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Yeast Biogeography and the Effects of Species Recognition Approaches: The Case Study of Widespread Basidiomycetous Species from Birch Forests in Russia

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Abstract Understanding diversity and distribution patterns of fungi, including yeasts, ultimately depends on accuracy of species recognition. However, different approaches to yeast species recognition often result in different entities or operational taxonomic units. We studied the effects of using different yeast species recognition approaches, namely morphological species recognition (MSR) and phylogenetic species recognition (PSR), on the distribution patterns of widespread basidiomycetous yeasts. Hence, we have revised a collection of yeast fungi isolated from spatially remote birch forests in the Moscow Region and Western Siberia with molecular typing and identification tools. PCR fingerprinting and rDNA sequencing analyses of strains of nine species previously identified on the basis of morphological and physiological tests (MSR) yielded 21 phylogenetic species (PSR), including three currently undescribed taxa. The number of distinct phylogenetic species comprised within a single morphospecies ranged from one to seven. A total of ten species were found in both regions, whereas the distribution of 11 yeasts was restricted to a single region only. Both geographical region and type of substrate (plant or soil) influence yeast distribution. *Cryptococcus wieringae*, *C. victoriae*, *C. magnus*, and *Leucosporidium scottii* were frequently found on plant substrates, whereas *C. terricola* and *C. podzolicus* were associated to soil substrates. Occurrence of *C. magnus*, *C. albidus* and *Sporobolomyces roseus* was found to depend on the geographical region. Microsatellite-PCR fingerprinting, MSP-PCR, applied to studying yeast intraspecific variability revealed three different types of distribution: (a) variability that depends on geographical factors (*Curvibasidium cygneicollum*, *C. podzolicus*, *C. victoriae*), (b) genetic identity irrespectively of the region of isolation (*Rhodotorula pinicola*, *C. terricola*), and (c) high degree of genetic variability that did not correlate with region of sampling (*C. albidus* and *C. magnus*).

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Introduction

Yeasts are reported to be inhabitants on different kinds of plant substrates, whether living or decaying, in different biotopes of many regions [2, 12]. A particular approach to yeast ecology has been undertaken by Bab'eva et al.: instead of focusing on specific substrates, i.e. plant species, they have chosen to analyze the spatial structure and biogeography of yeast populations obtained from large numbers of samples in the plant–soil system (phylloplane, litter and soil, e.g. [2, 34]). The structure of yeast communities

changes throughout different geographical zones, from tundra to desert, when determined according to their phenotypic characteristics [6]. Along the latitudinal gradient, both higher yeast densities and species richness were found in boreal forest biotopes, for which some differences in community composition were noted depending on the forest type, spruce, alder or birch [34]. However, it was difficult to conclude whether the observed differences are solely determined by the vegetation type of or just by spatial separation. Therefore, a survey of yeast communities associated with a single forest type, birch forests, in two spatially, geographically, separated regions was performed [60]. The analysed forests in Moscow region (studied by Maksimova and Chernov [34]) and in Novosibirsk (Western Siberia) presented similar climatic conditions (average summer and winter temperatures, rainfall), soil type, and vegetation (dominating plant species). Yeast communities as determined from phenotypic keys [4, 27] were notably alike, even though the two sampling regions were 3,500 km apart. Yeast abundance and composition were found to depend on the type of substrate, namely live plant parts, litter and soil. Phylloplane yeasts were mostly represented by *Cryptococcus albidus*, *C. laurentii*, *C. terricola*, *C. podzolicus*, *Rhodotorula glutinis*, *R. minuta* and *Sporobolomyces roseus* which were found in the same abundance in both regions. Topsoil was inhabited by *C. terricola* and *C. podzolicus*. A single species, *C. diffluens*, was significantly more abundant in Western Siberia, particularly in soils.

Molecular identification methods have mainly replaced physiological assimilation tests to recognise distinct species on yeast diversity studies, as commonly used assimilation tests are not necessarily able to distinguish between closely related species. In fact, the use of molecular markers for taxa differentiation has vastly increased the number of novel recognised yeast species in the last decade, e.g. [28, 29, 44]. Yet, the mechanisms determining distribution of microorganisms, and yeasts in particular, in their natural habitats are still poorly understood. This is partly due to the complexity of some systems, like soil and phyllosphere, which limits the possibility to uncover mechanisms influencing abundance, composition, structure and distribution patterns of microbial communities, e.g. [2, 5, 24, 62].

Due to the small cell size, microorganisms were long proposed to have boundless dispersal abilities and, consequently, the development of microbial populations was assumed to be rather limited by the environment than spatial barriers, e.g. [24]. However, distinct biogeographical patterns have been demonstrated for bacteria [55] and fungi [33, 48]. Also, Taylor et al. [48] have demonstrated that the distribution range of a fungal species may depend upon the method of species recognition, morphological (MSR), biological (BSR) and phylogenetic (PSR). While some morphologically defined fungal species showed global distribution, species defined by phylogenetic species recognition approaches typically showed smaller geographical ranges with many endemic species. Despite the growing number of studies using PSR approaches to identify yeast species from the environment, still little is known about the distribution patterns and ranges of many phylogenetic species. In contrast, widespread occurrence of yeast species identified with MSR has been extensively reported.

In this study, we assessed the effects of spatial factors on yeast communities inhabiting phylloplane and soil substrates. We report on the PSR reassessment of MSR data on the distribution of dominating basidiomycetous yeast species in two geographically separated forests in Moscow and Novosibirsk (Western Siberia) regions, using microsatellite-PCR fingerprinting and rDNA sequencing strategies. We compared the results obtained using both MSR and PSR approaches for yeast identification in order to: (i) evaluate the effect of geographical barriers on yeast species distribution and population structure; (ii) compare the ecology of identified species with previous surveys that also used molecular identification methods; and (iii) propose updated hypotheses regarding previous studies that only employed phenotypic characterisation of the yeast isolates.

Materials and Methods

Sample Collection

Samplings were carried out in the month of August of three consecutive years (2002–2004). Studied biotopes were located in two geographically isolated regions of Russia, namely Western Siberia and Moscow region. Climatic conditions of these regions are similar as they are located at nearly the same latitude. Main sampling sites were situated in Novosibirsk (approximate coordinates 54.86N, 83.05E) and western part of Moscow region, near Burtsevo settlement (approximate coordinates 55.98N, 33.60E). During August 2003, samples were collected in Western Siberia, in Novosibirsk and at the border of the Altay State Natural Reserve (approximate coordinates 51.34N, 87.78E). During August 2004, sampling was carried out mainly in Moscow region, i.e. Burtsevo, Biological station "Malinki" of A.N. Severtsov Institute of Ecology and Evolution RAS (approximate coordinates 55.46N, 37.18E) and at Losiny Ostrov National Park (approximate coordinates 55.85N, 37.75E).

Samples were collected in mixed birch forests with main stands of *Betula verrucosa* (together with *B. alba* in

Siberia) and *Sorbus aucuparia* (*S. sibirica* in Siberia). The herbs *Fragaria vesca*, *Deschampsia cespitosa*, *Potentilla* spp., *Stellaria media*, *Aegopodium podagraria*, and *Equisetum sylvaticum* were common to the two studied regions. Coniferous trees found regularly in birch forests, namely *Pinus sylvestris* (*P. sibirica*, in Siberia) and *Picea abies* (in European Russia) were also sampled. All plants were sampled in equal proportions during each sampling survey. In addition, a few other plants were exclusively collected in Moscow region, *Acer* spp., *Aesculus hippocastanum*, *Swida* spp., *Corylus avellana*, *Dactylis glomerata*, and *Taraxacum officinale* as they were observed growing in mixed birch forests. Plant substrates, i.e. green leaves, dried plant material and litter, and soil were collected in the corners of five randomly selected 100 × 100 m plots. Each of the four composite samples (50–200 g) collected per plot was composed of material derived from a single plant species (but different individuals), with green leaves, dry leaves and litter sampled and analysed separately. All composite samples were prepared in the field. For soil samples, coarse woody debris, roots and stones larger than 5 mm were removed in the field.

Yeast Isolation

From each composite sample, three to five sub-samples were randomly taken for yeast isolation. Yeast cultures were isolated by the conventional plating method, in which leaf washings and soil suspensions were plated onto solid medium. Substrate samples were placed in 100 ml glass flasks, suspended 1:10 to 1:50 (w/v) in sterile demineralised water and crushed with tissue homogeniser (3,000 rpm, 3 min). Aliquots of 0.1–0.2 ml of each sample suspension were plated on the surface of malt agar medium acidified with 40 % lactate (final pH 4.0–4.5). Each sample was plated in triplicate. Plates were incubated at room temperature for 2–3 days and then kept at lower temperatures, 6–10 °C, to prevent fast development of moulds. Plates were examined after 7, 14 and 21 days of incubation. Colonies were differentiated into macro-morphological types using dissection microscopy, counted, and 1–2 representatives of each colony type per plate were transferred into pure culture. Strains were maintained on the same medium as slants at 4 °C. Strains used in this study are listed in Table 1. Reference yeast strains were obtained from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands) and the Portuguese Yeast Culture Collection (PYCC, Caparica, Portugal). A few additional cultures representing widespread phenotypic species from older studies [2, 7, 34] were obtained from KBP culture collection (Moscow State University, Russia).

Strains Selection

This study was aimed to reassess the identification of yeasts previously identified using the morphological species recognition approach (MSR) as *Cryptococcus laurentii*, *C. albidus*, *C. diffluens*, *C. terricola*, *C. podzolicus*, *Rhodotorula minuta*, *R. fujisanensis*, *R. glutinis*, and *Sporobolomyces roseus* isolated in two remote regions of Russia and originally studied by Yurkov et al. [60]. Because not all original cultures analysed by Yurkov et al. [60] were available for this study, further sampling efforts took place at the years 2002–2003, to re-sample yeasts in Western Siberia, and at 2004, mainly focused on isolating cultures in the Moscow region. The isolates used in this study have been chosen to represent cultures isolated in both geographical regions from the main plant species (Table S1). Our selection of cultures was partly based on the physiological profiles formerly determined by Yurkov et al. [60]. Using this preliminary grouping, we reduced the number of strains selected for molecular characterization studies by taking one to three isolates of each phenotypic species from the same substrate or plant species.

Assimilation profiles were determined according to Yarrow [57] and utilisation of six aldaric acids and 11 aromatic compounds was tested for members of the genus *Cryptococcus* according to Fonseca [11] and Sampaio [38]. Strains identified as *Rhodotorula fujisanensis* were tested for compatibility with the mating type strains of *Curvibasidium cygneicollum*. Mating experiments were performed as described by Sampaio et al. [43].

Microsatellite-PCR Fingerprinting

Genomic DNA extraction and microsatellite-PCR fingerprinting (MSP-PCR) using primers M13 (5′-GAG GGT GGC GGT TCT-3′), $(GAC)_5$ and $(GTG)_5$ followed the protocols described by Sampaio et al. [42]. Gel electrophoresis images were acquired with the BioRad Gel Image system (Richmond, USA) and analyzed with GELCOMPAR, version 4.1 (Applied Maths, Belgium). Dendrograms were computed using the UPGMA clustering method. Ordination of the amplified DNA fragment profiles was performed using principal components analysis implemented in the Statistica 6.0 software (StatSoft Inc., USA) and is based on similarity matrix produced using bands position (length) information. In agreement with other studies, isolates demonstrating very high similarity (>90 %) of microsatellite electrophoretic profiles and identical physiological properties are believed to be conspecific, e.g. [22, 23]. Therefore, only one representative strain per microsatellite profile was used for further identification by rDNA sequencing.

Table 1 Results of molecular identification: species inventory and relative abundance of yeasts isolated in Moscow region and Western Siberia. Relative abundance (average values) is calculated separately for each substrate category and for each region, and is based on a total number of samples (provided in clams)

Original identification	Molecular identification	Geographical location	Strains studied	Relative abundance, %			
				Plant			Soil (40)
				Herb (100)	Tree (70)	Litter (38)	
Rhodotorula sp.	*L. scottii* (anamorph)	Moscow region	AY-39	41.4	46.9	0	0
		Western Siberia	AY-37, 38, 40	1.9	0.6	0	0
Cryptococcus diffluens[a]	*C. albidus* var. *albidus*	Moscow region	AY-44, 45, 48, 52	<0.01	0.6	0	0.05
		Western Siberia	AY-49, 50	0.8	1.7	0	7.5
	C. albidus var. *kuetzingii*	Western Siberia	AY-43, 51	1.5	0	0	0
	C. adeliensis	Moscow region	AY-46	s.s.	0	0	0
	C. albidosimilis	Moscow region	AY47, 55	0.38	0	0	0
Cryptococcus podzolicus[b]	*C. podzolicus*	Moscow region	AY-32, 36; PYCC 4488	0	0	0	6.0
		Western Siberia	AY33, 34, 35	0	0	0	3.8
Cryptococcus terricola	*C. terricola*	Moscow region	AY-15, 16, 17	1.5	0	0	65.5
		Western Siberia	AY-18–AY-24	1.3	0	0	86.6
Sporobolomyces roseus	*S. roseus*	Moscow region	AY-11, 12, 13, 14	11.3	0	0	0
Rhodotorula glutinis	*R. babjevae* (anamorph)	Western Siberia	AY-6, 7, 8, 9, 10	24.0	19.0	15.4	0
Rhodotorula fujisanensis	*Curv. cygneicollum*	Moscow region	AY-25, 29	1.0	0	1.2	0
		Western Siberia	AY-26, 27, 28	1.0	4.7	4.9	1.2
Rhodotorula minuta	*R. pinicola*	Moscow region	AY-1, 4, 5	1.0	1.3	0	0
		Western Siberia	AY-3	1.52	0.21	0	0
	R. slooffiae	Western Siberia	AY-2	5.67	0	1.8	0
Cryptococcus albidus	*C. wieringae*	Moscow region	AY-57, 58, 61, 63, 64, 67	19.9	39.8	0	0
		Western Siberia	AY-53, 69, 80, 90	19.1	21.6	14.3	0
	C. magnus	Moscow region	AY-54, 62, 65, 66	1.3	2.7	0	0
		Western Siberia	AY-72	3.6	8.7	11.6	0
	C. oeirensis	Western Siberia	AY-75	0.8	0	0	0
	Cryptococcus sp. CBS 8630	Western Siberia	AY-59	0	2.4	0	0
Cryptococcus laurentii	*C. victoriae*	Moscow region	AY-74, 84, 86, 93	12.9	5.0	0	0
		Western Siberia	AY-68, 70, 71 76, 77, 79, 81, 82, 88, 89, 91, 92, 96, 97, 99	27.6	16.7	50.9	0.0
	C. tepherensis	Moscow region	AY-95, 100	0	0.2	0	0
		Western Siberia	AY-94, 102, 103, 104	0.9	s.s.	1.7	0
	C. carnescens	Moscow region	AY-87	0	s.s.	0	0
	C. heimaeyensis	Moscow region	AY-73, 98	0.9	0	0	0
	C. "foliicola"	Moscow region	AY-78	s.s.	0	0	0
	Cryptococcus cf. *aureus*	Moscow region	AY-83	s.s.	0	0	0
	C. magnus	Western Siberia	AY-101	s.s.	0	0	0

s.s. single strain

[a] Strains isolated previously from Negev desert (Israel), KBP 3824 (plant material) and KBP 3826 (soil) were involved to re-assess distribution of *C. albidus*

[b] Type strain of *C. podzolicus* PYCC 4488 (CBS 6819; KBP 6) was used both as reference and the representative of European Russia isolates

DNA Sequence Analysis

PCR amplification prior to sequencing employed the forward primer ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G) and the reverse primer LR6 (5′-CGC CAG TTC TGC TTA CC) using a Uno II Thermal Cycler (Biometra, Germany) and the resulting amplicons were purified with the GFX Band Purification Kit (Amersham Biosciences). Cycle sequencing of the D1/D2 variable domains of the 26S rDNA employed the forward primer NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG) and the reverse primer NL4 (5′-GGT CCG TGT TTC AAG ACG G) and that of the ITS region employed forward primer ITS1 (5′-TCC GTA GGT GAA CCT GCG G) and reverse primer ITS4 (5′-TCC TCC GCT TAT TGA TAT GC), following protocols described by Inácio et al. [22, 23]. Sequences were obtained with an ALFexpress II DNA Analyser (Amersham Pharmacia Biotech), according to the manufacturer's instructions.

Alignment of nucleotide sequences was performed by the MAAFT algorithm [26]. Phylogenetic trees were computed using the maximum likelihood method with RAxML, version 7.0.3, and isolates' placement was further validated with 1,000 rounds of bootstrap replicates [46]. Model of DNA substitution (GTR) and parameters for maximum likelihood analysis were derived from Modeltest, version 3.7 [37]. Nucleotide sequences were deposited in GenBank under the accession numbers given in the phylogenetic trees (Figs. 1, 2 and 3). Additional sequences were retrieved from GenBank and CBS databases (accession numbers and strain numbers are indicated on the phylogenetic trees).

Statistical Analysis

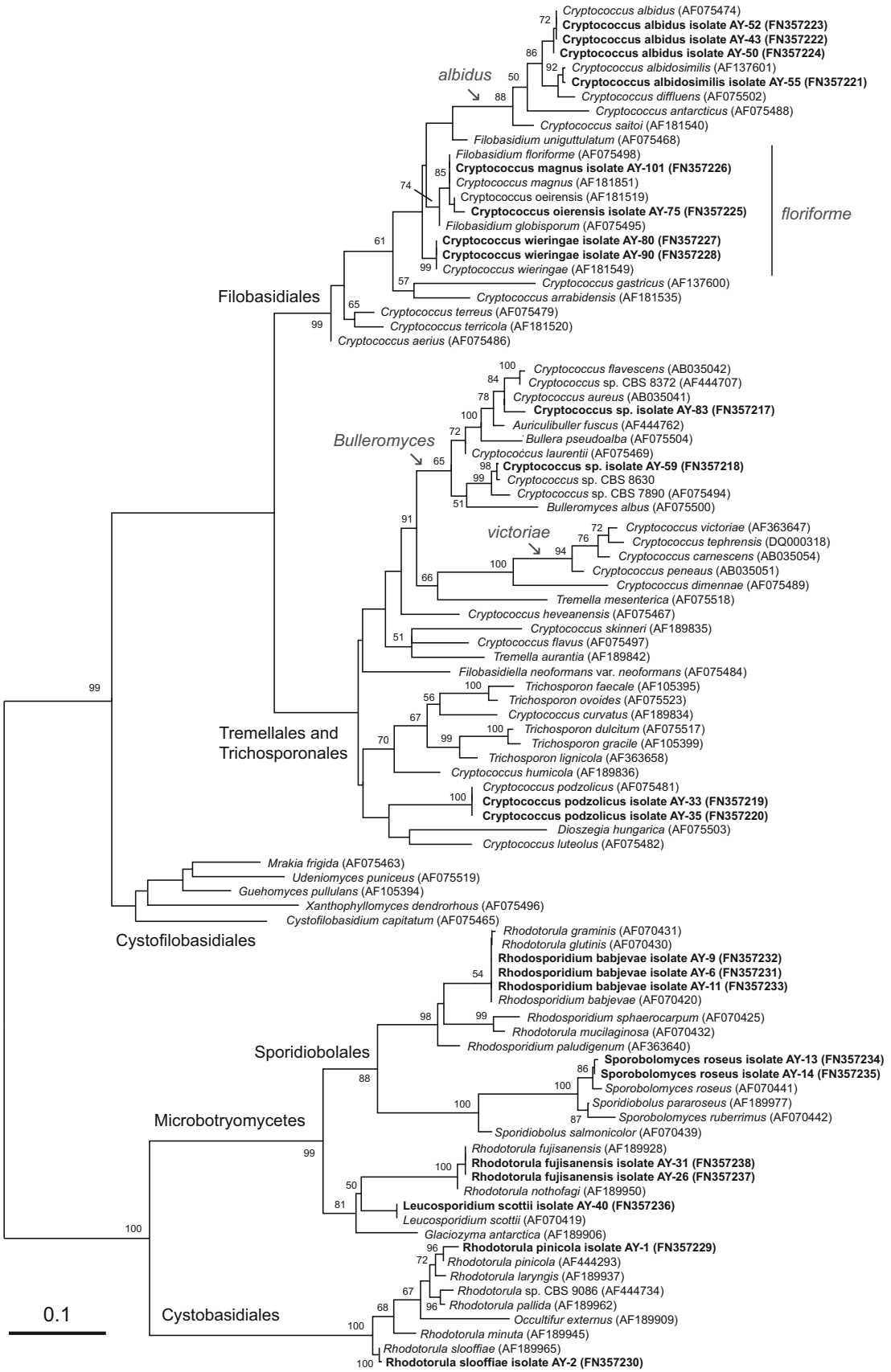
For each sub-sample, yeast quantity and community structure were determined. Yeast quantity was calculated as colony-forming units (CFU) per gram of dry plant material or soil at natural humidity. The community structure was characterised by relative abundance and frequency of occurrence of every observed species in the sample, see [62]. Distribution of basidiomycetous yeasts was analysed using previously published data referring to sampling surveys 1998, 2001, and 2002 [60], and results obtained in this study, sampling years 2003–2004. We analysed yeast communities composition (based on species' presence or absence) and structure (based on relative abundance values) extrapolating identification results (PCR fingerprinting and rDNA sequencing) on sub-samples, for which it was possible to attribute molecular identification results to the original yeast cultures or groups of cultures (Table S1) re-identified in the current study. Because not all phenotypically characterised cultures were available for the reassessment, we substantially reduced the original dataset, which was based on phenotypic identification results (MSR) and contained information of 200 sub-samples (sampling years 2003–2004) and 150 sub-samples (sampling 2002) analysed previously by Yurkov et al. [60]. After the reassessment of the data on the distribution of analysed basidiomycetous yeast species, the dataset was reduced to 248 sub-samples, most of which were sampled in 2002-2004 (Table S1).

The influence of spatial and environmental factors on the yeast communities' structure was analysed by ANOVA/MANOVA block implemented in the Statistica 6.0–9.0 software (StatSoft Inc., USA). Statistical analyses were performed to assess effects of geographical region (Western Siberia and Moscow region), and substrate (plant material and soil). Effects of a particular plant species were analysed for the plants that were common to both regions, namely *Betula* spp., *B. verrucosa* (and *B. alba*), *Sorbus* spp., *S. aucuparia* (and *S. sibirica*), *Fragaria vesca*, *Deschampsia cespitosa*, *Aegopodium podagraria* and *Equisetum sylvaticum*. The homogeneity of variance was assessed with Levene's test. Distribution of all dependent variables was close to normal, unimodal but asymmetric in some cases. Results of multivariate analysis of variance, MANOVA, were confirmed by ANOVA analyses. Effects were considered to be statistically significant at the level $P \leq 0.05$. Significant effects were additionally confirmed with χ^2 test.

Results

Species Inventory

A total of 100 isolates were studied and out of them a total of 57 strains were selected for sequence-based identification. The following 17 yeast species were identified using PCR fingerprinting (results not shown) and rDNA sequencing (Table 1; Figs. 1, 2 and 3): *Cryptococcus victoriae*, *C. carnescens*, *C. foliicola*, *C. heimaeyensis*, *C. tephrensis* (*victoriae* clade), and *C. podzolicus* in the Tremellales; *C. magnus*, *C. wieringae*, *C. oeirensis* (*floriforme* clade), *C. albidus*, *C. albidosimilis, C. adeliensis* (*albidus* clade), and *C. terricola* in the Filobasidiales; *Sporobolomyces roseus* and *Rhodosporidium babjevae*, in the Sporidiobolales; *Rhodotorula pinicola* and *R. slooffiae*, in the Cystobasidiales and *Curvibasidium cygneicollum* and *Leucosporidium scottii* in the Microbotryomycetes. Two isolates, AY-59 and AY-83 showed only low similarity to known species and may thus represent novel taxa (Fig. 1). Specifically, AY-59 was a close relative of *Cryptococcus* spp. CBS 7890 and CBS 8630 (Fig. 1), showing 9 and 2 nucleotide substitutions in the D1/D2


Cryptococcus albidus (AF075474)
Cryptococcus albidus isolate AY-52 (FN357223)
Cryptococcus albidus isolate AY-43 (FN357222)
Cryptococcus albidus isolate AY-50 (FN357224)
albidus
Cryptococcus albidosimilis (AF137601)
Cryptococcus albidosimilis isolate AY-55 (FN357221)
Cryptococcus diffluens (AF075502)
Cryptococcus antarcticus (AF075488)
Cryptococcus saitoi (AF181540)
Filobasidium uniguttulatum (AF075468)
Filobasidium floriforme (AF075498)
Cryptococcus magnus isolate AY-101 (FN357226)
Cryptococcus magnus (AF181851)
Cryptococcus oeirensis (AF181519)
Cryptococcus oierensis isolate AY-75 (FN357225)
Filobasidium globisporum (AF075495)
Cryptococcus wieringae isolate AY-80 (FN357227)
Cryptococcus wieringae isolate AY-90 (FN357228)
Cryptococcus wieringae (AF181549)
floriforme
Cryptococcus gastricus (AF137600)
Cryptococcus arrabidensis (AF181535)
Filobasidiales
Cryptococcus terreus (AF075479)
Cryptococcus terricola (AF181520)
Cryptococcus aerius (AF075486)
Cryptococcus flavescens (AB035042)
Cryptococcus sp. CBS 8372 (AF444707)
Cryptococcus aureus (AB035041)
Cryptococcus sp. isolate AY-83 (FN357217)
Auriculibuller fuscus (AF444762)
Bulleromyces
Bullera pseudoalba (AF075504)
Cryptococcus laurentii (AF075469)
Cryptococcus sp. isolate AY-59 (FN357218)
Cryptococcus sp. CBS 8630
Cryptococcus sp. CBS 7890 (AF075494)
Bulleromyces albus (AF075500)
victoriae
Cryptococcus victoriae (AF363647)
Cryptococcus tephrensis (DQ000318)
Cryptococcus carnescens (AB035054)
Cryptococcus peneaus (AB035051)
Cryptococcus dimennae (AF075489)
Tremella mesenterica (AF075518)
Cryptococcus heveanensis (AF075467)
Cryptococcus skinneri (AF189835)
Cryptococcus flavus (AF075497)
Tremella aurantia (AF189842)
Filobasidiella neoformans var. neoformans (AF075484)
Trichosporon faecale (AF105395)
Trichosporon ovoides (AF075523)
Cryptococcus curvatus (AF189834)
Trichosporon dulcitum (AF075517)
Trichosporon gracile (AF105399)
Tremellales and
Trichosporonales
Trichosporon lignicola (AF363658)
Cryptococcus humicola (AF189836)
Cryptococcus podzolicus (AF075481)
Cryptococcus podzolicus isolate AY-33 (FN357219)
Cryptococcus podzolicus isolate AY-35 (FN357220)
Dioszegia hungarica (AF075503)
Cryptococcus luteolus (AF075482)
Mrakia frigida (AF075463)
Udeniomyces puniceus (AF075519)
Guehomyces pullulans (AF105394)
Xanthophyllomyces dendrorhous (AF075496)
Cystofilobasidium capitatum (AF075465)
Cystofilobasidiales
Rhodotorula graminis (AF070431)
Rhodotorula glutinis (AF070430)
Rhodosporidium babjevae isolate AY-9 (FN357232)
Rhodosporidium babjevae isolate AY-6 (FN357231)
Rhodosporidium babjevae isolate AY-11 (FN357233)
Rhodosporidium babjevae (AF070420)
Rhodosporidium sphaerocarpum (AF070425)
Rhodotorula mucilaginosa (AF070432)
Rhodosporidium paludigenum (AF363640)
Sporidiobolales
Sporobolomyces roseus isolate AY-13 (FN357234)
Sporobolomyces roseus isolate AY-14 (FN357235)
Sporobolomyces roseus (AF070441)
Sporidiobolus pararoseus (AF189977)
Sporobolomyces ruberrimus (AF070442)
Microbotryomycetes
Sporidiobolus salmonicolor (AF070439)
Rhodotorula fujisanensis (AF189928)
Rhodotorula fujisanensis isolate AY-31 (FN357238)
Rhodotorula fujisanensis isolate AY-26 (FN357237)
Rhodotorula nothofagi (AF189950)
Leucosporidium scottii isolate AY-40 (FN357236)
Leucosporidium scottii (AF070419)
Glaciozyma antarctica (AF189906)
Rhodotorula pinicola isolate AY-1 (FN357229)
Rhodotorula pinicola (AF444293)
Rhodotorula laryngis (AF189937)
Rhodotorula sp. CBS 9086 (AF444734)
Rhodotorula pallida (AF189962)
0.1
Cystobasidiales
Occultifur externus (AF189909)
Rhodotorula minuta (AF189945)
Rhodotorula slooffiae (AF189965)
Rhodotorula slooffiae isolate AY-2 (FN357230)

◀**Fig. 1** Phylogenetic tree of selected isolates obtained by maximum likelihood analysis of 26S rRNA gene (D1/D2 domains). The numbers given on branches are frequencies (>50 %) with which a given branch appeared in 1,000 bootstrap replications. Sequences determined in the present study are typed in *boldface*. The *scale* indicates the number of expected substitutions accumulated per site. Names of the clades are given according to Fonseca et al. (2011)

domains of the 26S rDNA gene, respectively. Isolate AY-83 was found to be related to *C. aureus*, but differed in more than 20 nucleotide positions in the D1/D2 domain from the latter species (Fig. 1). Among the strains available in public collections, AY-83 has the closest match with the strain *Cryptococcus* cf. *aureus* NRRL Y-30215 [10],

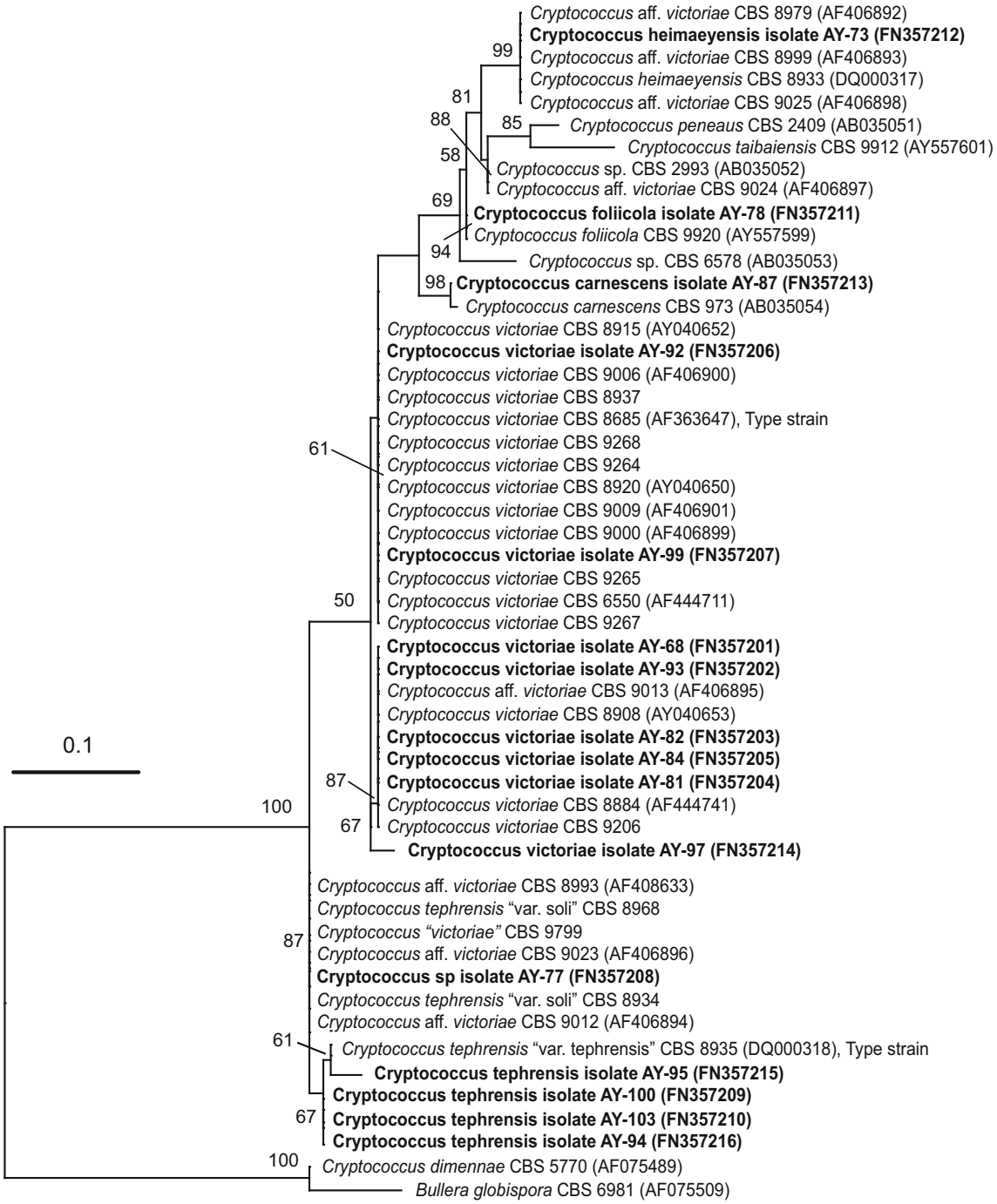


Fig. 2 Phylogenetic placement of isolates related to *C. victoriae* obtained by maximum likelihood analysis of 26S rRNA gene (D1/D2 domains). Nucleotide sequences with missing GenBank accession numbers were retrieved from CBS database. Other details as for Fig. 1

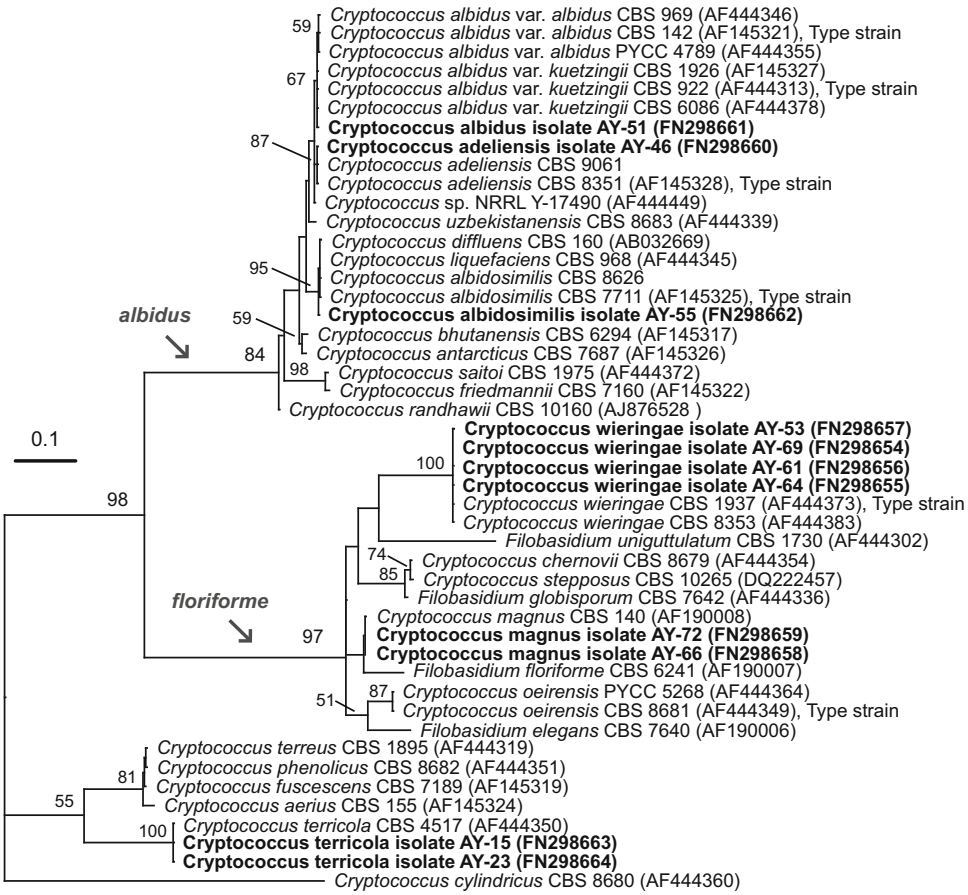


Fig. 3 Phylogenetic placement of *Cryptococcus* species in the Filobasidiales lineage obtained by maximum likelihood analysis of ITS region sequences. Nucleotide sequences with missing GenBank accession numbers were retrieved from CBS database. Names of the clades are given according to Fonseca et al. (2011). Other details as for Fig. 1

showing only 3 indels in the D1/D2 domains. The number of phylogenetic species comprised within a single morphospecies ranged from 1 to 7 (Table 1). The use of a PSR approach revealed that 3, 4 and 7 distinct species were comprised within the most diverse MSR-based species *C. diffluens*, *C. albidus* and *C. laurentii*, respectively (Table 1).

Among dominating red-coloured yeasts, isolates that produced ballistoconidia were identified as *S. roseus* and those that fitted the standard phenotypic diagnosis of *R. glutinis* were found to represent *Rhodosporidium babjevae* (Table 1; Fig. 1). *R. babjevae* belongs to the *Rhodotorula glutinis* sensu stricto group, together with *R. graminis* [15]. Strains previously identified as *R. minuta* were found to represent two distinct closely related species, namely *R. pinicola* and *R. slooffiae* (Table 1; Fig. 1). Sequencing results confirmed the phenotypic identification of *R. fujisanensis* and showed that non-pigmented strains of *Rhodotorula* sp. belong to the species *Leucosporidium scottii* (Table 1; Fig. 1). Isolates representing *R. fujisanensis* were additionally studied using mating tests with reference strains of *Cu. cygneicollum*, the sexual state of *R. fujisanensis* [43]. The three strains of *R. fujisanensis* (AY-25, 27, and 28) mated with MAT A2 test culture and thus represent the opposite mating type of *Cu. cygneicollum*.

Phylogenetic placement of our isolates and strains from CBS collection revealed a complex structure of the *victoriae* clade (Fig. 2). This group comprises several currently recognised species [14], but also strains originally identified and presently listed in GenBank as *Cryptococcus* aff. *victoriae*. A single strain, AY-78, was identified in our study as *C. foliicola* [54] (Table 1; Fig. 2). Other isolate, AY-87, was identified as *C. carnescens* (Table 1; Fig. 2), with one nucleotide substitution from the type strain CBS 973. Phylogenetic analysis revealed two clusters of *C. victoriae* strains (Fig. 2). Isolates AY-92 and AY-99 showed D1/D2 sequences identical to those of the type strain of *C. victoriae* CBS 8685 (Fig. 2). A second group of strains (AY-68, 81, 82, 84, and 93) differs from the type strain of *C. victoriae* in two nucleotide positions, as well as CBS 8908 [49], CBS 9013 [20], and CBS 9206 [51] (Fig. 2). Isolate AY-97 presented four nucleotide substitutions in the D1/D2 region when compared to members of the two above-mentioned clusters and thus showed an isolated position in the phylogenetic tree (Fig. 2).

Similarly to *C. victoriae*, cultures of *C. tephrensis* analysed in this study formed two major groups. The first subcluster includes the isolates AY-94, 95, 100, and 103, which are closely related to the type strain of *C. tephrensis* CBS 8935. Isolate AY-77 grouped in the second subcluster with strains CBS 8993, CBS 9012, and CBS 9023 [20]; CBS 8934 and CBS 8968 [51]; and CBS 9799, isolated from a plant in Norway (according to CBS database), which have three nucleotide substitutions in the D2 domain from the type strain of *C. tephrensis*. Strains CBS 8935 and CBS 8934 are listed in the CBS database as type strains of distinct varieties, namely *C. tephrensis* var. *tephrensis* and *C. tephrensis* var. *soli*, respectively. However, these varieties have apparently not been validly described, see [14].

Physiological tests provided a good differentiation of the closely related species *C. wieringae* and *C. magnus* in the Filobasidiales lineage due to differences in assimilation of ribitol and D-glucosamine (data not shown). We were also able to distinguish *C. tephrensis* from other members of the *C. victoriae* clade. Our isolates of *C. tephrensis* did not assimilate mucic and saccharic acids as sole carbon sources, whereas other members of the *C. victoriae* clade demonstrated growth using these compounds. Data available at the CBS yeast database and the last revision of the genus *Cryptococcus* [14] confirm this observation since *C. tephrensis* assimilates these compounds weakly or not at all. However, physiological profiles of *C. victoriae*, *C. carnescens*, *C. heimaeyensis* and *C. foliicola* are very similar and, thus, do not provide good characters for differentiation.

Distribution and Ecology

During the yeast isolation, the number of yeast colonies assigned to a species identified using phenotypic characters (MSR) was recorded and expressed as a proportion of the total number of yeast colonies (or CFU) observed in a subsample. After the reassessment of species identification, we were able to track back strains origin and match relative abundance values with a phylogenetic (PSR) yeast species. Despite the limited number of strains re-identified in this study, each culture included in the study represented a subset of isolates identical on the basis of MSR approach, so we were able to included data from a total of 248 samples in the final dataset (Table S1). However, considering the limitation of our analyses, we present and discuss here about the average values of relative abundance and incidence reflecting the large-scale distribution patterns of basidiomycetous yeast species re-identified in the present study.

The relative yeast abundance data, average values at both sample collection regions and for each studied substrate, is reported in Table 1. The most frequently isolated yeast species in both regions was *C. wieringae*. Other frequently isolated species were *C. victoriae*, *C. magnus* and *L. scottii*. *Cryptococcus terricola* was the most frequent and abundant species found in soil samples. Statistical analyses showed that both type of substrate (plant vs. soil) and geographic region (Moscow vs. Western Siberia) are essential factors determining distribution of yeast species (Table 2). Asexual states of *L. scottii* and several pigmented basidiomycetes, *S. roseus*, *R. babjevae*, *R. pinicola* and *R. slooffiae*, were found on plant material only (Table 1). Members of the *floriforme* clade of Filobasidiales [14] were mostly restricted to live plant materials (Table 1; Fig. 3). While *C. wieringae* and *C. magnus* occurred frequently in the phylloplane, *C. oeirensis* was represented only by a single isolate in this substrate (Table 1; Fig. 3). Members of the *victoriae* clade of Tremellales [14] were frequent and abundant in plants and in litter (Table 1; Fig. 2). The majority of these isolates were identified as *C. victoriae* and *C. tephrensis* (Table 1). Three other species, *C. carnescens*, *C. heimaeyensis*, and *C. foliicola* were represented by single isolates only. Interestingly, for both of the above-mentioned clades, more than 90 % of the respective isolates belonged to two most frequent species pairs: a dominant (*C. wieringae* or *C. victoriae*) and a sub-dominant (*C. magnus* or *C. tephrensis*) species. Three strains isolated from live plants were identified as the closely related species *C. adeliensis* and *C. albidosimilis* (Table 1; Fig. 3). Soil samples were populated by *C. terricola*, *C. podzolicus* and *C. albidus* (Fig. 1). The last species was also observed on living plant parts collected in Moscow region (Table 1). *Cu. cygneicollum* was isolated from plant, litter and soil samples.

Geographical Signal in Populations & Intraspecific Variability

Next to the application of PSR approach for species identification, special attention was given to analyses of

Table 2 Two-way analysis of variance (ANOVA) for relative abundance of yeasts in studied regions (Moscow region, Western Siberia) and substrates (plants, soil)

Variation source	Degree of freedom	Sum of squares	Total variance, %	F-level and *P* level
Total	248	14.8	100	
Substrate (S)	1	1.1	7.22	52.3***
Region (R)	1	3.9	26.11	7.3***
S × R	1	4.0	27.26	6.6***
Error	245	5.8	39.41	

ns not significant

Statistical significance: *** $P < 0.001$

microsatellite-PCR fingerprinting profiles of the studied basidiomycetous yeasts in relation to geographical origin of the strains. Three different types of distribution were found: (a) variability that depends on geographical factors (*Cu. cygneicollum*, *C. podzolicus*, *C. victoriae*), (b) genetic identity independently of the region of isolation (*C. terricola*, *R. pinicola*), and (c) high degree of genetic variability that did not correlate with region of sampling (Filobasidiales: *C. albidus* and *C. magnus*).

The members of the first group formed clusters corresponding to the geographic origin (Fig. 4b). Strains of *C. podzolicus* displayed groupings both with $(GTG)_5$ and M13 primers. Two reference strains of *C. podzolicus* were included in the study, as they were isolated from Moscow region, AY-36 (KBP 3365) and the type strain CBS 6819 (= PYCC 4488; KBP 6). It is interesting to note that the latter strain isolated by Bab'eva and Reshetova [3] and stored in collection for over 25 years grouped together with the other European isolates of *C. podzolicus*. Thus, the banding patterns revealed by MSP-PCR, which reflect genetic variability, appear to be stable over a long period of time. Similarly, strains of *Cu. cygneicollum* grouped according to their geographic origin (data not shown). Isolates identified as members of the *victoriae* clade (*C. victoriae* and *C. tephrensis*) showed diverse MSP-PCR fingerprint patterns. Quantitative comparison of the MSP-PCR profiles performed using ordination with principal component analysis (PCA) resolved a trend for separation of the isolates according to geographical origin and phylogenetic relatedness (Fig. 4c).

The second trend is represented by isolates showing low intraspecific variability as inferred by the highly similar microsatellite-PCR profiles with all primers. As an example, the similarity level among the *C. terricola* isolates was above 90 %, irrespectively of the isolation site (Fig. 4a). Similarly to isolates of *C. terricola*, all four strains of *R. pinicola* demonstrated almost identical profiles with $(GAC)_5$ primer.

The third trend corresponds to strains belonging to the *floriforme* and *albidus* clades of Filobasidiales [14]. MSP-PCR revealed high levels of intraspecific variability, but the origin of the observed genetic divergence is unknown. Strains of *C. albidus* and its varieties formed two different groups that did not reflect the geographical origin, type of substrate or phylogenetic relationship. Similarly, isolates of the main dominating species *C. wieringae* demonstrated high levels of genetic variability that did not correlate with any of the analysed factors.

An ordination with PCA was found to be more appropriate for analysis of microsatellite-PCR profiles of the widespread phylloplane species, members of *floriforme* and *victoriae* clades, than conventional one-dimensional dendrograms. Due to high dissimilarity of profiles, no significant groupings were observed by direct analysis of the dendrograms. Ordination with PCA enables differentiation of fingerprints with at least two extracted components (axes). For example, the first two extracted components, factors describe together 36 % of the genetic variability between strains of *C. victoriae* and *C. tephrensis*, whereas the first component describes only 21 % alone (Fig. 4c). Thereby, ordination retains larger proportion of the information available for analyses than a one-dimensional dendrogram.

Discussion

Spatial Trends

On the community level, geographical region and type of substrate (phylloplane vs. soil) have been found to determine yeast species distribution, i.e. species richness and abundance (Table 2). Even though both factors significantly influence communities' structure, effects of the substrate were found to be more pronounced in accordance with previous observations of Yurkov et al. [60]. Several species were only isolated from a single type of substrate (Table 1), which explains the difference between yeast communities in soils and live plant parts. Specifically, *C. victoriae*, *C. magnus*, *C. wieringae*, *S. roseus*, *R. babjevae*, *Rh. pinicola*, *Rh. slooffiae* and *L. scottii* are associated with live plant parts, whereas *C. terricola*, *C. podzolicus* and *C. albidus* are restricted to soil samples. In agreement with the results of Yurkov et al. [60] and regardless of the species recognition approach used, no association between yeasts species and individual plant species was found.

Upon utilizing the PSR approach, three significant differences in the phylloplane community reflecting spatial separation of the analysed forests were observed. Firstly, among the members of the *floriforme* clade, *C. wieringae* was isolated in both regions with similar abundance, whereas *C. magnus* was significantly more abundant in Western Siberia (Table 1). Secondly, the average relative abundance of *C. albidus* and its varieties was significantly higher in Western Siberia than European Russia, 5.1 and 0.4 %, respectively (Table 1). This trend in species distribution was also the only significant difference reported previously by Yurkov et al. [60]. Thirdly, the so-called "red yeasts" group appeared to consist of different species depending on the region studied, *S. roseus* in Moscow region and *R. babjevae* (not *R. glutinis*) in Western Siberia, respectively. Therefore, *S. roseus* might not have a wide occurrence in temperate zone (compare with [2, 6]), as it was not found in Western Siberia during this study. Also, opposing to the prevailing views on the worldwide

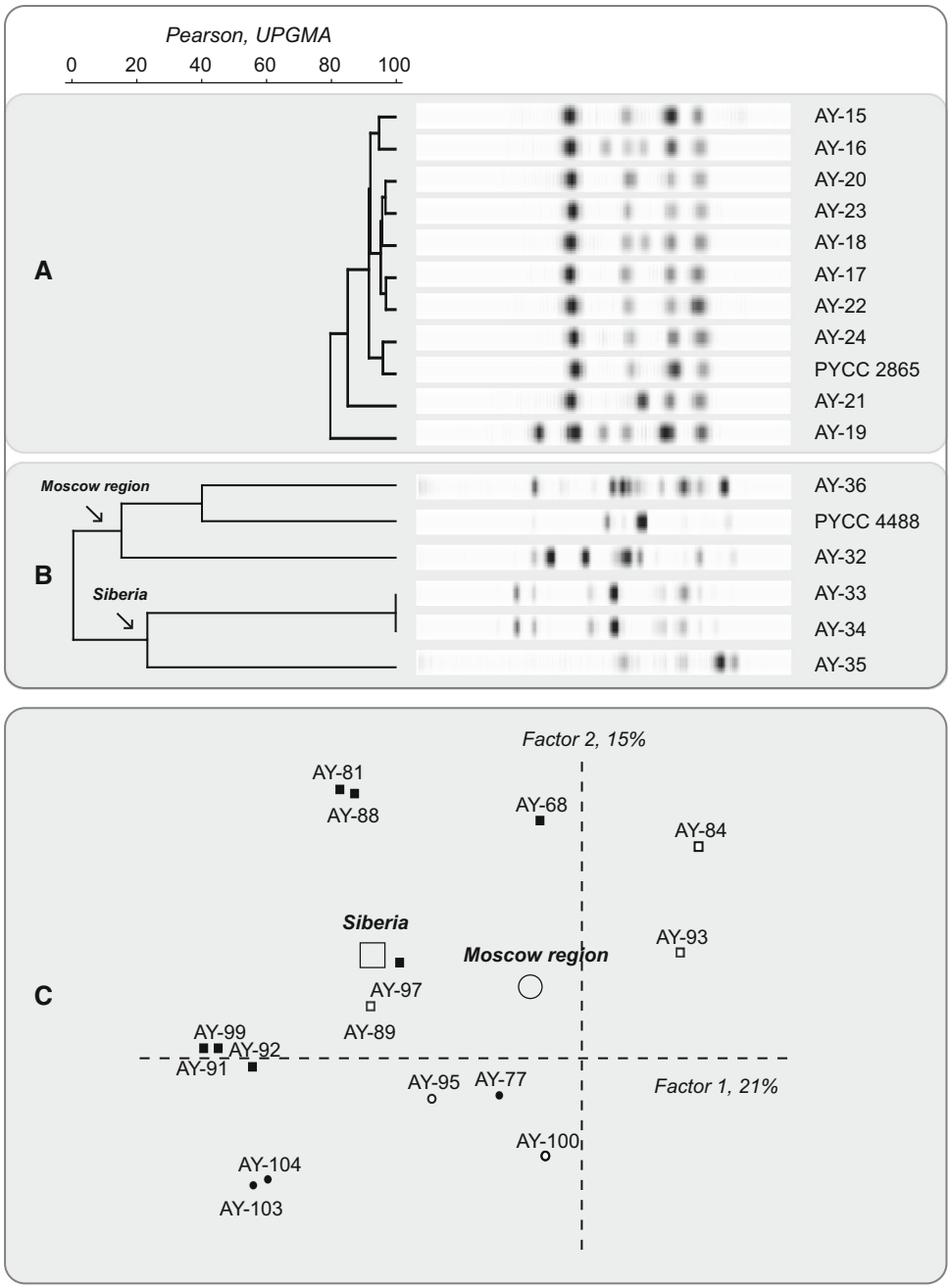


Fig. 4 Examples of different intraspecific variability patterns observed in present study: **a** no variability (*C. terricola*), **b** geographical populations (*C. podzolicus*), **c** high variability with geographical trend (*C. victoriae* and *C. tephrensis*). **a** and **b** DNA banding patterns obtained with M13 primer and resulting dendrogram using Pearson's coefficient and the UPGMA clustering method, co-phenetic correlation coefficient ($r = 0.74$). **c** DNA banding patterns obtained with $(GAC)_5$ primer and resulting ordination using Pearson's coefficient and principal components analysis (PCA). First two extracted components, factors describing together 36 % of the genetic variability between strains. A total of 75 % of data are described by six extracted components. *Black markers* designate isolates from Western Siberia and *white* ones from Moscow region. Strains of *C. victoriae* are marked with *circles* and those of *C. tephrensis* with *squares*. *Large markers* are centroids for groups according to geographical region of isolation

distribution of *R. glutinis*, it was found that none of the studied strains belong to that species (Table 1, Table S1). Only five isolates are currently assigned to *R. glutinis* [39]. Similarly, *R. graminis* is known at this moment from eight isolates only [8, 39, 63].

On the population level, geographical signal varied considerably between different phylogenetic groups of species (Fig. 4). In general members of the Filobasidiales (*C. albidus*, *C. terricola*, *C. wieringae* and *C. magnus*) demonstrated high levels of genetic variability as determined by MSP-PCR that did not correlate with geographical region, type of substrate or phylogenetic position. In contrast, members of the Tremellales (*C. podzolicus*, *C. victoriae*) appeared to form geographical populations. To date, influence of spatial factors on yeast community composition in the same substrate type was demonstrated for simple and rather homogeneous substrates, like floral nectar [30], necrotic tissues of cacti [47], and berries [35]. Intraspecific variability that resulted from spatial separation of conspecific isolates has been previously observed in ascomycetous yeasts, for example *Saccharomyces* spp. [32, 33, 36], *Candida sonorensis* [16],

and some other species [29, 31]. Previous analysis of ascomycetous yeasts *Hanseniaspora guilliermondii*, *Torulaspora delbrueckii*, and *Debaryomyces hansenii* isolated in Moscow region and Novosibirsk revealed distinct MSP-PCR fingerprint patterns with, $(GTG)_5$ and $(ATG)_5$ primers depending on the region of isolation, which demonstrated the existence of geographic populations [58]. Results obtained in the present study demonstrate that a similar pattern is apparently present not only within ascomycetous yeasts, but could be also observed among widespread basidiomycetes, namely *C. podzolicus*, *C. victoriae* and *Cu. cygneicollum* (Fig. 4).

Ecology and Distribution

Species concept has a great influence on diversity studies. Different approaches applied to species recognition may result in detecting different entities. A previous review aimed at resolving the impact of the species concept on biodiversity studies showed remarkable differences, with surveys based on a phylogenetic species concept detecting 48 % more species (300 % more for fungi) and an associated decrease in population size and range [1]. Although identification methods based on ribosomal DNA sequencing gained more and more importance in the last decade [48, 61, 62], some yeast ecologists repeatedly argued for using "natural" or "phenotypic" species in their studies as opposed to "genotypic" as the adaptation to environmental conditions results mainly from the phenotype, e.g. [17, 34]. However, these entities defined on morphological attributes (sometimes referred as sensu lato species) might not be restricted to a single phylogenetic lineage but include a broad set of unrelated taxa, e.g. [13]. Using a large collection of strains and different approaches to species identification, we discuss the phylogenetic relationships of yeasts comprised within the borders of species previously identified using only MSR approaches, namely *C. laurentii*, *C. diffluens* and *C. albidus*. Even though the selection strategy employed may not account for the full extent of species richness in these biotopes, it offers the possibility of re-assessment of species identification reported in earlier studies, e.g. [2, 17, 19, 34]. Thereby, we revise the distribution of identified phylogenetic species based on the comparison with earlier studies.

C. albidus, defined as a composite species complex, was considered an eurybiont species, as no significant trends in its distribution in time and space had been observed [2, 17, 19]. Our results suggest that mainly members of the *floriforme* clade (Filobasidiales, Tremellomycetes) are comprised within *C. albidus* (as identified with conventional physiological tests). Out of the two most frequent species in this group, *C. magnus* had been frequently reported from the phylloplane [12], but data regarding the distribution of the closely related species *C. wieringae* is scarce [14]. However, a limited number of molecularly identified strains suggest association of this yeast with plants [14]. Interestingly, *C. wieringae* was more abundant than *C. magnus* in our study and according to these results, *C. wieringae* appeared to be an important component of the phylloplane community, probably overlooked and accounted as *C. albidus* due to the use of less accurate phenotypic identification approaches in previous studies, e.g. [17, 34].

Molecular reassessment of the strains identified as *C. laurentii* placed most of the studied isolates within the *victoriae* clade (Tremellales, Tremellomycetes), with *C. victoriae* being the most frequently found species (Table 1; Fig. 2). In our study, the occurrence of this species was restricted to the plant material, which contrasts with previous reports where *C. victoriae* was also isolated from soil substrates [9, 21, 52, 56]. A few reports also describe the isolation of *C. victoriae* from apples, flower nectar and leaves of Mediterranean plants [14]. Our results additionally support phylloplane-related origin of this yeast, which was found in both studied regions and from various plants (Table 1; Table S1). We suggest that a large part of previous reports on *C. laurentii* isolates from plant surfaces [2, 17, 19] probably refer to *C. victoriae*. According to the origin of strains identified with molecular methods (this study; [14]), *C. victoriae* seems to have a wide distribution range. Although global distribution patterns of microbial species are currently debatable (e.g. [48]), *C. victoriae* might represent a good example of a globally spread phylogenetic species. According to our results, this species is extremely polymorphic. It displays interspecific variability of morphological and physiological characteristics as well as considerable genetic variability (see also [9]). Studies utilizing additional genetic markers are required to answer the question whether *C. victoriae* in its current circumscription is indeed widespread or it represents a complex of cryptic species.

About 20 % of isolates previously assigned to *C. laurentii* were identified as *C. tephrensis* in the present study. Although this species was originally isolated from soil [51], and latter isolated from this substrate by other authors [21, 52], the probable soil origin of *C. tephrensis* is controversial. An extensive study of different soils in Germany reported *C. tephrensis* as being a transient species in these substrates, due to its rare occurrence and low abundance [62]. In other example, *C. tephrensis* was reported in a large-scale study on soil yeasts but with a very low frequency, except for Iceland soils [52]. Thus, the ecology of this species is still unclear (see also [14]). However, evidence for association of *C. tephrensis* with plant material is provided in this study (Table 1). Additionally, several CBS collection strains (CBS 8993, CBS 9012, CBS 9023 and

CBS 9799) representing this species were also isolated from plants (e.g. [20]). We suggest that *C. tephrensis* may be associated with the phylloplane, from where it can reach neighbour substrates, like soil.

Yeasts identified as *C. diffluens* have been repeatedly observed in dry habitats, like deserts [2, 7], seasonally dried peat and house dust ([18] and references therein). Bab'eva and Chernov [2] suggested that the occurrence of *C. diffluens* may indicate a low water content in the environment. We found that isolates identified as *C. diffluens* using MSR approaches were mostly conspecific, but belong to a phylogenetically distinct species, *C. albidus* (Fig. 3). Remarkably, two reference strains of phenotypic *C. diffluens* (KBP 3824 and 3826) isolated from sub-tropical deserts by Chernov et al. [7] were also identified as *C. albidus* (Table 1; Fig. 3). The presence of *C. albidus* in dry habitats agrees with results of previous experiments, which demonstrated a good survival of these yeasts in sandy soil due to the abundant production of polysaccharide capsules [50]. Interestingly, the assessment of soil yeast distribution along a large latitudinal gradient using molecular identification tools showed that *C. albidus* occurrence was negatively correlated with the rainfall [52]. We suggest that a large proportion of previous reports describing the isolation of *C. diffluens* [2, 7, 18, 34] may in fact refer to *C. albidus*.

Interestingly, the identification of the soil-borne yeasts *C. terricola* and *C. podzolicus* using conventional physiological criteria was consistent with the identification using molecular tools. Recently, Yurkov et al. [61] demonstrated that yeast communities in forest soils largely consist of distantly related species, which are likely to be successfully identified using physiological tests. Unlike under-sampling, the presence of cryptic species does not cause significant underestimation of soil yeast diversity [61]. In the present study, we found soils to be inhabited by a few number of yeasts, *C. albidus*, *C. terricola* and *C. podzolicus* (Table 1). In contrast, plant substrates harbour diverse physiologically indistinguishable yeast species, such as *C. victoriae*, *C. carnescens*, *C. heimaeyensis*, *C. tephrensis* and *C. foliicola*. The occurrence of cryptic species seem to pose a real problem for assessing the biodiversity in the phylloplane when using only MSR approaches to identify yeast isolates.

Red yeasts were repeatedly reported as dominant phylloplane inhabitants and the two species *R. babjevae* and *S. roseus* were commonly isolated from plant material worldwide, e.g. [2, 12, 25, 34]. In contrast, the association of the other two *Rhodotorula* species isolated in this work, *R. pinicola* and *R. slooffiae*, with any habitat is currently unclear. While *R. slooffiae* is a rather frequent species [39], *R. pinicola* was known from a few isolates only. Our results suggest that *R. pinicola* might regularly inhabit plant substrates and have a wide distribution range (Table 1). Furthermore, this species might be mislabelled and accounted as *R. minuta* in earlier studies. However, it should be stressed out that additional studies are required to reassess distribution of red-coloured yeasts, as they are often difficult to differentiate using assimilation tests, e.g. [39].

Although the precise habitat of *Cu. cygneicollum* (*R. fujisanensis*) remains unclear, the majority of known strains were isolated from different types of plant material [25, 34, 40]. Our results support this observation as we found this yeast across all stages of plants decomposition, from live parts to litter and the topsoil (Table 1). Several anamorphic non-pigmented strains originally identified as *Rhodotorula* spp. were assigned to *L. scottii*. This species occupies diverse habitats in the temperate zone and were mostly reported from soils and water habitats [41]. To our knowledge, high abundance of this yeast in the phylloplane of vascular plants has not been reported before.

Species Recognition Approaches

Phylogenetic species boundaries do not necessarily coincide with the species boundaries based on morphological or biochemical characters or reproductive isolation, e.g. [48]. Despite their current popularity, biodiversity assessments using species recognition solely based on phylogenetic approaches are known to be sensitive to the sampling effort [45, 53, 61], since PSR is able to resolve smaller entities with more restricted geographic ranges and decreased abundances [1]. Re-identification of *Cryptococcus* yeasts performed with molecular tools demonstrated that taxonomical units determined with MSR correspond mostly to the level of phylogenetic clades or roughly genera when considering polyphyly of this genus [14]. Even though MSR and PSR seem to result in entities corresponding to two different taxonomic levels in yeast species, linking "phenotypic" and phylogenetic species is hardly feasible due to widespread mislabelling of polymorphic species identified with MSR as *C. laurentii* and *C. albidus*. Even though our results showed the majority of PSR-based species identified in MSR-based *C. laurentii* isolates were mostly placed within a monophyletic *victoriae* clade in Tremellales (Table 1; Fig. 1), this clade is distantly related to the Bulleromyces clade that includes the type strain of *C. laurentii* [14, 59]. The use of conventional phenotypic methods alone in biodiversity studies is feasible to some extent only. Our results suggest that these methods could provide reliable resolution at the level of phylogenetic clades and can be utilized for the analyses of functional traits in yeasts.

Conclusions

Appropriate re-identification of species previously resolved with phenotypic characters enabled comparisons with

previous surveys that also used molecular methods and revealed frequent occurrence and wide distribution of *C. wieringae*, *C. tephrensis* and *R. pinicola*, species known previously from only a few isolates, and *C. victoriae* and *C. albidus* being long reported under incorrect names due to the inaccuracy of phenotypic identification. Comparison with previous studies confirms certain trends and reveals others that clearly deserve further studies, including the definition of yeast characteristics that determine their vertical (substrates) and horizontal (spatial) distribution. Even though our study revealed some congruence between distribution patterns of yeasts identified with MSR and PSR approaches, strain profiling using PCR fingerprinting techniques suggests a rather complex interspecific structure of many basidiomycetous yeast species that should be addressed in further studies.

Molecular typing may additionally help to uncover interspecific heterogeneity and population structure and, thus, reveal possible correlations between genotype and geographical origin or other ecologically relevant parameters. Geographical population structure and allopatric speciation, previously observed in ascomycetous fungi, appears to be present to some extent among basidiomycetes as well.

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