Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Mika Helene Kirkhus

Hidden diversity and host specificity of *Pertusaria*-residing *Tremella* fungi in Norway

Master's thesis in Biodiversity and Systematics
Supervisor: Mika Bendiksby
Co-supervisor: Andreas Frisch, Marie Davey, Ana Maria Romero Millanes, Ann Evankow

May 2022

NTNU
Kunnskap for ei betre verd

Mika Helene Kirkhus

Hidden diversity and host specificity of *Pertusaria*-residing *Tremella* fungi in Norway

Master's thesis in Biodiversity and Systematics
Supervisor: Mika Bendiksby
Co-supervisor: Andreas Frisch, Marie Davey, Ana Maria Romero Millanes, Ann Evankow
May 2022

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of Science and Technology

Abstract

Hidden from plain view, the diversity of lichen-inhabiting *Tremella* fungi may be underestimated. *Tremella* occasionally form fruiting bodies on lichens and the more common yeast stage is asymptomatic on lichen thalli. Lichenicolous *Tremella* in the filamentous stage (symptomatic; forming fruiting bodies) is known to be host specific, while the host specificity and ecology of the yeast stage (asymptomatic) is poorly studied. Prior to this study, only one species of *Tremella* was known to reside symptomatically in the Pertusariaceae, namely *T. pertusariae*. However, while DNA barcoding species of the Pertusariaceae, contaminant ITS sequences of *Tremella* were obtained from asymptomatic specimens. Through this project, I have (1) studied the diversity of asymptomatic *Pertusaria*-residing *Tremella* fungi in Norway, (2) investigated their host specificity to *Pertusaria*, and 3) tested if there was a correlation between *Tremella* presence and various climatic variables. I used high-throughput amplicon sequencing targeting the ITS2, using both general fungal- and *Tremella* specific primers in two different PCR setups, on samples collected from various locations in Norway. I assessed the diversity with phylogenetic analyses, and I visualized host specificity using circos plots. I used a generalized linear mixed model (GLMM) to compare results obtained with the various primer combinations and PCR setups. Finally, I used a generalized linear model (GLM) to test whether *Tremella* presence may depend on climatic conditions. Most of the tremellalean species diversity obtained from the various *Pertusaria* hosts was found using the general fungal primers rather than the *Tremella*-specific primers. My phylogenetic results show that several tremellalean amplicon sequence variants (ASVs) group with a variety of tremellalean species, also outside of the genus *Tremella*, which is observed for the first time in *Pertusaria.* Most of the obtained ASVs do, however, group with the *T. pertusariae* reference. These ASVs appear as several rather distinct genetic lineages, suggesting *T. pertusariae* represents a species complex. The presence of *Tremella* ASVs in the *Pertusaria* specimens studied herein do not indicate host specificity and is negatively correlated with temperature. My study clearly shows that the tremellalean yeast stage is more common than its filamentous stage in species of *Pertusaria.* From this follows that different habitat preferences and limitations apply for the yeast stage of lichenicolous *Tremella* than for the filamentous species. The same seems to be the case regarding host specificity and climate preference.

Sammendrag

Usynlig for det blotte øyet, er mangfoldet av lavboende *Tremella* sopper ganske sikkert sterkt undervurdert. *Tremella* sopper kan noen ganger observeres med fruktlegemener på laver (symptomatisk). Dens vanligste livsform er dog usynlig som en asymptomatisk gjær inne i lavens kropp (thallus). Lavboende *Tremella* i dens filamentøse stadium (produserer fruktlegemer) er kjent for å være vertsspesifikk, mens vertsspesifisiteten og økologien til gjærstadiet er lite studert. Ved oppstart av denne studien, var kun en art kjent for å leve symptomatisk i Pertusariaceae, nemlig *T. pertusariae.* I tillegg har det blitt oppdaget *Tremella* ITS sekvenser, som forurensing, i forbindelse med DNA-strekkoding av asymptomatiske *Pertusaria* arter. I dette prosjektet har jeg (1) studert artsmangfoldet av asymptomatiske lavboende *Tremella* i *Pertusaria* i Norge, (2) undersøkt deres vertsspesifisitet til *Pertusaria,* og (3) testet om det kan være en korrelasjon mellom tilstedeværelse av *Tremella* og ulike klimatiske variabler. Jeg har brukt såkalt high-throughput sekvensering av DNA fragmentet ITS2 fra *Pertusaria* objekter innsamlet fra hele Norge, oppformert med både generelle sopp-primere og spesifikke primere for *Tremella* i to ulike PCR oppsett. Jeg har vurdert artsmangfoldet ved hjelp av fylogenetiske analyser og visualisert vertsspesifisiteten med cirkos plott. Videre brukte jeg ulike statiske analyser for å sammenlikne resultater produsert med de ulike primer kombinasjonene og PCR oppsett (GLMM), samt for å teste om tilstedeværelse av *Tremella* er avhengig av klimatiske forhold (GLM). Det største artsmangfoldet av *Tremella* i *Pertusaria* ble funnet med de generelle sopp primerne fremfor de *Tremella*-spesifikke primerne. Mine fylogenetiske resultater viser at de fleste amplikon sekvens variantene (ASVene) grupperer med ulike *Tremella* arter, men også med arter i andre slekter av ordenen Tremellales, noe som her er observert for aller første gang i slekta *Pertusaria.* Når det er sagt, så grupperer dog de fleste ASVene med en referanse av *T. pertusariae.* Blant disse ASVene opptrer flere distinkte genetiske linjer, noe som antyder at *T. pertusariae* representerer et artskompleks. Tilstedeværelsen av asymptomatisk *Tremella* i *Pertusaria* viser i denne studien få tegn til vertsspesifisitet og en negativ korrelasjon med temperatur. Min studie viser tydelig at gjærformen til *Tremella* er mer vanlig enn dens filamentøse form i arter av *Pertusaria.* Følgelig må det antas at *Tremella* arters ulike livsformer (gjær vs. filamentøs) har ulike habitat preferanser og begrensninger. Det samme ser ut til å gjelde for vertsspesifisitet og klimapreferanse.

Acknowledgements

I want to thank my team of supervisors Mika Bendiksby (UiO), Andreas Frisch (NTNU), Marie Davey (NINA), Ana Millanes (URJC), and Ann Evankow (UiO). I have benefited immensely from our educational discussions and for the many times you all read-through and edited my thesis manuscript. Mika, I am very glad you pitched this cool project to me, as I honestly do not think I could have found a better one. Your detailed feedback, phylogenetic skills, and fascination for taxonomy and fungi has both taught and inspired me to become a better researcher. Andreas, after our first fieldtrip I thought your knowledge on lichens and lichenicolous fungi was endless, and now after almost two years, I still do. Thank you for including me on fieldtrips and for sharing your knowledge. Marie, your guidance in metabarcoding and statistical analyses has been essential, and somehow you always seemed to have the answer to the most difficult question, to which I am very grateful. Ana, having a true *Tremella* expert on my side has helped me better understand (and love) this awesome group of fungi. Annie, I really benefited from our good talks, and for you never hesitating to offer a helping hand (without you I would probably still be arguing with R about coordinates). I would also like to thank Dr. Sarah Martin for her assistance in the lab at the NTNU University Museum, and the technicians at NINA for aiding me with the library preparation and sequencing. I greatly valued the initiative of NINA in Trondheim for organizing an educational metabarcoding workshop. It should also be mentioned that all the teachers and organizers of the NABiS program did a great job providing my class with a solid education in biosystematics. The remaining knowledge gaps were filled by excellent courses given by ForBio (Research School in Biosystematics). One of the highlights of my master was the Annual ForBio meeting, which made me feel like a true scientist. Thanks to all its organizers, especially Hugo de Boer and Quentin Mauvisseau. I want to thank my family, including the cats, chickens, Baloo and Rocky, for helping me recharge my batteries and for the mountains of love and support (and food). Thea, thank you for always being in my corner and for listening to my extensive chatter about lichens and horses. You truly are a high-quality best friend. Jakob, I feel so lucky to have someone as honest and caring as you. You are the sunshine in my life! Lastly, thanks to all others who supported me and helped me read-through and edit my thesis, you know who you are.

Contents

Abbreviations

AIC	Akaike Information Criterion
AICc	Akaike Information Criterion corrected for small sample sizes
ASDSF	Average Standard Deviation of Split Frequencies
ASV	Amplicon sequence variant
ASV-all	Alignment with sequences in *Tremella pertusariae* s. l., unfiltered
BG	Fungarium, University of Bergen, Bergen
Bio1	Mean annual temperature
Bio6	Minimum temperature of the coldest month
Bio8	Mean temperature of wettest quarter
Bio12	Mean annual precipitation
Bio15	Seasonality of precipitation
bp	Base pairs
BS	Bootstrap
CG	Guanine-cytosine
C-PCR	Conventional polymerase chain reaction
ESS	Effective sample size
FISH	Fluorescent in situ hybridization
GLM	Generalized linear model
GLMM	Generalized linear mixed model
HTS	High-throughput sequencing
MC3	Metropolis-Coupled Markov Chain Monte Carlo
ML	Maximum likelihood
nITS	Internal transcribed spacer of nuclear ribosomal DNA
nLSU	Nuclear large subunit ribosomal DNA
N-PCR	Nested polymerase chain reaction
nSSU	Nuclear small subunit ribosomal DNA
O	Fungarium, University of Oslo, Oslo
OTU	Operational taxonomic unit
PIC	Parsimony informative characters
PP	Posterior probability
REF-all	Alignment containing multiple tremellalean reference sequences
SE	Standard Error
TRH	Fungarium, NTNU University Museum, Trondheim

1 Introduction

The latest estimate of fungal species is 2.2 to 3.8 million, of which only about 144,000 currently are accepted (Willis 2018). The high number of undescribed fungi is a consequence of the hidden nature of fungal diversity. The fungal kingdom is rich in cryptic fungi, unresolved species complexes, as well as fungi that we cannot see. Conservation efforts are only applied to accepted species that we know exist. Therefore, discovering hidden fungi should be a top priority, as they can be essential to ecosystem functioning, as well as to us human beings in our daily lives (Singh 2017). Recent advances in molecular identification techniques highlight that fungi are all around us. They can be found in the air, soil, water, and other organisms such as plants, animals, or lichens.

A lichen is traditionally thought of as an ecologically stable mutualism between a dominant fungal partner known as the mycobiont, and one or more photosynthesizing populations of algae and/or cyanobacteria, the photobiont. The mycobiont provides protection, by sheltering its primary producer extracellularly in a matrix of fungal hyphae (Hawksworth & Honegger 1994). Lichens are colonized also by other organisms, such as prokaryotes and additional fungi (Aschenbrenner et al. 2016). It has been known for quite some time that the lichen thallus can host multiple fungal species, as some fungi cause clearly visible symptoms on its lichen host (Lawrey & Diederich 2003). Recent advances in molecular technologies have further highlighted the species richness of the mycobiome that exists in the thallus (Muggia & Grube 2010). These fungi, excluding the mycobiont, are categorized into three groups of microbes correlated to lichens: (1) *endolichenic fungi* are asymptomatic and, as such, similar to plant endophytes; (2) *extraneous* fungi are incorporated into the thallus without having a clear contribution to the symbiosis; and (3) *lichenicolous fungi* are recognized by their visible fruiting bodies and conidiomata. When present in the asexual stage, they may resemble endolichenic fungi, hidden in the host without any visible outer signs. Some lichenicolous fungi can also form conspicuous asexual structures producing mitospores, like pycnidia or sporodochia (Arnold et al. 2009; Banchi et al. 2018; Fernández-Mendoza et al. 2017). Lichenicolous fungi are the group of interest in this study.

Lichenicolous fungi are a highly specialized group of organisms that live associated with lichens, either as parasites, parasymbionts, or saprophytes. This group is biologically defined and taxonomically diverse. Lichenicolous fungi depend on their hosts and are often specific to certain species of lichens (Millanes et al. 2014). Currently, ca. 1,800 species of lichenicolous

fungi are recognized worldwide (Diederich et al. 2018). About 96% of these belong to the ascomycetes, whilst the remainder are basidiomycetes (Lawrey & Diederich 2003; Holien et al. 2016; Holien & Frisch 2022). There are currently 346 lichenicolous fungi found in Norway (Ekman et al. 2019; Klepsland 2020). Many of the lichenicolous species found in Norway have been reported for this country only recently, which may explain why there is so far little information on their distribution, ecology, and conservation status (Frisch et al. 2020; Westberg et al. 2021; Frisch et al. in prep.).

Many species of lichenicolous fungi belong to the Tremellomycetes, which is the largest class of lichenicolous basidiomycetes. The Tremellomycetes consist of five major orders: Cystofilobasidiales, Filobasidiales, Holtermanniales, Trichosporonales, and Tremellales (Millanes et al. 2011). Tremellales includes eight families, including the Tremellaceae, which has a cosmopolitan distribution. This family comprises 18 genera, of which *Tremella* is the largest genus of lichenicolous basidiomycetes (Diedrich et al. 2018; Lindgren et al. 2015). Both morphological and molecular studies have shown that neither the Tremellaceae nor *Tremella* represent monophyletic groups as currently circumscribed (Boekhout et al. 2011). The genus *Tremella*, in its narrow sense (s. str.), includes only taxa in the former mesenterica and fuciformis groups, none of which are lichenicolous. However, several other taxa, including all lichenicolous *Tremella* species described, are still placed in the wider sense of the genus (*Tremella* s. l.), mainly for convenience, until sounder phylogenies are available (Liu et al. 2016). *Tremella* s. l. comprises approximately 90 species worldwide. More than half of these are known to be lichenicolous (Zhao et al. 2019). In the Nordic countries, currently 26 lichenicolous *Tremella* species are registered, of which 13 have been found in Norway (Westberg et al. 2021).

The Tremellomycetes comprises fungi with a dimorphic life cycle, including an asexual haploid unicellular yeast stage and a sexual dikaryotic filamentous stage (Bandoni 1995). Fungi with this lifestyle can switch between the two stages, sometimes based on environmental factors (Boyce & Andrianopoulos 2015). Presence of filamentous *Tremella* may depend on the host's secondary chemicals or be limited by habitat and climatic conditions (i.e., temperature and humidity) or a combination of these factors, as suggested by Lawrey & Diederich (2003) and Werth et al. (2013). There are so far no in-depth studies on the preferred habitat and climatic preferences of lichenicolous filamentous *Tremella*, indicating the need for more research targeting the ecology of these fungi. Even less is known about the ecology of the yeast stage of lichenicolous *Tremella*. Results by Sampaio et al. (2004) suggest that basidiomycete yeasts

could occupy habitats different from that of the filamentous yeasts. There are, however, no studies so far targeting *Tremella* on this matter.

It is mainly the filamentous stage of lichenicolous *Tremella* that has been studied in previous literature, as the asymptomatic yeast stage is hidden from plain sight and thereby often overlooked. Pippola et al. (2009) suggested that the yeast stage of *Tremella* may be more common than originally thought. Tuovinen et al. (2019) showed that this was indeed the case for *Tremella lethariae* Diederich, as it could be present with a common and widespread yeast stage in species of *Letharia*. Their study further shows that *T. lethariae* rarely enters the sexual stage. A later study showed that the yeast phase of *Tremella macrobasidiata* J.C. Zamora, Pérez-Ortega & V.J. Rico (a lichen-inhabiting species whose filamentous phase is restricted to *Lecanora chlarotera* Nyl.) had a wider host range, including both *L. chlarotera* and *Lecanora variae* J.C. Zamora, Pérez-Ortega & V.J. Rico (Tuovinen et al. 2021). Studies that claim *Tremella* to be host specific are based on symptomatic filamentous specimens (Millanes et al. 2011, 2014).

Pertusaria is a genus of crustose lichens, usually found on trees, rocks, and in soils from sea level to alpine habitats in Norway. Species of *Pertusaria* often have either isidia or soredia, and either an exposed disc or perithecia-like fruiting bodies (Oset 2021). Currently, only one species of *Tremella* is known to be associated with *Pertusaria*, namely, *Tremella pertusariae* Diederich. During the sexual stage, this species induces formation of galls that are first regularly convex and cocolorous with the lichen thallus and later become brownish and irregular (Diederich 1996). *Tremella pertusariae* is known in Norway from a recent collection in Larvik, as well as from four internal transcribed spacer (ITS) sequences obtained from healthy looking, asymptomatic *Pertusaria hymenea* Schaerer (A. Frisch, pers. comm.). Further, *Tremella* ITS barcode sequences have been obtained as contaminants from barcoded species of the Pertusariaceae from Norway within the OLICH project of NorBOL. These findings indicate the presence of tremellalean fungi in asymptomatic thalli of the Pertusariaceae. Similar observations have been made also for other groups of lichens, for example the Parmeliaceae (Lindgren et al. 2015; Tuovinen et al. 2019, 2021).

Gaining knowledge of lichenicolous fungi has been proven difficult, due to (1) their small size, (2) the scarcity of morphological diagnostic characters, (3) often being hidden within the thallus of their hosts, (4) few specialists that study them, and (5) limited available collections. The number of lichenicolous fungi is expected to be greater than currently known, and *Tremella* is

no exception. The discovery of hidden diversity has been revolutionized by the advancement of DNA based molecular techniques (Cheek et al. 2020).

Metabarcoding enables the simultaneous high-throughput sequencing (HTS) of multiple components of the compound lichen, and has been able to capture more fungal diversity than traditional Sanger sequencing, even when only few cells are present (Banchi et al. 2018; Paul et al. 2018). This method also aids in increasing knowledge on host specificity, which can answer questions regarding the evolutionary and demographic history of lichenicolous fungi and their respective hosts (Werth et al. 2013). Primers specifically designed for lichenicolous tremellalean fungi targeting nuclear ribosomal DNA markers (nSSU, nITS, and nLSU) have been used extensively in cases where it has not been possible to physically separate the parasite and the host (e.g., Millanes et al. 2011).

In this project, I (1) study the diversity of lichenicolous *Tremella* in asymptomatic thalli (i.e., the presumed yeast stage of *Tremella*) of *Pertusaria* species in Norway, (2) investigate the specificity of lichenicolous *Tremella* sequences to species of *Pertusaria*, and (3) test for a potential correlation between climate and presence of *Tremella.* I use metabarcoding of the ITS2 barcode for detecting the yeast stage of one or more species of *Tremella* in asymptomatic *Pertusaria* thalli. I look at the various hosts of common amplicon sequence variants (ASVs) to assess host specificity, and use statistical tests to see whether the general fungal primers or the *Tremella*-specific primers detect the most tremellalean fungi. I also perform statistical tests to assess the impact of various climatic variables on diversity patterns of *Tremella* in *Pertusaria* in Norway.

2 Materials and methods

2.1 Sampling

In this project, I used 192 asymptomatic specimens from 14 species of *Pertusaria* borrowed from three fungaria in Norway (i.e., TRH, O, and BG) (Supplementary Table S1). Most samples were collected from 2007 onwards. I included older specimens of *Pertusaria bryontha* Nylander (1980-1990), as there were no recent specimens available. I chose specimens from various counties for each species, to ensure a broad geographic coverage in Norway (Fig. 1). I sampled apothecia or soredia, usually together with a small amount of plain thallus, in 2.0 ml micro tubes for DNA extraction. I sampled tissue based on structures present on individual specimens, as some only had apothecia or soralia. Some specimens had neither, in which case I only sampled plain thallus. I did the sampling with sterile razor blades and tweezers, sterilizing the equipment with ethanol and burning it off between each sampling. I used additional sequences from other projects (Supplementary Table S2) and references from GenBank (Supplementary Table S3).

2.2 Molecular data production

I extracted DNA from the asymptomatic *Pertusaria* specimens using the E.Z.N.A Plant DNA Kit (Omega Bio-tek, Inc. Norcross, USA) following the manufacturer's manual. I pulverized the samples on a TissueLyser II (Qiagen, Hilden, Germany) using two sterile metal beads in each 2.0 ml micro tube containing the lichen material. I included a negative and a positive control per 23 specimens of *Pertusaria*. My positive controls were from fruiting *Tremella coppinsii* Diederich & G. Marson and *Tremella hypogymniae* Diederich.

I have used the ITS2 for this study, which is the second half of the standard fungal DNA barcode marker, ITS. In its complete length (ca. 800 bp), the ITS is suitable for discriminating between closely related species in general (Nilsson et al. 2008) and Basidiomycota in particular (Badotti et al. 2017; Garnica et al. 2016). Using short-read HTS, however, allowed me to target only one of the two ITS sub-regions (ITS1 or ITS2) due to the relatively short read lengths (2 x 250 bp). While many studies demonstrate greater interspecific variation among most fungal lineages for the ITS1, there is evidence for greater variation in the ITS2 among Basidiomycota (Garnica et al. 2016; Tedersoo et al. 2015), which is why this marker was chosen in this study.

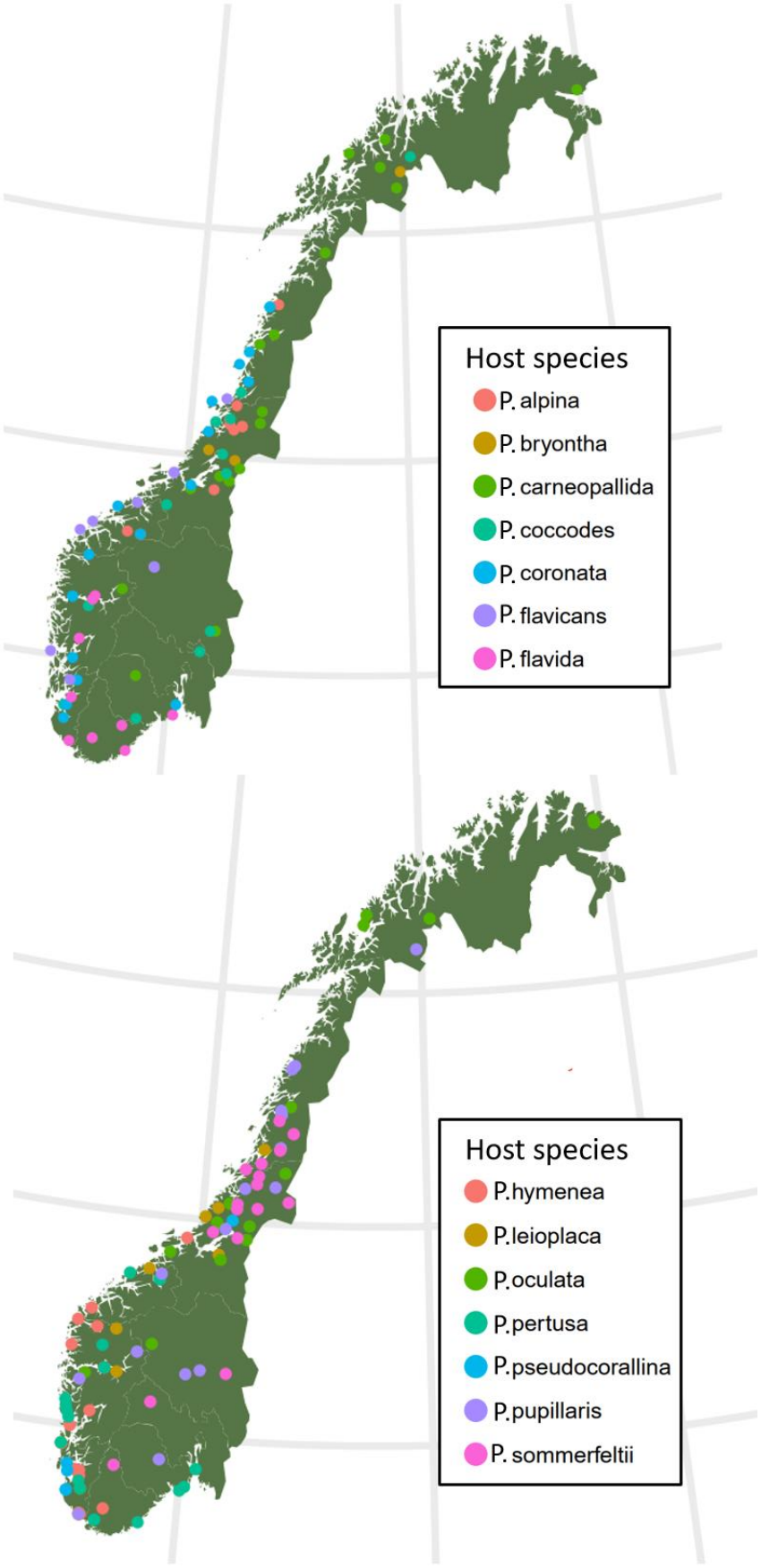


Figure 1. Distribution maps of Norway showing the sampling sites of the 192 fungarium specimens of asymptomatic *Pertusaria* included in this study. Specimens are colored based on species, see legend.

In addition to conventional PCR, I performed nested PCR using *Tremella*-specific primers followed by general fungal primers (Table 1). I used *Tremella*-specific primers first within a nested PCR to limit amplification of host DNA, which otherwise could cause signals from the lichenicolous DNA to drown in high abundances of host reads. Traditionally, the general primers are applied before specific primers in a nested PCR, in order to improve detection rates (Badiee et al. 2015; Hsu et al. 2021). However, I expected that by applying specific primers first, and thereby reducing amplification of host DNA, the detection rate of *Tremella* will still increase, as more of the sequencing effort would be spent on the lichenicolous fungi and not the host. An additional and intentional gain with this approach is that I would obtain small enough gene fragments for sequencing on the NovaSeq 6000 platform (2 x 250 bp). The *Tremella*-specific primers would not have had the appropriate length for this platform when applied alone.

For PCR with general fungal primers, I used the fungal primer, fITS7 (Ihrmark et al. 2012), and the general primer, ITS4 (White et al. 1990). I used the fungal primer, ITS1F (Gardes & Bruns 1993), and the *Tremella*-specific primer, BasidLSU3-3 (Millanes et al. 2011), as a *Tremella*-specific primer combination for the first round of PCR within the nested PCR approach. All primers had a concentration of 10 µM. The fITS7-ITS4 primer pair included terminal overhangs to allow downstream indexing with the Nextera XT DNA Library Prep Kit (Illumina, San Diego, USA). I also completed a separate round of PCR with the general fungal primers, fITS7 and ITS4, on all samples using DNA template instead of PCR product from the first round of PCR to account for primer bias. For this I used the same reaction mix and PCR conditions as with the second round of PCR in the nested PCR (Table 1).

Table 1. The nested PCR approach, including primers, PCR reactants. and PCR conditions. MM = Mastermix, FP = forward primer, and RP = reverse primer.

PCR method	Primers	PCR reactants	PCR conditions
1st round of nested PCR	fITS7/ITS4 (general fungal primers)	1 µl DNA, 10 µl MM, 7 µl dH_20, 1 µl of FP and RP.	98 °C for 30 s, thirty cycles of [98 °C for 7 s, 56.8 °C for 20 s, and 72 °C for 20 s], and a final extension at 72 °C for 7 min.
2nd round of nested PCR	ITS1F/ BasidLSU3-3 (*Tremella*-specific primers)	1 µl PCR product as template from 1st round of nested PCR, 10 µl MM, 4 µl of FP and RP.	94 °C for 5 s, ten cycles of [94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s], and a final extension at 72 °C for 7 min.

I accounted for the presence of *Tremella* amplicons by agarose gel electrophoresis. *Tremella* DNA was quantified on an Agilent 4200 TapeStation (Agilent Technologies, Waldbronn, Germany) and all samples amplified with ITS1F-BasidLSU3-3 from the nested PCR that did not contain measurable amounts of DNA were excluded. To test for primer bias, these were instead replaced by samples amplified with fITS7-ITS4 from the PCR using general fungal primers. A total of 190 samples from the nested PCR using *Tremella*-specific primers and 116 samples from PCR using only general fungal primers were included for sequencing. The Nextera XT DNA Library Prep Kit was used for adding IDT for Illumina DNA/RNA UD indices to the 5' and 3' ends of the amplicons according to the manufacturer's instructions, before diluting the amplicons to 7ng/uL, and using magnetic beads to remove fragments <500 base pairs (bp). Finally, amplicons were pooled in equimolar amounts and sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, USA) at the Norwegian Sequencing Center.

2.3 Data analysis

2.3.1 Pipeline

The data was demultiplexed on the Illumina NovaSeq platform. The Cutadapt v.1.18 software was used for primer removal, which required a minimum length match of 17 bp with 0.15 expected errors. The DADA2 pipeline was applied to process reads. This included quality filtering, error correction, merging, mapping and chimera removal. The final output of DADA2 was as ASVs, which were preferred over operational taxonomic units (OTUs). ASVs usually are recommended when there are many sequences that are closely related, which was the case for my data. Taxonomic information based on individual ASVs was generated in two ways. Firstly, the IDTAXA algorithm implemented in the DECIPHER package of R was used with the UNITE database for fungi (Murali et al. 2018). Secondly, because many ASVs did not get any matches against the UNITE database, the MegaBlast BLASTn algorithm was used to compare ASVs to the GenBank nucleotide-nr database.

2.3.2 Alignment and phylogeny

I aligned the resultant ASV sequences together with tremellalean ITS2 reference sequences from GenBank using MAFFT v.7.490 (Katoh et al. 2002) (Supplementary Table S1). The included reference sequences originated from both free-living and lichenicolous species, aimed at representing various families within the Tremellales, selected based on the phylogeny by Liu et al. (2016). I also included ITS2 sequences of *Tremella* that were obtained as contaminations

when DNA barcoding *Pertusaria* species in the OLICH project (see Marthinsen et al. 2019). I manually edited alignments using Aliview v.1.28 (Larsson 2014). I included different sets of obtained Tremellales ASVs into two separate alignments, the REF-all and the ASV-all. In the REF-all dataset, I excluded ASVs given the following: 1) the ASV was obtained from only one specimen, and 2) the ASV was not the most dominant in that specimen. I also deleted from the REF-all dataset the ASVs that had very low abundance (<99 reads) and that appeared in the same clade as other closely related ASVs with higher abundances from the same hosts. I tested various alignments of REF-all and compared phylogenetic results, in order to check for the impact of ambiguously aligned regions. I tested trimming away these ambiguously aligned regions of the most divergent sequences, to assess its potential impact on clade support and phylogenetic placement of the reference sequences relative to a published Tremellomycete phylogeny by Liu et al. (2016). For the ASV-all alignment, I included only ASVs that matched well with the *T. pertusariae* reference sequence. I made this separate alignment to explore if low abundant ASVs is true variation within the *T. pertusariae* s. l. species complex. I looked for parsimony informative characters (PIC) of both alignments using the WinClada v.1.00.08 software (Nixon 2002).

I performed phylogenetic analyses for both the ASV-all and REF-all dataset. I constructed Bayesian 50% majority rule consensus trees with MrBayes v.3.2.7a, via the CIPRES Science Gateway v.3.3 (Miller et al. 2010). I completed model selection using jModelTest (Darriba et al. 2012) based on the lowest Bayesian information criterion (BIC) value for both datasets. Each Bayesian analysis included two individual runs with eight separate chains, and the substitution model was set according to results from jModelTest. I ran the Metropolis-Coupled Markov Chain Monte Carlo (MC3) for seven million generations on the REF-all dataset, saving trees every 300th generation. I set *Trichosporon ovoides* as the outgroup for this phylogenetic analysis, as it was the most distantly related among all included Tremellomycete reference sequences according to the published Tremellomycete phylogeny by Liu et al. (2016). I ran the ASV-all dataset for 20 million generations, saving trees every 2000th generation. No specific outgroup was set for this phylogenetic analysis. I discarded as burn-in the generations before the stage where the Average Standard Deviation of Split Frequencies (ASDSF) had fallen to 0.01. The BEAGLE library (Ayres et al. 2012) ran for both analyses. I used Tracer v.1.7.1 (Rambaut et al. 2018) to assess convergence, trace plots, and effective sample size (ESS) values.

I constructed Maximum Likelihood (ML) phylogenies for both alignments using RaxML v.8.2.12 (Kozlov et al. 2014), with the GTRCAT substitution model, 1000 bootstrap replicates

and a 70% cut-off value for condensed trees. I used iTOL v.6.5.2 (Letunic & Bork 2021) for further editing and final visualization of phylogenetic trees that included information on branch support, abundance of reads, applied PCR method, and host data.

I regard as contaminants amplified fungi not residing within the lichen thallus, such as single spores attached to the lichen thallus. The abundance of reads (i.e., identical sequences) may be of help to distinguish between possible contaminations and the true presence of lichen-inhabiting fungi. For this reason, I included only ASVs with > 99 reads, as amplicons with few reads are in general more likely to be contaminants (Fernández-Mendoza et al. 2017). This is because contaminants typically have a relatively small mass compared to the actual lichen-inhabiting yeasts or filaments, and therefore a smaller chance of rendering high read abundance.

2.4 Host specificity

I visualized host specificity using the software Circos v.0.63-9 (Krzywinski et al. 2009). I constructed a circos plot based on only the ASVs that had above 100 reads within the genus *Tremella* and linked them to their respective asymptomatic *Pertusaria* hosts. I included only individual ASVs that had been obtained from four or more *Pertusaria* specimens to see patterns of potential specificity.

2.5 Statistical analyses

2.5.1 PCR method comparison

I used RStudio v.1.4.1106 (RStudio Team, 2020) to examine to what extent the presence of tremellalean ITS2 sequences in *Pertusaria* specimens depends on the applied primer pair. I tested presence/absence based on the PCR method by doing a generalized linear mixed model (GLMM) analysis with a binomial error distribution. This model had presence of Tremellales ITS2 sequences as binary response variable and PCR method as explanatory variable.

2.5.2 Effect of climate

I used an information-theoretic approach (Burnham & Anderson 2002) to create and test potential models that explore the correlation between environmental variables and occurrence data of tremellalean fungi in *Pertusaria* thalli. Climatic conditions were extrapolated from the worldclim dataset (Fick & Hijmans 2017), from which I extracted the bioclim variables gridded at 2.5 km resolution for all coordinates of the *Pertusaria* specimens. I avoided analyzing correlated explanatory variables by making a correlation plot and using it to select bioclim

variables to use in generating models for hypothesis testing. I used presence/absence of Tremellales, regardless of PCR method, as binary response variable and used the extracted bioclim data as explanatory variables. I made a generalized linear model (GLM) with each of the following bioclim variables: (1) mean annual temperature (Bio1), (2) minimum temperature of the coldest month (Bio6), (3) mean temperature of wettest quarter (Bio8), (4) mean annual precipitation (Bio12), and (5) seasonality of precipitation (Bio15). I also tested various variables together to search for interactive effects. I used the Akaike Information Criterion corrected for small sample sizes (AICc) for model selection. I made a predictor effects plot of the model with the best AICc score. I used RStudio to construct distribution maps of the top-five ASVs with highest *Tremella* occurrences (Supplementary Fig. 1S - 5S).

3 Results

3.1 Phylogenetic trees

I noted no improvement in clade support of preliminary trees after having trimmed away highly variable regions from the final REF-all dataset. Therefore, I used REF-all including its variable regions for further phylogenetic analyses. The final REF-all and the ASV-all datasets contained 94 and 219 sequences, respectively. Lengths in basepairs (bp) for the aligned REF-all and ASV-all datasets were 352 bp and 294 bp, respectively. REF-all had 166 parsimony informative characters, whilst ASV-all had 98. The estimated best fit model according to jModelTest was TIM2ef + G for REF-all and K80 + G for ASV-all.

The Bayesian MC3 runs on REF-all dataset reached convergence after approximately five million generations and at termination, the ASDSF had fallen to 0.011408. The first 23,333 saved trees (i.e., 25%) were discarded as burn-in. The Bayesian MC3 runs on ASV-all dataset reached convergence after approximately 12 million generations and at termination, the ASDSF had fallen to 0.014863. The first 10,000 saved trees (i.e., 25%) were discarded as burn-in. The ESS values were above 200 for all parameters for both the REF-all and ASV-all phylogenetic analyses.

The REF-all Bayesian 50% majority rule consensus tree of Tremellales is not resolved and reference sequences for the various families are recovered on a basal polytomy (Fig. 2). The various reference sequences cluster with other reference sequences or with ASVs, with or without statistical support set to ≥ 70% bootstrap (BS) and ≥ 0.95 posterior probabilities (PP). The majority of ASVs were found in clade 1O, the *T. pertusariae* s. l. species complex. ASVs with abundances above 100,000 reads were not found outside this clade (Fig. 2: clade 1O). Clade 1O also contained the majority of ASVs that had multiple occurrences in different host specimens, and all OLICH sequences (Fig. 2). Some occurrences of closely related ASVs originated from the same specimen, such as ASV74 and ASV527 (Fig. 2: clade 1N), which both were found in Ca17. Most clades in REF-all phylogeny had both BS values of ≥ 70% and PP of ≥ 0.95, except for clade 1M (47% BS, 0.95 PP) and clade 1Q (54% BS, 0.98 PP). Several ASVs grouped with reference sequences in highly supported clades outside of *T. pertusariae* s. l. species complex (Fig. 2) and Tremellaceae. ASV596 grouped with *T. coppinsii* (clade 1A; 90% sequence similarity), ASV77 with *Vishniacozyma carnescens* Verona & Luchetti (clade 1B; 100% sequence similarity), ASV2868 with *Tremella anaptychia* Zamora, Diederich,

Millanes & Wedin (clade 1E; 94% sequence similarity), ASV145 with *Tremella diploschistina* Millanes, Westberg, Wedin & Diederich (clade 1F; 92% sequence similarity), ASV339 with *Tremella wirthii* Diederich (clade 1G; 88% sequence similarity), ASV1683 with *Naematelia aurantia* (Schwein.) Brut. (clade 1H; 91% sequence similarity), and ASV94 with *Tremella mayrhoferi* Zamora, Millanes, Etayo & Wedin (clade 1L; 98% sequence similarity). Clade 1M contains a number of ASVs grouped with *T. macrobasidiata* with relatively high sequence similarities: ASV476 with 94% sequence similarity, ASV163 with 97% sequence similarity, ASV202 with 99% sequence similarity, and ASV85 with 97% sequence similarity. The only reference that got grouped with ASVs in clade 1O is *T. pertusariae* (Fig. 2). ASV3471, ASV246, ASV3312, ASV1876, ASV846, ASV711 did not appear in highly supported clades. For all BLAST results, see supplementary Table S4.

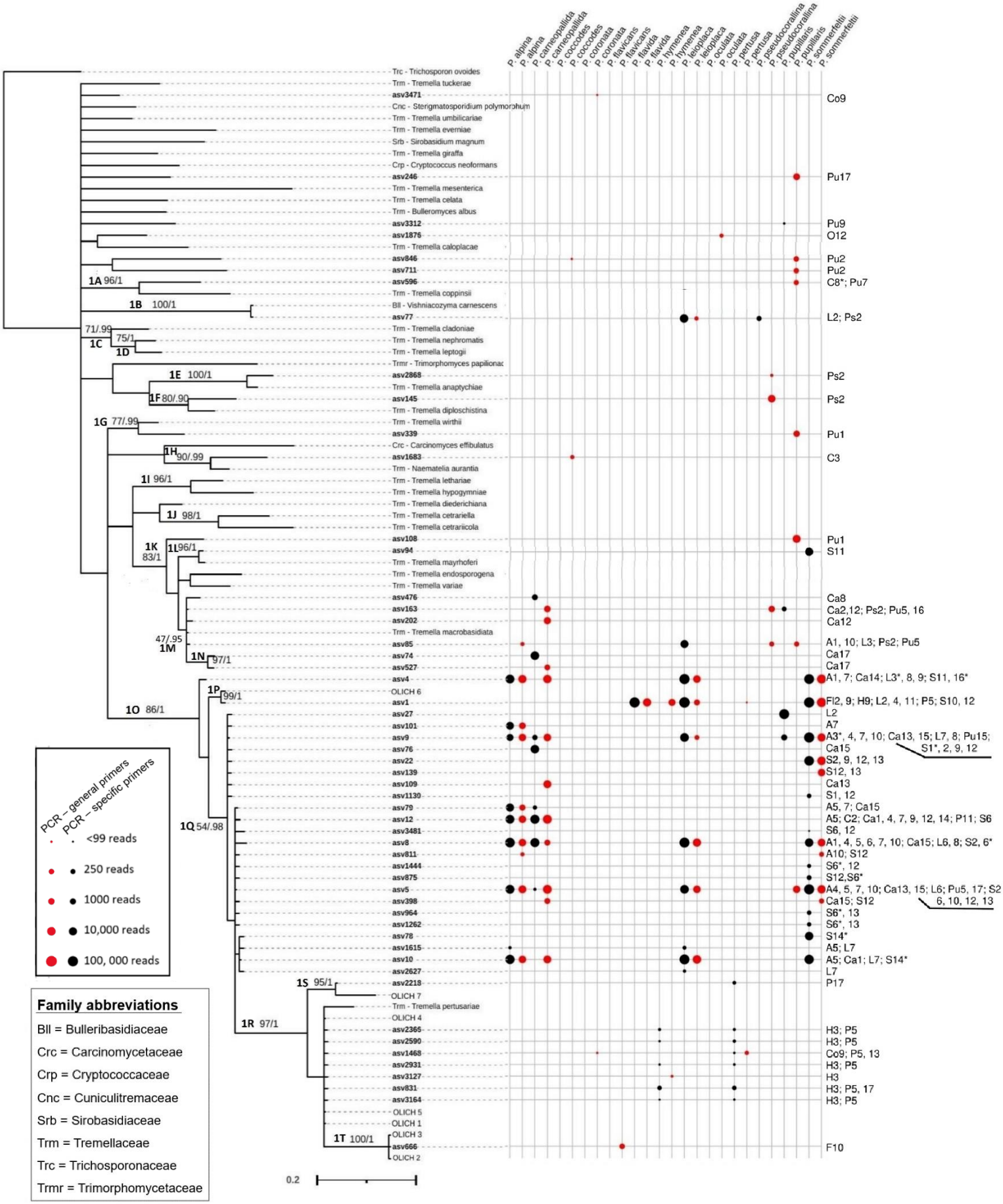
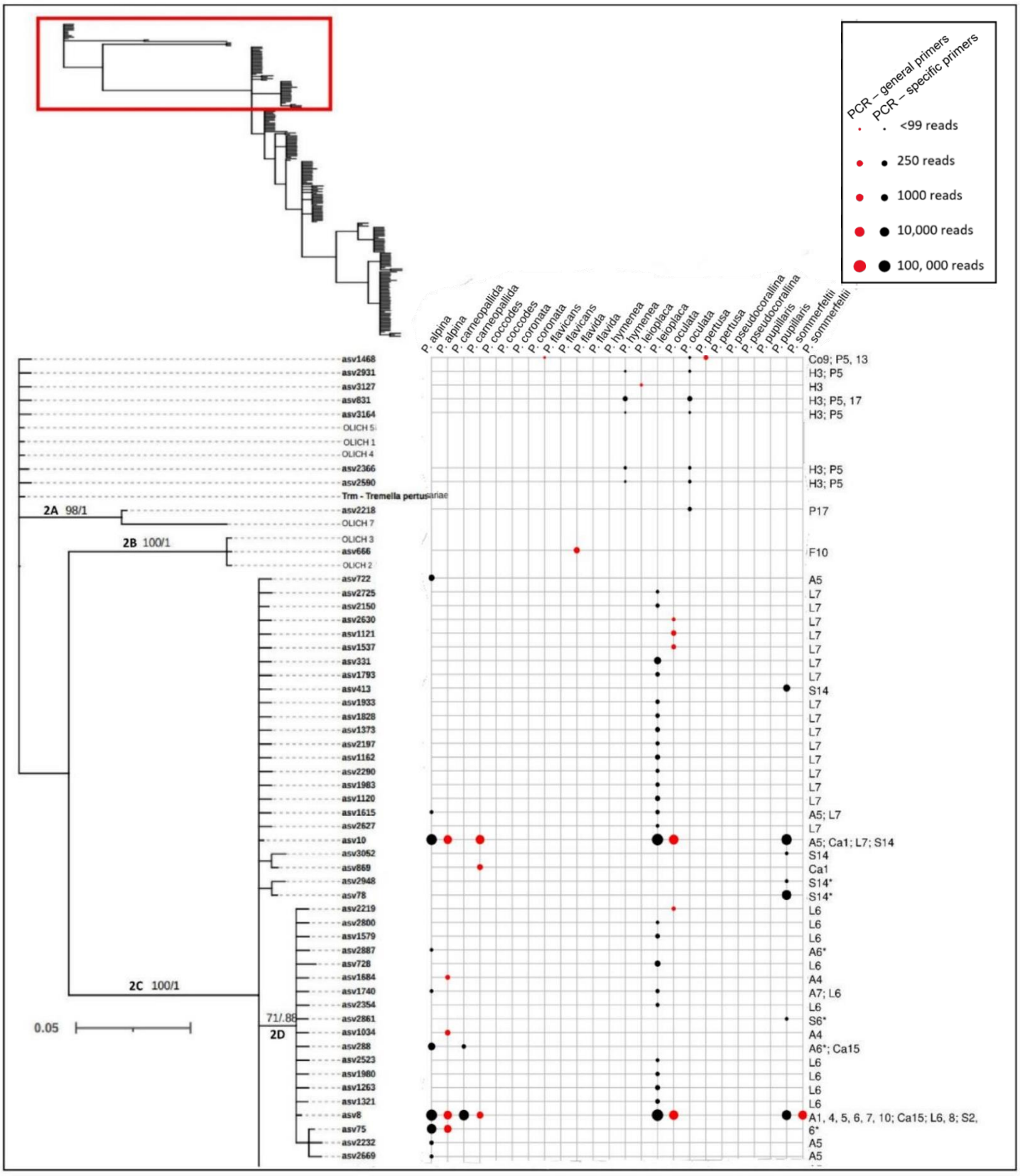

P. alpina
P. alpina
P. carneopallida
P. carneopallida
P. coccodes
P. coccodes
P. coronata
P. flavicans
P. flavida
P. flavida
P. hymenea
P. hymenea
P. leioplaca
P. oculata
P. pertusa
P. pertusa
P. pseudocorallina
P. pseudocorallina
P. pupillaris
P. sommerfeltii
P. sommerfeltii
Trc - Trichosporon ovoides
Trm - Tremella tuckerae
asv3471
Cnc - Sterigmatosporidium polymorphum
Trm - Tremella umbilicariae
Trm - Tremella everniae
Srb - Sirobasidium magnum
Trm - Tremella giraffa
Crp - Cryptococcus neoformans
asv246
Trm - Tremella mesenterica
Trm - Tremella celata
Trm - Bulleromyces albus
asv3312
asv1876
Trm - Tremella caloplacae
asv846
asv711
asv596
Trm - Tremella coppinsii
Bll - Vishniacozyma carnescens
asv77
Trm - Tremella cladoniae
Trm - Tremella nephromatis
Trm - Tremella leptogii
Trmr - Trimorphomyces papilionac
asv2868
Trm - Tremella anaptychiae
asv145
Trm - Tremella diploschistina
Trm - Tremella wirthii
asv339
Crc - Carcinomyces effibulatus
asv1683
Trm - Naematelia aurantia
Trm - Tremella lethariae
Trm - Tremella hypogymniae
Trm - Tremella diederichiana
Trm - Tremella cetrariella
Trm - Tremella cetrariicola
asv108
asv94
Trm - Tremella mayrhoferi
Trm - Tremella endosporogena
Trm - Tremella variae
asv476
asv163
asv202
Trm - Tremella macrobasidiata
asv85
asv74
asv527
asv4
OLICH 6
asv1
asv27
asv101
asv9
asv76
asv22
asv139
asv109
asv1130
asv79
asv12
asv3481
asv8
asv811
asv1444
asv875
asv5
asv398
asv964
asv1262
asv78
asv1615
asv10
asv2627
asv2218
OLICH 7
Trm - Tremella pertusariae
OLICH 4
asv2365
asv2590
asv1468
asv2931
asv3127
asv831
asv3164
OLICH 5
OLICH 1
OLICH 3
asv666
OLICH 2
1A 96/1
1B 100/1
71/.99
1C
75/1
1D
1E 100/1
1F 80/.90
1G 77/.99
1H 90/.99
1I 96/1
1J 98/1
1K
83/1
1L 96/1
47/.95
1M
1N 97/1
1O 86/1
1P 99/1
1Q 54/.98
1R 97/1
1S 95/1
1T 100/1
0.2
Co9
Pu17
Pu9
O12
Pu2
Pu2
C8*; Pu7
L2; Ps2
Ps2
Ps2
Pu1
C3
Pu1
S11
Ca8
Ca2,12; Ps2; Pu5, 16
Ca12
A1, 10; L3; Ps2; Pu5
Ca17
Ca17
A1, 7; Ca14; L3*, 8, 9; S11, 16*
Fl2, 9; H9; L2, 4, 11; P5; S10, 12
L2
A7
A3*, 4, 7, 10; Ca13, 15; L7, 8; Pu15;
Ca15
S1*, 2, 9, 12
S2, 9, 12, 13
S12, 13
Ca13
S1, 12
A5, 7; Ca15
A5; C2; Ca1, 4, 7, 9, 12, 14; P11; S6
S6, 12
A1, 4, 5, 6, 7, 10; Ca15; L6, 8; S2, 6*
A10; S12
S6*, 12
S12,S6*
A4, 5, 7, 10; Ca13, 15; L6; Pu5, 17; S2
Ca15; S12
6, 10, 12, 13
S6*, 13
S6*, 13
S14*
A5; L7
A5; Ca1; L7; S14*
L7
P17
H3; P5
H3; P5
Co9; P5, 13
H3; P5
H3
H3; P5, 17
H3; P5
F10
PCR – general primers
PCR – specific primers
<99 reads
250 reads
1000 reads
10,000 reads
100, 000 reads
Family abbreviations
Bll = Bulleribasidiaceae
Crc = Carcinomycetaceae
Crp = Cryptococcaceae
Cnc = Cuniculitremaceae
Srb = Sirobasidiaceae
Trm = Tremellaceae
Trc = Trichosporonaceae
Trmr = Trimorphomycetaceae

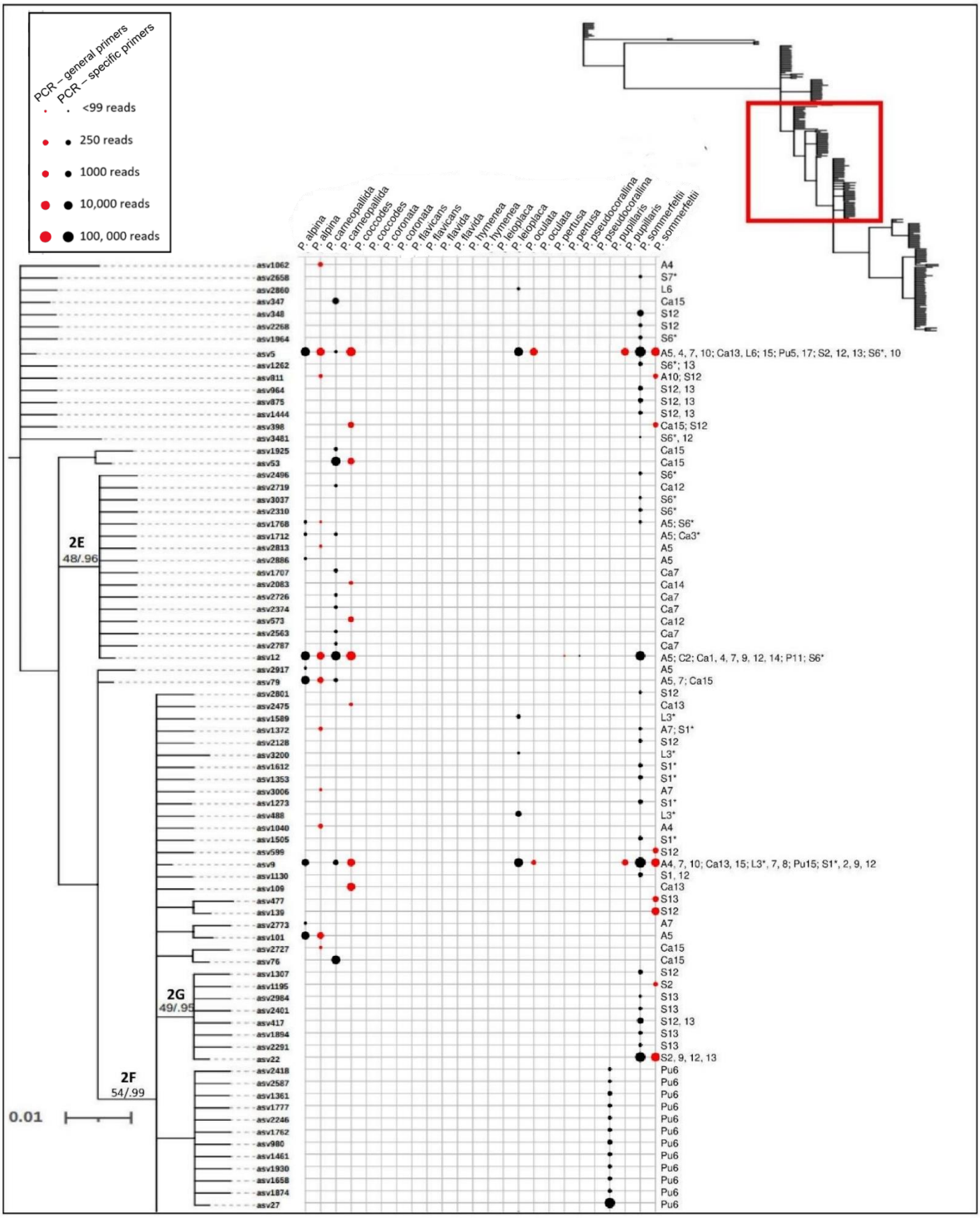
Figure 2. A Bayesian 50% majority rule consensus phylogeny of ITS2 of all remaining ASVs after subjected to filtering step. Branch support values ≥ 70% BS and ≥ 0.95 PP are indicated. Highly supported clades are numbered 1A-1T. Abundance of reads is visualized in dot plot with full grid, where size of dot indicates circa abundance of reads (see legend). Black dots indicate ASVs amplified with PCR using *Tremella*-specific primers, and red dots ASVs amplified with PCR using general fungal primers. Text to the right of the dot plot shows the specimen number of the *Pertusaria* hosts (see S2, S3, and S4 for specimen and reference overview). Asterix's indicate *Pertusaria* specimens that have only been sequenced with *Tremella*-specific primers, not general fungal primers. The second legend shows abbreviations of family names within Tremellales. The scale bar indicates the number of substitutions per site.

Most clades in the ASV-all phylogeny had support values of both ≥ 70% BS and ≥ 0.95 PP, except for clades 2D (71% BS, 0.88 PP), 2E (48% BS, 0.96 PP), 2F (54% BS and 0.99 PP), and 2G (49% BS and 95 PP) (Fig. 3).

My results from the ASV-all phylogeny (Fig. 3) show more ASVs originating from the same host specimen as observed for the REF-all phylogeny (Fig. 2), due to different filtering methods. For example, ASV1933, ASV1828, ASV1373, ASV2197, ASV1162, ASV2290, ASV1983, ASV1120, and ASV2627 are all sequenced from L7 and appear in same clade (Fig. 3: clade 2C). These patterns are found throughout the ASV-all phylogeny.

When an ASV was detected by both primer combinations, the *Tremella*-specific primers gave higher abundances per specimen than the general primers (Figs. 2 and 3: dot plots). In total 47 ASVs had considerably higher read abundances when amplified with *Tremella*-specific primers. For example, ASV77 in *Pertusaria leioplaca* DC. (L2) had an abundance of 10,000-100,000 reads when amplified with *Tremella*-specific primers and 250-1000 reads when amplified with general fungal primers (Fig. 2: clade 1A). Another example is ASV75 in *Pertusaria alpina* Hepp ex Ahles (specimen A1), which had 10,000-100,000 reads when amplified with *Tremella*-specific primers and 1000-10,000 reads when amplified with general fungal primers (Fig. 3: clade 2D). Only six ASVs had considerably higher read abundances when amplified with general fungal primers. For example, ASV9 in *Pertusaria carneopallida* (Nyl.) Anzi. (specimen Ca13) had an abundance of 250-1000 reads when amplified with *Tremella*-specific primers and 1000-10,000 reads when amplified with general fungal primers (Fig.2: clade 1Q). Another example is ASV5 in *P. carneopallida* (specimen Ca13), which had < 99 reads when amplified with *Tremella*-specific primers and 1000-10,000 reads when amplified with general fungal primers (Fig. 3: clade 2C).


PCR – general primers
PCR – specific primers
<99 reads
250 reads
1000 reads
10,000 reads
100, 000 reads
P. alpina
P. alpina
P. carneopallida
P. carneopallida
P. coccodes
P. coccodes
P. coronata
P. coronata
P. flavicans
P. flavicans
P. flavida
P. flavida
P. hymenea
P. hymenea
P. leioplaca
P. leioplaca
P. oculata
P. oculata
P. pertusa
P. pertusa
P. pseudocorallina
P. pseudocorallina
P. pupillaris
P. pupillaris
P. sommerfeltii
P. sommerfeltii
asv1468
asv2931
asv3127
asv831
asv3164
OLICH 5
OLICH 1
OLICH 4
asv2366
asv2590
Trm - Tremella pertusariae
asv2218
OLICH 7
OLICH 3
asv666
OLICH 2
asv722
asv2725
asv2150
asv2630
asv1121
asv1537
asv331
asv1793
asv413
asv1933
asv1828
asv1373
asv2197
asv1162
asv2290
asv1983
asv1120
asv1615
asv2627
asv10
asv3052
asv869
asv2948
asv78
asv2219
asv2800
asv1579
asv2887
asv728
asv1684
asv1740
asv2354
asv2861
asv1034
asv288
asv2523
asv1980
asv1263
asv1321
asv8
asv75
asv2232
asv2669
2A 98/1
2B 100/1
2C 100/1
2D
71/.88
0.05
Co9; P5, 13
H3; P5
H3
H3; P5, 17
H3; P5
H3; P5
H3; P5
P17
F10
A5
L7
L7
L7
L7
L7
L7
L7
S14
L7
L7
L7
L7
L7
L7
L7
L7
A5; L7
L7
A5; Ca1; L7; S14
S14
Ca1
S14*
S14*
L6
L6
L6
A6*
L6
A4
A7; L6
L6
S6*
A4
A6*; Ca15
L6
L6
L6
L6
A1, 4, 5, 6, 7, 10; Ca15; L6, 8; S2, 6*
A5
A5


PCR – general primers
PCR – specific primers
<99 reads
250 reads
1000 reads
10,000 reads
100, 000 reads
P. alpina
P. alpina
P. carneopallida
P. carneopallida
P. coccodes
P. coccodes
P. coronata
P. flavicans
P. flavicans
P. flavida
P. flavida
P. hymenea
P. hymenea
P. leioplaca
P. leioplaca
P. oculata
P. oculata
P. pertusa
P. pertusa
P. pseudocoralina
P. pseudocoralina
P. pupillaris
P. pupillaris
P. sommerfeltii
P. sommerfeltii
asv1062
asv2658
asv2860
asv347
asv348
asv2268
asv1964
asv5
asv1262
asv811
asv964
asv875
asv1444
asv398
asv3481
asv1925
asv53
asv2496
asv2719
asv3037
asv2310
asv1768
asv1712
asv2813
asv2886
asv1707
asv2083
asv2726
asv2374
asv573
asv2563
asv2787
asv12
asv2917
asv79
asv2801
asv2475
asv1589
asv1372
asv2128
asv3200
asv1612
asv1353
asv3006
asv1273
asv488
asv1040
asv1505
asv599
asv9
asv1130
asv109
asv477
asv139
asv2773
asv101
asv2727
asv76
asv1307
asv1195
asv2984
asv2401
asv417
asv1894
asv2291
asv22
asv2418
asv2587
asv1361
asv1777
asv2246
asv1762
asv980
asv1461
asv1930
asv1658
asv1874
asv27
2E
48/.96
2G
49/.95
2F
54/.99
0.01
A4
S7*
L6
Ca15
S12
S12
S6*
A5, 4, 7, 10; Ca13, L6; 15; Pu5, 17; S2, 12, 13; S6*, 10
S6*; 13
A10; S12
S12, 13
S12, 13
S12, 13
Ca15; S12
S6*, 12
Ca15
Ca15
S6*
Ca12
S6*
S6*
A5; S6*
A5; Ca3*
A5
A5
Ca7
Ca14
Ca7
Ca7
Ca12
Ca7
Ca7
A5; C2; Ca1, 4, 7, 9, 12, 14; P11; S6*
A5
A5, 7; Ca15
S12
Ca13
L3*
A7; S1*
S12
L3*
S1*
S1*
A7
S1*
L3*
A4
S1*
S12
A4, 7, 10; Ca13, 15; L3*, 7, 8; Pu15; S1*, 2, 9, 12
S1, 12
Ca13
S13
S12
A7
A5
Ca15
Ca15
S12
S2
S13
S13
S12, 13
S13
S13
S2, 9, 12, 13
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6
Pu6

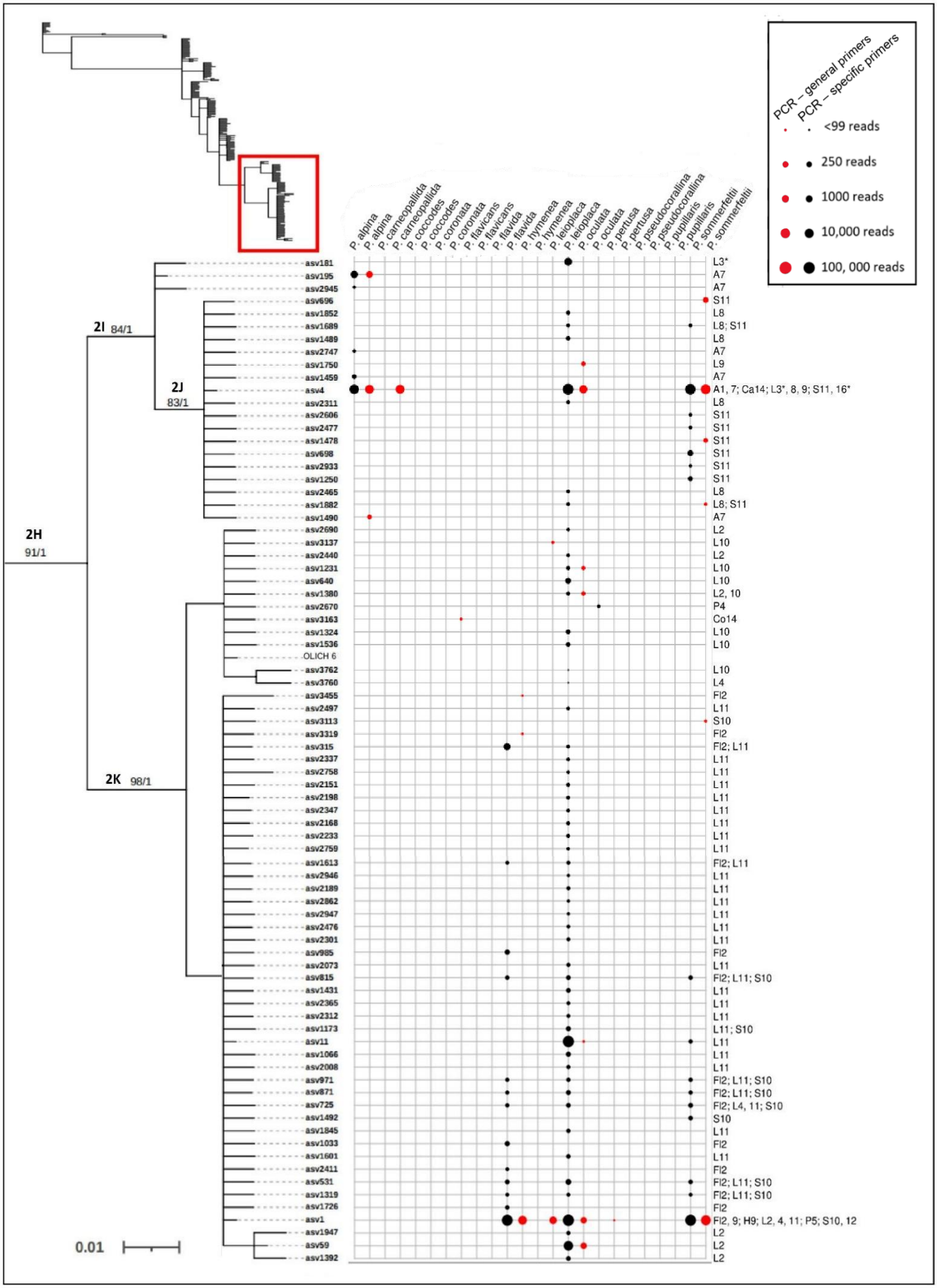

PCR – general primers
PCR – specific primers
<99 reads
250 reads
1000 reads
10,000 reads
100, 000 reads
P. alpina
P. alpina
P. carneopallida
P. carneopallida
P. coccodes
P. coccodes
P. coronata
P. coronata
P. flavicans
P. flavicans
P. flavida
P. flavida
P. hymenea
P. hymenea
P. leioplaca
P. leioplaca
P. oculata
P. pertusa
P. pertusa
P. pseudocorallina
P. pseudocorallina
P. pupillaris
P. pupillaris
P. sommerfeltii
P. sommerfeltii
2I 84/1
2J 83/1
2H 91/1
2K 98/1
asv181 L3*
asv195 A7
asv2945 A7
asv696 S11
asv1852 L8
asv1689 L8; S11
asv1489 L8
asv2747 A7
asv1750 L9
asv1459 A7
asv4 A1, 7; Ca14; L3*, 8, 9; S11, 16*
asv2311 L8
asv2606 S11
asv2477 S11
asv1478 S11
asv698 S11
asv2933 S11
asv1250 S11
asv2465 L8
asv1882 L8; S11
asv1490 A7
asv2690 L2
asv3137 L10
asv2440 L2
asv1231 L10
asv640 L10
asv1380 L2, 10
asv2670 P4
asv3163 Co14
asv1324 L10
asv1536 L10
OLICH 6
asv3762 L10
asv3760 L4
asv3455 Fl2
asv2497 L11
asv3113 S10
asv3319 Fl2
asv315 Fl2; L11
asv2337 L11
asv2758 L11
asv2151 L11
asv2198 L11
asv2347 L11
asv2168 L11
asv2233 L11
asv2759 L11
asv1613 Fl2; L11
asv2946 L11
asv2189 L11
asv2862 L11
asv2947 L11
asv2476 L11
asv2301 L11
asv985 Fl2
asv2073 L11
asv815 Fl2; L11; S10
asv1431 L11
asv2365 L11
asv2312 L11
asv1173 L11; S10
asv11 L11
asv1066 L11
asv2008 L11
asv971 Fl2; L11; S10
asv871 Fl2; L11; S10
asv725 Fl2; L4, 11; S10
asv1492 S10
asv1845 L11
asv1033 Fl2
asv1601 L11
asv2411 Fl2
asv531 Fl2; L11; S10
asv1319 Fl2; L11; S10
asv1726 Fl2
asv1 Fl2, 9; H9; L2, 4, 11; P5; S10, 12
asv1947 L2
asv59 L2
asv1392 L2
0.01

Figure 3. A Bayesian 50% majority rule consensus phylogeny of ITS2 from ASVs in *T. pertusariae* s. l. complex (corresponding to clade 1O in Fig. 2, but without filtering step). The phylogeny was split into three parts. Location in phylogeny is visualized with a red box on the skeleton. Branch support values ≥ 70% BS and ≥ 0.95 PP are indicated on the phylogenetic tree. Highly supported clades are numbered 2A-2K. Abundance of reads is visualized in dot plot with full grid, where size of dot indicates circa abundance of reads (see legend). Black dots indicate ASVs amplified with PCR using *Tremella*-specific primers, and red dots ASVs amplified with PCR using general fungal primers. Text on the right of the dot plot shows specimen number of the *Pertusaria* hosts (see Table S2 and S3 for specimen overview). Asterix indicates that the respective *Pertusaria* specimen has only been subjected to *Tremella*-specific primers, not general fungal primers. Scale bar indicates number of substitutions per site.

3.2 Host specificity

Host specificity was visualized on a circos plot including the most common ASVs (Fig. 4). All included *Tremella* ASVs, apart from ASV22, were detected in three to five species of *Pertusaria*. ASV22 was detected only in individuals of *Pertusaria sommerfeltii* (Sommerf.) Flörke. The 24 sampled specimens of *P. sommerfeltii*, however, hosted all other ASVs except ASV6. ASV12 had 80% of its occurrences in *P. carneopallida*, and ASV8 had 55% of its occurrences in *P. alpina.* The remaining ASVs had less than 50% of their occurrences in a single host species. Only ASV6 was detected in *Pertusaria coronata* (Ach.) Th. Fr. and *Pertusaria pertusa* (L.) Tuck., only ASV165 in *Pertusaria coccodes* (Ach.) Nyl., and only ASV1 in *Pertusaria flavida* (DC.) Laundon; all host species with only two investigated specimens.

Host specificity can also be inferred from the REF-all phylogeny (Fig. 2). For example, ASV476, ASV163, ASV202, ASV74, and ASV527 in clade 1M have *P. carneopallida* as host, while ASV85 is the only sequence in clade 1M that was found in different host species. Some of the OLICH sequences also group with ASVs that were sequenced from the same host species (Table S3). ASV1 and OLICH1 in clade 1P were sequenced from *P. pertusa*. Note that ASV1 was also found in many other host species. ASV2218 and OLICH2 (Table S3) in clade 1S were both sequenced from *P. pertusa*. Clade 1T contains ASV666, OLICH6, and OLICH7. Both ASV666 and OLICH6 were sequenced from *Pertusaria flavicans* Lamy, whilst OLICH7 from *Pertusaria amarescens* Nyl. No clades in the ASV-all phylogeny have more than 50% of ASVs originating from the same host species. Host specificity was not visualized on the ASV-all phylogeny (Fig. 3) because it includes a majority of ASVs that were recovered from only a single specimen of *P. leioplaca* (L11).

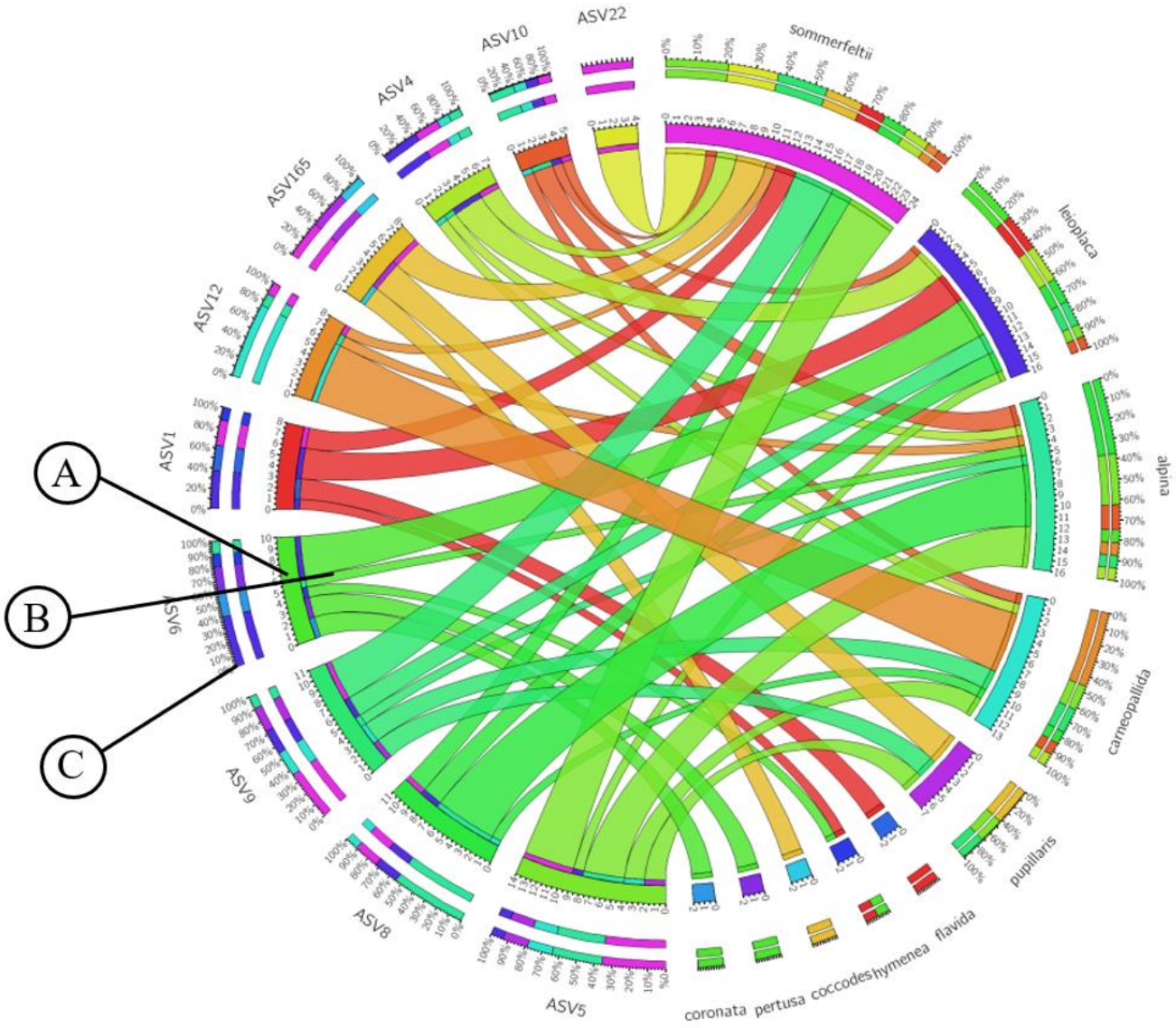


Figure 4. Circos plot linking individual ASVs with *Pertusaria* hosts. (A) The angular size of circularly arranged segments is proportional to the number of tremellalean ASVs for the different host species. (B) The ribbons visualize which individual ASVs were found in which host species. (C) The three outer rings are stacked bar plots which show the relative contribution of the number of tremellalean ASVs to the total of the different host species.

3.3 Comparison of PCR method

The results from the generalized linear mixed model (GLMM) analysis are based on 190 samples from PCR using *Tremella*-specific primers and 116 samples from PCR using general fungal primers. Tremellalean ASVs are more likely to be detected using the general fungal primers compared to the *Tremella*-specific primers, which is supported by the following statistics from the GLMM: $\beta = 9.35$, SE = 1.12, $z(292) = 8.31$, $p<0.0001$. The GLMM had reached an Akaike Information Criterion (AIC) value of 296.64. Detection rates of tremellalean ASVs differed between host species and methods (Fig. 2 and 3: dot plot, and Fig. 5). Tremellalean ASVs in *P. coccodes* and *Pertusaria pseudocorallina* (Lilj.) Arnold were only amplified with PCR using general fungal primers, whilst no hosts had only ASVs amplified with PCR using *Tremella*-specific primers. Ratios of *Tremella* presence was higher with only PCR using general fungal primers than only PCR using *Tremella*-specific primers for *P. carneopallida* (41% vs. 6%), *P. coccodes* (43% vs. 0%), *Pertusaria oculata* (Dickson) Th. Fr. (25% vs. 6%), *Pertusaria pupillaris* (Nyl.) Th. Fr. (47% vs. 6%), and *P. flavida* (22% vs. 0%). Ratios of *Tremella* presence was higher with only PCR using *Tremella*-specific primers than only PCR using general fungal primers for *P. leioplaca* (18% vs. 0%) and *P. sommerfeltii* (37% vs. 19%). Ratios of *Tremella* detection on same specimens by both methods was higher than detection with only PCR using *Tremella*-specific primers or PCR using general fungal primers for *P. alpina* (30% vs. 20% for general fungal primers and 10% for *Tremella*-specific primers) and *P. leioplaca* (64% vs. 0% for general fungal primers and 18% for *Tremella*-specific primers). All hosts had specimens which could only be detected by one of the methods. Amplification success also varied between species (Fig. 5). For example, *P. coronata* had an overall *Tremella* amplification success of ca. 19%, whilst *P. sommerfeltii* had an overall success of ca. 81%.

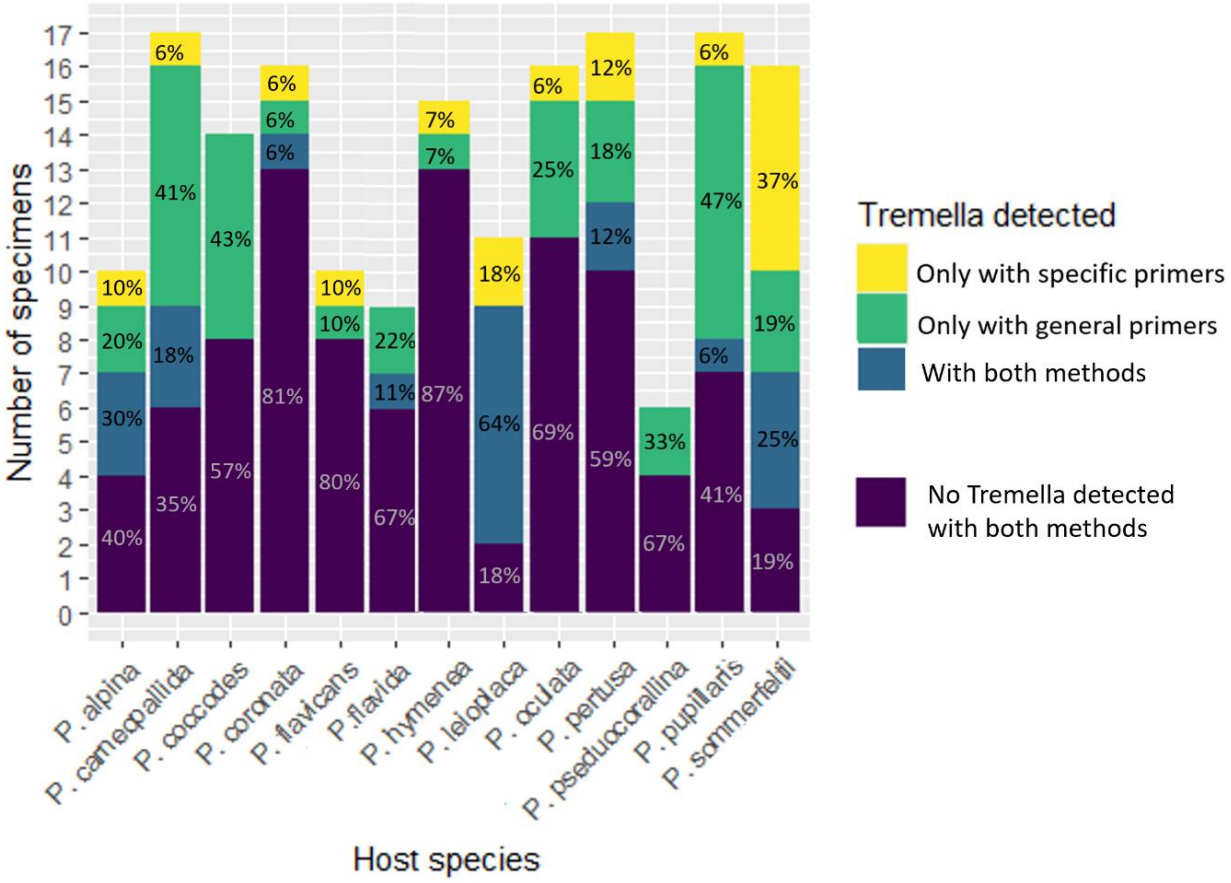


Figure 5. The numbers of specimens of each *Pertusaria* species with *Tremella* detected. The bars are divided into detection by PCR using *Tremella*-specific primers, PCR using general fungal primers, both approaches, and none of them, respectively. Ratios are displayed within bars.

3.4 Effect of climate

Among the five bioclim variables tested, only Bio6 (minimum temperature of the coldest month) was statistically significant as an explanatory variable for the occurrence of Tremellales (Table 2). Tests were deemed statistically significant when the P-value was ≤ 0.05. Bio6 without additional precipitation variables also had the lowest AICc value, making it the best model tested. This variable also proved to be significant when combined with Bio8 (mean temperature of wettest quarter). Bio6 was less significant when combined with Bio15 (seasonality of precipitation) but still had a low enough AICc score to be within the top three ranking models. Only the top-ranking models are discussed.

Table 2. Summary of relevant statistics from generalized linear models of presence of *Tremella* in *Pertusaria*, dependent on various climatic variables. Boldface indicates significance values. The boldface lines show start and end of top-ranking models. Bioclim variables are minimum temperature of the coldest month (Bio6), mean temperature of wettest quarter (Bio8), seasonality of precipitation (Bio15), mean annual temperature (Bio1), and mean annual precipitation (Bio12).

Bioclim variables	**Estimate (Intercept)**	**Estimate (covariates)**	**P-value Pr(>|z|)**	**AICc**
Bio6	-1.096035	-0.009794	**0.022871**	231.60
Bio6 + Bio8	-0.871715	-0.010286; -0.005038	**0.0186** + 0.2769	232.47
Bio6 + Bio15	-0.016247	-0.007950; -0.035637	0.0979 + 0.3802	232.89
Bio1	0.02462	-0.01283	0.0536	233.14
Bio6 + Bio12	-1.5066357	-0.0118357; 0.0002041	0.056 + 0.643	233.45
Bio15	1.31521	-0.06702	0.0697	233.54
Bio1 + Bio15	1.055105	-0.009260; -0.043311	0.216 + 0.292	234.08
Bio12	0.0615293	-0.0004057	0.187	235.17
Bio8	-0.302504	-0.004105	0.369	236.12

The predictor effect plot of Bio6 (Fig. 6) shows a clear decline in the presence of tremellalean fungi in *Pertusaria* when temperature of the coldest month increases, with the highest abundance when the minimum temperature is around -15 °C. This plot also shows that the majority of *Pertusaria* specimens with tremellalean fungi were collected in areas between -10 and 0 °C, and the standard error (SE) increases in areas with low sampling. Distribution maps over Norway and the top-five most common ASVs (Fig. S1 - S5) did not reveal any particular patterns of preferred localities among ASVs.

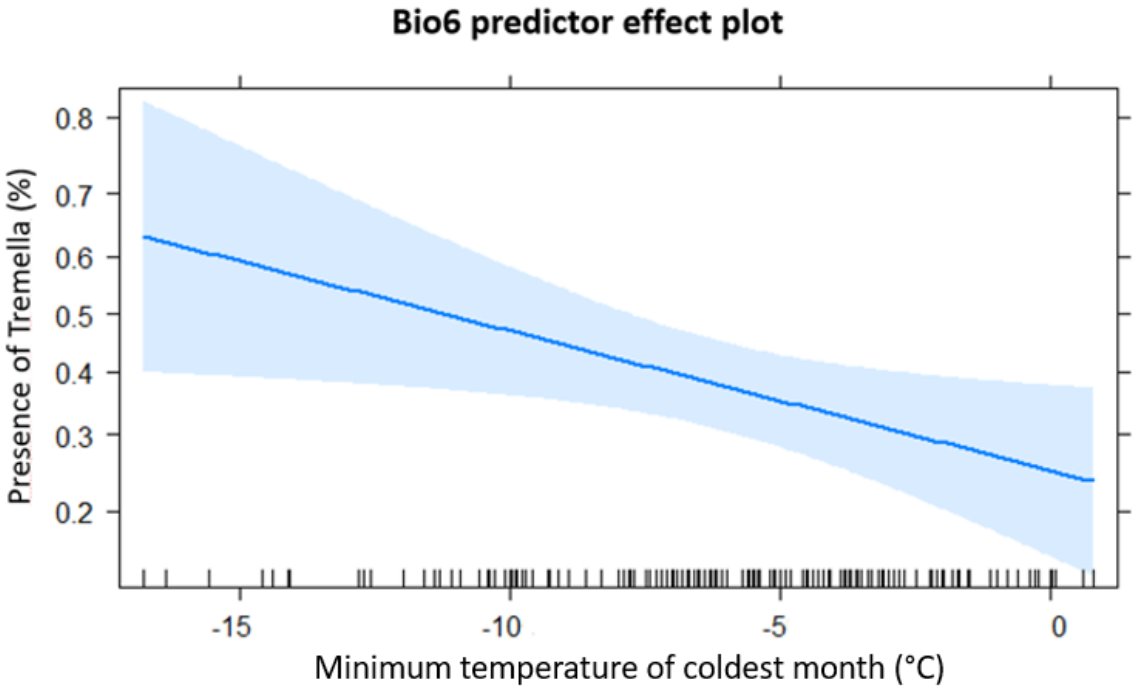


Figure 6. Predictor effects plot based on generalized linear model, that shows the percentage of *Pertusaria* samples containing reads of *Tremella* based on Bio6. The shaded area shows a pointwise confidence band and is based on SE. The rug plot at the bottom of the graph shows the temperature location of the sampled *Pertusaria* specimens, regardless of whether it contained tremellalean fungi.

4 Discussion

Herein, I have (1) studied the diversity of lichenicolous *Tremella* in asymptomatic thalli of *Pertusaria* species in Norway, (2) investigated the specificity of lichenicolous tremellalean sequences obtained from asymptomatic thalli to species of *Pertusaria*, and (3) tested for a potential correlation between climate and presence of *Tremella*. I discovered that the chosen barcode marker, PCR, and sequencing methods have their limitations, and that these should be chosen with care. Choice of primer also needs careful consideration, as primer bias was shown to have a major effect on species detection. My research illuminates previously unknown tremellalean diversity in *Pertusaria* hosts and reveals genetic lineages within the *Tremella pertusariae* s. l. species complex that may eventually qualify as new species. Opposed to the host specificity observed in filamentous *Tremella*, my results indicate generalist behavior among tremellalean yeasts. Finally, I found a decline in the presence of *Tremella* in asymptomatic *Pertusaria* when the temperature of the coldest month increases.

4.1 Methodological aspects

There are multiple instances, mainly in our phylogeny of the *T. pertusariae* s. l. complex, when highly similar low abundance ASVs appear in a single specimen (Fig. 3). These low abundance variants may represent PCR errors (i.e., polymerase errors or PCR stochasticity), sequencing errors, or the presence of a non-dominant ITS copy, representing true intraspecific variation, all of which are almost impossible to tell apart. For example, the ASVs 2984, 2401, 417, 1894, and 2219 are potential variants of ASV22 (Fig. 3: clade 2G), as they differ with only one bp from each other as well as from ASV22. I regard ASV22 as the "parent sequence" since it had the highest abundance and was amplified by both PCR methods. I believe most errors in our data were acquired during PCR, as these types of errors are more common and have a random distribution (Potapov & Ong 2017), which was reflected in our alignment of the ASV-all phylogeny (Fig. 3). These potential PCR errors, which were not detected in the DADA2 pipeline, could either be polymerase or stochastic errors (Kebschull & Zador 2015). The former type of error is caused by the polymerase enzyme inserting the wrong nucleotide during DNA replication library construction prior to short-read HTS (Pray 2008). We used a high-fidelity polymerase, which has a proofreading ability and suppresses these errors to a certain degree, but some errors may yet be missed (Filges et al. 2019). As replication of each nucleotide occurs with a probability < 1 (Kebschull & Zador 2015), PCR stochasticity may alone explain the low abundance variants in our dataset. However, some of these errors may yet have occurred during

the short-read HTS. The main sources of error during Illumina sequencing are substitution type miscalls (Stoler & Nekrutenko 2021) and indels (Nagai et al. 2022).

Distinguishing between PCR/sequencing errors and true intraspecific variation is challenging, but the ASV-all phylogeny holds some cases that I strongly believe represent intraspecific diversity. For example, clade 2E (Fig. 3) contains many ASVs (e.g., 1707, 2726, and 2374) that likely represent PCR/sequencing errors of the true sequence ASV12. ASV1768, on the other hand, is most likely not erroneous, as the exact same ASV was amplified with both PCR methods, which would be highly unlikely if it was a random PCR/sequencing error. Furthermore, there are several cases in the ASV-all phylogeny where the same low abundant and non-dominant ASV appear in multiple host specimens, indicating the presence of intraspecific variation rather than PCR/sequencing errors, for example ASV1740 (Fig. 3: clade 2D) which is present in both *P. alpina* (A7) and *P. leioplaca* (L6). The presence of intraspecific, or even intraindividual, variation in the ASV-all phylogeny is highly probable, as ITS is often prone to having multiple paralogous copies, which are practically indistinguishable from PCR and/or sequencing errors (Estensmo et al. 2021; Orton et al. 2015; Schoch et al. 2012).

Number of PCR cycles affected the amount of PCR errors in the dataset. The majority ASVs in the ASV-all phylogeny suspected of being errors were amplified with *Tremella*-specific primers (Fig. 3: PCR-specific primers). I distinguished erroneous ASVs from true genetic variation as explained above. The specific primers were part of a nested PCR and had a greater cycle number compared to PCR using only the general fungal primers (Fig. 3: PCR-general primers). This result was expected, as PCR errors increase with increasing number of PCR cycles (D'Amore et al. 2016; Patin et al. 2013).

Primers that are too specific can fail to detect genetic variation within a highly diverse genus. My results showed that, after taking varying sample size from both PCR methods into account, that more tremellalean ASVs were detected with PCR using only general fungal primers (Fig. 5), which most likely is due to primer bias. The *Tremella*-specific primers may have had more primer mismatches, due to not covering the vast diversity of this genus. This study thereby points out that there is a need for more primer testing in order to find the optimal general tremellalean primers, which should be diverse enough to not miss any of the genetic variation.

Previous studies comparing the two sub-regions, with regard to amplifying fungal diversity and providing taxonomic resolution, do not provide a one for all solution. Some studies found relatively little difference between the two (e.g., Blaalid et al. 2013). However, the majority of

studies prefer one above the other. For example, Mbareche et al. 2020 demonstrated a greater taxonomic coverage and species richness of fungal aerosols when using ITS1, in addition to some taxa being exclusively detected by either ITS1 or ITS2. The authors suggest that some of this discrepancy may be due to primer mismatch or differences in GC content, which may be in favor of ITS1. Another aspect to keep in mind is that short-read HTS has more difficulties in sequencing long amplicons. Since basidiomycetes have generally longer ITS2 sequences than ascomycetes, this can result in over-representation of ascomycetes in ITS2 datasets (Bellemain et al. 2013). A higher GC content in ITS2 compared to ITS1 can also negatively affect PCR and sequencing results (Wang et al. 2015). However, other studies have shown to favor ITS2. Yang et al. (2018) used pyrosequencing data for comparing ITS1 and ITS2. They found that ITS2 overall was the best marker, as ITS1 tended to overestimate species richness, even if both markers occasionally yielded different taxa. Based on the varying results between studies ITS1 ought to also be tested as a marker for asymptomatic lichenicolous *Tremella*.

Traditional Sanger sequencing could amplify both ITS1 and ITS2, which would give a better resolution compared to using a single marker. However, a downside of using Sanger sequencing is that it cannot detect any sequence variation if there are multiple variants in the DNA. This was my main reason for choosing short-read HTS, which also resulted in the detection of multiple *Tremella* variants from single specimens (Fig. 2 and 3). Long-read HTS techniques, such as PacBio and Nanopore sequencing, might clarify the unrecognized diversity in the *T. pertusariae* s. l. species complex, as it would enable obtaining longer sequence variants from the same host specimens. However, previous studies have experienced low base-calling accuracy when using long-read HTS compared to traditional Sanger sequencing and short-read HTS (Petersen et al. 2019; Tedersoo et al. 2018). Furthermore, Furneaux et al. (2021) attempted to combine the entire ITS and parts of the LSU region with PacBio, but they had trouble with the current laboratory and bioinformatics methods for these regions. On the other hand, Tedersoo et al. (2020) got a better resolution of unidentified fungal taxa by using this sequencing technique on multiple genes in combination with analyses of common enigmatic sequences in terms of taxonomic placement based on ITS. It has also been shown that long-read HTS (PacBio) is able to better resolve relative abundances in fungal communities rather than short-read HTS techniques (Castaño et al. 2020).

4.2 Tremellalean diversity

In this study, I find evidence for many species of *Tremella* never before found in *Pertusaria*. Several of the amplicon sequence variants (ASVs) belong to taxa unrelated to *T. pertusariae* (Fig. 2) and are closely related to sequences available from GenBank (Table S4). For example, *Vishniacozyma carnescens* of the Bulleribacidiaceae in Tremellales, has never before been found residing in *Pertusaria*, but my results show ASV77 to be in a highly supported clade with 100% sequence similarity to *V. carnescens*, according to the NCBI BLAST tool (Fig. 2: clade 1B). ASV596 appears in a highly supported clade together with *T. coppinsii* (Fig. 2: clade 1A), which so far has been detected only in species of *Platismatia* (Diederich 1996). ASV596 is separated from *T. coppinsii* by a long branch, with only 90% sequence similarity, and likely represents a different species. Before this study, only one species of *Tremella*, namely *T. pertusariae*, had been demonstrated in species of *Pertusaria* and only a single symptomatic specimen had been collected in Norway. Lichenicolous *Tremella* have previously been found as contaminations during DNA barcoding of asymptomatic *Pertusaria* using Sanger sequencing. However, no other studies have explored *Tremella* species diversity in asymptomatic *Pertusaria*. The ASVs from this study serve as evidence for previously undocumented diversity of tremellalean lichenicolous fungi in species of *Pertusaria*.

I want to point out that delimiting fungal species based on threshold values is problematic. Both phylogenetic trees (Fig. 2 and 3) contain ASVs with varying sequence similarities, yet some are grouped with relatively short branches in the same clade. For example, ASV496 has a 94% sequence similarity to *T. macrobasidiata* (Table S4), but appears in a highly supported clade with a short branch length (Fig. 2: clade 1M). Assigning taxonomy based on these results is problematic, as there is no standardized cut-off value for delimiting fungal species. Although it has been common to apply 97% sequence similarity for fungal ITS datasets (Geml et al. 2014). There are two types of errors that can happen when using genetic threshold values alone (Garnica et al. 2016). Firstly, the intraspecific variation could go deeper than the set threshold value, resulting in the splitting of true species (false positives). Secondly, interspecific variation could be shallower than the set threshold value, thereby failing to detect species (false negatives). Furthermore, Yurkov et al. (2015) conducted a study on basidiomycete yeasts within the Tremellales. They stated that a single cut-off value between 90-99% sequence similarity is hardly applicable to large yeast lineages, as intraspecific variability can differ greatly. This could also be the case for my tremellelean ASVs. Furthermore, simply using sequence similarity may not be enough for accurate species delimitations of fungi. The shortcomings of

delimitation with molecular identification methods can be solved with integrative taxonomy, by including information on host, ecology, and morphology.

The origin and growth form of the ASVs discovered in this study is not always clear. ASV596 may represent barcoded *Tremella* species that have not been included in the analysis. Metabarcoding also detects actively growing fungi, as well as fungal structures incidentally occurring on or in the lichen thallus, meaning that presence of a species in our dataset cannot be taken as definitive proof of active growth and colonization of the host.

I found a large amount of intraspecific variation and undescribed diversity closely related *to T. pertusariae* (Fig. 3), which I term the *T. pertusariae* s. l. species complex. Tremellalean diversity was especially apparent in the ASV-all phylogeny (Fig. 3). Based on a combination of high abundance ASVs, long branch length, and branch support (≥ 70 BS and ≥ 95 PP), I demonstrate the presence of six separate phylogenetic lineages (Fig. 3: clades 2A, B, D, E, G, K). The megablast algorithm that compared ASVs in these clades to the GenBank nucleotide-nr database resulted in taxonomic assignments that in most cases received "uncultured fungus" as top-hit (Table S4). The next species hit of a multitude of sequences in the ASV-all phylogeny was often below 90% in sequence similarity, indicating that there are no conspecific sequences in the GenBank database. Hence, the clades 2A, B, D, E, G, K (Fig. 3) cannot yet, due to the incompleteness of the fungal DNA barcode library, be taxonomically assigned. Some of the ASVs revealed herein may yet represent undescribed *Tremella* diversity in the *T. pertusariae* s. l. species complex.

This study is based on a single marker, the ITS2 barcode, which complicates interpretation of the observed molecular diversity. This means that the resulting phylogenies are gene trees, not species trees (Fig. 2 and 3). A gene tree may have very different topologies and levels of resolution than a species tree, as it cannot convey the entire species history, just the process of replication at a more local level (Szöllősi et al. 2015). The topology and resolution may change, given that additional markers are included, which could potentially bring us closer to a true species tree. However, for the purpose of species detection, a single marker can be useful. I underline the need for more sequencing and taxonomic studies targeting the *T. pertusariae* s. l. species complex, in order to build a more complete DNA barcode library and reference database. More tremellalean diversity may also be discovered with increased sampling, as the sampling of this study is limited geographically to Norway and only 14 out of 199 described *Pertusaria* species.

The yeast stage of fungi closely related to *T. pertusariae* is much more common in *Pertusaria* than first thought. I tested for presence of *Tremella* in 14 different asymptomatic *Pertusaria* hosts, and discovered ASVs belonging to the *T. pertusariae* s. l. species complex in 13 of the tested host species. Symptomatic *T. pertusariae* is previously only found in *P. hymenea* and *P. pertusa* (Millanes et al. 2014). This contrast begs the question if detecting ASVs from the yeast stage is the cause of my unexpectedly high amounts of diversity. According to Tuovinen et al. (2021), the haploid yeast stage behaves differently than the dikaryotic filamentous stage. Results based on different stages should therefore be treated cautiously.

4.3 Haploid yeast stage and host specificity

My results showcase generalist behavior among the majority of tremellalean ASVs (Figs. 2, 3 and 4). Note that tremellalean ASVs in the *T. pertusariae* s. l. species complex may still be restricted to species of *Pertusaria*. These ASVs might also occur in other lichens, but this cannot be known for sure, due to lack of appropriate studies specifically targeting Tremellales. I did observe some ASVs with higher levels of host specificity, such as ASV22 and ASV12 (Fig. 4). However, ASV22 occurred in only four specimens of *P. sommerfeltii*, adding uncertainty to the result due to the low sample number. ASV12 was present in six specimens of *Pertusaria carneopallida* and only two occurrences in other species. These results are not strong evidence of host specificity in tremellalean yeasts, but they can serve as indicators of stochasticity in specificity among asymptomatic lichenicolous lineages of *Tremella*.

Little is known about the various lichenicolous basidiomycete species, including whether they are host specific or not, due to technical difficulties when searching for hidden species. Only a few studies have targeted the hidden presence of basidiomycete yeasts in macrolichens (Černajová & Škaloud 2019; Mark et al. 2020; Spribille et al. 2016), indicating that these yeasts are common occurrences in lichens. For example, in a recent study, Tuovinen et al. (2019) showed that *Tremella lethariae* has a common and widespread yeast stage, only entering the sexual filamentous stage on rare occasions.

Questions regarding specificity of filamentous lichenicolous *Tremella* have received attention in previous papers (Millanes et al. 2011, 2018; Tuovinen et al. 2021). According to Lawrey and Diederich (2003), around 95% of all lichenicolous fungi are specific to a single lichen genus, which they explain may be due to the lichen host's secondary phenolic compounds. These compounds have inhibitory abilities which limit spore germination, and growth of fungi that cannot tolerate these compounds will be prevented (Merinero et al. 2015). Werth et al. (2013)

speculated if the lichen host has created an evolutionary selective environment, which only grants access to especially adapted lichenicolous fungi. It is not currently known if the host's secondary phenolic compounds affect the lichenicolous tremellalean yeasts. Furthermore, exactly how much of host specificity in lichenicolous filamentous *Tremella* is caused by secondary chemistry remains unknown, and more research is required to understand if there are other affecting factors.

The differences in host specificity between the asymptomatic yeast stage and the symptomatic filamentous stage may be due to physiological and morphological differences. For example, the tremellalean yeast stage differs from the filamentous stage by not forming tremelloid haustorical connections. The filamentous fungus uses these connections to interact with the mycobiont to gain nutrition, and these structures have been used as an indicator of having a parasitic lifestyle (Diederich 1996). This may be one reason why yeasts are less host specific, as a lack of these connections decreases direct contact between lichenicolous *Tremella* and the host lichen.

The behavior of the yeast stage of lichenicolous *Tremella* seems to vary between species, whilst lichenicolous filamentous *Tremella* are usually host specific. The majority of ASVs in my study showed no signs of host specificity, of which ASV22 and ASV12 were the only exceptions (Fig. 4). A study by Tuovinen et al. (2021) is the only previous research that targets host specificity of lichenicolous tremellalean yeasts. They used fluorescent in situ hybridization (FISH) and cell wall staining to study tremellalean yeasts and found that the yeast stage of *T. macrobasidiata* (which filamentous stage is restricted to *Lecanora chlarotera*) is also present in *Lecanora varia*. Tuovinen et al. (2021) also found that all life stages of *Tremella variae* seemed to be specific towards *L. varia.* Both the results from my study and from the study of Tuovinen et al. (2021) suggest that host specificity varies between the asymptomatic yeast stage and the symptomatic filamentous stage. Furthermore, our results may indicate that host specificity of the yeast stage also varies according to species. Still, I highlight I mainly have generalist behavior among tremellalean lichenicolous yeasts, which may indicate host specificity to be less common in *Pertusaria*-residing *Tremella*. A study by Spribille et al. (2016) revealed that some basidiomycete yeast lineages showed high levels of specificity towards host species, as with some of my and Tuovinen's data. These variable results highlight the need for additional studies investigating specificity of the lichenicolous tremellalean yeast stage in various species.

4.4 Climate data

A GLM testing various climatic variables (Table 1; Fig. 6) showed that presence of tremellalean ASVs in species of *Pertusaria* is negatively correlated with minimum temperature of the coldest month (Bio6). This suggests that the amount of presence of tremellalean species decreases when winter temperatures increase. The Bio6 variable was included in all top-three models, and was significant in all of them. The second and third model (Bio6 + Bio8 and Bio6 + Bio15, respectively) included interactive effects between temperature and precipitation (Table 1). However, both Bio8 and Bio15 had poorer explanatory power based on AICc values and neither were significant on their own in explaining the presence of *Tremella*. Hence, based on these results, the primary factor driving *Tremella* occurrence appears to be temperature of the coldest month.

It is important to keep in mind that demographic histories of a pathogen often are linked with that of its host, due to coevolution and connected population dynamics (Werth et al. 2013). With that logic, the presence or absence of *Tremella* would depend on the climatic preferences of *Pertusaria*. However, it is also possible that *Tremella* resides in *Pertusaria* simply due to a weakness of the host and not co-evolution. Lichens are highly sensitive to their environment due to exposure and the inability to actively control water uptake and storage (Green et al. 2011). If *Pertusaria* were to thrive and have a better defense in warmer and more humid climates, it might be more susceptible to invasion by *Tremella* in colder climates. Following from this, one would expect that the *Pertusaria* specimens that contained tremellalean ASVs were in a vulnerable state and had worse defense systems in colder and drier areas. However, *Pertusaria* has a broad distribution in Norway (Fig. 1), making it difficult to pinpoint its exact preferred climate.

It is important to note that there are physiological differences between a lichenicolous fungus' yeast and filamentous stages. These different stages, such as in *Tremella*, may occupy different habitats depending on whether they are asexual yeast forms or sexually reproducing and filamentous (Sampaio et al. 2004; Tuovinen et al. 2021). It is highly possible that the tremellalean yeast stage is present under a wide range of ecological conditions, and that it only shifts to its filamentous stage if the conditions are particularly favorable. *Tremella* has sometimes induced dimorphic switches based on temperature under laboratory conditions (Pippola & Kytoviita 2009). The favorable conditions in situ for any of the life stages of lichenicolous *Tremella* is, however, unknown.

To my knowledge, there is only one previous study that has looked at the effect of climatic factors on lichenicolous yeasts in situ (Mark et al. 2020), but this was not the main focus of their study. The authors found some degree of climatic effect on yeast community structure. Their dataset, however, was too limited at the macroclimatic scale, which did not allow for any strong conclusions. The lack of knowledge on the climatic preference of lichenicolous yeasts indicates the need for more studies on this topic.

4.5 Future perspectives

I anticipate that an increasing amount of the hidden fungal diversity will be discovered as molecular identification techniques continue to improve. Future studies targeting tremellaean yeasts should consider designing more generalized *Tremella* primers and applying additional markers in combination with long-read sequencing technologies to capture their vast species diversity. Once optimized, long-read HTS is a possible solution for resolving the taxonomy of lichenicolous *Tremella*. A potential limitation in my study is sampling size. Expanding the sampling of lichen specimens would likely reveal further unrecognized tremellalean species diversity. Furthermore, lichenicolous fungi present only as yeasts are not easily delimited to species, and similar studies targeting hidden fungi would benefit from an integrative taxonomic approach. For example, combining morphological data from FISH analyses with DNA sequence data would improve species delimitation efforts. FISH analyses could also resolve whether a tremellalean ASV originated from the yeast stage, or a premature filamentous *Tremella*. This was a minor limitation in this study. More research is needed to understand why the dikaryotic filamentous stage of lichenicolous *Tremella* shows more host specificity than the haploid yeast stage, potentially starting by exploring the morphology (e.g., haustoria) and physiology (e.g., source of carbohydrates) of both lifeforms. The study of correlations between climatic variables and the presence of *Tremella* would gain from denser sampling and assessing the species- rather than merely the genus level of asymptomatic *Pertusaria*. Finally, to assess whether the yeast- and filamentous stages behave differently towards climate conditions, future studies should target climatic preferences on both lifeforms of the lichenicolous *Tremella*.

Lichens were traditionally thought to be a symbiosis between a single fungus and photobiont. However, the emergence of molecular identification techniques proved that a lichen thallus could hide away a multitude of other fungi. This relatively new discovery requires yet a great deal of attention to reveal the true number of hidden fungi. I now have evidence that *Tremella* has been part of these overlooked fungi, by finding them as common occurrences in *Pertusaria*.

Note that the rapidly evolving molecular identification techniques can only reach their true potential of species discovery if researchers keep in mind the other aspects of mycology. The different rule sets for the yeast and filamentous stage in lichenicolous *Tremella* implies that we cannot fully grasp their underestimated diversity, without also understanding their physiology, ecology, life cycles, and morphology.

5 References

Arnold, A. E., Miadlikowska, J., Higgins, K. L., Sarvate, S. D., Gugger, P., Way, A., . . . Lutzoni, F. (2009). A Phylogenetic Estimation of Trophic Transition Networks for Ascomycetous Fungi: Are Lichens Cradles of Symbiotrophic Fungal Diversification? *Systematic Biology, 58*(3), 283-297. doi: 10.1093/sysbio/syp001

Aschenbrenner, I. A., Cernava, T., Berg, G. & Grube, M. (2016). Understanding Microbial Multi-Species Symbioses. *Frontiers in Microbiology, 7*, 180. doi:10.3389/fmicb.2016.00180

Ayres, D. L., Darling, A., Zwickl, D., Beerli, P., Holder, M., Lewis, P., . . . Suchard, M. (2012). BEAGLE: An application programming interface and high-performance computing library for statistical phylogenetics. *Systematic Biology, 61*(1), 170-173. doi:10.1093/sysbio/syr100

Badiee, P., Gandomi, B., Sabz, G., Khodami, B., Choopanizadeh, M. & Jafarian, H. (2015). Evaluation of nested PCR in diagnosis of fungal rhinosinusitis. *Iranian Journal of Microbiology, 7*(1), 62-66.

Badotti, F., Silva de Oliveira, F., Garcia, C. F., Vaz, A. B., Fonseca, P. L., Nahum, L. A., . . . Góes-Neto, A. (2017). Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). *BMC Microbiology, 17*, 42. doi:10.1186/s12866-017-0958-x

Banchi, E., Stankovic, D., Fernández-Mendoza, F., Gionechetti, F., Pallavicini, A. & Muggia, L. (2018). ITS2 metabarcoding analysis complements lichen mycobiome diversity data. *Mycological Progress, 17*, 1049-1066. doi: 10.1007/s11557-018-1415-4

Bandoni, R. J. (1995). Dimorphic heterobasidiomycetes: taxonomy and parasitism. *Studies in Mycology, 38*, 13-27.

Bellemain, E., Davey, M. L., Håvard, K., Epp, L. S., Boessenkool, S., Coissac, E., . . . Brochmann, C. (2013). Fungal palaeodiversity revealed using high-throughput metabarcoding of ancient DNA from arctic permafrost. *Environmental Microbiology, 15*(17), 2146-2146. doi:10.1111/1462-2920.12020

Blaalid, R., Kumar, S., Nilsson, R. H., Abarenkov, K., Kirk, P. M. & Kauserud, H. (2013). ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources, 13*(2), 218-224. doi:10.1111/1755-0998.12065

Boekhout, T., Sampaio, J., Kwon-Chung, K. & Bandoni, R. (2011). Discussion of teleomorphic and anamorphic basidiomycetous yeasts. Kurtzman, C.P., Fell, J.W., Boekhout, T. (Eds.), *The Yeasts: A Taxonomic Study.* Elsevier: London. doi: 10.1016/B978-0-444-52149-1.00100-2

Boyce, K. J. & Andrianopoulos, A. (2015). Fungal dimorphism: the switch from hyphae to yeast is a specialized morphogenetic adaptation allowing colonization of a host. *Microbiology Reviews, 39*(6), 797–811. doi:10.1093/femsre/fuv035

Burnham, K. P. & Anderson, D. R. (2002). *Model selection and multimodel inference: A Practical Information-Theoretic Approach* (2nd. ed.). Springer.

Castaño, C., Berlin, A., Durling, M., Ihrmark, K., Lindahl, B., Stenlid, J., . . . Olsen , A. (2020). Optimized metabarcoding with Pacific biosciences enables semi-quantitative analysis of fungal communities. *New Phytologist, 228*, 1149-1158. doi:10.1111/nph.16731

Černajová, I., & Škaloud, P. (2019). The first survey of Cystobasidiomycete yeasts in the lichen genus *Cladonia*; with the description of *Lichenozyma pisutiana* gen. nov., sp. nov. *Fungal Biology, 123*(9), 625-637. doi:10.1016/j.funbio.2019.05.006

Cheek, M., Lughadha, E. N., Kirk, P., Lindon, H., Carretero, J., Looney, B., . . . Niskanen, T. (2020). New scientific discoveries: Plants and fungi. *Plants, People, Planet, 2*(5), 371-388. doi:10.1002/ppp3.10148

D'Amore, R., Zeeshan Ijaz, U., Schirmer, M., Kenny, J. G., Gregory, R., Darby, A. C., . . . Hall, N. (2016). A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. *BMC Genomics, 17*, 55. doi: 10.1186/s12864-015-2194-9

Darriba, D., Taboada, G., Doalla, R. & Posada, D. (2012). JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods, 9*(8), 772. doi:10.1038/nmeth.2109

Diederich, P. (1996). The lichenicolous heterobasidiomycetes. *Bibliotheca Lichenologica, 61,* 3-198.

Diederich, P., Lawrey, J. D. & Ertz, D. (2018). The 2018 classification and checklist of lichenicolous fungi,with 2000 non-lichenized, obligately lichenicolous taxa. *The Bryologist, 121*(3), 340-425. doi:10.1639/0007-2745-121.3.340

Ekman, S., Svensson, M., Westberg, M. & Zamora, J. C. (2019). Additions to the lichen flora of Fennoscandia III. *Graphis Scripta, 31*(5), 34-46.

Estensmo, E. F., Maurice, S., Morgado, L., Martin-Sanchez, P. M., Skrede, I., & Kauserud, H. (2021). The influence of intraspecific sequence variation during DNA metabarcoding: A case study of eleven fungal species. *Molecular Ecology Resources, 21*, 1141-1148. doi:10.1111/1755-0998.13329

Fernández-Mendoza, F., Fleischhacker, A., Kopun, T., Grube, M. & Muggia, L. (2017). ITS1 metabarcoding highlights low specificity of lichen mycobiomes at a local scale. *Molecular Ecology, 26*(18), 4811-4830. doi:10.1111/mec.14244

Fick, S. & Hijmans, R. (2017). WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology, 37*(12), 4302-4315. doi:10.1002/joc.5086

Filges, S., Yamada, E., Ståhlberg, A. & Godfrey, T. E. (2019). Impact of Polymerase Fidelity on Background Error Rates in Next-Generation Sequencing with Unique Molecular Identifiers/Barcodes. *Scientific Reports, 9*, 3503. doi:10.1038/s41598-019-39762-6

Frisch, A., Klepsland, J., Palice, Z., Bendiksby, M., Tønsberg, T. & Holien, H. (2020). New and noteworthy lichens and lichenicolous fungi from Norway. *Graphis Scripta, 32*(1), 1-47.

Furneaux, B., Bahram, M., Rosling, A., Yorou, N. S. & Ryberg, M. (2021). Long- and short-read metabarcoding technologies reveal similar spatiotemporal structures in fungal communities. *Molecular Ecology Resources, 21*(6), 1833-1849. doi:10.1111/1755-0998.13387

Gardes, M. & Bruns, T. (1993). ITS primers with enhanced specificity for basidiomycetes, application to the identification of mycorrhizae and rusts. *Molecular Ecology, 2*(2), 113-118. doi:10.1111/j.1365-294x.1993.tb00005.x.

Garnica, S., Schön, M. E., Aberenkov, K., Riess, K., Liimatainen, K., Niskanen, T., . . . Ammirati, J. F. (2016). Determining threshold values for barcoding fungi: lessons from *Cortinarius* (Basidiomycota), a highly diverse and widespread ectomycorrhizal genus. *Microbiology Ecology, 92*(4), fiw045. doi:10.1093/femsec/fiw045

Geml, J., Gravendeel, B., van der Graag, K. J., Neilen, M., Lammers, Y., Reels, N., . . . Noordeloos, M. E. (2014). The contribution of DNA metabarcoding to fungal conservation: diversity assessment, habitat partitioning and mapping red-listed fungi in protected coastal *Salix repens* communities in the Netherlands. *PLoS One, 9*(6), e99852. doi:10.371/journal.pone.0099852

Green, T., Sancho, L. & Pintado, A. (2011). Ecophysiology of desiccation/rehydration cycles in mosses and lichens. I. U. Lüttge, E. Beck, & D. Bartels (Eds.), *Plant Desiccation Tolerance* (ss. 89-120). Springer Science & Business Media.

Hawksworth, D. L. (2015). Lichenization: The Origins of a Fungal Life-Style. I *Recent Advances in Lichenology* (ss. 1-10). New Dehli: Springer. doi:10.1007/978-81-322-2235-4_1

Hawksworth, D. L. & Lücking, R. (2021). Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiology spectrum, 5*(4). doi:10.1128/microbiolspec.FUNK-0052-2016

Hawksworth, D. & Honegger, R. (1994). The lichen thallus: A symbiotic phenotype of nutritionally specialized fungi and its response to gall producers. I M. Williams (Red.), *Systematics Association Special Volume* (ss. 77-98). Oxford: Clarendon Press.

Holien, H. & Frisch, A. (2022). *Perigrapha superveniens* (Nyl.) Hafellner, a lichenicolous fungus new to Fennoscandia from Norway. *Graphis scripta, 34*(3), 36-41.

Holien, H., Frisch, A., Jonsson, F., Klepsland, J. T., Millanes, A. M., Jurga, M., . . . Bendiksby, M. (2016). Interesting lichenized and lichenicolous fungi found during the Nordic Lichen Society excursion in Nord-Trøndelag, Norway 2015. *Graphis Scripta, 28*(1-2), 40-49.

Hsu, T.-K., Chen, J.-S., Tsai, H.-C., Tao, C.-W., Yang, Y.-Y., Tseng, Y.-C., . . . Hsu, B.-M. (2021). Efficient nested-PCR-based method development for detection and genotype identification of Acanthamoeba from a small volume of aquatic environmental sample. *Scientific Reports, 11*, 21740. doi:10.1038/s41598-021-00968-2

Ihrmark, K., Bödeker, I. T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., . . . Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology, 82*(3), 666-677. doi:10.1111/j.1574-6941.2012.01437.x

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research, 30*(14), 3059-3066. doi:10.1093/nar/gkf436

Kebschull, J. M. & Zador, A. M. (2015). Sources of PCR-induced distortions in high-throughput sequencing data sets. *Nucelic Acids Research, 43*(21). doi:10.1093/nar/gkv717

Klepsland, J. (2020). Thirty lichens and lichenicolous fungi new to Norway. *Graphis Scripta, 32*(7), 120-143.

Kozlov, A. M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2014). RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics, 35*(21), 4453-4455. doi: 10.1093/bioinformatics/btz305

Krzywinski, M. I., Schein, J. E., Birol, I., Connors, J., Gascoyne, R., Horsman, D., . . . Marra, M. A. (2009). Circos: An information aesthetic for comparative genomics. *Genome Research, 32*(3). doi:10.1101/gr.092759.109

Larsson, A. (2014). AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics, 30*(22), 3276-3278. doi:10.1093/bioinformatics/btu531

Lawrey, J. D. & Diederich, P. (2003). Lichenicolous Fungi: Interactions, Evolution, and Biodiversity. *The bryologist, 106*(1), 80-120. doi:10.1639/0007-2745(2003)106[0080:LFIEAB]2.0.CO;2

Letunic, I. & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids, 49*(W1), W293–W296. doi:10.1093/nar/gkab301

Lindahl, B. D., Nilsson, H. R., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., . . . Kauserud, H. (2013). Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. *New Phytologist, 199*(1), 288-299. doi:10.1111/nph.12243

Lindgren, H., Diederich, P., Goward, T. & Myllys, L. (2015). The phylogenetic analysis of fungi associated with lichenized ascomycete genus *Bryoria* reveals new lineages in the Tremellales including a new species *Tremella huuskonenii* hyperparasitic on *Phacopsis huuskoneni*. *Fungal Biology, 119*(9), 844-856. doi:10.1016/j.funbio.2015.06.005

Liu, X., Wang, Q., Göker, M., Groenewald, M., Kachalkin, A., Lumbsch, H., . . . Bai, F. (2016). Towards an integrated phylogenetic classification of theTremellomycetes. *Studies in Mycology, 81*, 85-147. doi:10.1016/j.simyco.2015.12.001

Mark, K., Laanisto, L., Bueno, C. G., Niinemets, Ü., Keller, C. & Scheidegger, C. (2020). Contrasting co-occurrence patterns of photobiont and cystobasidiomycete yeast associated with common epiphytic lichen species. *New Phytologist, 227*(5), 1362-1375. doi:10.1111/nph.16475

Marthinsen, G., Rui, S. & Timdal, E. (2019). OLICH: A reference library of DNA barcodes for Nordic Lichens. *Biodiversity Data Journal, 7*, e36252. doi:10.3897/BDJ.7.e36252

Mbareche, H., Veillette, M., Bilodeau, G. & Duchaine, C. (2020). Comparison of the performance of ITS1 and ITS2 as barcodes in amplicon-based sequencing of bioaerosols. *PeerJ, 8*, e8523. doi:10.7717/peerj.8523

Merinero, S., Bidussi, M. & Gauslaa, Y. (2015). Do lichen secondary compounds play a role in highly specific fungal parasitism? *Fungal Ecology, 14*, 125-129. doi:10.1016/j.funeco.2014.12.002

Millanes, A. M., Diederich, P., Ekman, S. & Wedin, M. (2011). Phylogeny and character evolution in the jelly fungi (Tremellomycetes, Basidiomycota, fungi). *Molecular phylogenetics and evolution, 61*(1), 12-28. doi:10.1016/j.ympev.2011.05.014

Millanes, A., Diederich, P., Westberg, M., Knutsson, T. & Wedin, M. (2014). *Tremella rhizocarpicola* sp. nov. and other interesting lichenicolous Tremellales and Filobasidiales in the Nordic countries. *MycoKeys, 8*, 31-41. doi:10.3897/mycokeys.8.8176

Millanes, A., Zamora, J., Keizer, P. & Wedin, M. (2018). New perspectives on the Tremellomycetes. *Coolia, 61*(1).

Miller, M., Pfeiffer, W. & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 1-8. doi: 10.1109/GCE.2010.5676129

Muggia, L. & Grube, M. (2010). Fungal composition of lichen thalli assessed by single strand conformation polymorphism. *The lichenologist, 42*(4), 461-473. doi:10.1017/S0024282909990752

Murali, A., Bhargava, A. & Wright, E. S. (2018). IDTAXA: A novel approach for accurate taxonomic classification of microbiome sequences. *Microbiome, 6*, 140. doi:10.1186/s40168-018-0521-5

Nagai, S., Sildever, S., Nishi, N., Tazawa, S., Basti, L., Kobayashi, T. & Ishino, Y. (2022). Comparing PCR-generated artifacts of different polymerases for improved accuracy of DNA metabarcoding. *Metabarcoding and Metagenomics, 6*, e77704. doi:10.3897/mbmg.6.77704

Nilsson, H. R., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K. H. (2008). Intraspecific ITS Variability in the Kingdom Fungi as Expressed in the International Sequence Databases and Its Implications for Molecular Species Identification. *Evolutionary Bioinformatics, 4*, 193-201. doi:10.4137/ebo.s653

Nixon, K. (2002). WINCLADA (Beta) 1.00.08. Ithaca, New york: Software published by author.

Orton, R. J., Wright, C. F., Morelli, M. J., King, D. J., Paton, D. J., King, D. P. & Haydon, D. T. (2015). Distinguishing low frequency mutations from RT-PCR and sequence errors in viral deep sequencing data. *BMC Genomics, 16*, 229. doi:10.1186/s12864-015-1456-x

Oset, M. (2021). Two new synonyms of *Pertusaria coccodes* (Pertusariales, Ascomycota). *Folia Cryptogamica Estonica, 58*, 61-63. doi:10.12697/fce.2021.58.08

Patin, N. V., Kunin, V., Lidström, U. & Ashby, M. N. (2013). Effects of OTU Clustering and PCR Artifacts on Microbial Diversity Estimates. *Microbial Ecology, 65*(3), 709-719. doi:10.1007/s00248-012-0145-4

Paul, F., Otte, J., Schmitt, I. & Dal Grande, F. (2018). Comparing Sanger sequencing and high-throughput metabarcoding for inferring photobiont diversity in lichens. *Scientific reports, 8*, 8624. doi:10.1038/s41598-018-26947-8

Petersen, L. M., Martin, I. W., Moschetti, W. E., Kershaw, C. M. & Tsongalis, G. J. (2019). Third-Generation Sequencing in the Clinical Laboratory: Exploring the Advantages and Challenges of Nanopore Sequencing. *Journal if Clinical Microbiology, 58*(1), e01315-19. doi:10.1128/JCM.01315-19

Pippola, E. & Kytoviita, M.-M. (2009). Growth and dimorphism of the mycoparasite *Tremella encephala* as affected by different nitrogen and carbon sources and the host presence. *Cryptogamie, 30*(1), 3-19.

Pray, L. A. (2008). DNA Replication and Causes of Mutation. *Nature Education, 1*(1), 214.

Rambaut, A., Drummond, A., Xie, D., Baele, G. & Suchard, M. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology, 67*(5), 901-904. doi:10.1093/sysbio/syy032

RStudio Team. (2020). RStudio: Integrated Development for R. Boston, MA. From http://www.rstudio.com/.

Sampaio, J., Agerer, R., Piepenbring, M. & Blanz, P. (2004). Diversity, phylogeny, and classification of basidiomycetous yeasts. *Frontiers in Basidiomycote Mycology, 1*, 49-80. doi:10.1016/j.simyco.2020.01.002

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, A. C. & Wen Chen and Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode makrer for Fungi. *National Acadamy of Sciences, 109*(16). doi:10.1073/pnas.1117018109

Singh, S. (2017). Ex situ Conservation of Fungi: A Review. I T. D. Satyanarayana (Red.), *Developments in Fungal Biology and Applied Mycology* (ss. 543-562). Singarpore: Springer. doi:10.1007/978-981-10-4768-8_27

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M. C., . . . McCutcheon, J. P. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science, 353*, 6298, 488-492. doi:10.1126/science.aaf8287

Stoler, N. & Nekrutenko, A. (2021). Sequencing error profiles of Illumina sequencing instruments. *NAR Genomics and Bioinformatics, 3*(1), lqab019. doi: 10.1093/nargab/lqab019

Szöllősi, G. J., Tannier, E., Daubin, V. & Boussau, B. (2015). The Inference of Gene Trees with Species Trees. *Systematic Biology, 64*(1), e42-e62. doi:10.1093/sysbio/syu048

Tedersoo, L., Anslan, S., Bahram, M., Kõljalg, U. & Abarenkov, K. (2020). Identifying the 'unidentified' fungi: a global-scale long-read third-generation sequencing approach. *Fungal Diversity, 103*, 273-293. doi:10.1007/s13225-020-00456-4

Tedersoo, L., Anslan, S., Bahram, M., Põlme, S., Riit, T., Liiv, I., . . . Abarenkov, K. (2015). Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycoKeys, 10*, 1-43. doi:10.3897/mycokeys.10.4852

Tedersoo, L., Tooming-Klunderud, A. & Anslan, S. (2018). PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives. *New Phytologist, 217*(3), 1370-1385. doi:10.1111/nph.14776

Tuovinen, V., Ekman, S., Thor, G., Vanderpool, D., Spribille, T. & Johannesson, H. (2019). Two Basidiomycete Fungi in the Cortex of Wolf Lichens. *Current Biology, 29*(3), 476-483. doi:10.1016/j.cub.2018.12.022

Tuovinen, V., Millanes, A. M., Freire-Rollo, S., Rosling, A. & Wedin, M. (2021). *Tremella macrobasidiata* and *Tremella variae* have abundant and widespread yeast stages in *Lecanora* lichens. *Environmental Microbiology, 23*(5), 2484-2498. doi:10.1111/1462-2920.15455

Wang, X.-C., Liu, C., Huang, L., Bengtsson-Palme, J., Chen, H., Zhang, J.-H., . . . Li, J.-Q. (2015). ITS1: a DNA barcode better than ITS2 in eukaryotes? *Molecular Ecology Resources, 15*(3), 573-586. doi:10.1111/1755-0998.12325

Werth, S., Millanes, A. M., Wedin, M. & Scheidegger, C. (2013). Lichenicolous fungi show population subdivision by host species but do not share population history with their hosts. *Fungal Biology, 117*(1),71-84. doi:10.1016/j.funbio.2012.11.007

Westberg, M., Moberg, R., Myrdal, M., Nordin, A. & Ekman, S. (2021). *Santesson's checklist of Fennoscandian lichen-forming and lichenicolous fungi.*

White, T., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols* (ss. 315-322). San Diego: Academic Press.

Willis, K. (2018). *State of the World's Fungi* . London: Royal Botanical Gardens.

Yang, R.-H., Su, J. H., Shang, J. J., Wu, Y. Y., Li, Y., Bao, D. P. & Yao, Y. J. (2018). Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. *PLoS One, 13*(10), e0206428. doi:10.1371/journal.pone.0206428

Yurkov, A., Guerreiro, M. A., Sharma, L., Carvahlo, C. & Fonseca, Á. (2015). Multigene Assessment of the Species Boundaries and Sexual Status of the Basidiomycetous Yeasts *Cryptococcus flavescens* and *C. terrestris* (Tremellales). *Plos One, 10*(3), e0126996. doi:10.1371/journal.pone.0120400

Zhao, Y., Liu, X. Z. & Bai, F. Y. (2019). Four new species of *Tremella* (Tremellales, Basidiomycota) based on morphology and DNA sequence data. *Mycokeys, 47*, 75-95. doi:10.3897/mycokeys.47.29180

6 Supplementary

Table S1. All reference sequences that were used in this study.

Reference species	GenBank accession number
Bulleromyces albus	KY101819
Carcinomyces effibulatus	JN053499
Cryptococcus neoformans	EF211144
Naematelia aurantia	AY866425
Sirobasidium magnum	JN053497
Sterigmatosporidium polymorphum	AF444320
Tremella anaptychiae	NR159047
Tremella caloplacae	JN053469
Tremella celata	NR159019
Tremella cetrariella	KT334562
Tremella cetrariicola	JN053491
Tremella cladoniae	JN053478
Tremella coppinsiia	JN053496
Tremella diederichiana	KT334581
Tremella diploschistina	JN790587
Tremella endosporogena	KT334579
Tremella everniae	JN053493
Tremella giraffa	NR137069
Tremella hypogymniae	MW115407
Tremella leptogii	JN053476
Tremella lethariae	MG774388
Tremella macrobasidiata	KT334583
Tremella mayrhoferi	MH168093
Tremella mesenterica	JN053463
Tremella nephromatis	JN053475
Tremella pertusariae	JN053494
Tremella tuckerae	KT334585
Tremella umbilicariae	KM507564
Tremella variaeam	KT334587
Tremella wirthii	JN053492
Trichosporon ovoides	AF444439
Trimorphomyces papilionaceus	AF444483
Vishniacozyma carnescens	MK648477

Table S2. *Pertusaria* specimens that were included in this study, including specimen ID, species name, and fungarium number.

Specimen ID	Species	Fugarium number
A1	*P. alpina*	TRH-L-36894
A2	*P. alpina*	TRH-L-11655
A3	*P. alpina*	TRH-L-14115
A4	*P. alpina*	TRH-L-12738
A5	*P. alpina*	TRH-L-13084
A6	*P. alpina*	O-L-197933
A7	*P. alpina*	O-L-200312
A8	*P. alpina*	O-L-177130
A9	*P. alpina*	O-L-165090
A10	*P. alpina*	O-L-186277
B1	*P. byontha*	TRH-L-13420
B2	*P. byontha*	TRH-L-13802
B3	*P. bryontha*	O-L-107139
Ca1	*P. carneopallida*	TRH-L-5732
Ca2	*P. carneopallida*	TRH-L-12491
Ca3	*P. carneopallida*	TRH-L-12556
Ca4	*P. carneopallida*	TRH-L-17834
Ca5	*P. carneopallida*	TRH-L-10291
Ca6	*P. carneopallida*	TRH-L-11684
Ca7	*P. carneopallida*	TRH-L-15519
Ca8	*P. carneopallida*	TRH-L-16363
Ca9	*P. carneopallida*	TRH-L-15886
Ca10	*P. carneopallida*	TRH-L-15246
Ca11	*P. carneopallida*	BG-L98692
Ca12	*P. carneopallida*	BG-L92420
Ca13	*P. carneopallida*	BG-L94661
Ca14	*P. carneopallida*	BG-L89489
Ca15	*P. carneopallida*	O-L-177160
Ca16	*P. carneopallida*	O-L-164852
Ca17	*P. carneopallida*	O-L-151383
C1	*P. coccodes*	TRH-L-12174
C2	*P. coccodes*	TRH-L-15160
C3	*P. coccodes*	TRH-L-14073
C4	*P. coccodes*	TRH-L-15551
C5	*P. coccodes*	TRH-L-651008
C6	*P. coccodes*	TRH-L-651758
C7	*P. coccodes*	TRH-L-16432
C8	*P. coccodes*	TRH-L-10066
C9	*P. coccodes*	TRH-L-9847
C10	*P. coccodes*	TRH-L-13334
C11	*P. coccodes*	BG-L-96642
C12	*P. coccodes*	O-L-199396
C13	*P. coccodes*	O-L-135269
C14	*P. coccodes*	O-L-164311
Co1	*P. coronata*	TRH-L-13374
Co2	*P. coronata*	TRH-L-15159
Co3	*P. coronata*	TRH-L-13019
Co4	*P. coronata*	TRH-L-14043
Co5	*P. coronata*	TRH-L-9676

Co6	*P. coronata*	TRH-L-17063
Co7	*P. coronata*	TRH-L-13074
Co8	*P. coronata*	TRH-L-651756
Co9	*P. coronata*	TRH-L-16955
Co10	*P. coronata*	TRH-L-13384
Co11	*P. coronata*	O-L-183707
Co12	*P. coronata*	O-L-199420
Co13	*P. coronata*	BG-L-98869
Co14	*P. coronata*	BG-L-98998
Co15	*P. coronata*	BG-L-98347
Co16	*P. coronata*	BG-L-92385
F1	*P. flavicans*	TRH-L-12983
F2	*P. flavicans*	TRH-L-16254
F3	*P. flavicans*	TRH-L-16973
F4	*P. flavicans*	BG-L-79504
F5	*P. flavicans*	BG-L-87948
F6	*P. flavicans*	BG-L-99074
F7	*P. flavicans*	O-L-161434
F8	*P. flavicans*	O-L-183729
F9	*P. flavicans*	O-L-161355
F10	*P. flavicans*	O-L-201495
Fl1	*P. flavida*	TRH-L-651746
Fl2	*P. flavida*	O-L-199393
Fl3	*P. flavida*	O-L-200563
Fl4	*P. flavida*	O-L-158259
Fl5	*P. flavida*	O-L-186093
Fl6	*P. flavida*	O-L-165187
Fl7	*P. flavida*	BG-L-96950
Fl8	*P. flavida*	BG-L-89639
Fl9	*P. flavida*	BG-L-92080
H1	*P. hymenea*	TRH-L-651737
H2	*P. hymenea*	TRH-L-28759
H3	*P. hymenea*	TRH-L-37188
H4	*P. hymenea*	TRH-L-651747
H5	*P. hymenea*	TRH-L-651719
H6	*P. hymenea*	O-L-176892
H7	*P. hymenea*	O-L-174349
H8	*P. hymenea*	O-L-159111
H9	*P. hymenea*	O-L-194473
H10	*P. hymenea*	O-L-205867
H11	*P. hymenea*	O-L-194340
H12	*P. hymenea*	BG-L-95078
H13	*P. hymenea*	BG-L-92421
H14	*P. hymenea*	BG-L-94441
H15	*P. hymenea*	BG-L-94408
L1	*P. leioplaca*	TRH-L-37105
L2	*P. leioplaca*	TRH-L-37344
L3	*P. leioplaca*	TRH-L-36845
L4	*P. leioplaca*	TRH-L-37162
L5	*P. leioplaca*	TRH-L-37061
L6	*P. leioplaca*	TRH-L-15119
L7	*P. leioplaca*	TRH-L-17693
L8	*P. leioplaca*	TRH-L-15691

L9	*P. leioplaca*	TRH-L-16828
L10	*P. leioplaca*	TRH-L-651641
L11	*P. leioplaca*	TRH-L-651732
O1	*P. oculata*	TRH-L-11484
O2	*P. oculata*	TRH-L-14181
O3	*P. oculata*	TRH-L-14744
O4	*P. oculata*	TRH-L-15328
O5	*P. oculata*	TRH-L-16914
O6	*P. oculata*	TRH-L-16693
O7	*P. oculata*	TRH-L-17210
O8	*P. oculata*	TRH-L-15804
O9	*P. oculata*	TRH-L-9818
O10	*P. oculata*	TRH-L-15749
O11	*P. oculata*	O-L-195871
O12	*P. oculata*	O-L-159881
O13	*P. oculata*	BG-L-89301
O14	*P. oculata*	BG-L-100084
O15	*P. oculata*	BG-L-89358
O16	*P. oculata*	BG-L-89150
P1	*P. pertusa*	TRH-L-37070
P2	*P. pertusa*	TRH-L-24273
P3	*P. pertusa*	TRH-L-37330
P4	*P. pertusa*	TRH-L-37050
P5	*P. pertusa*	TRH-L-37160
P6	*P. pertusa*	TRH-L-3698
P7	*P. pertusa*	TRH-L-17201
P8	*P. pertusa*	TRH-L-652213
P9	*P. pertusa*	TRH-L-651744
P10	*P. pertusa*	TRH-L-651845
P11	*P. pertusa*	BG-L-94206
P12	*P. pertusa*	BG-L-96429
P13	*P. pertusa*	O-L-165186
P14	*P. pertusa*	O-L-176960
P15	*P. pertusa*	O-L-175009
P16	*P. pertusa*	O-L-198948
P17	*P. pertusa*	O-L-208024
Ps1	*P. pseudocorallina*	TRH-L-36691
Ps2	*P. pseudocorallina*	TRH-L-36688
Ps3	*P. pseudocorallina*	BG-L-93476
Ps4	*P. pseudocorallina*	BG-L-38519
Ps5	*P. pseudocorallina*	O-L-203911
Pu1	*P. pupillaris*	TRH-L-37122
Pu2	*P. pupillaris*	TRH-L-24424
Pu3	*P. pupillaris*	TRH-L-24324
Pu4	*P. pupillaris*	TRH-L-24274
Pu5	*P. pupillaris*	TRH-L-37349
Pu6	*P. pupillaris*	TRH-L-17632
Pu7	*P. pupillaris*	TRH-L-10301
Pu8	*P. pupillaris*	TRH-L-16347
Pu9	*P. pupillaris*	TRH-L-14265
Pu10	*P. pupillaris*	TRH-L-652056
Pu11	*P. pupillaris*	BG-L-98925
Pu12	*P. pupillaris*	BG-L-89145

Pu13	*P. pupillaris*	BG-L-99529
Pu14	*P. pupillaris*	BG-L-98633
Pu15	*P. pupillaris*	BG-L-98684
Pu16	*P. pupillaris*	BG-L-98623
Pu17	*P. pupillaris*	O-L-200315
S1	*P. sommerfeltii*	TRH-L-24322
S2	*P. sommerfeltii*	TRH-L-14758
S3	*P. sommerfeltii*	TRH-L-650460
S4	*P. sommerfeltii*	TRH-L-10325
S5	*P. sommerfeltii*	TRH-L-12108
S6	*P. sommerfeltii*	TRH-L-16779
S7	*P. sommerfeltii*	TRH-L-12712
S8	*P. sommerfeltii*	TRH-L-12111
S9	*P. sommerfeltii*	TRH-L-36593
S10	*P. sommerfeltii*	TRH-L-11656
S11	*P. sommerfeltii*	O-L-131736
S12	*P. sommerfeltii*	O-L-158211
S13	*P. sommerfeltii*	O-L-197962
S14	*P. sommerfeltii*	O-L-158368
S15	*P. sommerfeltii*	O-L-176858
S16	*P. sommerfeltii*	O-L-164730

Table S3. Sequences used in this study of *Tremella* that were obtained as contaminations when DNA barcoding *Pertusaria* in the OLICH project.

OLICH ID	**Host species**	**Herbarium number**
OLICH1	*P. albescens*	O-L-175288
OLICH2	*P. amarescens*	O-L-230217
OLICH3	*P. flavicans*	O-L-201495
OLICH4	*P. hymenea*	O-L-194340
OLICH5	*P. hymenea*	O-L-205867
OLICH6	*P. pertusa*	TRH-L-37070
OLICH7	*P. pertusa*	TRH-L-37330

Table S4. BLAST results of the 50 highest abundance ASVs, within Tremellales. BLAST results from remaining lower abundance ASVs from REF-all phylogeny are also included. When top-hit for various ASVs was "unculutured fungus" the next hit that specifies species was also included.

AS	Family	Genus	Species	GenBank accession number	Sequence identity (%)	Query cover
ASV1	NA	NA	uncultured fungus	KC965534	87,259	100
ASV1	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,194	55
ASV4	NA	NA	uncultured fungus	KC965534	86,486	100
ASV4	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	90,141	54
ASV5	NA	NA	uncultured fungus	KC965534	88,76	100
ASV5	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,971	50
ASV6	NA	NA	uncultured fungus	KC965534	87,259	100
ASV6	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	85,561	68
ASV8	NA	NA	uncultured fungus	KC965534	88,76	100
ASV8	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,634	68
ASV9	NA	NA	uncultured fungus	KC965534	89,922	100
ASV9	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68
ASV10	NA	NA	uncultured fungus	KC965534	89,147	100
ASV10	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,112	53
ASV11	NA	NA	uncultured fungus	KC965534	86,873	100
ASV11	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	87,5	55
ASV12	NA	NA	uncultured fungus	KC965534	88,76	100
ASV12	Tremellaceae	*Tremella*	*Tremella diederichiana*	KT334580	85,227	66
ASV17	Tremellaceae	*Tremella*	*Tremella hypogymniae*	JN053485	100	86
ASV22	NA	NA	uncultured fungus	KC965534	89,535	100
ASV22	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	86,559	68
ASV27	NA	NA	uncultured fungus	KC965534	88,942	90
ASV27	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	86,559	63
ASV53	NA	NA	uncultured fungus	KC965534	88,76	100
ASV53	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,06	51
ASV59	NA	NA	uncultured fungus	KC965534	86,873	100
ASV59	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,194	55
ASV74	NA	NA	uncultured fungus	MG827690	95,149	100
ASV74	Tremellaceae	*Tremella*	*Tremella macrobasidiata*	MG209530	92,4	93
ASV75	NA	NA	uncultured fungus	KC965534	88,372	100
ASV75	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68
ASV76	NA	NA	uncultured fungus	KC965534	89,535	100
ASV76	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68
ASV77	NA	NA	uncultured fungus	MF156092	100	100
ASV77	Bulleribasidiaceae	*Vishniacozyma*	*Vishniacozyma carnescens*	MK648477	100	100
ASV78	NA	NA	uncultured fungus	KC965534	89,147	100
ASV78	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,806	51
ASV79	NA	NA	uncultured fungus	KC965534	89,147	100

ASV79	Tremellaceae	*Tremella*	*Tremella diederichiana*	KT334580	85,795	66
ASV85	NA	NA	uncultured fungus	MG827690	99,624	100
ASV85	Tremellaceae	*Tremella*	*Tremella macrobasidiata*	MG209530	97,177	93
ASV94	NA	NA	uncultured fungus	KC965962	99,624	100
ASV94	Tremellaceae	*Tremella*	*Tremella mayrhoferi*	NR_161065	98,696	86
ASV101	NA	NA	uncultured fungus	KC965534	89,535	100
ASV101	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	86,559	68
ASV108	NA	NA	uncultured fungus	MG827690	91,418	100
ASV108	Tremellaceae	*Tremella*	*Tremella macrobasidiata*	MG209530	88,8	93
ASV109	NA	NA	uncultured fungus	KC965534	89,922	100
ASV109	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68
ASV139	NA	NA	uncultured fungus	KC965534	89,535	100
ASV139	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,634	68
ASV145	NA	NA	uncultured fungus	KT195739	98,253	98
ASV145	Tremellaceae	*Tremella*	*Tremella diploschistina*	JN790586	92,746	82
ASV163	NA	NA	uncultured fungus	MG827690	100	100
ASV163	Tremellaceae	*Tremella*	*Tremella macrobasidiata*	MG209530	97,581	93
ASV181	NA	NA	uncultured fungus	KC965534	86,923	100
ASV181	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,811	54
ASV195	NA	NA	uncultured fungus	KC965534	87,308	100
ASV195	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	89,51	54
ASV202	NA	NA	uncultured fungus	MG827690	99,248	100
ASV202	Tremellaceae	*Tremella*	*Tremella macrobasidiata*	KT334582	99,565	86
ASV246	NA	NA	uncultured fungus	MN152049	99,585	98
ASV246	Cryptococcaceae	*Cryptococcus*	*Cryptococcus sp.*	MH718303	86,973	100
ASV288	NA	NA	uncultured fungus	KC965534	88,372	100
ASV288	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68
ASV315	NA	NA	uncultured fungus	KC965534	86,822	100
ASV315	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,194	55
ASV331	NA	NA	uncultured fungus	KC965534	88,76	100
ASV331	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	87,413	53
ASV339	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,94	82
ASV347	NA	NA	uncultured fungus	KC965534	88,372	100
ASV347	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,06	51
ASV348	NA	NA	uncultured fungus	KC965534	88,372	100
ASV348	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,235	50
ASV398	NA	NA	uncultured fungus	KC965534	88,372	100
ASV398	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,235	50
ASV413	NA	NA	uncultured fungus	KC965534	88,76	100
ASV413	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	87,413	53
ASV417	NA	NA	uncultured fungus	KC965534	89,147	100
ASV417	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	86,559	68
ASV476	NA	NA	uncultured fungus	MG827690	97,388	100
ASV476	Tremellaceae	*Tremella*	*Tremella macrobasidiata*	MG209530	94,8	93
ASV477	NA	NA	uncultured fungus	KC965534	89,147	100
ASV477	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68

ASV488	NA	NA	uncultured fungus	KC965534	89,535	100
ASV488	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68
ASV527	NA	NA	uncultured fungus	MG827690	96,241	100
ASV527	Tremellaceae	*Tremella*	*Tremella macrobasidiata*	MG209530	93,548	93
ASV531	NA	NA	uncultured fungus	KC965534	86,873	100
ASV531	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	87,5	55
ASV573	NA	NA	uncultured fungus	KC965534	88,372	100
ASV573	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,235	50
ASV596	NA	NA	uncultured fungus	MN152054	95,885	98
ASV596	Tremellaceae	*Tremella*	*Tremella coppinsii*	JN053496	90,476	67
ASV599	NA	NA	uncultured fungus	KC965534	89,535	100
ASV599	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	86,559	68
ASV666	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	89,545	81
ASV711	NA	NA	uncultured Tremellomycetes	KR265957	93,651	100
ASV711	Phaeotremellaceae	*Phaeotremella*	*Phaeotremella frondosa*	MK643441	91,304	100
ASV811	NA	NA	uncultured fungus	KC965534	88,372	100
ASV811	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,971	50
ASV831	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	99,574	87
ASV846	NA	NA	uncultured fungus	KP897203	100	100
ASV846	Tremellaceae	*Tremella*	*Tremella* sp.	DQ242632	86,228	64
ASV875	NA	NA	uncultured fungus	KC965534	88,372	100
ASV875	Tremellaceae	*Tremella*	*Tremella diederichiana*	KT334580	85,227	66
ASV964	NA	NA	uncultured fungus	KC965534	88,372	100
ASV964	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68
ASV1130	NA	NA	uncultured fungus	KC965534	89,535	100
ASV1130	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	86,559	68
ASV1262	NA	NA	uncultured fungus	KC965534	88,372	100
ASV1262	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,235	50
ASV1444	NA	NA	uncultured fungus	KC965534	88,372	100
ASV1444	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,235	50
ASV1468	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	99,574	87
ASV1615	NA	NA	uncultured fungus	KC965534	88,76	100
ASV1615	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,112	53
ASV1683	Naemateliaceae	*Naematelia*	*Naematelia aurantia*	NR_155873	91,339	100
ASV1876	Tremellaceae	*Tremella*	*Tremella subalpina*	NR_155908	96,538	100
ASV2218	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	92,373	87
ASV2365	NA	NA	uncultured fungus	KC965534	86,873	100
ASV2365	Tremellaceae	*Tremella*	*Tremella wirthii*	JN053492	88,194	55
ASV2590	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	99,574	87
ASV2627	NA	NA	uncultured fungus	KC965534	88,76	100
ASV2627	Tremellaceae	*Tremella*	*Tremella cetrariicola*	KT334565	88,112	53
ASV2868	NA	NA	uncultured Basidiomycota	GU328582	94,444	100
ASV2868	Tremellaceae	*Tremella*	*Tremella anaptychiae*	NR_159047	94,444	86
ASV2931	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	99,574	87
ASV3127	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	99,574	87

ASV3164	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	99,574	87
ASV3312	NA	NA	uncultured fungus	MG828064	100	100
ASV3312	Cryptococcaceae	*Cryptococcus*	*Cryptococcus sp.*	MG020322	96,654	100
ASV3471	NA	NA	uncultured fungus	KU188715	96,642	100
ASV3471	Tremellaceae	*Tremella*	*Tremella umbilicariae*	KM507564	87,778	100
ASV3481	NA	NA	uncultured fungus	KC965534	88,372	100
ASV3481	Tremellaceae	*Tremella*	*Tremella pertusariae*	JN053494	87,097	68

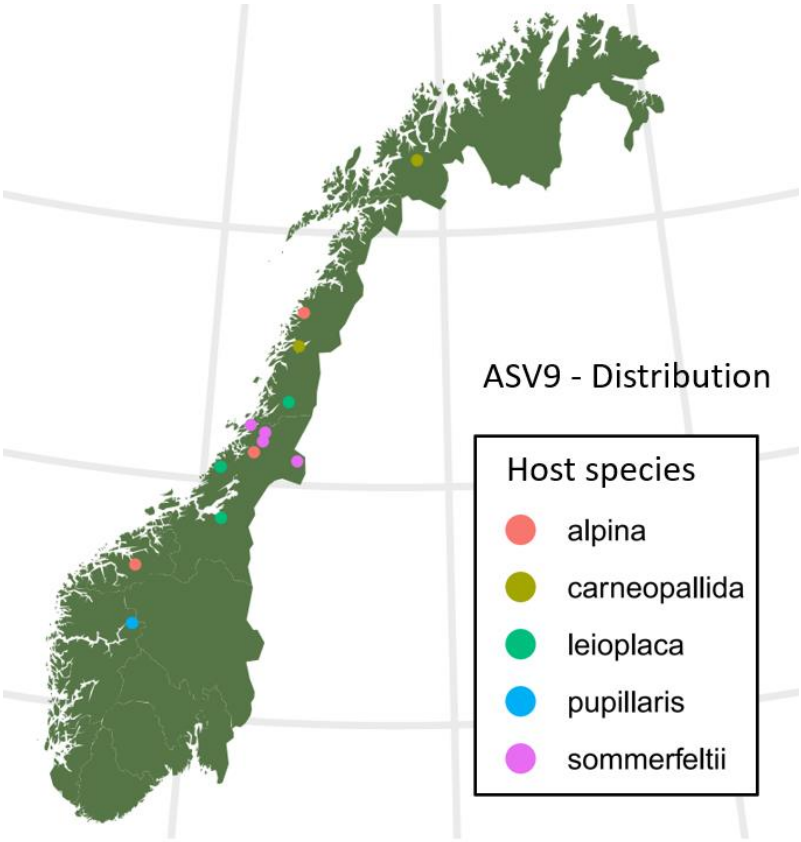


Figure S1. Distribution of *Pertusaria* specimens which contained ASV9.

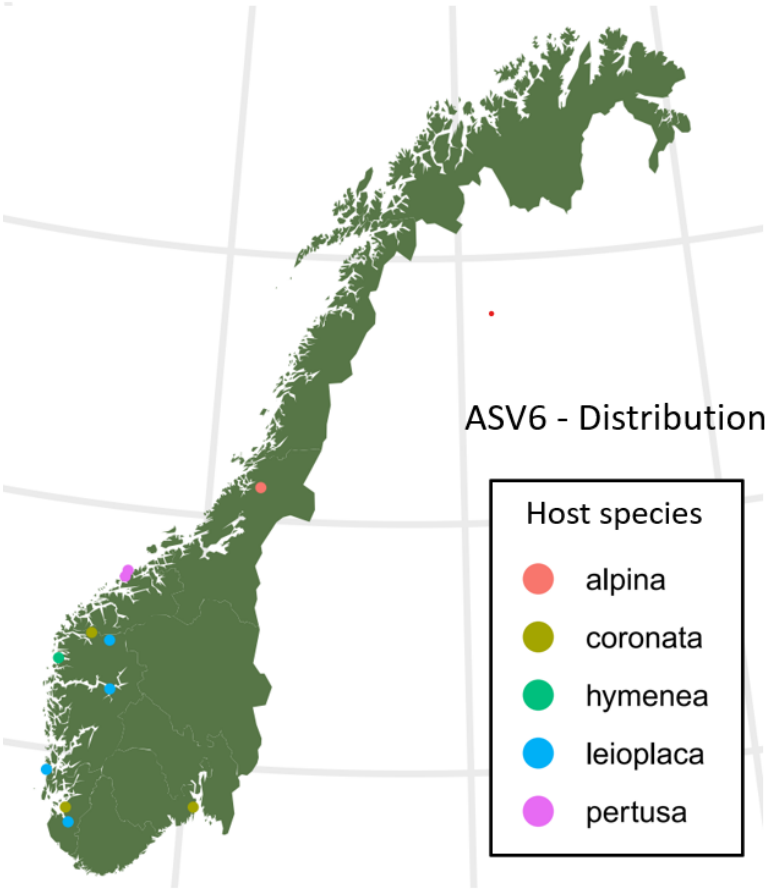


Figure S2. Distribution of *Pertusaria* specimens which contained ASV6.

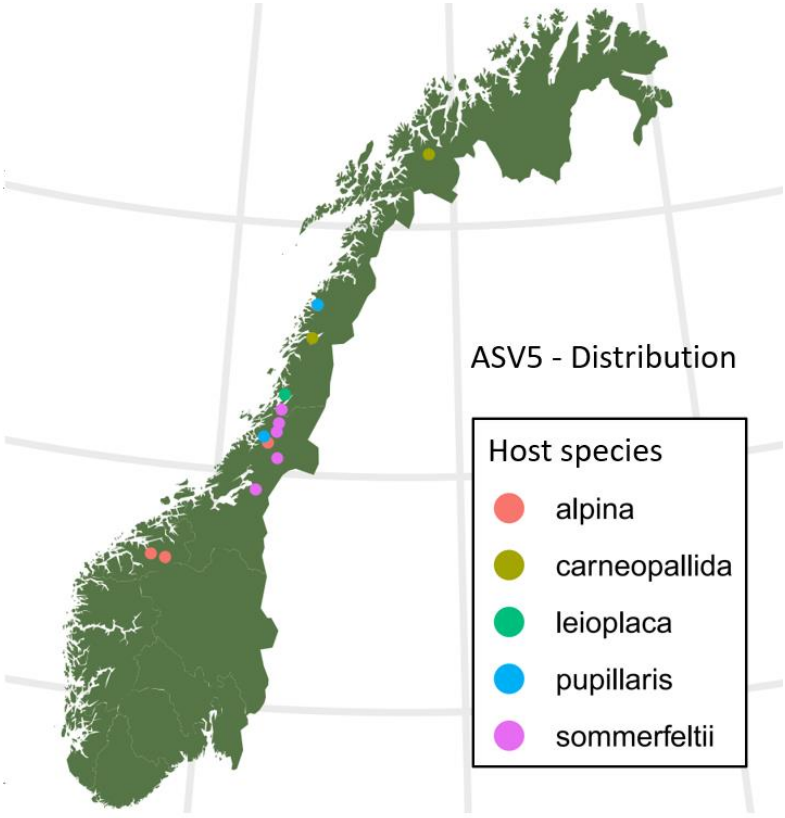


Figure S3. Distribution of *Pertusaria* specimens which contained ASV5.

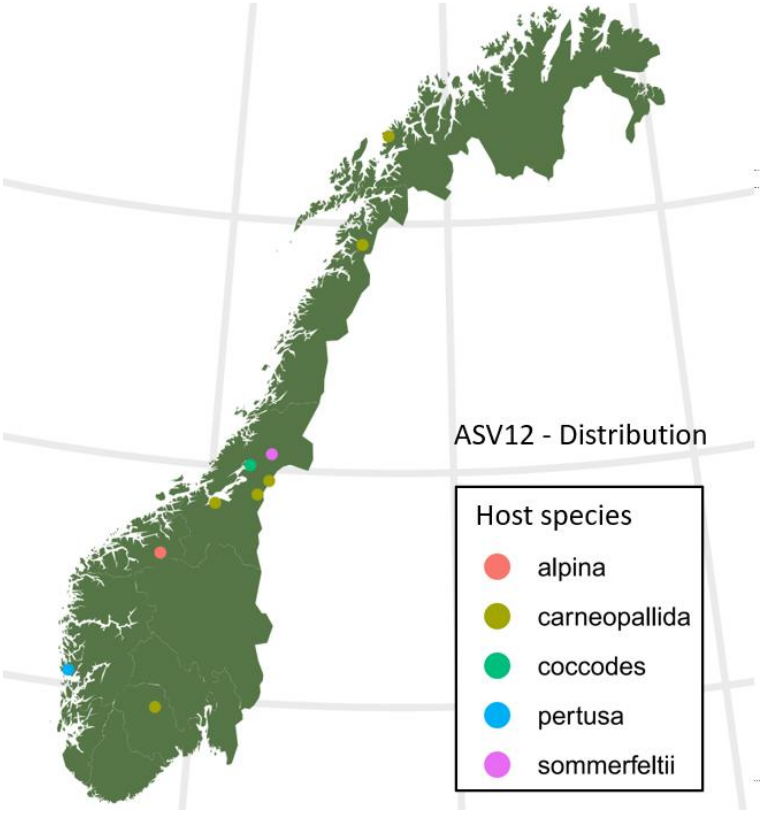


Figure S4. Distribution of *Pertusaria* specimens which contained ASV12.

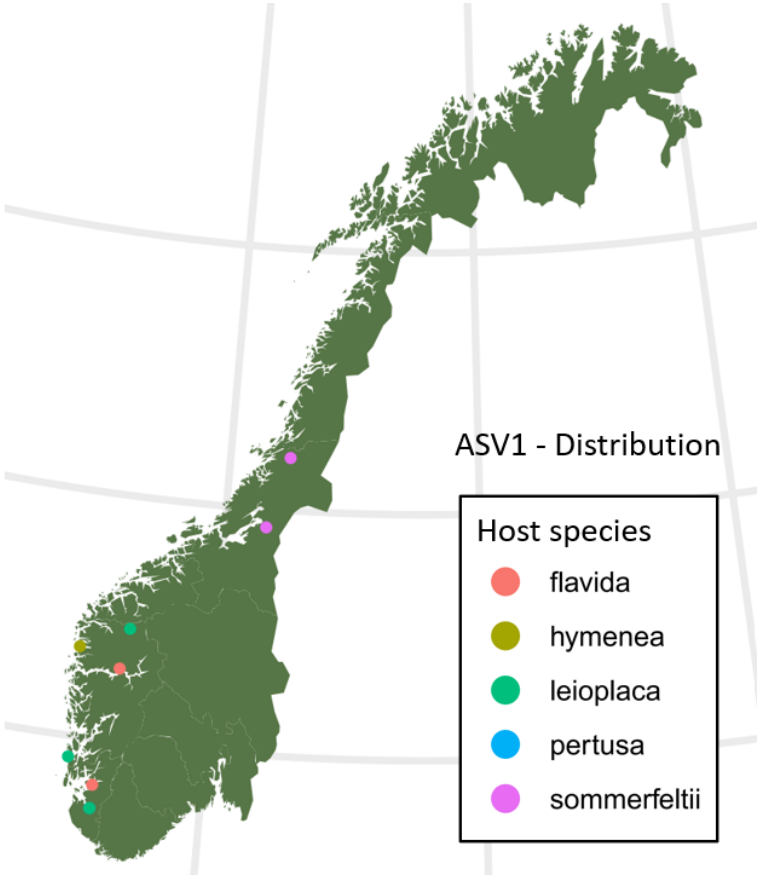
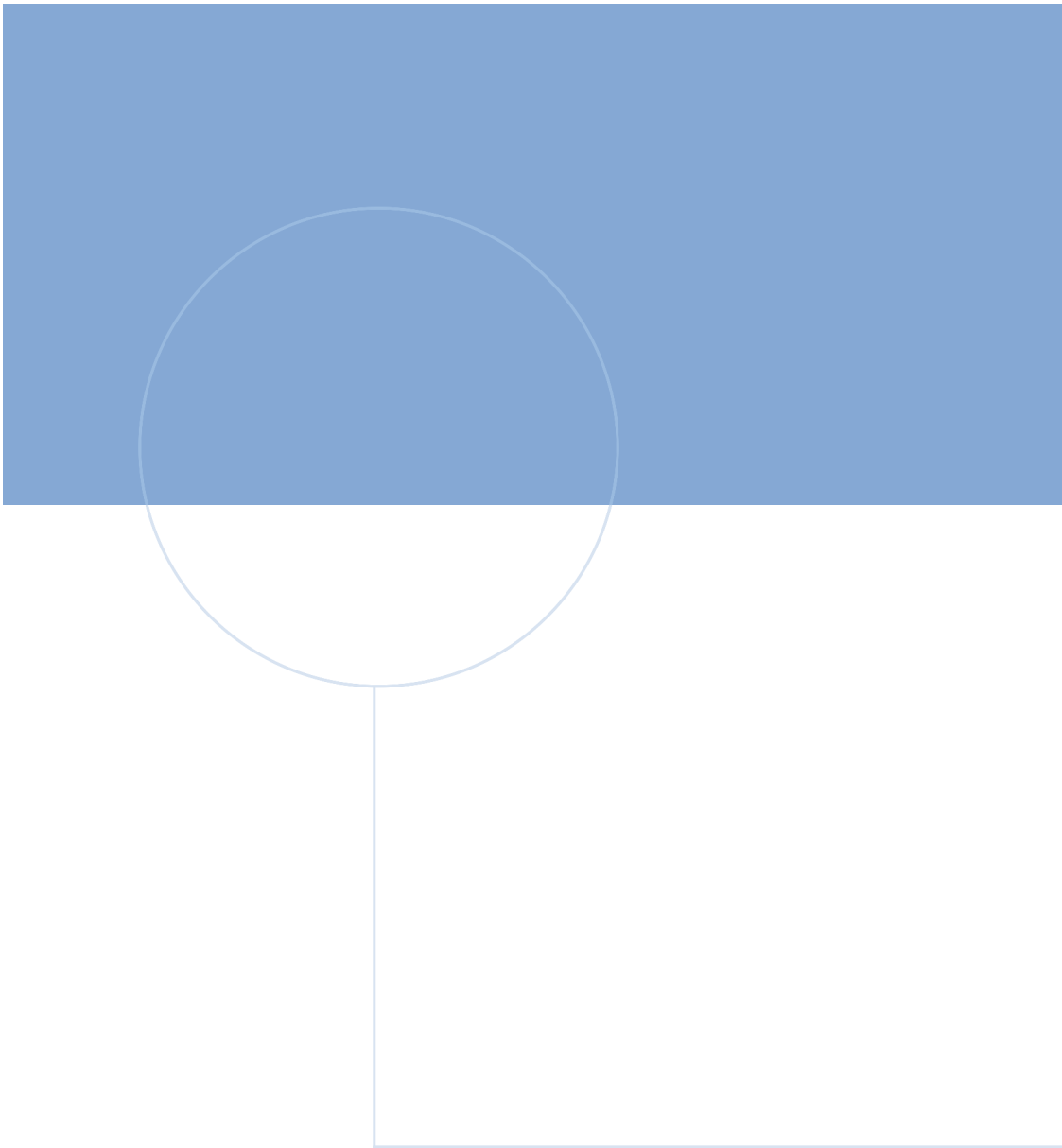


Figure S5. Distribution of *Pertusaria* specimens which contained ASV1.



NTNU

Kunnskap for ei betre verd