Using 454 sequencing for exploring diversity, host specificity and tissue specificity of the fungal genus *Galerina* associated with four boreal mosses

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Abstract

Bryophytes are a dominant vegetation component of the boreal forest, and an important part of the ecosystem, yet little is known about the microbial community associated with them. The aims of this thesis were 1) to analyze the diversity of *Galerina* species associated with four different boreal moss species, and 2) to examine how they are distributed between the mosses' photosynthetic versus senescent tissues. DNA sequences with affinity to *Galerina* were extracted from two comprehensive fungal diversity 454 pyrosequencing datasets and analyzed further. A total of 11,746 *Galerina* reads were clustered into 26 non-singleton OTUs that were attributed to 15 different *Galerina* species. Six of the *Galerina* OTUs were dominant and accounted for about 96% of the reads, and were found in most of the shoot fragments (>95%). Almost all the *Galerina* OTUs occurred more frequently in the brown senescent tissue than the green photosynthetic tissue, indicating that the OTUs are almost exclusively saprophytes. Some of the *Galerina* OTUs were host specific to one moss host, while others were not. This shows that the *Galerina* genus include species that probably are specialists to specific microniches, and also species that are non-specific generalists. This host specificity could be used as a tool to identify *Galerina* species apart from each other when morphological characters are very similar.

Introduction

Bryophytes are a dominant and functionally important vegetation component of the boreal forest floor vegetation, producing organic matter, hosting nitrogen-fixing bacteria, stabilizing soils or debris, and trapping sediments and water (Turetsky, 2003; DeLuca et al., 2002). Bryophytes contribute considerable bio mass in boreal biomes (Bach et al., 2009; Benscoter and Vitt, 2007; Turetsky, 2003), exceeding that of vascular plants (excluding trees) in a mixed boreal forest (Palviainen et al., 2005). Bryophytes represent a multitude of microhabitats for microorganisms that form a gradient from the green photosynthetic tissue at the top to the brown senescent tissue underneath and finally connecting to the rich fungal community of the soil (Lindahl et al., 2007). The green tissues have an active and broad diversity of chemical defenses similar to those found in vascular plants, protecting them from attacks by microorganisms (Ferreira et al., 2006; Frahm, 2004; Hammerschmidt, 1999; Wang et al., 2005). The brown tissues, on the other hand, are a mixture of storage (Hakala and Sewòn, 1992; Skre et al. 1983), senescent and dying cells presumably with lower defensive capabilities than the green tissues.

Bryophilous Fungi

Bryophilous fungi are fungi associated with bryophytes. They play various ecological roles such as saprotrophs, parasites, pathogens and mutualists, affecting the health of or even killing their bryophyte hosts (reviewed by Davey and Currah, 2006). As parasites and pathogens on bryophytes they can be effective to maintain the diversity of moss and higher plant communities (Hoshino et al., 2001). As saprotrophs on bryophytes, fungi can play an important role in the carbon cycle because the cell walls of mosses decompose slowly, probably because they contain polyphenolic compounds that resemble lignin. However, some microfungi are able to degrade mosses, and seems to have an important role in bryophyte decomposition (Thormann et al., 2002; Day and Currah, 2011). Many bryophilous fungi species are adapted to specific microsites and microniches, e.g. hyaline hair points, subterranean rhizoids or antheridial cups (Döbbeler, 2002; reviewed by Davey and Currah, 2006). However, in general terms little is known about the diversity and function of fungi associated with bryophytes. During recent years, the knowledge about the composition of the fungal community in dead bryophytes and humus (Lindahl et al., 2007) has increased, but still very little is known about the diversity of fungi associated with the photosynthesizing living green parts of boreal forest bryophytes (Kauserud et al., 2008).

Galerina

The basidiomycete genus *Galerina* is known to include more than 300 small agarics worldwide, but they are predominately described from the Northern Hemisphere (Gulden et al., 2005). Most *Galerinas* are thought to be saprotrophic and associated with bryophytes, probably as saprophytes of the senescent brown parts (Gulden et al., 2005), and produce a variety of degrading enzymes. *Galerina* species are known to produce laccase and peroxidase enzymes that breaks down lignin and lignin like compounds (Tortella et al., 2008), and resembles laccases in *Trametes* white rot fungi species (Ibrahim et al., 2011). Also, *Galerina* produces cellulase and hemicellulase enzymes that break down cellulose and hemicellulose (Nagendran et al., 2009; Wolfe et al., 2012). Other *Galerina* species are assumed to be biotrophic (Gulden, 2008) i.e. parasites or endophytes. Molecular phylogenetic analyses have revealed that the genus is polyphyletic, including at least four independent clades (Gulden et al., 2005). Furthermore, the genus includes many species complexes (Gulden et al., 2005) making taxonomic assignment difficult.

454 sequencing

During the last few years high throughput DNA sequencing techniques like 454 pyrosequencing have changed the field of microbiology, because sequences can be generated in much greater numbers than ever before. The vast amount of sequences allows us to detect organisms present in low abundances, and those not culturable (Begerow et al., 2010, Ekblom and Galindo, 2011). Some of the pioneering studies using pyrosequencing for fungal diversity inventories (Buèe et al., 2009; Amend et al., 2010) demonstrate the utility of this technology in studying fungal diversity and patterns in fungal distribution based on environmental samples. Jumpponen and Jones (2009) were the first to implemented these techniques to investigate the fungal communities in the phyllosphere of *Quercus macrocarpa*, where the phyllosphere being the living leaf as a whole including the inter- and intracellular interior spaces as well as the surface (Carroll *et al*., 1977). From 54 samples they detected ~18,000 sequences, and these were tentatively associated with almost 700 phyllosphere fungal species. Davey et al. (2012) analyzed the diversity and seasonal variance of fungi associated with boreal bryophytes using 454 pyrosequencing, and like Jumpponen and Jones (2009) found a high diversity of fungi in their samples; about 2,676 species (OTUs) in 294 samples.

Aims:

The main aim of this study was to investigate the diversity of *Galerina* species associated with four different bryophytes (*Dicranum scoparium, Hylocomium splendens, Pleurozium schreberi,* and *Polytrichum commune)*. This was done by extracting *Galerina* sequences from two 454 pyrosequencing studies dealing with the moss phyllospheres (Davey et al. Submitted; Davey et al. in prep). The ecology of the *Galerina* species was analyzed by investigating preferences for one or more of the four host mosses, and also for the green or brown tissue.

Materials and methods

Data mining and Bioinformatics

Sequences with taxonomic affinity to *Galerina* were mined from two ITS2 pyrosequencing datasets: (I) a dataset investigating the effects of nitrogen fertilization on the fungal communities associated with three bryophyte hosts (Davey et al. Submitted; Davey et al. in prep.) and (II) a dataset characterizing variation in bryophyte-associated fungal communities across an elevation and vegetation gradient (Davey et al. Submitted; Davey et al. in prep.). The two datasets included sequences obtained from the bryophyte hosts *Dicranum scoparium, Hylocomium splendens, Pleurozium schreberi. Polytrichum commune* was also included in

dataset (II), but not in dataset (I). In both studies, individual shoots of the bryophyte hosts were collected, cleaned, divided into photosynthetic and senescent tissues as described in Davey et al. (2012). DNA was extracted from a total of 588 shoot fragments, and for both datasets, the ITS2 region of rDNA was amplified by nested PCR, and the amplicons were pyrosequenced as described in Davey et al. (2012). Sequences were quality-filtered and denoised as described in Davey et al. (2012) before extraction of sequences identified as *Galerina* by their best BLAST match against the NCBI GenBank database. In total, 5,418 and 6,328 sequences with taxonomic affinity to *Galerina* were extracted from the two studies, respectively, representing putative *Galerina* incidences in 345 shoot fragments. The 11,746 sequences were clustered into Operational Taxonomic Units (OTUs) using the uclust algorithm as implemented in Qiime v.1.3.0 (Caporaso et al., 2010; Edgar, 2010) with a 96% similarity threshold. The most abundant sequence from each OTU was selected as the representative sequence, compared against the NCBI-nr nucleotide database using BLAST, and a taxonomic assignment given based on the best BLAST match to a known organism. Singleton OTUs were removed from the dataset as putative sequencing errors (Tedersoo et al., 2010).

Phylogenetic Analyses
A nucleotide matrix consisting of all the representative sequences and a collection of *Galerina* ITS2 reference sequences retrieved from GenBank (mainly from Gulden et al., 2005) was aligned using MAFFT v 6.717 (Katoh et al., 2009). Ambiguously aligned regions were removed using Gblocks version 0.91b (Castresana, 2000) and the resulting alignment subjected to maximum likelihood analysis using GARLI version 1.0 (Zwickl, 2006).

Statistical Analyses
EstimateS (Colwell, 2005) was used to calculate Mao Tao species accumulation curves, for the whole dataset, and for each hosts and tissue type (Fig 1). A bootstrap richness estimate (Smith and van Belle, 1984) was also calculated, which gives us an estimate of the total number of species expected in our samples. For analyzing host and tissue preferences, only OTUs occurring in more than 50 shoot fragments and with total abundance >1,000 reads were analyzed. For these six widespread OTUs, the total abundance and frequency of occurrence
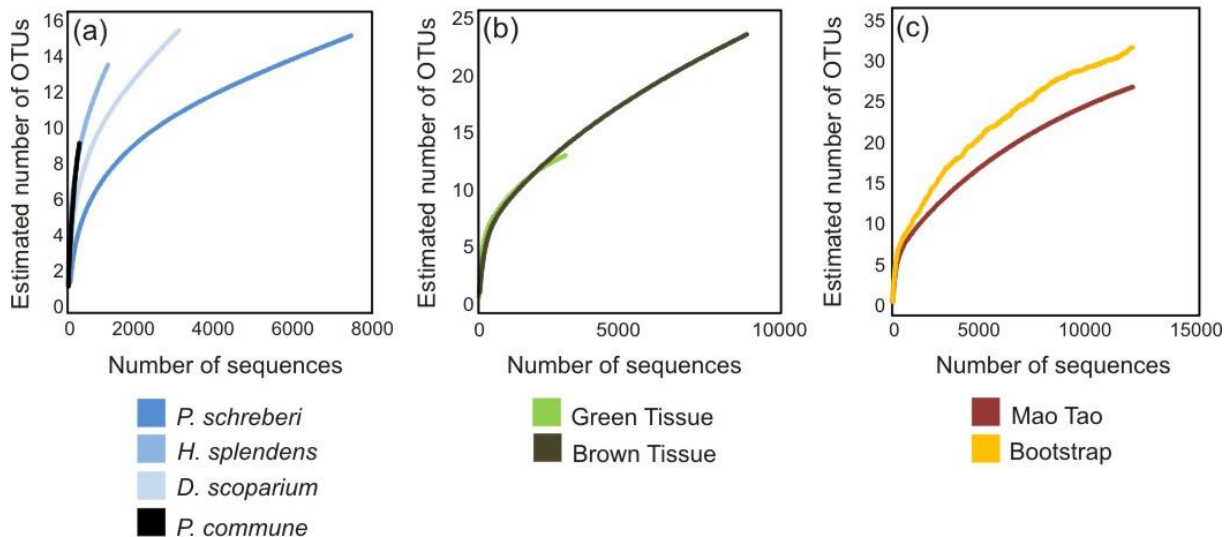
Fig. 1: Accumulation curves for *Galerina* OTUs using Mao Tao calculated observations showing the number of OTUs found in each of the four moss hosts (a), in the two tissue types (b) and a comparison of the Mao-Tau calculated number of OTUs detected, and a bootstrap richness estimate of the total *Galerina* species richness (c).

was compared in the different host and tissue types. Pearson's chi-squared tests were used to compare the frequency of occurrence of each OTU in the four hosts, and in the two tissue types. For each of the six most abundant OTUs, histograms were plotted based on number of reads detected in each shoot fragment divided by host and tissue class (Fig. 2).

Results

Data characteristics and taxonomic affinity

Galerina was found in 58.7% of the shoot fragments. The 11,746 *Galerina* reads extracted from the two datasets were clustered into 26 non-singleton OTUs. The *Galerina* OTUs accumulation curve were not saturated however (Fig. 1), indicating that not all OTUs present were recovered in this study. The bootstrap richness estimate (Smith and van Belle 1984) gives an estimate of ~30 OTUs occurring in the moss hosts. Most shoot fragments contained only one *Galerina* OTU (61.4%, range:1-6). The 26 OTUs were identified as belonging to *Galerina* based on the best BLAST match from the NCBI-nr nucleotide database, and were attributed to 15 different *Galerina* species. Twenty of the 26 OTUs had ≥97% sequence similarity to a known *Galerina* reference sequence. Multiple OTUs were assigned to *G. pumila*, *G. fallax, G. cephalotricha, G. atkinsoniana, G. stylifera* and *G. mniophila*. The OTUs did not group together into one lineage, but rather were affiliated with separate lineages across the *Galerina* tree (Fig. 3). The six most abundant OTUs (Table 1, shaded) account for

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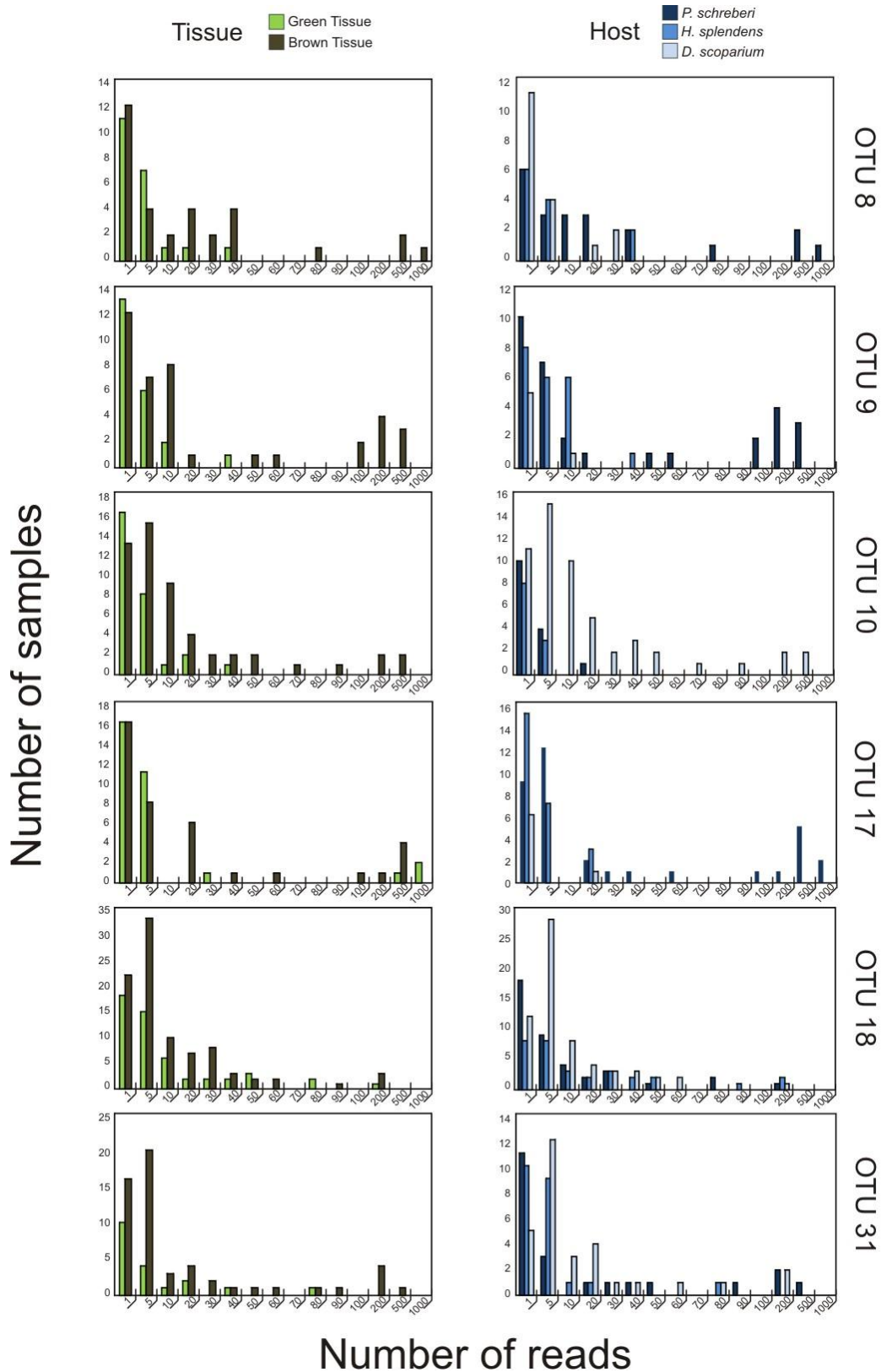
Fig. 2: Histograms showing the number of *Galerina* reads found in individual samples (shoot fragment) divided into categories ranging from 1-1000 reads. Distribution according to tissue type is shown on the left, and according to hosts to the right.
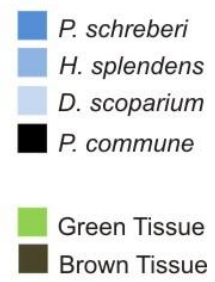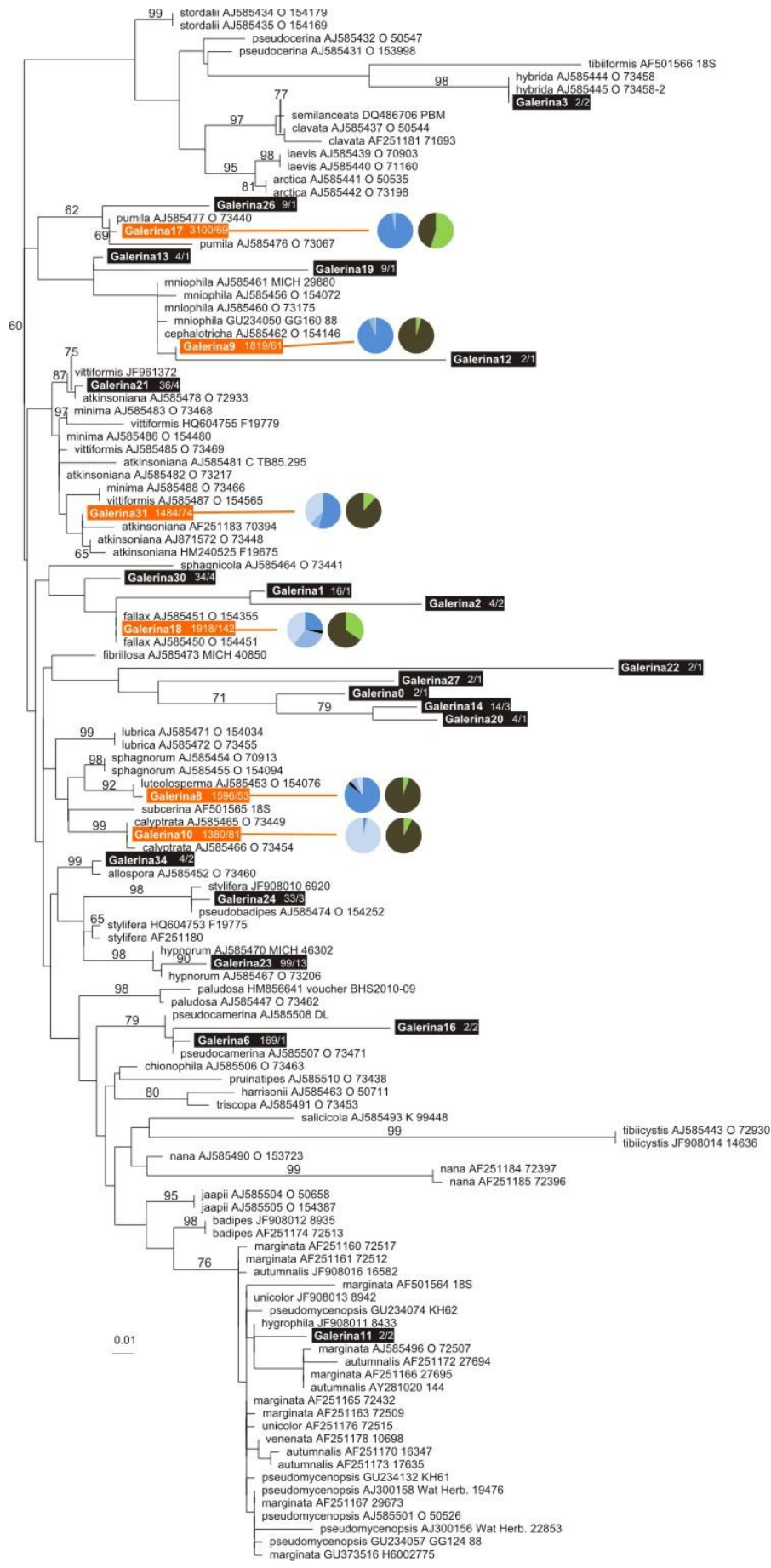
Fig. 3: Maximum likelihood tree showing the genetic diversity of *Galerina* OTUs in the dataset. The tree combines GenBank ITS2 reference sequences and the representative sequences of the 26 OTUs (highlighted). The six most abundant OTUs are colored in orange with attached pie charts showing the distribution of reads between the hosts and tissue types. All 26 OTU names are written as: OTU ID, total abundance and frequency.

Table 1: The 26 most abundant OTUs sorted from the largest number of reads to the smallest. The OTUs were given taxonomic assignments based on the best BLAST matches from NCBI-nr nucleotide database. The six most abundant OTUs (shaded) account for most of the reads (>96%), and are found in most of the samples (>95%).

OTU	#Reads	#Samples [1]	%Coverage [2]	%ID [3]	Consensus Lineage	Ecology [4]
17	3100	69	85	99	*pumila*	bryophilous
18	1918	142	89	100	*fallax*	bryophilous
9	1819	61	89	99	*cephalotricha*	bryophilous
8	1596	53	88	99	*luteolosperma*	bryophilous
31	1484	74	98	98	*atkinsoniana*	bryophilous
10	1380	81	88	99	*calyptrata*	bryophilous
6	169	1	89	99	*pseudocamerina*	conifer debris
23	99	13	88	100	*hypnorum*	bryophilous
21	36	4	89	99	*atkinsoniana*	bryophilous
30	34	4	91	94	*fallax*	bryophilous
24	33	3	89	98	*pseudobadipes*	conifer debris
1	16	1	99	91	*stylifera*	lignicolous
14	14	3	70	99	*fallax*	bryophilous
19	9	1	72	99	*mniophila*	bryophilous
26	9	1	89	95	*pumila*	bryophilous
2	4	2	99	92	*stylifera*	lignicolous
13	4	1	89	97	*cephalotricha*	bryophilous
20	4	1	70	99	*pumila*	bryophilous
34	4	2	89	99	*allospora*	bryophilous
0	2	1	71	99	*atkinsoniana*	bryophilous
3	2	2	87	99	*hybrida*	sphagnicolous
11	2	2	98	97	*unicolor*	ligni- & terri- colous
12	2	1	88	91	*cephalotricha*	bryophilous
16	2	2	46	95	*harrisonii*	bryophilous
22	2	1	59	100	*mniophila*	bryophilous
27	2	1	70	100	*atkinsoniana*	Bryophilous

[1] The number of samples (shoot fragments) where the OTU was present.
[2] Percent similar nucleotide coverage between the OTU representative sequence and BLAST match sequence from the NCBI-nr nucleotide database.
[3] Percent nucleotide match between the OTU representative sequence and the BLAST match sequence from the NCBI-nr nucleotide database.
[4] The OTU main known ecology (Gulden, 2008).

about 96% of the reads and were found in most of the 345 shoot fragments (>95%). The ecology of the detected OTUs, as judged by current literature knowledge of the best BLAST hits, is thought to be mostly bryophilous (Table 1). Among the more infrequent OTUs, species are known to have different substrate and host affinities appear, including species that are lignicolous, terricolous and associate with conifer debris (Table 1). The number of reads per OTU detected in each shoot fragment ranged from one to several hundred (Fig. 2). For each OTU, there were a high number of samples in which just a few reads were detected (1-5), and fewer samples with a high number of reads detected (100-1,000). Because of the wide dispersion of the abundance data and correspondingly large standard deviation, meaningful statistical analysis of the abundance data is difficult.

Host

A total of 7,452 sequences with affinity to *Galerina* occurred in 118 *P. schreberi* shoot fragments, 2,925 sequences in the 124 *D. scoparium* fragments, 1,059 sequences in the 88 *H. splendens* fragments, and 310 sequences s in the 15 *P. commune* fragments (Fig. 4a). Because of the low number of samples the *P. commune* data was removed from the host and tissue analyses (Fig. 2 and Fig. 4), but was used in the OTU table (Table 1), phylogenetic tree (Fig. 3) and accumulation curves (Fig. 1). When it comes to the frequency data (Fig. 4b), four of the six most abundant *Galerina* OTUs (9, 10, 17, 18) occurred significantly more frequently in one host than in the others (chi-square test, p <0.05). OTU 9 and 17 were most frequent in *P. schreberi*, while OTU 10 and 18 were most frequent in *D. scoparium*. The last two OTUs (8, 31) were not significantly more frequent in one host than in the others, but rather had a more even distribution between the hosts. In four of the six OTUs (8, 9, 10, 17) there was clearly higher abundance of reads in one host over the others (Fig. 4a), which mostly is due to a few samples having a very high number of reads (Fig. 2). OTU 8, 9 and 17 were most abundant in *P. schreberi,* while OTU 10 was most abundant in *D. scoparium*. The last two OTUs (18, 31) had an even distribution of reads across the hosts.

Tissue

A total of 2,874 sequences were detected in 141 green (photosynthetic) shoot fragments, and 8,872 sequences in 204 brown (senescent) shoot fragments (Fig. 4c). When it comes to the frequency data (Fig. 4d), the six most abundant *Galerina* OTUs occurred consistently more frequently in the brown tissue than in the green tissue, and there were significant differences in frequency for OTU 9, 10, 18 and 31 between the two tissue types (chi-square tests, p
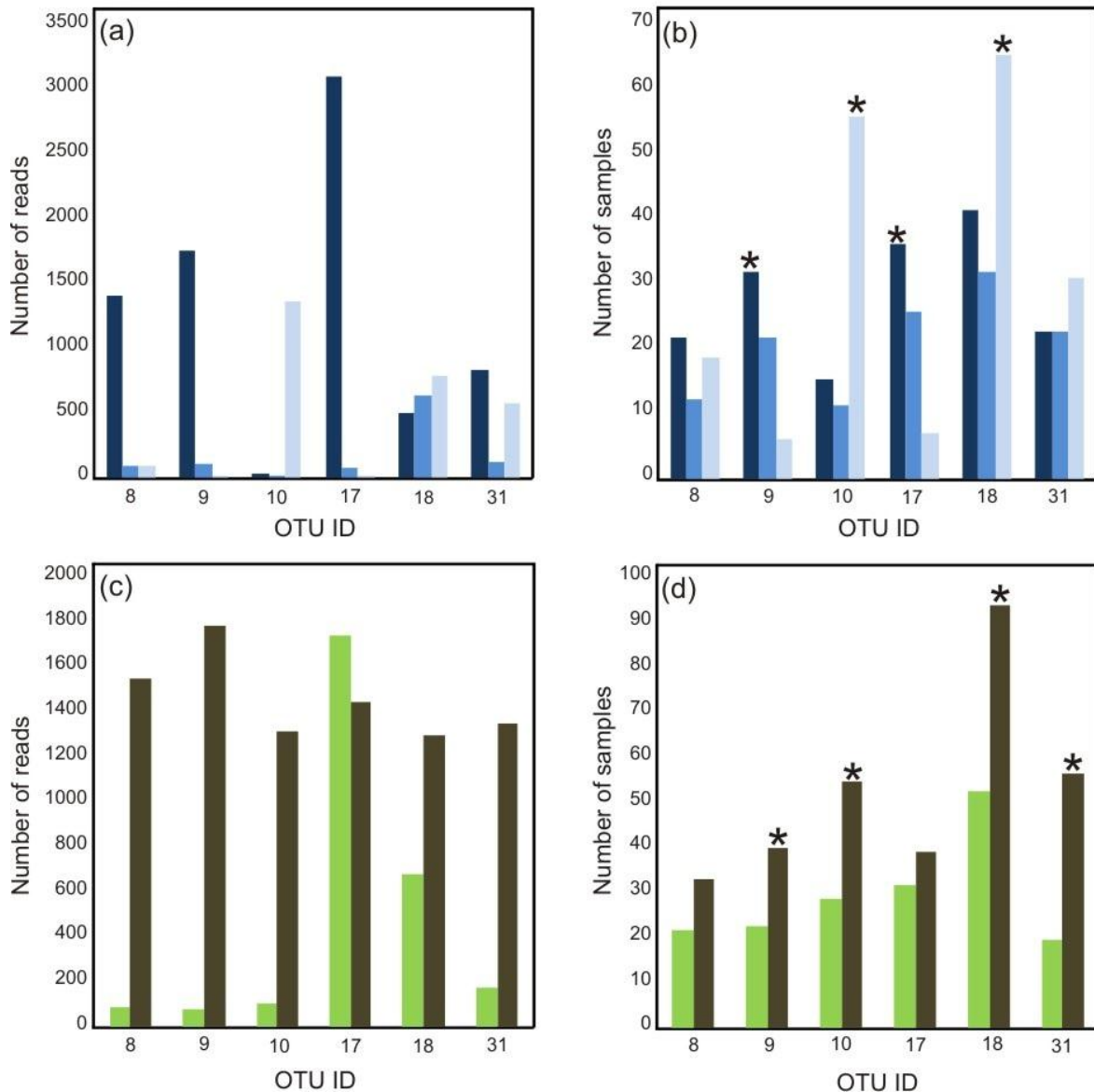
Fig. 4: Frequency (b,d) and abundance (a,c) of the six most abundant OTUs in each of the three host species (*P. schreberi*, D*. scoparium* and *H. splendens*) (a, b), and in the green and brown tissues (c, d). The asteriks indicate OTUs with a significant difference in frequency between tissue types (b), and host mosses (d) (chi-square tests, p <0.05).

<0.05). Correspondingly, in five OTUs (OTU 8, 9, 10, 18, 31) there were more reads detected in the brown tissue than in the green tissue, while OTU 17 had more reads in the green tissue. Also, the histograms of per-sample abundance demonstrate that those samples with a high number of reads were detected almost exclusively in the brown tissue (Fig. 2). OTU 17 was the only one with a high number of reads detected in both the green and brown shoot fragments (Fig. 4c).

Discussion

Diversity of Galerina in mosses

A high diversity of fungi is known to be associated with bryophytes (Felix, 1988). These are mainly ascomycetes (Döbbeler, 1997), but also some basidiomycetes (Kost, 1988), including the genus *Galerina* (Gulden et al., 2005). Gulden et al. (2005) showed that *Galerina* is polyphyletic, and includes as at least four independent main clades. The phylogenetic tree (Fig. 3) shows that the *Galerina* OTUs do not group together, but are rather scattered all over the tree. This suggests that there has not been one unique evolutionary transition for the genus to live on moss substrate, but several independent transitions.

In this study 26 OTUs with taxonomic affinity to *Galerina* were detected (Table 1). Twenty of these were detected in very low abundances and frequencies compared to the six most abundant ones, indicating that they may not be strongly associated with the moss hosts analyzed in this study. One would expect that only OTUs attributed to species with known bryophilous life histories would be found, but other ecologies were detected as well, including species associated with conifer debris, lignin and terricolous ones. Why were putative non-bryophilous *Galerina* species found? A possible hypothesis is that these OTUs were air-borne contaminants, or small numbers of residual spores not removed during the cleaning process. This is reasonable considering that the reads occur in low numbers and in very few shoot fragments (mostly only 1-4 samples).

Host affinity

Some bryophilous fungi are known to be generalists, i.e. not host specific, and are associated with a number of bryophyte species (Davey and Currah, 2006). Presumably these species have an effective enzymatic apparatus enabling them to grow across various hosts and tissues. Two of the six most abundant OTUs, OTU 18 (with 100% similarity to a *G. fallax* GenBank accession) and OTU 31 (with 98% similarity to a *G. atkinsoniana* GenBank accession), are presumably generalists, as both have reads occurring in an even distribution between at least two of the moss hosts (Fig. 4a). The frequency data shows that OTU 31 (~*G. atkinsoniana*) also occur in an even distribution between the host samples (Fig. 4b). OTU 18 (~*G. fallax)* has a significantly higher frequency of occurrence in moss host *D. scoparium*, which could indicate a preference for the host. However, most of the samples of *D. scoparium* include OTU 18 in a low abundance. Assuming that low abundance incidences represent spores, while high abundance incidences represent mycelium (Fig. 2), most of these reads may be spores. If so the reads are not colonizing tissue that gives an indication of preference for the host.

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Other bryophilous fungi are specialists, only colonizing one genus or even a single species of bryophyte moss (reviewed by Davey and Currah, 2006). They often have special traits like enzymes or pegs that help them break through the cell walls and survive fungal defenses (reviewed by Davey and Currah, 2006). The remaining four of the six most abundant OTUs are presumably host specific, because they have an uneven distribution of reads between the moss hosts. OTU 8 (with 99% similarity to a *G. luteolosperma* GenBank accession), OTU 9 (with 99% similarity to a *G. cephalotricha* GenBank accession) and OTU 17 (with 99% similarity to a *G. Pumila* GenBank accession) have a high number of reads found in *P. schreberi* samples, and OTU 10 (with 99% similarity to a *G. calyptrata* GenBank accession) has a high number of reads in *D. scoparium* samples. One way to investigate host specificity further would be to isolate the fungi at the lab and perform common garden experiments to explore their preference of various hosts.

P. commune was present only in one of the two original datasets. But even taking this in to consideration, there were few *Galerina* reads from *P. commune* compared to the other three moss hosts. When it comes to numbers of colonized samples, only approximately one seventh as many *P. commune* samples was colonized compared to the other hosts, suggesting *Galerina* is largely unable to colonize this species. *P. commune* is known to host less fungal biomass than *H. splendens* and *P. schreberi,* which suggest that *P. commune* presents a hostile environment for fungi, and might have effective fungal defense mechanisms (Davey et al., 2009). Whether this could be due to particularly effective host anti-fungal defenses, or if *P. commune* represents a nutritionally unsuitable host/substrate for *Galerina* is not known.

Colonization of green versus brown tissue

Most *Galerina* species are thought to be saprotrophic and associated with bryophytes, probably as saprophytes of the senescent brown parts (Gulden et al., 2005). One may speculate that this is because the brown tissue is a mixture of storage, senescent and dying cells (Hakala and Sewòn, 1992; Skre et al., 1985) making it a good source of nutrients. Also, the green tissues have chemical defenses against fungal infections (Ferreira et al., 2006; Frahm, 2004; Hammerschmidt, 1999; Wang et al., 2005) that prevent or restrict fungal colonization. One can assume that the brown tissues have no active defense mechanisms, which makes it easier to colonize then the green tissue.

Of the six most abundant OTUs, five occurred more frequently in the brown tissue (Fig. 4c). Also, some *Galerina* is also known to have the enzymatic apparatus to degrade moss tissue (Tortella et al., 2008; Nagendran et al., 2009; Ibrahim et al., 2011; Wolfe et al.,

2012), supporting the theory that they are saprotrophic (Gulden et al., 2005). However the OTUs also appeared in the green shoot fragments which indicate that they might also be biotrophic, and have multiple ecologies and are able to occupy several niches. One hypothesis is that they could be opportunists that do not colonize specific tissues, but rather try to colonize any available tissue. Another possible explanation is that the green tissue reads do not represent mycelium, but fungal spores. In most of the tissue samples (Fig. 2) few reads (1-5) were detected, while some had a high number of reads (100-1,000), and a few samples had a value in between (10-90). Assuming that low abundance incidences represent spores, and high abundance incidences represent mycelium, the majority of green shoot fragments contained only spores of *Galerina*, while the brown shoot fragments contain both spores and mycelium tissue of *Galerina*. *Galerina* species are known to produce laccase and peroxidase enzymes that breaks down lignin and lignin like compounds (Tortella et al., 2008; Ibrahim et al., 2011), and cellulase and hemicellulase enzymes that breaks down cellulose and hemicelluloses (Nagendran et al., 2009; Wolfe et al., 2012). Since they produce enzymes that should be able to degrade the cell walls of the green tissue, but seems to be less able to, it further supports the presence of hostile and defensive capabilities of the green tissue against fungal infections.

The only exception to the trend of brown tissue having more reads than the green is OTU 17 (~*G. pumila*), which has a high number of reads in the green tissue samples (Fig. 4c). OTU 17 (~*G. pumila*) might be a specialist on the green tissue of *P. schreberi*, being able to cope with the fungal defense mechanisms of the bryophyte. It might be a pathogen in the green tissue, like *Tephrocybe palustris,* which penetrates living chlorophyllous cells of *Sphagnum* (Redhead, 1981). However, all bryophytes collected for this study had a healthy appearance, and as reviewed by Davey and Currah (2006), bryophytes with pathogen infections often have macroscopic black, brown or yellow patches on them. It seems most likely that *G. pumila* is a weak parasite or an endophyte in the green tissue, adapted to the fungal defenses, and has found a microniche not used by the other OTUs.

Methodological considerations

Considering all 26 OTUs (Table 1), one can see that some *Galerina* morphospecies are linked to more than one OTU, whereas ideally we should have one OTU per species. One explanation could be that the within-species ITS2 divergence is lower than 96%, splitting a single species into several OTUs, resulting in multiple OTUs having the same species as their best BLAST match. The opposite would be that the within-species similarity is higher than

96%, resulting in different species getting lumped together into one OTU, making the best BLAST match for the OTU only representing one of several species. In these clustering based analyses there is a compromise to not split biological species in two or more OTUs with a threshold that is too high, or lump biological species together with a threshold that is too low. To minimize the effects of this, the OTU representative sequences were clustered with reference sequences from GenBank using different similarity thresholds, and 96% sequence similarity was selected because it minimized the number of splitting and lumping events. A second explanation may be that some OTUs have been compared with reference sequences that are not correctly named, i.e. the representative sequence for some OTUs may BLAST to a sequence that was deposited in GenBank with incorrect taxonomy listed. *Galerina* species assignment has hitherto been determined based on morphology/fruiting bodies (Gulden et al., 2005), and is a group with some species notoriously difficult to distinguish from one another, meaning there is high likelihood that some sequences with incorrect taxonomy have been deposited in the database. A third explanation may be that the OTUs represent rare or unknown *Galerina* species. Therefore, the best BLAST match is the closest match in the database, but still not correct because no reference sequence is available. Further the *Galerina* genus is known to include numerous species complexes (Gulden et al., 2005), making it a challenge to draw clear lines between species.

Conclusions

This study exemplifies how high throughput sequencing data can be used to explore fungal species diversity and also their ecology independent of fruit bodies. From large datasets one can extract data of interest with a sequencing depth sufficient to make new discoveries and support theories. This study extracted 11,746 *Galerina* sequences which were clustered into 26 OTUs (attributed to 15 unique species). Investigating the six most abundant OTUs showed that four out of six most abundant OTUs were host specific while the other two were not. *P. commune* was found to be less associated with *Galerina* than the other moss hosts, probably having a more hostile environment, and maybe effective mechanisms against fungal infections. Likewise, five out of the six most abundant OTUs where tissue specific while the last one was not. Hence the main ecology of the *Galerina* genus seems to be growth on brown tissue, probably as saprotrophs. However, there was also one OTU with affinity to *G. pumila* witch was found abundantly associates with both the green tissue, probably as parasites or endophytes, and the brown. This suggests that some *Galerina* species have multiple strategies, and are able to cope with several microniches.

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