## ORIGINAL PAPERS

# Fungi in Bottom Sediments of the Chukchi Sea

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Abstract—We present the first study on the quantity and diversity of the mycobiota in bottom sediments of the Chukchi Sea. During implementation of the RUSALCA-2012 program, 22 samples of bottom sediments from depths of 44–110 m were collected in late August—early September 2012. Fungi were isolated on agarized media (salinity 35 psu, temperature 6°C). Species identification was performed by morphological-cultural and molecular-genetic methods based on the nucleotide sequences of ITS1-ITS2 rDNA. A total of 128 colonies of mycelial fungi were isolated; 0 to 22 propagules were obtained from each 1 cm<sup>3</sup> sample. The overall diversity was represented by 48 morphotypes belonging to 32 genera in 15 orders of ascomycetes and basidiomycetes. Ascomycetes predominated both in number and diversity. A number of species were found in marine ecosystems for the first time. The taxonomic position of nonsporulating isolates (13% of the total) was established using molecular techniques. Among them, high diversity was observed in the orders Pleosporales and Helotiales (Ascomycota).

Keywords: marine fungi, Ascomycota, Basidiomycota, diversity, molecular methods, ITS, Arctic seas, Chukchi Sea, bottom sediments

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### **INTRODUCTION**

Marine mycology is developing fairly rapidly; however, fungi of the Arctic seas have been given little attention thus far. More than 30 works presenting the results of studies of fungi in the Arctic seas [27] have been published since the middle of the 19th century. Most investigations were conducted in the European sector of the Arctic. Only three works are known for the seas to the north of Siberia. One work provides data on fungi in water of the Arctic seas, including the Kara and Laptev seas [4]; the other two deal with fungi in bottom sediments of some areas of the Barents and Kara seas [2, 9]. The cultivated mycobiota of bottom sediments of the Arctic seas has a number of characteristic features: a low density of fungal propagules and a large proportion of nonsporiferous cultures, as well as of species of the genera *Tolypocladium*, *Penicillium*, and Cladosporium [2, 9, 27]. The taxonomic position of up to 30-40% of sterile isolates is difficult to determine by routine morphological-cultural methods. Their identification requires molecular genetic analysis, which has not been used in studies on the diversity of mycobiota in bottom sediments of the Arctic seas.

The purpose of the present research was to investigate the diversity of mycobiota in bottom sediments of the previously unstudied Chukchi Sea using morphological-cultural and molecular genetic methods of identification.

## MATERIALS AND METHODS

The material was 22 samples of bottom sediments collected during the period from August 30 to September 13, 2012 in the Chukchi Sea during the cruise of the R/V *Professor Khromov* within the framework of the RUSALCA-2012 program (Fig. 1). Sampling was carried out at depths of 44 to 110 m in two areas: northeast of Wrangel and Herald islands and in the southern part of the sea closer to the Bering Strait. The sediments studied were silts. The Chukchi Sea is characterized by harsh conditions, relatively small freshwater discharge, and a marked influence of two oceans, that is, the Arctic and Pacific [3, 21].

A 1 cm<sup>3</sup> sample from an upper 1-cm layer of bottom sediments was taken three times with a sterile syringe and placed in a sterile paper bag. Bags with samples were dried at room temperature. Inoculation was carried out every 60 days after final collection. For isolation of fungi, all sediment in sample was mixed and 1 cm<sup>3</sup> of the sediment from each sample was inoculated by the method of Warcup [10] on a malt–agar medium (total sugar content 0.2%, salinity 35 psu, ampicillin 1 g/L). After two months of incubation at a temperature of 6°C, pure cultures were isolated.

Primary identification was carried out based on morphological and cultural features in pure culture [10]. Apart from the identified cultures, nonsporulating and nonconforming morphotypes were fairly numerous in the samples.

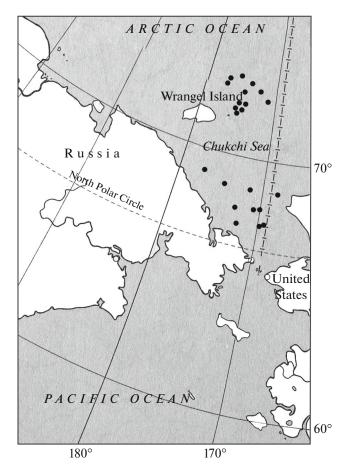


Fig. 1. The positions of sampling sites.

Representatives of all morphotypes that could not be identified based on the morphological and cultural features were used for molecular genetic analysis. DNA was isolated from 40 pure cultures with a Diatom Pro kit (Isogen, Moscow). The polymerase chain reaction of the ITS1-5.8S-ITS2 region was carried out using a ScreenMix kit (Evrogen, Moscow) and the ITS1-F and ITS4 primers (http://lutzonilab. org/nuclear-ribosomal-dna/) on a Verity thermocycler (Applied Biosystems, United States). The amplification products were sequenced in two directions according to the BigDye protocol using an ABI Prism 3500 sequencer (Applied Biosystems, United States). Sequence assembly and alignment, as well as hylogenetic tree construction, were performed using the CodonCod Aligner software package (www.codoncod.org). Sequence identity was determined with the BLAST program (blast.ncbi.nih.gov). Twelve sequences of ITS1-5.8S-ITS2 of isolates that were identified to species were deposited to GenBank under the accession numbers from KP739870 to KP739881. For taxonomic identification of the other sequences, phylogenetic trees based on the ITS1-ITS2 region were constructed in two variants for Ascomycota and Basidiomycota, respectively, using the MUSCLE [14] and

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statistically supported by the neighbor-joining method [15] in the MEGA 6.0 program [31, 32].

## RESULTS

## Number of Isolated Fungi

A total of 128 colonies of mycelial fungi were isolated (Table 1). We obtained from 0 to 22 propagules from a sample; most samples yielded no more than 10 propagules and several samples yielded one propagule each.

#### Morphological Diversity

According to morphological features, 48 morphotypes were distinguished (Table 1). Among them, 17 were identified: most species (16) were from the anamorphic ascomycete genera Penicillium, Cladosporium, Tolypocladium, Aspergillus, Fusicolla, Pseudogymnoascus, Lecanicillium, and Trichoderma; only one species. Pseudeurotium hygrophilum, produced telomorphic sporulating structures. Seventy-two isolates belonged to morphotypes that were identified by this method. The genus Penicillium was the most frequent and abundant (44 colonies in 19 samples). P. glabrum (21 colonies in 10 samples), P. aurantiogriseum (13 colonies in 9 samples), and P. nalgiovense (6 colonies in 4 samples) were more frequent than others. For species of the genus *Cladosporium* (C. cladosporioides and C. macrocarpum), we isolated 12 colonies in six samples, while for Aspergillus flavus we isolated 8 colonies in three samples. Other species were found less frequently and 11 of them were only encountered as single colonies in some samples.

The remaining 56 isolates were assigned to 31 unidentified morphotypes (Table 1). Of these, 16 isolates were sterile, 6 isolates had teleomorphic structures of ascomycetes, 2 were anamorphic picnidial sporulating, and 7 had structures resembling the hyphomycete-type anamorphs. Despite the fact that the number of unidentified isolates was lower, their morphological diversity was higher than that of known species.

### Molecular Identification and Taxonomic Diversity

Using molecular genetic methods, we identified 40 cultures that were referred to 31 doubtful morphotypes. For species-level identification, GenBank data were only available for 14 morphotypes (Figs. 2, 3). Most of the cultures that were identified to species, except the Pleosporales ascomycete *Nematostoma par-asiticum (Herpotrichia parasitica)* and the polypore basidiomycete *Bjerkandera adusta*, had any sporebearing structures (Table 1). For 17 morphotypes, only genus- or even order-level identification was available. In some cases, identity was found with phylotypes that were identified using molecular techniques were mostly represented by single colonies in

Table 1.	The taxonomic	composition	of the	identified fungi
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Morphological identification	Genetic identification	BLAST	Num	ber of
			colonies	samples
ASCOMYCOTA				
PEZIZOMYCOTINA				
Incertae sedis			1	
Mycelia sterilia 14	Slimacomyces isiolus (R.T. Moore) G.Z. Zhao	99	1	1
DOTHIDEOMYCETES				
Botryosphaeriales				
Botryosphaeriaceae		1	1	1 .
Ascomycete gen. sp. 3	<i>Lasiodiplodia pseudotheobromae</i> A.J.L. Phillips		1	1
Capnodiales				
Cladosporiaceae			1	
Cladosporium cladosporioides Fresen.) G.A. de Vries			6	4
C. macrocarpum Preuss			6	3
Pleosporales				
Mycelia sterilia 5	Fungal sp. MG20Sn7L3x	89	1	1
Phoma cf. eupyrena	Uncultured fungus clone CMH252	100	1	1
Aycelia sterilia 10	Uncultured fungus clone S238	99	1	1
Aycelia sterilia 11	Uncultured fungus clone S352	100	1	1
Aycelia sterilia 12	Uncultured soil fungus clone RS5M5c23P	94	1	1
Didymellaceae				•
Phoma sp.	Phoma herbarum Westend.	100	1	1
Lophiostomataceae				
Mycelia sterilia 9	Lophiostoma cynaroidis Marincowitz, M.J. Wingf. & Crous	99	1	1
Pleosporaceae				
Alternaria cf. alternata	Alternaria alternata (Fr.) Keissl.	99, 100	2	2
Dothideomycetes Incertae sedis				
Pseudoperisporiaceae				
Mycelia sterilia 3	<i>Nematostoma parasiticum</i> (R. Hartig) M.E. Barr	96	2	1
EUROTIOMYCETES		·		
Eurotiales				
Trichocomaceae		1	1	I
Ispergillus flavus Link			8	3
Penicillium atramentosum Thom			1	1
P. aurantiogriseum Dierckx			13	9
P. chermesinum Biourge			1	1
P. dierckxii Biourge			1	1
P. glabrum (Wehmer) Westling			21	10
P. nalgiovense Laxa			6	4
P. thomii Maire			1	1

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## FUNGI IN BOTTOM SEDIMENTS

Table I. (Collid.)	Table	1.	(Contd.	)
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Morphological identification	Genetic identification	BLAST	Num	ber of
Morphological Identification	Generic Identification	DLAST	colonies	sample
LEOTIOMYCETES				
Thelebolales				
Thelebolaceae				
Ascomycete gen. sp. 1	Thelebolus microsporus (Berk. & Broome) Kimbr.		1	1
Ascomycete gen. sp. 2	Thelebolus stercoreus Tode	98	1	1
Helotiales	1		1	1
Mycelia sterilia 2	<i>Cadophora</i> sp.	92	1	1
Mycelia sterilia 8	<i>Cyathicula</i> sp.	94	2	1
Mycelia sterilia 16	Hymenoscyphus sp. FC-2727	99	1	1
Mycelia sterilia 13	Helotiales sp. WMM-2012c isolate 24m	96	1	1
Mycelia sterilia 15	Lachnum sp. FR-F3	95, 96	5	2
Mycelia sterilia 7	Uncultured Lachnum clone R2_17	96	1	1
Mycelia sterilia 4	Helotiales sp. MU-2009-3	94, 97	1	1
Leotiomycetes Incertae sedis	1		I	I
Myxotrichaceae				
Pseudogymnoascus pannorum (Link) Minnis & D.L. Linder			1	1
Pseudeurotiaceae	1		1	1
Pseudeurotium hygrophilum			1	1
(Sogonov, W. Gams, Summerb. Schroers) Minnis D.L. Linder				
SORDARIOMYCETES	1		1	1
Coniochaetales				
Coniochaetaceae				
Anamorphic gen. sp. 2	Coniochaeta ligniaria (Grev.) Massee		1	1
Hypocreales				I
Cordycipitaceae				
<i>Lecanicillium lecanii</i> (Zimm.) Zare & W. Gams			1	1
Нуросгеасеае	1		I	1
Trichoderma viride Pers.			1	1
Nectriaceae	1		1	1
<i>Fusicolla aquaeductuum</i> (Radlk. & Rabenh.) Gräfenhan, Seifert & Schroers			1	1
Ophiocordicipitaceae	1			I
Tolypocladium cylindrosporum			2	2
W. Gams				
T. inflatum W. Gams			1	1
Microascales				
Microascaceae			1 .	1
Wardomyces sp. 1	Pseudoscopulariopsis hibernica (A. Mangan) Sandoval-Denis, Gené & Cano	97, 98	7	3

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Mampalagiaalidantification	Genetic identification BL		Number of	
Morphological identification	Genetic identification	BLAST	colonies	samples
Hypocreales Incertae sedis				
Acremonium-like anam. sp. 2	Acremonium charticola (Lindau) W. Gams	90	1	1
Sordariales		1		
Chaetomiaceae				
Chaetomium sp.	Chaetomium globosum Kunze ex Fr.	91, 95, 98	3	3
Ascomycete gen. sp. 4	Chaetomium sp. E02	87, 91, 94	3	3
Ascomycete gen. sp. 5	Chaetomium sp. 6/97–55	94	1	1
Xylariales				
Acremonim-like anam. sp. 1	Xylariales sp. 1	91	3	2
BASIDIOMYCOTA		I		
AGARICOMYCETES				
Agaricales				
Mycelia sterilia 6	Psilocybe sp. KR22	95	1	1
Cantharellales		1		
Beauveria-like anam. sp. 1	Sistotrema raduloides (P. Karst.) Donk	99	1	1
Polyporales		1		
Mycelia sterilia 1	Bjerkandera adusta (Willd.) P. Karst.	99	5	3
Anamorphic gen. sp. 1	Trametes versicolor (L.) Lloyd	99	1	1

 Table 1. (Contd.)

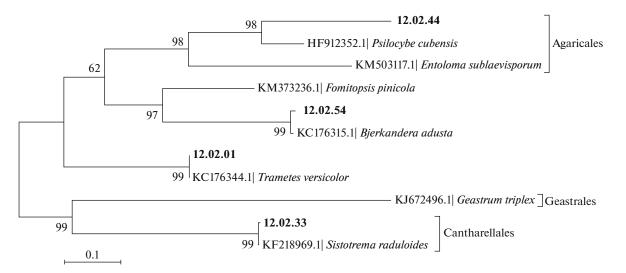
BLAST, percentage similarity of the obtained sequences to the closest sequences available from GenBank.

one to two samples, but sometimes by several colonies in two to three samples. The most frequent were the anamorphic ascomycete *Pseudoscopulariopsis hibernica* (seven colonies in three samples), the basidiomycete *B. adusta* (five colonies in three samples), *Lachnum* sp. FR-F3 (five colonies in two samples), as well as two species of the genus *Chaetomium* (*C. globosum* and *Chaetomium* sp. E02) with a total of six colonies in five samples.

## DISCUSSION

### Number of Isolated Fungi

The number of the obtained fungal colony-forming units approximately corresponded to the data reported for off-shore remote Arctic areas [2, 9]. Closer to the shore, the number of fungal propagules is generally higher [9], as is the case for marine bottom sediments of warmer regions [1, 5-7]. The high number of fungal



## Fig. 2. A phylogenetic tree of isolated basidiomycetes constructed based on ITS1-5.8S-ITS2 gene sequences.

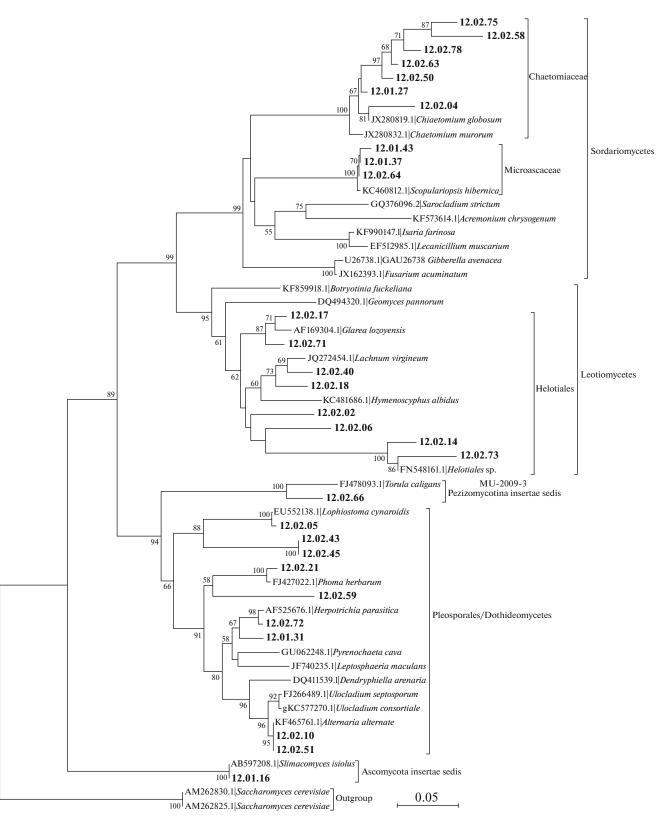


Fig. 3. A phylogenetic tree of isolated ascomycetes constructed based on ITS1-5.8S-ITS2 gene sequences.

Species	Distribution	References	GenBank number
ASCOMYCOTA			·
Slimacomyces isiolus	Plant remains (New Guinea and China)	Moore, 1957; Zhao et al., 2007	AB597208
	Pine needle litter (Hokkaido, Japan)	GenBank, unpublished (Hirose D., Tokumasu S., Ogawa Y.)	AB620068
Lasiodiplodia pseudotheobromae	Parasitic on various trees: Citrinae, acacia, <i>Coffea</i> (The Netherlands and Costa-Rica); <i>Grevillea robusta</i> , Kenya	Alves et al., 2008; GenBank, unpublished (Njuguna J.W., Barklund P., Ihrmark K., Stenlid J.)	FJ904834
Nematostoma parasiticum	Parasitic on conifers (North Africa, Poland, and Germany)	Rossman et al., 2002; Kowalski, Andruch, 2010	AF525676
Lophiostoma cynaroidis	Parasitic on flowering plants in Fijnboch-type shrub ecosystems (South Africa)	Marincowitz et al., 2008	EU552138
Fungal sp. MG20Sn7L3x	Endophytic on gypsophilous plants	Porras-Alfaro et al., 2011	KF752699
Uncultured fungus clone CMH252	Indoor environments (Kansas, United States)	Rittenour et al., 2014	KF800343
Uncultured fungus clone S238	Air dust	Fröhlich-Nowoisky et al., 2009	FJ820726
Uncultured fungus clone S352	Air dust	Fröhlich-Nowoisky et al., 2009	FJ820839
Uncultured soil fungus clone RS5M5c23P	Soil of semiarid grassland	Porras-Alfaro et al., 2011	EU479983
Cadophora sp. AU_BD06	Unknown	GenBank, unpublished (Griffith G.)	JN995648
Cyathicula sp. 34_100A	Winter wheat Triticum aestivum	GenBank, unpublished (Grudzinska- Sterno M., Djurle A., Yuen J., Stenlid J.)	KC989059
<i>Hymenoscyphus</i> sp. FC-2727	Unknown	GenBank, unpublished (Zhao Y.J., Hosoya T., Baral H.O., Hosaka K., Kakishima K.)	AB705232
Helotiales sp. WMM-2012c isolate 24m	Qinghai—Tibet Plateau, psychrophilic communities	GenBank, unpublished (Wang M.)	JX001621
Lachnum sp. FR-F3	Deschampsia flexuosa, subarctic islands	GenBank, unpublished (Poosakkannu A., Nissinen R., Kytoviita MM.)	KJ529001
Uncultured <i>Lachnum</i> clone R2_17	Winter wheat Triticum aestivum	GenBank, unpublished (Grudzinska- Sterno M., Djurle A., Yuen J., Stenlid J.)	KC753434
Helotiales sp. MU-2009-3	Leaves of the common beech <i>Fagus sylvatica</i>	Unterseher and Schnittler, 2010	FN548161
Thelebolus microsporus	Bird droppings, bird rookeries, probably, a parasite. Psychrophile	Leotta et al., 2002; De Hoog et al., 2005	AY942191.1
Thelebolus stercoreus	(Antarctica) Bird droppings, bird rookeries. Psychrophile (Anractica)	De Hoog et al., 2005	AY942194
Scopulariopsis hibernica	Soils of various regions	Sandoval-Denis et al., 2016	FJ946484
Chaetomium sp. E02	Seeds of Puccinellia distans	GenBank, unpublished	KC867277
Chaetomium sp. 6/97–55	Roots, stems, and leaves of common reed <i>Phragmites australis</i>	Wirsel et al., 2001	AJ279466
Coniochaeta lignaria	Parasitic or endophytic on wood	Damm et al., 2010	AJ496242

 Table 2. Data on fungi identified by molecular methods

Species	Distribution	References	GenBank number
BASIDIOMYCOTA	- <b>I</b>	•	
Psilocybe sp. KR22	Roots of Pinus sylvestris	Menkis and Vasaitis, 2011	HM036648
Sistotrema raduloides	Saprotrophic on wood (Finland)	Kotiranta and Larsson, 2013	KF218969
Bjerkandera adusta	Common in soil and on wood; found in Antarctic soils	Thorn et al., 1996; GenBank, unpublished (Vasilenko et al.)	MF120203

Data on the identified species are derived from papers referenced in GenBank. In addition, species distribution and habitat data, if available in surveys in the literature, are also provided. Only GenBank data are given for phylotypes identified on the genus or order level.

propagules in the ocean is connected with the proximity to the coastline and the presence of permanent freshwater runoff. Samples for our study were collected far from the coast; the freshwater discharge into the Chukchi Sea is smaller than in the other Arctic seas. Therefore, a low number of fungal propagules was expected. Surprisingly, there was no north-south gradient in the number of fungal propagules. We believed that the southern area of the Chukchi Sea might have a higher diversity of fungi due to the exchange with the Pacific Ocean via the Bering Strait [3, 17]. It is well known that the temperate Pacific seas are rich in fungi [5, 7]. However, the input of material, particularly fungal spores, to the southern area of the Chukchi Sea is probably not as large as was thought. Another explanation may be low survival rates of fungal propagules that were transferred from the south into the harsh Arctic conditions.

#### Taxonomic Diversity of Mycobiota

On the whole, the picture of mycobiota identified by morphological methods was typical for the bottom sediments of the northern seas [1, 2, 6, 9]. This also applies to the presence of a large number of sterile and ambiguous cultures [27].

We discovered 32 species of fungi using morphological-cultural and molecular methods. The taxonomic structure of the mycobiota consists of species of basidiomycetes (3 orders of one class) and ascomycetes (11 orders of four classes), as well as one group of uncertain taxonomic position. Ascomvcetes predominated both in the number of isolates and in the diversity of morphotypes. Species of the order Eurotiales were the most abundant, followed by species of the orders Helotiales and Pleosporales. Moreover, the order Eurotiales was the most diverse; the orders Helotiales and Pleosporales, as well as Hypocreales and Sordariales showed a marked diversity. Using molecular methods, we were able to reveal the high diversity of the Pleosporales and Helotiales, to which a large number of sterile and unidentified isolates were assigned. Helotiales species probably play a significant but underestimated role in Arctic marine ecosystems. They are, as a rule, rare in morphological-cultural studies; however, their relatively high taxonomic diversity and number was demonstrated using molecular methods [27, 36]. Members of the division Basidiomycota, which were only revealed by molecular methods, are a minor component of the total species complex.

## Autecology of the Isolated Fungi

All the fungi found in the present study can be divided into two large groups: common species to various marine habitats, which are mentioned in numerous publications on marine mycobiota, as well as unusual "guests" that were found in the ocean for the first time. The first group mainly includes abundantly sporulating anamorphs of ascomycetes identified based on morphological features. These all are typical inhabitants of soils and other substrates [13] and are also frequently encountered in marine habitats [1, 4–7, 9]; some of them were identified by molecular methods [25, 27]. Interestingly, most of these fungi do not generally occur in cold regions; they might be transferred via a current from the Pacific Ocean rather than via river runoff.

The second group of fungi were mainly sterile isolates and were identified by molecular methods (Table 2). Information on most of them is scarce, and the geographic distribution is wide and ambiguous; some were previously known only as phylotypes from molecular studies. Many of these fungi are xylotrophs, parasites, or endophytes associated with higher plants (Table 2). It may be the remains of plants (from wood fragments to microscopic pieces of grass and leaves) that provide the opportunity for these fungi to travel long distances through the Pacific Ocean from the Antarctic and Australia to the Arctic. The Pacific Ocean is the most likely source of these interesting species. Currently, too little information is available on the diversity of mycobiota in different geographic localities, and there are many isolates that do not correspond to any of the described species; therefore, the ranges of the fungi we found seem to be disjunct.

Thus, the mycobiota in bottom sediments of the harsh Chukchi Sea demonstrates similar features to those of the previously studied Arctic seas. These are the extremely low number of fungal propagules and the abundance of species of the genus Penicillium and nonsporulating isolates. Using molecular genetic methods, we were able to determine the systematic position of nonsporulating and other ambiguous cultures, which substantially expands the understanding of the taxonomic structure of the studied mycobiota. In particular, members of Basidiomycota, as well as Pleosporales and Helotiales of Ascomvcota were revealed. Remarkably, a noticeable part of the identified fungi are known from various localities from the Antarctic and Australia to China and Japan but were not known from the Arctic. Hence, it can be supposed that the current from the Pacific Ocean influences the mycobiota of the Arctic seas, increasing its species richness. Some of the transferred species can survive and possibly propagate under the Arctic conditions.

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#### COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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