Rare and undersampled dimorphic basidiomycetes

Kachalkin, A.V. †; Turchetti, B. †; Inácio, J. †; Carvalho, C.; Mašínová, T.; Pontes, A.; Röhl, O.; Glushakova A.M.; Akulov, A.; Baldrian, P.; Begerow, D.; Buzzini, P.; Sampaio, J.P.; Yurkov A.M.*

* corresponding author: Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Inhoffenstrasse 7B, 38124 Braunschweig, Germany; andrey.yurkov@dsmz.de

† equal contribution

Affiliations:

Kachalkin, A.V.;

Lomonosov Moscow State University, 119991 Moscow, Russia

All-Russian Collection of Microorganisms (VKM), G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142290 Pushchino, Russia

Glushakova A.M.;

Lomonosov Moscow State University, 119991 Moscow, Russia

Turchetti, B.; Buzzini, P.;

Department of Agricultural, Food and Environmental Sciences & Industrial Yeasts Collection DBVPG, University of Perugia, 06121 Perugia, Italy

Inácio, J.;

School of Pharmacy and Biomolecular Sciences, University of Brighton, Lewes road, Brighton BN2 4GJ, United Kingdom

Global Health and Tropical Medicine, GHTM, Unit of Medical Microbiology, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisbon, Portugal

Carvalho, C.; Pontes, A.; Sampaio, J.P.;

UCIBIO-REQUIMTE, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

Mašínová, T.; Baldrian, P.;

Laboratory of Environmental Microbiology, Institute of Microbiology of the CAS, 14220 Praha 4, Czech Republic

Akulov, A;

V. N. Karazin Kharkiv National University, 61022 Kharkiv, Ukraine

Röhl, O.; Begerow, D.;

Geobotany, Faculty of Biology and Biotechnology, Ruhr-University Bochum, 44801 Bochum, Germany

Yurkov A.M.;

Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, 38124 Braunschweig, Germany

Abstract

The diversity of yeasts has grown rapidly as the discovery of new species has benefited from intensified sampling and largely improved identification techniques. An environmental study typically reports the isolation of yeast species, some of which are new to science. Rare species represented by a few isolates often do not result in a taxonomic description. Nucleic acid sequences from these undescribed yeasts remain in public sequence databases, often without a proper taxonomic placement. This study presents a constrained phylogenetic analysis for many rare yeasts from unpublished but publicly available DNA sequences and from studies previously conducted by the authors of this work. We demonstrate that single isolates are an important source of taxonomic findings such as including new genera and species. Independent surveys performed during the last 20 years on a large geographic scale yielded a number of single strains, which were proved to be conspecific in the phylogenetic analyses presented here. The following new species were resolved and described: *Vustinia terrea* Kachalkin, Turchetti & Yurkov gen. nov. et sp. nov., *Udeniomyces caspiensis* Kachalkin sp. nov., *Udeniomyces orazovii* Kachalkin sp. nov., *Tausonia rosea* Kachalkin sp. nov., *Itersonilia diksonensis* Kachalkin sp. nov., *Krasilnikovozyma fibulata* Glushakova & Kachalkin, *Kwoniella fici* Turchetti sp. nov., *Heterocephalacria fruticeti f.a.* Carvalho, Roehl, Yurkov & Sampaio sp. nov., *Heterocephalacria gelida f.a.* Turchetti & Kachalkin sp. nov., *Heterocephalacria hypogea f.a.* Carvalho, Roehl, Yurkov & Sampaio sp. nov., *Heterocephalacria lusitanica f.a.* Inacio, Carvalho, Roehl, Yurkov & Sampaio sp. nov., *Piskurozyma arborea* Yurkov, Kachalkin, Mašínová & Baldrian sp. nov., *Piskurozyma silvicultrix* Turchetti, Mašínová, Baldrian & Yurkov sp. nov., *Piskurozyma stramentorum* Yurkov, Mašínová & Baldrian sp. nov., *Naganishia nivalis* Turchetti sp. nov., *Yurkovia nerthusi* Yurkov & Begerow, sp. nov. In addition, two new combinations were proposed *Krasilnikovozyma curviuscula* (Babeva, Lisichkina, Reshetova & Danilevich) Yurkov, Kachalkin & Sampaio comb. nov., *Hannaella taiwanensis* (F.L. Lee & C.H. Huang) Yurkov comb. nov. The order Cyphobasidiales T. Spribille & H. Mayrhofer is rejected in favour of the older name Erythrobasidiales R. Bauer, Begerow, J.P. Sampaio, M. Weiss & Oberwinkler. Other potential novel species identified in this paper await future description. Phylogenetic placement of yet unpublished sequences is believed to facilitate species descriptions and improve classification of yeasts from environmental sequence libraries.

Keywords: 1 new genus, 17 new species, 2 combinations, yeasts, taxonomy, Tremellomycetes, Pucciniomycotina, Microbotryomycetes, Cystobasidiomycetes

Introduction

Our knowledge of a yeast stage of members of the phylum Basidiomycota dates back to the 19th century and the pioneering work of Brefeld, who observed the germination of teliospores of the corn smut *Mycosarcoma maydis* with budding yeast-like cells (reviewed by Oberwinkler 2017). Subsequent mating experiments confirmed the presence of the filamentous-yeast switch in several smuts (reviewed by Oberwinkler 2017). Later, Kluyver and van Niel (1927) pointed to the similarity between forcibly ejected buds of a red yeast belonging to the genus *Sporobolomyces* and basidiospores of basidiomycetes. The most conclusive evidence of a basidiomycete connection was the discovery of mating and a sexual state in strains of *Rhodotorula glutinis* by Banno (1963, 1967), followed by the discoveries of teleomorphs of several yeasts and description of the genera *Filobasidiella*, *Cuniculitrema* and *Bulleromyces*. A number of morphological, biochemical, ultrastructural, and physiological criteria indicated the basidiomycetous affinity of many asexual yeasts. These characters include a positive diazonium blue B reaction, urease activity, enteroblastic mode of budding, presence of ballistoconidia, red carotenoid pigments, a lamellate cell wall ultrastructure, presence of a dolipore septum (in hyphae), the biochemical composition of the cell wall, and a high GC content of genomic DNA (Boekhout et al. 2011; Kurtzman and Boekhout 2017). However, evolutionary relationships between yeasts, whether asexual or sexual, and teleomorphic filamentous taxa remained unknown. Early molecular evolutionary studies of 5S rRNA distinguished two phylogenetic lineages, Agaricomycotina and Pucciniomycotina, and these results correlated well with septal ultrastructure (reviewed by Kurtzman and Boekhout 2017; Oberwinkler 2017). Later, studies of the SSU rRNA gene revealed the third lineage of basidiomycetes, the Ustilaginomycotina.

Studies of dimorphic heterobasidiomycetes followed two different directions (reviewed by Begerow et al. 2017). One approach taken by traditional mycologists, who sampled fungi in the field and investigated them in the laboratory. From these studies we know that sexual structures of some mycoparasites (e.g. *Tremella*, *Rhynchogastrema*, *Trimorphomyces*) and plant parasites (e.g. *Microbotryum*, *Mycosarcoma*) germinate with yeast states. Another approach was undertaken by yeast researchers and included mating experiments to obtain teleomorphic states on laboratory media (e.g. *Curvibasidium*, *Leucosporidium*, *Papiliotrema*, and *Rhodosporidium*) and the subsequent description of the relevant morphological characters, including basidial and hyphal morphology. It transpired that species commonly considered as yeasts form a sexual cycle *ex situ* and display features previously described as an adaptation to a parasitic lifestyle, for example appressoria, colacosomes and haustoria.

Yeasts were among the first fungi for which nucleic acids were sequenced and phylogenetically analysed, and their presence in all three subphyla in Basidiomycota was demonstrated by many molecular phylogenetic studies (reviewed by Kurtzman and Boekhout 2017). Pioneering studies from the 1980s showed that sexual and asexual taxa are intermixed in many clades suggesting that morphological dimorphism is a common feature among basidiomycetous yeasts. Yeasts were also the first group of fungi subjected to DNA-barcoding using ribosomal gene fragments such as LSU rRNA gene (Fell et al. 1995, 2000; Begerow et al. 1997; Fonseca et al. 2000; Sampaio 2004) and ITS (Scorzetti et al. 2002).

These studies provided a solid background for fast and reliable identification of yeasts and facilitated the discovery of new species. Although the number of known yeast species in the Agaricomycotina and Pucciniomycotina was growing rapidly, the number of available sequences of teleomorphic taxa remained low (Fell et al. 2000; Millanes et al. 2011; Liu et al. 2015a; Wang et al. 2015b). Recent changes in fungal taxonomic rules have resulted in a unified classification system for naming sexual and asexual taxa, including lineages containing yeasts. Consequently, large polymorphic and polyphyletic anamorphic genera like *Bullera*, *Cryptococcus*, *Rhodotorula*, *Sporobolomyces* and *Trichosporon* were reclassified, which resulted in 35 new genera and 296 new combinations (Liu et al. 2015a; Wang et al. 2015b). Similarly, several teleomorphic species of *Cystobasidium*, *Tremella* and *Syzygospora* distantly related to clades that contain type species were reclassified (Liu et al. 2015a; Wang et al. 2015b; Millanes et al. 2016; Spirin et al. 2018). These studies demonstrated that undersampling and the lack of reference sequences hamper both taxonomical works and biodiversity assessments. It has been also shown that the lack of reference sequences and inaccessibility of material (culture or specimen) can result in taxonomic redundancy, as exemplified by the yeast genus *Bandoniozyma* (Tremellomycetes), which coincided with *Rhynchogastrema*, an earlier discovered, but not sequenced, filamentous genus (Liu et al. 2015a).

To date, most of our knowledge of diversity in Cystobasidiomycetes, Microbotryomycetes and Tremellomycetes is derived from studies reporting the discovery of new yeast species (Liu et al. 2015; Wang et al. 2015; Kurtzman and Boekhout 2017). Yeasts are isolated worldwide from a multitude of habitats and substrates (Peter et al. 2017). Many of them remain, however, undescribed because of an unclear phylogenetic placement or because they are known from a limited number of isolates (e.g., Seifert and Rossman 2010). Constrained phylogenetic analyses of LSU rRNA proved their usefulness and identified potential new species clustering in already recognized (sometimes monotypic) lineages (Liu et al. 2015a; Wang et al. 2015b). A few from these potential new species have been described recently (e.g., Yurkov et al. 2016a), and others still await a description in spite of having been discovered for a long time. Moreover, every new biodiversity study adds a few potential novel yeasts to the already existing pool of unnamed and unplaced species. Sometimes the number of potential novel yeasts reported in a study can be as large as 25-30% of the total diversity (e.g., Yurkov et al. 2012a, 2016b; Mašínová et al. 2017a, 2017b). Thus, it becomes challenging for researchers to maintain an overview of the ever-growing diversity of undescribed yeasts.

The aim of this paper is to provide updated phylogenies of yeasts in Agaricomycotina and Pucciniomycotina, and identify new clades in these groups. The work includes previously isolated but yet undescribed species and more recent isolates obtained from a number of studies performed by us. Many of these species belong to the so-called heterobasidiomycetes, a group of fungi extensively studied by Franz Oberwinkler and his collaborators, including Robert Bauer and Robert Bandoni. Also, several strains from the former collection of the University of Tübingen served a reference for teleomorphic taxa for which no sequence was available. Several new species and one new genus identified in this study are described.

Material and Methods

Sampling and isolation of yeasts have been independently performed in previous studies. Isolations from soils in Czech Republic, Germany and Portugal followed protocols described by Mašínová et al. (2017b) and Yurkov et al. (2012a, 2016b), respectively. Strains from Russia were obtained using sampling and isolation protocols described by Glushakova et al. (2015). Yeasts on plant material in Portugal were studied by Inácio et al. (2002) and Inácio (2003). Isolations from glacial sampling and plant materials in Italy followed protocols described in Turchetti et al. (2013) and Franca et al. (2016).

Sequences of D1/D2 domains of the 26S rRNA gene (LSU) were aligned into multiple sequence alignments, previously used by Liu et al. (2015a) and Wang et al. (2015b), by utilising MAFFT online service (version 7) and *--add* and *--keeplength* functions (Katoh et al. 2017). New sequences were either obtained from public databases or produced in previous studies. Resulting alignments contained a total of 860 sequences, 301 for Pucciniomycotina and 559 for Agaricomycotina. Trees were constructed with raxmlGUI 1.5b software using the maximum-likelihood algorithm and GTRGAMMA (GTR substitution model with gamma-distributed rate heterogeneity) model with 100 bootstrap replicates; topological constrains were enforced as was described previously by Liu et al. (2015a) and Wang et al. (2015b). A parsimony network was constructed from aligned LSU and ITS sequences with the program TCS 1.21 (Clement et al. 2000) using a 95% connection limit and gaps treated as missing data. A fragment of the gene encoding translation elongation factor 1 alpha (*TEF1*) was amplified and sequenced following the previously described protocols (Yurkov et al. 2015a, 2015b; Spirin et al. 2018). Multiple sequence alignments were performed with the genomic sequences using online version of MAFFT algorithm (Katoh et al. 2017). Phylogenetic relationships were inferred from a concatenated dataset of the ITS region, LSU rRNA and *TEF1* genes as described above.

Assimilation tests were performed on solid and in liquid media following the procedures described by Kurtzman et al. (2011), with API 50CH test strips (bioMérieux) and YT and FF MicroPlates (Biolog) as described previously (Yurkov et al. 2017; Mašínová et al. 2018). For observing micro-morphology, cultures were grown at 10-22°C on YM, PD, GPY and 0.5% glucose-YNB agars and studied with phase-contrast optics.

Results and discussion

Phylogenetic analyses included 860 sequences of which 185 were not studied by Liu et al. (2015a) or Wang et al. (2015b). The overall topology was consistent with the two previous constrained LSU analyses (Liu et al. 2015a; Wang et al. 2015b), although several lineages were enlarged with potential novel species (Figs. 1, 2). We discuss some of these clades below.

Tremellomycetes

Cystofilobasidiales

The order Cystofilobasidiales comprises two families, Mrakiaceae and Cystofilobasidiaceae (Liu et al. 2015a). The study of Liu et al. (2015a) indicated a number of sequences corresponding to potential new species in the genera *Itersonilia*, *Krasilnikovozyma*, *Tausonia* and *Udeniomyces*. Also, two novel *Cystofilobasidium* species were recently described from Mediterranean soils (Pontes et al. 2017). Three yeasts isolated from cold soils in Russia (Altay region), Italy (Alps) and Kyrgyzstan (Byshkek) showed highly similar sequences and were placed close to the genus *Krasilnikovozyma* (Fig. 3). Our phylogenetic analyses based on LSU, ITS and *TEF1* suggest these yeasts are likely to represent a new genus in Mrakiaceae (see Taxonomy below).

The genus *Krasilnikovozyma* was proposed by Liu et al. (2015a) to accommodate members of the so-called *Cryptococcus huempii* clade, which also included the species *Mrakia curviuscula* and *Cryptococcus tahquamenonensis*. *C. huempii* and *M. curviuscula* were sequenced from the material available at the CBS culture collection (strains CBS 8168 and CBS 9136, respectively) and showed identical D1/D2 sequences (GenBank AF189844 and EF118826, respectively) suggesting these yeasts belong to the same species. ITS sequence of *Mrakia curviuscula* strain CBS 9136 was first published by Liu et al. (2015b). Because both LSU (see above) and ITS (GenBank KY103891 and KY103892, respectively) sequences of the two species were identical, *Mrakia curviuscula* was considered to be a taxonomic synonym of *Krasilnikovozyma huempii* by Liu et al. (2015a, 2015b).

Cryptococcus tahquamenonensis (presently *Krasilnikovozyma tahquamenonensis*) was described based on a single strain from soil; its LSU sequence (KM408125) shows 98% similarity to the type strain of *Cryptococcus huempii* (AF189844) and differs in 9 substitutions (including two N base calls in and 1 gap in KM408125 using GenBank Blastn). The similarity of the ITS sequences of the two species is 92% (GenBank KM384610 and AF444322), thus allowing a proper differentiation of these yeasts with either LSU or ITS sequencing.

The type material of *Mrakia curviuscula* strain Oz-358 was preserved in the yeast collection of the Lomonosov Moscow State University as KBP Y-3618 (holotype) and deposited (ex-type) in the VKM collection as VKM Y-2953 (Babjeva et al. 2002). The original culture Oz-358, from which the holotype Oz-358 was derived, was revived in 2003 and sub-cultured, following a request from the Portuguese Yeast Culture Collection. The progeny of Oz-358 was preserved in and later transferred to PYCC as PYCC 5836. Sequencing, performed independently, of cultures derived from the type material, i.e. VKM Y-2953 (GenBank MK244628) and PYCC 5836 (GenBank MF372143, MF372124), demonstrated that the two strains share identical LSU and ITS sequences, which showed high similarity to sequences of the type strain of *Krasilnikovozyma* (*Cryptococcus*) *tahquamenonensis* (ITS: KM384610, LSU: KM408125). Specifically, comparison of ITS sequences showed 3 mismatches in ITS2, corresponding to 1 base pair difference and two gaps. Our results suggest that the presumptive ex-type CBS 9136 is not identical to two other ex-types VKM Y-2953 and PYCC 5836. In

our opinion, VKM Y-2953 and PYCC 5836 are the correct ex-type cultures, whereas CBS 9136 is a strain of *K. huempii*. Sequencing of ex-types VKM Y-2953 and PYCC 5836 suggests that *M. curviuscula* is conspecific with *K. tahquamenonensis*. However, *Mrakia curviuscula* Babeva, Lisichkina, Reshetova & Danilevich (2002) is older than *Cryptococcus tahquamenonensis* Q.M. Wang, A.B. Hulfachor, K. Sylvester & C.T. Hittinger (2015) and, thus, has a taxonomic priority. Therefore, we resurrect and recombine *Mrakia curviuscula* Babeva, Lisichkina, Reshetova & Danilevich (2002) as *Krasilnikovozyma curviuscula* comb. nov. and put *Krasilnikovozyma tahquamenonensis* (Q.M. Wang, A.B. Hulfachor, K. Sylvester & C.T. Hittinger) A.M. Yurkov (2015) in taxonomic synonymy with the former (see Taxonomy).

A GenBank search resulted in a number of sequences labelled as *Mrakia frigida* and *Mrakia gelida*. Many of these strains have ITS sequences that differ from those of type strains of *M. frigida* and *M. gelida*. Although nucleotide sequences of the ITS region were successfully used to delimit species in the genus *Mrakia*, these results suggest that additional genes should be used in the future to reassess the most useful criteria for species delimitations in this genus.

Tremellales

Family Bulleraceae. A single-species lineage represented by *Cryptococcus mujuensis* in a 7-gene phylogeny (Liu et al. 2015b) was enlarged with two more species in a LSU-phylogeny (Liu et al. 2015a). These tree species were transferred to the newly erected genus *Fonsecazyma* (Liu et al. 2015a). This study also indicated that this clade contains four potential new species. Yeasts of this genus have been isolated from diverse regions mostly from plant material (Herzberg et al. 2002; Inácio 2003; Mittelbach et al. 2016; Sylvester et al. 2015). The *Tremella* clade I (sensu Millanes et al. 2011) comprises not yet re-classified species of the genera *Tremella* and *Sirobasidium*, some of which are also known from living cultures. We identified two more cultures in public collections, namely a yet undescribed *Tremella* species represented by two cultures, DSM 104578 (Germany, FO 24396) and NBRC 32520 (Japan). Another species in this clade, closely related to *Cryptococcus cuniculi pro. temp.*, is represented by strain KBP Y-5716 (MH697756). The genus *Pseudotremella* was proposed to accommodate the well-supported clade including *T. moriformis* by Liu et al. (2015a). These authors also reported some inconsistency between sequences of *T. indecorata*, which clustered in *Pseudotremella* and *Tremella* clade I. In addition to the aforementioned inconsistency, we found some heterogeneity between sequences of specimens identified as *P. moriformis* (Fig. 1A). Specifically, *Tremella moriformis* specimen UBC F13838 (= RB284b, sometimes wrongly cited as RB2846 and RJB2846), collected by Wells from UC Davis (USA), was sequenced by Chen in Tübingen, Germany (GenBank AF042426, AF042244). Sequences of this specimen were highly similar to another recently collected *Tremella* specimen CWU (MYC) 136 from Kharkiv (Ukraine) and a yeast isolate from Italy (DBVPG 10729, GenBank MK634540) (Fig. 1A). Phylogenetic analyses demonstrated that they are placed in the genus *Pseudotremella* but very distant from the reference specimen of *P. moriformis* UBC F13868 (= CBS 7810, GenBank AF075493, AF444331) used by Liu et al.

(2015a). Another sequence representing a potentially conspecific isolate was found in GenBank (MG190052); this yeast was isolated from an insect frass on an olive tree in South Africa.

Family Carcinomycetaceae. The mycoparasitic genus *Carcinomyces* (Oberwinkler & Bandoni 1982) presently comprises two sexual and one asexual species. Our analysis identified another, yet undescribed, species in this genus isolated from an insect gallery in the USA (Fig. 1A).

Family Cryptococcaceae. Our analyses revealed several potential new species in the genera *Kwoniella* and *Cryptococcus* from soil (Yurkov et al. 2016b, Mašínová et al. 2018), plant (Glushakova and Kachalkin 2017) and insect sources (Fig. 1).

Family Phaeotremellaceae. *Gelidatrema*, a monotypic genus proposed by Liu et al. (2015a), was expanded with an additional in our phylogenetic analysis (Fig. 1A). The recently described from a microbial mat in the Canadian High Arctic *Gelidatrema psychrophila* was previously observed by Turchetti et al. (2013). This culture, though the sequence was available for in Genbank (KC433781), was not considered in the description by Tsuji et al. (2018).

Family Sirobasidiaceae. The genus *Fibulobasidium* is distinguished from closely related *Sirobasidium magnum*, based on its unusual basidium development (Bandoni 1979). The genus received strong support in recent phylogenetic analyses (Liu et al. 2015a). *Sirobasidium magnum* (Liu et al. 2015a) and *Sirobasidium japonicum* (Liu et al. 2015b) were placed sister to the genus *Fibulobasidium* (Fig. 1B). In the present study, we identified a new closely related sub-clade represented by seven yeasts (Fig. 1B). These strains originate from diverse habitats and regions, all of which are characterized by a substantial water limitation, i.e. from Mediterranean soils, Greenland sandstone, moss-dominated desert soil crusts, grape berries and floral nectar (Cadez et al. 2010; Selbman et al. 2014; Mittelbach et al. 2015; Yurkov et al. 2016b). A sequence of an unspecified *Sirobasidium* sp. (GenBank LC203429) formed a well-supported cluster with the genus *Fibulobasidium* (Fig. 1B), while *S. intermedium* is placed in *Tremella* clade I (Fig. 1A). Delimitation of the two genera will be difficult in the future because the sequence of the type species of the genus *Sirobasidium*, *S. rubrofuscum* (syn. *S. sanguineum*, Dämon and Hausknecht 2002), is not yet available.

Family Tremellaceae. The genus *Tremella* was re-defined to include only members of the *T. mesenterica* clade. A new lineage consisting of three isolates from soils and insect-related sources was identified close to the *Tremella* clade, though statistical support for this placement was low (Fig. 1A).

Family Trimorphomycetaceae. The monotypic genus *Sugitazyma* was proposed to accommodate *Bullera miyagiana* (Liu et al. 2015a). Our results show that several yeasts were placed close to *Sugitazyma miyagiana* and *Tremella parmeliarum*, though statistical support for this clustering was low (Fig. 1B). These yeasts were isolated from soils (Czech Republic, Portugal), an insect (Bulgaria) and a plant (Taiwan). Two strains independently isolated in Serra da Arrábida, Portugal (Inácio 2003; Yurkov et al. 2016b) represent potentially new

species, which were placed in the genus *Saitozyma*, though statistical support for this placement was low (Fig. 1B).

Filobasidiales

After recent re-classification of *Cryptococcus* species in Filobasidiales, yeasts in this lineage are accommodated in two existing (i.e. *Filobasidium*, *Syzygospora*), three new (i.e. *Goffeauzyma*, *Piskurozyma*, *Solicoccozyma*) and two resurrected (i.e. *Heterocephalacria*, *Naganishia*) genera (Liu et al. 2015a).

Family Filobasidiaceae. The genus *Filobasidium* has been restricted to the clades Magnus and Floriforme (sensu Fonseca et al. 2000, 2011; Scorzetti et al. 2002), which contain the type species *F. floriforme* (Liu et al. 2015a). Similarly, Liu et al. (2015a) resurrected the genus *Naganishia*, with the type species *N. globosa*, to accommodate members of the Albidus clade of the order Filobasidiales (sensu Fonseca et al. 2000, 2011; Scorzetti et al. 2002). Sixteen species are presently accepted in the genus considering the latest member, *Naganishia qatarensis*, isolated from a hypersaline marine environment (Fotedar et al. 2018). Our analysis revealed a new member of the genus isolated from cold environments. The study by Liu et al. (2015a) revealed four potential new species in the genus *Heterocephalacria*. Our analyses expand the number of yet undescribed species in this genus to eight (Figs. 1, 4). According to the available literature and GenBank records, these species were isolated from Mediterranean soils and plants (Inácio 2003; Yurkov et al. 2016b), supraglacial sediments (Turchetti et al. 2013), boreal swamp plants, tundra plants, and New Zealand soil.

Family Piskurozymaceae. The genus *Piskurozyma* was proposed to accommodate the well-supported Cylindricus clade of the order Filobasidiales (Scorzetti et al. 2002; Fonseca et al. 2011), a single-species lineage *F. capsuligenum*, and the mycoparasite *Syzygospora sorana*. The analysis performed by Liu et al. (2015a) showed two well-supported sub-clades in the genus, one comprising members of the Cylindricus clade and another one represented by *P. fildesensis* and two undescribed species, later described as *P. yama* and *P. tuonelana* (Yurkov et al. 2016b). Our study substantially expanded the size of the genus with seven potentially new species as suggested by phylogenetic analyses (Figs. 1, 5).

Pucciniomycotina

Similarly to studies in Tremellomycetes, recent taxonomic changes in yeasts belonging to Cystobasidiomycetes and Microbotryomycetes were directed towards the re-classification of large polyphyletic genera such as *Bensingtonia*, *Rhodotorula*, and *Sporobolomyces* (Yurkov et al. 2015; Wang et al. 2015b).

In the Cystobasidiales, sequencing and subsequent phylogenetic analyses showed that the parasitic lichen-inhabiting teleomorphic genus *Cystobasidium* is polyphyletic (Yurkov et al. 2015b; Millanes et al. 2016) and distributed between two clades. The first clade, containing the type species *C. fimentarium*, also includes numerous yeast species from the so-called *Rhodotorula minuta* clade (Yurkov et al. 2015b). The second clade, with *C. hypogymniicola*

and *C. usneaicola*, is placed outside Cystobasidiales and to *Cyrenella elegans* in the Erythrobasidiales (Fig. 2). It is important to document that *Cyrenella elegans* was not included in the phylogenetic analyses that addressed the taxonomic position of the genus *Cyphobasidium* (Millanes et al. 2016; Spribille et al. 2016). Below we discuss taxonomy of this genus in more detail. Recent studies expanded *Cystobasidium* and the closely genus *Occultifur* with three and four new yeast species, respectively (Wang et al. 2015b; Šibanc et al. 2018; Turchetti et al. 2018).

In the Microbotryomycetes, a number of new genera and species were described since the re-classification of *Bensingtonia*, *Rhodotorula*, and *Sporobolomyces* by Wang et al. (2015b). Two genera, *Libkindia* and *Yurkovia*, were proposed to accommodate new yeasts from forest soils in the Czech Republic (Mašínová et al. 2017b). The genus *Heitmania* was recently described to include three novel yeasts from plants in China (Liu et al. 2017). Despite being based on multi-gene analyses, the taxonomic position of these genera remained unclear and they were placed in the Microbotryomycetes as '*incertae sedis*'. Wang et al. (2015b) noted that using the signal of the LSU rRNA gene alone is not sufficient to resolve the high-level phylogenetic relationships in Microbotryomycetes. Our analyses indicated two loosely placed clusters in Microbotryomycetes close to genera *Curvibasidium*, *Pseudoleucosporidium* and *Sampaiozyma* (Fig. 2). These weakly supported clades contained yeasts isolated from Germany (forest soil, Yurkov et al. 2016a), Portugal (plant, Inacio 2003) and Russia. A robust multi-gene analysis is required to resolve the phylogenetic relationships and taxonomic position of these yeasts. The constrained LSU rRNA gene phylogenetic analysis performed by Wang et al. (2015 b) failed to resolve placement of several psychrophilic yeasts, including *Rhodotorula svalbardensis* and a few undescribed species. In agreement with this observation, our results showed the unclear placement of CRUB 1733 (GenBank FJ841888) and DBVPG 10048 (GenBank KC433880) close to the filamentous fungus *Camptobasidium* (Fig. 2). The analysis also suggested a potential new species in this yet monotypic genus represented by an antarctic yeast, CBS 8941 (Fig. 2). Two species of *Hamamotoa* and one species of *Colacogloea*, *Leucosporidium* and *Slooffia* were described recently from soils in Europe (Yurkov et al. 2016a; Mašínová et al. 2017b). A few more novelties in genera *Bannozyma* (KY558342, Mašínová et al. 2017b, 2018; this study), *Chrysozyma* (AB552933, KX067789, Endoh et al. 2011; Wang et al. 2015b), *Colacogloea* (EU002850, FN428953, Kachalkin et al. 2008; Wang et al. 2015b), *Fellozyma* (FN868158, Wang et al. 2015b), *Hamamotoa* (AM039679, KU609479, AB462346, EF585181, Wang et al. 2015b; this study), *Oberwinklerozyma* (FN401525, Wang et al. 2015b; Yurkov et al. 2016b), *Slooffia* (AF444728, DQ531946, EF450537, Sampaio et al. 2011; Wang et al. 2015b) and *Yurkovia* (FN428970, Mašínová et al. 2017b) were reported and analysed in previous studies and await a formal description. For the first time our analysis also revealed the diversity of the genus *Yunzhangia* (previously known from two species only) suggesting three novel species close to *Yunzhangia sonckii* (Fig. 2).

Taxonomy

Tremellomycetes, Cystofilobasidiales

Description of *Vustinia* Kachalkin, Turchetti & Yurkov, gen. nov. (MB 829115)

Etymology: the genus is named in honor of the Russian zymologist Dr. Michael Vustin (VKPM culture collection, State Research Institute of Genetics and Selection of Industrial Microorganisms, Russia) for his contributions to the study of soil yeasts and yeasts producing carotenoid pigments.

This monotypic genus is proposed to accommodate a new species represented by two isolates, which are phylogenetically close to the genus *Krasilnikovozyma* in a well-supported clade (Fig. 3).

Phylogenetic position: Fungi, Dikarya, Basidiomycota, Agaricomycotina, Tremellomycetes, Cystofilobasidiales, Mrakiaceae.

Basidiocarps absent. True hyphae and pseudohyphae not observed. Sexual reproduction not observed. Budding cells present. Budding is on the wide basis, polar with sympodial proliferation. Ballistoconidia absent. Arthroconidia absent. Urea hydrolysis and Diazonium Blue B reaction are positive. Nitrate is utilized.

Type species: *Vustinia terrae* Kachalkin, Turchetti & Yurkov, sp. nov. (MB 829116)

Notes: New species of the genus *Vustinia* can be distinguished from known *Krasilnikovozyma* species by orange-colored pigmentation, which is an important character in Tremellomycetes. This is the third pigmented genus in Cystofilobasidiales after *Cystofilobasidium* and *Phaffia*. The genus *Vustinia* was distant from known *Krasilnikovozyma* species in a concatenated ITS-LSU and ITS-LSU-*TEF1* phylogenies (Figure 3).

Description of *Vustinia terrae* Kachalkin, Turchetti & Yurkov, sp. nov. (MB 829116)

Etymology: The species epithet *terrae* is derived from terra (L. gen. sing. f., n., of the earth) and refers to the original substrate of isolation, namely soil.

After 1 week at 20 °C on PD, GPY and 2% Glucose YNB agars, streak is orange, butyrous with a smooth glistening surface. Margins are smooth and entire. Cells are ellipsoidal to cylindrical 6–10 × 2–3 µm in size, occurring singly or in pairs, and proliferating by polar budding (Figure 7 a). Lipid-like bodies can be present in cells. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, D-ribose (weak), D-xylose, L-arabinose, D-arabinose (weak and delayed), L-rhamnose, sucrose, maltose (weak), trehalose, cellobiose, salicin (weak), arbutin (delay for some strains), melibiose (variable, delayed), lactose, raffinose (variable), melezitose (variable), soluble starch (variable, delayed), glycerol, erythritol, ribitol, xylitol (delay), D-mannitol, D-glucitol, 2-keto-D-gluconate, 5-keto-D-gluconate, D-gluconate, D-glucoronate (variable), succinate (delayed) and citrate (variable). No growth on D-glucosamine, methyl-alpha-D-glucoside,

inulin, inositol, methanol, ethanol, galactitol, DL-lactate, L-malate and ethyl acetate. Utilization of nitrogen sources: positive growth on potassium nitrate, sodium nitrite, lysine and cadaverine. No growth in the presence of 10% sodium chloride, 50% D-glucose and 0.01% cycloheximide. Growth on vitamin-free medium is weak. 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) rRNA, and partial sequence of *TEF1* deposited in NCBI/EMBL (GenBank) under the accession numbers: MH685196, MH697747 and LS992156, respectively.

Deposits: holotype, strain K833b isolated from mountain soil collected in June 2015 near Kosh-Agach village, Republic of Altay, Russia, preserved in a metabolically inactive state in the KBP collection of Department of Soil Biology, Faculty of Soil Sciences, Lomonosov Moscow State University (WDCM 1173) as KBP Y-5245^{T}. Ex-type cultures are deposited in the All-Russian Collection of Microorganisms (VKM), Pushchino, Russia (VKM Y-3018), the Russian National Collection of Industrial Microorganisms (VKPM), Moscow, Russia (VKPM Y-4321) and the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 105056).

Strains studied: K833b (= KBP Y-5245^{T}); paratypes: DBVPG 10597 from forest soil collected in Kleiner Priol (Montiggl), South Tyrol, Alps, Italy (GenBank KU745368, KU745306, MH986600) and KBP Y-5336 (= VKM Y-3019) from soil collected in a park in Bishkek, Kyrgyzstan (GenBank MH697748, LS992158).

Notes: New species of the genus *Vustinia* can be distinguished from known *Krasilnikovozyma* species by orange-colored pigmentation, positive growth on glycerol and erythritol and inability to grow on D-glucosamine.

Description of *Udeniomyces caspiensis* Kachalkin, sp. nov. (MB 829119)

Etymology: The species epithet *caspiensis* is derived from Latin Caspius (L. adj. m., Caspian) and refers to the region, where the species was isolated.

After 1 week at 20 °C on PD, GPY and 2% Glucose YNB agars, streak is pinkish-white, soft with a dull to semi-shiny surface. Margins are smooth and entire. Cells are ovoid to ellipsoidal, 6–11 × 4–5 µm in size, occurring singly or in pairs, and proliferating by polar budding on a broad base (Figure 7 b). Budding scars may be present. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-ribose (weak), L-arabinose, D-arabinose (weak), sucrose, maltose, trehalose, cellobiose, salicin (weak), arbutin (delay for some strains), raffinose, melezitose, glycerol, D-mannitol (weak), D-glucitol (weak), D-glucoronate (weak), D-gluconate (weak), 2-keto-D-gluconate (weak) and 5-keto-D-gluconate. No growth on D-galactose, L-sorbose, D-glucosamine, D-xylose, L-rhamnose, methyl-alpha-D-glucoside, melibiose, lactose, inulin, soluble starch, ethanol, erythritol, ribitol, galactitol, inositol, DL-lactate, succinate and citrate. Utilization of nitrogen sources: positive growth on

potassium nitrate and lysine. No growth in the presence of 10% sodium chloride and 50% D-glucose. Growth on vitamin-free medium is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 23 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession number MH697745.

Deposits: holotype, strain K686-1b isolated from leaves of *Camphorosma sp*. (Amaranthaceae) collected in May 2014 at the Djanybek Research Station of Institute of Forest Science RAS, Volgograd Oblast, Russia, preserved in a metabolically inactive state in the KBP collection of Department of Soil Biology, Faculty of Soil Sciences, Lomonosov Moscow State University (WDCM 1173) as KBP Y-5036^{T}. Ex-type cultures are deposited in the All-Russian Collection of Microorganisms (VKM), Pushchino, Russia (VKM Y-3016), the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 15357) and the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 106747).

Description of *Udeniomyces orazovii* Kachalkin, sp. nov. (MB 829120)

Etymology: The species epithet *orazovii* (NL. gen. sing. masc. n.) is in honor of the mycologist Prof. Hodjanazar Orazov (Institute of Botany of the Academy of Sciences of Turkmenistan) for his contributions to the study of fungi in arid soils. Prof. Orazov also provided the sample from which this species was isolated.

After 1 week at 20 °C on PD, GPY and 2% Glucose YNB agars, streak is white, butyrous with a smooth glistening surface. Margins are undulating with some outgrowth in a medium underneath the streak. Cells are sub-globose, ellipsoidal, elongate to cylindrical, 6–15 × 3–6 µm in size, occurring singly, in pairs or chains, and proliferating by polar budding on a broad base (Figure 7 d). Ballistoconidia were not observed. Pseudohyphae and short true hyphae occur. Terminal, lateral and intercalary spherical chlamydospores, 18–20 µm in size, with refractile granules from a single cell on true hyphae formed on PDA and 2% Glucose YNB agar after 8-10 d at 20 °C (Figure 7 e, f). Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, L-sorbose (variable), D-glucosamine (variable), D-ribose (weak), D-xylose (variable), L-arabinose, D-arabinose (weak), sucrose, maltose, trehalose, cellobiose, salicin, arbutin, lactose (variable), raffinose, melezitose, soluble starch (weak), glycerol (weak), ribitol (variable), D-glucitol, D-mannitol, inositol (weak), ethanol, 2-keto-D-gluconate, 5-keto-D-gluconate, D-glucoronate (weak), succinate (weak) and citrate (weak). No growth on D-galactose, L-rhamnose, methyl-alpha-D-glucoside, melibiose, inulin, erythritol, galactitol, D-gluconate and DL-lactate. Utilization of nitrogen sources: positive growth on potassium nitrate and lysine. No growth in the presence of 50% D-glucose. Growth on vitamin-free medium is positive. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 24 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA, and partial sequence of *TEF1* deposited in NCBI/EMBL (GenBank) under the accession numbers: MH734791, MH697744 and LS998026, respectively.

Deposits: holotype, strain K515b isolated from leaves of *Halocharis hispida* (Amaranthaceae) collected in May 2013 near Babadurmaz village, Turkmenistan (approx. coordinates 37.65 N, 59.15 E), preserved in a metabolically inactive state in the KBP collection of Department of Soil Biology, Faculty of Soil Sciences, Lomonosov Moscow State University (WDCM 1173) as KBP Y-4766^{T}. Ex-type cultures are deposited in the All-Russian Collection of Microorganisms (VKM), Pushchino, Russia (VKM Y-3014), the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS xxxxx) and the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 100168).

Strains studied: K515b (= KBP Y-4766^{T}); paratype KBP Y-4704 (GenBank MH697743) from soil near Babadurmaz village, Turkmenistan.

Description of *Tausonia rosea* Kachalkin, sp. nov. (MB 829122)

Etymology: The species epithet *rosea* is derived from roseus (L. adj. f., pink) and refers to the culture color.

After 2 weeks at 20 °C on PD, GPY and 2% Glucose YNB agars, streak is light pink, butyrous, with a dull and smooth surface. Margins are smooth and entire. Cells are globose, sub-globose and ovoid, 7–8 × 4.5–7 μm in size, occurring singly or in pairs, and proliferating by polar budding on a broad base (Figure 7 c). Budding scars may be present. Spherical chlamydospore-like cells, 8–10 μm in size, may be present in older culture. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, L-arabinose, D-arabinose, sucrose, maltose, trehalose, cellobiose (weak), salicin, arbutin, raffinose, melezitose, soluble starch (weak), glycerol (weak), ribitol (weak), D-glucitol, D-mannitol, 2-keto-D-gluconate, 5-keto-D-gluconate (weak), D-glucoronate (weak) and ethanol (weak). No growth on D-galactose, L-sorbose, D-glucosamine, D-ribose, D-xylose, L-rhamnose, methyl-alpha-D-glucoside, melibiose, lactose, inulin, erythritol, galactitol, inositol, D-gluconate, DL-lactate, succinate and citrate. Utilization of nitrogen sources: positive growth on potassium nitrate and lysine. No growth in the presence of 10% sodium chloride and 50% D-glucose. Growth on vitamin-free medium is positive. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 23 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA, and partial sequence of *TEF1* deposited in NCBI/EMBL (GenBank) under the accession numbers: LN871177 and LS998027, respectively.

Deposits: holotype, strain K744-1b isolated from leaves of *Salicornia sp*. (Amaranthaceae) collected in August 2014 on the shore of lake Elton, Volgograd Oblast, Russia, (approx.

coordinates 49.15 N, 46.68 E), preserved in a metabolically inactive state in the KBP collection of Department of Soil Biology, Faculty of Soil Sciences, Lomonosov Moscow State University (WDCM 1173) as KBP Y-4584^{T}. Ex-type cultures are deposited in the All-Russian Collection of Microorganisms (VKM), Pushchino, Russia (VKM Y-3007), the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS xxxxx) and the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 100302).

Description of *Itersonilia diksonensis* Kachalkin, sp. nov. (MB 829123)

Etymology: The species epithet *diksonensis* (NL. gen. sing. masc. n.) is derived from Dikson, the urban-type settlement on Russia's Arctic Ocean coast and refers to the region where the species was isolated.

After 2 weeks at 20 °C on GPY and 2% Glucose YNB agars, streak is salmon-pink, butyrous, with a glossy wrinkled surface. Margins are crenulate and fringed with tiny colonies resulting from discharged ballistoconidia. Cells are ellipsoidal, sub-globose to ovoid, 6–9 × 4.5–6 μm in size, occurring singly or in pairs, and proliferating by polar and lateral budding (Figure 7 g). Ballistoconidia are lunate, 5.5–6 × 3.5–4 μm size, formed abundantly on cells with 1–2 occasionally sympodially branching sterigmata (Figure 7 h). After 2 weeks at 20 °C on PDA, streak is light salmon-pink, mucoid, raised with a smooth and glossy surface. Margin is entire. Cells are ovoid, ellipsoidal or elongate, 6–12 × 5–6 μm in size, occur singly, in pairs or chains, and proliferating by polar budding. Rare ballistoconidia formed at the end of 10–30 μm long sterigmata. Pseudohyphae and true hyphae were not observed on any media. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, L-sorbose, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, trehalose, cellobiose, salicin, arbutin, raffinose, melezitose (weak), soluble starch (weak), glycerol, ribitol, D-glucitol, D-mannitol, 5-keto-D-gluconate (weak), D-gluconate, D-glucoronate (weak), DL-lactate (weak), succinate, citrate and ethanol. No growth on D-galactose, D-glucosamine, maltose, methyl-alpha-D-glucoside, melibiose, lactose, inulin, erythritol, galactitol and inositol. Utilization of nitrogen sources: positive growth on potassium nitrate and lysine. No growth in the presence of 50% D-glucose. Growth on vitamin-free medium is positive. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are not produced. Maximum growth temperature: 36 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA, and partial sequence of *TEF1* deposited in NCBI/EMBL (GenBank) under the accession numbers: MH697741, MH734790 and LS998025, respectively.

Deposits: holotype, strain K343b isolated from a mixed sample of leaves of flowering arctic plants collected in July 2012 near the settlement Dikson, Taymyr peninsular, Krasnoyarsk Krai, Russia, preserved in a metabolically inactive state in the KBP collection of Department of Soil Biology, Faculty of Soil Sciences, Lomonosov Moscow State University (WDCM 1173) as KBP Y-4765^{T}. Ex-type cultures are deposited in the All-Russian Collection of

Microorganisms (VKM), Pushchino, Russia (VKM Y-3013), the Russian National Collection of Industrial Microorganisms (VKPM), Moscow, Russia (VKPM Y-4017), the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS xxxxx) and the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 100169).

Description of *Krasilnikovozyma fibulata* Glushakova & Kachalkin sp. nov. (MB 829124)

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 2 weeks at 20 °C on PD, GPY and 2% Glucose YNB agars, streak is cream colored, butyrous with a smooth glistening surface and hyphe produced at the margin. Cells are fusiform, 6–10 × 2–3 μm in size, and occurring singly or in pairs, and proliferating by polar budding (Figure 7 i). Ballistoconidia were not observed. Pseudohyphae and true hyphae with clamp connections occur. Spherical teliospores, 15–16 μm in size, are produced laterally or terminally (Figure 7 j, k). No teliospore germination was observed and basidia morphology remains unknown.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, D-glucosamine, D-xylose, L-rhamnose, sucrose, trehalose, cellobiose, salicin (weak), arbutin, lactose, ethanol (weak), ribitol (weak), D-glucitol, 2-keto-D-gluconate, 5-keto-D-gluconate, citrate (weak) succinate (weak) and ethanol. No growth on L-sorbose, D-ribose, L-arabinose, D-arabinose, maltose, methyl-alpha-D-glucoside, melibiose, raffinose, melezitose, inulin, soluble starch, glycerol, erythritol, D-mannitol, galactitol, inositol, D-gluconate, D-glucoronate and DL-lactate. Utilization of nitrogen sources: positive growth on potassium nitrate and lysine. No growth in the presence of 50% D-glucose. Growth on vitamin-free medium is positive. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 24 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA, and partial sequence of *TEF1* deposited in NCBI/EMBL (GenBank) under the accession numbers: MH685197, MH697754 and LS992159, respectively.

Deposits: holotype, strain A528 isolated from roots of *Impatiens parviflora* (Balsaminaceae), in September 2010 in the Losiny Ostrov (Rus. Elk Island) National Park, Moscow, Russia, preserved in a metabolically inactive state in the KBP collection of Department of Soil Biology, Faculty of Soil Sciences, Lomonosov Moscow State University (WDCM 1173) as KBP Y-5098^{T}. Ex-type cultures are deposited in the All-Russian Collection of Microorganisms (VKM), Pushchino, Russia (VKM Y-3017), the Russian National Collection of Industrial Microorganisms (VKPM), Moscow, Russia (VKPM Y-3827), the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS xxxxx) and the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 105054).

Description of *Krasilnikovozyma curviuscula* (Babeva, Lisichkina, Reshetova & Danilevich) Yurkov, Kachalkin & Sampaio comb. nov. (MB 829125)

Basionym: *Mrakia curviuscula* Babeva, Lisichkina, Reshetova & Danilevich MB 529873

Holotype: KBP Y-3618 isolated from moss *Bryum* sp., dry white moss-pine forest, Oka Nature Reserve, Ryazan Oblast, Russia, preserved in a metabolically inactive state (dried) in the KBP collection of Department of Soil Biology, Faculty of Soil Sciences, Lomonosov Moscow State University (WDCM 1173).

Ex-type cultures are deposited in the All-Russian Collection of Microorganisms (VKM), Pushchino, Russia (VKM Y-2953) and the Portuguese Yeast Culture Collection (PYCC), Caparica, Portugal (PYCC 5836).

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: MK244628 (VKM Y-2953), and MF372124 and MF372143 (PYCC 5836), respectively.

Ecology: The species is widespread. It was found in soil (Japan, Canada, USA, Denmark; GenBank AB462342, KM384610, MH655679, MG914803), in mushroom (Taiwan; GenBank FJ873516), pine forest litter (Russia; GenBank MK244629, MK244630), rotten wood (Argentina), Sphagnum moss (Russia), and orchid flower (North America; GenBank EU218880) (e.g. Taylor and McCormick 2008, Glushakova et al. 2015, Sylvester et al. 2015).

Tremellomycetes, Tremellales

Description of *Hannaella taiwanensis* (F.L. Lee & C.H. Huang) Yurkov comb. nov. (MB 829114)

Basionym: *Cryptococcus taiwanensis* F.L. Lee & C.H. Huang , Fungal Science 26 (1): 61 (2011); MB 560182

Note: The species was overlooked in the analysis by Liu et al. (2015). Sequences D1/D2 domains of LSU (HQ591443) of the type strain BCRC 23252 show 97% similarity to *Hannaella zeae*, *H. kunmingensis*, *H. siamensis*, and 96% similarity to the type species of the genus *H. sinensis* (Fig. S1).

Validation of *Tremella basidiomaticola* X.Z. Liu & F.Y. Bai, MycoKeys 47:80 (2018); MB 827184

Zhao et al. (2018) described four new *Tremella* species from China. Type material of the three sexual species (*Tremella cheejenii*, *T. erythrina*, and *T. salmonea*) was deposited in the Herbarium of the Chinese Academy of Sciences (acronym HMAS). The species *Tremella basidiomaticola* is known from its asexual (yeast) state, and the holotype (a strain) was deposited in the China General Microbiological Culture Collection Center. The description of *Tremella basidiomaticola* does not conform with the ICN Shenzhen Code. According to the Article 40.8 of the ICN Shenzhen Code, for the name of a new species or infraspecific taxon

published on or after 1 January 2019 of which the type is a culture, the protologue must include a statement that the culture is preserved in a metabolically inactive state. This requirement was not fulfilled in the original description by Zhao et al. (2018). Therefore, the description is validated here in accordance with the Article 9.2 of the ICN Shenzhen Code.

Holotype, strain CGMCC 2.5724^{T} isolated from a basidioma of *Tremella fuciformis* collected in July 2017 by X.Z. Liu in Gutian county, Ningde city, Fujian Province, China, preserved in a metabolically inactive state at the China General Microbiological Culture Collection Center, Beijing, China. Ex-holotype culture is deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 15261).

Comments to the genus *Gelidatrema*

The description of the second species of the genus *Gelidatrema*, *Gelidatrema psychrophila*, was published simultaneously with the preparation of the present manuscript (Tsuji et al. 2018). This species was described based on two isolates from a microbial mat in the Canadian High Arctic. Another culture of *Gelidatrema psychrophila* (DBVPG 5459, GenBank KC433781) was isolated from a snow sample in Italian Alps (Turchetti et al. 2013). This culture has not been considered in the description by Tsuji et al. (2018). We compared physiological profiles of *Gelidatrema psychrophila* from the original description (Tsuji et al. 2017) with those of the strain DBVPG 5459 (this study). In contrast to results published by (Tsuji et al. 2018), the strain *G. psychrophila* DBVPG 5459 grew on D-gluconate but not on D-glucoronate, N-acetyl-D-glucosamine, and succinate. Assimilation of glycerol and ethanol was weak or negative (Tsuji et al. 2018; this study). Similarly to results obtained for the type species of the genus, *G. spencermartinsiae*, and in contrast to results published by Tsuji et al. (2018), the strain DBVPG 5459 did not assimilate potassium nitrate and sodium nitrite.

The two species of the genus *Gelidatrema* (*G. spencermartinsiae* and *G. psychrophila*) are polytrophic utilizing a total of 22 carbon sources, namely arbutin, cellobiose, D-arabinose, D-galactose, D-xylose, D-glucose, galactitol, L-arabinose, L-rhamnose, D-ribose, D-mannitol, lactose, maltose, melezitose, melibiose, myo-inositol, raffinose, ribitol, salicin, sorbitol, trehalose, and xylitol. Both species do not grow on citrate, erythritol, ethanol, ethyl-acetate, glycerol, hexadecane, inulin, lactate, L-sorbose and methanol. Amino acids lysine and cadaverine are utilised as a source of nitrogen, but no growth occurs on potassium nitrate and sodium nitrite. Taking together, the results of the present study and descriptions of *G. spencermartinsiae* and *G. psychrophila* (de Garcia et al. 2010; Tsuji et al. 2018), the two species can be only distinguished on the basis of utilisation of D-ribose, sucrose, D-glucosamine and ethylamine.

Description of *Kwoniella fici* Turchetti & Buzzini sp. nov. (MB 829127)

Etymology: The species epithet *fici* is derived from ficus (L. gen. sing. n. n., of fig tree) and refers to the substrate of isolation.

After 1 week at 25 °C on MEA, PDA and GPYA, streak culture is greyish-white (MEA, PDA) to cream-colored (GPYA), viscous to butyrous with a dull smooth surface. Margins are smooth and entire and the profile is flat. Cells are spherical to globose, 5–5.5 µm, occurring singly or in pairs, and proliferating by multilateral budding. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose (weak, delayed), D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl-alpha-D-glucoside (weak), cellobiose, salicin (weak, delayed), lactose, raffinose, melezitose, glycerol, erythritol, ribitol, xylitol, D-glucitol, D-mannitol, galactitol, myo-inositol, glucono-delta-lactone, D-gluconate, succinate and ethanol. No growth on L-sorbose, arbutin, melibiose, D-glucoronate, D-galacturonate, DL-lactate, citrate, methanol, L-malate, hexadecane, N-acetyl-D-glucosamine, and ethyl acetate. Utilisation of nitrogen sources: positive growth on potassium nitrate, sodium nitrite, ethylamine, lysine and cadaverine. No growth in the presence of 0.01% cycloheximide. Growth in the presence of 10 % NaCl and 50 % glucose is negative. Growth on vitamin-free medium is weak. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are abundantly produced. Maximum growth temperature: 30 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: MK070336 and MK070318, respectively.

Deposits: holotype, strain AH-10 isolated from a fruit of the common fig (*Ficus carica*) collected in September 2011 in Umbria, Italy, preserved in a metabolically inactive state in the Industrial Yeasts Collection DBVPG, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy as DBVPG 10122^{T}.

Tremellomycetes, Filobasidiales

Description of *Heterocephalacria fruticeti f.a.* Carvalho, Roehl, Yurkov & Sampaio sp. nov. (MB 829128)

Etymology: The species epithet *fruticeti* is derived from fruticetum (L. gen. sing. n. n., of a thicket) and refers to the substrate of isolation, which was a dense *maqui* scrubland.

After 1 week at 25 °C on GPY agar and PDA, streak culture is whitish to cream-coloured, mucoid with a glistering smooth surface. Margins are smooth and entire and the profile is flat. Cells are oval to ellipsoidal 3–5 × 5–7 µm in size, occurring singly or in pairs, and proliferating by polar and multilateral budding. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl-alpha-D-glucoside, cellobiose, salicin, lactose, melezitose, inulin (weak), soluble starch (delayed), ribitol, xylitol, D-glucitol, D-mannitol, inositol, D-glucuronate, succinate, citrate, L-malate, L-tartrate, methanol (weak) and ethanol. No growth on D-glucosamine, D-ribose, D-xylose, melibiose, raffinose, glycerol,

erythritol, galactitol, glucono-delta-lactone, D-gluconate and DL-lactate. Utilisation of nitrogen sources: positive growth on potassium nitrate, sodium nitrite, lysine and cadaverine. Growth in the presence of 0.01% and 0.1% cycloheximide is positive. Growth in the presence of 10 % NaCl is negative. Growth on vitamin-free medium is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: MK307720 and KT314192, respectively.

Deposits: holotype, strain OR 402 isolated from dry scrubland soil collected in the Arrábida Natural Park, Serra da Arrábida, Portugal, preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, Caparica, Portugal as PYCC 8314^{T}.

Description of *Heterocephalacria gelida f.a.* Turchetti & Kachalkin sp. nov. (MB 829129)

Etymology: The species epithet *gelida* is derived from gelidus (L. f. adj., frosty) and refers to the climatic conditions of localities, from which the species was isolated.

After 1 week at 25 °C on GPY agar and PDA, streak culture is whitish to cream-colored, mucoid and viscous with a glistering smooth surface. Margins are smooth and entire, and the profile is flat. Cells are sub-globose, oval to ellipsoidal 3–5 × 7–9 µm in size, occurring singly, in pairs or short chains, and proliferating by polar and multilateral budding. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Sediment is produced when the strain grows in ME and GPY broth at 25°C after 7 days. After 14 days, a superficial ring is also present. Teleomorph not observed.

Glucose is not fermented. Positive growth on glucose, D-galactose, L-sorbose, D-glucosamine, D-ribose (weak), D-xylose, L-arabinose, D-arabinose (weak), L-rhamnose, sucrose, maltose, trehalose, methyl alpha-methyl-D-glucoside, cellobiose, salicin, arbutin, melibiose (weak), lactose, raffinose, melezitose, ribitol, xylitol, D-glucitol, D-mannitol, galactitol, myo-inositol, glucono-delta-lactone, D-gluconate, and D-galacturonate (weak). No growth on glycerol, erythritol, D-glucoronate, DL-lactate, succinate, citrate, ethanol, methanol, L-malic acid, hexadecane, N-acetyl-D-glucosamine and ethyl acetate. Utilisation of nitrogen sources: positive growth on potassium nitrate, sodium nitrite, ethylamine and lysine. Growth in the presence of 0.01% is weak; no growth in the presence of 0.1% cycloheximide. Growth in the presence of 10 % NaCl and 50 % glucose is negative. Growth on vitamin-free medium is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: KC455903 and KC433839, respectively.

Deposits: holotype, strain 20.9 LB.6 isolated from supraglacial sediments collected in July 2009 in Miage glacier, Mont Blanc massif, Alps, Italy (45.783333N, 6.866667E), preserved

in a metabolically inactive state in the Industrial Yeasts Collection DBVPG, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy as DBVPG 5868^{T}.

Strains studied: DBVPG 5868^{T}, paratype KBP Y-5466 from leaves of *Dryas punctata* (Rosaceae) collected near the settlement Dikson, Taymyr peninsular, Krasnoyarsk Krai, Russia.

Description of *Heterocephalacria hypogea f.a.* Carvalho, Roehl, Yurkov & Sampaio sp. nov. (MB 829130)

Etymology: The species epithet *hypogea* is derived from hypogeus (L. f. adj., underground) and refers to the substrate of isolation.

After 1 week at 25 °C on GPY agar and PDA, streak culture is whitish to cream-coloured, mucoid with a glistering smooth surface. Margins are smooth and entire and the profile is flat. Cells are oval to ellipsoidal 3–5 × 5–7 µm in size, occurring singly or in pairs, and proliferating by polar and multilateral budding. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, D-glucosamine, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl-alpha-D-glucoside, cellobiose, salicin, melibiose (weak), lactose, raffinose, melezitose, inulin (weak), soluble starch (weak), ribitol, xylitol, D-glucitol, D-mannitol, galactitol, inositol, D-glucuronate, succinate, citrate, L-malate, L-tartrate, methanol (weak) and ethanol (weak). No growth on D-ribose, D-xylose, glycerol, erythritol, glucono-delta-lactone, D-gluconate and DL-lactate. Utilisation of nitrogen sources: positive growth on potassium nitrate, sodium nitrite, lysine and cadaverine. Growth in the presence of 0.01% and 0.1% cycloheximide is positive. Growth in the presence of 10 % NaCl is negative. Growth on vitamin-free medium is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: MK307721 and KT253539, respectively.

Deposits: holotype, strain OR 262 isolated from semihumid chaparral forest soil collected in the Arrábida Natural Park, Serra da Arrábida, Portugal, preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, Caparica, Portugal as PYCC 6805^{T}.

Description of *Heterocephalacria lusitanica f.a.* Inacio, Carvalho, Roehl, Yurkov & Sampaio sp. nov. (MB 829131)

Etymology: The species epithet *lusitanica* (L. f. adj.) is derived from the name of the ancient Roman province Lusitania, which included the territory of modern Portugal. The epithet refers to the locality, where the species was isolated.

After 1 week at 25 °C on GPY agar and PDA, streak culture is whitish to cream-coloured, mucoid with a glistering smooth surface. Margins are smooth and entire and the profile is flat. Cells are oval to ellipsoidal 3–5 × 5–7 μm in size, occurring singly or in pairs, and proliferating by polar and multilateral budding. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, D-glucosamine, D-ribose (variable), D-xylose (variable), L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl-alpha-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose (delayed), melezitose, inulin (variable), soluble starch (variable, delayed), ribitol, xylitol, D-glucitol, D-mannitol, inositol, glucono-delta-lactone, D-gluconate, D-glucuronate, succinate (variable), citrate, L-malate, L-tartrate, saccharate and galactarate. No growth on glycerol, erythritol, DL-lactate, methanol, ethanol, D-tartrate and m-tartrate. Growth on low-weight aromatic compounds is negative. Utilisation of nitrogen sources: positive growth on potassium nitrate, sodium nitrite, ethylamine (variable), lysine and cadaverine. Growth in the presence of 0.01% and 0.1% cycloheximide is positive. Growth in the presence of 10 % NaCl is negative. Growth on vitamin-free medium is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: MK307716 and EU002809, respectively.

Deposits: holotype, strain 2MV5 isolated from fruits of the strawberry tree (*Arbutus unedo*) collected in the Arrábida Natural Park, Serra da Arrábida, Portugal, preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, Caparica, Portugal as PYCC 6104^{T}. Ex-type culture is deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 10185).

Strains studied: 2MV5 (= CBS 10185), paratypes 4ExS4 (= CBS 10186) from an oak exudate, OR 78 (= PYCC 8313) from soil under humid sclerophyll forest. All known strains were isolated from Nature Park of Arrábida on northern and southern slopes of the Serra da Arrábida mountain range from plants and soil (Inacio 2003; Yurkov et al. 2016).

Comments to the genus *Heterocephalacria*

The genus *Heterocephalacria* proposed by Liu et al. (2015) comprises two teleomorphic mycoparasites *H. bachmannii* and *H. physciacearum* and asexual *H. arrabidensis*, which is known from yeast state only. Although no teleomorph was discovered for newly described *H. gelida* and *H. lusitanica*, a mycoparasitic lifestyle cannot be excluded. No culture is available for *H. bachmannii* and *H. physciacearum*. Physiological profiles are only available for asexual species, which can be distinguished based on assimilation of ethanol, glycerol, DL-lactate, succinate, citrate, D-glucoronate, L-malate, nitrate and lysine. However, closely related *H. gelida* and *H. lusitanica* only differ in growth on succinate, citrate, D-glucoronate

and L-malate. *H. hypogea* can be distinguished from the closely related *H. arrabidensis* in assimilation of D-xylose, D-ribose, methanol, glycerol, DL-lactate, D-glucoronate, nitrate, ethylamine, and lysine.

Description of *Piskurozyma arborea* Yurkov, Kachalkin, Mašínová & Baldrian sp. nov. (MB 829132)

Etymology: The species epithet arborea refers to the habitat of isolation.

After 1 week at 25 °C on GPY agar and PDA, streak culture is white to cream-coloured, butyrous with a glistering smooth surface. Margins are smooth and entire. Cells are ovoid, cylindrical or somewhat sausage-shaped, 6-7 × 2-4 μm. Budding is polar or occurs, in the case of lateral budding, near the poles of the cells. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, D-ribose (variable), D-xylose, L-Arabinose, D-Arabinose, L-rhamnose, sucrose, maltose, trehalose, cellobiose, salicin (variable), arbutin, melibiose, lactose, raffinose, melezitose, inulin, soluble starch, glycerol (variable), ribitol, xylitol, arabitol, D-glucitol, D-mannitol, galactitol, inositol, 2-Keto-D-Gluconate, 5-Keto-D-Gluconate, D-gluconate, D-glucuronate, DL-lactate, succinate, citrate and L-malate. No growth on D-glucosamine and erythritol. Utilization of nitrogen sources: positive growth on potassium nitrate and lysine. Growth in the presence of 10 % NaCl and 50 % glucose is negative. Growth on vitamin-free medium is positive. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are not produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession number KY558349.

Deposits: holotype, strain KT168 isolated from spruce litter collected in Kladeruby nad Oslavou, Třebíč District, Vysočina Region, Czech Republic, preserved in a metabolically inactive state in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany as DSM 103202^{T}.

Strains studied: DSM 103202^{T}, paratype KBP Y-4682 (= VKM Y-3011) from Sphagnum moss collected in Moscow Region, Russia.

Description of *Piskurozyma silvicultrix* Turchetti, Mašínová, Baldrian & Yurkov sp. nov. (MB 829133)

Etymology: The species epithet *silvicultrix* (L. f. adj., inhabiting forest) refers to the habitat of isolation.

After 1 week at 25 °C on GPY agar and PDA, streak culture is white to cream-colored, mucoid with a glistering smooth surface. Margins are smooth and entire and the profile is flat. Cells are globose 7-7.5 μm in diameter or ovoid 6-7 × 2-4 μm, occurring singly, in pairs or

short chains, and proliferating by polar budding. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, D-xylose, L-arabinose, sucrose, maltose, trehalose, methyl-alpha-D-glucoside, arbutin, melezitose, soluble starch, erythritol, D-glucitol, D-mannitol, myo-inositol, glucono-delta-lactone (delayed), D-gluconate (delayed), D-glucoronate (delayed), D-galacturonate (delayed), DL-lactate (delayed) and succinate. No growth on D-ribose, D-arabinose, L-rhamnose, cellobiose, salicin, melibiose, lactose, raffinose, glycerol, ribitol, xylitol, galactitol, citrate, ethanol, methanol, L-malate, hexadecane, N-acetyl-D-glucosamine and ethyl acetate. Utilisation of nitrogen sources: positive growth on ethylamine; no growth on potassium nitrate, sodium nitrite, L-lysine, and cadaverine. Growth in the presence of 0.01% and 0.1% cycloheximide is positive. Growth in the presence of 10 % NaCl and 50 % glucose is negative. Growth on vitamin-free medium is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (type strain): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: KU745333 and KU745299, respectively.

Deposits: holotype, strain 20.14 A S4 2R isolated from forest soil collected in June 2014 in Kleiner Priol (Montiggl), South Tyrol, Alps, Italy, preserved in a metabolically inactive state in the Industrial Yeasts Collection DBVPG, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy as DBVPG 10557^{T}.

Strains studied: DBVPG 10557^{T}, paratypes DSM 103194 and DSM 103201 (GenBank KY558341 and KY558348, respectively) from beech litter collected in Czech Republic (Mašínová et al. 2017b).

Description of *Piskurozyma stramentorum* Yurkov, Mašínová & Baldrian sp. nov. (MB 829134)

Etymology: The species epithet *stramentorum* is derived from stramentum (L. gen. plu. n. n., litter) and refers to the substrate of isolation.

After 1 week at 25 °C on GPY agar and PDA, streak culture is white to cream-coloured, butyrous with a glistering smooth surface. Margins are smooth and entire. Cells are ovoid, cylindrical or somewhat sausage-shaped, 6-7 × 2-4 μm. Budding is polar or occurs, in the case of lateral budding, near the poles of the cells. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Teleomorph was not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, L-sorbose, D-glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, D-trehalose, methyl-alpha-D-glucoside (weak), cellobiose, melibiose, lactose, raffinose, melezitose, ribitol, D-glucitol, D-mannitol (weak), D-sorbitol (weak), galactitol, inositol, D-Gluconate, L-malate (weak) and arbutin. No growth on salicin, inulin, glycerol, erythritol, xylitol, citrate, DL-lactate and succinate. Utilization of nitrogen sources: positive growth on

potassium nitrate, ethylamine and L-lysine. Growth on vitamin-free medium is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (type strain): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession number KY558344.

Deposits: holotype, strain KT146 isolated from spruce litter collected in Kladeruby nad Oslavou, Třebíč District, Vysočina Region, Czech Republic, preserved in a metabolically inactive state in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany as DSM 103197^{T}.

Description of *Naganishia nivalis* Turchetti & Buzzini sp. nov. (MB 829135)

Etymology: The species epithet *nivalis* (L. f. adj., snowy) refers to the substrate of isolation.

After 1 week at 25 °C on MEA, PDA and GPYA, streak culture is white to cream-colored (becoming brownish upon aging), viscous to butyrous with a dull wrinkled surface. Margins are smooth and entire and the profile is raised. Cells are spherical to ovoid 2.5–3 × 2–2.5 µm in size, occurring singly or in pairs, and proliferating by polar budding. Pseudohyphae and true hyphae were not observed. Ballistoconidia were not observed. Sediment is produced when the strain grows in ME and GPY broth at 25°C after 7 days. After 14 days, a superficial ring is also present. Teleomorph not observed.

Glucose is not fermented. Positive growth on D-glucose, D-galactose, D-ribose, D-xylose, L-arabinose (weak), D-arabinose (delayed), sucrose (delayed), maltose, trehalose, methyl-alpha-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melezitose, soluble starch, glycerol, erythritol, ribitol, xylitol, D-glucitol, D-mannitol, galactitol, inositol, methanol, ethanol, hexadecane, N-acetyl-D-glucosamine and ethyl acetate. No growth on L-sorbose, L-rhamnose, arbutin, glucono-delta-lactone, D-gluconate, D-glucoronate, D-galacturonate, DL-lactate, succinate, citrate and L-malate. Utilisation of nitrogen sources: positive growth on potassium nitrate, sodium nitrite, ethylamine, lysine and cadaverine (weak). Growth in the presence of 0.01% is negative. Growth in the presence of 10 % NaCl and 50 % glucose is negative. Growth on vitamin-free medium is positive. Urea hydrolysis and Diazonium Blue B reaction are positive. Starch-like compounds are produced. Maximum growth temperature: 25 ºC.

Molecular characteristics (holotype): nucleotide sequences of ITS and LSU (D1/D2 domains) rRNA deposited in NCBI/EMBL (GenBank) under the accession numbers: MK070337 and KC433768, respectively.

Deposits: holotype, strain MB 10.12 isolated from snow collected in July 2010 on Glacier du Geant, Mont Blanc massif, Alps, Italy (45.833333N, 6.916667E), preserved in a metabolically inactive state in the Industrial Yeasts Collection DBVPG, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy as DBVPG 5693^{T}.

Strains studied: DBVPG 5693^{T}, paratype DBVPG 5706 from the same locality.

Microbotryomycetes

Description of *Yurkovia nerthusi* Yurkov & Begerow, sp. nov. (MB 828552)

Etymology: The specific epithet *nerthusi* is derived from a Latinized form (Nerthus) (NL. gen. sing. m. n., snowy) of Germanic goddess of earth and fertility Nerþuz.

After growth on YM agar plates for 1 mo at 16 °C and 22 ºC, the streak culture is off-white to tan, butyrous to mucoid with a smooth and glistening surface. The margin is entire or rarely wrinkled. After growth on YM agar plates for 7 d at 16 °C and 22 ºC, cells are ellipsoidal or cylindrical (2–4 × 8–10 µm), occurring singly or in pairs, and proliferating by polar budding. Pseudohyphae and true hyphae were not observed after 1 mo in Dalmau plate culture on CMA at 16–22 °C. Fermentation is absent.

Assimilation of carbon compounds: Growth on D-glucose, D-galactose, L-sorbose, L-arabinose (weak), D-arabinose (weak), sucrose, maltose, trehalose, cellobiose, melezitose, inulin, starch, glycerol, ribitol, DL-lactate, succinate, citrate, ethanol (weak), L-tartaric acid, D-saccharic acid, 3,4-dyhydroxybesoic acid and 4-hydroxybencoic acid. No growth on raffinose, D-ribose, L-rhamnose, salicin, melibiose, lactose, erythritol, xylitol, galactitol and myo-inositol. Assimilation of nitrogen compounds: growth on potassium nitrate, sodium nitrite, ethylamine and lysine. Growth in the presence of 0.01% cycloheximide is positive. Growth in in the presence of 10 % NaCl and in medium with 50% and 60% glucose is negative. Urea hydrolysis and Diazonium Blue B reaction are positive. Maximum growth temperature: 25 ºC.

Molecular characteristics (holotype): nucleotide sequences of LSU (D1/D2 domains) rRNA gene and ITS deposited in NCBI/EMBL (GenBank) under the accession number FN428970 and KY083054, respectively.

Deposits: holotype, strain HEW-2-3 isolated from spruce forest soil collected in Stadtwald Mühlhausen, Thuringia, Germany (51.21N, 10.37E), preserved in a metabolically inactive state in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany as DSM 26788^{T}. Ex-type cultures are deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 11560) and the Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL), Louvain-la-Neuve, Belgium (MUCL 53962).

Strains studied: DSM DSM 26788^{T}; paratype AEG-2-20 (= CBS 11560) isolated from grassland soil in Swabian Alb, in the proximity of Sternberg Wanderheim, Gomadingen, Baden-Wuerttemberg, Germany (48.391389N, 9.376889E); GenBank FN428969, KY083054.

Notes: Strain AEG-2-20 was recovered on modified Browns' nitrogen deficient media (Yurkov et al. 2011), whereas strain HEW-2-3 was isolated on nutrient-rich acidified with lactic acid YPD agar (Yurkov et al. 2012a).

Validation of *Leucosporidium drummii* Yurkov, A.M. Schäfer & Begerow, International Journal of Systematic and Evolutionary Microbiology 62: 730 (2012); MB 563455

Leucosporidium drummii was described from soil by Yurkov et al. (2012b). However, the description does not conform to the ICN Melbourne Code Article 40.7, which requires that the single herbarium or collection or institution in which the type is conserved must be specified. We here fulfil the requirements for valid publication of *Leucosporidium drummii*.

Holotype: strain DSM 106046^{T}, isolated from grassland soil collected near Günterberg, Angermünde, Brandenburg, Germany, preserved in a metabolically inactive state in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany. Ex-type cultures are deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 11562) and the Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL), Louvain-la-Neuve, Belgium (MUCL 52878).

Validation of *Leucosporidium krtinense* Mašínová, A. Pontes, C. Carvalho, J.P. Samp. & Baldrian, International Journal of Systematic and Evolutionary Microbiology 67: 904 (2017); MB 815370

Leucosporidium krtinense was described from soil by Mašínová et al. (2017b). However, the description does not conform to the ICN Melbourne Code Article 40.7, which requires that the single herbarium or collection or institution in which the type is conserved must be specified. We here fulfil the requirements for valid publication of *Leucosporidium krtinense*.

Holotype: strain PYCC 6879^{T} isolated from beech litter collected in October 2013 in the Křtiny forest area (49.303278N, 16.747389E), Czech Republic, preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, Caparica, Portugal. Ex-type cultures are deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 14304) and the in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 101892).

Validation of *Libkindia masarykiana* Mašínová, A. Pontes, C. Carvalho, J.P. Samp. & Baldrian, International Journal of Systematic and Evolutionary Microbiology 67: 906 (2017); MB 815373

Libkindia masarykiana was described from soil by Mašínová et al. (2017b). However, the description does not conform to the ICN Melbourne Code Article 40.7, which requires that the single herbarium or collection or institution in which the type is conserved must be specified. We here fulfil the requirements for valid publication of *Libkindia masarykiana*.

Holotype: strain PYCC 6886^{T} isolated from oak litter collected in April 2014 in the Křtiny forest area (49.266944N, 16.721167E), Czech Republic, preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, Caparica, Portugal. Ex-type cultures are deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht,

the Netherlands (CBS 14275) and the in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 101891).

Validation of *Yurkovia mendeliana* Mašínová, A. Pontes, C. Carvalho, J.P. Samp. & Baldrian, International Journal of Systematic and Evolutionary Microbiology 67: 907 (2017); MB 815372

Yurkovia mendeliana was described from soil by Mašínová et al. (2017b). However, the description does not conform to the ICN Melbourne Code Article 40.7, which requires that the single herbarium or collection or institution in which the type is conserved must be specified. We here fulfil the requirements for valid publication of *Yurkovia mendeliana*.

Holotype: strain PYCC 6884^{T} isolated from beech litter collected in October 2013 in the Křtiny forest area (49.324944N, 16.652750E), Czech Republic, preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, Caparica, Portugal. Ex-type cultures are deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 14273) and the in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany (DSM 101889).

Cystobasidiomycetes

CyphobasidialesT. Sprib. & H. Mayrhofer (2016) Index Fungorum 309: 1 (MB 552589) nomen dubium

The order Cyphobasidiales T. Sprib. & H. Mayrhofer (2016) and family Cyphobasidiaceae T. Sprib. & H. Mayrhofer (2016) were proposed to accommodate lichenicolous parasite *Cyphobasidium* Millanes, Diederich & Wedin (2016) and lichen-inhabiting fungi represented by environmental sequences (Spribille et al. 2016). The monophyly of *Cyphobasidium* was not supported in the ML analysis of rRNA genes by Millanes et al. (2016). The analysis was also lacking *Cyrenella elegans*, which is the closest relative of *Cyphobasidium* according to sequence similarities from NCBI Blast and our phylogenetic analysis (Fig. 2). According to Bauer et al. (2006) *Cyrenella elegans* is placed in order Erythrobasidiales R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006). The order Cyphobasidiales coincides with Erythrobasidiales and the latter is older name for the order. Although the principle of taxonomic priority does not apply above the rank of family (ICN Shenzhen Code, Art. 11.10), the name Cyphobasidiales T. Sprib. & H. Mayrhofer (2016) is a duplicate and *nomen dubium*. Its delimitation from the order Erythrobasidiales is unclear and was not supported in our analyses. The order Erythrobasidiales includes genera *Bannoa*, *Buckleyzyma*, *Cyrenella*, *Hasegawazyma*, *Erythrobasidium* and *Symmetrospora* (Aime et al. 2006; Bauer et al. 2006; Wang et al. 2015a, 2015b). Erythrobasidium is an older generic name (ICN Shenzhen Code, Recommendation 16A.1) and the order Erythrobasidiales has been widely used by yeast taxonomists, mycologists and botanists (e.g. Aime et al. 2006; Bauer et al. 2006; Ruggiero et al. 2015; Oberwinkler 2017; Zhao et al. 2017). In our opinion, the name Cyphobasidiales should not be used in favour of the name Erythrobasidiales.

Similarly, the proposal of the family CyphobasidiaceaeT. Sprib. & H. Mayrhofer (2016) is premature since its delimitation from Buckleyzymaceae Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout (2015), Erythrobasidiaceae Denchev (2009) and Symmetrosporaceae Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout (2015) was not supported by a robust phylogenetic analysis.

Discussion

Two important evolutionary characters, such as the ability of basidiospores to germinate by secondary spore formation and active spore discharge, connected yeasts with some Basidiomycetes (reviewed by Oberwinkler 1987). The evolution of filamentous habit was essential for penetration and growth in the substrate and production of fruiting bodies as well as for the emergence specialised organs like haustoria and sporangia. Although basidiomycetous yeast species with a sexual state do not form macroscopic fruiting bodies (basidiocarps) in culture, filaments and haustoria have been observed in several yeasts. Filamentous heterobasidiomycetes display diverse germination patterns, including budding, secondary spores, microconidia and hyphae. A close connection between filamentous parasites like *Carcinomyces*, *Syzygospora* and *Tremella*, and teleomorphic yeasts like *Filobasidium* and *Filobasidiella* (presently *Cryptococcus*), has been suggested based on the basidial morphology, presence of haustoria and on ultrastructural markers (Bandoni 1987; Oberwinkler 1987). The first molecular studies already confirmed close relationships between some filamentous Heterobasidiomycetes and yeasts, and subsequent works demonstrated that many basidiomycetous yeasts represent cultivable morphs of Heterobasidiomycetes presently classified in the classes Cystobasidiomycetes, Microbotryomycetes and Tremellomycetes (Boekhout et al. 1993; Fell et al. 2000; Scorzetti et al. 2002; Sampaio 2004; Bauer et al. 2006; Millanes et al. 2011; Liu et al. 2015a; Wang et al. 2015b). Also, large filamentous genera *Tremella*, *Sirobasidium*, *Syzygospora* and *Cystobasidium* were shown to be polyphyletic. The implementation of rRNA and multi-gene phylogenies has provided valuable information for the re-evaluation of the classification of these fungi (Liu et al. 2015a; Wang et al. 2015b). However, despite substantial progress in species sequencing, taxon sampling for teleomorphic taxa is meagre. Very few sequences were obtained for species of genera like *Carcinomyces*, *Holtermannia*, *Sirobasidium*, *Sirotrema*, *Syzygospora*, *Tetragoniomyces* and several lineages of *Tremella*. Sequences of type species of genera like *Mycogloea*, *Occultifur*, *Sirobasidium* and *Spiculogloea* are lacking. No sequences are available for *Phragmoxenidium*, *Phyllogloea*, *Sigmogloea*, *Sigmotrema*, *Tremellina*, *Xenolachne* and *Zygogloea*. Unfortunately, few culture experiments have been carried out to isolate and preserve these fungi. In addition, many species are rarely collected. New collections studied molecularly and preserved *ex-situ* would improve our understanding of the diversity and systematics of this group. The recent reassessment of the genus *Phaeotremella* provides a good example of the relevance of such taxonomic studies. Along with the re-collection the availability of cultures, isolates from several *Tremella* species collected by Franz Oberwinkler and his co-workers were authenticated and re-sequenced (Spirin et al. 2018). Another recent study by Pontes et al. (2017) studied the culture of *Tetragoniomyces uliginosus*, which was earlier obtained by

Franz Oberwinkler (PYCC database). Nucleotide sequences from this culture suggest that the phylogenetic placement of this species in the Trichosporonales (Liu et al. 2015a) is likely to be an artefact of a short sequence, which was originally obtained from a herbarium material (Millanes et al. 2011).

Our phylogenetic analyses included sequences from single strains from different studies. Interestingly, some of them represent conspecific isolates, e.g. in genera *Cryptococcus*, *Heterocephalacria*, *Piskurozyma* and *Pseudotremella* (Figs. 1, 4, 5). A number of potential new species were shown to enlarge small or previously monotypic genera, e.g. *Carcinomyces*, *Fibulobasidium*, *Gelidatrema*, *Heterocephalacria*, *Sugitazyma* and *Yurkovia* (Figs. 1, 2). This observation legitimises to some extent the re-classification of large polymorphic genera into smaller, sometimes monotypic entities. A considerable number of species awaiting description would be otherwise assigned to genera *Bensingtonia*, *Bullera*, *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces*, increasing taxonomic complexity of many groups. Newly erected genera of Microbotryomycetes accommodated species previously classified as *Bensingtonia*, *Rhodotorula*, and *Sporobolomyces* and, in some cases, closely related species were assigned to different genera (Wang et al. 2015a, 2015b). Similarly, GenBank sequences of yeasts belonging to such problematic groups were mislabelled or had a doubtful taxonomic assignment up to the rank of a family. This situation is often confusing; it does not facilitate description of new species nor does it help to identify them in the environment. Sequences obtained from Genbank and a few other repositories were placed into the two LSU rRNA datasets used by Liu et al. (2015a) and Wang et al. (2015b) in order to provide an update on phylogenetic relationships in Tremellomycetes, Cystobasidiomycetes and Microbotryomycetes. The presented phylogenetic analyses (Figs. 1, 2) show the diversity of basidiomycetous yeasts and provide authors of potential new species with a robust phylogenetic analysis to ease future species descriptions. Discovery of a single strain representing a new species seldom warrants a publication. However, public sequences representing yet undescribed species become an extremely important source of rare fungi and urge researchers to release unpublished data of potential new species to facilitate their formal description. In the present study, sharing sequence data helped us to identify many closely related and conspecific strains originating from a number of independent studies.

Rare and rarely sampled

Yeasts thrive in the environment not as pure cultures but as a part of a microbial community which varies in time and space. Physico-chemical characteristics of the environment, substrate colonisation, dissemination of propagules, competition with other species, and interactions with potential vectors and predator-prey relationships determine composition of the community (reviewed in Yurkov 2017; Yurkov and Pozo 2017). As a result, most of known yeast communities consist of a few dominating species and a large number of species which are found in low numbers. While isolation of dominant species is easy to achieve, cultivation of less numerous species requires a larger sampling and cultivation effort. Consequently, it is well documented and understandable that some species are recovered from

the environment as single strains. These yeasts represent rare species, which are also rarely sampled.

Yeasts used in this study were isolated from different substrates and regions, suggesting that basidiomycetous yeasts are rather widespread and not restricted to a specific location or substrate. Recent surveys of soils and cold habitats reported a substantial proportion of potential new yeasts (Buzzini et al. 2017; Sannino et al. 2017). These habitats are often characterized by very uneven structure of yeast communities with a few dominating species and a large number of rare yeasts. Most of the new soil-borne basidiomycetes recently described from soils represent rare taxa (e.g. Yurkov et al. 2016a, 2016b; Mašínová et al. 2017a, 2017b; Pontes et al. 2017). Mediterranean plants sampled in Serra da Arrábida (Portugal) during years 1997-1999 yielded diverse yeast communities, including many new species known from very few isolates (Inacio 2003). A number of these yeasts remained undescribed and were not found again until the recent resampling of the same biotopes (Yurkov et al. 2016b).

The number of known basidiomycetous species has increased dramatically in recent years. It is important to highlight that a number of species and genera in some groups were described from a very few strains and that this trend has increased in the last few years. For example, 80% of species descriptions published in IJSEM since 2018 have been based on 1-3 strains. It is also important to mention that taxonomic publications often do not provide essential information on the number of samples, isolated strains, species abundance or sampling depth. Therefore, it is not possible to conclude whether the sampling effort was sufficient in the aforementioned studies and these novel yeasts are rare species. Descriptions of either prokaryotic or eukaryotic species are expected to include a careful examination of species properties and ecology, which is difficult (if possible at all) to achieve on a limited number of strains. In our opinion, publication policies need to be modified to ensure that descriptions of novel taxa, especially genera, include (considering availability of both the material and data) all related strains and sequences.

Non-pigmented Microbotryomycetes, previously classified in the genera *Bensingtonia*, *Rhodotorula* and *Sporobolomyces*, are slow-growing species that are rarely isolated from the environment. Some species and genera are psychrophilic which makes their cultivation more complicated. During our past studies, we have also observed yeasts which grow slowly after isolation but lose their viability after a few passages. Therefore, fast and efficient preservation of yeast cultures is essential to keep the material for further investigations. Public databases, GenBank and culture collections contain nucleotide sequences of potential novel species which were isolated long time ago but await a formal description. Despite ongoing studies of European forest soils, no additional strain of species recently described by Yurkov et al. (2016a) such as *Colacogloea*, *Hamamotoa* and *Slooffia* were recovered even though they were isolated in 2008. Likewise, *Yurkovia nerthusi* described here is known from only two strains, and no additional culture of this species was found since the first isolation. However, several yeasts isolated by Fonseca et al. (2000) and Inacio (2003) from the Nature Park of Arrábida, were not found elsewhere. *Heterocephalacria* (formerly *Cryptococcus*) *arrabidensis* was isolated from plants and more species of this genus were identified and

described in this study (Figs. 1, 4). One of them is represented by two strains isolated from the same locality with a gap of 20 years. Similarly, *Cystofilobasidium alribaticum* was described after a recent resampling in Serra da Arrábida (Pontes et al. 2016). These examples show that rare species can persist in the environment for a long period. However, since these yeasts are rare and cultured as single isolates, a substantial sampling effort is required to resample them or to isolate them from a new source. We do not call for vast species descriptions based on a single culture but it is important to note that an effort to obtain additional isolates of some yeasts can be far too difficult.

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Figure captions

Figure 1 – Phylogenetic relationships of yeasts and related taxa in Agaricomycotina obtained by maximum-likelihood analysis of LSU (D1/D2 domains) rRNA gene. Tree topology was constrained according to the topology of the seven genes-based tree (for details see Liu et al. 2015a, 2015b) with nodes showing bootstrap values >85 % inforced to be monophyletic. Taxa not included in the previous analysis by Liu et al. (2015) are in red. The numbers provided on branches are frequencies (> 50 %) with which a given branch appeared in 100 bootstrap replications. The scale bars indicate the numbers of expected substitutions accumulated per site.

Figure 2 – Phylogenetic relationships of yeasts and related taxa from Pucciniomycotina lineages obtained by Maximum-Likelihood analysis of the LSU (D1/D2 domains) rRNA gene. Tree topology was constrained according to the topology of the seven genes-based tree (for details see Wang et al. 2015a, 2015b) with nodes showing bootstrap values >85 % inforced to be monophyletic. Taxa not included in the phylogenetic analysis of the seven genes (Wang et al. 2015a) are indicated in red. The numbers provided on branches are frequencies (> 50 %) with which a given branch appeared in 100 bootstrap replications. The scale bars indicate the numbers of expected substitutions accumulated per site.

Figure 3 – Phylogenetic relationships of yeasts and related taxa from the order Cystofilobasidiales in Tremellomycetes obtained by maximum-likelihood analysis of a concatenated alignment of the (A) ITS region and LSU (D1/D2 domains) rRNA gene and (B) ITS region, LSU rRNA gene and *TEF1*. The numbers provided on branches are frequencies (> 50 %) with which a given branch appeared in 100 bootstrap replications. The scale bars indicate the numbers of expected substitutions accumulated per site.

Figure 4 – Maximum likelihood analysis of a concatenated alignment of the ITS region and LSU (D1/D2 domains) rRNA gene for the genus *Heterocephalacria*. The numbers provided on branches are frequencies (> 50 %) with which a given branch appeared in 100 bootstrap replications. The scale bars indicate the numbers of expected substitutions accumulated per site.

Figure 5 – Maximum likelihood analysis of a concatenated alignment of the ITS region and LSU (D1/D2 domains) rRNA gene for the genus *Piskurozyma*. The numbers provided on branches are frequencies (> 50 %) with which a given branch appeared in 100 bootstrap replications. The scale bars indicate the numbers of expected substitutions accumulated per site.

Figure 6 – Maximum likelihood analysis of a concatenated alignment of the ITS region and LSU (D1/D2 domains) rRNA gene for the genus *Naganishia*. The numbers provided on branches are frequencies (> 50 %) with which a given branch appeared in 100 bootstrap replications. The scale bars indicate the numbers of expected substitutions accumulated per site.

Figure 7 – Micrographs showing morphology of new species: *Vustinia terrae* (a), vegetative cells on PDA after 7 d at 20 °C; *Udeniomyces caspiensis* (b), vegetative cells on 2% Glucose YNB agar, after 7 d at 20 °C; *Tausonia rosea* (c), vegetative cells on PDA, after 7 d at 20 °C; *Udeniomyces orazovii* (d-f), vegetative cells (d), microcolony (e), and chlamydospores (f) on PDA after 10 d at 20 °C; *Itersonilia diksonensis* (g, h), vegetative cells (g) and sympodially branched sterigmata with ballistoconidia (h) on 2% Glucose YNB agar, after 14 d at 20 °C; *Krasilnikovozyma fibulata* (i-k), vegetative cells (i), hyphae with clamp connections and teliospores (j, k) on PDA after 14 d at 20 °C. Scale bars: 10 µm (a, b, c, d, g, h, i, k), 20 µm (f), 60 µm (e) and 70 µm (j).