ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 5, pp. 690–698. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © A.B. Antropova, E.N. Bilanenko, V.L. Mokeeva, L.N. Chekunova, A.V. Kachalkin, O.V. Shtaer, O.V. Kamzolkina, 2014, published in Mikrobiologiya, 2014, Vol. 83, No. 5, pp. 605–614.

EXPERIMENTAL ARTICLES

Report of *Quambalaria cyanescens* in Association with the Birch (*Betula pendula*)

A. B. Antropova[a, 1], E. N. Bilanenko[b], V. L. Mokeeva[b], L. N. Chekunova[b], A. V. Kachalkin[b], O. V. Shtaer[b], and O. V. Kamzolkina[b]

[a] *Mechnikov Research Institute for Vaccines and Sera, Russian Academy of Medical Sciences, Moscow, Russia*
[b] *Lomonosov Moscow State University, Moscow, Russia*

Received December 10, 2013

Abstract—Long-term microbiological investigation of the pollen of silver birch (*Betula pendula*) in the Moscow and Moscow oblast areas revealed that almost one-third of the analyzed samples contained the fungus identified by morphological, cultural, and molecular genetic techniques as *Quambalaria cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer. This species was previously known mostly as a symbiont of tropical plants of the genera *Eucalyptus* and *Corymbia* and have not been isolated in Russia. We revealed a close association between *Quambalaria cyanescens* and silver birch. The micromycete was regularly detected in pollen samples, as well as on the inside and outside of the aments, and on the surface of leaves and branches. It was never isolated from other plant species in the investigated area. The data on the morphological and cultural characteristics of the fungus, its cell ultrastructure, and occurrence are presented, as well as the phylogenetic analysis of the isolated strains.

Keywords: *Quambalaria cyanescens*, *Betula pendula*, birch, birch pollen, *Microstromatales*, *Ustilaginomycotina*
DOI: 10.1134/S0026261714050038

It is well known that micromycetes frequently enter into close relationships with plants during their life-cycle. Biocenotic interactions between micromycetes and plants vary from symbiosis to parasitism. However, the area occupied by the fungus does not always depend on that of the plant host. Much attention is constantly given to the issue concerning the strength of a fungus' attachment to a particular plant species and the pattern of their relationships. In 2007, our studies on micromycetes which occur on the pollen of various plants yielded data on the presence of significant numbers of colony-forming units (CFU) of the fungus *Quambalaria cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & Bauer in birch (*Betula pendula*) pollen samples collected in various districts of the Moscow city and the Moscow Region [1]. This fungus was earlier isolated from the human skin and described as *Sporothrix cyanescens* de Hoog & G. A. de Vries in 1973 [2]. In 1987, the cells of this fungus were shown to contain dolipore septa and coenzyme Q-10, and, therefore, the fungus was classified into a new basidiomycete genus (*Cerinosterus cyanescens* (de Hoog & G.A. de Vries) R.T. Moore, *Basidiomycota*). In 2003, the genus *Fugomyces* with the type species *Fugomyces cyanescens* (de Hoog & G.A. de Vries) Sigler was placed in the order *Microstomatales*, based on LSU rDNA sequence. Further molecular-level studies of the ITS region of the rDNA in conjunction with research on the LSU rDNA and ultrastructural analysis data made it possible to discern a new family, *Quambalariaceae* (*Microstromatales*, *Exobasidiomycetidae*, *Exobasidiomycetes*, *Ustilaginomycotina*, *Basidiomycota*), in which the species *F. cyanescens* was placed; its new name was *Q. cyanescens* [3]. Until present, the species has been detected in the air and the soil as well as in association with insects in various countries; clinical isolates have been obtained (www.cbs.knaw.nl). However, the micromycete is predominantly known as a symbiont of plants belonging to two closely related genera, *Eucalyptus* and *Corymbia* [3–6]. Unexpectedly, the fungus was also detected on the birch, and this encouraged us to conduct further research concerning its area, frequency, abundance, and location.

This work is the first full-size report on the detection of *Q. cyanescens* in association with the birch *B. pendula*. The goal of this work was (i) to carry out a phylogenetic analysis and to conduct comparative studies with various *Q. cyanescens* strains isolated from the birch *B. pendula* and (ii) to investigate the ultrastructural characteristics and (iii) the frequency of the fungus on birch trees in the city of Moscow and in the Moscow Region.

[1] Corresponding author; e-mail: antropova-a@yandex.ru

MATERIALS AND METHODS

The studies were conducted during the 2007–2012 period. We analyzed 112 samples of the pollen of the birch *B. pendula* that grows in various areas and biotopes within the Moscow city and the Moscow Region. The tested trees had no visible signs of fungal infection. Pollen samples were collected as follows. Shortly before the onset of flowering (late April-early May), twigs with closed thyrsi were detached from birch trees at a height of less than 2.5 m 5–20 twigs with 20–50 thyrsi were obtained from one tree. The twigs were put in sterile vessels with water and stored in a presterilized laminar box in which sterile paper sheets were placed. As the flowers opened, the pollen was collected in sterile microtubes for further studies. The pollen collected from one tree was regarded as one pollen sample. Pollen was suspended in water, diluted (1 : 10, 1 : 20, 1 : 50, 1 : 100), and inoculated. For comparison, pollen samples of other plants with thyrsi-like inflorescences were analyzed, such as the common hazel (82 samples) and the grey and black alder (22 samples), as well as the cock's-foot (37 samples) and the common timothy grass (17 samples). Approximately 100–300 stalks with unexpanded inflorescences of graminaceous plants were collected from adjacent sods in each bunch.

In order to establish the location of *Q. cyanescens* on the birch and the possible pathways of its migration to its pollen, male inflorescences, twigs, and leaves were inoculated. Branches from 50 birches and thyrsi from 50 birches were sampled either synchronously with pollen collection or in a different season; leaves were collected from 34 birches in spring (after their budding), summer, or the fall. The samples collected were placed on nutrient media. We also analyzed the internal content of male inflorescences. For this purpose, we selected a model birch tree whose pollen samples contained *Q. cyanescens*. Surface sterilization of the thyrsi collected from the model tree was carried out for 4.5 min using 5% sodium hypochlorite solution; thereupon, the thyrsi were washed two times with water, ground in a mortar and placed on nutrient media.

Pollen, twigs, inflorescences, and leaves were inoculated (i) on Czapek agar containing the following: $NaNO_3$ 2 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4$ 0.01 g, sucrose 20 g, agar 20 g, and distilled H_2O 1 L and (ii) on the xerophile medium containing the following: 16°B wort 200 mL, NaCl 100 g agar 20 g, and distilled H_2O 800 mL. The results were monitored after 7–10 days of incubation in a thermostate at $t = 25 \pm 1$°C that is optimum or close to optimum for most mycelial fungi. A pure culture of micromycetes was obtained, and they were identified according to morphological and cultural criteria and molecular genetic traits.

The number of micromycetes per 1 g of pollen was calculated. Frequency was determined as the percentage of fungi-containing samples relative to the total number of samples.

Morphological and cultural features were assessed on Czapek agar, malt agar (16°B wort 150 mL, agar 20 g, and distilled H_2O 850 mL), oat meal agar (oat meal 30 g, agar 20 g, and distilled H_2O 1 L), potato–dextrose agar (potatoes 200 g, glucose 20 g, agar 20 g, and distilled H_2O 1 L), and glucose yeast extract agar (glucose 5 g, yeast extract 0.5 g, KH_2PO_4 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, agar 20 g, and distilled H_2O 1 L).

The genetic identification of the isolated strains that were classified as *Q. cyanescens* according to morphological criteria was performed by analyzing the nucleotide sequences of the ITS1-5.8S-ITS2 region and the D1/D2 domain of the LSU rDNA. The total DNA was isolated from 4–6 day cultures using 300–500 μm glass beads; the samples were incubated for 1 h at 65°C in the lysing buffer (Tris Base 50 mM, NaCl 250 mM, EDTA 50 mM, SDS 0.3%, pH 8) and frozen. The amplification of rDNA regions was carried out using ITS1f (5'-CTTGGTCATTTAGAGGAAGTA) and NL4 (5'-GGTCCGTGTTTCAAGACGG) primers and ScreenMix (Evrogen Closed Joint-Stock Company, Moscow) for the PCR. The PCR product was purified using the BigDye XTerminator Purification Kit (Applied Biosystems, USA). Thereupon, the reaction products were analyzed with an Applied Biosystems 3130xl Genetic Analyzer at the Syntol Research and Development Company (Moscow). The MAFFT 6 [7] and MEGA 4 [8] software packages were used for a phylogenetic analysis of the results obtained. The nucleotide sequences for constructing a phylogenetic tree were taken from descriptive works and NCBI data (http://www.ncbi.nlm.nih.gov/).

The nucleotide sequences of the ITS1-5.8S-ITS2 region and the D1/D2 domains of the LSU rDNA for *Q. cyanescens* strains that were obtained during our studies are available at the ENA and NCBI gene banks; the accession numbers are HG799001–HG799003.

The tested strains were compared in genetic terms by DNA-profiling these strains using PCR analysis with the M13 non-specific primer. DNA isolation was performed as described above. Amplification and electrophoresis were carried out according to the method published earlier [9].

Samples for scanning electron microscopy (1 cm^3 blocks of the upper agar layer with the fungus) were fixated for 1 h in 2.5% glutaraldehyde solution prepared with 0.1 M phosphate buffer (pH 7.2) and washed three times (each washing period was 20 min) with the buffer. The samples were dehydrated with 30, 50, 70, and 96% ethanol solutions and with acetone, desiccated at the critical point under CO_2, and coated with metal. The fungus was investigated using a JSM-6380 scanning electron microscope (Japan).

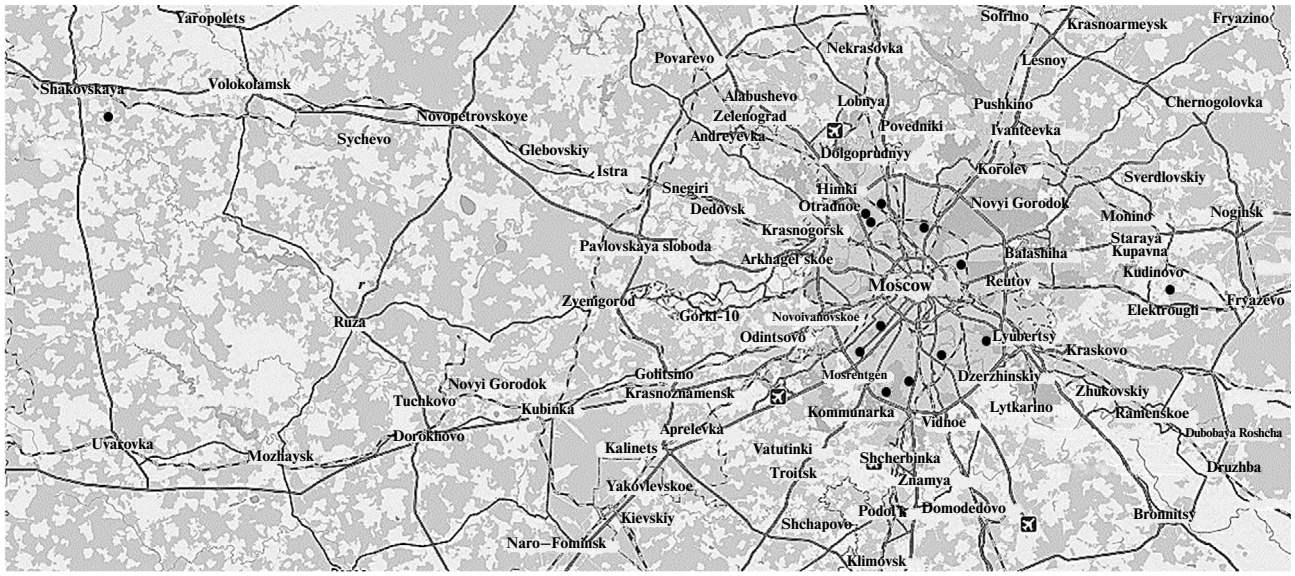


Fig. 1. Locations in which *Q. cyanescens* was detected.

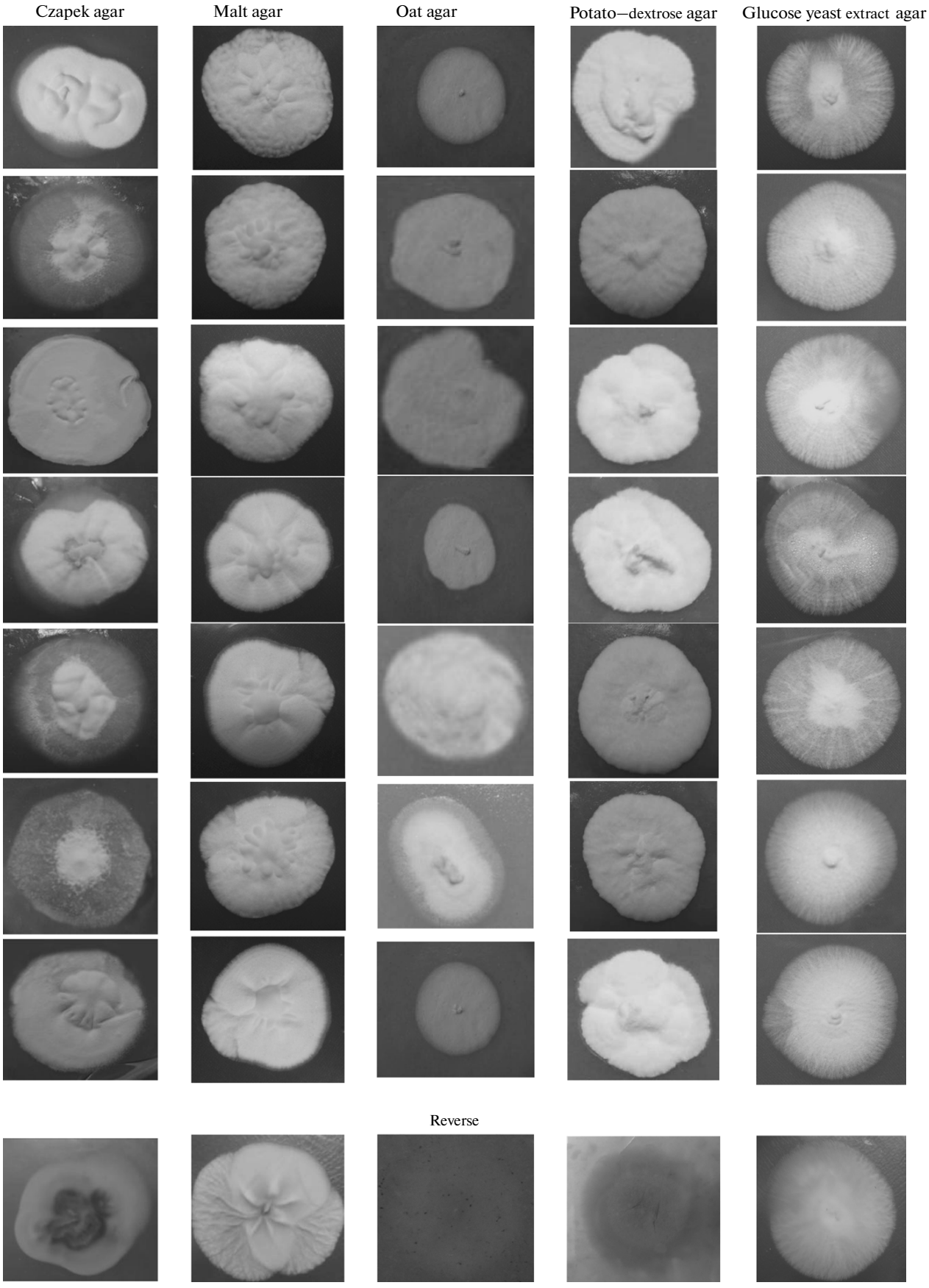
Transmission electron microscopy was performed with samples of surface mycelium and/or yeast cells cultivated on malt agar for 3–5 days that were fixated in 4% aqueous $KMnO_4$ solution for 4 h at room temperature. Upon fixation, the samples were washed three times with water; each washing period was 5–10 min. Yeast cell samples were centrifuged at 3000 rpm for 10 min. Upon fixation, the samples were dehydrated with ethanol (30, 50, 70, and 96%; 10 min) and acetone (two 30-min cycles). Thereupon, the samples were placed in the Epon resin (Ferak)-acetone mixture (1 : 1) without a catalyst and incubated during a night in darkness at room temperature. After that the samples were placed in the Epon resin with catalyst and incubated in a thermostat at 37°C for 1 day and at 60°C for 2 days. Ultrathin sections were obtained using an LKB-8800 ultratome with diamond knives, placed on copper nets, stained with aqueous uranylacetate solution for 60 min, counterstained by Reynolds' method [10], and examined with a Jeol (JEM-100B) microscope. 2–3 nets with sections were analyzed for each sample.

The number of nuclei in fungal cells was determined by luminescent microscopy. A block with the mycelium of the *Q. cyanescens* isolate was inoculated on Petri plates with agar-containing nutrient medium (malt agar); sterile cover slips were placed near the block. The Petri plates were incubated in a thermostat for several days at 25 ± 1°C. The glass-overgrowing mycelium was fixated; thereupon, its nuclei were stained with DAPI (4,6-diamidino-2-phenylindole dihydrochloride) reagent. The samples were fixated in modified Carnoy solution (chloroform : 96% ethanol : acetic acid = 7 : 5 : 2) for 10 min, washed with 50% ethanol, and placed in water. The staining of the nuclear DNA was carried out for 10 min in 500 ng/mL DAPI solution in Na-phosphate buffer (pH 6.9) [11]. The samples were investigated with an Axioscope 40 FL light microscope (lens magnification ×40 or ×100) using No.01 color filter (Zeiss). At least 10 fields were scanned and photographed by an AxioCam MRc digital camera. Samples of mycelia, conidia, and yeast cells used for microscopy were taken from the growth zone of the colony.

RESULTS AND DISCUSSION

The studies conducted in 2007–2012 revealed that the *Q. cyanescens* is distinctly associated with the birch *B. pendula*. The frequency of this fungal species was 29.5%, i.e., the pollen of one birch in three within various areas in Moscow city and the Moscow Region contained *Q. cyanescens* propagules, based on averaged data (Fig. 1). The fungus' distribution was uneven: we discerned a microlocal distribution pattern that may result from infecting adjacent birch trees. The numbers of fungal cells drastically varied (from

Fig. 2. The colonies of different *Q. cyanescens* strains on various media. Strains (from top to bottom): 1, VKM F-4082, CBS 127351, Moscow, Khovrino District, North Administrative Division; 2 and 3, Moscow, Levoberezhnyi District, North Administrative Division; 4, Moscow, Sokolinaya Gora District, East Administrative Division; 5, Moscow, Kuz'minki District, South-East Administrative Division; 6, VKM F-4083, CBS 127352, a quarry near the Kupavna urban settlement, Noginsk District, Moscow Region; 7, VKM F-4084, CBS 127353, a forest near the Burtsevo village, Shakhovskaya District, Moscow Region.


Czapek agar
Malt agar
Oat agar
Potato–dextrose agar
Glucose yeast extract agar
Reverse

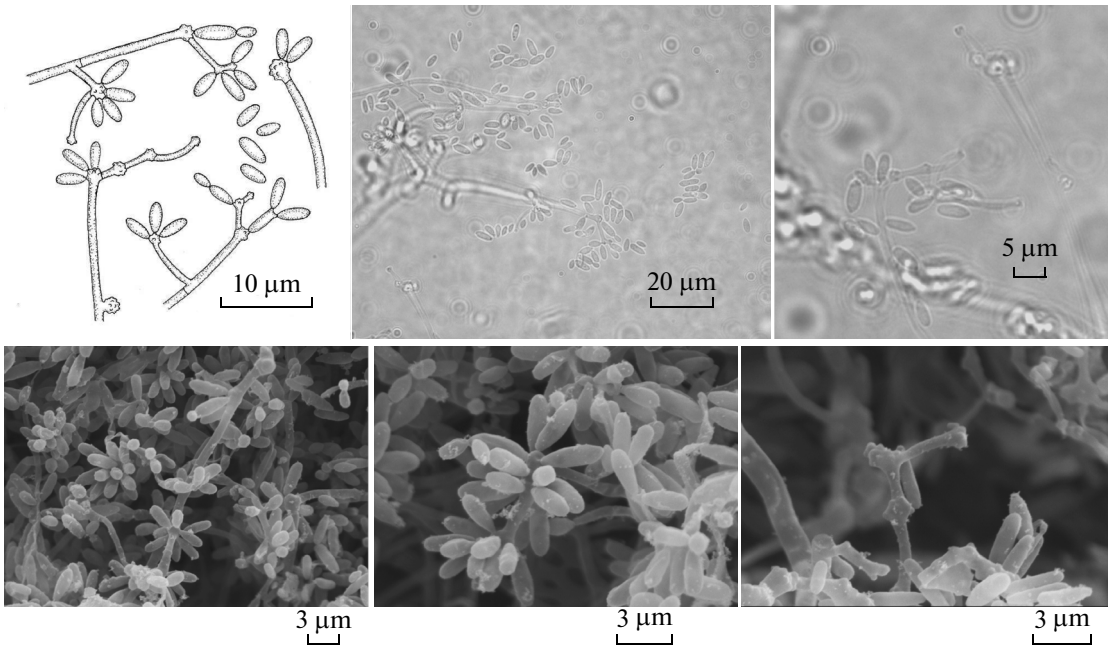


Fig. 3. Micromorphology of *Q. cyanescens*.

83 to 1.2×10^5 CFU/g) and amounted to $1.5 \times 10^4 \pm 9.0 \times 10^3$ CFU/g of pollen on an average.

It was established that *Q. cyanescens* can also be isolated from the surface and the interior of male inflorescences, the leaves, and the branches of *B. pendula*. The frequency of *Q. cyanescens* on male inflorescences was 30%, which virtually coincides with its frequency in pollen samples. The frequency of the fungus on birch leaves was significantly lower (8.8%); it was lower still on branches (4%). The low frequency of *Q. cyanescens* on branches prevents us from drawing any conclusions on its seasonal rhythm. This micromycete is isolated from leaves in spring, summer, and autumn. Importantly, *Q. cyanescens* occurs on male thyrsi during all seasons including the winter. The analysis of the samples taken from the model birch tree revealed the presence of *Q. cyanescens* not only on the surface, but also in the interior of the thyrsi. Plausibly, it is inside thyrsi that the fungus survives the winter. In the pollen samples of other tested trees and grasses (the common hazel, the grey and black alder, the cock's-foot, and the common timothy grass), no *Q. cyanescens* was detected. Apparently, the life cycle of the micromycete is closely related to that of the birch, and *Q. cyanescens* migrates to the pollen from the inflorescences. Interestingly, the literature available for us lacked data on the presence of *Q. cyanescens* on any substrate in the Moscow Region or within the whole Russia.

Despite the paucity of the data available in the literature concerning the micromycete that is to be considered a rare species, the variety of the substrates from which it is isolated is sufficiently wide. Its thermotolerance and lipolytic activity apparently account for its clinical importance [12], although *Q. cyanescens* is considered a weak human pathogen [13]. Most researchers agree that plant substrates are the *Q. cyanescens's* main habitats. This statement is consistent with the fact that the micromycete is capable of hydrolyzing plant cuticle and assimilating various compounds of plant origin, including gallic, tannic, vanillic, and *p*-coumaric acid [12, 14]. Nevertheless, *Q. cyanescens*, unlike other representatives of the same genus, is predominantly envisaged as an endophyte rather than a phytopathogen [4]. In some systems, it can compete with phytopathogens for the plant substrate and, therefore, suppress their growth, presumably in combination with other phylloplane fungi [14]. It is known that some strains even produce a fungicide, the sesquiterpenoid (+)-globulol [15]. Since *Q. cyanescens* exists in association with insects [16, 17], some researchers believe that it is the insects that facilitate the penetration of the fungus into plant tissues [5]. Nonetheless, *Q. cyanescens* is predominantly regarded as a symbiont of eucalypti and corymbia, and Australia is considered the fungus' "homeland" [3–6]. Of special interest, therefore, is the fact that the fungus is associated with *B. pendula* on a different continent.

The anamorphic genus *Quambalaria* is easy to cultivate on nutrient media. The strains isolated by us displayed the morphological and cultural features that are characteristic of the type isolate of *Q. cyanescens*. On all tested media, the fungus formed restricted, white, farinose or velvety colonies, often furrowed and forming sectors. The mycelium–yeast dimorphism, which is typical of this species, is particularly manifest on

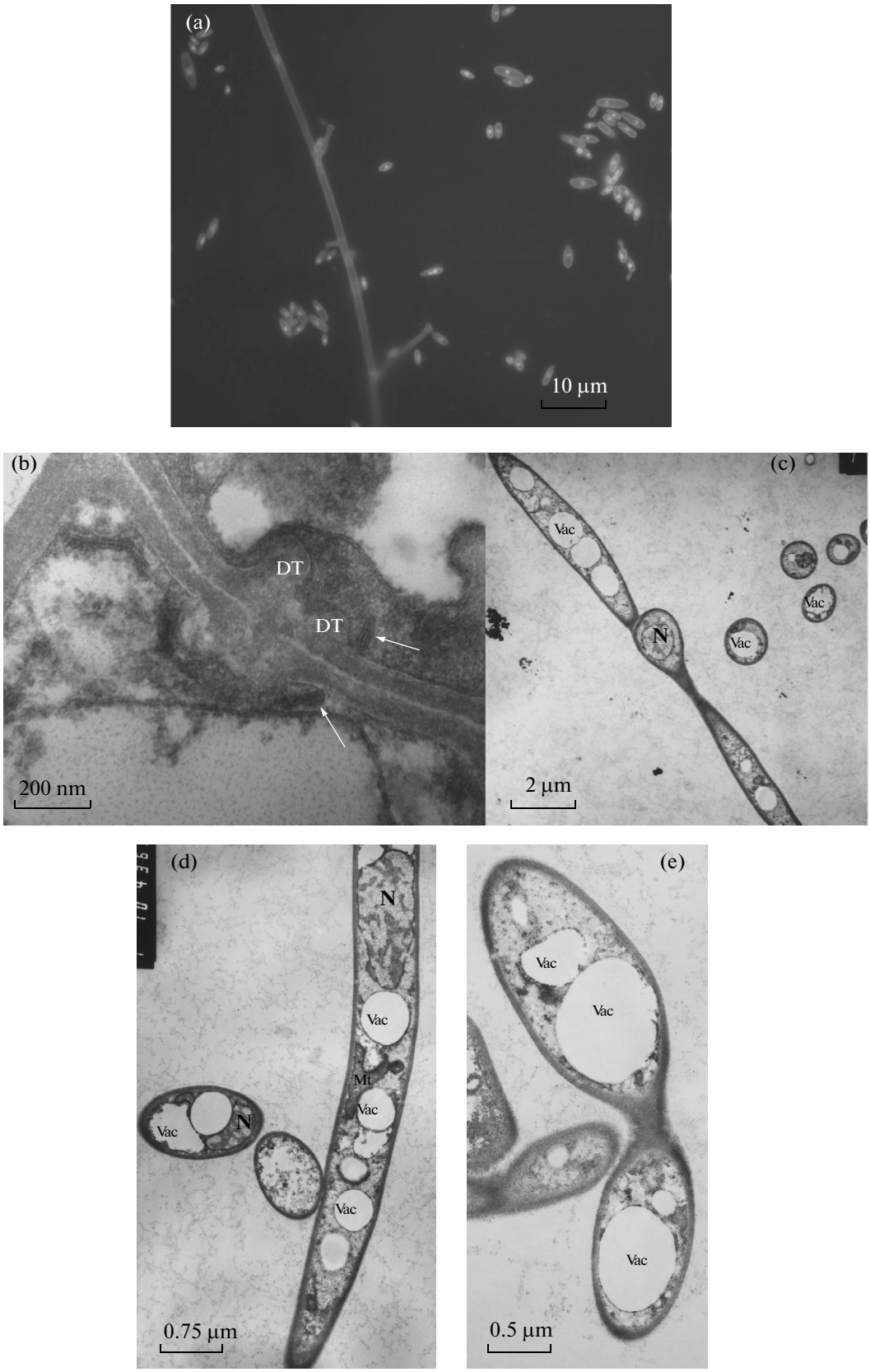


Fig. 4. Cytology of *Q. cyanescens*: a, a conidiophore, conidiogenous cells, and conidia (the nuclei are stained with DAPI reagent). b, dolipore septa. Membrane cisterns (arrows) are located at the base of the thickenings. Designation: DT, dolipore thickening. c, a conidiophore, conidiogenous cells, and conidia. Designations: Vac, vacuole; N, nucleus. d, mycelium fragment and conidia. Designations: Vac, vacuole; Mt, mitochondrion; N, nucleus; e, budding conidium.

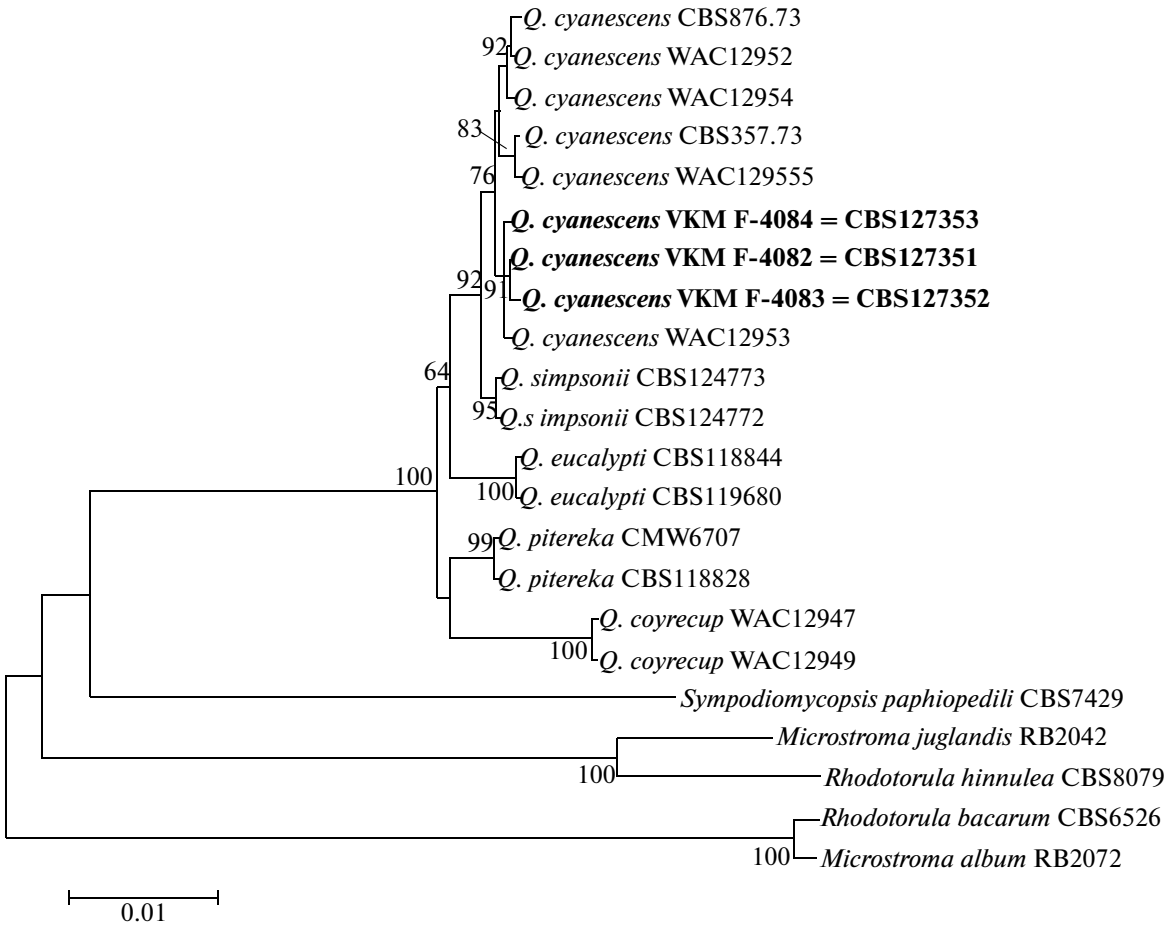


Fig. 5. Phylogenetic position of the isolated *Q. cyanescens* strains, based on the neighbor-joining method [21]. Numbers above branches, frequencies (>55%) of intertaxon conjunctions obtained with 1000 constructs. Scale, substitution number per nucleotide. The aligned sequences consisted of 663 bp for the ITS1-5.8S-ITS2 region and 523 bp for the D1/D2 domains of the LSU rDNA.

Czapek agar. The characteristic violet pigment of the reverse was detected on Czapek agar and on potato–dextrose agar. An exudate was present on glucose yeast extract agar (Fig. 2).

A distinctive feature of *Q. cyanescens's* morphology is the frequently observed repeated proliferation of conidiogenous cells; primary conidia forming on their denticles carry secondary conidia (Fig. 3).

Most vegetative mycelium cells and conidia have one nucleus or, very seldom, two nuclei, which is probably due to the nuclei division (Fig. 4a). Both the mycelia and the yeast-like growth stages are characterized by mononuclear cells whose nuclei are likely to be haploid. Conidiogenesis is abundant with mononuclear primary and secondary conidia. The teleomorphic stage has not been detected up to now. Presumably, a prerequisite for its formation is interaction with the tissues of the host plant.

Of paramount importance for the taxonomy of basidial fungi is the structure of the septal apparatus. The mycelium of the tested isolate contained dolipore septa with characteristic thickenings around the pore channel. At the base of the dolipore thickening, there are membrane cisterns that are likely to be related to the endoplasmic reticulum (arrows, Fig. 4). This structural pattern of the septal apparatus is characteristic of the species *Sporothrix cyanescens* (syn. *Q. cyanescens*) [18]. Two opinions have been expressed in the literature concerning the details of the structure of the septa of *Q. cyanescens* (syn. *Sporothrix cyanescens* and *Fugomyces cyanescens*). Some researchers emphasize that the septal apparatus contains the double-membrane "dolipore cap", which surrounds the dolipore septum and is symmetrically arranged on its both sides [3, 19]. Other researchers argue that the dolipore lacks the "cap" [18, 20]. This discrepancy calls for additional research on the ultrastructure of the pore apparatus of *Q. cyanescens's* strains in various collections.

The mycelium and the yeast-like cells of *Q. cyanescens* cultivated on malt agar are surrounded by single- or double-layer cell walls and contain numerous vacuoles and mitochondria (Figs. 4b–4d).

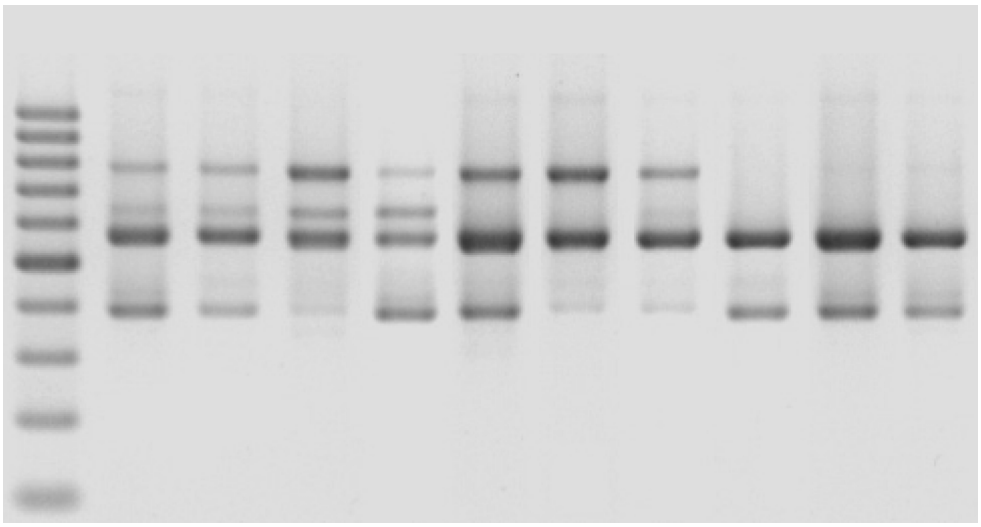


Fig. 6. DNA profiling of the tested strains based on the results of amplifying DNA sequences with the M13 primer. M, molecular weight marker (100 bp Low Range, Fermentas); *Q. cyanescens* strains: *1*, Moscow, Kuz'minki District, South-East Administrative Division; *2*, *3*, and *8*, Moscow, Levoberezhnyi District, North Administrative Division; *4*, Moscow, Sokolinaya Gora District, East Administrative Division; *5*, (VKM F-4082, CBS 127351), Moscow, Khovrino District, North Administrative Division; *6*, (VKM F-4084, CBS 127353), and *7*, a forest near the Burtsevo village, Shakhovskaya District, Moscow Region.; *9* (VKM F -4083, CBS 127352) and *10*, Kupavna urban settlement, Noginsk District, Moscow Region.

The analysis of the nucleotide sequences of the ITS1-5.8S-ITS2 regions and the D1/D2 domains of the LSU rDNA in three of the strains isolated by us revealed their 99.8-100% similarity to the strain *Q. cyanescens* WAC12953 (DQ823422, DQ823443). The phylogenetic analysis carried out by us demonstrated that the *Q. cyanescens* species consists of three clearly delimited phylogenetic groups. The differences of *Q. cyanescens* WAC12953 and our strains from the type strain *Q. cyanescens* CBS 357.73 amounted to 1.4% in the ITS1-5.8S-ITS2 region and 0.4% in the D1/D2 domains of the rDNA (Fig. 5).

Data on tested strains from various habitats that were obtained by DNA profiling based on PCR with non-specific primer M13 provided evidence for the population heterogeneity of the *Q. cyanescens* species within the Moscow city and the Moscow Region (Fig. 6). The isolated strains from two remote districts of the Moscow Region (the village Burtsevo and the urban settlement Kupavna) are significantly different. Moreover, the Moscow Region strains of *Q. cyanescens* differ from most of the tested strains isolated within the Moscow city. This suggests a phylogeographical structure of the *Q. cyanescens* species within Moscow and the Moscow Region. The existence of such a structure is an important distinctive feature of the *Q. cyanescens* species; it may be due to the close relationship between *Q. cyanescens* and the host plant because *B. pendula* is also characterized by a certain phylogeographical differentiation pattern [22].

Hence, the *B. pendula*–*Q. cyanescens* association was revealed for the first time in the world; *Q. cyanescens* is a well-known symbiont of eucalypti and the closely related taxon of corymbia in Australia. It was established that the fungus is present in almost one third of the birch population of the Moscow Region. The morphological and cultural characteristics and the micromorphological traits of the isolates obtained are similar to those of the type strain; the structure of the pore apparatus with dolipore septa enables us to classify the isolates as basidiomycetes. The species is characterized by a specific phylogeographical structure within Moscow and the Moscow Region. The isolated strains of *Q. cyanescens* were deposited in the All-Russian Collection of Microorganisms (VKM F-4082, VKM F-4083, and VKM F-4084) and Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 127351, CBS 127352, and CBS 127353).

ACKNOWLEDGMENTS

The authors wish to thank V.S. Mukhina and M.A. Mukhina, students of the Mycology & Algology Department of the School of Biology of the Lomonosov Moscow State University, for their assistance in conducting these studies.

This work was supported by grant 11-04-01063_a from the Russian Foundation for Basic Research.

REFERENCES

1. Antropova, A.B., Bilanenko, E.N., Mokeeva, V.L., Chekunova, L.N., and Zheltikova, T.M., Micromycetes associated with birch (Betula pendula Roth)

pollen, in *Tezisy dokladov Vtorogo S''ezda mikologov Rossii "Sovremennaya mikologiya v Rossii"* (Proc. 2nd Russ. Congr. Mycol. "Modern Mycology in Russia"), Moscow: Natl. Akad. Mikol., 2008, p. 216.

2. De Hoog, G.S. and De Vries, G.A., Two new species of *Sporothrix* and their relation to *Blastobotrys nivea, Antonie van Leeuwenhoek*, 1973, vol. 39, pp. 515–520.
3. De Beer, Z.W., Begerow, D., Bauer, R., Pegg, G.S., Crous, P.W., and Wingfield, M.J., Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia, *Stud. Mycol.*, 2006, vol. 55, pp. 289–298.
4. Paap, T., The incidence, severity and possible causes of canker disease of *Corymbia calophylla* (marri) in the southwest of Western Australia, *Ph. D. Thesis*, Perth, Murdoch Univ., 2006.
5. Pegg, G.S., O'Dwyer, C., Carnegie, A.J., Burgess, T.I., Wingfield, M.J., and Drenth, A., *Quambalaria* species associated with plantation and native eucalypts in Australia, *Plant Pathol.*, 2008, vol. 57, pp. 702–714.
6. Paap, T., Burgess, T.I., McComb, J.A., Shearer, B.L., and Hardy, G.E.St.J., *Quambalaria* species, including *Q. coyrecup* sp. nov., implicated in canker and shoot blight diseases causing decline of *Corymbia* species in the southwest of Western Australia, *Mycol. Res.*, 2008, vol. 112, pp. 57–69.
7. Katoh, K., Asimenos, G., and Toh, H., Multiple alignment of DNA sequences with MAFFT, *Methods Mol. Biol.*, 2009, vol. 537, pp. 39–64.
8. Tamura, K., Dudley, J., Nei, M., and Kumar, S., MEGA4: molecular evolutionary genetics analysis (Mega) software version 4.0, *Mol. Biol. Evol.*, 2007, vol. 24, pp. 1596–1599.
9. Kachalkin, A.V., Glushakova, A.M., Yurkov, A.M., and Chernov, I.Yu., Characterization of yeast groupings in the phyllosphere of *Sphagnum* mosses, *Microbiology (Moscow)*, 2008, vol. 77, no. 4, pp. 474–481.
10. Reynolds, E.S., The use of lead citrate at high pH as an electron opaque stain in electron microscopy, *J. Biophys. Biochem. Cytol.*, 1963, vol. 17, p. 208.
11. Ota, Y., Fukuda, K., and Suzuki, K., The nonheterothallic life cycle of japanese *Armillaria mellea, Mycologia*, 1998, vol. 90, no. 3, pp. 396–405.
12. Middelhoven, W.J., Guého, E., and De Hoog, G.S., Phylogenetic position and physiology of *Cerinosterus cyanescens, Antonie van Leeuwenhoek*, 2000, vol. 77, pp. 313–320.
13. Sigler, L., Harris, J.L., Dixon, D.M., Flis, A.L., Salkin, I.F., Kemna, M., and Duncan, R.A., Microbiology and potential virulence of *Sporothrix cyanescens*, a fungus rarely isolated from blood and skin, *J. Clin. Microbiol.*, 1990, vol. 28, pp. 1009–1115.
14. Middelhoven, W.J., Identity and biodegradative abilities of yeasts isolated from plants growing in an arid climate, *Antonie van Leeuwenhoek*, 1997, vol. 72, pp. 81–89.
15. Stodůlková, E., Šulc, M., Císařová, I., Novák P., Kolařík, M.,and Flieger, M., Production of (+)-globulol needle crystals on the surface mycelium of *Quambalaria cyanescens, Folia Microbiol.*, 2008, vol. 53, pp. 15–22.
16. Kolařík M., Sláviková E., and Pažoutová, S. The taxonomic and ecological characterisation of the clinically important heterobasiodiomycete *Fugomyces cyanescens* and its association with bark beetles, *Czech Mycol.*, 2006, vol. 58, pp. 81–98.
17. Kubátová, A. and Kolařík, M., Culture collection of fungi (CCF) in Prague—original isolates accessed during 2003-05, *Novit. Bot. Univ. Carol.*, 2007, vol. 18, pp. 91–111.
18. Smith, M.T. and Batenburg-Van der Vegte, W.H., Ultrastructure of septa in *Blastobotrys* and *Sporothrix, Antonie van Leeuwenhoek*, 1985, vol. 51, pp. 121–128.
19. Cannon, P.F. and Kirk, P.M., *Fungal families of the world, Wallingford: CAB International*, 2007.
20. *The Yeasts: A Taxonomic Study, 5th ed.*, Kurtzman, C., Fell, J.W., and Boekhout, T., Eds., Amsterdam: Elsevier, 2011.
21. Tamura, K., Nei, M., and Kumar, S., Prospects for inferring very large phylogenies by using the neighbor-joining method, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, vol. 101, pp. 11030–11035.
22. Maliouchenko, O., Palmé, A.E., Buonamici, A., Vendramin, G.G., and Lascoux, M., Comparative phylogeography and population structure of european *Betula* species, with particular focus on *B. pendula* and *B. pubescens, J. Biogeogr.,* vol. 34, pp. 1601–1610.

Translated by A. Oleskin