределения видов по таксономическим ветвям, что проявлялось в доминировании видов семейства *Aspergillaceae* (78.9% видов, класс *Eurotiomycetes*), 2 вида, принадлежали к классу *Sordariomycetes*, и по одному виду к классам *Saccharomycetes* и *Microbotryomycetes* из отдела *Basidiomycota*. Следовательно, были обнаружены статистически значимые различия между таксономическими структурами комплексов грибов восточной экваториальной Атлантики и других регионов Мирового океана, которые обусловлены недостаточным количеством полученных данных о видовом составе грибов в донных отложениях этого р-на. Исследование не выявило какой-либо закономерности в изменении количества видов грибов и их обилия в зависимости от параметров воды (температура, pH, соленость), глубины горизонта в керне отложений, типа отложений или расположения станции отбора проб в зонах разломов Романш и Чейн.

**Ключевые слова:** абиотические факторы, индекс таксономической отличительности, индекс вариативности таксономической отличительности, морские грибы, подстилающие горизонты осадка, *Aspergillaceae*