



Peribolosporomycetes class. nov.: description of a new heat resistant and osmotolerant basidiomycete lineage, represented by *Peribolospora* gen. nov., *P. kevripleyi* sp. nov., and *P. baueri* sp. nov.

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Abstract

Heat resistance is the ability to survive short, extreme temperature stresses, exceeding the own growth temperature by far. Despite their occurrence in natural substrates and their relevance for the food and healthcare industry, the diversity of fungi with heat resistance abilities is poorly studied. Sampling of boreal forest soils in Canada in combination with a heat-shock treatment (75 °C, 30 min) yielded, among others, four heat resistant, mesophilic fungal isolates. Based on rDNA barcode sequences, the novel isolates were assigned to *Basidiomycota*. In this study, we use macromorphological and micromorphological observations, cultivation assays and comparative genomics for physiological characterization, interspecific differentiation, and phylogenetic placement of these isolates. A phylogeny of 38 single-copy orthologous genes, an orthology analysis, and septal pore type analysis revealed the isolates as representatives of two new basidiomycetous species of the novel class *Peribolosporomycetes*, a sister lineage to all other members of *Ustilaginomycotina*. Further genomic and phenotypic data support two different species (*Peribolospora kevripleyi*, *Peribolospora baueri*), which are heat resistant and osmotolerant.

Keywords *Basidiomycota*₁ · *Ustilaginomycotina*₂ · *Peribolosporomycetes*₃ · *Peribolospora*₄ · Heat resistance₅ · Chlamydospores₆

Introduction

Several ecosystems and habitats are characterized by spontaneous or reoccurring high temperatures. The increasing frequency and intensity of heat waves due to climate change, common phenomena like forest or bush fires in

natural habitats, and the application of slash-and-burn methods in agriculture and forestry, flare pits in the oil and gas industry, or even the thermal changes during the process of composting cause heat stress to organisms in the respective ecosystems. Thus, substrates like soil (Piecková et al. 2020), plant debris, or compost (Witfeld et al. 2021),

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which are exposed to such fluctuating temperatures are often inhabited by fungi with high temperature adaptations, having a potential ecological advantage towards less heat resistant counterparts.

Most fungi are adapted to moderate temperatures up to maximum 40 °C, while some heat resistant species show remarkable resistance to temporary, extremely high temperatures. The physiological trait of heat resistance can be described as a function of stress intensity and time. Usually, a fungus can survive less intense heat-stresses for longer time periods, while extremely high temperatures can only be survived for shorter durations. For example, *Aspergillus niger* is reported to survive exposure to 60 °C for 60 min but shows no survival when exposed to 70 °C for 10 min (Jesenská et al. 1993). Due to this interaction of the two factors temperature and time, Samson et al. (2004) defined heat resistant fungi as such, which survive 75 °C for at least 30 min. And although survival of even higher temperatures is known for some fungi, e.g., *Talaromyces avelaneus* survives 90 °C for 10 min (Jesenská et al. 1993), this definition has been widely accepted and applied in the description and isolation of heat resistant fungi (Houbraken and Samson 2006; Yaguchi et al. 2012; Nguyen et al. 2013; Day et al. 2020; Ulusoy et al. 2022). Fungal heat resistance can be derived by various physiological and morphological adaptations, including especially heat-stable cell types and structures. The most heat resistant fungal structures known so far are thick-walled ascospores (Conner and Beuchat 1987; Dijksterhuis and Teunissen 2004; Dijksterhuis 2007; Rachon et al. 2021). Other examples are heat resistant sclerotia (Hind-Lanoiselet et al. 2005) and chlamydospores with thickened cell walls (Seifert et al. 2004). Moreover, heat and stress resistance of these fungal structures can be increased by the production and accumulation of compatible solutes with thermo-protective function, like glycerol, mannitol, trehalose, or trehalose-based oligosaccharides (Dijksterhuis 2007; Wyatt et al. 2013). Besides the synthesis of cell-stabilizing compounds, heat resistant fungi can produce toxic secondary metabolites (Tournas 1994; Puel et al. 2005), also called mycotoxins. Producers of such are, e.g., *Byssochlamys* species, which are among the most common fungal contaminants of heat processed food products (e.g., tomato paste, apple juice) (Tournas 1994; Kotzekidou 1997; Salomão et al. 2014; Santos et al. 2018). Therefore, heat resistant fungi represent a great concern for the food and healthcare industry. The natural occurrence of this ubiquitous and saprophytic soil fungi in agricultural substrates often leads to contamination of fruits and crops. Due to their increased stress and heat resistance, high-temperature treatments during food processing are insufficient to remove the contaminating fungi, or even trigger an activation of resting propagules (Dijksterhuis and Teunissen 2004). Subsequently, the occurrence and growth in food products and the

production of toxins lead to economic losses (Piecková et al. 2020) and exposure of customers to potentially opportunistic pathogens and respective toxins (Yang et al. 2014).

While the known diversity of heat resistant fungi comprises predominantly soil inhabiting and food spoiling ascomycetes, descriptions of stress- and heat resistant basidiomycetes are rare. Despite the great diversity of the *Agaricomycotina* and *Ustilaginomycotina* lineages (Hibbett 2006; Begerow et al. 2014), most records of stress-resistant basidiomycetes account to the small subphylum of *Walle-miomycotina*. This group contains the two orders *Geminibasidiales* and *Wallemiales*, and its phylogenetic position is recently discussed, e.g., to retain the lineage of *Walle-miomycetes* within the *Agaricomycotina* or to represent a fourth subphylum of the *Basidiomycota* (Mishra et al. 2018, Naranjo-Ortiz and Gabaldón 2019). The species described for the *Wallemiales* were isolated from hypersaline environments (e.g., salted cod, solar salterns, sea water), sweets, soil, and air (Zalar et al. 2005), and include some of the most halophilic, osmophilic, and xerotolerant fungal species worldwide (Zajc and Gunde-Cimerman 2018). The few described species for the *Geminibasidiales* are exclusively soil-borne fungi and are moderately heat resistant and xerotolerant (Nguyen et al. 2013; Nasr et al. 2014). As seen in those taxa, physiological resistance often includes the resilience to multiple abiotic factors simultaneously like heat, drought, salinity, or osmolarity (Nguyen et al. 2013; Zajc and Gunde-Cimerman 2018). However, research on the diversity of stress- and heat resistant basidiomycetes, their evolution, and the ecological roles and physiological mechanisms is still underrepresented.

In this study, we describe and characterize four novel basidiomycetous mesophilic isolates with heat resistance abilities. The isolates were obtained from forest soils in Canada (Nova Scotia) by following a commonly recommended procedure to isolate heat resistant fungi from natural substrates (Seifert et al. 2004). BLAST searches using the ITS sequences of the isolates against the GlobalFungi database (Větrovský et al. 2020) and NCBI led to few hits of uncultured fungi from environmental samples, taken from forest soils in Australia and Taiwan (Teder-soo et al. 2014) and a forest fire region in Portugal (Buscardo et al. 2015). We provide, based on 38 orthologous single-copy genes using 63 fungal genomes, the phylogenetic hypothesis that these isolates represent a novel lineage named *Peribolosporemycetes* class. nov. within the *Ustilaginomycotina*, as well as two new species: *Peribolospora kevringleyi* sp. nov. and *Peribolospora baueri* sp. nov. Our taxonomic conclusions are supported by additional data from ultrastructure of the septal pore type of *P. kevringleyi*. We performed microscopic and colony observations, as well as scanning electron microscopy to assess the macromorphology and micromorphology and obtain insights into the putative

lifestyle. Additionally, the relationship of the four isolates was studied, by analysis of barcode sequences, microsatellite fingerprinting, and the comparison of orthologous gene clusters with other fungal genomes.

Material and methods

Sampling

Forest soils associated with stands of *Pinaceae* trees were sampled at four locations in Canada (Shelburne, Nova Scotia) in 2001 and 2002 (Online Resource 1). For isolation of heat resistant, mesophilic fungi, a flotation approach with heat-treatment was used (Seifert et al. 2004). In brief, the soil substrate was put in a 0.1% (w/v) proteose peptone solution, heated to 75 °C in a water bath and then incubated at 75 °C for 30 min under constant shaking. Aliquots of the substrate solutions were mixed with melted and cooled (50 °C) potato dextrose agar (PDA, Difco) containing chloramphenicol (40 mg/l) and poured into Petri dishes. The plates were incubated at room temperature (RT) and monitored for growth. Fungal colonies were subcultured and purified from the environmental plates.

Morphological characterization

Macromorphology

For morphological description of cultures, fungal isolates were cultivated on potato dextrose agar (PDA) (39 g/l potato dextrose agar (Carl Roth); pH 6.2), malt extract agar (MEA) (30 g/l malt extract, 3 g peptone, 20 g/l agar; pH 6.2), corn meal agar (CMA) (30 g/l malt extract, 20 g/l agar, cornmeal water (25 g/l cornmeal in tap water at 60 °C overnight, filtered with cloth tissue); pH 6.2) and malt yeast 40% sucrose agar (M40Y) (20 g/l malt extract, 5 g/l yeast extract, 400 g/l sucrose, 15 g/l agar; pH 6.2) for 7 days at 25 °C. Inoculation of plates was performed with spore solutions of defined concentration. To obtain spore solutions, fungal isolates were grown on malt extract agar for 21 days. The cultures were covered with 1% (v/v) Tween 20 and 0.85% (w/v) NaCl solution and spores were carefully detached by scratching with an inoculation loop. The spore solutions were centrifuged (4000 g, 10 min, RT), and the sedimented spores were washed with 0.85% (w/v) NaCl solution. After a second centrifugation, the washed spores were diluted in 0.85% (w/v) NaCl solution and the spore titer was determined and standardized using a counting chamber (Neubauer improved) following the manufacturer's recommendations. Cultures were pictured, and colony sizes were measured using an optical stereo microscope (Zeiss Axio Zoom.V16; Carl Zeiss Microscopy GmbH, Göttingen, Germany), a PlanApo

Z 0.5× objective, and Zen software (Zeiss, Oberkochen, Germany). Diameters of colonies on each growth medium were measured in triplicates. To test further growth characteristics in simulated natural conditions, the two species were grown in pots with twice autoclaved (first: 1 h, 121 °C; second: 25 min, 121 °C) pine forest soil. The substrate was taken from the O- and A-horizon containing both litter (e.g., needles, twigs) and soil. The samples were inoculated with spore solutions, incubated at RT, and growth was observed for three months.

Micromorphology

For assessment of micromorphological structures, fungal isolates were grown on MEA and CMA medium for 2–3 weeks at RT. Scanning electron microscopy (SEM) was performed with a Sigma VP electron microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany) on colony sections. After freezing in liquid nitrogen, samples were sublimated at –90 °C for 8 min, platinum sputtered for 90 s at 30 mA, and recorded with the Zeiss-smartSEM-User-Interface. Measurements were performed with SmartTiffV3 software (v.02.01) from Zeiss. For septal pore type analysis, transmission electron microscopy (TEM) was performed. High-pressure freezing and freeze substitution were conducted as described by Bauer et al. (1995).

To check for indicators of meiosis, the nuclear behavior of the four fungal isolates was studied by using DAPI staining. The isolates were grown in liquid PD medium for up to 1 week on RT. Mycelium was taken with a sterile inoculation loop, mixed with 10 µl of ddH₂O on a glass slide, and the cells were quickly heat fixed by using a Bunsen burner. The slides were cooled down, and 5 µl of 1× DAPI solution was added to the cells, which were afterwards covered by a coverslip. The cells were examined by fluorescence microscopy using an Axioplan microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany).

Physiological characterization

Cardinal growth temperatures of the isolates were tested on PDA and yeast peptone dextrose agar with additional 10% glucose (YPD + 10) (10 g/l yeast extract, 20 g/l peptone, 120 g/l dextrose, 20 g/l agar; pH 6.2) in five replicates with one point inoculation. Plates were inoculated with spore solutions and incubated at 10, 16, 20, 25, 30, and 36 °C (PD) and 25, 27, 30, and 33 °C (YPD + 10). Colonies were photographed after 14 days with a Samsung J5 smartphone, and growth was measured using Image J (Abramoff et al. 2004). Heat resistance of the fungal isolates was investigated with different approaches. First, maximum 3-week-old culture sections with mycelium and spores from CMA medium were transferred into 1.5 ml reaction tubes and heat-treated at 60 °C

and 75 °C for 30 min in a heating shaker. Heated material was spread on PDA, incubated at RT, and examined periodically for growth. Second, soil, inoculated with spore solutions and incubated (RT) for 3 months, was mixed with 0.1% (w/v) protease peptone solution and heated at 75 °C (30 min) in a water bath as described previously. Plating of the solution on PDA was followed by incubation (RT) for 21 days and periodical examination for fungal growth. To test the fermentation abilities of the strains, phenol red assays were performed using liquid media, which contained protease peptone (10 g/l), sodium chloride (5 g/l), phenol red (0.018 g/l), and a single carbon source trehalose, sucrose, or xylose (each 10 g/l). For sterilization, 25 ml of the phenol red carbohydrate broth were filled in test tubes containing an inverted Durham tube and autoclaved (121 °C, 3 min). Each test tube was inoculated with three inoculation loops of freshly grown mycelium, and incubated for three days at RT. The tests were performed in triplicates. The osmotolerance, xerotolerance, and halotolerance of the four isolates was tested on MEA plates with increasing sucrose, sodium chloride (NaCl) and magnesium chloride (MgCl₂) gradients (Nguyen et al. 2013), and glycerol medium (5 g/l peptone, 1 g/l KH₂PO₄, 200 g/l glycerol, 10 g/l glucose, 0.5 g MgSO₄, 16 g/l agar; pH 5.8), similar to DG18 agar (Hocking and Pitt 1980).

Molecular studies

DNA extraction, barcode amplification, and fingerprinting

For genome sequencing, gDNA was extracted from 150 mg mycelium per sample using an adapted cetyl-trimethylammonium-bromide (CTAB) protocol, provided in scope of the 1000 fungal genomes project (Grigoriev et al. 2014) by Kohler et al. (2011), modified from Fulton et al. (1995). Mycelium (150 mg), grown on MEA (2 weeks, RT), was cooled in liquid nitrogen and homogenized with a ball mill (Retsch). Homogenized material was roughly mixed with 5.25 ml CTAB (2%) lysis buffer and incubated at 65 °C for 30 min, under frequent inverting of the tubes. Then, 0.33 volumes of potassium acetate (5 M) were added and samples were incubated on ice (30 min) and centrifuged (40 min, 3.220 g, 4 °C). The supernatant was transferred in a fresh tube and mixed with one volume of chloroform:isoamylalcohol (24:1) and centrifuged again (30 min, 3.220 g, 4 °C). Then, the aqueous phase was mixed with 20 µl RNase A (20 mg/ml) (NEB) and incubated (2 h, 35 °C). For precipitation, the samples were mixed with 1/10 volume of sodium acetate (3 M) and 1 volume isopropanol and incubated at RT (5 min). After centrifugation (30 min, 10.000 g, 4 °C), the supernatant got discarded, and the pellet washed with 0.6 ml EtOH (70%) and centrifuged (10 min, 10.000 g, 4 °C). After air drying (5 min, RT), the DNA pellet was dissolved in

100 µl EDTA TE buffer at 65 °C and stored at – 80 °C for further processing. For barcode analysis and microsatellite fingerprinting, gDNA was extracted by a phenol–chloroform-based approach, as in Witfeld et al. (2021). Amplification of the ITS-rDNA region (Schoch et al. 2012; Stielow et al. 2015) was performed with the primer combination ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), as described in Witfeld et al. (2021). The LSU was amplified using the primers LROR (Cubeta 1991) and LR6 (Vilgalys and Hester 1990). Partial amplification of *RPB1* was performed with the primers *RPB1-af* and *RPB1-cr* (Stiller and Hall 1997; Matheny et al. 2002), the *RPB2* gene was partially amplified with the primers *RPB2-5f* and *RPB2-7cr* (Liu et al. 1999), and partial *MCM7* gene region was amplified using the primers *MCM7-709f* and *MCM7-1348rev* (Schmitt et al. 2009). Amplicons were purified using Exonuclease I and Shrimp alkaline phosphatase (NEB) with 1:5 diluted enzyme concentration and sequenced with a capillary sequencer (3130xl Genetic Analyzer, Applied Biosystems) by the sequencing facility of the Ruhr-University Bochum. Sequences were manually checked and corrected with BioEdit (v.7.0.5.3 28.10.2005) (Hall 1999). For alignments, MEGA X (v. 10.1.8, Build 10200331) was used to perform a multiple sequence alignment by MUSCLE on standard settings. Additionally, sequences were checked using BLASTn against the GenBank database (<https://blast.ncbi.nlm.nih.gov>).

For assessment of the global distribution, ITS barcode sequences of the four isolates were used in a BLAST search in the GlobalFungi Database (Větrovský et al. 2020). For genetic fingerprinting of the isolates, microsatellite-primed PCRs (MSP-PCR) were conducted using the primers (ATG)₅, (GTG)₅ (Lieckfeldt et al. 1993; Vuyst et al. 2008), and (GAC)₅ (Baleiras Couto et al. 1996). The amplicon fingerprints of the different samples were compared by agarose gel electrophoresis with standardized conditions. Barcode sequences of the four isolates were deposited at the European Nucleotide Archive (ENA) database and received accession numbers, which are associated with the study PRJEB50042 (Online Resource 1). Genome assemblies and genome raw reads are publicly available at NCBI (Online Resource 1). The annotated genomes will be provided by request. Cultures of the four isolates were deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) (DSM 113856, DSM 113857, DSM 113858, DSM 113859) and Canadian Collection of Fungal Cultures (DAOMC) (DAOMC 252470, DAOMC 252471, DAOMC 252472, DAOMC 252473). Moreover, dried specimens were deposited in the Canadian National Mycological Herbarium (DAOM) and received respective accession numbers (DAOM 984983, DAOM 984984, DAOM 984985, DAOM 984986) (Online Resource 1).

Genomic analysis

Genome sequencing, assembly, and annotation

The quality and quantity of extracted gDNA were measured with Qbit (Life Technologies, Burlington, Canada). For library preparation, the NxSeq AmpFREE Low DNA Library Kit (Lucigen) was used according to manufacturer conditions. Whole-genome sequencing (2 × 300 bp paired end) was done at the Agriculture and Agri-Food Canada (AAFC) on an Illumina MiSeq. Read quality was assessed with FastQC (v.0.11.8), and Trimmomatic (v.0.39) was applied to remove Illumina adaptors, low quality bases, and reads shorter than 36 bp. Genomes were assembled de novo using SPAdes (v.3.12.0), and assembly statistics were obtained with QUAST (v.5.0.2) (Gurevich et al. 2013). Genome completeness was calculated using BUSCO (v.5.2.2) against the fungal database (v.odb10) (Manni et al. 2021). Average nucleotide identities were calculated by pairwise whole-genome alignment with NUCmer (v3.23) (MUMmer; Kurtz et al. 2004) with the “-mum” parameter. A delta filtering step with “-1” and “-1100” was applied to select 1-to-1 alignments to allow rearrangements and to select a minimal alignment length of 100 bp, respectively. Gene predictions of the genomes and other species were performed with AUGUSTUS (v.3.3.3), trained with *Cryptococcus neoformans* (San Felice) Vuillemin or *Ustilago maydis* (DeCandolle) Corda and for ascomycetes *Schizosaccharomyces pombe* Lindner, *Saccharomyces cerevisiae* (Desmazières) Meyen, *Chaetomium globosum* Kunze, or *Aspergillus fumigatus* Fresenius, as appropriate. Secreted proteins were calculated using SignalP (v.6.0) (Teufel et al. 2022), and tRNAs and CAZymes were predicted using tRNAscan-SE (v.2.0.9) (Chan et al. 2021) and dbCAN2 (v.10) (Zhang et al. 2018), respectively.

Phylogenomics

Genome assemblies of fungal species were obtained from GenBank (<https://www.ncbi.nlm.nih.gov/assembly>) and MycoCosm (<https://mycoCosm.jgi.doe.gov/mycoCosm/home>). Accession numbers are provided (Online Resource 2). Orthologous protein sequences among the *Peribolosporymycetes* genomes and 59 fungal species from the *Basidiomycota* and *Ascomycota* were calculated using OrthoFinder (v.2.5.2) (Emms and Kelly 2019). A set of 38 single-copy orthologous genes was found to be present in all species, and these were individually aligned with MAFFT (v.7.273) (Kato and Standley 2013). These alignments were subsequently concatenated, and a maximum likelihood tree was calculated with RAxML (v.8.2.12) (Stamatakis 2014), using 500 bootstrap replicates, the PROTGAMMAWAG model, 123 as seed number for the parsimony inferences and a

random seed of 321. Shared orthologous genes were calculated with ComplexUpset (v.1.3.1) package (Krassowski et al. 2021) and the “exclusive intersection” option in R (v.4.1.0; R Core Team 2021).

Results

Molecular studies

Illumina (MiSeq) sequencing yielded 4.48–6.29 million paired end reads for each isolate. After quality control and trimming, the reads were used for de novo assemblies. Between 750 and 1288 contigs were obtained for the four genomes (Table 1), and genome coverages were between 89,4X and 112,6X. The genome size of *P. baueri* is 22.7 Mb, while *P. kevripleyi* has a genome size of 23.2 Mb. The GC content varies slightly between the two species (*P. baueri* 58.7%; *P. kevripleyi* 59.1%) but shows a distinctly higher GC content in coding regions than in non-coding regions (Table 1). BUSCO calculated a genome completeness of 92.9% (*P. baueri*) and 92.6% (*P. kevripleyi*). Gene predictions lead to 7054 and 7024 protein-coding genes for *P. baueri* isolates and 7283 and 7224 for *P. kevripleyi*.

Calculations of the similarity of the genomic sequences of the *Peribolosporymycetes* revealed that the similarity between the *P. baueri* isolates (99.98%) and *P. kevripleyi* isolates (99.69%) is distinctly higher than between the species (95.37–95.40%) (Table 2). Maximum likelihood phylogenetic analyses were performed using 38 single-copy orthologous genes of 63 species to resolve the phylogenetic relationship of the *Peribolosporymycetes* isolates within *Basidiomycota* (Fig. 1). The phylogeny indicates that the four isolates represent a new monophyletic lineage, which we named *Peribolosporymycetes* class. nov., and this group is a sister lineage to all classes in *Ustilaginomycotina*. Moreover, the four isolates seem to be members of two closely related sister species, for which we propose the names *Peribolospora baueri* sp. nov. (DAOMC 252473, DAOMC 252471) and *Peribolospora kevripleyi* sp. nov. (DAOMC 252472, DAOMC 252470).

We compared the gene contents of species representing distinct classes within the *Basidiomycota* and the *Ascomycota* with the *Peribolospora* species. We identified orthologous groups (OGs) based on the predicted proteomes of the 63 species used for the phylogeny (Fig. 2). Orthology analyses identified 18,913 OGs for all species used in our data set. From these, 956 OGs were shared between all analyzed classes of both *Ascomycota* and *Basidiomycota*, while in total, 965 genes were shared between all basidiomycetous classes and the *Peribolosporymycetes*. A total of 2,072 OGs were shared among all *Ascomycota*. The number of class-unique OGs varied according to the taxonomic groups.

Table 1 Genome assembly statistics of *Peribolosporomyces* isolates

	<i>P. baueri</i> (DAOMC 252471)	<i>P. baueri</i> (DAOMC 252473)	<i>P. kevringleyi</i> (DAOMC 252472)	<i>P. kevringleyi</i> (DAOMC 252470)
Assembly statistics				
Total assembly size (Mb)	22.7	22.7	23.2	23.2
Number of contigs	776	750	1211	1288
L50 contig (kb)	166.4	201.2	121.3	139.9
GC content (%)	58.7	58.7	59.1	59.1
GC content coding (%)	60.9	60.9	61.2	61.2
GC content non-coding (%)	56.0	56.1	56.5	56.5
BUSCO (%)	92.9	91.9	92.0	92.6
Gene annotation				
Protein-coding genes	7054	7024	7283	7224
Secreted proteins	335	334	355	349
Coding genome (%)	54.8	54.9	55.3	55.2
Average gene size (bp)	1764	1773	1765	1771
Number of tRNA	114	99	103	99
Total CAZymes	163	163	168	171

For instance, 2,734 OGs were solely found in the *Agaricomycetes* (represented by 8 species), 565 OGs only in the *Exobasidiomycetes* (8), and the *Wallemiomycetes* (2) had 233 unique OGs, not shared with other fungal classes. The four *Peribolosporomyces* shared 530 OGs, which were not present in other taxonomic groups. The counts of OGs shared between the *Peribolosporomyces* and the closest related taxa or taxa of putatively similar lifestyle are low. For instance, 41 OGs are shared exclusively with the *Exobasidiomycetes*, two OGs with the *Ustilaginomycetes*, and 9 OGs with the *Geminibasidiomycetes*. Comparison of OGs within the *Peribolospora* species revealed almost no strain specific OGs. However, comparisons between the *Peribolospora* species showed substantial amounts of shared OGs within the species *P. baueri* and *P. kevringleyi*, indicated by the phylogeny (Fig. 1). Within *P. baueri* (DAOMC 252473, DAOMC 252470) 362 unique OGs are shared, while within *P. kevringleyi* (DAOMC 252472, DAOMC 252470) 309 OGs are shared.

The higher genetic similarity within the two isolates of *P. baueri* (DAOMC 252473 and DAOMC 252470) and *P. kevringleyi* (DAOMC 252472, DAOMC 252471) was also

observed in barcode sequence alignments. While the ITS, *MCM7*, *RPB1*, and *RPB2* sequences were highly similar or identical among the two isolates of each species, the similarity between the species was between 97.78% (*RPB1*) and 99.67% (ITS). The LSU sequences were identical for all isolates. Microsatellite-primed PCR-fingerprints revealed no differences between the isolates within one species but showed distinct patterns between the two species *P. baueri* and *P. kevringleyi* (Online Resource 3). Additionally, BLAST searches for mating-type genes and comparative analyses led to the detection of the A2 allele of the pheromone-receptor gene in all four *Peribolospora* strains.

Morphological and physiological studies

Colony growth The *Peribolospora* species show relatively slow growth and a high phenotypic variability (Fig. 3). Young colonies are white or light beige. Older cultures are often gray-brown with purplish tints and tough, sometimes coriaceous, hyphal growth. After 1 week on PDA, flatly grown colonies of *P. baueri* consist of light beige,

Table 2 Average nucleotide identity (%) based on whole-genome pairwise comparisons for the *Peribolosporomyces* isolates

	<i>P. baueri</i> (DAOMC 252471)	<i>P. baueri</i> (DAOMC 252473)	<i>P. kevringleyi</i> (DAOMC 252472)	<i>P. kevringleyi</i> (DAOMC 252470)
<i>P. baueri</i> (DAOMC 252471)				
<i>P. baueri</i> (DAOMC 252473)	99.98			
<i>P. kevringleyi</i> (DAOMC 252472)	95.39	95.37		
<i>P. kevringleyi</i> (DAOMC 252470)	95.40	95.37	99.69	

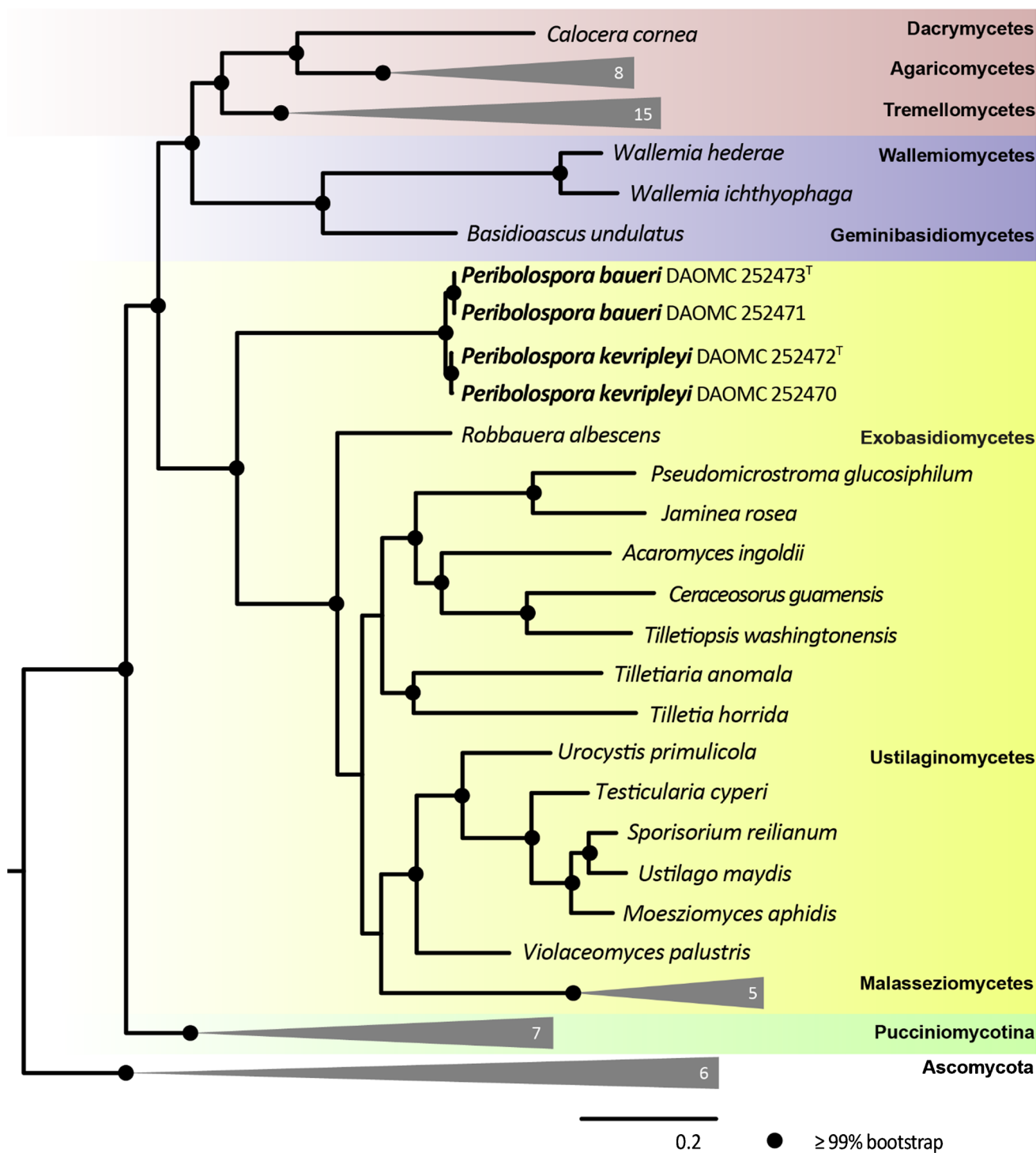


Fig. 1 Phylogenetic position of the *Peribolosporomycetes* and *Peribolospora* species within *Basidiomycota*. The phylogenetic position of the isolates was calculated based on 38 single-copy orthologous protein coding genes. The number of species included in collapsed groups are indicated by white numbers. Lineages are color-coded and correspond to the subphyla of *Agaricomycotina* (red), *Wallemiomycotina* (purple), *Ustilaginomycotina* (yellow), and *Pucciniomycotina* (green). *Ascomycota* (white) was used as the outgroup. Nodes with a bootstrap of ≥99% support are indicated by a black dot. Scale bar represents the number of substituted aminoacids per site. A phylogeny without collapsed groups (Online Resource 6), strain IDs of used species (Online Resource 2), and the original alignment file are provided (Online Resource 7)

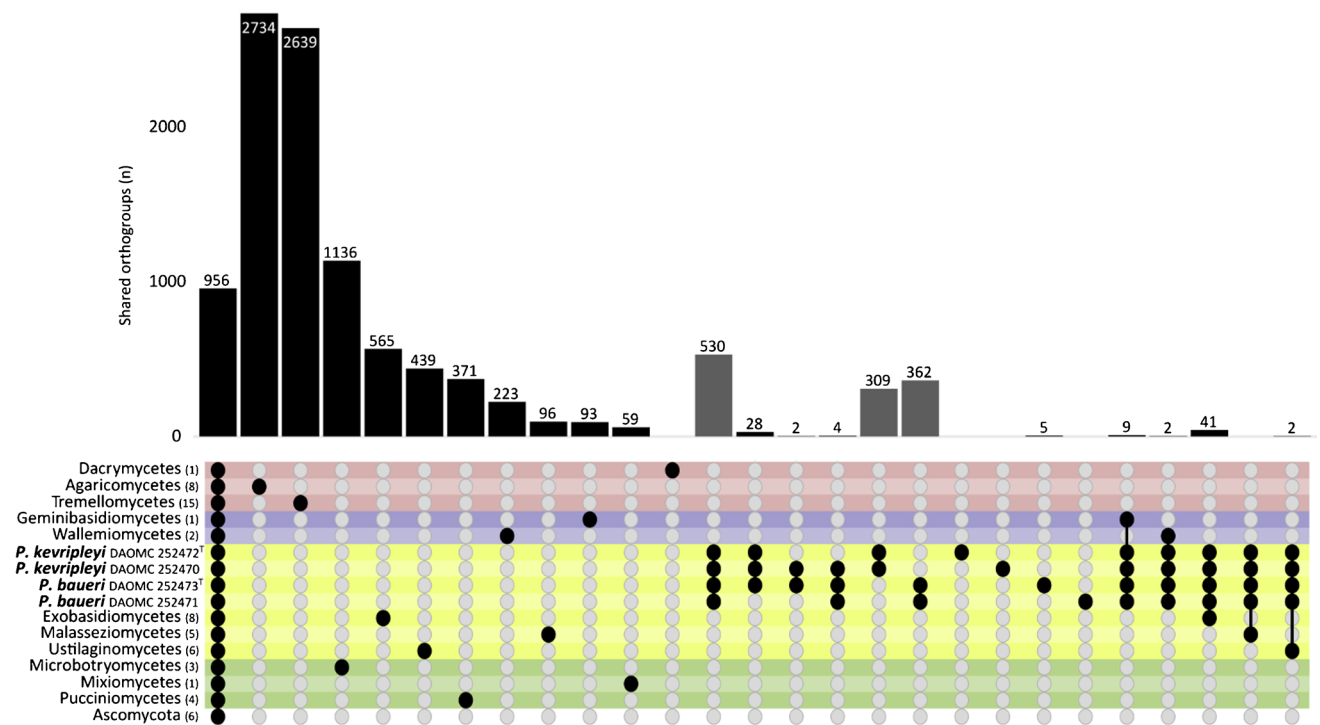


Fig. 2 Orthology analysis of the *Peribolospora* species, representing the *Peribolosporomycetes*, and related fungal lineages. Total number of orthogroups shared among datasets are indicated by bars. Bars representing orthogroups exclusively found in the *Peribolospora* isolates are highlighted in gray. Background colors correspond to *Agaricomycotina*

(red), *Wallemiomycotina* (purple), *Ustilaginomycotina* (yellow), *Pucciniomycotina* (green), and *Ascomycota* (white). Taxonomic groups sharing a specific set of orthologous genes are highlighted by black dots, while not included taxa are grayed out. The number of species included in each group is indicated after the lineage name

agglutinated hyphae, while *P. kevringleyi* colonies show more centrally convex growth. On MEA and CMA, *P. baueri* appears gray and *P. kevringleyi* gray-brownish. Characteristically, both species depict convex and grooved rosettes, and especially for *P. kevringleyi*, the sulcate growth continues peripherally. On M40Y, both species appear as flat-growing white hyphae, with *P. kevringleyi* showing centrally denser growth.

Abiotic stress resistance Growth on M40Y indicated osmotolerance for both *Peribolospora* species, but growth on DG18-like glycerol medium was strongly reduced and no growth was observed for *P. kevringleyi* strain DAOMC 252472. Addition of different compounds, which lower the water activity (a_w) of a medium, was used to assess the degree of xerotolerance, osmotolerance and halotolerance of the *Peribolospora* species (Fig. 4). Increasing amounts of sodium chloride (NaCl) and glycerol strongly inhibited growth of *P. baueri* and *P. kevringleyi*. In comparison, better growth on sodium chloride was observed up to an a_w of 0.95 (7.8% NaCl, 1.3 M), while on glycerol, growth was slower and observed up to an a_w of 0.98 (16.5% glycerol, 1.8 M). In contrast to that, reduction of the a_w by addition of sucrose had a positive effect on the growth of both *Peribolospora*

species, up to an a_w of 0.95 (26% sucrose, 0.7 M), and growth on sucrose was observed up to a concentration of 60.8% (1.8 M), respectively, an a_w of 0.87. Both species showed growth on media with tested pH values between 3.5 and 9. Thereby, growth was only marginally affected by pH values between 4.5 and 7.5, and growth at the rather extreme pH values 3.5 and 9 was slower, but not weakened. Notably, only a small growth reduction of *P. kevringleyi* strain DAOMC 252470 was observed at pH 9, while the other strains showed clearly inhibited growth.

Temperature adaptation Growth of *P. baueri* and *P. kevringleyi* was observed from 10 to 35 °C. Growth of *Peribolospora kevringleyi* strain DAOMC 252472 was observed up to 36 °C. On PDA, the optimum temperature of all isolates is between 25 and 30 °C. Therefore, all *Peribolospora* isolates are considered as mesophilic. Heat resistance tests (75 °C, 60 °C; 30 min) using spore solutions or stamped mycelia from 3-week-old solid-state cultivations failed. However, for both *P. baueri* and *P. kevringleyi* (DAOMC 252473, DAOMC 252470), cultures which grew up to 3 months on sterilized pine forest soil with a pH of 3.65 (Fig. 5) survived the heat-treatment at 75 °C (30 min), were re-isolated from the soil substrate, and regrew on PDA.

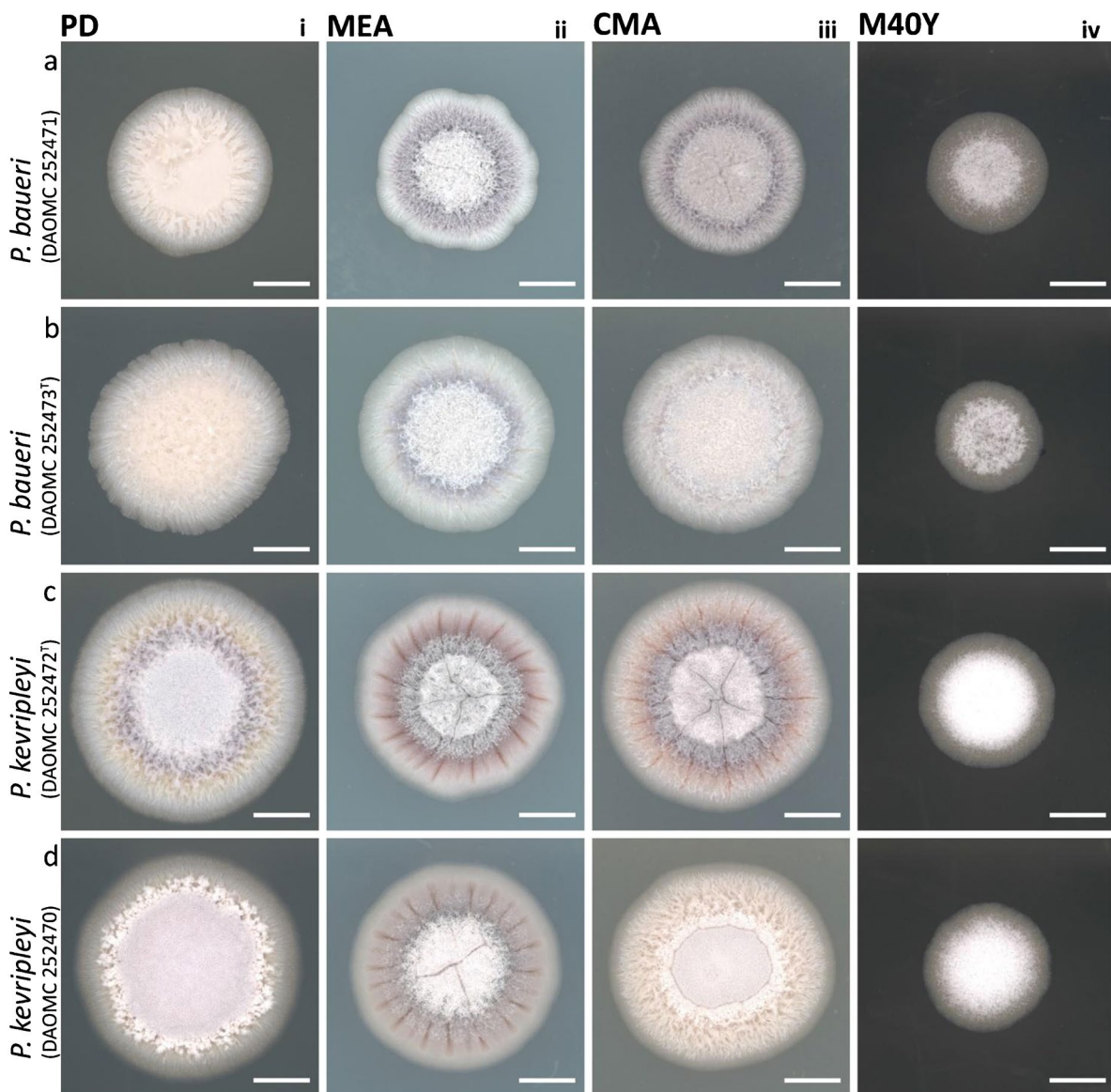


Fig. 3 Colonies of *Peribolospora baueri* and *Peribolospora kevripleyi*. **a–b** Colonies of *P. baueri* strains (DAOMC 252471, DAOMC 252473) on PDA, MEA, CMA, and M40Y after 7 days at 25 °C. **c–d**

Colonies of *P. kevripleyi* strains (DAOMC 252472, DAOMC 252470) on PDA, MEA, CMA, and M40Y after 7 days at 25 °C. Scale bars = 5 mm

Spore morphology Chlamydospores and conidia were observed for cultures grown on corn meal agar (CMA), malt extract agar (MEA) with or without addition of sucrose for at least 2 weeks (Fig. 7), and on pine forest soils after 3 months (Fig. 5). Micromorphological features of *Peribolospora baueri* and *Peribolospora kevripleyi* are highly similar. In both species, chlamydospores are terminally produced and are carried by a coiled hypha, often wrapping around the fully developed spore. Characteristically, mature chlamydospores are hyaline, triangular, pear-like shaped with a narrow proximal side and broadening distally (Fig. 8). Chlamydospores of *P. baueri* had a length of $7.2\text{--}11.4\ \mu\text{m}$ (mean \pm SE = 9.0 ± 0.8) and a width of

$7\text{--}11.4\ \mu\text{m}$ (mean \pm SE = 9.4 ± 0.4), while the measurements for *P. kevripleyi* were $7.6\text{--}11.7\ \mu\text{m}$ (mean \pm SE = 9.8 ± 0.9) and $7.1\text{--}10.2\ \mu\text{m}$ (mean \pm SE = 8.7 ± 0.7). Both species produce ovoid or cylindrical, petiolate conidia, sometimes with apical scars after bearing secondary conidia (Fig. 6; Fig. 8; Online Resource 4). Primary conidia of *P. baueri* are $4.3\text{--}9.7\ \mu\text{m}$ (mean \pm SE = 6.6 ± 1.3) in length and $2\text{--}3\ \mu\text{m}$ (mean \pm SE = 2.4 ± 0.2) in width, while *P. kevripleyi* primary conidia have a size of $4.3\text{--}13.7\ \mu\text{m}$ (mean \pm SE = 7 ± 2) and $1.8\text{--}3.8\ \mu\text{m}$ (mean \pm SE = 2.4 ± 0.4). Secondary conidia of *P. baueri* have a length of $2.6\text{--}5\ \mu\text{m}$ (mean \pm SE = 3.7 ± 0.5) and a width of $1.9\text{--}3.4\ \mu\text{m}$ (mean \pm SE = 2.5 ± 0.4), and secondary conidia of *P.*

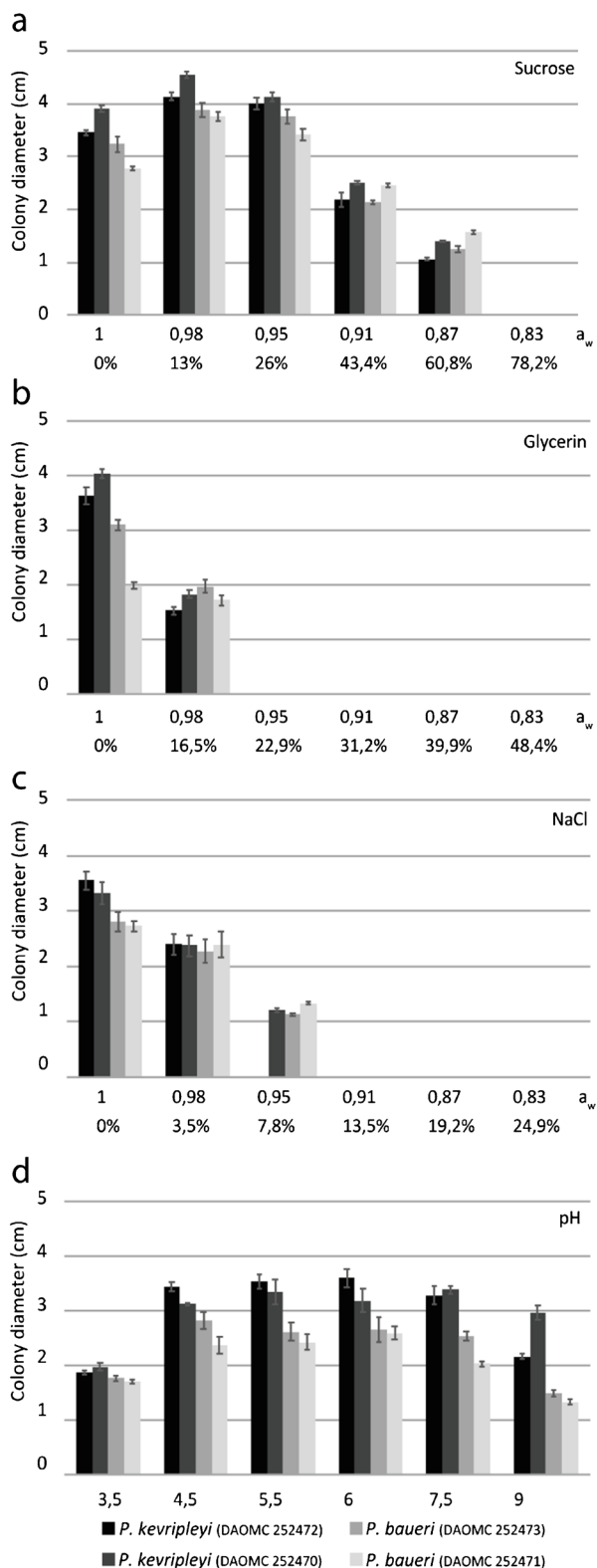


Fig. 4 Growth of *Peribolospora baueri* and *Peribolospora kevrileyi* at different water activities and pH. Water activities between 1.00 and 0.83 were reached by adding defined amounts of **a** sucrose, **b** sodium chloride, and **c** glycerin to the media. **d** Growth on pH 3.5–9. Cultures were incubated at room temperature for 14 days

kevrileyi have a size of 2–5.1 μm (mean \pm SE = 3.4 ± 0.6) length and 1.8–3.2 μm (mean \pm SE = 2.2 ± 0.3) width. Nuclear staining and fluorescence microscopy of the micro-morphological structures did not lead to the observation of any indicators of meiosis.

Septal pore morphology The septal pores of *P. kevrileyi* (DAOMC 252470) were observed with transmission electron microscopy, as a representative for all *Peribolosporomycetes* (Fig. 7). *P. kevrileyi* has relatively simple pore type characteristics. Septum membranes, adjacent to the pore openings, are not thickened, but unequally layered. Parenthesomes, forming distinct outer septal pore caps or tubular extensions, are absent. However, low contrasted elements margin the pore openings and could be related to the septal pore functions. Additionally, supposedly non-membranous areas of increased electron-density are visible in the pore openings and occasionally slightly extend towards the cytoplasm of connected cells (Fig. 7A, C). The imaged septal pores of *P. kevrileyi* had widths between 0.06 and 0.09 μm .

Taxonomy

Peribolosporomycetes Witfeld, M. A. Guerreiro, H.D.T. Nguyen, Begerow, class. nov.

Mycobank: 843630

Description: Class of mesophilic, heat resistant and osmotolerant basidiomycetes with slow hyphal growth and high phenotypic variability. Characteristically, triangular shaped chlamydospores are distally produced on coiled hyphae. Sympodial, ovoid conidia are produced. Relatively simple septal pores, without thickened septum membranes. Parenthesomes, forming distinct septal pore caps are absent, but low contrasted elements indicate non-membranous structures around the pore openings and within the pore. Based on the analysis of 38 protein coding genes, orthology analysis, and septal pore type analysis, this class is placed within the *Ustilaginomycotina*.

Type species: *Peribolospora baueri*

Peribolosporales Witfeld, M. A. Guerreiro, H.D.T. Nguyen, Begerow, ord. nov.

Mycobank: 843631

Description: According to the characters given above for the class *Peribolosporomycetes*.

Peribolosporaceae Witfeld, M. A. Guerreiro, H.D.T. Nguyen, Begerow, fam. nov.

Mycobank: 843632

Description: According to the characters given above for the class *Peribolosporomycetes*.

Peribolospora Witfeld, M. A. Guerreiro, H.D.T. Nguyen, Begerow, gen. nov.

Mycobank: 842991

Etymology: “Peribolos” refers to the characteristic enclosure or embracement of the chlamydospores (“spora”) by the coiled, spore-carrying hyphae.

Type species: *Peribolospora baueri*

Description: Class of mesophilic, heat resistant, and osmotolerant basidiomycetes with high phenotypic variability. Characteristically, the hyaline, triangular shaped chlamydospores are distally produced on coiled hyphae. Basidiomata or basidiospores are not observed. Sympodial, hyaline, and ovoid conidia are produced. Species have relatively simple septal pores, without thickened septum membranes. Parentheses, forming distinct septal pore caps, are absent, but low contrasted elements indicate non-membranous structures around the pore openings and within the pore. White–gray or beige mycelia are formed by slow growing, young colonies. A high phenotypic variability, depending strongly on cultivation medium and culture age, is observed. In older colonies brown and purplish pigmentation and coriaceous growth of agglutinated hyphae are often observed. Xylose, trehalose, or sucrose are not fermented. Based on the analysis of 38 protein coding genes, orthology analysis, and septal pore type analysis, this class is placed within the *Ustilaginomycotina*.

Notes: The two described species *P. baueri* and *P. kevrileyi* can be identified and distinguished using molecular barcodes (e.g., *RPB1*, *RPB2*, *MCM7*, ITS). Additionally, a reliable differentiation of the two species is possible based on MSP-PCR fingerprinting (e.g., *ATG*₅, *GAC*₅, *GTG*₅) and orthology analysis.

Peribolospora baueri Witfeld, M. A. Guerreiro, H.D.T. Nguyen, Begerow, sp. nov.

Mycobank: 843634

Etymology: Named after the mycologist Robert Bauer for his extensive research in mycology and simple pored fungi.

Type: Canada: Nova Scotia, Shelbourne County, Colquist Road, isolated from soil associated with a stand of *Pinus resinosa* Aiton by a 75 °C heat-shock (30 min) approach, isolation date: Oct 1, 2002. Holotype: *P. baueri* DAOM 984984, deposition as dried culture of DAOMC 252473; Ex-type: *P. baueri* DAOMC 252473. DSMZ: DSM 113857. ENA database: Study: PRJEB50042, ITS: OW455115, LSU: OW455119, Genome assembly: JAMDHS000000000. Strains examined: DAOMC 252473 (ex-type), DAOMC 252471.

Description: Colony growth to a diameter of 1.73 cm ± 0.03 cm on PDA, 1.76 cm ± 0.04 cm on MEA, 1.76 cm ± 0.02 cm on CMA, and 0.99 cm ± 0.02 cm on M40Y after 1 week at RT, was observed. Cardinal temperatures are 10–35 °C, and optimum temperature is between 25 and 30 °C with colony sizes of 3.68 ± 0.21 cm at 30 °C. Increasing pigmentation from white and beige colored young colonies to

gray, gray-brown, or purplish colored mycelia in older colonies. Growth is often coriaceous or shows agglutinated hyphae forming sulcate growing colonies with sharp margins. Chlamydospores and conidia are produced on MEA and CMA after 7–14 days. Chlamydospore development is observed to start with a distally swelling hyphae tip. During chlamydospore formation, spore carrying hyphae increase coiling. Mature, triangular shaped chlamydospores have a length of 7.2–11.4 μm (mean ± SE = 9 ± 0.8) and a width of 7–11.4 μm (mean ± SE = 9.3 ± 0.4). Ovoid or cylindrical conidia are petiolate with apical scars when secondary conidia detached. Primary conidia are 4.3–9.7 μm (mean ± SE = 6.6 ± 1.3) × 2–3 μm (mean ± SE = 2.4 ± 0.2) and secondary conidia are 2.6–5 μm (mean ± SE = 3.6 ± 0.5) × 1.9–3.4 μm (mean ± SE = 2.5 ± 0.4).

Peribolospora kevrileyi Witfeld, M. A. Guerreiro, H.D.T. Nguyen, Begerow sp. nov.

Mycobank: 843633

Etymology: Named after Kevin Ripley, the partner of one of the co-authors (H.D.T. Nguyen) of the study.

Type: Canada: Nova Scotia, Shelbourne County, Colquist Road, isolated from soil associated with a stand of *Pinus banksiana* Lambert by a 75 °C heat-shock (30 min) approach, isolation date: 17 Oct 2001. Holotype: *P. kevrileyi* DAOM 984985, deposition as dried culture of DAOMC 252472; Ex-type: *P. kevrileyi* DAOMC 252472. DSMZ: DSM 113856. ENA database: Study: PRJEB50042 ITS: OW455118, LSU: OW455122, Genome assembly: JAMDHT000000000. Strains examined: DAOMC 252472 (ex-type), DAOMC 252470.

Description: Colony growth to a diameter of 2.10 cm ± 0.06 cm on PDA, 1.81 cm ± 0.04 cm on MEA, 2.06 cm ± 0.02 cm on CMA, and 1.23 cm ± 0.01 cm on M40Y after 1 week at RT was observed. Cardinal temperatures are 10–36 °C, and optimum temperature is between 25 and 30 °C, with colony sizes of 3.18 ± 0.43 cm at 30 °C. Chlamydospore and conidia production on MEA and CMA after 7–14 days. During ontogenesis, increasing colony pigmentation from white and beige young colonies to gray-brown or purplish older colonies. Agglutinated hyphae with the tendency to form coriaceous appearing colonies with sulcate growth and often centrally rosette growth with sharp margins. Formation of chlamydospores begins with swelling hyphae tips and coiling of the spore-carrying hyphae. Mature, triangular shaped chlamydospores have a length of 7.6–11.7 μm (mean ± SE = 9.8 ± 0.9) and a width of 7.1–10.2 μm (mean ± SE = 8.7 ± 0.7). For ovoid or cylindrical, petiolate conidia apical scars are observed when secondary conidia detached. From proximal to distal, primary conidia are 4.3–13.7 μm (mean ± SE = 7 ± 2) and the width varies between 1.8–3.8 μm (mean ± SE = 2.4 ± 0.4). Secondary conidia are 2–5.1 μm (mean ± SE = 3.4 ± 0.6) × 1.8–3.2 μm (mean ± SE = 2.2 ± 0.3).

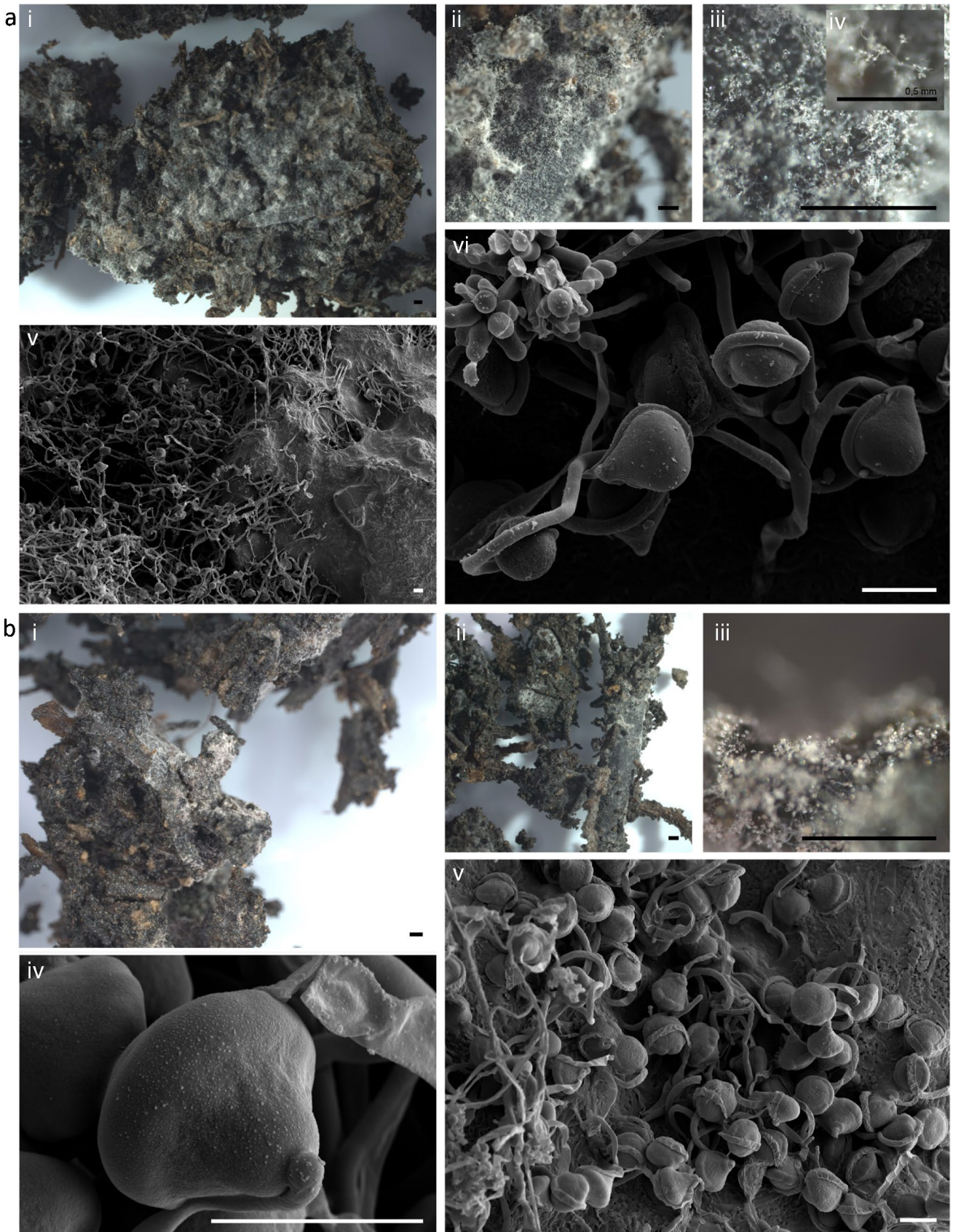


Fig. 5 Growth of *Peribolospora* on sterilized coniferous forest soil. **a** *P. kevripleyi* DAOMC 252472 and **b** *P. baueri* DAOMC 252473 growing on soil samples, including coniferous litter. **a** (i–ii) Growth of *P. kevripleyi* on a piece of wood within the soil sample. (iii–iv) Chlamydo spores and conidiophores of *P. kevripleyi*. (v–vi) SEM pictures of *P. kevripleyi* growing on soil substrate and spore formation. **b** (i–ii) Growth of *P. baueri* on soil samples, including pine needles and wood. (iii) Chlamydo spores and conidiophores of *P. baueri* on soil samples. (iv–v) SEM pictures of *P. baueri* growing on soil samples and chlamydo spores. Inoculated soil samples were incubated (RT) in moderate light for 90 days. Unlabeled black scale bars = 1 mm, white scale bars = 10 μ m

Discussion

A heat treatment approach (Seifert et al. 2004) used on Canadian boreal forest soils led to the isolation of four heat resistant, undescribed fungal strains, which based on molecular barcodes were classified as basidiomycetes. Morphological, physiological, phylogenetic, and genomic analyses provide evidence for the description of a novel class, *Peribolosporomycetes*, and two new species, *Peribolospora baueri* and *P. kevripleyi*.

Heat- and stress-resistance of *Peribolospora* species

Due to their high morphological similarity and overall phenotypic variability (Figs. 3, 6, and 8), we aimed for additional features for species differentiation of *P. baueri* and *P. kevripleyi*. Physiological traits are often used to differentiate morphologically similar and cryptic species (Peterson and Jurjević 2013; Jančić et al. 2015; Peintner et al. 2019). However, the growth temperature ranges of both species are typical for mesophilic fungi and highly similar (Online Resource 5), and the stress resistance to differently lowered water activities is also comparable. Xerotolerance or osmotolerance are the ability to grow at a water activity of 0.85 or lower (Pitt 1975). Both *Peribolospora* species grow on low NaCl concentration up to 7.8% (a_w 0.95) (Fig. 4), which is high for the majority of terrestrial basidiomycetes, while ascomycetes often tolerate higher salt concentrations (Tresner and Hayes 1971; Jaouani et al. 2014). Theoretically, that enables *Peribolospora* to colonize saline soils and share habitats with halotolerant plants, which flourish at sea water salinity of 3.5% NaCl. Nonetheless, the ability to withstand high sucrose concentrations is far more pronounced in both *Peribolospora* species, showing osmotolerant capabilities with growth on M40Y and even up to 60.8% sucrose (a_w 0.87) (Fig. 4). Although several common mechanisms are involved in adaptation to high salinity and sugar concentrations, the lopsided tolerance to high osmotic pressure caused by one of these factors is a common phenomenon in fungi (Kunčić et al. 2010, 2013; Bubnová et al. 2014). The ability to grow at high sugar concentrations is typical for many food spoilage fungi (e.g., *Zygosaccharomyces* or *Xeromyces*) (Snyder et al. 2019). Other traits of spoilage fungi, isolated frequently from industrially processed food products

(e.g., canned fruits, juices), are tolerance of low pH values and heat resistance (Scaramuzza and Berni 2014; Santos et al. 2018). Both, *P. kevripleyi* and *P. baueri* show growth at pH 3 (Fig. 4) and colonized coniferous soils of pH 3.65 in an in vitro experiment (Fig. 5).

While heat treatments (60 °C, 75 °C), using maximum 3-week-old spore solutions and mycelium stamps obtained from *Peribolospora* species grown on PDA were not survived, exposure to a heat treatment of the colonized soil substrate, imitating natural growth conditions and the commonly applied procedure for the isolation of heat resistant fungi (Seifert et al. 2004; Yaguchi et al. 2012; Nguyen et al. 2013), verified the heat resistance of *Peribolospora*. It is well known that the heat resistance abilities of fungi are highly dependent on manifold intrinsic and extrinsic factors. For instance, culture age can positively correlate with the resilience of spores to high temperature stresses (Conner and Beuchat 1987; Dijksterhuis and Teunissen 2004). Conner et al. (1987) demonstrated the increased heat resistance of older spores and the accumulation of thermoprotective sugars, morphological changes of the cell wall, and extractable proteins in aging spores. Further, a dependency between the heat resistance abilities of fungi and the availability of certain solutes (e.g., salts, sugars), organic acids, or the pH value (Bayne and Michener 1979) in the cultivation substrate or the heated test substrate has been observed before (Conner and Beuchat 1987; Engel and Teuber 1991; Tournas 1994). This variation of the heat resistance of a fungus is a major problem in food industry, since different food products require different temperature treatments to successfully inactivate contaminants of the same fungal species (Dijksterhuis 2007).

SEM of the colonized soils revealed exceedingly numerous amounts of chlamydo spores in the samples (Fig. 5). We speculate that either the prolonged cultivation time itself, the low pH value of the soil substrate, or compounds within the soil led to physiological changes during the maturation of the numerous spores, which allowed the survival of the heat treatment at 75 °C for 30 min. The possibility of soil accumulates, which protected the fungal structures from the heat treatment can be excluded, due to the high dilution of the substrate and the constant shaking during the heat exposure. Notably, the trait of heat resistance is more commonly known for ascomycetous fungi, producing extremely heat resistant asco- or chlamydo spores (Seifert et al. 2004; Berni et al. 2017; Biango-Daniels et al. 2019), while descriptions of heat resistant basidiomycetes are rare, with only a few species described so far (Nguyen et al. 2013, 2014). Further investigations (e.g., cell wall composition, thermoprotective compound accumulation, heat-shock proteins) are necessary to elucidate the potential mechanisms behind the heat resistance, presumably derived by heat resistant chlamydo spores of the novel *Peribolospora* species. Also, additional sampling campaigns aiming for mesophilic, heat resistant

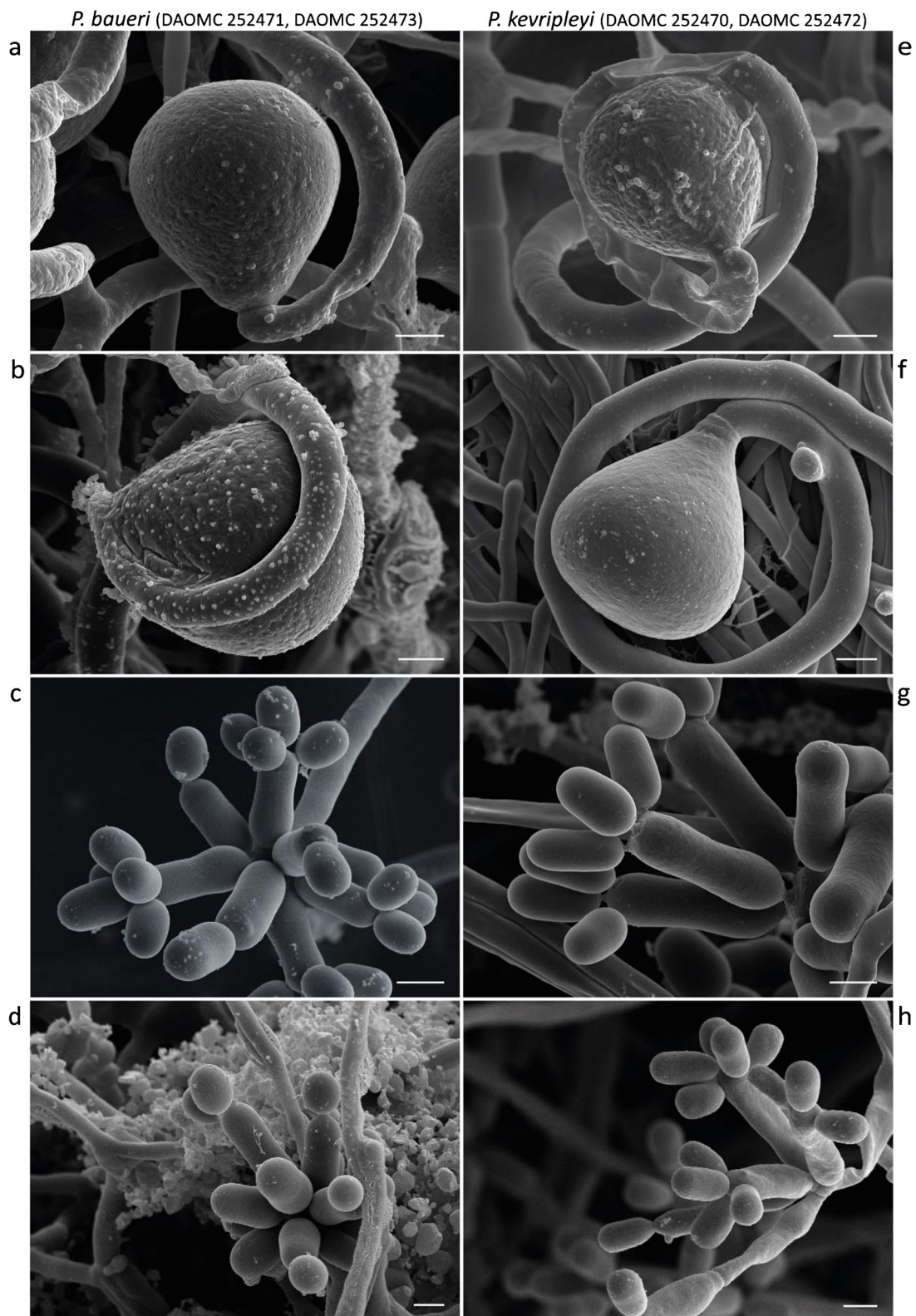


Fig. 6 Chlamydospores and conidiogenous cells with conidia of *Peribolospora baueri* and *Peribolospora kevripleyi*. Scanning electron micrographs of *P. baueri* chlamydospores (**a** DAOMC 252473, **b** DAOMC 252471) and conidiophores (**c** DAOMC 252743, **d** DAOMC

252471), and *P. kevripleyi* chlamydospores (**e** DAOMC 252472, **f** DAOMC 252470) and conidiophores (**g** DAOMC 252472, **h** DAOMC 252470). Cultures were grown for at least 2 weeks on CMA or MEA at RT. Scale bars = 2 μ m

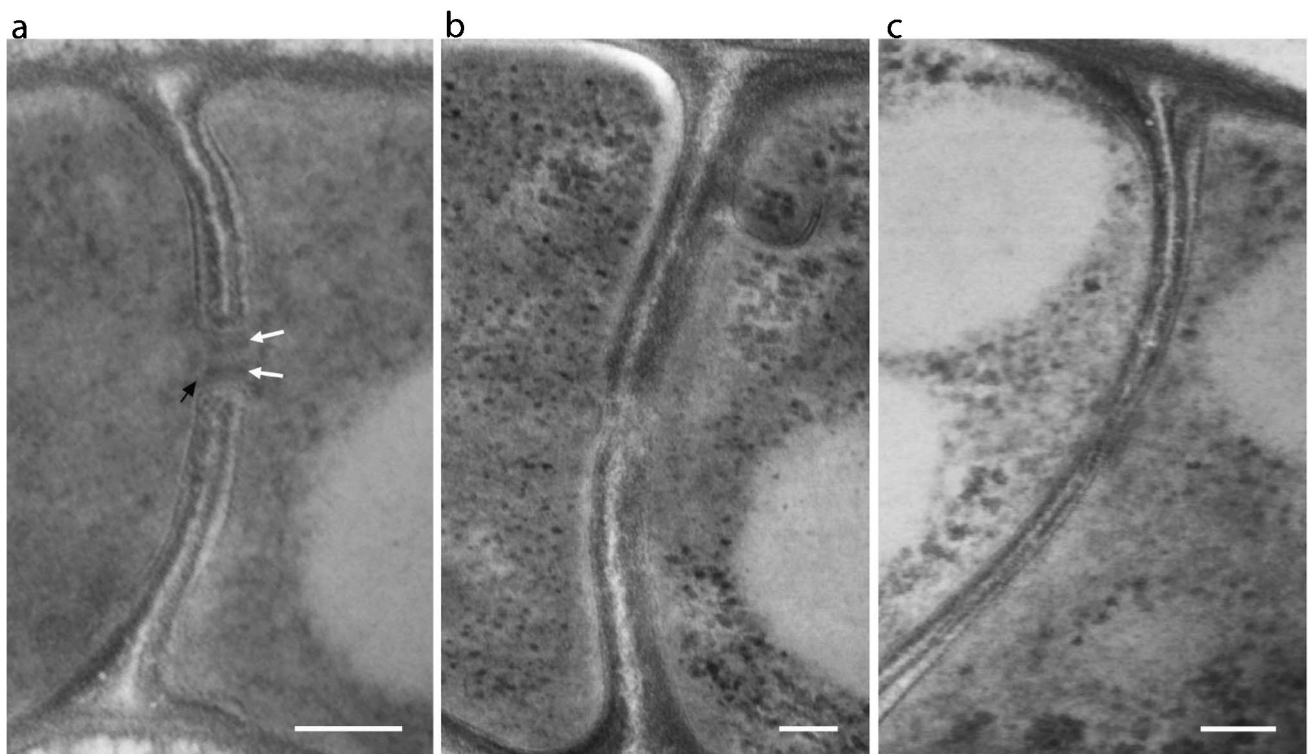


Fig. 7 Septal pore morphology of *Peribolospora*. Transmission electron micrographs of *P. kevrileyi* (DAOMC 252470) septal pores (a–c). A tubular structure, visible in the pore, is indicated by white

arrows. Low contrasted, non-membranous structures around the pore opening are highlighted by a black arrow. Cultures were grown for at least two weeks on CMA or MEA at RT. Scale bars = 100 nm

basidiomycetes might reveal further heat resistant species belonging to the *Peribolosporomycetes*.

Taxonomic position of the new class *Peribolosporomycetes*

The isolates described in this study share certain physiological traits (e.g., heat resistance, pH growth range) with the *Gemini-basidiales* within the subphylum of *Wallemiomycotina*, which is a sister lineage to the *Agaricomycotina* (Naranjo-Ortiz and Gabaldón 2019). Our phylogenetic analysis is congruent with the current knowledge of fungal taxonomy (Spatafora et al. 2017; Naranjo-Ortiz and Gabaldón 2019) and supports the placement of the *Peribolospora* isolates in a sister lineage to all other existing *Ustilaginomycotina* (Fig. 1; Online Resource 6). Additionally, it revealed two different *Peribolospora* species in our study. This is supported by the similarity of analyzed barcode sequences and the identical fingerprints within the *P. baueri* and *P. kevrileyi* isolates (Online Resource 3). Microsatellite-primed PCR fingerprinting has been already successfully used by several studies to differentiate isolates both on species or strain level, leading to the unraveling of genetic diversity and species complexes within the *Ascomycota* (Suh et al. 2013; Ramírez-Castrillón et al. 2014), and differentiation

of basidiomycetous genera (Caldeira et al. 2009). Given that lifestyle adaptations and relatedness are reflected in genomic differences and gene content (Buijs et al. 2021), the orthology analysis further strengthens the creation of new lineage within the *Basidiomycota*, represented by two different species (Figs. 1 and 2). Differentiation of *P. baueri* and *P. kevrileyi* based on their orthologous groups is possible, and each chosen fungal class, as well as the combined *Peribolospora* isolates, had a unique set of orthologs, not shared with species from other groups. Moreover, individual comparisons of the set of orthologs of *Peribolospora* to the other classes revealed no compelling overlap, with the biggest overlap observed for the *Exobasiodiomycetes*. Respectively, this indicates unique genetic traits and a lower degree of relatedness of the *Peribolospora* species towards already existing fungal taxa. To substantiate the idea of a novel lineage within the *Basidiomycota* and to answer the question of the rank of the taxonomic group, for which the *Peribolospora* isolates are representative species, we investigated the septal pore type of a *Peribolospora* isolate (*P. kevrileyi* DAOMC 252470) (Fig. 7). Since a long time, septal pore morphology is essential for the arrangement of the major basidiomycetous taxa (Bauer et al. 1997) and is still used as an important micromorphological marker to solve phylogenetic relationships and describe novel classes and species (Scheuer

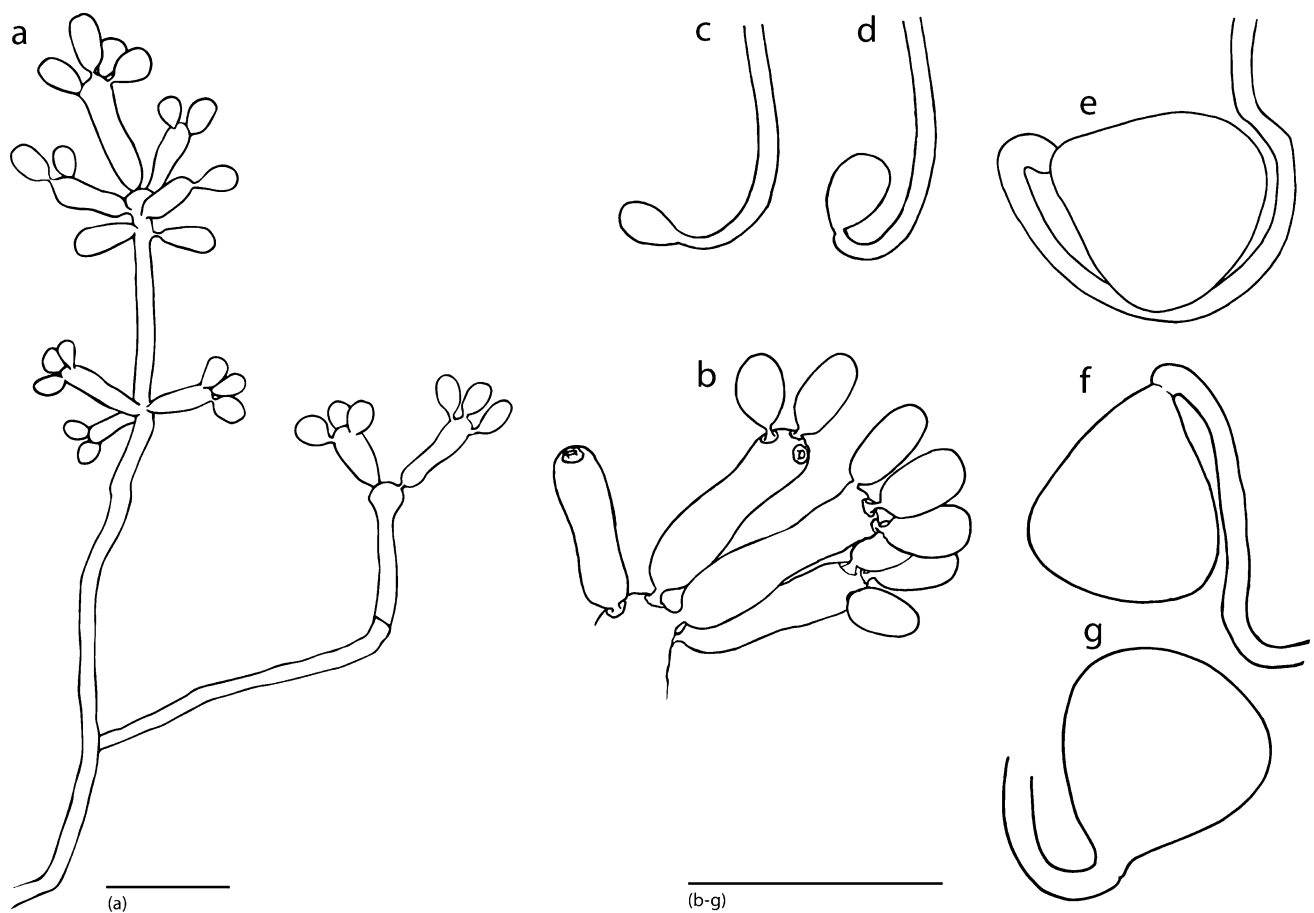


Fig. 8 Micromorphological traits of *Peribolospora*. **a–b** Hyphae and conidiophores with primary and secondary conidia. After conidia release, apical scars are visible. **c–d** Young, developing chlamydo-

spores with an increase in hyphal coiling. **e–g** Fully developed chlamydospores at coiled hyphae. Scale bars = 10 μ m

et al. 2008; Nguyen et al. 2015). The simple septal pore type of *Peribolospora* without membrane swelling differs from the characteristic dolipores found in the *Agaricomycotina* and *Wallemiomycotina* (Van Driel et al. 2009; Nguyen et al. 2015). Instead, *Peribolospora* septal pores resemble rather the morphology of *Ustilaginomycotina* or *Pucciniomycotina* species, which have simple septal pores. Especially the members of the *Ustilaginomycotina* are characterized by outer pore caps and often variably intense, electron-dense areas or bands associated to the pore openings, as frequently described for various taxa in the *Ustilaginomycetes* and *Exobasidiomycetes* (Bauer et al. 1997; de Beer et al. 2006; Lutz et al. 2012). Notably, the septal pores of these classes usually contain very distinct, membranous outer septal pore caps. However, we observed only extremely low contrasted, thin structures in association to the pore opening (Fig. 7), which could be homologous to the septal pore caps, similar to Lutz et al. (2012). In addition, a tubular structure in the pore seems highly similar to the tubular ring described for *Exobasidiomycetes* (Begerow et al. 2014). Therefore, and based on the genetic analyses, further

morphological observations and the physiological properties, we conclude that the *Peribolospora* isolates represent at least a novel class within the *Ustilaginomycotina*, called *Peribolosporomycetes*, and are a sister lineage to the existing classes of *Ustilaginomycotina*. Further, we describe the four *Peribolospora* isolates of our study as two different species (*P. baueri* and *P. kevrileyi*) of the novel genus *Peribolospora*.

In further studies, detailed genome analysis assessing genome composition and structure, functional gene annotation, transcriptomic, and CAZyme analyses could provide insights into the physiological adaptations and the lifestyle of the *Peribolospora* species. With their heat resistance, growth at low pH values, and osmotolerant properties, the *Peribolospora* species represent a novel taxonomic lineage within the *Basidiomycota*. These capabilities could serve as an ecological advantage and be conducive for *Peribolosporomycetes* to successfully live in environments with pronounced physiological stresses. Several BLAST hits for its sequences against fungal databases putatively indicate a global distribution of species related to *Peribolospora*. Notably, all hits were from environmental samples

taken from forest soils, partially in forest fire regions. This supports the idea of forest soils, exposed to severe temperature stresses, as potentially one of the natural habitats of *Peribolosporymyces* and explains the heat resistance as an adaptation to an ecological niche.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11557-023-01879-4>.

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Author contribution DB, HN, and FW designed the study. FW conducted the molecular work, light microscopy, cultivation experiments, and micromorphological drawings. FW conducted the scanning electron microscopy together with undergraduate students, and the transmission electron microscopy, and supported by technicians. MAG and FW analyzed the genomic data. FN prepared the samples for transmission electron microscopy. QMW provided the genome of *Robbauera albescens*. FW wrote the draft manuscript and edited it. All authors read, reviewed, and approved the final version of the manuscript.

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Data availability All data and material generated during this study are included in this manuscript, on its supplementary information, or publicly available on databases or microbial culture collections. The study, including sampling, research work as well as culture and data transfer was conducted in compliance with the Nagoya protocol.

Declarations

Conflict of interest The authors declare no competing interests.

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References

- Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. *Biophotonics Int* 11:36–42
- Baleiras Couto CMM, Eijnsma B, Hofstra H, Huis in't VJH, van der Vossen JM (1996) Evaluation of molecular typing techniques to assign genetic diversity among *Saccharomyces cerevisiae* strains. *Appl Environ Microbiol* 62(1). <https://doi.org/10.1128/aem.62.1.41-46.1996>
- Bauer R, Mendgen K, Oberwinkler F (1995) Septal pore apparatus of the smut *Ustilicystis waldsteiniae*. *Mycologia* 87(1):18–24
- Bauer R, Oberwinkler F, Vánky K (1997) Ultrastructural markers and systematics in smut fungi and allied taxa. *Can J Bot* 75(8):1273–1314. <https://doi.org/10.1139/b97-842>
- Bayne HG, Michener HD (1979) Heat resistance of *Byssoschlamys* ascospores. *Appl Environ Microbiol* 3:449–453
- Begerow D, Schäfer AM, Kellner R, Yurkov A, Kemler M, Oberwinkler F et al (2014) “11 *Ustilaginomycotina*” in systematics and evolution. The *Mycota* (a comprehensive treatise on fungi as experimental systems for basic and applied research), eds. McLaughlin D, Spatafora J (Springer Berlin Heidelberg), pp 295–329 https://doi.org/10.1007/978-3-642-55318-9_11
- Berni E, Tranquillini R, Scaramuzza N, Brutti A, Bernini V (2017) Aspergilli with *Neosartorya*-type ascospores: heat resistance and effect of sugar concentration on growth and spoilage incidence in berry products. *Int J Food Microbiol* 258:81–88. <https://doi.org/10.1016/j.ijfoodmicro.2017.07.008>
- Biango-Daniels MN, Snyder AB, Worobo RW, Hodge KT (2019) Fruit infected with *Paecilomyces niveus*: a source of spoilage inoculum and patulin in apple juice concentrate? *Food Control* 97:81–86. <https://doi.org/10.1016/j.foodcont.2018.10.020>
- Bubnová M, Zemančíková J, Sychrová H (2014) Osmotolerant yeast species differ in basic physiological parameters and in tolerance of non-osmotic stresses. *Yeast* 31(8):309–321. <https://doi.org/10.1002/yea.3024>
- Buijs VA, Groenewald JZ, Haridas S, LaButti KM, Lipzen A, Martin FM et al (2021) Enemy or ally: a genomic approach to elucidate the lifestyle of *Phyllosticta citrichinaensis*. *bioRxiv*. <https://doi.org/10.1101/2021.11.27.470207>
- Buscardo E, Rodríguez-Echeverría S, Freitas H, de Angelis P, Pereira JS, Muller LAH (2015) Contrasting soil fungal communities in Mediterranean pine forests subjected to different wildfire frequencies. *Fungal Divers* 70(1):85–99. <https://doi.org/10.1007/s13225-014-0294-5>
- Caldeira AT, Salvador C, Pinto F, Arteiro JM, Martins MR (2009) MSP-PCR and RAPD molecular biomarkers to characterize *Amanita ponderosa* mushrooms. *Ann Microbiol* 59(3):629–634. <https://doi.org/10.1007/BF03175156>
- Chan PP, Lin BY, Mak AJ, Lowe TM (2021) tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic Acids Res* 49(16):9077–9096. <https://doi.org/10.1093/nar/gkab688>
- Conner DE, Beuchat LR (1987) Heat resistance of ascospores of *Neosartorya fischeri* as affected by sporulation and heating medium. *Int J Food Microbiol* 4(4):303–312. [https://doi.org/10.1016/0168-1605\(87\)90005-5](https://doi.org/10.1016/0168-1605(87)90005-5)
- Conner DE, Beuchat LR, Chang CJ (1987) Age-related changes in ultrastructure and chemical composition associated with changes in heat resistance of *Neosartorya fischeri* ascospores. *Trans Br Mycol Soc* 89(4):539–550. [https://doi.org/10.1016/S0007-1536\(87\)80088-8](https://doi.org/10.1016/S0007-1536(87)80088-8)
- Cubeta MA (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified Ribosomal RNA gene. *Phytopathology* 81(11):1395–1400. <https://doi.org/10.1094/Phyto-81-1395>

- Day NJ, Cumming SG, Dunfield KE, Johnstone JF, Mack MC, Reid KA, Turetsky MR, Walker XJ, Baltzer JL (2020) Identifying functional impacts of heat-resistant fungi on boreal forest recovery after wildfire. *Front For Glob Change* 9(3):68. <https://doi.org/10.3389/ffgc.2020.00068>
- de Beer ZW, Begerow D, Bauer R, Pegg GS, Crous PW, Wingfield MJ (2006) Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. *Stud Mycol* 55:289–298. <https://doi.org/10.3114/sim.55.1.289>
- Dijksterhuis J, Teunissen PG (2004) Dormant ascospores of *Talaromyces macrosporus* are activated to germinate after treatment with ultra high pressure. *J Appl Microbiol* 96(1):162–169. <https://doi.org/10.1046/j.1365-2672.2003.02133.x>
- Dijksterhuis J (2007) Heat resistant ascospores. In: *Mycology series*. CRC Press, pp 115–132
- Dos Santos JL, Samapundo S, Biyikli A, Van Impe J, Akkermans S, Höfte M, Abatih EN, Sant'Ana AS, Devlieghere F (2018) Occurrence, distribution and contamination levels of heat resistant moulds throughout the processing of pasteurized high-acid fruit products. *Int J Food Microbiol* 281:72–81. <https://doi.org/10.1016/j.ijfoodmicro.2018.05.019>
- Emms DM, Kelly S (2019) OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biol* 20(1):1–14. <https://doi.org/10.1186/s13059-019-1832-y>
- Engel G, Teuber M (1991) Heat resistance of ascospores of *Byssoschlamys nivea* in milk and cream. *Int J Food Microbiol* 12(2–3):225–233. [https://doi.org/10.1016/0168-1605\(91\)90073-X](https://doi.org/10.1016/0168-1605(91)90073-X)
- Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Mol Biol Rep* 13:207–209. <https://doi.org/10.1007/BF02670897>
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. *Mol Ecol* 2(2):113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F, Smirnova T (2014) MycoCosm portal: gearing up for 1000 fungal genomes. *Nucleic Acids Res* 42(1):699–704
- Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUAST: quality assessment tool for genome assemblies. *Bioinform* 29(8):1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Hibbett DS (2006) A phylogenetic overview of the *Agaricomycotina*. *Mycologia* 98(6):917–925. <https://doi.org/10.3852/mycologia.98.6.917>
- Hind-Lanoiselet TL, Lanoiselet VM, Lewington FK, Ash GJ, Murray GM (2005) Survival of *Sclerotinia sclerotia* under fire. *Austral Plant Pathol* 34(3):311–317. <https://doi.org/10.1071/AP05048>
- Hocking AD, Pitt JI (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. *Appl Environ Microbiol* 39(3):488–492. <https://doi.org/10.1128/AEM.39.3.488-492.1980>
- Houbraken J, Samson RA (2006) Standardization of methods for detecting heat resistant fungi. In: Hocking AD, Pitt JI, Samson RA, Thrane U (Eds) *Adv Food Mycol. Adv Exp Med Biol*. Springer, pp 107–111. https://doi.org/10.1007/0-387-28391-9_5
- Jančić S, Nguyen HDT, Frisvad JC, Zalar P, Schroers HJ, Seifert KA et al (2015) A taxonomic revision of the *Walleimia sebi* species complex. *PLoS ONE* 10(5):e0125933. <https://doi.org/10.1371/journal.pone.0125933>
- Jaouani A, Neifar M, Prigione V, Ayari A, Sbissi I, Ben Amor S et al (2014) Diversity and enzymatic profiling of halotolerant micromycetes from Sebkhah El Melah, a Saharan salt flat in southern Tunisia. *BioMed Res Int* 1–11. <https://doi.org/10.1155/2014/439197>
- Jesenská Z, Piecková E, Bernát D (1993) Heat resistance of fungi from soil. *Int J Food Microbiol* 19(3):187–192. [https://doi.org/10.1016/0168-1605\(93\)90076-S](https://doi.org/10.1016/0168-1605(93)90076-S)
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30(4):772–780. <https://doi.org/10.1093/molbev/mst010>
- Kohler A, Murat C, Costa M (2011) High quality genomic DNA extraction using CTAB and Qiagen genomic-tip. *Nat Inst Agric Res (INRA)*, France
- Kotzekidou P (1997) Heat resistance of *Byssoschlamys nivea*, *Byssoschlamys fulva* and *Neosartorya fischeri* isolated from canned tomato paste. *J Food Sci* 62(2):410–412. <https://doi.org/10.1111/j.1365-2621.1997.tb04014.x>
- Krassowski M, Arts M, Lagger C (2021) krassowski/complex-upset: V1.3.3. Zenodo. <https://doi.org/10.5281/zenodo.3700590>
- Kunčić MK, Kogej T, Drobne D, Gunde-Cimerman N (2010) Morphological response of the halophilic fungal genus *Walleimia* to high salinity. *Appl Environ Microbiol* 76(1):329–337. <https://doi.org/10.1128/AEM.02318-09>
- Kunčić MK, Zajc J, Drobne D, Tkalec ŽP, Gunde-Cimerman N (2013) Morphological responses to high sugar concentrations differ from adaptation to high salt concentrations in the xerophilic fungi *Walleimia* spp. *Fungal Biol* 117(7–8):466–478. <https://doi.org/10.1016/j.funbio.2013.04.003>
- Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C et al (2004) Versatile and open software for comparing large genomes. *Genome Biol* 5(2):1–9. <https://doi.org/10.1186/gb-2004-5-2-r12>
- Lieckfeldt E, Meyer W, Börner T (1993) Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. *J Basic Microbiol* 33(6):413–425. <https://doi.org/10.1002/jobm.3620330609>
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Mol Biol Evol* 16(12):1799–1808. <https://doi.org/10.1093/oxfordjournals.molbev.a026092>
- Lutz M, Vánky K, Bauer R (2012) *Melanoxa*, a new genus in the *Urocytidales* (*Ustilaginomycotina*). *Mycol Prog* 11(1):149–158. <https://doi.org/10.1007/s11557-010-0737-7>
- Manni M, Berkeley MR, Seppely M, Simão FA, Zdobnov EM (2021) BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol Biol Evol* 38(10):4647–4654. <https://doi.org/10.1093/molbev/msab199>
- Matheny PB, Liu YJ, Ammirati JF, Hall BD (2002) Using *RPB1* sequences to improve phylogenetic inference among mushrooms (*Inocybe*, *Agaricales*). *Am J Bot* 89(4):688–698. <https://doi.org/10.3732/ajb.89.4.688>
- Mishra B, Choi YJ, Thines M (2018) Phylogenomics of *Bartheletia paradoxa* reveals its basal position in *Agaricomycotina* and that the early evolutionary history of basidiomycetes was rapid and probably not strictly bifurcating. *Mycol Prog* 17:333–341. <https://doi.org/10.1007/s11557-017-1349-2>
- Naranjo-Ortiz MA, Gabaldón T (2019) Fungal evolution: diversity, taxonomy and phylogeny of the fungi. *Biol Rev Camb Philos Soc* 94(6):2101–2137. <https://doi.org/10.1111/brv.12550>
- Nasr S, Soudi MR, Nasrabadi SMZ, Nikou MM, Salmanian AH, Nguyen HDT (2014) *Basidioascus persicus* sp. nov., a yeast-like species of the order *Geminibasidiales* isolated from soil. *Int J Syst Evol Microbiol* 64(9):3046–3052. <https://doi.org/10.1099/ijs.0.062380-0>
- Nguyen HDT, Nickerson NL, Seifert KA (2013) *Basidioascus* and *Geminibasidium*: a new lineage of heat resistant and xerotolerant basidiomycetes. *Mycologia* 105(5):1231–1250. <https://doi.org/10.3852/12-351>

- Nguyen HDT, Tanney JB, Chabot D, Nickerson NL, Seifert KA (2014) *Paratritirachium curvibasidium*, a new heat resistant basidiomycete from flare pit soils in Alberta, Canada. *Mycol Prog* 13(3):575–587. <https://doi.org/10.1007/s11557-013-0941-3>
- Nguyen HDT, Chabot D, Hirooka Y, Roberson RW, Seifert KA (2015) *Basidioascus undulatus*: genome, origins, and sexuality. *IMA Fungus* 6(1):215–231. <https://doi.org/10.5598/imafungus.2015.06.01.14>
- Peintner U, Kuhnert-Finkernagel R, Wille V, Biasioli F, Shiryayev A, Perini C (2019) How to resolve cryptic species of polypores: an example in *Fomes*. *IMA Fungus* 10(1):1–21. <https://doi.org/10.1186/s43008-019-0016-4>
- Peterson SW, Jurjević Ž (2013) *Talaromyces columbinus* sp. nov., and genealogical concordance analysis in *Talaromyces* clade 2a. *PLoS ONE* 8(10):e78084. <https://doi.org/10.1371/journal.pone.0078084>
- Piecková E, Lehotská R, Globanová M (2020) Heat resistant fungi, toxicity and their management by nanotechnologies. In: *Nanomycoecotoxicology*. Academic Press, pp 217–237
- Pitt J (1975) Xerophilic fungi and the spoilage of foods of plant origin. In: Duckworth RB (Ed) *Water relations of foods*. Academic Press, London, pp 273–308
- Puel O, Tadrist S, Galtier P, Oswald IP, Delaforge M (2005) *Byssochlamys nivea* as a source of mycophenolic acid. *Appl Environ Microbiol* 71(1):550–553. <https://doi.org/10.1128/aem.71.1.550-553.2005>
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rachon G, Raleigh CP, Pawlowsky K (2021) Heat resistance of yeast ascospores and their utilisation for the validation of pasteurisation processes for beers. *J Inst Brew* 127(2):149–159. <https://doi.org/10.1002/jib.646>
- Ramírez-Castrillón M, Mendes SDC, Inostroza-Ponta M, Valente P (2014) (GTG)₅ MSP-PCR fingerprinting as a technique for discrimination of wine associated yeasts? *PLoS ONE* 9(8):e105870. <https://doi.org/10.1371/journal.pone.0105870>
- Salomão BD, Muller C, Amparo HC, Aragão GM (2014) Survey of molds, yeast and *Alicyclobacillus* spp. from a concentrated apple juice productive process. *Braz J Microbiol* 45:49–58. <https://doi.org/10.1590/S1517-83822014000100008>
- Samson RA, Hoekstra ES, Lund F, Filtenborg O, Frisvad JC (2004) Methods for the detection, isolation and characterisation of food-borne fungi. In: Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (Eds) *Introduction to food- and airborne fungi*, 7 edn. Centraal-bureau voor Schimmelcultures, pp 283–297
- Scaramuzza N, Berni E (2014) Heat resistance of *Hamigera avellanea* and *Thermoascus crustaceus* isolated from pasteurized acid products. *Int J Food Microbiol* 168:63–68. <https://doi.org/10.1016/j.ijfoodmicro.2013.10.007>
- Scheuer C, Bauer R, Lutz M, Stabenheiner E, Grube M (2008) *Bartheletia paradoxa* is a living fossil on *Ginkgo* leaf litter with a unique septal structure in the *Basidiomycota*. *Mycol Res* 112(11):1265–1279. <https://doi.org/10.1016/j.mycres.2008.06.008>
- Schmitt I, Crespo A, Divakar PK, Fankhauser JD, Herman-Sackett E, Kalb K et al (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. *Pers: Mol Phylogeny Evol Fungi* 23:35–40. <https://doi.org/10.3767/003158509X470602>
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA et al (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci U S A* 109(16):6241–6246. <https://doi.org/10.1073/pnas.1117018109>
- Seifert KA, Nickerson NL, Corlett M, Jackson ED, Louis-Seize G, Davies RJ (2004) *Devriesia*, a new hyphomycete genus to accommodate heat resistant, cladospore-like fungi. *Can J Bot* 82(7):914–926. <https://doi.org/10.1139/b04-070>
- Snyder AB, Churey JJ, Worobo RW (2019) Association of fungal genera from spoiled processed foods with physicochemical food properties and processing conditions. *Food Microbiol* 83:211–218. <https://doi.org/10.1016/j.fm.2019.05.012>
- Spatafora JW, Aime MC, Grigoriev IV, Martin F, Stajich JE, Blackwell M (2017) The fungal tree of life: from molecular systematics to genome-scale phylogenies. *Microbiol Spectr*, pp 1–34. <https://doi.org/10.1128/microbiolspec.FUNK-0053-2016>
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinform* 30(9):1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stielow JB, Lévesque CA, Seifert KA, Meyer W, Irinyi L, Smits D et al (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Pers: Mol Phylogeny Evol Fungi* 35(1):242–263. <https://doi.org/10.3767/003158515x689135>
- Stiller JW, Hall BD (1997) The origin of red algae: Implications for plastid evolution. *Proc Natl Acad Sci U S A* 94(9):4520–4525. <https://doi.org/10.1073/pnas.94.9.4520>
- Suh SO, Gujjari P, Beres C, Beck B, Zhou J (2013) Proposal of *Zygosaccharomyces parabailii* sp. nov. and *Zygosaccharomyces pseudobailii* sp. nov., novel species closely related to *Zygosaccharomyces bailii*. *Int J Syst Evol Microbiol* 63(5):1922–1929. <https://doi.org/10.1099/ijs.0.048058-0>
- Tedersoo L, Bahram M, Pöhlme S, Kõljalg U, Yorou NS, Wijesundera R et al (2014) Fungal biogeography Global diversity and geography of soil fungi. *Science* 346(6213):1256688. <https://doi.org/10.1126/science.1256688>
- Teufel F, Almagro Armenteros JJ, Johansen AR, Gíslason MH, Pihl SI, Tsigirgos KD et al (2022) SignalP 6.0 predicts all five types of signal peptides using protein language models. *Nat Biotechnol* 1–3. <https://doi.org/10.1038/s41587-021-01156-3>
- Tournas V (1994) Heat resistant fungi of importance to the food and beverage industry. *Crit Rev Microbiol* 20(4):243–263. <https://doi.org/10.3109/10408419409113558>
- Tresner HD, Hayes J (1971) Sodium chloride tolerance of terrestrial fungi. *Appl Microbiol* 22(2):210–213
- Ulusoy BH, Hamed NS, Yıldırım FK (2022) Heat-resistant moulds: Assessment, prevention and their consequences for food safety and public health. *Czech J Food Sci* 40(4):273–280. <https://doi.org/10.17221/26/2022-CJFS>
- Van Driel KG, Humbel BM, Verkleij AJ, Stalpers J, Müller WH, Boekhout T (2009) Septal pore complex morphology in the *Agaricomycotina* (*Basidiomycota*) with emphasis on the *Cantharellales* and *Hymenochaetales*. *Mycol Res* 113(5):559–576. <https://doi.org/10.1016/j.mycres.2008.12.007>
- Větrovský T, Morais D, Kohout P, Lepinay C, Algora C, Awokunle Hollá S et al (2020) GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. *Sci Data* 7:1–14. <https://doi.org/10.1038/s41597-020-0567-7>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172(8):4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Vuyst L, Camu N, Winter T, Vandemeulebroecke K, van de Perre V, Vancanneyt M et al (2008) Validation of the (GTG)₅-rep-PCR fingerprinting technique for rapid classification and identification of acetic acid bacteria, with a focus on isolates from Ghanaian fermented cocoa beans. *Int J Food Microbiol* 125:79–90. <https://doi.org/10.1016/j.ijfoodmicro.2007.02.030>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR*

- protocols: A guide to methods and applications, vol 18(1). Academic Press, pp 315–322. <https://doi.org/10.1016/b978-0-12-372180-8.50042-1>
- Witfeld F, Begerow D, Guerreiro MA (2021) Improved strategies to efficiently isolate thermophilic, thermotolerant, and heat resistant fungi from compost and soil. *Mycol Prog* 20(3):325–339. <https://doi.org/10.1007/s11557-021-01674-z>
- Wyatt TT, Wösten HAB, Dijksterhuis J (2013) Fungal spores for dispersion in space and time. *Adv Appl Microbiol* 85:43–91. <https://doi.org/10.1016/b978-0-12-407672-3.00002-2>
- Yaguchi T, Imanishi Y, Matsuzawa T, Hosoya K, Hitomi J, Nakayama M (2012) Method for identifying heat-resistant fungi of the genus *Neosartorya*. *J Food Prot* 75(10):1806–1813. <https://doi.org/10.4315/0362-028X.JFP-12-060>
- Yang J, Li J, Jiang Y, Duan X, Qu H, Yang B et al (2014) Natural occurrence, analysis, and prevention of mycotoxins in fruits and their processed products. *Crit Rev Food Sci Nutr* 54(1):64–83. <https://doi.org/10.1080/10408398.2011.569860>
- Zajc J, Gunde-Cimerman N (2018) The Genus *Wallemia* - From contamination of food to health threat. *Microorganisms* 6(2):46. <https://doi.org/10.3390/microorganisms6020046>
- Zalar P, Sybren HG, Schroers HJ, Frank JM, Gunde-Cimerman N (2005) Taxonomy and phylogeny of the xerophilic genus *Wallemia* (*Wallemiomycetes* and *Wallemiales*, cl. et ord. nov.). *Antonie van Leeuwenhoek* 87(4):311–328. <https://doi.org/10.1007/s10482-004-6783-x>
- Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z et al (2018) dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res* 46(1):95–101. <https://doi.org/10.1093/nar/gky418>

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