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Mrakia fibulata sp. nov., a psychrotolerant yeast from temperate and cold habitats

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Abstract Tree fluxes are sugar-rich, sometimes ephemeral, substrates occurring on sites where tree sap (xylem or phloem) is leaking through damages of tree bark. Tree sap infested with microorganisms has been the source of isolation of many species, including the biotechnologically relevant carotenoid yeast *Phaffia rhodozyma*. Tree fluxes recently sampled in Germany yielded 19 species, including several psychrophilic yeasts of the genus *Mrakia*. Four strains from tree fluxes represented a potential novel *Mrakia* species previously known from two isolates from superficial glacial melting water of Calderone Glacier (Italy). The Italian isolates, originally identified as *Mrakia aquatica*, and two strains from Germany did not show any sexual structures. But another culture collected in Germany produced clamped hyphae with teliospores. A detailed examination of the five isolates (three from Germany and two from Italy) proved them to be a novel yeast species, which is described in this manuscript as *Mrakia fibulata* sp. nov. (MB 830398), holotype DSM 103931 and isotype DBVPG 8059. In contrast to other sexually reproducing *Mrakia* species, *M. fibulata* produces true hyphae with clamp connections. Also, this is the first psychrotolerant *Mrakia* species which grows above 20 °C. Spring tree fluxes are widespread and can be recognized and sampled by amateurs in a Citizen Science project. This substrate is a prominent source of yeasts, and may harbor unknown species, as demonstrated in the present work. The description of *Mrakia fibulata* is dedicated to our volunteer helpers and amateurs, like Anna Yurkova (9-years-old daughter of Andrey Yurkov), who collected the sample which yielded the type strain of this species.

Keywords 1 new species · Basidiomycete · Mrakia · Psychrophilic · Tree fluxes

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Introduction

The yeast genus *Mrakia* comprises sexual and asexual yeast species. They share a remarkable adaptation to low temperatures (Buzzini et al. 2018), the ability to ferment sugars (Fell 2011) and to produce diverse extracellular enzymes such as lipases, amylases,

proteases, pectinases, cellulases, chitinase and lignolytic enzymes (Tasselli et al. 2017). These abilities were applied to wastewater treatment, bioethanol and beer production at low temperature (Tsuji et al. 2013a; Tsuji et al. 2013b; De Francesco et al. 2018).

The majority of reported isolations of yeasts of the genus *Mrakia* were made from cold habitats such as polar soils (Arctic, Antarctic), glaciers (ice, sediment, cryoconite, meltwater), caves (cave ice), permafrost and snow (Buzzini et al. 2017; Sannino et al. 2017). A more exotic habitat of these yeasts includes frozen foods (fish and meet) and arctic goose feathers (Fell 2011; Kabisch et al. 2016; Singh et al. 2016). Reports of isolation of these psychrophilic yeasts outside cold habitats are scarce and limited to a few studies in European boreal and temperate forests, from either plant material or soils (e.g. Glushakova and Chernov 2004; Maksimova and Chernov 2004; Yurkov et al. 2016). However, it must be mentioned that isolation of *Mrakia* yeasts in association with plants and lichens in polar climates was repeatedly published (Babjeva and Reshetova 1998; Fell 2011; Ferreira et al. 2019; Santiago et al. 2015; Zhang et al. 2015).

Tree (slime) fluxes are sugar-rich substrates occurring on sites where phloem or xylem sap is leaking through damages of tree bark. High sugar (glucose, fructose and sucrose) concentration makes tree sap a suitable substrate for propagation of microorganisms, including yeasts (reviewed in Péter et al. 2017). Slime fluxes (also referred to as wetwood by phytopathologists) are caused by an infection (often bacterial) which hinders injury (mechanical or by insects) healing. These fluxes are not temporarily restricted and may last for years. Conversely, spring sap flows in some temperate tree species like birch are restricted to a short period of time in spring when roots create substantial pressure that pushes tree sap upstream (Essiamah 1980). Yeast communities found in different types of tree exudates differ accordingly (reviewed in Péter et al. 2017). Numerous basidiomycetous yeasts were isolated in the past from injured trees (often birches, *Betula* spp.) (reviewed in Péter et al. 2017). The accuracy of techniques employed for yeast identification increased greatly allowing more reliable identification of phenotypically indistinguishable (cryptic) species. Tree fluxes caused by tree injuries (pruning or selected tree removal) during winter time were collected in spring of years 2013, 2016 and 2019 in Braunschweig (Lower Saxony, Germany). Several psychrophilic yeasts were isolated from xylem sap and thick microbial matts on stems and branches. Three species of the genus *Mrakia* were identified in this study. One of them represented a potential novel species highly similar to two isolates from superficial glacial melting water of Calderone Glacier, Italy (Branda et al. 2010) originally identified as *Mrakia aquatica*. A detailed examination of the five isolates (three from Germany and two from Italy) proved the strains to be a novel yeast species, which is described in this manuscript as *Mrakia fibulata* sp. nov.

Materials and methods

Tree fluxes caused by tree injuries during winter time were collected in spring of years 2013 and 2016 in the city Braunschweig, Lower Saxony, Germany (Fig. S1). In March 2013, two locations in Stöckheim were sampled: a stump from a recently cut down silver birch *Betula pendula* (52.209635 N, 10.527774 E) and from a young beech *Fagus sylvatic*a pruned in the winter (52.213777 N, 10.531985 E). In April 2016, stumps from recently cut down silver birch trees *B. pendula* in Gartenstadt (52.238836 N, 10.507971 E) were sampled. In April 2019, young birch, hornbeam *Carpinus betulus* and dogwood (*Cornus* sp.) plants, which were pruned during winter, were sampled in Stöckheim in the same locality as in 2013. Tree fluxes and thick mucous microbial matts (Fig. S1) were aseptically collected in 50 mL tubes and kept at 4 °C until processing.

Yeasts were isolated by streaking out samples on surface of YM plates supplemented with chloramphenicol or by serial dilution (1:10, 1:100, 1:1000) plating on the same medium. Plates were incubated 2 weeks at 10 °C and were inspected every 2–3 days. Colonies were differentiated into macro-morphological types with dissection microscopy, counted and 1–2 representatives of each colony type per plate were transferred into pure culture.

Calderone Glacier (42.470278 N, 13.565556 E) can be considered a debris-covered glacier. The lower part of the glacier is completely covered by white limestone rock debris, whereas the top is characterized by the presence of seasonal snow. Above the glacier two of the highest peaks are present with pioneer vegetation. Details on sampling and isolation of yeasts

from superficial glacial melting water of Calderone Glacier are provided by Branda et al. (2010).

Growth tests were performed in liquid media (according to Kurtzman et al. 2011) and in BIOLOG MicroStation and YT and FF MicroPlates (BIOLOG Inc., Hayward, CA, USA) at 20 °C. To inoculate BIOLOG MicroPlates, yeasts were pre-incubated on potato dextrose agar (PDA, Difco) at 20 °C, harvested from PDA, suspended in the inoculation solution IF-B (BIOLOG Inc., Hayward, CA, USA) and the turbidity was adjusted to the transmittance value provided by the manufacturer. MicroPlates were sealed to prevent desiccation and incubated at 22 °C and measured after 1, 2 and 3 weeks, with the optical density recorded at 490, 590 and 750 nm. The ability to utilise particular substrates by individual strains was recorded as positive, weak and negative.

DNA was isolated with the MasterPure Yeast DNA Purification Kit (Epicenter, Madison, WI, USA), following the manufacturer's instructions. Amplification of the ITS and partial LSU regions were performed with primers ITS1F and LR5, while internal primers ITS4 and NL4 were used for sequencing (Spirin et al. 2018). The assembly and editing of sequence data were performed with Sequencher 5.3 (Gene Codes, Ann Arbor, MI, USA). Sequences were compared with sequences deposited in the NCBI GenBank (full dataset and RefSeq) and MycoBank.

Phylogenetic relationships of yeasts of the genus *Mrakia* were analysed with ITS and LSU nucleotide sequences. Sequence alignments were performed with the online version of the MAFFT algorithm using the Q-INS-i option with the default parameters (Katoh et al. 2019). Sequences were trimmed to obtain strict flanking regions. Phylogenetic relationships were inferred by the maximum likelihood (ML) method based on the general time reversible (GTR) model with RAxML (version 8.1.22) using raxmlGUI 1.5b and the GTRGAMMA option with 1000 rounds of bootstrap replicates (Silvestro and Michalak 2012).

Pair-wise distances were analysed in a 1181 bp long ITS-LSU alignment among type strains of *Mrakia aquatica*, *Mrakia hoshinonis*, and *Mrakia niccombsii*, and strains identified as *M. aquatica* in other studies. Distances were estimated after trimming ITS and LSU sequences in multiple sequence alignments to obtain strict flanking regions. A parsimony network was constructed from aligned ITS and LSU (D1/D2 domains) sequences with the program TCS 1.21 (Clement et al. 2000) using 95% connection limit and gaps treated as fifth state.

Results

Tree fluxes caused by tree injuries sampled during 2013–2019 in Braunschweig (Lower Saxony, Germany) yielded 19 yeasts. In 2013, a stump of a silver birch tree (*Betula pendula*) yielded one yeast species, *Cystofilobasidium infirmominiatum* (Table 1). Fluxes dropping from branches of young beech (*Fagus sylvatic*a) trees which were trimmed during winter, yielded ten more species (Table 1). Sampled in 2016 stumps of recently cut down silver birch trees *Betula pendula* yielded only basidiomycetous yeasts, including three *Mrakia* species (Table 1). More recent sampling (April 2019) of birch, hornbeam and dogwood trees resulted in isolation of ten yeasts, five of which were not obtained from previous samplings (Table 1). *Cystofilobasidium capitatum*, *Cystofilobasidium macerans*, *Cystofilobasidium infirmominiatum,* and *Tausonia pullulans* were isolated in all three sampling years. Only one ascomycetous yeast, *Candida sake* was isolated from the sampled tree fluxes. Out of 19 isolated yeasts, 13 species were only isolated in one sampling year.

Identification of most of yeasts isolated from tree fluxes was successful using either nucleotide sequences of LSU or ITS fragments. A few strains showed one to three nucleotide substitutions to type strains of respective species (Fig. 1). Nucleotide sequences of the ITS region of strains DSM 27892 (GenBank MK693026) and DSM 103936 (GenBank MK693025) preliminary identified as *M. gelida* differed in five nucleotide positions from that of the type strain (GenBank AF144485). Sequences of strains recovered from tree fluxes matched with strains of *M. gelida* previously isolated from Calderone (Apennines) and Miage (Alps) glaciers, respectively (GenBank GQ911543, KC455909). A detailed investigation of strains identified as *M. gelida* will be made in a separate study. Four strains of *Mrakia* sp. from tree fluxes showed almost equal sequence similarity values to strains comprising the *M. aquatica* sub-clade, namely *M. aquatica*, *M. hoshinonis*, and *M. niccombsii*. Three of the four strains DSM 103931 (GenBank MK372216), DSM 109846 (GenBank MN597447), and DSM 109847

(MN597448) were studied further. Strain BS-7 isolated from birch in 2013 was identified but not preserved and not further studied. All four strains shared identical ITS and LSU sequences. The best match was obtained with strain DBVPG 4990, one gap in ITS and identical LSU sequences (GenBank GQ911547 and GQ911522, respectively). Strain DBVPG 4990 was isolated from superficial meltwater collected at Calderone glacier and preliminary identified as *Mrakia aquatica* by Branda et al. (2010). Among *Mrakia aquatica* strains preserved in the DBVPG collection, strain DBVPG 4999, which shared ITS and LSU sequences identical with DBVPG 4990 (data not shown), was included in the analysis. Phylogenetic analyses of concatenated ITS and LSU alignments using a maximum likelihood and parsimony network analyses were performed to determine the placement of this potential new yeast (Figs. 1, 2).

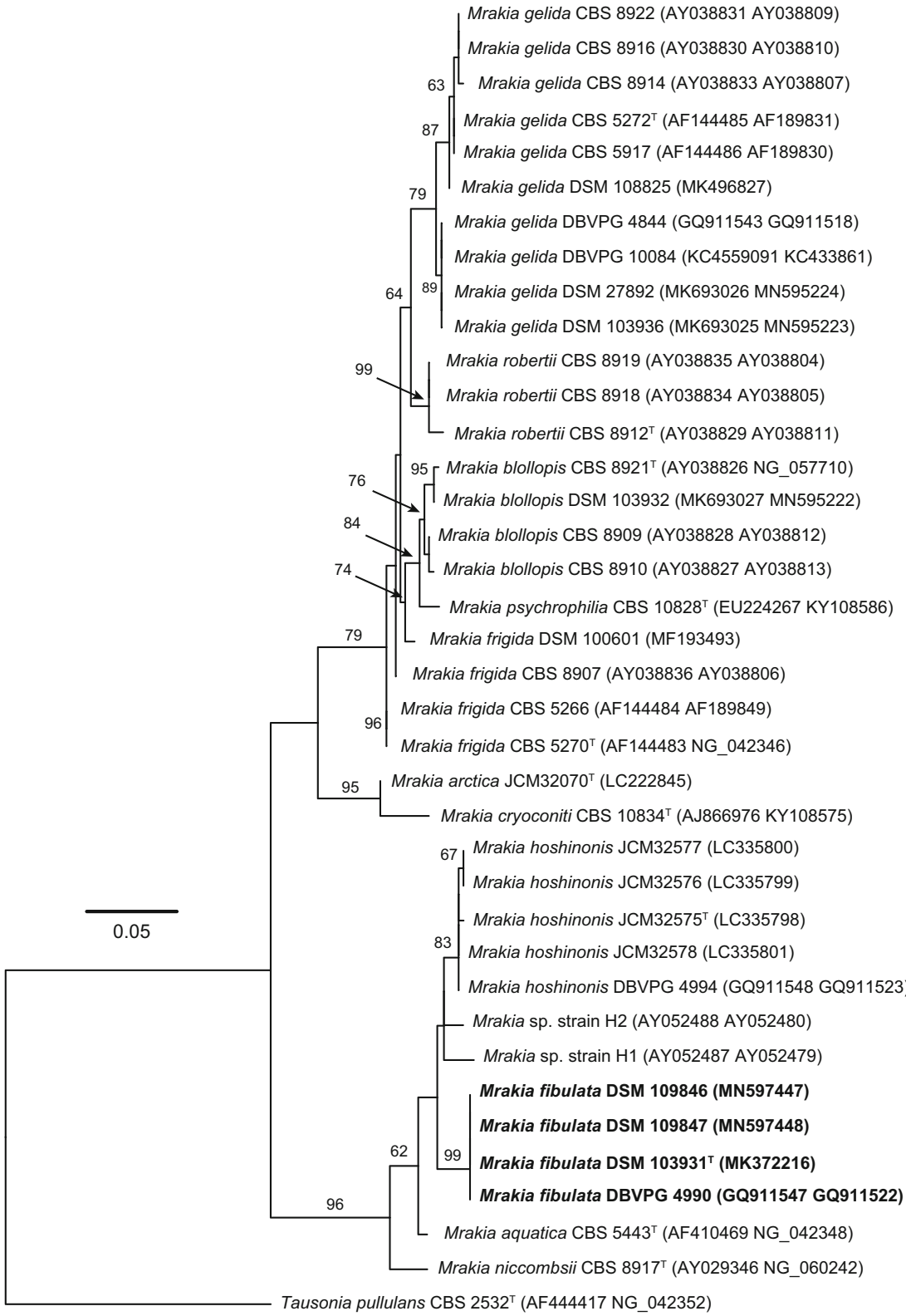
Fig. 1 Maximum likelihood analysis of concatenated sequences of the internal transcribed spacer (ITS) regions and D1/D2 domains of the LSU rRNA gene of the genus *Mrakia*. The numbers given on branches are frequencies (> 60%) with which a given branch appeared in 1000 bootstrap replications. The scale indicates the number of expected substitutions accumulated per site. The tree is rooted with *Tausonia pullulans* CBS 2532^{T} (AF444417, NG_042352)

Phylogenetic analysis showed that strains representing potential new species were placed in the *Mrakia aquatica* sub-clade comprised by *M. aquatica*, *M. hoshinonis* and *M. niccombsii* (Fig. 1). This clade received good (ML: 96%) statistical support in the analysis but internal nodes were not resolved. The most recent phylogenetic study of the genus *Mrakia* was performed by Tsuji et al. (2019), who described *M. hoshinonis* from a glacier in Canadian Arctic

Table 1 Yeast species isolated from spring fluxes of sampled trees during this study

Species	2013	2016	2019	Representative strains, DSM collection numbers
Candida sake	Beech			27891
Curvibasidium cygneicollum			Hornbeam	109506
Cystofilobasidium capitatum	Beech	Birch	Birch	27899, 103933
			Hornbeam	
Cystofilobasidium infirmominiatum	Birch	Birch	Birch	27770, 27771, 27888, 27889, 103935, 109505
	Beech		Hornbeam	
			Dogwood	
Cystofilobasidium macerans			Hornbeam	109504
Filobasidium wieringae			Hornbeam	109542
Filobasidium stepposum	Beech			27898
Goffeauzyma gastrica	Beech			27890
Holtermanniella festucosa	Beech			27887
Holtermanniella takashimae			Hornbeam	109503
Holtermanniella wattica			Hornbeam	109502
Leucosporidium creatinivorum		Birch		103934
Mrakia blollopis		Birch		103932
Mrakia gelida	Beech	Birch		27884, 27892, 27893, 27894, 103932, 103936
Mrakia fibulata		Birch	Hornbeam	103931, 109846, 109849
			Dogwood	
Rhodosporidiobolus colostri	Beech		Hornbeam	27885
Rhodotorula babjevae	Beech			27900
Tausonia pullulans	Beech	Birch	Birch	27882, 27883, 103930
			Hornbeam	
			Dogwood	
Vishniacozyma tephrensis	Beech			27886

Tree species, birch *Betula pendula*, beech *Fagus sylvatica*, hornbeam *Carpinus betulus* and dogwood *Cornus* sp


Mrakia gelida CBS 8922 (AY038831 AY038809)
Mrakia gelida CBS 8916 (AY038830 AY038810)
Mrakia gelida CBS 8914 (AY038833 AY038807)
Mrakia gelida CBS 5272T (AF144485 AF189831)
Mrakia gelida CBS 5917 (AF144486 AF189830)
Mrakia gelida DSM 108825 (MK496827)
Mrakia gelida DBVPG 4844 (GQ911543 GQ911518)
Mrakia gelida DBVPG 10084 (KC4559091 KC433861)
Mrakia gelida DSM 27892 (MK693026 MN595224)
Mrakia gelida DSM 103936 (MK693025 MN595223)
Mrakia robertii CBS 8919 (AY038835 AY038804)
Mrakia robertii CBS 8918 (AY038834 AY038805)
Mrakia robertii CBS 8912T (AY038829 AY038811)
Mrakia blollopis CBS 8921T (AY038826 NG_057710)
Mrakia blollopis DSM 103932 (MK693027 MN595222)
Mrakia blollopis CBS 8909 (AY038828 AY038812)
Mrakia blollopis CBS 8910 (AY038827 AY038813)
Mrakia psychrophilia CBS 10828T (EU224267 KY108586)
Mrakia frigida DSM 100601 (MF193493)
Mrakia frigida CBS 8907 (AY038836 AY038806)
Mrakia frigida CBS 5266 (AF144484 AF189849)
Mrakia frigida CBS 5270T (AF144483 NG_042346)
Mrakia arctica JCM32070T (LC222845)
Mrakia cryoconiti CBS 10834T (AJ866976 KY108575)
Mrakia hoshinonis JCM32577 (LC335800)
Mrakia hoshinonis JCM32576 (LC335799)
Mrakia hoshinonis JCM32575T (LC335798)
Mrakia hoshinonis JCM32578 (LC335801)
Mrakia hoshinonis DBVPG 4994 (GQ911548 GQ911523)
Mrakia sp. strain H2 (AY052488 AY052480)
Mrakia sp. strain H1 (AY052487 AY052479)
Mrakia fibulata DSM 109846 (MN597447)
Mrakia fibulata DSM 109847 (MN597448)
Mrakia fibulata DSM 103931T (MK372216)
Mrakia fibulata DBVPG 4990 (GQ911547 GQ911522)
Mrakia aquatica CBS 5443T (AF410469 NG_042348)
Mrakia niccombsii CBS 8917T (AY029346 NG_060242)
Tausonia pullulans CBS 2532T (AF444417 NG_042352)
0.05
63
87
79
64
89
99
95
76
84
74
79
96
95
67
83
62
99
96

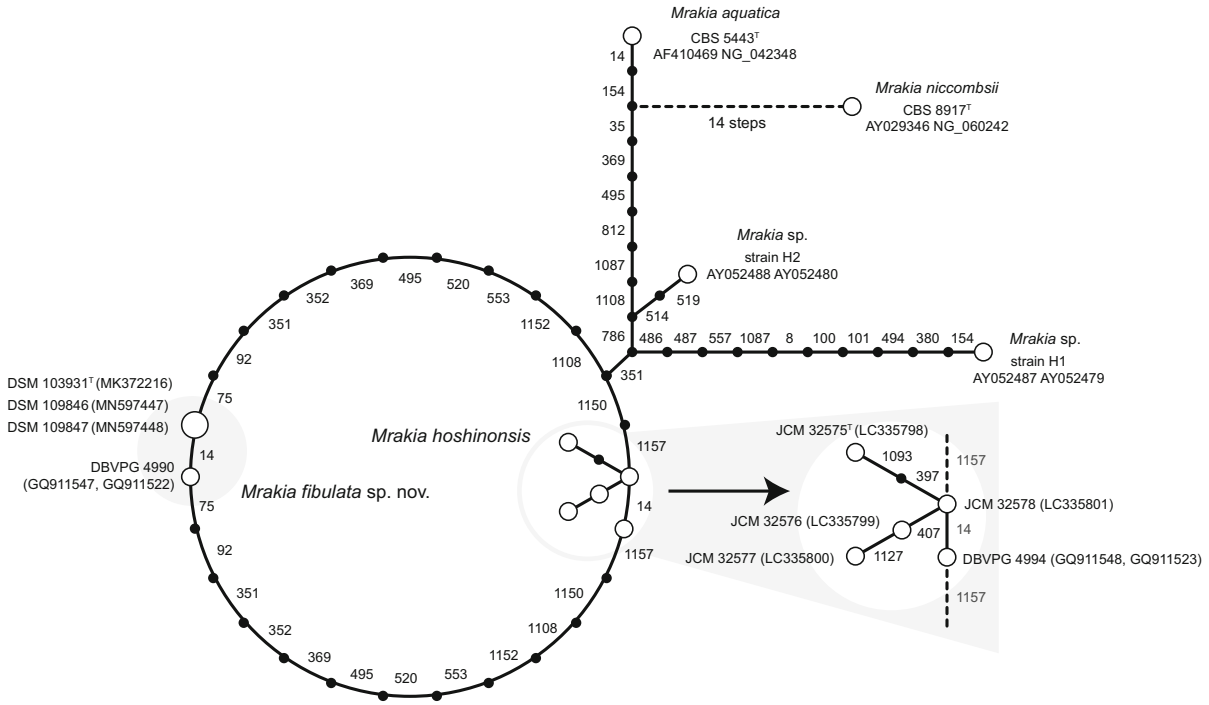


Fig. 2 Parsimony network analysis of the LSU (D1/D2 domains) rRNA gene and the internal transcribed spacer (ITS) regions of yeasts in *Mrakia aquatica* subclade (95% connection limit and gaps treated as fifth state). Each connecting line represents one substitution, numbers indicate a position in the alignment and each small circle represents a missing intermediate sequence. *Mrakia niccombsii* CBS 8917 was disconnected from the network at 95% connection limit but not at 90% connection limit (dashed line)

Archipelago. These authors considered only type strains and did not include other strains labelled in GenBank as *M. aquatica* or unidentified *Mrakia* sp. isolates. Three strains designated as *M. aquatica* (H1, H2, and DBVPG 4994) clustered close to *M. hoshinonis* (Fig. 1). To resolve phylogenetic relationships in this group, we analysed pair-wise distances in a trimmed 1181 bp long ITS-LSU alignment among type strains comprising the *M. aquatica* sub-clade and strains identified as *M. aquatica* with parsimony network analysis with 95% connection limit. This type of analysis proved to be useful to delimit ascomycetous (Lachance et al. 2010, 2011) and basidiomycetous (e.g. Yurkov and Golubev 2012) yeast species. Although Lachance et al. (2010, 2011) treated gaps as missing data in the aforementioned parsimony network analyses, we included gaps in the analysis in order to compare distances in the network with results of pair-wise sequence comparisons performed by Tsuji et al. (2019) to support the erection of the species *M. hoshinonis*.

All strains were connected in the network at 95% connection limit and gaps treated as missing data (Fig. S2). *Mrakia niccombsii* CBS 8917 was excluded from the network at 95% connection limit and gaps treated as fifth character state but not at 90% (Fig. 2). Parsimony network analysis showed that strains *Mrakia* sp. DSM 103931, 109846, 109847 and DBVPG 4990 differed from the type strain of *M. hoshinonis* JCM 32575 and *M. aquatica* CBS 5443 in 12-15 nucleotide positions and 19-20 nucleotide positions, respectively (Fig. 2). In the network, the type strain of *M. hoshinonis* JCM 32575 differed in eight positions from "*M. aquatica*" H2 (GenBank AY052488, AY052480); 13 positions from the type strain of *M. aquatica* CBS 5443 and in 15 positions from "*M. aquatica*" H1 (GenBank AY052487, AY052479). Type strains of *M. aquatica* CBS 5443 and *M. niccombsii* CBS 8917 differed in 16 positions at 90%

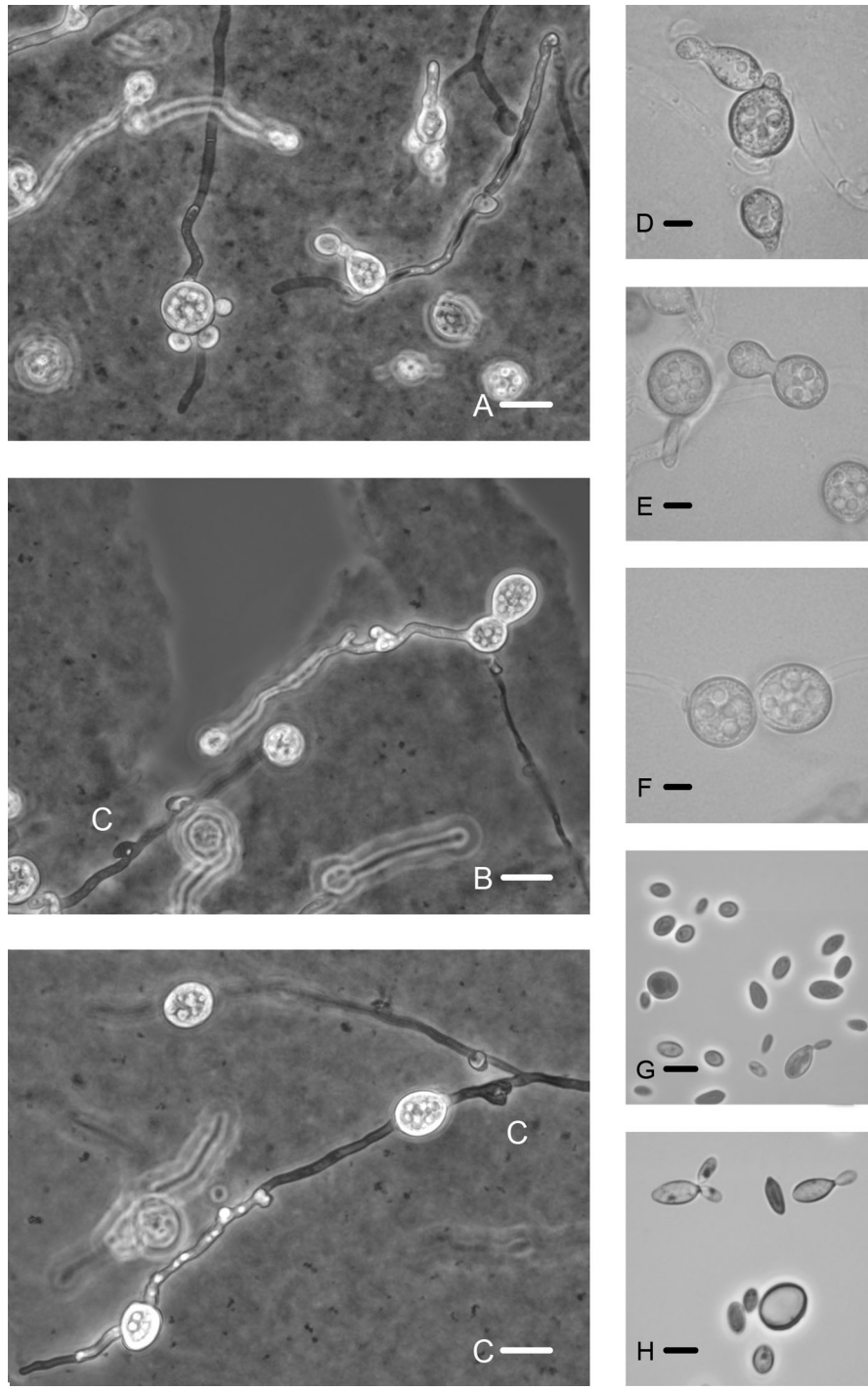


Fig. 3 Light microscopic images illustrating the different stages of *Mrakia fibulata* sp. nov. (strain DSM 103931) after 6 months at 10 °C on PDA: true hyphae with clamp connections and teliospores (**a**–**c**); germinating teliospores (**a**, **d**); teliospores produced with a bud-like projection (**b**, **e**); teliospores in pairs (**f**); asexual cells (**g**, **h**). Bars, 20 μm (**a**–**c**) and 10 μm (**d**–**h**)

connection limit (Fig. 2). It is important to note that these pair-wise distances are lower than those reported by Tsuji et al. (2019), possibly due to sequencing artefacts (read errors and gapped positions in homopolymer repeats) in flanking regions, which were trimmed in the present analysis. But even after trimming, nucleotide sequences of *M. hoshinonis* strains (GenBank LC335798-LC335801) still contained 4 unique positions (2 mismatches and two insertions) which were not present in any other analysed sequence (members of the genus *Mrakia* and outgroup *Tausonia pullulans*). Considering unclear nature (natural variation or sequencing artefacts) of these differences, *M. aquatica* strain from Iceland (H2; GenBank AY052488, AY052480) may represent *M. hoshinonis*. Another presumptive *M. aquatica* strains from Calderone glacier (DBVPG 4994: GQ911548, GQ911523) was identified as *M. hoshinonis* in our study (Fig. 2). According to these results, *M. hoshinonis* is not restricted to the Canadian high Arctic and has been previously isolated in Iceland (Birgisson et al. 2003) and Italy (Branda et al. 2010).

Our results showed that pair-wise distances between type strains of *M. hoshinonis, M. aquatica* and strain *Mrakia* sp. DSM 103931 are equal and lay within 14–20 nucleotide positions (Fig. 2). These results suggest that *Mrakia* sp. DSM 103931 represents a yet undescribed species in the genus *Mrakia*, for which the name *Mrakia fibulata* sp. nov. is proposed. We isolated six strains of this species (four from Germany and two from Italy) which showed identical LSU and similar (one gap) ITS sequences. These yeasts were isolated from tree fluxes (this study) and from superficial meltwater collected at Calderone glacier (Branda et al. 2010). Strain DSM 103931 produced true branched hyphae with regularly occurring septa and clamp connections and teliospores after a prolonged incubation at 10 °C on PDA (Fig. 3). Teliospores of a 3- to 6- month-old culture germinated in agar with hyphal segments in addition to the typical basidia-like structures (Fig. 3). Unfortunately, technical circumstances prohibited a clear-cut photo documentation of basidium formation because teliospore germination occured in agar blocks. Other strains reproduced strictly asexually. The sexually reproducing strain was selected as the type for the new species.

Description of *Mrakia fibulata* A.M. Yurkov & B. Turchetti sp. nov., MB 830398

Etymology The species epithet '*fibulata*' is derived from fibula (L. adj. f., with clamp) and refers to the presence of hyphae with clamp connections.

After 1 week at 15 °C on PDA, streak culture is whitish to cream-coloured, raised, dull, mucoid with a smooth surface. Margins are smooth and entire and the profile is flat. Cells are ovoid to elongate 2–4 × 4–7 µm in size, occur singly or in pairs, and proliferating by polar budding (Fig. 3). After 1 month at 15 °C, the streak culture is cream to light brownish-grey, raised, dull, butyrous with smooth surface. The margin may be fringed with true hyphae. Pseudohyphae and true hyphae with clamp connections are developed after 6 months at 10 °C on PDA (Fig. 3). True, branched hyphae with regularly occurring septa and clamp connections are present after 6 months at 15 °C on PDA. Blastoconidia are ovoid and occur singly, in chains or in clusters. Teliospores are spherical, 5–15 µm in diameter, terminal or intercalary, single and in short chains of 2–4 spores (Fig. 3). Teliospore germination mode is variable. Teliospores germinate with a one- to three-celled metabasidium, 8–12 µm long followed a transfer of an agar block to CMA and PDA. Teliospores also germinate with hyphal segments.

Fermentation of glucose is variable. Growth on D-glucose, D-galactose, L-sorbose, D-glucosamine, D-xylose (variable, weak), L-arabinose, L-rhamnose (weak), sucrose, maltose (variable), trehalose, methyl-alpha-D-glucoside (variable), cellobiose (variable), salicin (variable), arbutin, melibiose, lactose, raffinose, inulin, glycerol (variable), erythritol (variable), ribitol, xylitol, arabinitol, D-glucitol, D-mannitol, galactitol, myo-inositol, 2-keto-D-gluconate, 5-keto-D-gluconate, D-gluconate, D-glucuronate (variable), succinate, citrate (variable, weak), ethanol, quince acid (weak), D-glucarate, gentiobiose, palatinose, L-malate (variable) and Tween 80. No growth on D-ribose, D-arabinose, melezitose, starch, D-galacturonate, lactate, methanol and hexadecane. Utilization of nitrogen sources: growth on potassium nitrate and L-lysin but not on ethylamine and sodium nitrite. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 °C.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) are deposited in NCBI GenBank under the accession number MK372216.

Deposits: holotype, DSM 103931 isolated from xylem sap of a silver birch *Betula pendula* collected by Anna Yurkova in April 2016 in Braunschweig, Lower Saxony, Germany, permanently preserved as a metabolically inactive culture in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany. Isotype DBVPG 8059 is deposited in a metabolically inactive state in the Industrial Yeasts Collection DBVPG, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy.

Strains studied: DSM103931; paratypes: DSM 109846 from xylem sap of hornbeam *Carpinus betulus*, Braunschweig, Germany; DSM 109847 from xylem sap of birch *Betula pendula*, Braunschweig, Germany; DBVPG 4990 and DBVPG 4999 from superficial meltwater collected at Calderone glacier, Gran Sasso d'Italia (an Apennine mountain massif), Abruzzo, Italy (Branda et al. 2010).

Notes New species can be distinguished from closely related *Mrakia aquatica*, *Mrakia hoshinonis* and *M. niccombsii* by growth on inulin, L-rhamnose, myo-inositol, N-acetyl-D-glucosamine, ribitol, and succinate, and inability to grow on D-ribose. In contrast to other sexually reproducing *Mrakia* species, *M. fibulata* produces true hyphae with clamp connections.

Discussion

Tree (slime) fluxes are sugar-rich substrates. Here, we studied yeast communities on sites where xylem sap is leaking from cuts on limbs and stems. Fluxes resulting from tree injuries naturally occur in early spring. They result from winter damages occurring through freeze–thaw cycles and injuries caused by birds and animals. In cities and managed forested areas, much of fluxes result from artificial damages during silvicultural interventions like pruning and clearcutting. Numerous yeasts were isolated in the past from spring sap flows (reviewed in Péter et al. 2017). Yeast communities of the studied tree fluxes were strongly dominated by basidiomycetous species, including slowly fermenting species of the genus *Mrakia*. Despite repeated attempts, we were unable to isolate *Phaffia rhodozyma*, though various pigmented yeasts of genera *Cystofilobasidium*, *Filobasidium*, *Rhodosporidiobolus*, *Rhodotorula*, and *Vishniacozyma* were obtained from spring fluxes. Because spring sap fluxes usually occur early in spring when temperatures are still low, yeast communities were dominated by psychrophilic and psychrotolerant yeasts (reviewed in Buzzini et al. 2018) such as *Cystofilobasidium* spp., *Filobasidium* spp., *Goffeauzyma gastrica*, *Mrakia* spp., *Leucosporidium creatinivorum*, and *Holtermanniella wattica*.

Global warming has already significant effects on our lives causing heat waves, changing precipitation patterns, rising sea level, increasing the incidence of fires and shrinking areas covered by ice. Most of the world's ice is accumulated in Antarctica followed by ice and snow accumulated during winter in the Northern Hemisphere. Arctic temperatures have increased at almost twice the rate of the rest of the world (Overland et al. 2018). As a result, Arctic sea ice is now declining at a rate of 12.8% per decade since 1979 (NASA global climate change). Climate change affects glacier mass balance through variations in temperature and snowfall. Rising temperatures are responsible for a negative mass balance of most of glaciers and has led to near-global glacier retreat during the last century (Gardner et al. 2013; Parkes and Marzeion 2018; Roe et al. 2017; Sahade et al. 2015). In some regions such as the Alps and Patagonia glacier retreat rates have accelerated during last decades. In Southern Europe glacier retreat is dramatic. Glaciers in the Pyrenees and Apennines lost 84% and 90% of their ice volume, respectively (Grunewald and Scheithauer 2010). Warming determined the cryosphere vanishing and the devastation of habitats of cold-adapted organisms, changing ranges of animals and plants. The loss of ice cover and glaciers retreat also affects microorganisms (Hotaling et al. 2017).

Climate change and cryosphere reduction in space and time can negatively affect psychrophilic and psychrotolerant species, including prominent cold-dwelling yeast genera *Glaciozyma, Leucosporidium, Mrakia, Naganishia*. Most isolations of *Mrakia* yeasts were made in polar and non-polar cold environments such as threatened due to climate change Antarctic, Arctic and different glaciers. Strong glacier recession in the Alps, Apennines and Patagonia suggests that the

habitat of psychrophilic and psychrotolerant yeasts discovered during the decade (Branda et al. 2010; De García et al. 2007) may disappear soon (Zekollari et al. 2019). If the present deglaciation trend continues, Europe's southernmost glacier Calderone is unlikely to survive past 2020 (Grunewald and Scheithauer 2010; Zekollari et al. 2019).

Biodiversity and distribution of psychrophilic and psychrotolerant yeasts outside cold habitats are not well known. Frequent sources of isolation such as ice, glacier sediment, snow and meltwater do not tell much about ecological preferences of these yeasts and their dispersal abilities. Most of our knowledge about these yeasts comes from laboratory experiments, not from field studies. We have some understanding of traits helping these yeasts to survive (e.g. production of specific cold active extracellular enzymes) (Buzzini et al. 2012). However, the range of potential habitats and niches suitable for these yeasts remains largely unknown.

The present study reports isolation of psychrophilic yeasts *Mrakia* spp. (*Mrakia blollopis and M. gelida*) also from tree fluxes in the temperate zone. Below we review the distribution of *Mrakia* outside cold habitats. *Mrakia frigida* was occasionally isolated in Moscow region from vascular plants (Glushakova and Chernov 2010) and Sphagnum moss (Kachalkin et al. 2008). Because of the inability to grow above 20 °C and because no nucleotide sequences were available for any of these isolates, Fell (2011) suggested this was not *M. frigida* but a different species. However, other strains of *M. frigida* (for example representative sequence KM384372) were also isolated from an oak bark in different sites across the northern United States using an enrichment protocol and incubation at 10 °C (Sylvester et al. 2015). This species was also found in soils in Germany (Yurkov et al. 2016) and Japan (Nakagawa et al. 2004). *M. aquatica*, *M. blollopis*, *Mrakia robertii* were isolated in summer period from urban soils in Moscow, but not from adjacent low-managed soils (Tepeeva et al. 2018a). Same authors reported *M. aquatica* above and below ground in locations situated above hot water heating pipelines in Moscow urban areas (Tepeeva et al. 2018b). Similarly to culture-based experiments, culture-independent detected *Mrakia* yeasts in various cold habitats (Duo Saito et al. 2018; Rämä et al. 2016). An interesting observation was made in a culture-independent study, which investigated the effects of deglaciation on microbial communities (Rime et al. 2015). Sequence reads corresponding to *Mrakia* species were more characteristic (association strength) for barren (10 years) and sparsely vegetated (60 years) soils in the Damma glacier (Central Alps of Switzerland) forefield. These nutrient-poor soils were inhabited by metabolically versatile bacteria which are able to grow at oxygen-depleted and oligotrophic conditions.

These examples demonstrate the growing evidence that psychrophilic yeasts of the genus *Mrakia* live also outside cold habitats. So far only *M. fibulata* was able to propagate above 20 °C. This is the first record of *Mrakia* species that exhibits psychrotolerant, not strictly psychrophilic, aptitude. Sylvester et al. (2015) already supposed that although *Mrakia* seems to have a competitive advantage at 10 °C, it can grow at warmer temperature. Isolation of *M. aquatica*, *M. blollopis*, *M. gelida*, *M. robertii* and newly described *M. fibulata* from the temperate climate suggests these yeasts might be common inhabitants of boreal and temperate climates. Whether or not *Mrakia* spp. and other cold-adapted yeasts survive warm temperatures in a dormant state is yet unknown. Adaptations of such species to cold (e.g. enzymes and cryoprotectors) are better studied than the mechanisms to cope with warm temperatures.

Spring tree fluxes are an ephemeral but rather widespread substrate which can be easily recognized and sampled by amateurs in a Citizen Science project. A good example of such Citizen Science initiative has been reported in a recent study of yeasts from Dutch soils (Groenewald et al. 2018). We believe that new studies and isolates of psychrophilic and psychrotolerant yeasts from temperate climates would improve our understanding of yeast adaptation to the changing environment.

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Author contributions AY: Sampled, isolated and identified yeasts from spring fluxes in Germany; performed physiological tests for strains isolated in Germany; performed phylogenetic analyses; observed teleomorph and made microphotographs; wrote the manuscript. CS: Performed physiological tests for strains isolated in Italy. BT: Sampled, isolated and identified yeasts from glaciers in Italy; improved the dataset for phylogenetic analyses; wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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