ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 5, pp. 665–676. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © Ya.V. Kireev, O.P. Konovalova, N.S. Myuge, A.V. Shnyreva, E.N. Bubnova, 2015, published in Mikrobiologiya, 2015, Vol. 84, No. 5, pp. 582–594.

EXPERIMENTAL ARTICLES

Cultural Properties and Taxonomic Position of *Helminthosporium*-like Fungal Isolates from the White Sea

Ya. V. Kireev[a], O. P. Konovalova[b], N. S. Myuge[c], A. V. Shnyreva[a], and E. N. Bubnova[b, 1]

[a] *Department of Mycology and Algology, Biological Faculty, Lomonosov Moscow State University, Moscow, Russia*
[b] *Pertsov White Sea Biological Station, Biological Faculty, Lomonosov Moscow State University, Moscow, Russia*
[c] *Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia*

Received November 6, 2014

Abstract—Morphological, cultural, and physiological characteristics of 19 *Helminthosporium*-like hyphomycetes isolated from the White Sea were studied. Taxonomic status of the isolates was verified using molecular genetics techniques. One of the isolates was identified as *Alternatia* sp., while the rest of the marine isolates belonged to the species *Paradendriphiella salina* (G.K. Sutherl.) Woudenb. & Crous. The specific features of the isolates studied were characterized as adaptive. Optimum salinity for their growth was 1–2% NaCl, which is lower than the value for the known open ocean isolates. This is probably due to relatively low salinity of White Sea (22–24‰) as compared with the ocean water (35‰). While the temperature optimum for growth was 22°C, growth and sporulation occurred at 6°C, which has not been reported for marine fungi isolated from warmer seawater. All isolates studied grew and sporulated efficiently on the medium supplied with the *Fucus* algae extract and in the sea water layer. Conidia of the isolates submerged in the sea water were propagated efficiently, unlike the soil-born fungi. Holoblastic conidiogenesis was demonstrated by light and scanning electron microscopy, confirming the separation of *P. salina* from the genus *Scolecobasidium*.

Keywords: Paradendryphiella salina, Alternaria, Bipolaris, marine fungi, physiology, taxonomy and molecular systematics of fungi, White Sea

DOI: 10.1134/S0026261715050136

Ascomycetes of the order *Pleosporales* are most abundant in the ocean. Most of them are saprotrophs on plant debris. Moreover, they occur in soils and can be parasites of algae and higher plants or symbionts of algae (Jones et al., 2009; Kohlmeyer and Kohlmeyer, 1979; Suetrong et al., 2009).

It is often impossible to determine the taxonomic position of such fungi (even at the genus level) while studying their cultural and morphological properties because of the high variability of morphological characteristics of the cultures growing on nutrient media and the absence of teleomorphs (sexual stages) in most cultures. At the same time, the literature provides almost no accurate and detailed descriptions of their anamorphic (asexual) stages. There are two main causes for molecular genetic studies not always being successful: a small number of gene sequences of marine fungi in the databases (GenBank) and an ambiguous current situation in the taxonomy of pleosporalean fungi (Jones et al., 2009; Suetrong et al., 2009, Woudenberg et al., 2013).

Such intricate taxonomic history is exemplified by the two species currently referred to the genus *Paradendryphiella* Woudenb. & Crous: *P. salina* (G.K. Sutherl.) Woudenb. & Crous and *P. arenariae* (Nicot) Woudenb. & Crous. Originally, the species *P. salina* was described in the genus *Cercospora* as *C. salina* Sutherland, while the species *P. arenariae* was described in the genus *Dendryphiella* as *D. arenaria* Nicot. In 1964, Pugh and Nicot transferred *C. salina* to the genus *Dendryphiella* as *D. salina* (Pugh and Nicot, 1964). These species are best known in the literature under the names of *"D. salina"* and *"D. arenaria"* (Kohlmeyer and Kohlmeyer, 1979). The genus *Dendryphiella* comprised several species: together with marine species, it also included terrestrial species and those inhabiting plant substrates and soils of warm regions. In 1979, Ellis transferred the marine species from the genus *Dendryphiella* to the genus *Scolecobasidium* (like *S. arenarium* (Nicot) M.B. Ellis and *S. salinum* (Nicot) M.B. Ellis), regarding them as having the enteroblastic type of conidium formation (Ellis, 1979). Subsequent to him, many mycologists began to use these names; for quite a long time they were the recommended names in the Dictionary of the Fungi (2011). The molecular studies of the early 2000s did not confirm this transfer (Jones et al., 2009; Suetrong et al., 2009). In 2013, an extensive work on the molecular taxonomy of *Alternaria*-like fungi was published (Woudenberg et al., 2013), where the authors described a novel genus, *Paradendryphiella*, comprising only two species: the marine *P. salina*

[1] Corresponding author; e-mail: katya.bubnova@wsbs-msu.ru

and *P. arenariae*. It should be noted that the necessity of describing a separate genus for marine *Dendriphiella* had already been discussed in the literature (Jones et al., 2009). For phylogenetic construction, the DNA sequences of only two type cultures were used: *D. salina* and *D. arenaria*, as well as the type culture *Embellisia annulata* de Hoog, which was finally also assigned to the species *P. salina*. In their work the authors did not provide original descriptions of the cultures but used the descriptions published previously by other researchers (Woudenberg et al., 2013).

P. salina and *P. arenariae* are extremely widespread species. They have been found in all of the studied seas from the tropics to mid-latitudes. They occur in sea marshes, in various soils, on live algae and their dead thalli, on sea grasses and on wood; their conidia commonly occur in water and sea foam (Kohlmeyer and Kohlmeyer, 1979; Artemchuk, 1981; dela Cruz et al., 2006). These species have become the favorite objects of research due to very extensive occurrence, together with the simplicity of isolation and maintenance in culture. More than 40 works are devoted to different aspects of ecophysiology (dela Cruz et al., 2006; Curran, 1980; Duffy et al., 1991; Edvards et al., 1998; Jones and Jennings, 1964; Panebianco, 1994), enzymatic apparatus (Gessner, 1980; Grant and Rhodes, 1992; MacDonald and Speedie, 1982; Rohrmann and Molitoris, 1992), and antimicrobial activity (dela Cruz et al., 2006) of *P. salina* and *P. arenariae*. All of these works were carried out with the isolates obtained from warm and temperate waters; the number of isolates was usually small (up to 10). The maximum number of the studied isolates (32) is presented in the work of dela Cruz et al. (2006), but not all of these cultures were used in all experiments.

While studying fungal biodiversity in different ecotopes of the cold subarctic White Sea, we obtained a number of isolates of unclear taxonomic position, which were morphologically similar to the genera *Helminthosporium, Alternaria*, and some other anamorphs of the order *Pleosporales* (Bubnova et al., 2014). Originally we used the identification guides for dark-pigmented fungi (Sivanesan, 1987; Velikanov and Khasanov, 2003) and attributed most of the *Helminthosporium*-like fungi to the species *Bipolaris australiensis* (Bubnova et al., 2014). However, further studies, including the current work, allowed us to correct this error and to confidently attribute them to the genus *Paradendryphiella*.

This work was aimed at investigating the taxonomic position and physiological properties of *P. salina* isolates from the White Sea.

MATERIALS AND METHODS

The organisms used in research were 19 marine *Helminthosporium*-like fungi isolated in 2003 and 2006 in the vicinity of the Pertsov White Sea Biological Station of Moscow State University (WSBS MSU). They were originally attributed to the species *Bipolaris australiensis* and, hence, our study also involved three soil isolates of *B. australiensis* from the All-Russian Collection of Microorganisms of the Russian Academy of Sciences (VKM RAS) and one isolate, originally identified as *Curvularia lunata* (finally attributed to the genus *Bipolaris*), from the South China Sea. The isolates are characterized in the table.

The cultural and morphological properties of the isolates were assessed on day 7 of their growth on a potato glucose agar (PGA) at room temperature. All isolates were inoculated three times. The diameter, color, and texture of the colonies were described. The micromorphology of the isolates was studied under a light microscope. The following parameters were assessed: (1) micromorphology of the mycelium (color, diameter, and the presence of swellings or chlamydospores) and (2) the micromorphological features of conidiogenic structures and conidia (size, color, branching of conidiophores; the structure of conidiogenous nodes; conidium size and septation).

Scanning electron microscopy (SEM) was used to study conidiogenesis in fresh samples of the mycelium with conidiogenous structures prepared by the standard method with 2% glutaraldehyde (Ando and Nakamura, 2000).

The first stage of molecular genetic studies was biomass production in liquid culture in a glucose yeast medium. The flasks with the medium inoculated with the fungal cultures were incubated for 10–20 days at 22°C on a shaker. DNA was isolated by the standard technique of phenol/chloroform extraction (Lee et al., 1988). DNA concentration and the purity of DNA preparations were determined by spectrophotometry (Perkin-Elmer spectrophotometer) at the wavelengths of 230, 260, and 280 nm. DNA amplification was carried out in a programmable thermal cycler (Eppendorf Mastercycler) using a standard amplification kit (Sileks, Moscow, Russia).

The taxonomic positions of the isolates were defined more precisely by sequencing the ITS1-ITS2 region of the rRNA gene cluster. PCR products were purified by elution from agarose gels using the kit for DNA purification from agarose gels with $MPSiO_2$ magnetic particles (Sileks). The ITS-1(TCCGTAGGTGAACCTGCGG) and ITS-4(TCCTCCGCTTATTGATATGC) primers were used in the work; annealing was carried out at 55°C (Lee et al., 1988). The results of sequencing, alignment, and phylogenetic tree construction were processed with the DNASTAR software package (Lasergene). BLAST (Basic Local Alignment Search Tool) and GenBank database from http://www.ncbi.nlm.nih.gov/ were used for sequence analysis. The Maximum Likelihood algorithm was used for phylogenetic tree construction. The sequences of *P. salina* strains used in phylogenetic analysis were deposited in the GenBank database (table).

Characteristics of the isolates used in the work

Isolate	Sampling site	Time of sampling	Substrate	Species affiliation	VKM accession number of the strain	GenBank sequence number
White Sea *Helminthosporium*-like isolates						
03.3.19	Ermolinskaya Gulf 66°33′18″ N 33°01′18″ E	2003, August	Soil (silt, sand), lower littoral	*Paradendryphiella salina*	–	
03.3.26		2003, August	Soil (silt, sand), mid-littoral	*P. salina*	F-4557	KC 986955
B.h.4.01	Eremeevsky sill 66°33′07″ N 33°06′54″ E	2006, August	Thallus of *Ascophyllum nodosum* ecad. *muscoides*, upper littoral	*P. salina*	F-4556	KC 986958
A.n.k 2		2006, August	Thallus of *A. nodosum*, mid-littoral	*P. salina*	–	
F.c.23.11		2006, August	Thallus of *Fucus vesiculosus* ecad. *muscoides*. upper littoral	*P. salina*	–	
03.2.11		2003, August	Thallus of *Cladophora* sp., upper littoral	*P. salina*	–	
06.11.75		2006, August	Soil (silt, sand), mid-littoral	*P. salina*	–	
03.3.56		2003, August	Thallus of *A. nodosum*, mid-littoral	*P. salina*	–	
06.11.2.	Cape Kindo 66°32′17″ N 33°11′35″ E	2006, August	Thallus of *A. nodosum*, mid-littoral	*P. salina*	–	
06.11.3		2006, August	Soil (sand), upper littoral	*P. salina*	–	
06.11.4		2006, August	Mussel shells, upper littoral	*P. salina*	–	
1.3 B	Biofilters Bay 66°32′25″ N 33°09′56″ E	2006, August	Thallus of *F. vesiculosus*, upper littoral	*P. salina*	–	
1.5.B		2006, August	Thallus of *Cladophora* sp., mid-littoral	*P. salina*	–	
06.8.25		2006, August	Soil (sand, silt), mid-littoral	*Alternaria* sp.	–	

Table. (Contd.)

Isolate	Sampling site	Time of sampling	Substrate	Species affiliation	VKM accession number of the strain	GenBank sequence number
06.2.38	Plashkov Island 66°31′05″ N 33°09′24″ E	2006, March	Thallome of *F. serratus*, sublittoral	*P. salina*	–	
B.h.9H5	Kostyan Island 66°29′53″ N 33°23′48″ E	2006, August	Thallus of *F. vesiculosus*, mid-littoral	*P. salina*	–	
B.h.10		2006, August	Thallus of *F.distichus*, mid-littoral	*P. salina*	F-4556	KC 986958
06.4.4		2006, August	*Phaeophyceae*, mid-littoral	*P. salina*	–	
06.10.88	Velikaya Salma strait 66°32′ N 33°14′ E	2006, July	Ground (sandy aleuropelite), depth 77.5 m	*P. salina*	F-4558	KC 986954
Additional isolates						
6.241	Nha Trang Bay, South China Sea	2002, March	Thallus of *Padina* sp.	*Bipolaris* sp.	–	
F-3040*	Egypt	–	Desert soil	*B. australiensis*	–	
F-955*	Ukraine, Donetsk region	–	Soil	*B. australiensis*	–	
F-3704*	Republic of Karachayevo-Cherkesia, Teberdinsky State Natural Biosphere Reserve	–	gravelly soil	*B. australiensis*	–	

* Isolates obtained from VKM RAS.

The effects of abiotic factors on culture development were studied by assessing the linear growth rate of the colonies under different conditions. Inoculations were made by a single stab into the center of a dish in triplicates. In all tests, the diameter of colonies on the agar in petri dishes was measured on day 10 of their growth, and the mean diameter was calculated.

Halotolerance of the isolates was assessed in malt agar with sodium chloride concentrations of 0, 0.5, 1, 2, 3, 5, 10, 15, 20, and 25%. Thirteen isolates were tested at a salt content of 0–5 g/L and five isolates were tested at a salt content of 10–25 g/L.

The temperature optima were determined as follows: the petri dishes containing PGA inoculated with the isolates under study were placed into thermostats at 6, 22, and 37°C. The test was performed with 5 isolates.

The ability to assimilate carbon from brown algae was assessed as follows: the studied isolates were inoculated into an original medium, fucus agar (100 g of dry thalli of the White Sea species of the genus *Fucus* (*Phaeophyceae, Fucales*) were ground in a porcelain mortar into small crumbs; 16 g of the agar was added per 1 L of fresh water). The strains growing on starvation agar were used as the control.

Fifteen *Paradendryphiella* isolates from the White Sea, one *Bipolaris* sp. isolate from our collection, and three VKM cultures were inoculated into petri dishes with PGA; then 50 mL of autoclaved natural seawater from the White Sea was applied onto dish surface. The dishes were examined under a light microscope on day 10. Microscopy showed the presence of sporophores, chlamydospores, rhizomorphs, and other structures in the fungal colonies developed under the water layer.

The peculiar features of conidial germination in all strains under study were assessed as follows: the slides with conidial preparations in seawater were placed into moist chambers for 24 h and then examined under the microscope. The experiments were carried out with the natural seawater (24‰ salinity) from the Velikaya Salma strait of the White Sea (the vicinity of WSBS). Conidial preparations in distilled water were used as the control. The water was not sterilized. The preparations were examined to find out the germinating conidial cell (or cells), and the ratio of the sizes of germ tubes in fresh and sea water was determined. The first characteristic is considered important for the sys-

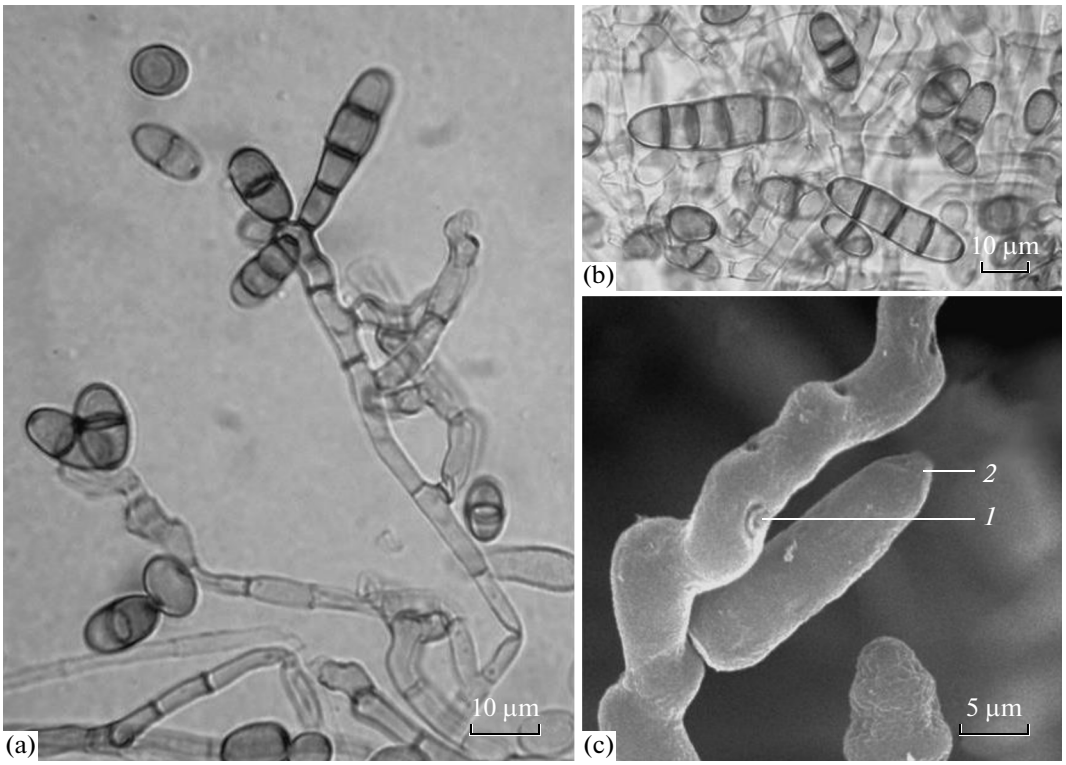

Fig. 1. Conidia and conidiophores of *P. salina*, light microscopy (a); conidia of *P. salina*, light microscopy (b); and conidiophore and conidium of *P. salina*, SEM (c). *1*, the pore of the conidiophore; *2*, the ridge on the conidium.

tematics of anamorphs of pleosporalean fungi (Velikanov and Khasanov, 2003). We considered the second parameter to be important with regard to assessment of species adaptation to marine environments.

RESULTS AND DISCUSSION

The absolute majority of isolates under study were identified as members of the species *Paradendryphiella salina*. We have provided the following descriptions of morphological and cultural features of the isolates of this species.

***Paradendryphiella salina* (G.K. Sutherl.) Woudenb. & Crous.**

***Basionym*:** *Cercospora salina* G.K. Sutherl.

≡ *Dendryphiella salina* (G.K. Sutherl.) Pugh & Nicot

≡ *Scolecobasidium salinum* (G.K. Sutherl.) M.B. Ellis

≡ *Embellisia annulata* de Hoog, Seigle-Mur., Steiman & K.-E. Erikss.

The *colonies* of the isolates are prostrate, 3–5 cm in diameter, olive brown, dark brown, up to nearly black, and velvety. The *mycelium* is septate, 2.5–8 μm thick, light to dark brown, mostly submerged; the aerial mycelium is darker than the submerged one. *Conidiophores* are single, straight, unbranched or irregularly (1- to 4-fold) branched, and smooth. They are often angular, with the terminal and intercalary conidiogenous nodular swellings (Fig. 1a), light brown, septate, up to 60 μm long and up to 6 μm thick. *Conidiogenous cells* are polytertiary, terminal or intercalary, proliferating, with dark rings at the points of attachment of conidia. *Conidia* are acropleurogenous poroconidia, oblong, straight, subcylindrical, with 1 or more septa (up to 8), usually slightly constricted at the septa, 8–52 μm long and 4–9 μm thick. For the most part slightly colored, light brown, smooth or a little rough. Have a distinct ridge that does not project beyond the conidium (Figs. 1a, 1b). Single, do not form chains in the standard medium.

All of the *P. salina* cultures under study were similar in most characteristics: growth rate, the color and texture of colonies, and the structural features of mycelium and conidiogenous apparatus. There were insignificant differences only in the size and number of septa in the conidia, these characteristics, however, are generally instable in this group of fungi (Jones et al., 2009).

The characteristics of the studied cultures correspond to the known descriptions (Pugh and Nicot, 1964; Kohlmeyer and Kohlmeyer, 1979). These characteristics are also quite similar to the descriptions provided by Ellis (1979), except for the type of conidiogenesis. The peculiar feature of the genus *Scolecobasidium* is enteroblastic conidia formed on the spines of different length (Ellis, 1979). Light microscopy of all

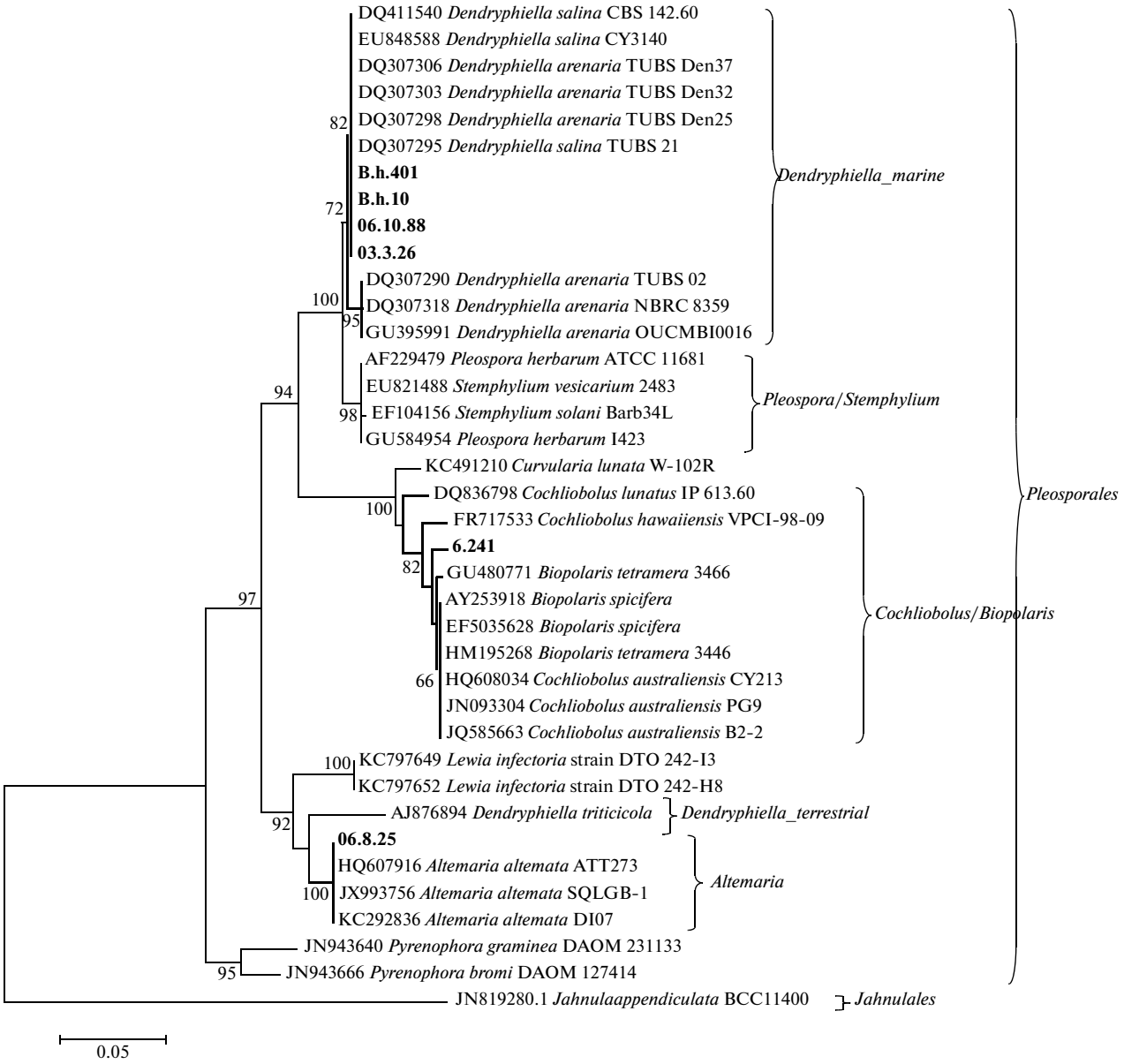

Fig. 2. The phylogenetic tree constructed on the basis of rDNA ITS1-5.8S-ITS2 sequences. Alignment length: 631 nucleotides. Scale bar: the number of substitutions per nucleotide. The bootstrap index is indicated near the base of the branches.

cultures showed that they had poroconidia and, accordingly, holoblastic conidiogenesis. It can be clearly seen that the conidia formed on dark-colored conidiophores are almost colorless and become darker over time. The SEM study (Fig. 1c) showed that the conidiophore had a rounded pore but not a spine at the site of conidium attachment. Thus, our research into the morphology of conidia formation confirms the correctness of separation of *P. salina* from the genus *Scolecobasidium*.

Molecular genetic studies demonstrated that most of the White Sea isolates that we have studied unambiguously belonged to the species *Paradendryphiella salina* (Fig. 2). In the database, this species is still referred to as *Dendryphiella salina*; however, taking into consideration that we have analyzed also the CBS 142.60 isolate used by Woudenberg for describing the novel genus, we can say that our isolates unambiguously belong to the genus *Paradendryphiella*. The constructed phylogram (Fig. 2) clearly shows the separate positions of marine *Dendryphiella* (*D. salina* and *D. arenaria*) relative to other representatives of the genus, e.g., *D. triticola*. Unfortunately, other species of this genus were not considered in the analysis for description of the genus *Paradendryphiella*. Nevertheless, our constructions only confirm the necessity of assigning these species to a separate genus. Apart from genetic differences, we should also mention that noth-

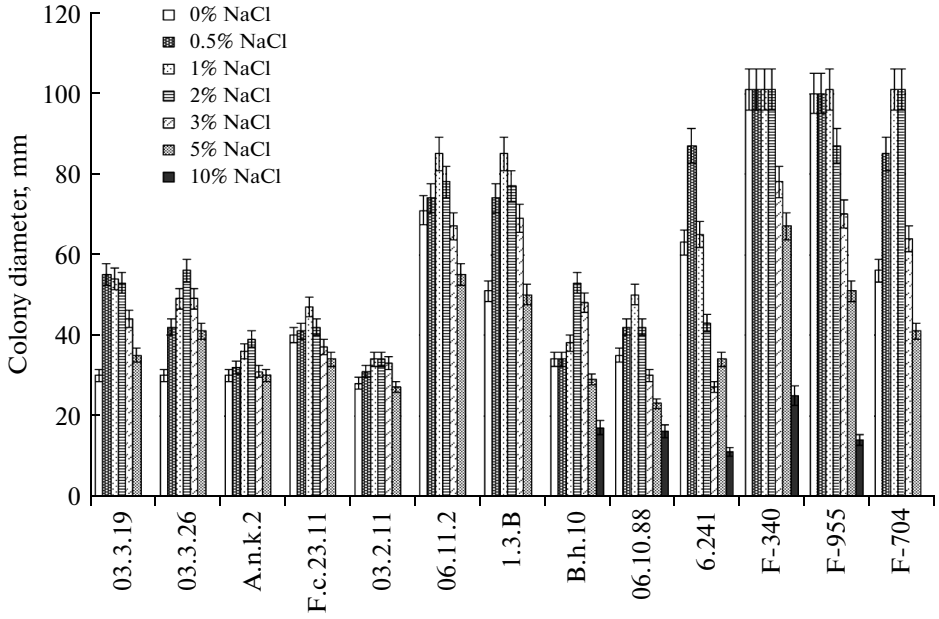

Fig. 3. Colony diameter of the isolates on day 10 of growth on the media with 0 to 100 g/L of NaCl.

ing is known about teleomorph stages in the former marine representatives of the genus *Dendryphiella*, in contrast to terrestrial species. The differences in ecology are obvious too. Thus, the former marine representatives of the genus *Dendryphiella* stand apart from nonmarine species.

All of the studied *P. salina* cultures grew well and sporulated at a salinity of 0 to 5% NaCl (Fig. 3). At 10% NaCl in the medium, the colony diameters of both isolates decreased drastically. Only one isolate (B.h.10) demonstrated slight growth at 15% NaCl: about 2 mm on day 10; at the same time, no sporophore was observed. None of the isolates grew at the higher values of salinity. The 1–2% NaCl content in the medium was optimal for most isolates. The increase in salt content had no noticeable effect on external appearance of the colonies, on sporophore morphology, or sporulation intensity. The only exception was B.h.10: on the medium with 5% NaCl, it formed distinct sectors with small (5–10 times less than in the rest of the colony) conidia formed in chains (Fig. 4).

The preference of average salinity for marine *Dendryphiella* has already been mentioned before (Edvards et al., 1998; Jones and Jennings, 1964). Nevertheless, dela Cruz et al. (2006) indicate that the salinity of 35 g/L is optimal for most of the 16 cultures studied by the authors, which is quite understandable, because this salt content corresponds to the average salinity of the ocean. Dela Cruz et al. did not reveal any peculiar features of the isolates from the regions with different values of natural water salinity. However, our data show that such preferences still may be observed, taking into account that the organisms used in our studies were isolated from the White Sea, where water salinity is below the oceanic value: 24‰ on average.

The analysis of culture growth at different temperatures (Fig. 5) showed that both White Sea isolates could not grow at 37°C, while the temperature of 22°C was optimal for them. Earlier studies demonstrated that the optimal temperature for growth and sporulation of marine species of the genus *Dendryphiella* was 20–30°C (Curran, 1980; Duffy et al., 1991; Edvards et al., 1998; Panebianco, 1994). Dela Cruz et al. (2006) showed that 22°C was the optimal temperature the growth of all 16 *D. arenaria* and *D. salina* isolates irrespective of their sampling site. At 37°C, the growth of all cultures was inhibited, which seems to be a common characteristic of these species. At 5°C, the growth was very weak, mostly without sporophore formation. In the cited work, the isolates from temperate and subtropical regions were used. Thus, the marine *Dendryphiella* seem to be characterized by temperature adaptations, since the White Sea isolates grew well and sporulated abundantly at 6°C, in contrast to the isolates from the warmer seas.

The studied cultures of *Paradendryphiella* were able to grow in the medium with fucus extract (Fig. 6). Most of them grew on fucus agar 1.5–2 times more rapidly than on starvation agar. It has already been shown that *P. salina* can synthesize laminarinase (Grant and Rhodes, 1992). Laminarin was also found in algae of the genus *Fucus* (Powell and Meeuse, 1964). Members of the genus *Paradendryphiella* probably have other enzymes for the cleavage of specific polysaccharides of marine algae. The exception was the B.h.10 isolate. It did not show any difference when

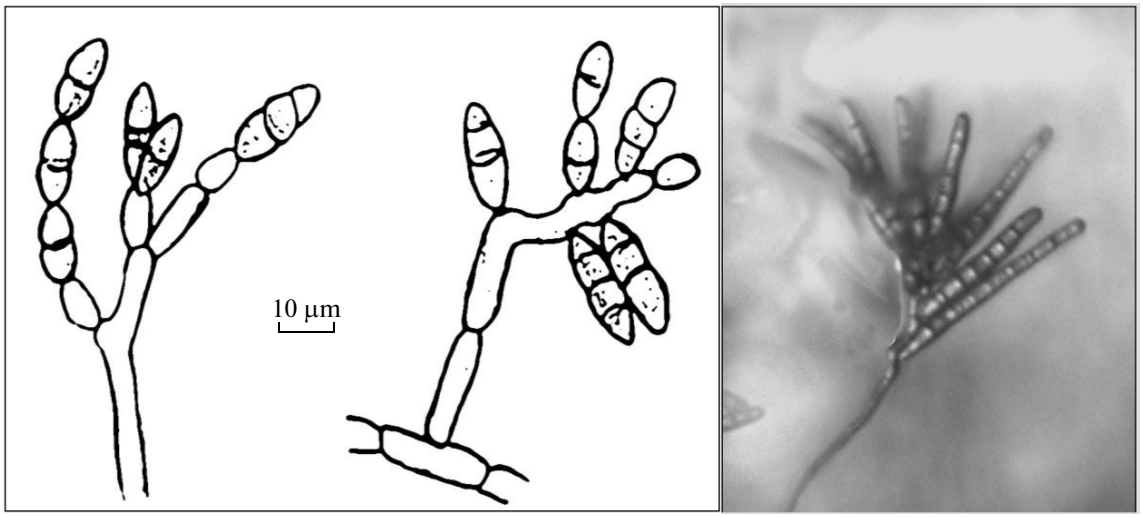

Fig. 4. The conidial sporophore of *D. salina* B.h.10 on the medium with 5% NaCl. In the sector with small conidia (on the left); ordinary sporophore (on the right).

growing on fucus and starvation media. We have no explanation for this fact, taking into consideration that this organism was isolated from the thallus of *Fucus distichus* (table).

The studied cultures were able to grow an agar medium under the layer of sea water. In 5–6 days, mycelium could be noticed already above the water surface. Here, in the air, typical conidiophores formed, as during their growth on an agar medium under standard conditions. Morphology of the mycelium and sporophores present in the water layer was of particular interest. The mycelium of all isolates under study was colorless or weakly pigmented. All isolates demonstrated abundant formation of chlamydospores and rhizomorphs. Conidia formation in the water layer was observed only in the Â.h.10 isolate. Moreover, very few conidia were formed, compared to the standard growth conditions; they were nonseptate and small, no more than 10 × 5 μm.

The study of conidial germination patterns revealed that the common regularities of germination in *P. salina* isolates resembled those known for the genus *Dreschlera*: these could be from any conidial cell with equal probability (Velikanov and Khasanov, 2003). Comparison of the features of their germination in seawater and fresh water showed that the conidia of all isolates gave rise to germ tubes of almost the same length in both cases.

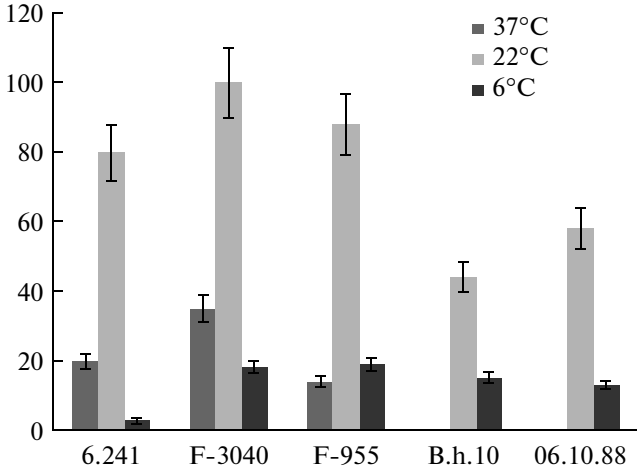

Fig. 5. Colony diameter of the isolates on day 10 of growth on PGA at different temperatures. The *Y*-axis: colony diameter in mm.

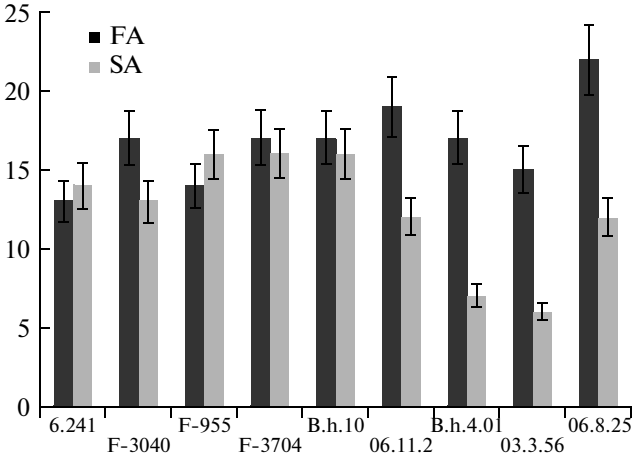

Fig. 6. Colony diameter of the isolates on fucus (FA) and starvation (SA) agar. The *Y*-axis: colony diameter in mm.

One of the studied White Sea isolates (06.8.25.) was slightly different from other isolates in its morphological and cultural characteristics. At the same time, the genetic differences between the former and the latter were considerable (at the genus level). Below we provide the description of its morphological, cultural, genetic, and physiological features.

Alternaria sp.

The isolate 06.8.25 showed small difference from the studied *P. salina* isolates in external appearance, colony texture and growth pattern, structural features of conidiophores and conidial nodes, and conidial formation. Its distinctive characteristic was the shorter rounded conidia with a smaller number of septa. They were 6–20 μm long and 4–8 μm thick; there were no more than two septa. Several short unbranched chains of conidia were also observed during its growth on the standard medium. It would be very difficult to attribute this isolate to the genus *Alternaria* on the basis of its morphological and cultural characteristics. At the same time, the differences from *Paradendryphiella* were so negligible that this culture could be erroneously attributed to this genus.

Molecular genetic investigation of this isolate showed that it was distant from the genus *Paradendryphiella*. It proved to be close to the species *Alternaria alternata*, but we refer to it as *Alternaria* sp., since *A. alternata* is a complex species (Suetrong et al., 2009; Woudenberg et al., 2013). This cluster combines the isolates with great morphological differences; at the same time, there may be substantial genetic differences between morphologically similar isolates, which is common for pleosporalean fungi in general (Crous et al., 2007). The isolate is morphologically different from the classical description of the species *A. alternata*. Therefore, so far we retained the name of *Alternaria* sp. for this culture.

The isolate 06.8.25 was used only for testing the utilization of algal polysaccharides, growth under seawater layer, and conidial germination. The growth pattern on the medium with fucus extract was similar to that of *P. salina* cultures (Fig. 6). On starvation agar medium, the mean diameter of the colonies was notably less compared to the growth on fucus agar. Hence we may conclude that the enzymatic apparatus of the isolate is potentially adapted to utilization of such polysaccharides. The pattern of growth below the seawater layer was not fundamentally different from that of *P. salina* cultures. Here, the mycelium also filled the whole dish by day 6 and appeared on the water surface; a sporophore typical of this culture growing on an agar under standard conditions was formed in the air. The submerged mycelium was almost colorless; there was abundant chlamydospore and rhizomorph formation; no conidial formation in the water layer was observed. Conidial germination occurred from any cell with equal probability, similar to the studied isolates of *P. salina*. Comparison of the patterns of their germination in seawater and fresh water showed that in both cases conidia gave rise to germ tubes of nearly the same length.

Apart from the White Sea isolates, the cultures from southern regions attributed to the genus *Bipolaris* were used in the study. Most of the cultures were isolated from soils and obtained from the All-Russian Collection of Microorganisms, Russian Academy of Sciences. They belong to the species *B. australiensis*:

***Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama**

***Basionym*:** *Drechslera australiensis* M.B. Ellis

≡ *Helminthosporium australiense* Bugnic.

≡ *Drechslera australiensis* (Bugnic.) Subram. & B.L. Jain

≡ *Curvularia australiensis* (M.B. Ellis) L. Manamgoda

***Teleomorph*:** *Cochliobolus australiensis* (Tsuda & Ueyama) Alcorn

≡ *Pseudocochliobolus australiensis* Tsuda & Ueyama

Colonies are prostrate, dark brown, velvety, 4–5 cm in diameter. *Mycelium* is septate, light to dark brown, smooth, 2 to 4 μm thick; the submerged mycelium is brighter than the aerial mycelium. *Conidiophores* are single, winding or angular, septate, smooth, cylinder-shaped, reddish brown, sometimes up to 150 μm long, mainly 3–7 μm thick. *Conidiogenous nodes* are terminal or intercalary, rough. *Conidia* are straight, ellipsoid or oblong, with rounded ends, light to medium reddish brown; most conidia have 3 pseudosepta, more rarely 4 or 5; the length of conidia is 13–40 (mainly 18–33) 6–11 (mainly 8–10) μm. Single, do not form chains on the standard medium.

The external characteristics of the cultures correspond exactly to those of the species *Bipolaris australiensis* (Sivanesan, 1987; Velikanov and Khasanov, 2003). Their molecular genetic investigation has not been carried out.

The cultures grew well and sporulated in a broad salinity range (Fig. 3). All three of them developed well at 0–2% NaCl in the medium. Their growth notably decreased at 3% NaCl and was not observed at all at more than 10%. These features distinguish *B. australiensis* isolates from the studied *P. salina* isolates.

In contrast to the studied cultures of *P. salina*, *B. australiensis* isolates grew well and sporulated at 37°C (Fig. 5). The F-3040 isolate from Egypt demonstrated the maximum growth rate at this temperature. The diameter of *B. australiensis* colonies cultivated at 6°C was approximately 4–5 times less compared to the growth at 22°C. This difference was greater than in the case of the White Sea isolates. It indicates that *B. australiensis* prefers warm and moderate temperature conditions.

The studied cultures were found to grow on the medium with fucus extract (Fig. 6). However, no reli-

able differences in colony size were observed during their growth on fucus and starvation agar, except for the F-3040 isolate. This fact leads to a conclusion that the members of this species are incapable of efficient extraction of carbon from the polysaccharides of brown algae. Moreover, fucus agar may have an unfavorable effect on the growth of soil isolates due to the mycostatic properties of brown algae extracts, which is in agreement with the literature data (Sieburth and Tootle, 1981).

The isolates of *B. australiensis* were capable of growth on agar under a layer of seawater. The general pattern was not different from what had been shown for the *P. salina* and *Alternaria* sp. isolates. Mycelium also covered the whole dish and appeared on the water surface, where it formed a sporophore typical of this species. Regular spores were not formed in the water layer; only different swellings and chlamydospore-like structures were formed.

The study of conidial germination of the isolates in fresh water and seawater demonstrated the following peculiar characteristics: monopolar germination was no less frequent than bipolar germination. The F-955 isolate demonstrated quite extraordinary types of germination, e.g., by the second or by the first and second out of four cells. Bipolar germination of conidia is considered typical of the genus *Bipolaris* (Velikanov and Khasanov, 2003). The germ tubes are on average 10 times shorter during the germination in seawater than during the germination in fresh water. Thus, the test for conidial germination in saline water clearly distinguishes between marine and nonmarine species.

The last of the studied isolates (6.241) was obtained from the thallus of the brown alga *Padina* from the South China Sea. According to its morphological and cultural characteristics, it belonged to the species *Bipolaris australiensis*; however, this identification has not been unambiguously confirmed by molecular analysis. Therefore, for the time being, we refer to it as *Bipolaris* sp. Its description is given below.

Bipolaris sp.

This isolate is most similar to the species *Bipolaris australiensis* by morphological and cultural characteristics. The external appearance of the *colonies* does not differ from that of the *B. australiensis* isolates we have studied. The structures of *conidiophores* and conidiogenous nodes are also similar to those in *B. australiensis* (Fig. 7a). Almost all *conidia* of this culture have 3 pseudosepta; the conidia with 4 pseudosepta are extremely rare and those with 5 pseudosepta have not been found at all, in contrast to the studied isolates of *B. australiensis* (Fig. 7b). The conidia are 15–36 × 7–10 μm in size.

Based on the results of molecular genetic analysis, this isolate has been attributed to the genus *Bipolaris*. According to the analysis of ITS1-ITS2 alignment, the species of the genera *Bipolaris* and *Cochliobolus* are not separated because of slight differences between the sequences (Fig. 2). In order not to complicate the situation, thus far we refer to this culture as *Bipolaris* sp.

The culture grew well and sporulated at a salinity of 0.5% NaCl (Fig. 5). At higher salinity values, the colony size decreased. This isolate was the worst adapted to saline environments compared to all other isolates under study. The study of growth temperature optima (Fig. 5) showed that this isolate grew well and sporulated at 37°C. It demonstrated the maximum growth rate at 22°C. At 6°C, the colony diameter decreased more than 25 times. All these data characterize the isolate as thermophilic, with the optima in the range of warm and moderate temperatures. The diameter of colonies was slightly smaller on the medium with fucus extract (Fig. 6) compared to starvation agar. This fact leads us to a conclusion that the enzymatic apparatus of this culture is poorly adapted to utilization of the polysaccharides of marine algae. The growth of colonies in a seawater layer showed no fundamental differences in development pattern from those of other tested cultures. Monopolar germination was no less frequent for the conidia of this isolate than bipolar germination. Germ tubes were on average 10 times shorter during the germination in seawater compared to the germination in fresh water. In general, the physiological properties characterize this isolate as poorly adapted to dwelling in the marine environment, although it has been isolated from a marine habitat.

In conclusion, we would like to mention the following. It is well known that quite a lot of terrestrial fungi can be found in the world ocean. Some of them not only survive but can also function in the marine environment. As is exemplified by the *Paradendryphiella* species, the evolution of such species seems to take place in this environment. With insignificant morphological differences from their terrestrial relatives, the fungi residing in the seas become genetically distinct from them at the species and genus levels and acquire adaptive physiological features (e.g., the ability to utilize typical marine polysaccharides). The White Sea is an isolated basin weakly connected with the Arctic Ocean. The peculiar features of the White Sea waters are their lower salinity compared to the ocean and permanently low temperatures. These hydrological characteristics of this basin probably resulted in emergence of a *Paradendryphiella salina* population with a number of adaptive features untypical of the isolates of this species from other regions of the global ocean.

ACKNOWLEDGMENTS

The authors are grateful to N.E. Ivanushkina (All-Russian Collection of Microorganisms, Russian Academy of Sciences) for her assistance in identification of the cultures, and to Dr. I. Druzhinina (Institute of Chemical Engineering, Vienna, Austria) and T.V. Neretina (White Sea Biological Station of Mos-

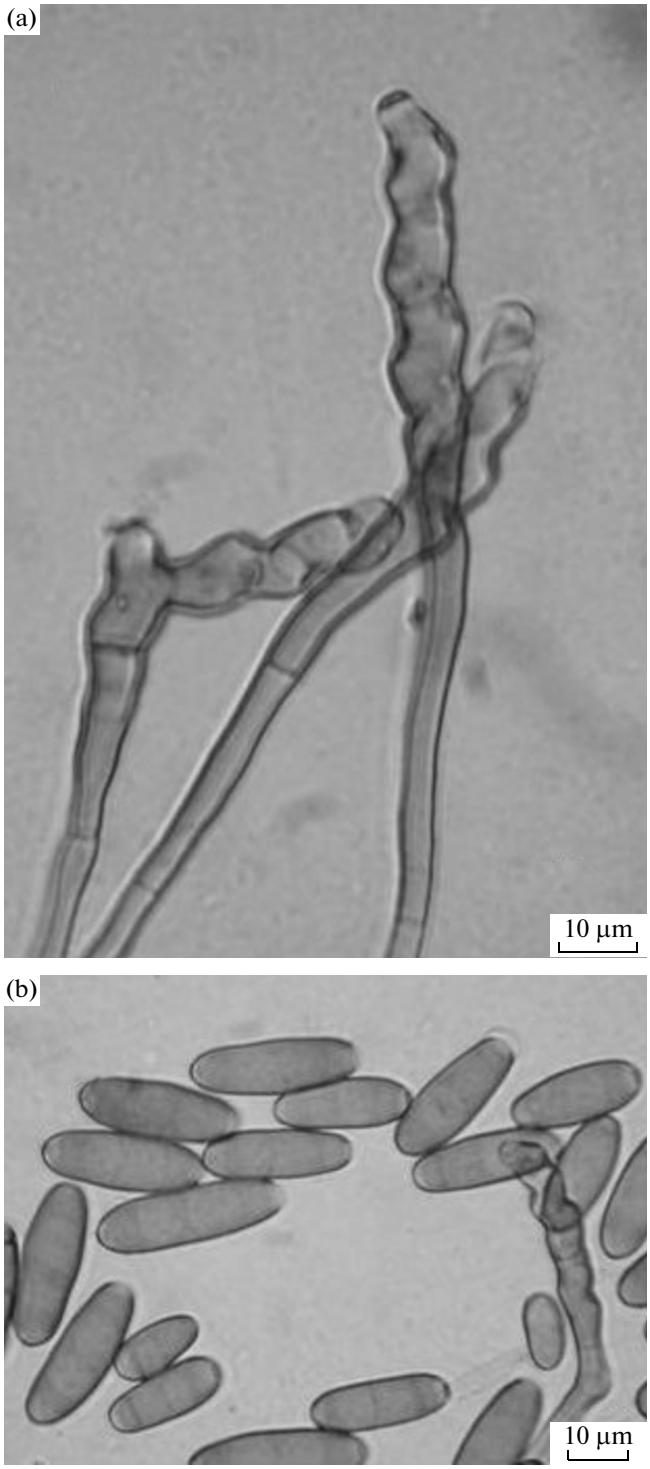

Fig. 7. Conidiophores of *Bipolaris* sp., light microscopy (a); and conidia of *Bipolaris* sp., light microscopy (b).

cow State University) for discussion of the results of molecular genetic studies.

The work was partially supported by the Russian Foundation for Basic Research (project no. 15-04-02722a; genetic research, data processing) and the Russian Science Foundation (project no. 14-50-00029; culture descriptions, physiological tests, data processing).

REFERENCES

Ando, K. and Nakamura, N., *Pseudosigmoidea*: a new genus for hyphomycete (ATCC 16660) formely identified as *Sigmoidea prolifera, J. Gen. Appl. Microbiol.*, 2000, vol. 46, pp. 51–57.

Artemchuk, N.Ya., *Mikoflora morei SSSR* (Microflora of the Seas of the USSR), Moscow: Nauka, 1981.

Bubnova, E.N., Grum-Grzhimaylo, O.A., Konovalova, O.P., and Marfenina, O.E., Fifty years of mycological studies at the White Sea Biological Station of Moscow State University: challenges, results, and outlook, *Moscow Univ. Biol. Sci. Bull.*, 2014, vol. 69., no. 1, pp. 23–39.

Crous, P.W., Braun, U., and Groenewald, J.Z., *Mycosphaerella* is polyphyletic, *Stud. Mycol.*, 2007, vol. 58, pp. 1–32.

Curran, P.M.T., The effect of temperature, pH, light and dark on the growth of fungi from Irish coastal waters, *Mycologia*, 1980, vol. 72, pp. 350–358.

Dictionary of the Fungi, 10th ed., Kirk, P.M., Cannon, P.F., Minter, D.W., and Stalpers, J.A., Eds., CABI Europe – UK, 2011.

dela Cruz, T.E., Wagner, S., and Schulz, B., Physiological responses of marine *Dendryphiella* species from different geographical locations, *Mycol. Progress.*, 2006, vol. 5, pp. 108–119.

Duffy, A.P., Curran, P.M.T., and Muircheartaigh, I.G.O., Effect of temperature and nutrients on spore germination in marine and non-marine fungi, *Crypt. Bot.*, 1991, vol. 2., no. 3, pp. 125–129.

Edwards, J., Chamberlain, D., Brosnan, G., West, D., Stanley,M.S., Clipson, N.J.W., and Hooley, P., A comparative physiological and morphological study of *Dendryphiella salina* and *D. arenaria* in relation to adaptation to life in the sea, *Mycol. Res.*, 1998, vol. 102, no. 10, pp. 1198–1202.

Ellis, M.B., *More Dematiaceous Hyphomycetes*, Surrey: Cab International, 1979.

Gessner, R.V., Degrading enzyme production by salt-marsh fungi, *Bot. Mar.*, 1980, vol. 23, pp. 133–139.

Grant, W.D. and Rhodes, L.L., Cell-bound and extracellular laminarinase activity in *Dendryphiella salina* and five other marine fungi, *Bot. Mar.*, 1992, vol. 35, pp. 503–511.

Jones, E.B.G. and Jennings, D.H., The effect of salinity on the growth of marine fungi in comparison with non-marine species, *Trans. Br. Mycol. Soc.*, 1964, vol. 47., no. 4, pp. 619–625.

Jones, E.B.G., Sakayaroj, J., Suetrong, S., Somrithipol, S., and Pang, K.-L., Classification of marine *Ascomycota*, anamorphic taxa and *Basidiomycota, Fungal Diversity*, 2009, vol. 35, pp. 1–203.

Kohlmeyer, J. and Kohlmeyer, E., *Marine Mycology, the Higher Fungi*, New York: Academic, 1979.

Lee, S.B., Milgroom, M.G., and Taylor, J.W., A rapid high yield mini-prep method for isolation of total genomic DNA, *Fungal Genet. Newsletter*, 1988, vol. 35, pp. 23–24.

MacDonald, M.J. and Speedie, M.K., Cell-associated and extracellular enzyme activity in the marine fungus *Dendryphiella arenaria, Can. J. Bot.*, 1982, vol. 60, pp. 838–844.

Panebianco, C., Temperature requirements of selected marine fungi, *Bot. Mar.*, 1994, vol. 37, pp. 157–161.

Powell, J.H. and Meeuse, B.J.D., Laminarin in some Phaeophyta of the Pacific Coast, *Economic Botany*, 1964, vol. 18., no. 2, pp. 164–166.

Pugh, G.J.F. and Nicot, J., Studies on fungi in coastal soils. V. *Denryphiella salina* (Sutherland) comb. nov., *Trans. Brit. Mycol. Soc.,* 1964, vol. 47., no. 2, pp. 263–367.

Rohrmann, S. and Molitoris, H.P., Screening for wood-degrading enzymes in marine fungi, *Can. J. Bot.*, 1992, vol. 70, pp. 2116–2123.

Sieburth, J.Mc.N. and Tootle, J.L., Seasonality of microbial fouling on *Ascophyllum nodosum* (L.) LeJol., *Fucus vesiculosus* L., *Polysiphonia lanosa* (L.) Tandy and *Chondrus crispus* Stackh., *J. Phycol.*, 1981, vol. 17, pp. 57–64.

Sivanesan, A., Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exerohilum* and their teleomorphs, *Mycol. Papers (CABI)*, 1987, no. 158, pp. 1–114.

Suetrong, S., Schoch, C.L., Spatafora, J.W., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sakayaroj, J., Phongpaichit, S., Tanaka, K., Hirayama, K., and Jones, E.B.G., Molecular systematics of the marine Dothideomycetes, *Stud. Mycol.*, 2009, vol. 64, pp. 155–173.

Velikanov, L.L. and Khasanov, B.A., Taxonomy of the formal species *Helminthosporium, Bipolaris, Drechslersa, Exserophilum*, and *Curvularia*, in *Novoe v sistematike i nomenklature gribov* (News on Fungal Systematics and Nomenclature), Moscow: Nat. Akad. Mikol., 2003, pp. 304–341.

Woudenberg, J.H.C., Groenewald, J.Z., Binder, M., and Crous, P., *Alternaria* redefined, *Stud. Mycol.*, 2013, vol. 75, pp. 171–212.

Translated by E. Makeeva