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BIO-COLLECTIONS

Diversity of filamentous fungi associated with *Sargassum miyabei* Yendo

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ABSTRACT

We present data on the diversity of culturable fungi associated with brown algae *Sargassum miyabei* (the Sea of Japan, Russia). By the plate method, 269 isolates were obtained using solid nutrient media and 31 species from 12 genera were identified based on phenotypic as well as molecular genetic traits (using ITS and beta-tubulin markers) in cases where identification based on morphological features alone was difficult. The structure of the species composition was analyzed and the list of species was given. The analysis showed that facultative marine fungi were the vast majority of the species diversity, namely *Penicillium*, *Cladosporium*, *Alternaria*, *Monodictys*, *Sarocladium*, *Trichoderma*, *Fusarium*, *Arthrinium*, *Botrytis* and *Aspergillus* species. Among obligate marine fungi, *Paradendryphiella* and *Piricauda* species were revealed by the method mentioned above. Also, taxonomic affiliation and phylogenetic position of some *Penicillium* representatives have been clarified.

Keywords: marine mycology, fungal diversity, marine fungi, brown algae, *Sargassum*, phylogenetic analysis, ITS, beta-tubulin gene

РЕЗЮМЕ

Киричук Н.Н., Чаусова В.Е., Пивкин М.В. Разнообразие мицелиальных грибов, ассоциированных с *Sargassum miyabei* Yendo. Представлены результаты изучения видового разнообразия микромицетов, ассоциированных с бурыми водорослями *Sargassum miyabei* (Японское море, Россия). Чашечным методом, с использованием плотных питательных сред, было выделено 269 изолятов грибов. На основе фенотипических, а также молекулярно-генетических признаков (генов ITS и бета-тубулина) идентифицирован 31 вид грибов из 12 родов. Проведен анализ структуры изученных грибных комплексов, а также приведен список видов. Анализ видового разнообразия показал доминирование факультативных морских грибов, в частности, выделены представители родов *enicillium*, *Cladosporium*, *Alternaria*, *Monodictys*, *Sarocladium*, *Trichoderma*, *Fusarium*, *Arthrinium*, *Botrytis* and *Aspergillus* species. Among obligate marine fungi, *Paradendryphiella* и *Aspergillus*. Из группы облигатных морских грибов выше упомянутыми методами были выявлены представители родов *Paradendryphiella* и *Piricauda*. Также, на основе молекулярных данных уточнены таксономическая принадлежность и филогенетическое положение некоторых видов *Penicillium*.

Ключевые слова: морская микология, разнообразие микромицетов, морские мицелиальные грибы, бурые водоросли, *Sargassum*, филогенетический анализ, ITS, бета-тубулин

Filamentous fungi have a wide distribution in different marine habitats. Their importance in marine ecosystems cannot be overestimated. Fungi take an active part in interactions with marine hydrobionts of both animal and plant origin. It is known that facultative marine fungi cause diseases of marine animals, as well as parasitize on algae (Dewey et al. 1983, Blaylock et al. 2001, Sterflinger et al. 2001). In addition, they are permanent components of the soil microbial assemblages, participating in the decomposition of organic matter as saprotrophs (Slinkina & Pivkin 2007, Slinkina et al. 2010, Kirichuk et al. 2012).

Compared to soils, macrophytes are more complex and changeable substrates. They are known to release nearly half of the substances they produce, into the environment. In this regard, marine plants are the more preferred substrate for fungi, which are an integral component of autotrophic community. Moreover, there is evidence that facultative marine fungi are able to perform protective functions and prevent algal infection by pathogenic microorganisms, including fungi, due to their production of secondary metabolites (Ding et al. 2008). Of seaweeds, brown algae, due to their resistance to degradation by bacteria and yeasts, are the most preferred for mycelial fungi colonization, as opposite to green and red algae (Pivkin et al. 2006). Kohlmeyer (1972) previously noted a rare occurrence of pathogenic fungi in the center of the Sargasso Sea and a much greater abundance of them on the periphery, on the coast of North Carolina. In recent years, it has been shown that brown algae *Sargassum* are characterized by fungicidal, defoliant and phytohormonal effects (Kavipriya 2011, Khaled et al. 2012, Thenmozhi et al. 2013). This allows the use of compounds from *Sargassum* as ecopesticides, and in this regards, determines the relevance of the study of the mycobiota of these algae. In addition, the determination of the

fungal diversity of *Sargassum* is of practical importance, since these algae serve as raw materials for the production of agar and alginates (Vozzhinskaya et al. 1971). Despite above-mentioned reasons, little information is available for fungi in the periphyton of brown algae (Withers et al. 1975, Moravskaya & Mikhailov 1990, Zuccaro et al. 2003, 2004, 2008, Bubnova & Kireev 2009, Kirichuk & Pivkin 2015a). Another reason is that facultative marine fungi are promising producers of new biologically active compounds (Bugni & Ireland 2004). Thus, the study of the fungal diversity is a starting point in solving many fundamental and applied problems, including understanding the role of this group of micromycetes in marine ecosystems. Today, in mycology, along with traditional methods, molecular genetic approaches are widely used. In particular, molecular markers, such as transcribed spacer regions of the rRNA gene cluster, internal transcribed spacers ITS1 and ITS2, and beta-tubulin gene are often used for phylogenetic investigations (Visagie et al. 2014, Jones et al. 2015).

In the article we present the results of a study of the fungal diversity associated with brown algae *Sargassum miyabei* Yendo using phenotypic and molecular genetic approaches.

MATERIAL AND METHODS

Characteristics of sampling sites

The samples of brown algae *Sargassum miyabei* were collected at Trinity Bay (the Sea of Japan, Russia) (coordinates: 42°37'14.07"N 131°07'42.47"E) in July–August 2011. The salinity of the seawater in Trinity Bay is 30–32‰. The temperature in the growth zone of *Sargassum* algae is from 20 to 26°C in August, and from 0 to -0.9°C in February. The submarine soil composition: silt, sand, pebbles, shells.

Sample collection and fungal isolation

The samples of brown algae were collected in sterile plastic bags. Prior to inoculation, the algae were washed five times in sterile seawater. Fungi were isolated according to the standard method by inoculation of pieces of algae thallus on wort agar prepared using seawater, Tubaki medium, and using filter paper (Litvinov & Dudka 1975). For sample inoculation, 30 Petri dishes with medium and 10 Petri dishes with filter paper were used, which were incubated at room temperature for 1–4 weeks. Colonies of fungi were transferred to the slant seawater wort agar, where they were kept in pure culture. The following media were used to identify the fungi: seawater wort agar, Czapek medium. The media were sterilized in an autoclave under 0.5 atm at 112°C for 30 minutes before use. To suppress bacterial growth, antibiotics penicillin and streptomycin were added to the media (500000 units/L and 0.5 g/L, respectively).

The isolated strains of fungi are stored in the Collection of Marine Microorganisms (official acronym KMM) of the G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS (http://www.wfcc.info/ccinfo/index.php/search/basic/).

Fungal species were identified on the basis of phenotypic features using manuals and original articles. Some species were identified using molecular genetic methods as well, as their correct identification based on morphology alone was problematic.

Morphological characterization

The following media were used to study the phenotypic characteristics of the strains: Czapek medium with yeast autolysate (CYA), malt extract (MEA), yeast extract with sucrose (YES), according to the recommendations (Visagie et al. 2014). Preparation of these media was detailed by Frisvad & Samson (2004). The strains were inoculated at three points on 9-cm Petri dishes and incubated for 7 days at 25°C in darkness. In addition, inoculated CYA plates were incubated for 7 days at 37°C according to Pitt (1979). Microscopic examination was performed with an Olympus CX41 microscope (Olympus Corporation, Japan).

DNA extraction, amplification and sequencing

The cultures used for the molecular studies were grown on MEA at 25°C for 7 days. Genomic DNA was isolated from the mycelium using the MagJET Plant Genomic DNA Kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. PCR was conducted using GoTaq Flexi DNA Polymerase (Promega, Madison, WI, USA). Fragments containing the ITS regions were amplified using the primers ITS1 and ITS4 (White et al. 1990). The reaction profile was 95°C for 300 s, 30 cycles of 95°C for 20s, 55°C for 20s, and 72°C for 90s, and finally 72°C for 300 s. For amplification of the partial β-tubulin gene, Bt2a and Bt2b primers were used (Glass & Donaldson 1995). The reaction profile was 95°C for 300 s, 30 cycles of 95°C for 20s, 55°C for 20s, and 72°C for 90s, and finally 72°C for 300 s. The amplified ITS and partial β-tubulin genes were purified with the ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing of PCR products was performed with the same primers in two directions on an Applied Biosystems SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) using the Big Dye Terminator reagent kit, version 3.1. Gene sequences were deposited in GenBank under accession numbers MW527122, ON751766–751777 for the ITS gene region and ON737911–ON737923 for the partial β-tubulin gene.

Phylogenetic analysis

The ITS gene and partial β-tubulin gene sequences were aligned by MEGA X software version 11.0.9 using MUSCLE and Clustal W algorithms, respectively (Kumar et al. 2018). The available homologs were searched in the GenBank database (http://ncbi.nlm.nih.gov) using the BLASTn algorithm. Phylogenetic analysis was conducted using MEGA X software (Kumar et al. 2018). The ITS gene and partial β-tubulin gene sequences were concatenated into one alignment. All sequence alignments were model-tested prior to tree constructions. A phylogenetic tree was constructed according to the Maximum Likelihood (ML) algorithm based on the Kimura-2 parameter model (Kimura 1980). The topologies of the trees were evaluated by 1000 bootstrap replicates.

RESULTS

The diversity of culturable micromycetes isolated from algae samples was mainly represented by fungi of terrestrial

origin (facultative marine fungi) and, to a lesser extent, by obligate marine fungi. Species number of the latter was only about 10 % of the total species diversity (Fig. 1).

In total, we isolated 269 isolates of mycelial fungi from algae samples in a pure culture. Of these, 179 isolates were identified by us on the basis of both morphological and molecular features and related to 31 species from 12 genera: *Penicillium*, *Cladosporium*, *Paradendryphiella*, *Alternaria*, *Monodictys*, *Piricauda*, *Sarocladium*, *Trichoderma*, *Fusarium*, *Arthrinium*, *Botrytis*, *Aspergillus*. Of all the species identified only 3 were obligate marine fungi – *Paradendryphiella arenaria*, *P. salina* и *Piricauda pelagica* (Table 1).

Fungi from the genus *Penicillium* prevailed in terms of the number of isolates (63 isolates), followed by species of *Cladosporium* and *Paradendryphiella* genera (49 and 40, respectively) (Fig. 2). *Paradendryphiella arenaria* and *Cladosporium cladosporioides* were the most frequently isolated species from the brown algae studied. More than 30 % of isolates belonged to *Mycelia sterilia* (90 isolates), and dematiaceous mycelium was the most abundant (82 isolates). The genus *Penicillium* also dominated in the number of species (22 species). The rest of the genera included 1–2 species. The diversity of fungi from the genus *Aspergillus* was represented by only 2 species (*A. aculeatus and A. penicillioides*), of which *A. penicillioides* was the most abundant. In addition, some representatives of well-known terrestrial phytopathogenic fungi were revealed, such as *Alternaria* spp. (6 isolates), *Botrytis cinerea* (3 isolates), *Fusarium* sp. (1 isolate). Besides, *Arthrinium* spp. (2 isolates), *Piricauda pelagica* (2 isolates) as well as *Monodyctis*, *Sarocladium* and *Trichoderma* species (in number of one isolate each) were revealed. Representatives of the genus *Acremonium* were not found.

The diversity of *Penicillium* species was represented by 22 species belonging to 12 sections. Identification of some representatives of the genus *Penicillium* on the basis of only phenotypic characters turned out to be difficult, so molecular data (ITS and partial β-tubulin sequences) were used to confirm their taxonomic affiliation (Table 2). Phylogenetic analysis showed sequenced strains to be 98–100 % similar to the following species: *P. antarcticum*, *P. murcianum*, *P. sajarovii*, *P. solitum*, *P. spinulosum*, *P. subspinulosum*, *P. roseomaculatum* and *P. mexicanum* (Fig. 3). One strain showed relation to the representatives of the section *Aspergilloides*, namely *P. glabrum*-clade (95 % of similarity) and phenotypically was characterized by limited growth on CYA and YES media and faster growth on MEA at 25°C. Under the frame of this investigation this strain was kept as *Penicillium* sp.

Among the *Penicillium* species, *P. glabrum*, *P. thomii*, *P. antarcticum*, and *P. roqueforti* were the most frequently isolated.

Table 1. The list of fungi isolated from brown algae *Sargassum miyabei*.

Taxon	Number of isolates	Abundance, %
Alternaria sp.	6	2.23
Arthrinium sp.	2	0.74
Aspergillus aculeatus Iizuka	2	0.74
A. penicillioides Speg.	8	3
Botrytis cinerea Pers.	3	1.12
Cladosporium cladosporioides (Fresen.) G.A. de Vries	31	11.53
C. sphaerospermum Penz.	9	3.35
Cladosporium sp.	8	3
Fusarium sp.	1	0.37
Monodictys castaneae (Wallr.) S. Hughes	1	0.37
Paradendryphiella arenariae (Nicot) Woudenb. *et* Crous	37	13.76
P. salina (G.K. Sutherl.) Woudenb. *et* Crous	3	1.12
Penicillium antarcticum A.D. Hocking *et* C.F. McRae	8	3
P. atramentosum Thom	1	0.37
P. brevicompactum Dierckx	3	1.12
P. chrysogenum Thom	2	0.74
P. corylophilum Dierckx	1	0.37
P. expansum Link	1	0.37
P. glabrum (Wehmer) Westling	11	4.01
P. jensenii K.W. Zaleski	1	0.37
P. lividum Westling	1	0.37
P. melinii Thom	1	0.37
P. mexicanum Visagie, Seifert *et* Samson	2	0.74
P. murcianum C. Ramírez *et* A.T. Martínez	1	0.37
P. roqueforti Thom	8	3
P. roseomaculatum Biourge	1	0.37
P. sajarovii Quintan.	1	0.37
P. simplicissimum (Oudem.) Thom	2	0.74
P. solitum Westling	1	0.37
P. spinulosum Thom	2	0.74
P. subspinulosum Houbraken	1	0.37
P. thomii Maire	9	3.35
P. vancouverense Houbraken, Frisvad *et* Samson	1	0.37
P. waksmanii K.W. Zaleski	2	0.74
Penicillium sp.1	1	0.37
Piricauda pelagica T. Johnson	2	0.74
Sarocladium kiliense (Grütz) Summerb.	1	0.37
Trichoderma aureoviride Rifai	1	0.37
T. koningii Oudem.	1	0.37
Mycelia sterilia	90	33.46
Total	**269**	**100**

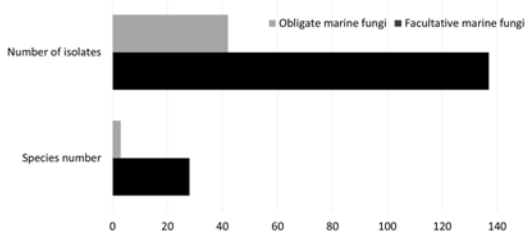

Figure 1 The ratio of the species number and number of isolates of obligate and facultative marine fungi, associated with brown algae *Sargassum miyabei*

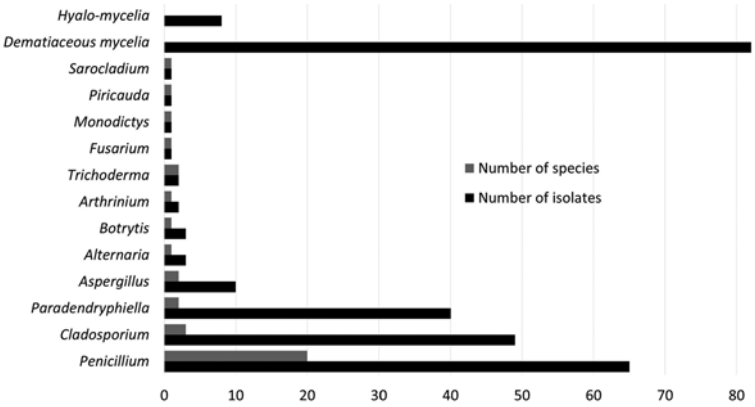

Figure 2 Species number and abundance of isolated micromycetes

Table 2. The list of *Penicillium* strains identified using molecular markers, and their percent of identity with ex-type strain sequences from Genbank.

Taxon	Collection number	ITS	TUB	More similar ex-type material sequences from GenBank	Percent of Identity, %
P. antarcticum	KMM 4720	ON751766	ON737912	*P. antarcticum* CBS 100492^T	100
P. antarcticum	KMM 4727	ON751768	ON737913	*P. antarcticum* CBS 100492^T	100
P. antarcticum	KMM 4685	MW527122	ON737911	*P. antarcticum* CBS 100492^T	100
P. antarcticum	KMM 4724	ON751767	ON737914	*P. antarcticum* CBS 100492^T	99.72
P. murcianum	KMM 4730	ON751776	ON737922	*P. murcianum* CBS 161.81^T	100
P. roseomaculatum	KMM 4729	ON751775	ON737921	*P. roseomaculatum* CBS 137962^T	99.75
P. sajarovii	KMM 4722	ON751770	ON737916	*P. sajarovii* CBS 277.83^T	99.07
P. solitum	KMM 4725	ON751772	ON737918	*P. solitum* CBS 424.89^T	99.74
P. spinulosum	KMM 4728	ON751774	ON737920	*P. spinulosum* CBS 374.48^T	100
P. spinulosum	KMM 4723	ON751771	ON737917	*P. spinulosum* CBS 374.48^T	100
P. subspinulosum	KMM 4721	ON751769	ON737915	*P. subspinulosum* CBS 137946^T	100
P. mexicanum	KMM 4726	ON751773	ON737919	*P. mexicanum* CBS 138227^T	98.31
Penicillium sp.1	KMM 4731	ON751777	ON737923	*P. glabrum* CBS 125543^T	95.07

In general, the representatives of the sections *Aspergilloides* and *Canescentia* prevailed over the other species in terms of species number and abundance. Almost 30 % of all *Penicillium* species related to monoverticillate fungi from the section *Aspergilloides* (6 species), followed by species from the section *Canescentia* (3 species). The most abundant species from these two sections were *Penicillium glabrum*, *P. thomii*, and *P. antarcticum*. Also, representatives of the *Paradoxa* (*P. atramentosum*, *P. mexicanum*), *Exilicaulis* (*P. corylophilum*, *P. melinii*), *Citrina* (*P. vancouverense*, *P. waksmanii*), *Fasciculata* (*P. solitum*), *Lanata-Divaricata* (*P. simplicissimum*), *Ramosa* (*P. sajarovii*), *Roquefortorum* (*P. roqueforti*), *Penicillium* (*P. expansum*), *Chrysogena* (*P. chrysogenum*) and *Brevicompacta* (*P. brevicompactum*) sections were isolated.

DISCUSSION

Thus, the analysis of the fungal assemblages showed the representatives of *Paradendryphiella*, *Cladosporium*, and *Penicillium* species composed the core group of micromycetes associated with *Sargassum miyabei*. An analysis of the data obtained and the results of our previous studies showed that the species composition of the fungal assemblages associated with *S. miyabei* differed significantly from those of submarine soils (Slinkina & Pivkin 2007, Slinkina et al. 2010, Kirichuk et al. 2012). Unlike the latter, the composition of fungi from the genus *Aspergillus* on brown algae is characterized by low abundance and species diversity. The most characteristic species of brown algae was *A. penicillioides* which was not found by us in the submarine soil samples. This species prefers habitats with low water activity caused by dry conditions or high concentrations of sugars or salts (Cantrell et al. 2006). Some strains of *A. penicillioides* have previously been shown to have maximal growth and normal formation of mycelia, vesicles, and also conidial germination, when 10 % NaCl was added to the medium (Nazareth & Gonsalves 2014). Previously, we isolated this species from the leaves of *Zostera marina*, but it was not found in the rhizoplane and rhizosphere of the seagrass (Kirichuk & Pivkin 2015b).

Another species that we have revealed, *Penicillium thomii*, is known as one of the active decomposers of organic matter of plant origin. In the terrestrial environment, this species is associated with plant, occurs on decaying wood and fruits (Urooj et al. 2018). In addition, it is a constant component of fungal assemblages of acidic soils, it is often found on plants of raised bogs (Grum-Grzhimaylo & Bilanenko 2012). Probably, in the marine environment, this species is also mostly confined to plant substrates. We isolated this species from the *Sargassum pallidum* thallus, as well as from the leaves, roots, rhizosphere of *Zostera marina* and from submarine soils in the place of its growth (Kirichuk & Pivkin 2015a,b). However, this species was not found by us in the submarine soils and sponges of the Sea of Japan and the Sea of Okhotsk (Pivkin et al. 2006, Slinkina & Pivkin 2007, Slinkina et al. 2010, Kirichuk et al. 2012).

Penicillium antarcticum, on the contrary, turn out to be the widespread eurybiontic species. It was first found in soil scraping near nest site of Southern fulmar (McRae et al. 1999). However, *P. antarcticum* also occurs in the sea on various substrates (sponges, sediment bottom) (Park et al. 2014), probably being a common component of the marine mycobiota.

Some species of micromycetes revealed were found sporadically. However, among them there were species that are widespread in the marine environment and are often isolated from various marine substrates including marine plants. Among them, there were both obligate marine fungi, for example, *Monodyctis* and *Piricauda* species, and facultative ones. For instance, a known phytopathogen of terrestrial plants *Botrytis cinerea* is a permanent component of the fungal communities of the brown algae *Fucus serratus* (Zuccaro et al. 2008). When we isolated the fungi from the algae samples, we noticed that *B. cinerea* strains grew well directly on the natural substrate and lost their viability very quickly growing on the laboratory medium in pure culture. Probably, this is due to the adaptation to existence in marine environment or to the specific host, in particular. It is known, that, due to the degrading enzyme repertoire, this species is able to inhabit different host plants and feed on their tissues (Zhang et al. 2010, Walker et al. 2011, Grand-Downton et al. 2014, Ferrada et al. 2016). Moreover, it was recently shown, that *B. cinerea* had some strain-specific virulence factors, which may be involved in the host adaptation of the strains (Choquer et al. 2007).

Arthrinium spp. are another micromycetes frequently found in marine habitats. They are permanent components of marine hydrobionts fungal communities. A number of terrestrial species of the genus *Arthrinium* are often isolated from marine sponges, seaweeds, seagrasses (Ebada et al.

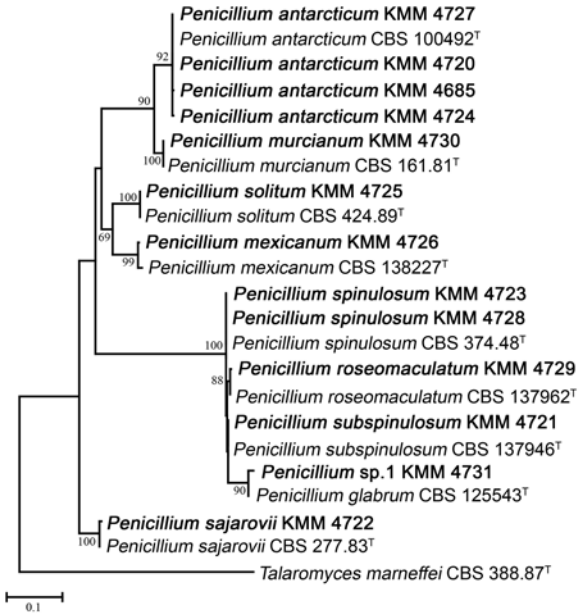

Figure 3 Phylogenetic tree based on maximum likelihood (ML) analysis of concatenated ITS gene and partial β-tubulin gene sequences confirming taxonomic affiliation and phylogenetic position of some *Penicillium* species. Bootstrap values (%) of 1000 replications. Nodes with confidence values greater than 50% are indicated. The scale bar indicates 0.1 substitutions per site

2011, Kirichuk & Pivkin 2015b, Jang et al. 2016). Earlier, a marine *Arthrinium* species was described by N.J. Artemczuk named as *Papularia algicola* N.J. Artemczuk (now *Arthrinium algicola* (N.J. Artemczuk) E.B.G. Jones, Sakay., Suetrong, Somrith. et K.L. Pang) (Artemchuk 1980, Jones et al. 2010). Later, an unknown *Arthrinium* species was discovered on a sand beach and described as new species *Arthrinium marii* Larrondo et Calvo (Larrondo & Calvo 1990). Recently, a phylogenetic analysis of marine-derived *Arthrinium* isolates was carried out, as a result of which 8 new species were described (Kwon et al. 2021). The most of them were isolated from seaweeds, including brown algae. The ability of *Arthrinium* spp. to exist in such a variety of conditions can be explained by a high level of metabolic activity, as well as by the presence of a rich enzymatic system, which is capable of acting depending on conditions according to the required scenario, ensuring the adaptation of the fungus to habitat conditions. It is not for nothing that species of this genus have recently been considered as promising producers of biologically active compounds (Ebada et al. 2011, Wang et al. 2015, Heo et al. 2018).

Species of *Trichoderma* were found sporadically on *Sargassum miyabei*. However, representatives of this genus frequently associate with marine autotrophs. They turned out to be the most characteristic of *Sargassum pallidum*, and were also found in large numbers on *Zostera marina*, namely on the leaves, roots and in the rhizosphere zone (Kirichuk & Pivkin 2015a). Previously, fungi of the genus *Trichoderma* were mentioned in the study of brown algae of the Sea of Japan (*Laminaria*, *Costaria*, *Pelvetia*, *Sargassum*), in particular, *T. viride* was recorded on *Laminaria japonica* (Moravskaya & Mikhailov 1990, Zvereva 1998, Kim et al. 2020). In addition, *Trichoderma* spp. have also been found in sponges (Pivkin et al. 2006, Abdel-Lateff et al. 2009). In addition, as a result of phylogenetic analysis of marine-derived *Trichoderma* spp. three potentially new species were revealed (Kim et al. 2020).

Thus, the species composition of cultureable micromycetes isolated from *S. miyabei* by the plate method was represented mainly by facultative marine fungi. In addition, two potentially new *Penicillium* species have been identified. A comparative analysis of the micromycetes diversity based on the obtained data and published earlier (Kirichuk & Pivkin 2015a) showed a difference in the composition of *Trichoderma* species, which turned out to be the most characteristic of *S. pallidum*. Many of the isolated fungi are often found in the marine environment on various substrates of plant and animal origin. Considering this fact and confirmed cases of active interaction of fungi of this group with various aquatic organisms it is wrong to ignore the role of facultative marine fungi in marine ecosystems. In this regard, some species of facultative marine fungi are justifiably added to the list of marine fungi (Jones 2019). Analysis of species diversity, supported by literature data, shows that the composition of facultative marine fungi associated with marine plants, including brown algae, is not accidental. Despite the fact that some species of micromycetes are rare, they are constantly isolated from different marine habitats. In addition, molecular genetic analysis of strains of facultative marine fungi often reveals ones with an indefinite species affiliation: even strains phenotypically similar to known micromycetes species show genetic differences at the species level. This suggests a much greater species diversity of micromycetes and their specific composition in marine ecosystems.

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