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Ectomycorrhizal Diversity on *Dryas octopetala* and *Salix reticulata* in an Alpine Cliff Ecosystem

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Abstract

The ectomycorrhizal communities in alpine habitats have been relatively little studied. As global change is predicted to have a large impact in Arctic and alpine environments, it is important to document the fungi of these climatic regions to monitor changes and to understand upcoming successions. This study investigates the ectomycorrhizal community of *Dryas octopetala* and *Salix reticulata* on cliff ledges in a mid-alpine setting using the internal transcribed spacer region of nuclear ribosomal DNA for the identification of the fungal component of ectomycorrhizal root tips. It is shown that the community is relatively species rich, with 74 molecular operational taxonomic units (MOTUs)/species, and that it is dominated by *Cenococcum geophilum*, Thelephoraceae spp., *Cortinarius* spp., and Sebaciniales spp. Furthermore, the dominating species have low specificity regarding the tested hosts and seem likely to be able to facilitate the succession of the alpine tundra to subalpine forest by serving as mycorrhizal partners for establishing pioneer trees.

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Introduction

Alpine ecosystems are predicted to be seriously affected by global warming (ACIA, 2005). One predicted, and already observed, change is that the tree line will advance above the present altitude (Kupfer and Cairns, 1996; Rochefort and Peterson, 1996). Most of the tree species forming the tree line are dependent on ectomycorrhiza (Kernaghan and Harper, 2001), and the ectomycorrhizal community is likely to play an essential role in the establishment of trees above the present tree line (cf. Nara et al., 2003; Nara, 2006). Despite their importance, the ectomycorrhizal communities in alpine habitats remain sparsely investigated.

Cliff ledges constitute key elements in alpine environments as they differ from the surrounding landscape in their microclimate. Due to lower albedo and higher inclination to the sun, many south-facing cliffs have higher temperatures than the surrounding landscape. To a varying degree they are also protected against grazing by mammals. These qualities make them prime sites for early establishment of trees above the present tree line. Such pioneer trees may serve as seed sources to the surrounding area and thereby accelerate the advancement of the tree line.

Several ectomycorrhizal shrubs and herbs are common in alpine and Arctic plant communities (Väre et al., 1992; Cripps and Eddington, 2005) and are potential sources of ectomycorrhizal fungal inoculum for trees. *Dryas octopetala* and *Salix reticulata* are two prominent members of the plant community on calcareous cliff ledges in alpine environments of northern Europe. Both species are well documented as ectomycorrhizal, but whereas *S. reticulata* (Salicaceae) belongs to a family where the majority of the species can form ectomycorrhiza, *D. octopetala* (Rosaceae) belongs to a family where most species do not (Wang and Qiu, 2006). Both *Dryas* and *Salix* have been found to have fruiting bodies of many different ectomycorrhizal fungi associated with them. Important genera include *Cenococcum*, *Cortinarius*, *Russula*, *Inocybe*, and *Hebeloma*, but also genera such as *Laccaria* and

Lactarius (Gulden et al., 1985; Gulden and Jenssen, 1988; Senn-Irlet et al., 1990; Gardes and Dahlberg, 1996). It has also been shown that Arctic and alpine ectomycorrhizal communities can be rather species rich with upwards of 60 fungal species (Gardes and Dahlberg, 1996). Fruiting-body formation does, however, often correspond poorly both to the composition of the below-ground community and to the abundance of the respective constituent species (Horton and Bruns, 2001). This study uses root-tip sampling to explore the ectomycorrhizal community of *D. octopetala* and *S. reticulata* occurring on cliff ledges in the mid-alpine zone in northern Sweden, and contrasts the communities of both species against each other to investigate patterns of species specificity. In addition, the importance of seasonal variation and cliff ledge size for the composition of the fungal communities is investigated.

Materials and Methods

STUDY SITE

This study is part of a long-term project on alpine cliff ecology based at the Abisko Scientific Research Station (The Royal Swedish Academy of Sciences) in northern Sweden. The field site is located near Lake Latnjajaure (68°21'N, 18°30'E; Fig. 1) and is situated in a U-shaped glacial valley in the mid-alpine region. The mean annual temperature is -2°C (1993–2005). The warmest month (July) has a mean temperature of 8.6°C and the coldest (February) has a mean temperature of -9.4°C . The mean annual precipitation is 850 mm (1990–2005) of which 206 mm falls during the growing season (approximately June–August). The sampled cliff ledges are located in a west-facing slope at an elevation of 1010–1040 m above sea level. The dominating bedrock at the site is garnet mica schist but there are also inclusions of marble and dolomite. For further description of the vegetation of the Latnjajaure catchment area, see Lindblad et al. (2006).

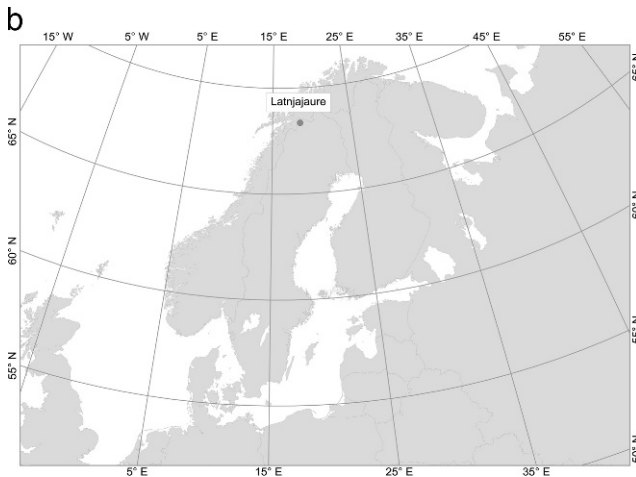


FIGURE 1. (a) The cliffs as seen from the west. (b) The study location plotted on a map depicting the northern part of Europe.

COLLECTION OF ROOT TIPS

The field work was conducted between 27 June and 5 July 2006, 26 June and 5 July 2007, and 16 and 27 August 2007. Five cliff ledges were sampled in 2006 and four in 2007 (Table 1). In 2006, the five cliff ledges comprised three different width categories (thin, approximately 0.5–1 m wide; medium, approximately 1.5 m; and wide, approximately 8 m). In 2007 the sample regime was altered to include four cliff ledges (three from the previous year) and one reference plot situated below the cliff ledges. If not otherwise stated, cliff ledges (including the reference plot) were used as sample units. Plants of *S. reticulata* and *D. octopetala* were collected following a transect parallel to the edge of the cliff (for reference, in the general direction of the cliff ledges). In the first year only *S. reticulata* were sampled while both species were sampled the second year. In 2006, 20 plants of *S. reticulata* were sampled from each width category, while during both field periods in 2007, six plants of each species were collected from each cliff ledge. The plants were collected at least 20 cm apart. *Dryas octopetala* and *S. reticulata* have a creeping habit often with subterranean stems. The stems were excavated by hand for up to 15 cm and great care was taken to excavate adventitious roots up to 15 cm of length. In the lab, the roots were removed from each plant separately and examined for ectomycorrhizae. Living ectomycorrhizal root tips longer than 1 mm were counted and four tips per plant were randomly selected for DNA extraction. Plants with fewer than four root tips were discarded and replaced by additional sampling. The root-tip samples were stored in lysis buffer until DNA extraction.

TABLE 1

The sampled cliff ledges and their approximate width and length.

Cliff ledge	Width (m)	Length (m)	Sampled year
A	0.8–1.4	3.2	2006
B	0.4–0.8	3.9	2006, 2007
C	1.4–1.7	5.1	2006
D	1.1–2.4	5.3	2006, 2007
E	1.3–1.5	7.5	2007
F	7.9–8.3	32.8	2006, 2007
Reference (R)			2007

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

The DNA extractions for the first year were performed using a CTAB-based protocol (Larsson and Jacobsson, 2004). For the second year, the E-Z 96 Plant DNA extraction kit was used following the manufacturers instructions (Omega Bio-Tek). PCR reactions were carried out using Illustra™ PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences AB). The primers ITS1F (Gardes and Bruns, 1993) and LR21 (Hopple and Vilgalys, 1999) were used to amplify the complete ITS region and about 375 bp of the 5' end of the nuclear large subunit (LSU).

The amplified products were purified using Qiaquick spin columns (Qiagen) with 35 µL elution buffer instead of 100 µL to increase the final DNA concentration. PCR products with a concentration of less than 12 µg DNA mL⁻¹ were re-amplified using internal primers ITS1 and ITS4 (White et al., 1990). Only PCR products with a concentration above 12 µg DNA mL⁻¹ were used for sequencing.

The sequences from the first year (2006) were obtained using the CEQ 8000 DNA analysis system and the DTCS Quick Start Kit (both Beckman Coulter). The second year of sequencing was conducted by Macrogen Inc. (Seoul, South Korea). The ITS3 primer (White et al., 1990) was used for all sequencing to obtain sequences of the ITS2 region and the 5' part of the LSU region.

SEQUENCE GROUPING AND THE ASSIGNMENT OF TAXONOMIC AFFILIATION FOR THE SEQUENCES

The root-tip sequences were queried for similar sequences in INSD (Benson et al., 2008) and UNITE (Kõljalg et al., 2005) using BLAST 2.2.18 (Altschul et al., 1997). Sequences best matched by ectomycorrhizal species were divided into taxonomic groups based on the annotation of the sequences in the BLAST output. Sequences best matched by species of other nutritional modes or that had dubious taxonomic affiliations were excluded from the rest of the analyses. Within the ectomycorrhizal taxonomic groups, the sequences were compared for similarity in the ITS2 region and clustered using a 3% cutoff value of sequence divergence (Hamming distances; Swofford et al., 1996). In addition, each taxonomic group (except for sequences clustering with *Cenococcum geophilum*) was aligned together with a selection of highly similar sequences from the BLAST outputs as well as sequences selected based on recent phylogenetic studies: Clavuliniaceae—Nilsson et al. (2006); *Cortinarius*—Garnica et al. (2005); *Hebeloma*—Yang et al. (2005) and Boyle et al. (2006); *Inocybe*—Matheny (2005) and Ryberg et al. (2008); Russulaceae—Shimono et al. (2004); and Sebaciales—Selosse et al. (2007). The alignments were subjected to estimation of maximum likelihood-based phylogenetic inference in RAXML 7.0.4 (Stamatakis, 2006). For each alignment, a search for the best scoring maximum likelihood tree was performed in combination with 100 bootstrap replicates. The similarity analysis was used in combination with

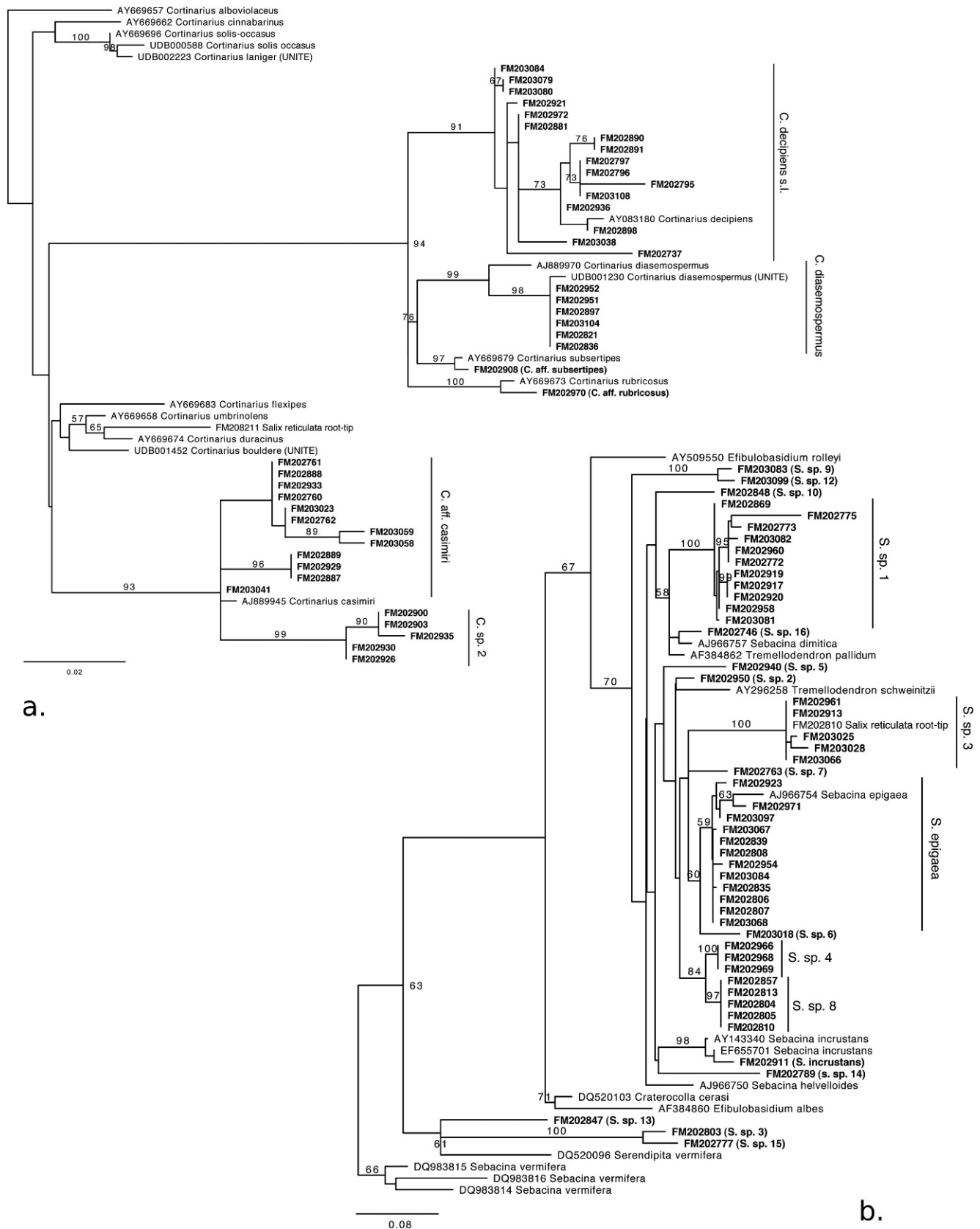


FIGURE 2. Maximum likelihood-based phylogenies depicting two particularly difficult groups: (a) *Cortinarius* subgenus *Telamonia*, and (b) Sebacinaceae. Bootstrap values over 50 are given above the branches, but some bootstrap values on very short branches are omitted for the sake of clarity. The outgroup taxa (*C. rubellus* and *Auricularia auricula-judae*, respectively) have been excluded in the interest of a clear presentation of the focal taxa (*Telamonia* and Sebacinaceae, respectively). Terminal taxa labeled with UNITE in parenthesis after the species name originate from the UNITE database (Köljalg et al., 2005). FM202730–FM203118 represent ectomycorrhizal root tips from this study. Sequences representing singletons of a MOTU/species have their species affinity given in parentheses. Lines mark sequences belonging to the same MOTU/species, and the species affinity is marked at the line. The scale bars serve to quantify the length of the branches as measured in expected number of substitutions per base (shown separately for each tree).

the phylogenies to define molecular operational taxonomic units (MOTUs; Floyd et al., 2002), and the taxonomic affinities of the MOTUs were inferred as completely as possible from the phylogenies.

STATISTICAL ANALYSIS

The species richness of the community was investigated using EstimateS 8.0 (Colwell, 2006) to construct mean species accumulation curves and to perform estimations of the real number of MOTUs/species.

To account for differences arising between the samples due to different DNA amplification success, the number of species per sample (cliff ledge) was rarefied to the same number of individuals using the vegan package (Oksanen, 2008) in R (R Development Core Team, 2008) for the comparisons of species richness. An individual was defined as to encompass all root tips of a MOTU/species made from one individual plant collection.

The difference in species richness on *S. reticulata* between the spring and autumn sampling periods of 2007 was compared with the differences between the spring sampling for the separate years using a paired *T*-test. Spearman's rank correlation was used to analyze the dependence of species richness on the cliff ledge size using the samples from 2007. To investigate if there were any differences in host preference for the fungi, Fishers exact test was applied to the samples of 2007 (following Tedersoo et al., 2008) using R. The species composition was also investigated using correspondence analysis (CA; using the vegan package) to explore if any apparent correlation with cliff ledge size could be found. Correspondence analysis was also done using year, season, cliff ledge, and plant species for separation of samples to investigate the influence of plant species and season on the ectomycorrhizal community.

Results

DNA sequences were obtained from 472 of the 720 root tips. Of these, 83 sequences were excluded since they could not be confirmed as belonging to ectomycorrhizal taxa, the majority (62) being associated with the ascomycete genus *Phialemonium*. There were also 11 sequences associated with various anamorphic ascomycete genera, of which one sequence was associated with *Rhizoscyphus ericae* that can form ericoid mycorrhiza and one with *Phialocephala fortinii* that can form pseudomycorrhizae in the form of dark septate hyphae (Smith and Read, 2008), but neither have been shown to be ectomycorrhizal. The basidiomycete sequences not confirmed to be mycorrhizal were found to be associated to groups such as *Cryptococcus*, *Malassezia*, *Polyporales*, *Trechispora*, and the tricholomatoid clade (*sensu* Matheny et al., 2006). The sequence associated with the tricholomatoid clade could not be confirmed as belonging to any of the ectomycorrhizal genera in that group.

To be able to create satisfactory alignments for *Cortinarius*, the sequences belonging to this genus were divided into two matrices: subgenus *Telamonia* and remaining *Cortinarius*. Russulaceae were similarly divided into *Lactarius* and *Russula*, and *Inocybe* were divided into four alignments considering a similar division in Ryberg et al. (2008). The 389 root tips (Appendix 1; available online only at BioOne <<http://www.bioone.org/loi/aare>> or at MetaPress <<http://instaar.metapress.com/content/120707>>) associated with ectomycorrhizal taxa were found to represent 74 MOTUs/species (45 spp. from 2006 and 49 spp. from 2007; Fig. 2; Appendix 2 [available online only at BioOne <<http://www.bioone.org/loi/aare>> or at MetaPress <<http://instaar.metapress.com/content/120707>>]; Table 2). Of the 74 MOTUs/species, 7 (9%) could be identified to species level, while the rest were named as aff. (when neighboring a fully identified species in the phylogenetic analysis) or cf. (when associated with a sequence annotated with a full, but dubious, species name) of a species, or a genus name plus sp. and a number. The community was found to be dominated by *Cenococcum geophilum* (1 MOTU/sp.), Thelephoraceae spp. (25), Sebaciniales spp. (18), and *Cortinarius* spp. (8). There were also MOTUs/species belonging to *Inocybe* (10), *Hebeloma* (4), Clavulinaceae (4), and Russulaceae (4; Fig. 3, Table 2). Only 21 MOTUs/species were found on more than one cliff ledge (Table 2) and 35 on more than one plant (Fig. 3). Of the 35 MOTUs/species found on more than one plant, 13 were found on only one host species but five of these were collected only during 2006 when only one host species was sampled.

The accumulation curve for the 2007 sampling does not level out and this holds true even if the sampling of 2006 is included (Fig. 4). Based on the 2007 samples, the estimated numbers of species ranges from 68 (Chao 1) to 159 (Michaelis Menten). When considering both years, the estimated number ranges from 93 (bootstrap) to 328 (Michaelis Menten; Table 3). The Michaelis Menten estimate calculated in this way is, however, sensitive to uneven sample sizes.

No seasonal difference in species richness was found between spring and autumn ($N = 3$, $P = 0.67$) and there was no large difference in species composition, either (Table 2). Spearman's rank correlation revealed no significant relation between cliff ledge size and species richness ($N = 5$, $P = 0.35$). The correspondence analysis revealed a good spread of the species along the two first axes but neither of them seem to be correlated with the cliff ledge size as cliff ledge F and D form the extreme points on the first axes and E and F, the largest and second largest cliff ledges, form the extreme on the second axes (Fig. 5). The second correspondence analysis, using year, season, cliff ledge, and plant to divide samples, did not reveal any clear clustering other than due to cliff ledge, i.e. spatial autocorrelation (Appendix 3; available online only at BioOne <<http://www.bioone.org/loi/aare>> or at MetaPress <<http://instaar.metapress.com/content/120707>>). When testing for host preference of the fungal species, no significant ($P = 0.63$) difference was found between *D. octopetala* and *S. reticulata*.

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Discussion

The tree line in alpine areas is generally formed by species that are obligatorily ectomycorrhizal. In the Scandes of northern Europe this is usually *Betula pubescens* ssp. *czerepanovii*. It has been observed that *Betula pubescens* establishes more readily on eroded soils when in the vicinity of *Salix* plants (Magnusson and Magnusson, 2001) and that *Salix* can provide ectomycorrhizal partners for establishing *Betula* seedlings (Nara and Hogetsu, 2004). The apparent lack of host preferences of the ectomycorrhizal fungi in this study suggests that there is ample fungal inocula, on these cliff ledges, for the establishment of ectomycorrhiza with pioneer trees. This is in accordance with Kernaghan and Harper (2001), who demonstrated that the ectomycorrhizal community of alpine habitats is less host specific than that of subalpine. Furthermore, a study by Harrington and Mitchell (2002) showed that *D. octopetala*, in a nonalpine habitat, was associated with a wide variety of nonhost-specific fungi that otherwise associate with forest trees. As *D. octopetala* and *S. reticulata* are mainly found on calcareous soils, the species pool available for ectomycorrhizal colonization is limited to species

TABLE 2

The distribution of the molecular operational taxonomic units (MOTUs)/Species between the year, host plant, and the seasons (spring sampling between 26 June and 5 July, autumn between 16 and 27 August). Counted as number of cliff ledges each species occurred on in each category. The numeration (No.) refers to the numbers in Figure 2. For *Hebeloma* the names in parentheses are from Eberhardt and Beker (personal communication).

No.	MOTUs/Species	<i>Salix reticulata</i>			<i>Dryas octopetala</i>		Total
		2006	2007		2007		
		Spring	Spring	Autumn	Spring	Autumn	
1	<i>Cenococcum geophilum</i>	5	5	4	5	1	7
10	<i>Tomentella</i> cf. <i>badia</i>	1	0	2	1	3	5
4	<i>Cortinarius</i> aff. <i>casimiri</i>	2	2	2	1	2	4
5	<i>Cortinarius decipiens</i> s.l.	3	1	2	2	0	4
19	<i>Inocybe</i> aff. <i>rufuloides</i> 1	2	0	0	1	1	3
7	<i>Sebacina epigaea</i>	2	0	2	0	1	3
9	<i>Sebacina</i> sp. 1	3	0	1	1	0	3
3	<i>Tomentella</i> aff. <i>ramossisima</i>	2	2	2	2	0	3
6	<i>Tomentella</i> sp. 1	1	2	1	2	3	3
12	<i>Cortinarius diasemospermus</i>	2	0	1	0	1	2
25	<i>Hebeloma</i> aff. <i>cavipes/vaccinum</i>	1	1	1	0	0	2
11	<i>Inocybe</i> cf. <i>rufuloides</i> 2	0	1	1	1	1	2
24	<i>Inocybe</i> sp. 2	0	0	1	0	2	2
26	<i>Sebacina</i> sp. 8	0	0	0	0	2	2
32	<i>Thelephora</i> sp. 2	1	0	1	0	0	2
18	<i>Tomentella</i> cf. <i>viridula</i>	1	1	0	1	0	2
17	<i>Tomentella</i> sp. 12	0	0	0	1	2	2
2	<i>Tomentella</i> sp. 13	1	2	2	1	1	2
30	<i>Tomentella</i> sp. 5	2	0	0	0	0	2
33	<i>Tomentella</i> sp. 6	0	1	0	1	0	2
27	<i>Tomentella</i> sp. 7	0	1	0	1	0	2
21	<i>Clavulinaceae</i> sp. 1	0	1	0	1	0	1
34	<i>Clavulinaceae</i> sp. 2	1	0	0	0	0	1
71	<i>Clavulinaceae</i> sp. 3	1	0	0	0	0	1
68	<i>Clavulinaceae</i> sp. 4	1	0	0	0	0	1
60	<i>Cortinarius</i> aff. <i>collinitus</i>	1	0	0	0	0	1
73	<i>Cortinarius</i> aff. <i>rubicosus</i>	1	0	0	0	0	1
37	<i>Cortinarius</i> aff. <i>sanguineus</i>	0	0	0	1	0	1
39	<i>Cortinarius</i> aff. <i>subsertipes</i>	1	0	0	0	0	1
31	<i>Cortinarius</i> (subg. <i>Telamonia</i>) sp. 2	1	0	0	0	0	1
62	<i>Hebeloma</i> aff. <i>leucosarx</i> (<i>velutipes</i>)	0	1	0	0	0	1
20	<i>Hebeloma</i> aff. <i>polarelmonticola</i>	1	0	1	0	0	1
14	<i>Hebeloma</i> aff. <i>vinosophyllum</i> (<i>hiemale</i>)	1	1	0	0	1	1
63	<i>Inocybe</i> aff. <i>egenula</i>	0	0	0	0	1	1
58	<i>Inocybe</i> cf. <i>leucoblema</i>	1	0	0	0	0	1
28	<i>Inocybe bulbosissima</i>	1	0	0	0	0	1
54	<i>Inocybe</i> sp. 1	1	0	0	0	0	1
43	<i>Inocybe</i> sp. 3	0	0	0	1	0	1
45	<i>Inocybe</i> sp. 4	1	0	0	0	0	1
55	<i>Inocybe</i> sp. 5	1	0	0	0	0	1
61	<i>Lactarius</i> sp. 1	1	0	0	0	0	1
52	<i>Lactarius</i> sp. 2	1	0	0	0	0	1
70	<i>Lactarius</i> sp. 3	1	0	0	0	0	1
22	<i>Russula</i> sp.	1	1	1	0	0	1
48	<i>Sebacina incrustans</i>	1	0	0	0	0	1
56	<i>Sebacina</i> sp. 10	0	0	0	0	1	1
66	<i>Sebacina</i> sp. 11	0	0	0	0	1	1
69	<i>Sebacina</i> sp. 12	0	0	1	0	0	1
47	<i>Sebacina</i> sp. 13	0	0	0	0	1	1
42	<i>Sebacina</i> sp. 14	0	0	0	1	0	1
38	<i>Sebacina</i> sp. 15	0	0	0	1	0	1
36	<i>Sebacina</i> sp. 16	0	0	0	1	0	1
46	<i>Sebacina</i> sp. 2	1	0	0	0	0	1
15	<i>Sebacina</i> sp. 3	1	1	1	0	0	1
35	<i>Sebacina</i> sp. 4	1	0	0	0	0	1
44	<i>Sebacina</i> sp. 5	1	0	0	0	0	1
40	<i>Sebacina</i> sp. 6	0	1	0	0	0	1
49	<i>Sebacina</i> sp. 7	0	0	0	1	0	1
53	<i>Sebacina</i> sp. 9	0	0	1	0	0	1

TABLE 2
Continued.

No.	MOTUs/Species	<i>Salix reticulata</i>			<i>Dryas octopetala</i>		Total
		2006	2007		2007		
		Spring	Spring	Autumn	Spring	Autumn	
8	<i>Thelephora</i> sp. 1	1	1	1	1	1	1
29	<i>Thelephora</i> sp. 3	0	1	0	1	0	1
13	<i>Tomentella</i> aff. <i>stuposa</i>	0	0	1	1	1	1
41	<i>Tomentella</i> cf. <i>cinerascens</i>	0	1	0	0	0	1
59	<i>Tomentella</i> cf. <i>umbrinospora</i>	1	0	0	0	0	1
50	<i>Tomentella</i> sp. 10	0	0	0	0	1	1
67	<i>Tomentella</i> sp. 11	0	0	0	0	1	1
65	<i>Tomentella</i> sp. 14	0	0	1	0	0	1
23	<i>Tomentella</i> sp. 15	1	1	0	0	1	1
51	<i>Tomentella</i> sp. 2	1	0	0	0	0	1
74	<i>Tomentella</i> sp. 3	1	0	0	0	0	1
64	<i>Tomentella</i> sp. 4	1	0	0	0	0	1
57	<i>Tomentella</i> sp. 8	0	0	0	1	0	1
16	<i>Tomentella</i> sp. 9	0	0	0	1	1	1
72	<i>Tomentella</i> <i>stuposa</i>	1	0	0	0	0	1

tolerant of these conditions. This may have limited the number of host-specific species particularly with respect to fungi restricted to *Salix*. It cannot be ruled out that there are host-specific fungi on the cliff ledges of this study since several species were found on only one host. These were, however, not abundant enough for any conclusion on their preference to be drawn (Table 2).

The well known relationship of increasing species richness with increased area (Arrhenius, 1921; Peay et al., 2007) was not found in this study in that there were no significant relationships between cliff ledge size and number of species. This could be due to lack of power in the statistical analyses but it may also be that the cliff ledges in these ecosystems are well connected by somatic structures transported by soil movement between cliff ledges or by wind- or animal-dispersed spores. This would mean that the individual cliff ledges should not be viewed as separate units but rather as parts of an integrated community. This is supported by the fact that the CA did not show any size-dependent spread of the cliff ledges in any of the two first axes, indicating that the cliff ledge size is not a gradient over which the ectomycorrhizal community change is correlated. The fact that the differences between the seasons were not significantly larger than the differences between the years corresponds well with Mühlmann et al. (2008), who showed there to be little variation between the

seasons in the ectomycorrhizal community of *Polygonum viviparum* on a successional site in an alpine environment.

The use of single cut-off values for species delimitation over a wide taxonomic scope has been put into question (Nilsson et al., 2008), but the joint approach adopted in the present study was devised to ensure that the MOTUs should correspond reasonably well to distinct species. There were, however, some cases where the delimitation of taxonomic units was difficult, and it cannot be ruled out that there were distinct species that were lumped together, especially within the *Cortinarius* subgenus *Telamonia* (e.g. *C. decipiens* s.l.). Within Sebaciniales there seem to be several evolutionary lineages that are not represented as sequences with a full species epithet in GenBank or UNITE. This makes it even more difficult to relate the root-tip samples to species names and to delimit taxa (Nilsson et al., 2009). As a consequence of this incomplete body of reference sequences, some species may have been split into two MOTUs. The extent of these problems should nevertheless be relatively limited (Fig. 2).

Both the accumulation curves and species richness estimators indicate that the communities investigated here hold more than the recovered 74 MOTUs/species. While species estimators are unreliable at low sample intensity (Colwell and Coddington, 1994), and the different estimators applied in this paper show

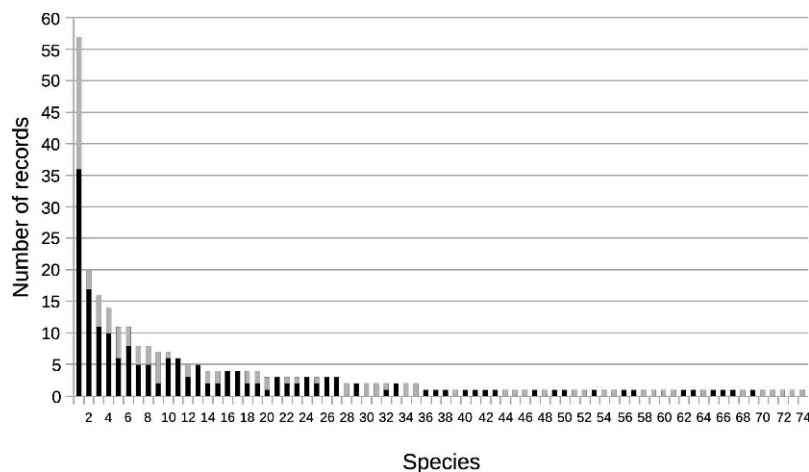


FIGURE 3. Diagram depicting the number of plants from which the ectomycorrhizal fungi MOTUs/species were collected. Black represents the sampling in 2007 while gray represents the sampling in 2006. The species are numbered according to Table 2.

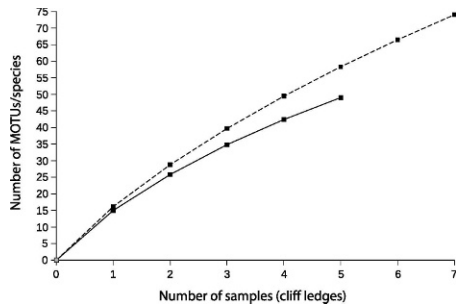


FIGURE 4. Mean species accumulation curves. The black line represents the sampling in 2007, the dashed line represents the sampling in 2006 and 2007 combined.

widely different results, it seems likely that the cliff ledge ecosystem of this study holds at least 100 species. Since it is rarely possible to sample ectomycorrhizal communities exhaustively, it is hard to compare species richness between studies (Taylor, 2002), but the richness found here seems to be similar to that of many temperate forest ecosystems (Rosling et al., 2003; Izzo et al., 2005; Kjølner, 2006). The dominating fungal taxa were *C. geophilum*, Thelephoraceae, *Cortinarius* (mostly from subgenus *Telamonia*), and Sebacinaleae. In addition, *Inocybe* and *Hebeloma* were relatively abundant, and there were also representatives of Clavulinaceae and Russulaceae. This is largely in accordance with

what Kernaghan and Harper (2001) and Mühlmann et al. (2008) found for other alpine ecosystems, using similar methods as this study, but the *Polygonum viviparum* community in the study of Mühlmann et al. (2008) seems to be even more dominated by Sebacinaleae while no such species were reported by Kernaghan and Harper (2001). This study also adds to the observation of Mühlmann and Peintner (2008) that Russulaceae, that is often a dominating component both above and below ground of other ectomycorrhizal communities (Horton and Bruns 2001), is not among the more abundant below ground in an alpine environment.

As in many other studies (e.g. Izzo et al., 2005; Kjølner, 2006; Nara, 2006; Mühlmann et al., 2008), many of the mycorrhizal fungi of the root tips remain unidentified to species level even after DNA sequencing. This may simply be a consequence of the incomplete coverage of fungal species in the international sequence databases; something that is especially true for alpine fungi (Ryberg et al., 2009). It could, however, also be an indication that many ectomycorrhizal species only rarely or perhaps never form fruiting bodies and therefore are undescribed. Together with the fact that many of the fungi found in this study belong to corticoid (forming crust-like fruiting structures) taxa that are often missed in fruiting-body-based surveys, this observation points to the importance of studies based on ectomycorrhizal root tips also in alpine ecosystems. However, for such studies to give a detailed picture of this diversity, more sequences from well identified

TABLE 3

The estimated species richness using different estimators as calculated in EstimateS. For 2007 (5 samples) and 2006–2007 combined (7 samples). Standard deviation given in parentheses when applicable.

Samples	ONS ¹	ACE	ICE	Chao 1	Chao 2	Jack 1	Jack 2	Bootstrap	MM ²	MM ³
2007	49	69.98	124.82	68.09 (11.05)	87.4 (18.56)	75.4 (8.63)	90.7	60.63	158.9	112.21
2006–2007	74	131.32	227.66	156.33 (38.28)	164.86 (36.22)	119.43 (16.5)	150.14	93.26	328.43	182.41

¹ Observed number of species.

² Michaelis Menten richness estimator based on mean number from 1000 different runs.

³ Michaelis Menten richness estimator based on mean species accumulation curve (MauTau).

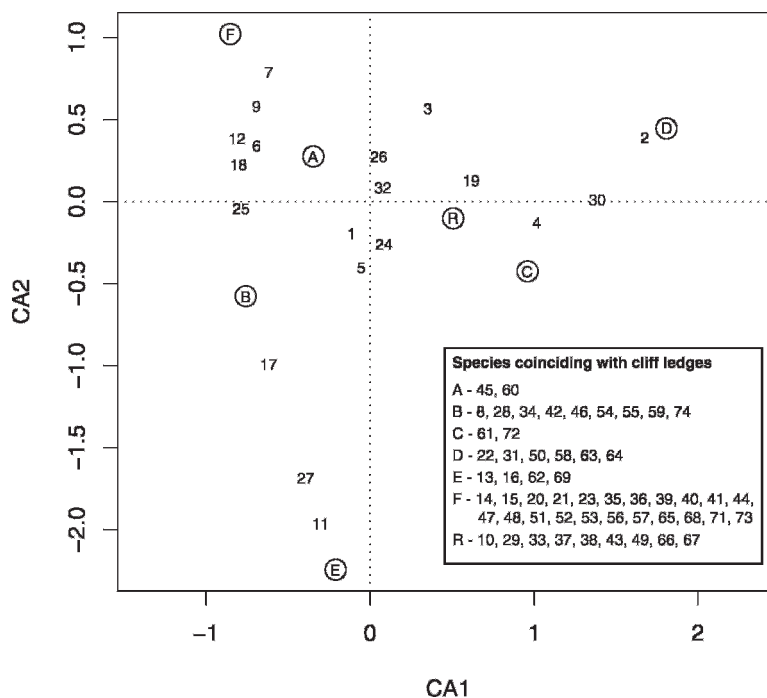


FIGURE 5. Correspondence analysis. The species are numbered in accordance with Table 2. The circles represent the cliff ledges (labeled according to Table 1). Eigenvalues for axes 1–6 are 0.63, 0.57, 0.46, 0.41, 0.31, and 0.27, respectively.

fruiting bodies are needed and further development of the taxonomy in some groups is desirable so that root-tip samples can be placed in an informative phylogenetic and taxonomic framework.

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