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TAXONOMIC DESCRIPTION

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Inopinatum lactosum gen. & comb. nov., the first yeast-like fungus in Leotiomycetes

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Abstract

Sporobolomyces lactosus is a pink yeast-like fungus that is not congeneric with other members of *Sporobolomyces* (Basidiomycota, Microbotryomycetes, Sporidiobolales). During our ongoing studies of pink yeasts we determined that *S. lactosus* was most closely related to *Pseudeurotium zonatum* (Ascomycota, Leotiomycetes, Thelebolales). A molecular phylogenetic analysis using sequences of the ITS region and the small and large subunit (SSU, LSU) rRNA genes, indicated that four isolates of *S. lactosus*, including three ex-type isolates, were placed in Thelebolales with maximum support. A new genus is proposed to accommodate *S. lactosus*, *Inopinatum*. This is the first pink yeast reported in Leotiomycetes.

Pink-pigmented yeasts in the order Sporidiobolales (Basidiomycota, Pucciniomycotina, Microbotryomycetes) produce lipid droplets with carotenoid pigments – mostly β-carotene and torulene – contributing to the pink to orange-reddish colour of colonies [1–3]. These pigments are thought to offer antimicrobial, anticancer, and anti-ageing activities and to protect against radiation [4, 5]. Because of these characteristics, pink-pigmented yeasts have gained interest from pharmaceutical, cosmetics, and biotechnology industries [6–8]. The pink yeasts were historically placed in two anamorphic basidiomycete genera, *Rhodotorula* and *Sporobolomyces*. In their traditional sense, both these asexual genera are polyphyletic, occurring in all three subphyla and several classes and orders of Basidiomycota [9–11].

Following the elimination of the use of dual naming systems for asexual and sexual morphs of fungi, *Rhodotorula* and *Sporobolomyces* are now retained only for those species within Sporidiobolales [12]. Efforts to reassign many of the species once classified into *Rhodotorula* and *Sporobolomyces* into natural genera are ongoing [8, 12, 13]. At present, the order Sporidiobolales is estimated at ca. 260 species of which 42 have been described [3]; *Sporobolomyces* currently includes ca. 22 species [8, 14, 15]. These are reported from diverse habitats including freshwater and marine ecosystems, fruit must, surfaces of buildings, food, soil, air, and—the most common habitat from which they are isolated—leaf surfaces [3, 15–22].

During our studies of pink yeasts in the genus *Sporobolomyces*, we noted that the internal transcribed spacer (ITS) barcode sequence of *Sporobolomyces lactosus* [23] was not similar to other species in the genus. Moreover, *S. lactosus* is not treated in Kurtzman *et al.* [24]. A general Nucleotide blast search (https://blast.ncbi. nlm.nih.gov/Blast.cgi?) of *S. lactosus* against ex-type sequences of all fungi resulted in *Pseudeurotium zonatum* CBS 329.36^T (Ascomycota, Leotiomycetes, Thelebolales) as the closest match with 90.84% shared identity. An ex-type culture of *S. lactosus* was obtained from the Culture Collection of Yeasts (CCY:19-21-1^T) [23] at the Slovak Academy of Sciences (Bratislava, Slovakia). Here we present the results of our phylogenetic analyses of *S. lactosus* and formally describe *Inopinatum* gen. nov. to accommodate it in the Thelebolaceae (Thelebolales), as the first known yeast-like species in the Leotiomycetes.

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Abbreviations: ITS, internal transcribed spacer; LSU, large subunit rRNA gene; ML, maximum likelihood; nt, nucleotides; PDA, potato dextrose agar; SSU, small subunit rRNA gene.
The GenBank/EMBL/DDBJ accession numbers of the SSU and ITS sequences of *Inopinatum lactosum* gen. & comb. nov. CCY 19-21-1^T=JCM 8510 are AB021676 and AB038132. The GenBank/EMBL/DDBJ accession number of the ITS sequence of *I. lactosum* C4 is EU551181. The GenBank/EMBL/DDBJ accession numbers of the newly generated SSU, ITS, and LSU sequences of *I. lactosum* CCY 19-21-1^T are MW471137, MW471138 (SSU), MW471139, MW471140 (ITS), MW471141, and MW471142 (LSU). The MycoBank accession numbers are MB835917 for *Inopinatum* gen. nov. and MB835918 for *I. lactosum* comb. nov. The aligned three-locus dataset used for ML phylogenetic inference is available from the figshare online repository with the URL https://doi.org/10.6084/m9.figshare.12495878.

Table 1. Sequences of Thelebolales used in phylogenetic analysis

Species	Isolate/strain	SSU	ITS	LSU	Reference(s)
Antarctomyces pellizariae	UFMG 12416^T	NA	NR_164245	NA	[62]
*Antarctomyces psychrotrophicus**	IMI 378528^T	NA	AJ133431	NA	[69]
*Inopinatum lactosum**	JCM 8510	AB021676	AB038132	NA	–
*Inopinatum lactosum**	C4	NA	EU551181	NA	[64]
*Inopinatum lactosum**	D. Haelew. F-3088a (ex-CCY 19-21-1^T)	MW471137	MW471139	MW471141	This study
*Inopinatum lactosum**	D. Haelew. F-3088b (ex-CCY 19-21-1^T)	MW471138	MW471140	MW471142	This study
*Cleistothelebolus nipigonensis**	CBS 778.70^T	NA	NR_164284	MH871738	[70]
*Connersia rilstonii**	CBS 537.74	AF096174	KJ755499	FJ176866	[71–73]
*Crinula caliciiformis**	AFTOL-ID 272	AY544729	KT225524	AY544680	–
Geomyces auratus	CBS 108.14^T	AB015785	NR_111872	NG_042776	[42, 74, 75]
Gymnostellatospora alpina	CBS 620.81^T	NA	MH861383	MH873132	[70]
*Gymnostellatospora japonica**	UAMH 9239	NA	DQ117454	NA	[76]
*Holwaya mucida**	TU 112863	KX090898	MH752062	KX090844	[44, 77]
*Leuconeurospora pulcherrima**	AFTOL-ID 1397	FJ176828	KF049206	FJ176884	[73]
Pleuroascus nicholsonii	CBS 345.73^T	AF096182	NR_156627	AF096196	[71]
Pleuroascus rectipilus	CBS 120411^T	NG_067690	NR_165899	NA	[78]
Pseudeurotium hygrophilum [as *Teberdinia hygrophila*]	CBS 102670^T	AY129282	AY129291	NA	[79]
Pseudeurotium sp.	01NH01	NA	JX270336	NA	[80]
Pseudeurotium zonatum	AFTOL-ID 1912^T	DQ471040	NR_111127	DQ470988	[75, 81]
Pseudogymnoascus destructans	JGI Genome	NA	JGI genome	NA	–
*Pseudogymnoascus roseus**	CBS 395.65^T	AB015778	NR_165894	MH870271	[70, 74]
Ramgea ozimecii	CNF 2/9997^T	NA	NR_164248	KY368753	[82]
Thelebolus balaustiformis	MUT 2357^T	NA	NR_159056	NG_067559	[83]
Thelebolus globosus	CBS 113940^T	NG_062682	NR_138367	NG_067263	[60, 73]
Thelebolus stercoreus	CBS 718.69^T	NA	MH859396	MH871167	[70]
*Thelebolus stercoreus**	JGI Genome	NA	JGI genome	NA	–

*, Type species; ^{T}, ex-type; NA, Not available.

Sporobolomyces lactosus, CCY:19-21-1^T, (Poland: Warsaw, Plock Refinery sewage treatment plant) [23], was grown on potato dextrose agar (PDA) with 2% agar, supplemented with 50 µg ml^{-1} chloramphenicol and 100 µg ml^{-1} ampicillin (BD, Franklin Lakes, New Jersey) to inhibit bacterial growth. Samples were removed for DNA isolation by using a J-hook to superficially scrape off pieces of fungal tissue. DNA was extracted using the Wizard Genomic DNA Purification kit (Promega Co., Madison, Wisconsin), following the manufacturer's instructions. Next, we amplified the internal transcribed spacer, and nuclear small and large subunits of the ribosomal DNA repeat (ITS, SSU, and LSU, respectively). Primer combinations used were NS1/NS4 for SSU [25], ITS1f/ITS4 for ITS [25, 26], and LR0R/LR5 and LR0R/LR7 for LSU [27, 28]. PCR reactions consisted of 12.5 µl of Promega 2×PCR Master Mix, 1.25 µl of each 10 µm primer, 9.0 µl of H_2O, and 1.0 µl of template DNA. All amplifications were done in an Eppendorf Mastercycler ep Thermal Cycler (Hauppauge, New

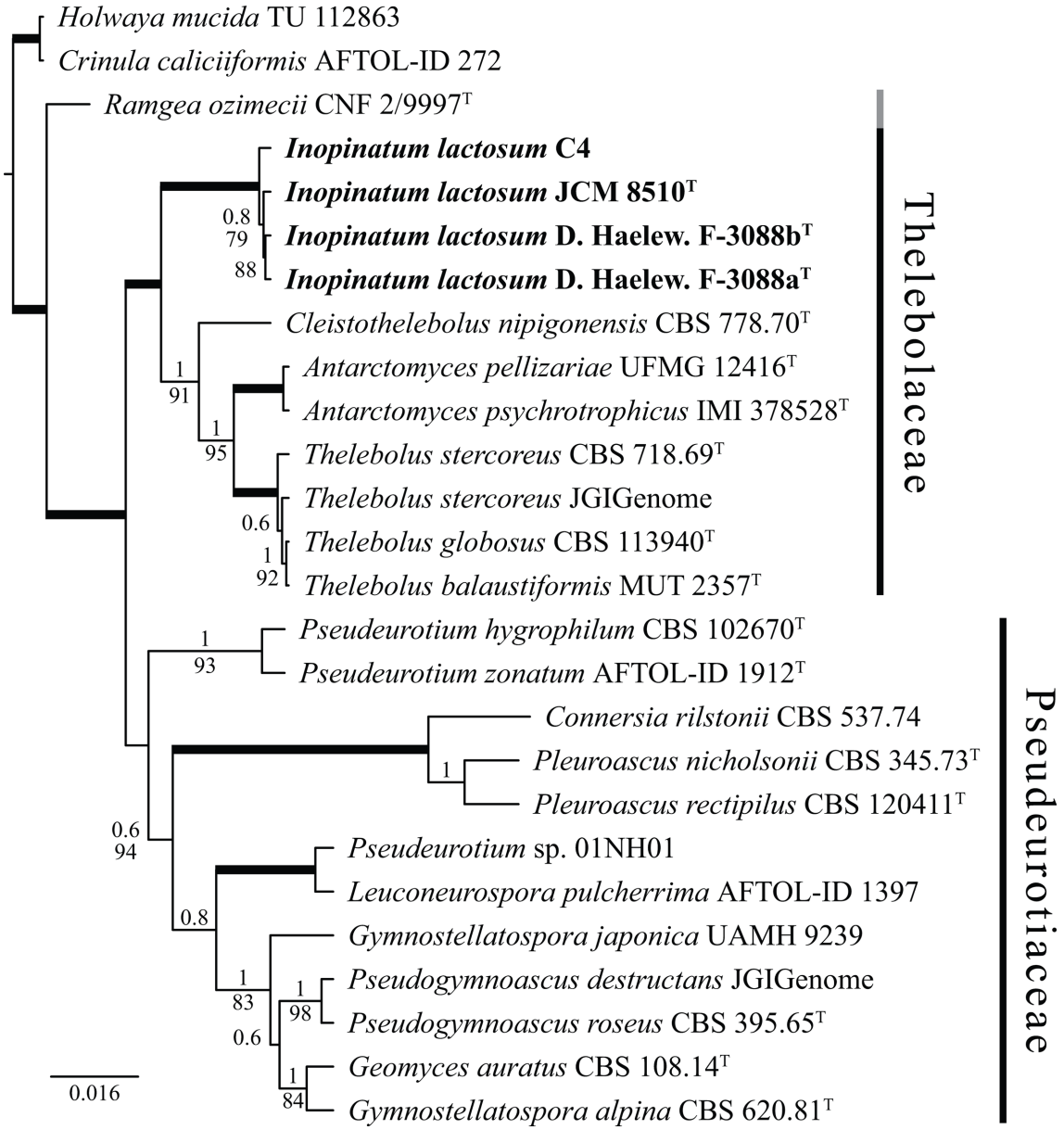

Fig. 1. Phylogenetic placement of *Inopinatum lactosum* gen. and comb. nov. within Thelebolales, reconstructed from a combined dataset of SSU, ITS, and LSU sequences (26 isolates, 2496 characters). The topology is the result of Bayesian inference performed with BEAST. *Crinula caliciiformis* AFTOL-ID 272 and *Holwaya mucida* TU 112863 were used as outgroups. For each node, pp ≥0.6 and ML bootstrap ≥60 are presented above/below the branch leading to that node. Thick branches, maximum support from both Bayesian and ML inference; T, ex-type; bar, number of substitutions per site.

York) under the same cycling conditions as in Haelewaters *et al.* [13].

Maximum likelihood (ML) analyses were done using IQ-TREE [29], on a multi-locus dataset of all three amplified loci. Representative sequences for all genera in Thelebolales were downloaded from NCBI GenBank (Table 1). Sequences for each locus were aligned using MUSCLE [30] available from the Cipres Science Gateway [31], and then trimmed using the command-line version of TrimAl 1.3 [32] with gap threshold of 0.6 and minimal coverage of 0.5. Substitution models were selected using ModelFinder [33] by considering the Akaike Information Criterion corrected for small sample size (AICc): TN+F+G4 for SSU (-lnL=2007.697), TIM2e+R2 for ITS (-lnL=2411.356), and TIM3 +F+R2 for LSU (-lnL=2300.016). ML was inferred for the concatenated SSU–ITS–LSU dataset under partitioned models, with rapid bootstrapping under 1000 replicates [34, 35].

Bayesian analyses were done using a Markov chain Monte Carlo (MCMC) approach implemented in the BEAST package [36], with a strict clock assuming a constant rate of evolution

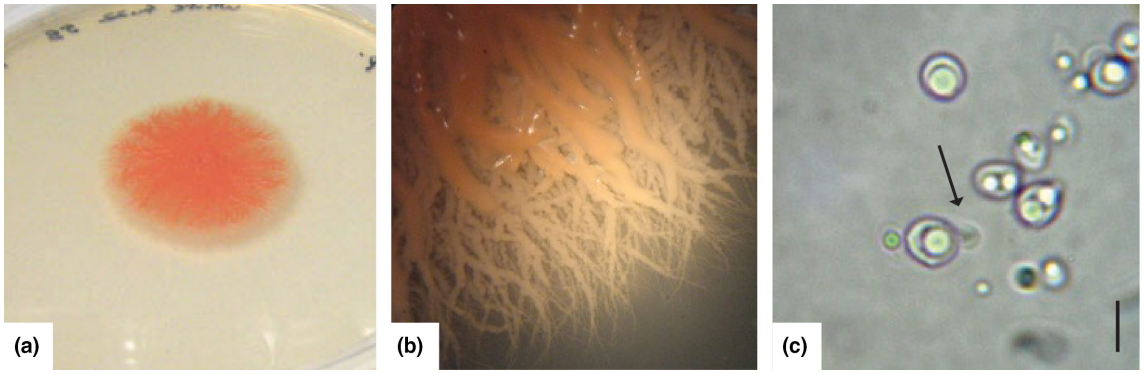

Fig. 2. *Inopinatum lactosum* gen. and comb. nov. Strain C4 from [64], growth on PDA supplemented with chloramphenicol (100 mg l^{-1}) and ampicillin (100 mg l^{-1}), after incubation of 5–7 days. (a) Colony, with (b) detail of colony with thick and 'veiny' undulating margin. (c) Vegetative cells, arrow pointing at daughter blastoconidium connected to its mother cell. Bar, 10 µm.

across the tree and a Yule Speciation tree prior [37, 38]. The nucleotide substitution models, as determined as by jModelTest 2.1.6 [39] under AICc, were as follows: HKY+G for SSU (-lnL=2008.6823), TIM2ef+G for ITS (-lnL=2406.8675), and TrN +I+G for LSU (-lnL=2300.4287). Two runs were undertaken from a random starting tree for 40 million generations, with a sampling frequency of 4000. Tracer 1.6 [40] was used to check MCMC trace plots. After removed of 10% as burn-in, trees files were combined, consensus trees were generated (with 0% burn-in), and the Maximum Clade Credibility (MCC) tree was inferred with the higher product of individual clade posterior probabilities (pp).

Intra- and interspecific divergence in the ITS and LSU regions was calculated using the Compute Pairwise Distances function in MEGA7 [41] with model/method set at 'No. of differences', gaps/missing data treatment set at 'pairwise deletion', and default settings for other parameters. The aligned, trimmed ITS sequences of our two ex-CCY 19-21-1^{T} isolates and of ex-type strain JCM 8510 were 100% identical. Isolate C4 differed in its ITS in four nucleotides (nt), followed by *Pseudeurotium zonatum* CBS 329.36^{T}=AFTOL-ID 1912^{T} with 42 nt differences in the ITS. The two ex-CCY 19-21-1^{T} isolates also shared 100% identity in their LSU sequences. The isolate from our dataset with least nt differences in the LSU region was *Thelebolus balaustiformis* MUT 2357^{T} (28 nt), followed by *Leuconeurospora pulcherrima* AFTOL-ID 1397 (29 nt), and *Cleistothelebolus nipigonensis* CBS 778.70^{T} and *Pseudeurotium zonatum* CBS 329.36^{T} (both 30 nt).

The phylogenetic reconstruction of Thelebolales based on the concatenated three-locus dataset is shown in Fig. 1. *Crinula caliciiformis* and *Holwaya mucida* (Leotiomycetes incertae sedis) were chosen as outgroup taxa. All included genera except *Ramgea* were placed in either Pseudeurotiaceae or Thelebolaceae as currently accepted [42–44]. In our three-locus phylogenetic reconstruction, *Ramgea ozimecii* CNF 2/9997^{T} was retrieved as the earliest diverging clade in the order with maximum support, resulting in a paraphyletic family Thelebolaceae. *Inopinatum lactosum* gen. and comb. nov. was maximally supported as sister to other members of Thelebolaceae.

Leotiomycetes are a diverse class within subphylum Pezizomycotina [44, 45] comprising ca. 6500 described species in 630 genera. These fungi are often found as major components of environmental samples. Nonetheless, many taxa remain unnamed or *incertae sedis* within the class. Leotiomycetes species appear to be predominantly saprotrophic and parasitic, including economically and ecologically important pathogens such as the powdery mildews (Erysiphaceae) and the causal agent of white-nose syndrome in bats (*Pseudogymnoascus destructans*, only known from its asexual morph) [43, 44]. Other species, however, are mycorrhizal mutualists (ectomycorrhizae and ericoid mycorrhizae) and plant endophytes [46–49].

Ascomycetous yeasts and yeast-like taxa are primarily found in the subphyla Saccharomycotina (Saccharomycetes) and Taphrinomycotina (Neolectomycetes, Pneumocystomycetes, Schizosaccharomycetes, Taphrinomycetes) [24], but have also been revealed in other lineages: Arthoniomycetes, Dothideomycetes, Eurotiomycetes, Xylonomycetes (subphylum Pezizomycotina), and *Gemmulina* (Ascomycota *incertae sedis*) [50–56]. Only recently, the black yeast genus *Phaeococcomyces* was placed in a newly erected order Lichenostigmatales (Arthoniomycetes) along with taxa forming colonies of stromatic ascomata or conidiomata (*Etayoa*, *Lichenostigma*)—a lineage that is unique within this class, which is otherwise composed primarily of lichenized species [51].

Inopinatum lactosum is the first described yeast-like fungus in Leotiomycetes, adding to the body of work that is expanding the known morphological and ecological diversity in the class. For example, the perithecioid apothecial *Annabella australiensis* (Leotiomycetes, Cordieritidaceae) was recently discovered from mangrove wood [57], a habitat that is otherwise mostly populated by Dothideomycetes and Sordariomycetes [58]. *Inopinatum* is placed in Thelebolaceae. This family includes many coprophilic and psychrophilic species [59–62] and, based on

genomic-scale data, was recently determined to be the sister to Pseudeurotiaceae—the order that contains the asexual fungus *P. destructans* [44, 63]. Although little is known of the ecology of *I. lactosum*, a coprophilous habit is likely given its isolation from animal faeces [64] and petrochemical wastewater [23].

Inopinatum lactosum is represented in NCBI GenBank by SSU, ITS, and LSU sequences of two ex-CCY 19-21-1^T isolates (this study); SSU, ITS, LSU, and cytochrome b (*cytb*) sequences of strain JCM 8510 (unpublished data); and an ITS sequence of isolate C4 [64], which shares 99.01% identity with the ex-type sequences. The C4 isolate was screened for production of enzymes on agar plates containing different substrates. Protease, amylase, mannanase, and variable xylanase activity was observed at 25 °C, while at 15 °C and 39 °C all enzymatic activity was either variable or absent [64].

DESCRIPTION OF *INOPINATUM* HAELEW. & AIME, GEN. NOV.

Inopinatum (Latin, meaning 'unexpected' and referring to the unexpected placement of this pink yeast genus in Leotiomycetes) MycoBank number: MB835917.

Type species: *Inopinatum lactosum* (E. Sláviková and Grab.-Łon.) Haelew. and Aime

Description: Yeast-like fungi belonging to Theobolaceae (Theobolales, Leotiomycetes). Teleomorph unknown. Anamorph pink-pigmented, forming pseudohyphae and hyphae; blastoconidia bilaterally symmetrical; no known fermentation. Isolated from animal faeces and wastewater.

Inopinatum lactosum (E. Sláviková and Grab.-Łon.) Haelew. and Aime, comb. nov. MycoBank number: MB835918. Fig. 2.

Basionym: *Sporobolomyces lactosus* E. Sláviková and Grab.-Łon., Anton Leeuw 61 (3): 246 (1992).

Inopinatum lactosum forms pink, glistening, ropey colonies on PDA (Fig. 2a). The colony margin is coarsely fimbriate, with a 'veiny' appearance (Fig. 2b) reminiscent of growth of some *Aureobasidium* Viala and G. Boyer and *Kabatiella* Bubák species in culture [65, 66]. Whereas *Aureobasidium* cultures become black with time, *I. lactosum* retains its pink pigementation (Fig. 2a). Growth is dimorphic, producing short chains of pseudohyphae and a few true hyphae near margins, and blastoconidia from older growth in the center (Fig. 2c). CCY 19-21-1^T, the holotype strain of *I. lactosum*, was isolated from an activated sludge in Poland [23]. The conidia were described as ballistoconidia in the protologue but are blastoconidia [67], analogous to *Aureobasidium* [68]. The C4 strain was isolated from koala faeces [64], a habitat that is consistent with that of other members of Thelebolaceae that are mainly known from dung [59].

The holotype is CCY 19-21-1, from petrochemical wastewater in Warsaw, Poland, and is permanently preserved in a metabolically inactive state in the Culture Collection of Yeasts, Bratislava, Slovakia. Ex-type cultures are preserved as JCM 8510 and JCM 10082 in the Japan Collection of Microorganisms, Tsukuba, Japan; and as NCYC 2618 in the National Collection of Yeast Cultures, Norwich, UK.

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Author contributions
Conceptualization, Formal Analysis, Visualization, Writing – original draft: D.H., Methodology: D.H. and M.C.A., Writing – review and editing: D.H., R.A.P., K.M.H.N., and M.C.A.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Valadon LRG. Carotenoids as additional taxonomic characters in fungi: A review. *Trans Brit Mycol Soc* 1976;67:1–15.
2. Ratledge C. Microorganisms for lipids. *Acta Biotechnol* 1991;11:429–438.
3. Urbina H, Aime MC. A closer look at *Sporidiobolales*: Ubiquitous microbial community members of plant and food biospheres. *Mycologia* 2018;110:79–92.
4. Davoli P, Mierau V, Weber RW. Carotenoids and fatty acids in red yeasts *Sporobolomyces roseus* and *Rhodotorula glutinis*. *Appl Biochem Microbiol* 2004;40:460–465.
5. Manimala MRA, Murugesan R. In vitro antioxidant and antimicrobial activity of carotenoid pigment extracted from *Sporobolomyces* sp. isolated from natural source. *J Appl Nat Sci* 2014;6:649–653.
6. Frengova G, Beshkova M. Carotenoids from *Rhodotorula* and *Phaffia*: yeasts of biotechnological importance. *J Ind Microbiolol Biotechnol* 2009;36:163–180.
7. Mannazzu I, Landolfo S, da ST, Buzzini P. Red yeasts and carotenoid production: outlining a future for non-conventional yeasts of biotechnological interest. *World J Microbiol Biotechnol* 2015;31:1665–1673.
8. AH L, Yuan FX, Groenewald M, Bensch K, Yurkov AM, *et al*. Diversity and phylogeny of basidiomycetous yeasts from plant leaves and soil: Proposal of two new orders, three new families, eight new genera and one hundred and seven new species. *Stud Mycol* 2020;96:17–140.
9. Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/ D2 domain sequence analysis. *Int J Syst Evol Microbiol* 2000;50:1351–1371.
10. Aime MC, Matheny PB, Henk DA, Frieders EM, Nilsson RH, *et al*. An overview of the higher-level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. *Mycologia* 2006;98:895–905.
11. Bauer R, Begerow D, Sampaio JP, Weiβ M, Oberwinkler F. The simple-septate basidiomycetes: a synopsis. *Mycol Prog* 2006;5:41–66.
12. Aime MC, Castlebury L, Abbasi M, Begerow D, Berndt R, *et al*. Recommendations of generic names competing for use: Pucciniomycotina and Ustilaginomycotina, Basidiomycota. *IMA Fungus* 2018;9:75–90.
13. Haelewaters D, Toome-Heller M, Albu S, Aime MC. Red yeasts from leaf surfaces and other habitats: three new species and a new combination of *Symmetrospora* (Pucciniomycotina, Cystobasidiomycetes). *Fungal Syst Evol* 2020;5:187–196.

14. Wang QM, Yurkov AM, Göker M, Lumbsch HT, Leavitt SD, *et al*. Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. *Stud Mycol* 2015;81:149–189.
15. Lorenzini M, Zapparoli G, Azzolini M, Carvalho C, Sampaio JP. *Sporobolomyces agrorum* sp. nov. and *Sporobolomyces sucorum* sp. nov., two novel basidiomycetous yeast species isolated from grape and apple must in Italy. *Int J Syst Evol Microbiol* 2019;69:3385–3391.
16. Derx HG. Etude sur les Sporobolomycetes. *Ann Mycol* 1930;28:1
17. Tubaki K. Studies on the *Sporobolomycetaceae* in Japan. III. On *Sporobolomyces* and *Bullera*. *Nagaoa* 1953;3:12–21.
18. Last FT. Seasonal incidence of *Sporobolomyces* on cereal leaves. *Trans Brit Mycol Soc* 1955;38:221–239.
19. Hamamoto M, Nakase T. Ballistosporous yeasts found on the surface of plant materials collected in New Zealand. 1. Six new species in the genus *Sporobolomyces*. *Anton Leeuw* 1995;67:151–171.
20. Nakase T. Expanding world of ballistosporous yeasts: distribution in the phyllosphere, systematics and phylogeny. *J Gen Appl Microbiol* 2000;46:189–216.
21. Molnár O, Wuczkowski M, Prillinger H. Yeast biodiversity in the guts of several pests on maize; comparison of three methods: classical isolation, cloning and DGGE. *Mycol Prog* 2008;7:111–123.
22. Debode J, Van Hemelrijck W, Creemers P, Maes M. Effect of fungicides on epiphytic yeasts associated with strawberry. *MicrobiologyOpen* 2013;2:482–491.
23. Sláviková E, Grabiňska-Loniewska A. *Sporobolomyces lactosus*, a new species of ballistosporous yeast equipped with ubiquinone-10. *Anton Leeuw* 1992;61:245–248.
24. Kurtzman CP, Fell JW, Boekhout T. *The Yeasts, a Taxonomic Study*. 5th ed. Amsterdam: Elsevier; 2011
25. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Innis M, Gelfand D, Sninsky J and White T (eds). In: *PCR Protocols: a Guide to Methods and Applications*. New York: Academic Press; 1990. pp. 315–322.
26. Gardes M, Bruns TD. ITS primers with enhanced specificity for Basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol Ecol* 1993;2:113–118.
27. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 1990;172:4238–4246.
28. Hopple JS. *Phylogenetic Investigations in the Genus Coprinus Based on Morphological and Molecular Characters (Phd Dissertation)*. Durham, North Carolina: Duke University; 1994.
29. Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol Biol Evol* 2015;32:268–274.
30. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
31. Miller M, Pfeiffer WT, Schwartz T. Creating the CIPRES Science Gateway for inferences of large phylogenetic trees. *Proc Gateway Comp Environ Workshop* 2010;14:1–8.
32. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 2009;25:1972–1973.
33. Kalyaanamoorthy K, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat Methods* 2017;14:587–589.
34. Chernomor O, Von Haeseler A, Minh BQ. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst Biol* 2016;65:997–1008.
35. Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol* 2017;35:518–522.
36. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 2012;29:1969–1973.
37. Gernhard T. Using birth-death model on trees. *J Theor Biol* 2008;253:769–778.
38. Yule GU. II.—A mathematical theory of evolution, based on the conclusions of Dr. JC Willis, F.R.S. *Phil Trans R Soc Lond B Biol Sci* 1925;213:21–87.
39. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 2012;9:772.
40. Rambaut A, Suchard MA, Xie D, Drummond AJ. Tracer v1.6 (internet). 2014. http://tree.bio.ed.ac.uk/software/tracer/
41. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
42. Minnis AM, Lindner DL. Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biol* 2013;117:638–649.
43. Baral HO. Inoperculate discomycetes. Jaklitsch W, Baral H, Lücking R, Lumbsch H and Frey W (eds). In: *Syllabus of Plant Families: A. Engler's Syllabus der Pflanzenfamilien Part 1/2*. Stuttgart: Borntraeger; 2016. pp. 157–205.
44. Johnston PR, Quijada L, Smith CA, Baral HO, Hosoya T, *et al*. A multigene phylogeny toward a new phylogenetic classification of leotiomycetes. *IMA Fungus* 2019;10:1.
45. LoBuglio KF, Pfister DH. Placement of Medeolaria farlowii in the Leotiomycetes, and comments on sampling within the class. *Mycol Prog* 2010;9:361–368.
46. Wang Z, Binder M, Schoch CL, Johnston PR, Spatafora JW, *et al*. Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): A nuclear rDNA phylogeny. *Mol Phylogenet Evol* 2006;41:295–312.
47. Wang Z, Johnston PR, Yang ZL, Townsend JP. Evolution of reproductive morphology in leaf endophytes. *Plos One* 2009;4:e4246.
48. Tedersoo L, Pärtel K, Jairus T, Gates G, Põldmaa K, *et al*. Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. *Environ Microbiol* 2009;11:3166–3178.
49. U'Ren JM, Lutzoni F, Miadlkowska J, Arnold AE. Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. *Microb Ecol* 2010;60:340–353.
50. Ruibal C, Gueidan C, Selbmann L, Gorbushina AA, Crous PW, *et al*. Phylogeny of rock-inhabiting fungi related to Dothideomycetes. *Stud Mycol* 2009;64:123–133.
51. Ertz D, Lawrey JD, Common RS, Diederich P. Molecular data resolve a new order of Arthoniomycetes sister to the primarily lichenized Arthoniales and composed of black yeasts, lichenicolous and rock-inhabiting species. *Fungal Divers* 2014;66:113–137.
52. Zupančič J, Novak Babič M, Zalar P, Gunde-Cimerman N. The black yeast *Exophiala dermatitidis* and other selected opportunistic human pathogens spread from dishwashers to kitchens. *Plos One* 2016;11:e0148166.
53. Réblová M, Hubka V, Thureborn O, Lundberg J, Sallstedt T, *et al*. From the tunnels into the treetops: New lineages of black yeasts from biofilm in the Stockholm metro system and their relatives among ant-associated fungi in the Chaetothyriales. *Plos One* 2016;11:e0163396.
54. Teixeira MM, Moreno LF, Stielow BJ, Muszewska A, Hainaut M, *et al*. Exploring the genomic diversity of black yeasts and relatives (*Chaetothyriales, Ascomycota*). *Stud Mycol* 2017;86:1–28.
55. Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL, Madrid H, *et al*. Notes for genera: Ascomycota. *Fungal Divers* 2017;86:1–594.
56. Baral HO, Weber E, Marson G, Quijada L. A new connection between wood saprobism and beetle endosymbiosis: the rarely reported saprobic discomycete *Tromeropsis* is congeneric with the symbiotic yeast *Symbiotaphrina* (*Symbiotaphrinales, Xylonomycetes*) and two asexual morphs misplaced in *Hyphozyma*. *Mycol Prog* 2018;17:215–254.

57. Fryar SC, Haelewaters D, Catcheside DE. *Annabella australiensis* gen. & sp. nov. (*Helotiales, Cordieritidaceae*) from South Australian mangroves. *Mycol Prog* 2019;18:973–981.
58. Jones EBG, Suetrong S, Sakayaroj J, Bahkali AH, Abdel-Wahab MA, *et al*. Classification of marine *Ascomycota, Basidiomycota, Blastocladiomycota* and *Chytridiomycota*. *Fungal Divers* 2015;73:1–72.
59. Landvik S, Kristiansen R, Schumacher T. Phylogenetic and structural studies in the *Thelebolaceae* (*Ascomycota*). *Mycoscience* 1998;39:49–56.
60. de Hoog GS, Gottlich E, Platas G, Genilloud O, Leotta G, *et al*. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Stud Mycol* 2005;51:33–76.
61. Robinson CH. Cold adaptation in Arctic and Antarctic fungi. *New Phytol* 2001;151:341–353.
62. de Menezes GCA, Godinho VM, Porto BA, Gonçalves VN, Rosa LH, *et al*. *Antarctomyces pellizariae* sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus from Antarctica. *Extremophiles* 2017;21:259–269.
63. Batista TM, Hilario HO, de Brito GAM, Moreira RG, Furtado C, *et al*. Whole-genome sequencing of the endemic Antarctic fungus *Antarctomyces pellizariae* reveals an ice-binding protein, a scarce set of secondary metabolites gene clusters and provides insights on Thelebolales phylogeny. *Genomics* 2020;112:2915–2921.
64. Peterson RA, Bradner JR, Roberts TH, Nevalainen KMH. Fungi from koala (*Phascolarctos cinereus*) faeces exhibit a broad range of enzyme activities against recalcitrant substrates. *Lett Appl Microbiol* 2009;48:218–225.
65. Zalar P, Gostinčar C, de Hoog GS, Uršič V, Sudhadham M, *et al*. Redefinition of *Aureobasidium pullulans* and its varieties. *Stud Mycol* 2008;61:21–38.
66. Bills GF, Menéndez VG, Platas G. *Kabatiella bupleuri* sp. nov. (*Dothideales*), a pleomorphic epiphyte and endophyte of the Mediterranean plant *Bupleurum gibraltarium* (*Apiaceae*). *Mycologia* 2012;104:962–973.
67. Boekhout T, Bai FY, Daniel HM, Groenewald M, Robert V, *et al*. The yeasts trust database [Internet]. 2020. https://theyeasts.org/
68. Pechak DG, Crang RE. An analysis of *Aureobasidium pullulans* developmental stages by means of scanning electron microscopy. *Mycologia* 1977;69:783–792.
69. Stchigel AM, Cano J, Mac Cormack W, Guarro J. *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. *Mycol Res* 2001;105:377–382.
70. Vu D, Groenewald M, Vries M, Gehrmann T, Stielow B, *et al*. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Stud Mycol* 2019;92:135–154.
71. Suh SO, Blackwell M. Molecular phylogeny of the cleistothecial fungi placed in Cephalothecaceae and Pseudeurotiaceae. *Mycologia* 1999;91:836–848.
72. Malloch D, Sigler L, Hambleton S, Vanderwolf KJ, Gibas CFC, *et al*. Fungi associated with hibernating bats in New Brunswick caves: the genus *Leuconeurospora*. *Botany* 2016;94:1171–1181.
73. Schoch CL, Sung GH, López-Giráldez F, Townsend JP, Miadlikowska J, *et al*. The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Syst Biol* 2009;58:224–239.
74. Sugiyama M, Ohara A, Mikawa T. Molecular phylogeny of onygenalean fungi based on small subunit ribosomal DNA (SSU rDNA) sequences. *Mycoscience* 1999;40:251–258.
75. Schoch CL, Robbertse B, Robert V, Vu D, Cardinali G, *et al*. Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. *Database* 2014;2014.
76. Rice AV, Currah RS. Two new species of *Pseudogymnoascus* with geomyces anamorphs and their phylogenetic relationship with *Gymnostellatospora*. *Mycologia* 2006;98:307–318.
77. Pärtel K, Baral HO, Tamm H, Põldmaa K. Evidence for the polyphyly of *Encoelia* and Encoelioideae with reconsideration of respective families in Leotiomycetes. *Fungal Divers* 2017;82:183–219.
78. Untereiner WA, Yue Q, Chen L, Li Y, Bills GF, *et al*. *Phialophora* section *Catenulatae* disassembled: New genera, species, and combinations and a new family encompassing taxa with cleistothecial ascomata and phialidic asexual states. *Mycologia* 2019;111:998–1027.
79. Sogonov MV, Schroers HJ, Gams W, Dijksterhuis J, Summerbell RC. The hyphomycete *Teberdinia hygrophila* gen. nov., sp. nov. and related anamorphs of Pseudeurotium species. *Mycologia* 2005;97:695–709.
80. Lorch JM, Lindner DL, Gargas A, Muller LK, Minnis AM, *et al*. A culture-based survey of fungi in soil from bat hibernacula in the Eastern United States and its implications for detection of *Geomyces destructans*, the causal agent of bat white-nose syndrome. *Mycologia* 2013;105:237–252.
81. Spatafora JW, Sung GH, Johnson D, Hesse C, O'Rourke B, *et al*. A five-gene phylogeny of Pezizomycotina. *Mycologia* 2006;98:1018–1028.
82. Hyde KD, Norphanphoun C, Abreu VP, Bazzicalupo A, Chethana KT, *et al*. Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. *Fungal Divers* 2017;87:1–235.
83. Bovio E, Garzoli L, Poli A, Prigione V, Firsova D, *et al*. The culturable mycobiota associated with three atlantic sponges, including two new species: *Thelebolus balaustiformis* and *T. Spongiae*. *Fungal Syst Evol* 2018;1:141–167.