

High diversity of fungi associated with living parts of boreal forest bryophytes

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Abstract: Bryophytes are a dominant and functionally important component of the forest floor vegetation in boreal forests, yet little is known about the fungal diversity associated with these abundant plants. Using molecular identification, we document an ecologically and phylogenetically diverse array of fungi associated with the living parts of three widespread and abundant boreal forest bryophytes, i.e., *Hylocomium splendens* (Hedw.) Schimp. in B.S.G., *Pleurozium schreberi* (Brid.) Mitt., and *Polytrichum commune* Hedw. From 376 cloned ITS sequences, 158 operational taxonomic units (OTUs), roughly corresponding to the number of species, were defined based on sequence similarity searches (Fasta) and phylogenetic analyses. A main portion (62.8%) of the OTUs belonged to Ascomycota, 32.0% to Basidiomycota, 3.9% to Chytridiomycota, and 1.3% to Glomeromycota. The most common orders were Helotiales (18.6%), Agaricales (11.5%), Chaetothyriales (9.6%), and Tremellales (9.0%). Frequently detected OTUs of potentially high ecologic importance included two with high sequence similarity (>99%) to the agaric *Entoloma conferendum* (Britzelm.) Noordel and the endophyte *Lophodermium piceae* (Fckl.) Hoehn., and various OTUs with affinity to the Helotian genus *Hyphodiscus*. Several ectomycorrhiza-forming basidiomycetes were also detected. Most OTUs (77.2%) were only detected once and a very small overlap in composition of OTUs was observed among the three bryophytes, indicating the occurrence of an immense fungal diversity associated with boreal forest bryophytes that deserves further study.

Key words: bryophytes, boreal forest, endophytes, environmental sampling, rRNA ITS sequence, moss.

Résumé : Les bryophytes constituent une part importante dominante et fonctionnelle de la végétation des parterres forestiers en forêt boréale, pourtant on connaît peu de choses sur la diversité fongique associée à ces plantes abondantes. À l'aide de l'identification moléculaire, les auteurs ont étudié un ensemble écologiquement et phylogénétiquement diversifié de champignons associés aux parties vivantes de trois bryophytes abondantes et largement distribuées dans la forêt boréale, soient les *Hylocomium splendens* (Hedw.) Schimp. in B.S.G., *Pleurozium schreberi* (Brid.) Mitt. et *Polytrichum commune* Hedw. À partir de 376 séquences ITS clonées et en appliquant des recherches de similarité de séquences (Fasta) et des analyses phylogénétiques, les auteurs ont défini 158 unités taxonomiques opérationnelles (OTU's) correspondant en gros au nombre d'espèces. La majorité (62,8 %) des OTU's appartiennent aux Ascomycota, 32,0 % aux Basidiomycota, 3,9 % aux Chytridiomycota et 1,3 % aux Glomeromycota. Les Hélotiales (18,6 %, les Agaricales (11,5 %), les Chaetothyriales (9,6 %) et les Tremellales, constituent les principaux ordres. Parmi les OTU's de grande importance écologique fréquemment répertoriés on en retrouve deux avec forte ressemblance de séquences (>99 %) avec l'agaric *Entoloma conferendum* (Britzelm.) Noordel et l'endophyte *Lophodermium piceae* (Fckl.) Hoehn., ainsi que divers OTU's ayant des affinités avec les Hélotiales du genre *Hyphodiscus*. On retrouve également plusieurs basidiomycètes mycorrhiziens. La plupart des OTU's n'apparaissent qu'une seule fois (72,2 %) et on observe un très faible recouvrement des OTU's pour l'ensemble des trois bryophytes, ce qui indique le besoin de poursuivre les études sur l'immense diversité fongique associée aux bryophytes de la forêt boréale.

Mots-clés : bryophytes, forêt boréale, endophytes, échantillon environnemental, séquence rARN ITS, mousse.

Introduction

Bryophytes are dominating constituents of the forest floor vegetation in boreal forests, which make up the world's second largest terrestrial biome covering ca. 1.4×10^7 km² of

the land surfaces of the earth. Certain bryophyte species, e.g., the feather mosses *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) Schimp. in B.S.G., are ubiquitous and remarkably abundant in the boreal forest (Bonan and Shugart 1989; Pharo and Vitt 2000; Benscoter and Vitt 2007) and play important roles in forest biomass production (Van Cleve et al. 1986; Bond-Lamberty and Gower 2007). Despite the importance of the abundant bryophytes, it is the fungi that are key determinants of carbon and nutrient cycling in boreal forests (Lindahl et al. 2007). The fungal community comprises two main functional groups, viz. a degrading component of saprobic fungi that decompose litter and wood (Rayner and Boddy 1986), and a nutrient-mobilizing component of mycorrhizal fungi that provide their host-plants with soil-derived nutrients (Smith

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and Read 1997). Little is known about the spatial distribution of these components, but recent research indicates that the degrading and nutrient-mobilizing components of the fungal community are spatially and temporally separated in boreal forests, since the saprobic fungi were primarily confined to recently shed litter in the surface of the forest floor, while the mycorrhizal fungi dominated in the underlying and older humus layers (Lindahl et al. 2007).

Mycorrhizal fungi efficiently colonize dead feather mosses (Carleton and Read 1991; Genney et al. 2006), which make up a major component of the humus in boreal forest soils. Actually, most of the mycelium in the humus below the uppermost moss and litter layer seems to originate from mycorrhizal fungi or otherwise root-associated fungi, as shown by DNA-based identification methods (Lindahl et al. 2007). As dead and living parts of boreal feather mosses are physically connected — with the dead parts below ground and the green living parts above ground — these parts together constitute a tight and direct link between the soil humus community and the photoassimilating above-ground community. Although detailed knowledge about the composition of the fungal community in dead mosses and humus has emerged recently, very little is known about the diversity of fungi associated with the living green parts of common and abundant boreal forest bryophytes. However, interactions between fungi and bryophytes is being reported with increasing frequency and it is evident that a taxonomically diverse suite of fungi interacts with bryophytes as pathogens, parasites, saprobes, commensals, and symbionts (Read et al. 2000; Davey and Currah 2006).

A vast diversity of endophytic fungi live hidden inside plant tissues apparently without causing injury to the host plant (Arnold 2007). These fungi belong to several fungal taxonomical groups and they appear to be ubiquitous (Arnold 2007; Higgins et al. 2007). Endophytic fungi may impact plant–herbivore relationships, as they are known to function as herbivore deterrents (Faeth 2002). While the functional ecology of endophytic fungi are rather well studied in grasses, and much less studied in herbaceous plants (Gange et al. 2007), it is virtually unstudied in bryophytes.

In this study we use DNA-based identification methods to document the diversity of fungi associated with living parts of three common and abundant bryophyte species within a boreal spruce forest landscape. We show for the first time that a diverse array of phylogenetically divergent fungi, including mycorrhizal, forest pathogenic, and saprobic fungi, characterizes the living parts of boreal forest bryophytes. We also discuss some possible functional roles of the fungal community associated with forest bryophytes.

Materials and methods

Field work and sampling of bryophytes

The analyzed bryophyte shoots were sampled in a spruce forest landscape located on the border between the south and middle boreal vegetation zone in Årums, southeast Norway (59°21'N, 9°45'E, ca. 500 m above sea level). Three sub-localities were defined subjectively within a 1 km² area (within old forest stands) and at each sub-locality three shoots of the species *Hylocomium splendens*, *Pleurozium*

schreberi, and *Polytrichum commune* Hedw. were sampled, giving a total of 27 shoots. Only shoots with a vigorous appearance were sampled. Further information about the forest landscape is available in Nielsen et al. (2007).

Molecular methods

The 1.5 cm green top sections of the collected shoots were cut off and used in further analyses. The shoots were first washed thoroughly with tap water and subsequently with distilled water (dsH₂O). DNA was extracted from the shoots using the 2% CTAB miniprep method described by Murray and Thompson (1980) with minor modifications: DNA was resuspended in 100 µL dsH₂O at the final step of extraction, and DNA templates were diluted 50-fold before PCR-amplification.

PCR amplification was accomplished using the fungal specific primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993). To minimize the problem with recombinant sequences, which can potentially be a problem during PCR amplification from mixed DNA templates (see e.g., Jumpponen 2007), the fidelity enzyme DyNAzyme EXT (Finnzymes Oy, Espoo, Finland) was employed according to the manufacturer's directions. PCR was run on an Eppendorf thermocycler in 30 µL reactions containing 17.5 µL of 50× diluted template DNA and 12.3 µL reaction mix. Final concentrations were 4 × 250 mmol/L dNTPs, 0.625 mmol/L of each primer, 2 mmol/L MgCl₂ and 1 unit polymerase enzyme. The PCR amplification program was as follows: 4 min at 94 °C, followed by 37 cycles of 25 s at 94 °C, 35 s at 54 °C, 72 °C for 90 s, and a final extension step at 72 °C for 10 min before storage at 4 °C. An elongated extension step (90 s) was used to minimize the problem with recombinant sequences (i.e., lower the frequency of incomplete ITS fragments present after each temperature cycle).

The ITS fragments were cloned with the TOPO TA Cloning kit (Invitrogen) using blue–white screening according to the manufacturer's manual. Positive colonies were subjected to direct PCR with the M13R/T7 primers using the same PCR conditions as described above (except that the elongation period was reduced to 35 s) and the PCR products run on gels. Twelve to 18 colonies were sequenced from each bryophyte shoot. The targeted fungal diversity in the shoots was maximized by selecting ITS amplicons of different lengths for further sequence analyses. All sequences were generated using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.) and visualized on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All sequence chromatograms were controlled manually and sequence alignments established in the program BioEdit (Hall 1999). All unique sequences have been accessioned in GenBank under the accession Nos. AM992621–AM992629 and AM999540–AM999763.

Bioinformatics and statistical inferences

All sequences were submitted to Fasta similarity searches (Pearson and Lipman 1988) to reveal their taxonomic affiliation. The taxonomic system implemented in GenBank–EMBL was employed for taxonomic grouping. Sequences having high similarity (>97%) to GenBank–EMBL accessions were considered nonchimeric. Likewise, sequences of high similarity (>99%) independently amplified from differ-

Fig. 1. Phylogenetic relationships of fungi commonly associated with *Hylocomium splendens* (Hylo), *Pleurozium schreberi* (Pleu), and *Polytrichum commune* (Poly), inferred from the comparison of rDNA ITS sequences. The numbers behind the moss abbreviations indicate which of the three sublocalities and which plant replicate (within sublocalities) the sequences were derived from (e.g., 2, 3). The accession numbers of GenBank sequences with highly similar sequences are indicated. Grey shading is used to delimit OTUs (groups of sequences that were at least 98% similar). Bootstrap support values are shown below nodes (1000 replicates).

ent bryophyte shoots were also considered nonchimeric. The remaining sequences were reinspected using the visual Fasta interface to detect potential chimeric sequences. Different parts of the sequences were analyzed independently by multiple searches, with the expectation that different constituents of a chimeric sequence will have affinity to different taxa and (or) accessions in GenBank. Hence, sequences including portions with affinity to different taxonomic groups were considered as putative chimeric sequences. Based on the phylogenetic analyses (see below), Fasta searches and direct sequence comparisons, the sequences were grouped into operational taxonomic units (OTUs). In general, 98% sequence similarity was used as cut-off when pooling similar sequences into the same OTU. However, most of the sequences represented distinct and unique lineages, making the delimitation of OTUs in general a straightforward operation. However, it must be emphasized that there is no threshold value of sequence similarity that is universally useful for distinguishing species of fungi. Hence, OTUs are only used as proxies for estimating species richness.

Similar ITS sequences were grouped into various subalignments that included the most similar GenBank accessions. Phylogenetic analyses were conducted using the maximum parsimony criterion as implemented in PAUP* version 4.02b (Swofford 2003), treating characters as unordered with equal weights and gaps as unknown. The heuristic search option, with the tree bisection–reconnection (TBR) branch swapping algorithm and the random addition sequence option with 100 replicates to find multiple islands, was employed for all searches for most parsimonious tree(s). All other settings were default. Bootstrap support for branching topologies was determined with the same parameter settings using 1000 search replicates.

Results

From the 27 bryophyte shoots, 376 ITS clones were sequenced. After removing 11 putative chimeric sequences and identical (replicate) ITS sequences obtained from the same bryophyte shoots, 233 sequences remained for further analyses (62%). Using the procedures mentioned above, these sequences were grouped into 158 OTUs. In Fig. 1, phylogenetic trees derived from five subalignments are shown, demonstrating the phylogeny-based delimitation of OTUs. All the defined OTUs are listed in the supplementary data².

As judged from the similarity to GenBank accessions, 62.8% of the OTUs belonged to Ascomycota, 32.0% to Basidiomycota, 3.9% to Chytridiomycota (*sensu lato*), and 1.3% to Glomeromycota, respectively (Fig. 2). The most common orders were Helotiales (18.6%), Agaricales (11.5%), Chaetothyriales (9.6%), and Tremellales (9.0%).

In Table 1, the most common OTUs detected three times or more are listed. Three OTUs with taxonomic affiliation to the omnipresent *Cladosporium* were among the most frequent. Sixteen sequences had highest similarity (91.2%–97.0%) to GenBank accessions of the Helotian species *Hyphodiscus hymeniophilus* (P. Karst.) Baral, and were delimited into eight OTUs (Fig. 1a). Another frequent putative Helotian OTU, detected in all three bryophytes, obtained only low similarity (89%) to an accession AF141190 of *Neofabrea alba*. Thirteen sequences had affinity to various accessions of the plant endophyte *Lophodermium piceae* (Fckl.) Hoehn., and three different OTUs were defined within the “*L. piceae* complex,” two of them derived from all the three bryophytes (Fig. 1d). Six sequences obtained from all three bryophytes had high similarity (99%) to an accession of the agaricoid *Entoloma conferendum* (Britzelm.) Noordel. (AF538624). One unidentified OTU with highest affinity to an accession of *Tremella* sp. (AY188379) was also detected in the three bryophytes. Six sequences (derived from *H. splendens* and *P. schreberi*, respectively) obtained low affinity to an accession of *Powellomyces* sp. (AY349104) of Spicellomycesales (Chytridiomycota).

Thirty-one OTUs (19.6%) had 98% or higher similarity to various GenBank accessions (Table 2). Among these were five ectomycorrhiza-forming basidiomycetes, namely *Cortinarius armillatus* (99.7% similarity), *C. caperatus* (98.6%), *Suillus variegatus* (99.5%), *Lactarius kauffmannii* (98.4%), and *Piloderma byssinum* (98.9%). Two sequences obtained 100% and 99.8% matches to accessions of the putatively saprobic agarics *Marasmius androsaceus* and *Galerina luteolosperrya*, respectively. GenBank accessions of several plant pathogens obtained high matches, including *Exobasidium vaccinii* (100%), *Sclerotinia sclerotiorum* (99.8%) and *Fusarium oxysporum* (99.3%) (Table 2).

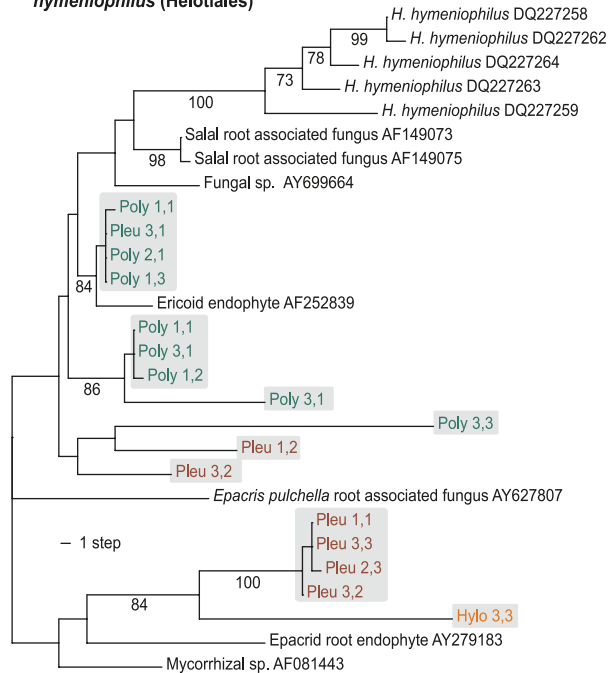
Overall, 122 OTUs (77.2%) were detected once only, 22 (13.9%) were encountered twice, while 14 OTUs (8.9%) were detected in 3–8 shoots. The 27 bryophyte shoots included from five (one shoot of *P. schreberi*) up to 13 OTUs (one shoot of *P. commune*), but no significant difference in number of OTUs appeared between the three bryophytes, nor between the three sublocalities (ANOVA, $p > 0.05$). Most OTUs were detected in *H. splendens* (73), followed by *P. schreberi* (59) and *P. commune* (53). *Hylocomium splendens* and *P. commune* had most OTUs in common (6) while *H. splendens* and *P. schreberi* shared least OTUs (3).

Discussion

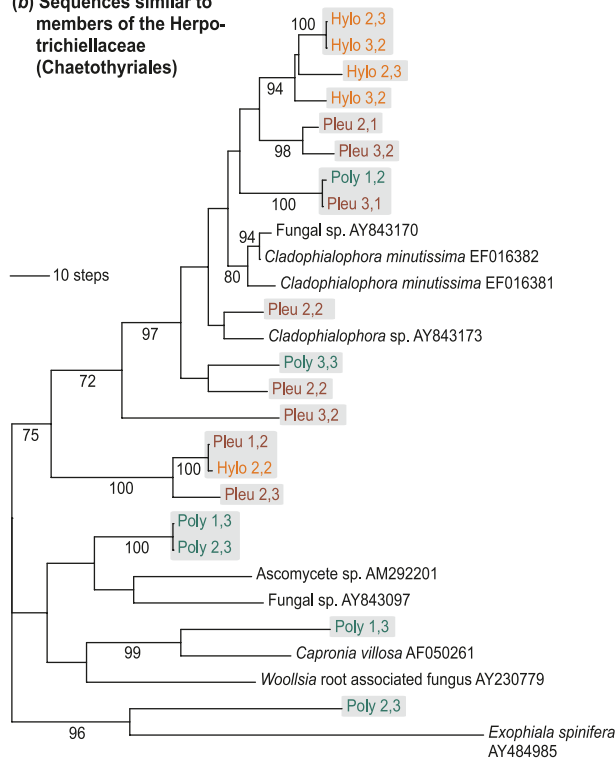
In this study we demonstrate that an ecologically and phylogenetically diverse array of fungi is associated with living parts of the boreal bryophytes *Hylocomium splendens*, *Pleu-*

²Supplementary data for this article are available on the journal Web site (<http://botany.nrc.ca>) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3841. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

(a) Sequences similar to *Hyphodiscus hymeniophilus* (Helotiales)



(b) Sequences similar to members of the Herpotrichiellaceae (Chaetothyriales)



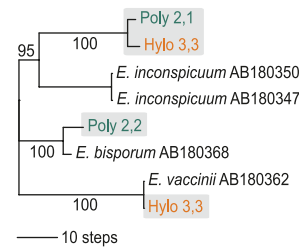
Host bryophytes for selected ITS sequences:

Hylo: *Hylocomium splendens*

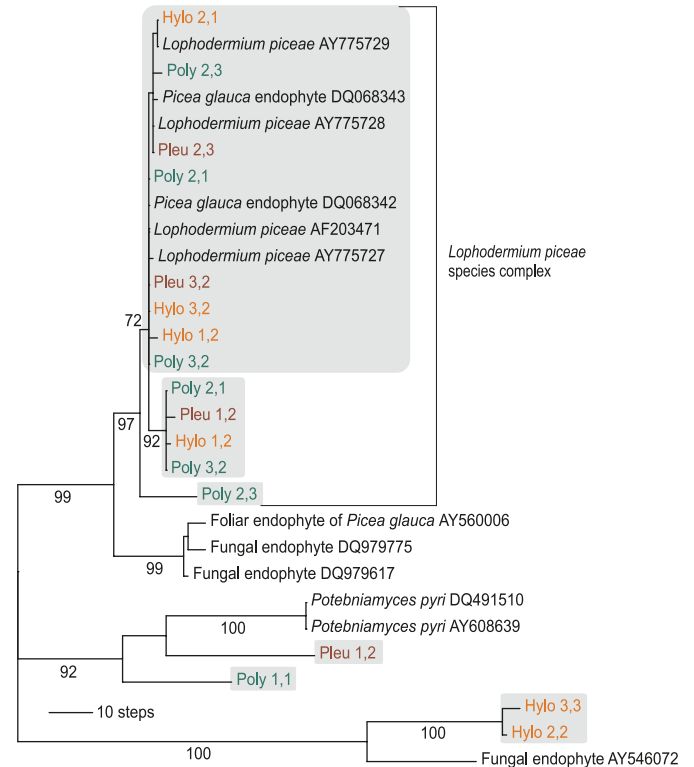
Pleu: *Pleurozium schreberi*

Poly: *Polytrichum commune*

(c) Sequences similar to species of *Exobasidium* (Exobasidiales)



(d) Sequences similar to *Lophodermium* and *Polybniamyces* (Rhytismatales)



(e) Sequences similar to members of the Capnodiales

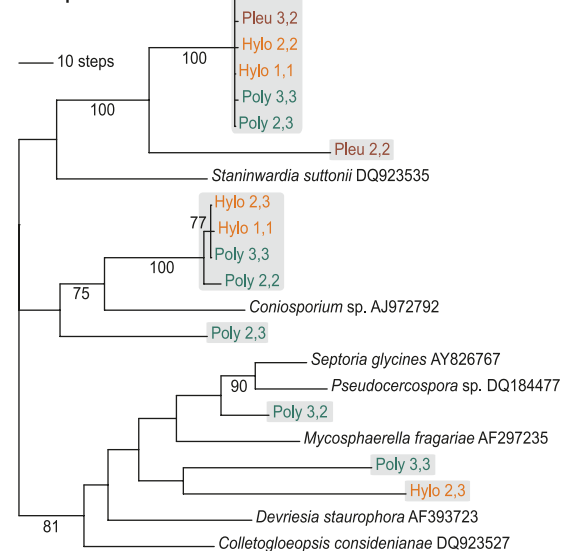
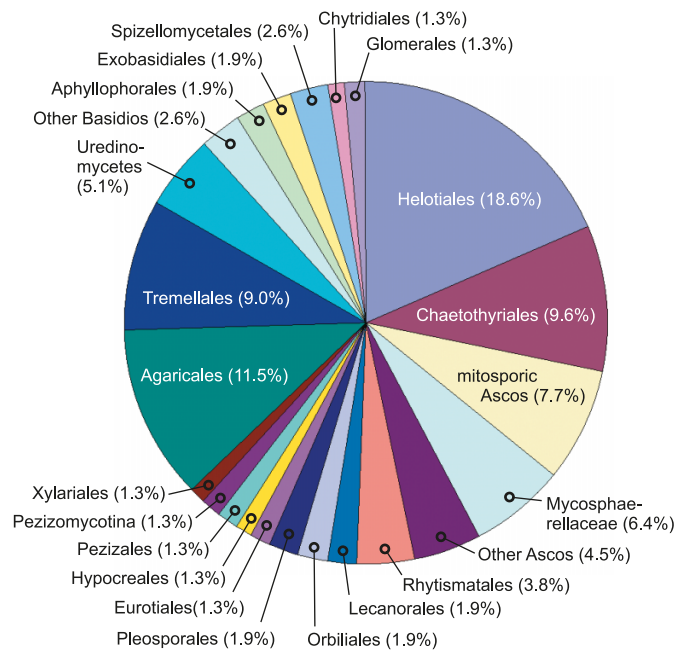


Table 1. Best matches in GenBank of the most frequently detected OTUs and their taxonomic affiliation (A, Ascomycota; B, Basidiomycota; C, Chytridiomycota s.l.).

Best match in GenBank	Taxonomy	Acc. No.	Similarity	Overlap	Hylo	Pleu	Poly
<i>Cladosporium magnusianum</i>	Capnodiales (A)	AF393704	99.8–100	577	1		2
<i>Devriesia staurophora</i>	Capnodiales (A)	AJ972792	84.4–87.6	542–573	2		2
<i>Devriesia staurophora</i>	Capnodiales (A)	AF393723	80.4–84.1	577–585	2	2	3
<i>Gyoeffyaella rotula</i>	mitosporic Asco. (A)	AY729937	98.5	520	1	1	1
<i>Hyphodiscus hymeniophilus</i>	Helotiales (A)	DQ227258	93.9–97.0	497–603		1	3
<i>Hyphodiscus hymeniophilus</i>	Helotiales (A)	DQ227258	94	603			3
<i>Hyphodiscus hymeniophilus</i>	Helotiales (A)	DQ227258	91.2–91.4	603		4	
<i>Lophodermium piceae</i>	Rhytismatales (A)	DQ068342	91.4–100	480–703	3	2	3
<i>Lophodermium piceae</i>	Rhytismatales (A)	DQ979775	90.6–98.9	480–702	1	1	2
<i>Neofabraea alba</i>	Helotiales (A)	AF141190	88.9–89.4	587–588	2	2	1
<i>Entoloma conferendum</i>	Agaricales (B)	AF538624	99.2–99.3	648–649	1	2	3
<i>Powellomyces</i> sp.	Spizellomycetales (C)	AY349104	74.9–75.5	431–433	3	3	
<i>Tremella</i> sp.	Tremellales (B)	AY188379	95.5–95.7	450–451	1	1	5
<i>Vermispora fusarina</i>	Orbiliiales (A)	AY776169	77.7–77.9	661			3

Note: The accession numbers of the most similar GenBank sequences, their similarity and overlap (in number of base pairs) to the OTUs are also shown. In the last three columns, the number of occurrences of the various OTUs distributed on the three bryophytes is shown (Hylo, *Hylocomium splendens*; Pleu, *Pleurozium schreberi*; Poly, *Polytrichum commune*).

Fig. 2. Taxonomic distribution of fungi associated with *Hylocomium splendens*, *Pleurozium schreberi*, and *Polytrichum commune*, based on the similarity of rRNA ITS sequences to GenBank–EMBL accessions.

roizium schreberi, and *Polytrichum commune*. Out of the 158 OTUs, 77.2% were detected only once, and a very low proportion was shared between the bryophyte species. This suggests that an immense fungal diversity is associated with these ubiquitous mosses, given that just 27 shoots originating from a single forest landscape were analyzed in the present study. Our results, suggesting a truly great fungal diversity in bryophytes, are corroborated in the recent review by Davey and Currah (2006), who summarized that a taxonomically diverse suite of fungi interacts with bryophytes. It

is, however, difficult to determine whether some of the OTUs detected in our study represent well-known bryophyte-inhabiting fungi because their previous identification has been based mainly on classical approaches (e.g., microscopy and staining) and these fungi are hitherto sparsely accessioned in GenBank.

The detected OTUs obviously have various life strategies and host dependences. Although the surface of the analyzed bryophytes were rinsed thoroughly, we realize that some of the detected fungi may not have intimate associations with the bryophytes, but might have been detected just because spores have landed and (or) germinated more or less accidentally on the bryophytes, appearing as “casual phylloplane fungi.” Moreover, morphological traits of the studied bryophytes may add to the appearance of casual phylloplane fungi, i.e., plicate leaves and paraphyllia in *Hylocomium splendens*; distinct lamellae on the leaf nerve in *Polytrichum commune*; and leaf incurve in *Pleurozium schreberi*, the latter which is known to harbor N-fixing epiphytic *Nostoc* (DeLuca et al. 2002). Mycorrhizal fungi have not been observed to form functional, nutrient-exchanging mycorrhizal interfaces with bryophytes, rather, it is believed that they function as saprobes on moribund and senescent gametophytes (Davey and Currah 2006). Hence, the detected OTUs with high sequence similarity to the ectomycorrhiza forming basidiomycetes *Cortinarius armillatus* (Fr.) Fr., *Cortinarius caperatus* (Pers: Fries) Fries, *Lactarius kauffmannii*, *Piloderma byssinum* (P.Karst.) Jül., and *Suillus variegatus* (Swartz: Fr.) O. Kunze (which were detected only once), could represent casual phylloplane fungi. It has also been hypothesized that alternate periods of drying and wetting may result in the release of nutrient-rich aqueous leachates from moss gametophytes, which can be used by mycorrhizal fungi (Carleton and Read 1991). Noteworthy, Carleton and Read (1991) used radioactive tracing experiments to demonstrate transfer of phosphate and carbon from *Pleurozium schreberi* to *Pinus contorta* via the ectomycorrhizal *Suillus bovinus*. However, the ecological relevance of

Table 2. Best matches in GenBank of the OTUs having 98% or higher sequence similarity to GenBank accessions, and their taxonomic affiliation (A, Ascomycota; B, Basidiomycota; Z, Zygomycota).

Best match in GenBank	Order	Acc. No.	Similarity	Overlap	Hylo	Pleu	Poly
Ascomycete sp. olrim457	Ascomycota (A)	AY805600	99.8	475		1	
Basidiomycete sp. 05–525	Aphyllorphales (B)	AM084802	99.5	558	1		
<i>Cladosporium magnusianum</i>	Mycosphaerell. (A)	AF393712	99.8–100.0	577	1		2
<i>Cladosporium tenuissimum</i>	Mycosphaerell. (A)	AJ300331	99.7	589	1		
<i>Cortinarius armillatus</i>	Agaricales (B)	DQ114744	99.7	607			1
<i>Cortinarius caperatus</i>	Agaricales (B)	DQ367911	98.6	730		1	
<i>Cryptococcus victoriae</i>	Tremellales (B)	AM160647	99.8	521			1
<i>Epicoecum</i> sp.	Pleosporales (A)	AJ279463	99.7	582	1		
<i>Exobasidium vaccinii</i>	Exobasidiales (B)	AB180362	100.0	552	1		
Foliar endophyte of <i>Picea</i>	Pezizales (A)	AY566887	98.2	625		1	
Fungal sp. TRN520	Pezizomycotina (A)	AY843191	99.4	513		1	
<i>Fusarium oxysporum</i>	Hypocreales (A)	AF322074	99.3	581	1		
<i>Galerina luteolosperra</i>	Agaricales (B)	AJ585453	99.8	612		1	
<i>Guehomyces pullulans</i>	Cystofilobasidiales	AF444417	99.7	620	1		
<i>Gyoeffia rotula</i>	mitosporic Asco. (A)	AY729937	98.5	519–520	1	1	1
Helotiales sp. sd2aN4b	Helotiales (A)	AY465452	98.5	470			1
<i>Hypocrea</i> sp.	Hypocreales (A)	AY241587	99.8	625	1		
<i>Lactarius kauffmanii</i>	Agaricales (B)	DQ097875	98.4	756	1		
<i>Lophodermium piceae</i>	Rhytismatales (A)	DQ068342	98.9–100.0	480	2		2
<i>Marasmius androsaceus</i>	Agaricales (B)	AF519893	100.0	610	1		
<i>Microdochium</i>	Xylariales (A)	AJ279481	100.0	586	1		1
<i>Entoloma conferendum</i>	Agaricales (B)	AF538624	99.2–99.4	648–649	1	2	3
<i>Phialophora</i> sp.	Helotiales (A)	AY805587	98.5	391		2	
<i>Piloderma byssinum</i>	Stereales (B)	AY010279	98.9	580		1	
<i>Rhizosphaera</i>	mitosporic Asco. (A)	AY183366	99.7	625	1		
<i>Sclerotinia sclerotiorum</i>	Helotiales (A)	AF455523	99.8	557			1
<i>Suillus variegatus</i>	Boletales (B)	AJ272421	99.6	688			1
<i>Zalerion arboricola</i>	mitosporic Asco. (A)	AF169308	98.2	498			1
Zygomycete sp.	Mortierellales (Z)	AY805544	99.6	543	1		

Note: The accession numbers of the most similar GenBank sequences, their similarity and overlap (in number of base pairs) to the OTUs are also shown. In the last three columns, the number of occurrences of the various OTUs, distributed on the three bryophytes is shown (Hylo, *Hylocomium splendens*; Pleu, *Pleurozium schreberi*; Poly, *Polytrichum commune*).

moss – mycorrhizal fungi associations, beyond saprobic interactions, seem little understood.

Some OTUs had high sequence similarity to known plant parasitic fungi and could have detrimental effects on the bryophytes. For example, three OTUs with affinity to the plant pathogenic *Exobasidium* spp. were detected, including one OTU with 100% match to an accession of *Exobasidium vaccinii* (AB180362). The small agarics of *Galerina* are often associated with mosses and one species, *Galerina paludosa* is known to parasitize *Sphagnum* (Redhead 1981). Two OTUs detected in this study had affinity to *Galerina* spp., one of them with high sequence similarity (99.8%) to an accession of *Galerina luteolosperra* A.H. Sm. & Singer (AJ585453).

One may argue that OTUs detected more frequently and from the three studied bryophytes potentially have a more intimate association with its host. One such example involves an OTU with high sequence similarity (99.2%) to the agaric *Entoloma conferendum*. This morphotaxon produces small basidiocarps, normally in mats of bryophytes, and we hypothesize that this fungus has obligate biotrophic interactions with various bryophytes. Other examples of fre-

quently detected OTUs were the three with high sequence similarity to GenBank accessions of *Lophodermium piceae*, which is a widespread needle endophyte of various conifers, believed to have little detrimental effect on the host plant (Lehtijärvi et al. 2001; Müller et al. 2001). Our analyses (Fig. 1c) indicated, rather surprisingly, that *L. piceae* is associated with bryophytes, as well as conifer trees. The possibility that *L. piceae* colonizes mosses from fallen needles, interspersed among the moss shoots, should be taken into account. Furthermore, *L. piceae* sporulates on the ground and the amount of spores on mosses is therefore likely to be high. Hence, we cannot exclude the possibility that the taxon is being detected simply because it is present in the conifers dominating the overstory.

Several OTUs had affinity to accessions of the Helotian species *Hyphodiscus hymeniophilus*. *Hyphodiscus hymeniophilus* often produces minute ascomata on dead wood, but both this and the other members of the genus are typically associated with fungi on this substrate. This species may be a hyperparasite of lignicolous fungi (see Hosoya 2002; Untereiner et al. 2006). Three of the OTUs with affinity to *H. hymeniophilus* were detected more than three times

(Fig. 1a), indicating putatively closely evolved associations with the bryophytes. The phylogenetic tree also indicated that the OTUs with taxonomic affinity to *H. hymeniophilus* have adapted to various bryophyte species since two of the common OTUs were restricted to only one bryophyte species. Several OTUs had affinity to various accessioned sequences of Tremellales, including one that was detected in all three bryophyte species, being most similar to a sequence in an unnamed species (AY188379) of the order Tremellales. This OTU, as well as the other OTUs with affinity to Tremellales, probably represent yeast forming species adapted to the humid environment that bryophytes represent.

A further step to demonstrate the mentioned OTUs putatively widespread occurrence and intimate association with bryophytes would be to develop taxon specific primers and screen a broader sample of bryophytes for their presence.

It has been emphasized that the generation of artificial chimeric sequences during PCR can be a huge problem in environmental sampling studies (e.g., Jumpponen 2007), where target sequences are derived from a mixture of various DNA templates. In our study we identified only a few potential chimeras (2.9%). This could be due to our usage of a proof-reading polymerase enzyme, as well as extended elongation period during PCR, which minimizes the number of unfinished fragments after each PCR cycle. Furthermore, the risk of generating chimeric sequences might be higher when the target sequence is more conserved compared to the ITS region and, hence, there is a higher similarity between the different DNA templates.

Bryophytes and fungi have been reported to engage in a number of commensal interactions (e.g., Hahn and Bopp 1972; During and Van Tooren 1990). One might speculate that some of the frequently occurring OTUs have a mutualistic relationship with bryophytes, with the latter benefiting through enhanced resistance to herbivores and (or) plant pathogens. Noteworthy, bryophytes are in general highly resistant to herbivores (Prins 1982) and this could, hypothetically, partly be due to the fungal symbionts. Such mutualistic relationships have been observed in other plant–fungus associations (e.g., Arnold et al. 2003). Endophyte effects on herbivorous insects range from negative to null to positive in grasses (Saikkonen et al. 2006), trees (Faeth and Hammon 1997; Wilson and Faeth 2001) and herbaceous plants (Gange et al. 2007). We believe that bryophytes will be characterized by corresponding species-specific effects and that there is a need for experimental studies to investigate the causal role of interactions between bryophytes and fungi in functional ecology.

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References

Arnold, A.E. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol. Rev.* **21**: 51–66. doi:10.1016/j.fbr.2007.05.003.

Arnold, A.E., Mejia, L.C., Kylo, D., Rojas, E.I., Maynard, Z.,

Robbins, N., and Herre, E.A. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 15649–15654. doi:10.1073/pnas.2533483100. PMID:14671327.

Benscoter, B.W., and Vitt, D.H. 2007. Evaluating feathermoss growth: a challenge to traditional methods and implications for the boreal carbon budget. *J. Ecol.* **95**: 151–158. doi:10.1111/j.1365-2745.2006.01180.x.

Bonan, G.H., and Shugart, H.H. 1989. Environmental factors and ecological processes in boreal forests. *Annu. Rev. Ecol. Syst.* **20**: 1–28. doi:10.1146/annurev.es.20.110189.000245.

Bond-Lamberty, B., and Gower, S.T. 2007. Estimation of stand-level leaf area for boreal bryophytes. *Oecologia (Berl.)*, **151**: 584–592. doi:10.1007/s00442-006-0619-5.

Carleton, T.J., and Read, D.J. 1991. Ectomycorrhizas and nutrient transfer in conifer – feather moss ecosystems. *Can. J. Bot.* **69**: 778–785. doi:10.1139/b91-101.

Davey, M.L., and Currah, R.S. 2006. Interactions between mosses (Bryophyta) and fungi. *Can. J. Bot.* **84**: 1509–1519. doi:10.1139/B06-120.

DeLuca, T.H., Zackrisson, O., Nilsson, M.-C., and Sellstedt, A. 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature (London)*, **419**: 917–920. doi:10.1038/nature01051. PMID:12410308.

During, H.J., and Van Tooren, B.F. 1990. Bryophyte interactions with other plants. *Bot. J. Linn. Soc.* **104**: 79–98. doi:10.1111/j.1095-8339.1990.tb02212.x.

Faeth, S.H. 2002. Are endophytic fungi defensive plant mutualists? *Oikos*, **98**: 25–36. doi:10.1034/j.1600-0706.2002.980103.x.

Faeth, S.H., and Hammon, K.E. 1997. Fungal endophytes in oak trees. I. Long-term patterns of abundance and associations with leafminers. *Ecology*, **78**: 810–819.

Gange, A.C., Dey, S., Currie, A.F., and Sutton, B.C. 2007. Site- and species-specific differences in endophyte occurrence in two herbaceous plants. *J. Ecol.* **95**: 614–622. doi:10.1111/j.1365-2745.2007.01245.x.

Gardes, M., and Bruns, T.D. 1993. TS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **2**: 113–118. doi:10.1111/j.1365-294X.1993.tb00005.x. PMID:8180733.

Genney, D.R., Anderson, I.C., and Alexander, I.J. 2006. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytol.* **170**: 381–390. doi:10.1111/j.1469-8137.2006.01669.x. PMID:16608462.

Hahn, H., and Bopp, M. 1972. Stimulation of protonema growth and bud formation in the moss *Funaria hygrometrica* by several fungi. *Z. Pflanzenphysiol.* **68**: 19–29.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**: 95–98.

Higgins, K.L., Arnold, A.E., Miadlikowska, J., Sarvate, S.D., and Lutzoni, F. 2007. Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. *Mol. Phylogenet. Evol.* **42**: 543–555. doi:10.1016/j.ympev.2006.07.012. PMID:17005421.

Hosoya, T. 2002. Hyaloscyphaceae in Japan 6: the genus *Hyphodiscus* in Japan and its anamorph *Catenulifera* gen. nov. *Mycoscience*, **43**: 47–57. doi:10.1007/s102670200008.

Jumpponen, A. 2007. Soil fungal communities underneath willow canopies on a primary successional glacier forefront: rDNA sequence results can be affected by primer selection and chimeric data. *Microb. Ecol.* **53**: 233–246. doi:10.1007/s00248-004-0006-x. PMID:17106807.

Lehtijärvi, A., Swertz, C., and Barklund, P. 2001. Co-occurrence of

- the ascomycete *Lophodermium piceae* and the rust fungus *Chrysomyxa abietis* in Norway spruce needles. *For. Pathol.* **31**: 25–31.
- Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högborg, P., Stenlid, J., and Finlay, R.D. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* **173**: 611–620. doi:10.1111/j.1469-8137.2006.01936.x. PMID:17244056.
- Müller, M.M., Valjakka, R., Suokko, A., and Hantula, J. 2001. Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. *Mol. Ecol.* **10**: 1801–1810. doi:10.1046/j.1365-294X.2001.01304.x. PMID:11472547.
- Murray, M.G., and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**: 4321–4325. doi:10.1093/nar/8.19.4321. PMID:7433111.
- Nielsen, A., Totland, Ø., and Ohlson, M. 2007. The effect of forest management operations on population performance of *Vaccinium myrtillus* on a landscape-scale. *Basic Appl. Ecol.* **8**: 231–241. doi:10.1016/j.baae.2006.05.009.
- Pearson, W.R., and Lipman, D.J. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. U.S.A.* **85**: 2444–2448. doi:10.1073/pnas.85.8.2444. PMID:3162770.
- Pharo, E.J., and Vitt, D.H. 2000. Local variation in bryophyte and macro-lichen cover and diversity in montane forests of western Canada. *Bryologist*, **103**: 455–466. doi:10.1639/0007-2745(2000)103[0455:LVIBAM]2.0.CO;2.
- Prins, H.H.T. 1982. Why are mosses eaten in cold environments only? *Oikos*, **38**: 374–380. doi:10.2307/3544680.
- Rayner, A.D.M., and Boddy, L. 1986. Fungal decomposition of wood, its biology and ecology. John Wiley and Sons, London, UK.
- Read, D.J., Duckett, J.G., Francis, R., Ligrone, R., and Russell, A. 2000. Symbiotic fungal associations in 'lower' land plants. *Philos. Trans. R. Soc. Lond., B*, **355**: 815–831. doi:10.1098/rstb.2000.0617.
- Redhead, S.A. 1981. Parasitism of bryophytes by agarics. *Can. J. Bot.* **59**: 63–67. doi:10.1139/b81-011.
- Saikkonen, K., Lehtonen, P., Helander, M., Koricheva, J., and Faeth, S.H. 2006. Model systems in ecology: dissecting the endophyte-grass literature. *Trends Plant Sci.* **11**: 428–433. doi:10.1016/j.tplants.2006.07.001. PMID:16890473.
- Smith, S.E., and Read, D.J. 1997. Mycorrhizal symbiosis. Academic Press, London, UK.
- Swofford, D.L. 2003. PAUP*. Phylogenetic analysis using parsimony *and other methods. Version 4. Sinauer, Sunderland, Mass.
- Untereiner, W.A., Naveau, F.A., Bachewich, J., and Angus, A. 2006. Evolutionary relationships of *Hyphodiscus hymeniophilus* (anamorph *Catenulifera rhodogena*) inferred from β -tubulin and nuclear ribosomal DNA sequences. *Can. J. Bot.* **84**: 243–253. doi:10.1139/B05-165.
- Van Cleve, K., Chapin, F.S., Flanagan, P.W., Vierek, L.A., and Dyrnes, C.T. 1986. Forest ecosystems in the Alaskan Taiga: A synthesis of structure and function. Springer, New York, N.Y.
- White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications* Edited by M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White. Academic Press, San Diego, Calif. pp. 315–321.
- Wilson, D., and Faeth, S.H. 2001. Do fungal endophytes result in selection for leafminer ovipositional preference? *Ecology*, **82**: 1097–1111.