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Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi

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Abstract

Through traditional culturing and molecular characterization, we have determined that five putative species and 2 polyphyletic assemblages of fungi produce ericoid mycorrhizae in *Gaultheria shallon*, other Ericaceae and Epacridaceae. Using phylogenetic analysis of ITS2 sequences in GenBank, we have confirmed that most of these fungi occur in North America, Europe, and Australia. The low recovery rate of culturable ericoid mycorrhizal fungi from *Gaultheria shallon* may partly be explained by the fact that most mycorrhizal root segments contain an unculturable basidiomycete, revealed by direct amplification, cloning, and sequencing of LSU fungal DNA from root. Molecular characterization and phylogenetic analysis are powerful tools in revealing the geographic distribution and identity of ericoid mycorrhizal fungi.

Introduction

Almost two decades ago, David Read had proposed that ericoid mycorrhizal fungi allow ericaceous plants to access nutrients locked in the organic matrix that would otherwise be unavailable (Read, 1983). So, when growth check was observed on conifer seedlings growing in dense stands of the Ericaceous shrub, salal (*Gaultheria shallon* Pursh), on northern Vancouver Island, Weetman et al. (1989) suggested that ericoid mycorrhizal fungi might be involved. The Salal Cedar Hemlock Integrated Research Project (SCHIRP) was established over a decade ago to understand and solve this problem (Prescott, 1996; Prescott and Weetman, 1994). A component of SCHIRP is the investigation of the role of ericoid mycorrhizal fungi of salal in conifer growth check.

Using traditional fungal culturing, Xiao and Berch (1996) showed that at least four different fungal groups formed ericoid mycorrhizae at the SCHIRP site at Pt. McNeil, Vancouver Island. Two groups sporulated in culture, *Acremonium strictum* W. Gams and *Oidiodendron maius* Barron, and two non-sporulating groups were described as Unknown 1 (exemplified by UBC S9) and Unknown 2 (exemplified by isolate UBC S246). Xiao (1994) described Unknown 2 as resembling Read's isolates 100 and 101 of *Hymenoscyphus ericae* (Read) Korf & Kernan. *Acremonium strictum*, a fungus of wide distribution, was found to form mycorrhizae by Xiao (1994) but has not been isolated or detected in salal roots by subsequent workers. Its abundance and role in the roots of salal remains to be clarified. For all ericoid mycorrhizal fungi from salal, the percent recovery of mycorrhizal fungi from roots was low, around 3 – 5% per fungal taxon (Table 1).

To identify sterile isolates from the SCHIRP site, Monreal et al. (1999) took advantage of high variability of the internal transcribed spacer regions ITS1 and ITS2 of ribosomal RNA repeats. The ITS regions were analyzed with restriction enzymes that digested the amplified DNA into discrete fragments. The resulting restriction fragment length polymorphisms (RFLPs) were unique banding patterns that allowed for cat-

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Table 1. Frequency of confirmed ericoid mycorrhizal fungi isolated from 1120 fine root segments of salal during first study[a]

Fungal taxon	Representative isolates[b]	Number of isolates	Percentage of root segments producing this fungus
Acremonium strictum	S220	41	4
Oidiodendron maius	S4, S18, S45, S80	59	5
Unknown 1	S9	36	3
Unknown 2	S246	41	4

[a] Xiao (1994).
[b] Deposited at UAMH.

Table 2. Frequency of confirmed ericoid mycorrhizal fungi isolated from 800 fine root segments of salal during this study

Fungal taxon	Representative isolates	Number of isolates	Percentage of root segments producing this fungus
Capronia sp.	UBCtra1046	10	1
Hymenoscyphus ericae[a]	M8, UBCtra 241	80	10
Hymenoscyphus ericae[a]	M5, UBCtra175	9	1
Oidiodendron maius	UBCtra133	49	6
Phialophora finlandia	UBCtra323	27	3
Rhytismatales	UBCtra300, UBCtra1018C	2	<1
Unknown 1	S9, UBCtra1128, UBCtra180	25	3
Unknown 2	S246, UBCtra270	23	3
	UBCtra290	19	2
	UBCtra466	9	1
	UBCtra179	6	1

[a] Taxa differentiated due to differences in RFLP patterns (Monreal et al., 1999).

egorization of sterile isolates into four groups capable of forming ericoid mycorrhizae (*Oidiodendron*, Unknown 2, and two new groups, Unknown 3 and 4). Then, using sequencing of the ITS2 region, Monreal et al. (1999) determined that Unknown 3 (isolate M8) and Unknown 4 (isolate M20) were similar to *Hymenoscyphus ericae*.

We returned to the same SCHIRP site, isolated more fungi from salal roots and confirmed their ability to form ericoid mycorrhizae. We found some of the fungi isolated by Xiao (*Oidiodendron maius*, Unknown 2) and by Monreal (Unknown 3). In addition, we found a number of isolates not identifiable as any of these fungi that were nonetheless able to form mycorrhizae on back-inoculation (Table 2). Again percent recovery of ericoid mycorrhizal fungi from mycorrhizal root segments was low.

Hymenoscyphus ericae has been consistently reported to be the major ericoid mycorrhizal fungus of Ericaceae such as *Calluna* and *Erica* in Europe since 1973 (Pearson and Read, 1973; Read, 1983, 1986; Strandberg and Johansson, 1999). *Scytalidium vaccinii* Dalpé, Sigler & Litten, which was found through molecular characterization to be the anamorph of *H. ericae* (Egger and Sigler, 1993), has also been isolated from the roots of *Vaccinium vitis-idaea* L. in Finland (Currah et al., 1999) and from *Vaccinium angustifolium* in Quebec, Canada (Dalpé, 1989). Fungal isolates from ericoid mycorrhizae, including those of *H. ericae*, are usually sterile in culture, or form arthroconidia as in *Scytalidium vaccinii* that might not be easily recognizable to the non-taxonomist, thus making their identification difficult using classical taxonomic approaches. Although *H. ericae*-like sterile or arthroconidial cultures have been isolated from Ericaceae in North America for awhile, only recently have Hambleton et al. (1999) induced apothecium production in a culture isolated from *Ledum groenlandicum* Oeder in Alberta, thus confirming the occurrence of *H. ericae* in Canada. Given this recalcitrance of *H. ericae* to sporulate in culture (Straker, 1996), molecular characterization is clearly the best way to identify this fungus. Molecular characterization can also be done directly on roots from the field, which allows for efficient and complete surveys of fungal communities.

Around the globe, research groups have begun to reveal a diversity of ericoid mycorrhizal fungi associated with ericaceous and epacridaceous plants (Chambers et al., 1999; Monreal et al., 1999; Perotto et al., 1996; Xiao and Berch, 1996), but the number of taxa of ericoid mycorrhizal fungi is still uncertain (Straker, 1996). The objective of this research was to use molecular characterization to determine the diversity and distribution of ericoid mycorrhizal fungi at two scales: in the fine roots of salal at the SCHIRP site on northern Vancouver Island, and of ericoid mycorrhizal fungi globally.

Methods

Ericoid mycorrhizal fungus isolation

Roots of salal (*G. shallon*) were collected over a number of years (Monreal, 1997; Xiao, 1994; this study)

from the SCHIRP installation on northern Vancouver Island, British Columbia (50° 60′ N, 127° 35′ W). Roots were kept cool, transported to the University of British Columbia, and held at 4 °C until cleaned. Fine roots were then removed from the rhizomes, soaked in cool tap water, washed gently to remove soil and plant debris, surface sterilized in 30% hydrogen peroxide for 30 – 60 s, and rinsed 3 times in sterile distilled water. The fine roots were cut into 0.5 cm segments and plated on potato dextrose agar (PDA).

Mycorrhiza synthesis

Each fungal isolate was tested for mycorrhizal synthesis on *G. shallon* in Petri dish growth chambers as described by Xiao and Berch (1995). Seeds of *G. shallon* were sterilized in 30% hydrogen peroxide for 1 min. and placed on water agar for germination. Seedlings were transplanted to a low nutrient medium, modified Melin-Norkrans agar (MMN), in plastic Petri dishes, and inoculated with a fungal isolate. Mycorrhizal colonization was confirmed with light microscopy. Fungal isolates that formed mycorrhizae were re-tested by culturing the fungus from sterilized roots of colonized plants and inoculating new plants. Isolates that did not immediately form mycorrhizae were maintained on salal in Petri dish growth chambers and checked for 1 year.

Molecular characterization

Genomic DNA from each fungal isolate was extracted from young mycelium (Lee and Taylor, 1990). Following the PCR amplification procedures of Monreal et al. (1999), the ribosomal DNA regions of the ITS1 and ITS2 were amplified with the fungal specific primer combination ITS1-F and ITS4 (White et al., 1990). The genomic DNA of each isolate was characterized by RFLP with three enzymes: *Msp*1, *Rsa*1, and *Cfo*1 following the manufacturer's instructions (New England Biolabs). The isolates were grouped by RFLP banding patterns. The ITS2 regions of selected isolates from each group were sequenced with primer ITS3. To find matching sequences in the GenBank database, ITS2 sequences were subjected to BLAST searches. Matching sequences obtained during the BLAST searches were combined with previously collected sequences of fungal isolates from the same field site to reconstruct phylogenetic relationships.

Fungi detected by direct amplification and cloning of fungal DNA from root

Total genomic DNA was extracted from 1 cm segments of sterilized field collected roots. Genomic DNA from sterile leaves of *G. shallon* was used as negative controls for DNA amplification and cloning. Total fungal DNA was amplified with primers ITS1-F and ITS4, or ITS1-F (Gardes and Bruns, 1993) and TW13 (5′-GGTCCGTGTTTCAAGACG-3′) (Tom White, personal communication), to target a 1200 bp region of the ribosomal RNA repeat including the ITS region and about 500 bp of the LSU. PCR products were cloned using Invitrogen TOPOTMTA Cloning kit following the manufacturers' instructions. Cloned material was characterized with three restriction enzymes *Msp*1, *Cfo*1, and *Rsa*1. For phylogenetic analysis, the ITS2 and 500 bp of the LSU region were sequenced from selected clones for each RFLP group.

Sequence analysis

Sequences from clones and cultures were manually aligned in SeqPup with sequences retrieved from GenBank and analyzed with PAUP 4.0b5. GenBank accession numbers for all isolates used in our analyses are listed in Appendices 1 (ITS2) and 2 (LSU).

Results

Phylogenetic tree of ericoid mycorrhizal fungi of salal

To reconstruct phylogenetic relationships for salal's ericoid mycorrhizal fungi, we combined isolates and clones from all our studies (Monreal, 1997; Xiao, 1994; this study). The highly diverse ITS2 region of the rDNA was used to determine relationships of fungal groups at species level. Our sequences of ericoid mycorrhizal fungi and mycorrhiza-inhabiting clones from salal comprise a number of groups (Figure 1) which are discussed in the following section. All of the groups found in salal roots have now been reported from elsewhere in the world and all groups of ericoid mycorrhizal fungus reported from elsewhere in the world have now also been found in the roots of salal on a single site on northern Vancouver Island (Figure 2).

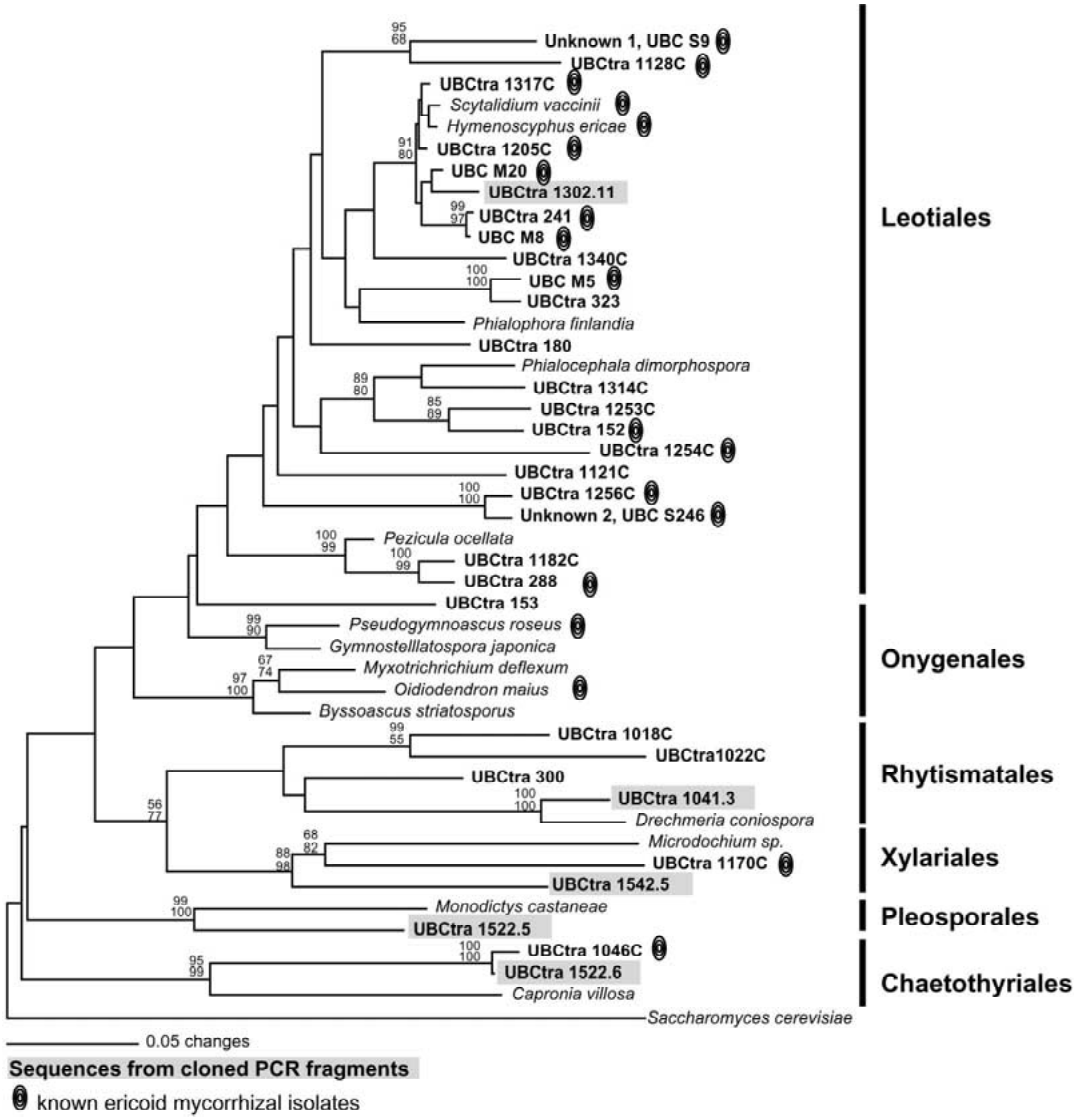

Figure 1. Relationships of cultured fungi and fungi detected by PCR amplification and cloning directly from ericoid mycorrhizal roots of *Gaultheria shallon*. Distances for this neighbour-joining tree were calculated using a Jukes-Cantor correction and were based on an alignment of about 350 nucleotides of the 5.8S and ITS2 rDNA regions. Branch lengths are proportional to base pair differences. Percentages from 500 bootstrap replicates are shown with neighbour-joining on top and fast parsimony without branch swapping on the bottom (values <50% not shown).

Phylogenetic tree of ericoid mycorrhizal fungi available in GenBank

Included in the phylogenetic analysis (Appendix 1) were ITS2 sequences from GenBank from June, 2001, selected to represent the diversity of fungal root endophytes of Ericaceae (Hambleton et al., 1998; Monreal et al., 1999; Sharples et al. 2000; this study) and Epacridaceae (Chambers et al., 2000; McLean et al., 1999). Also included were sequences of fungi from leafy liverworts in the Antarctic (Chambers et al., 1999) and from oak ectomycorrhizae in Italy (Bergero et al., 1999) that clustered with known ericoid mycorrhizal fungi.

The majority of ITS2 sequences sampled from GenBank belong to the Leotiales and many cluster in a species complex around *Hymenoscyphus ericae* (Figure 2). Sequences of this complex originate from Ericaceae in western Canada and eastern USA (Monreal et al., 1999; this study) and Europe (Chambers et al., 2000; Sharples, 2000), from Epacridaceae in Australia (McLean et al., 1999), and from the leafy

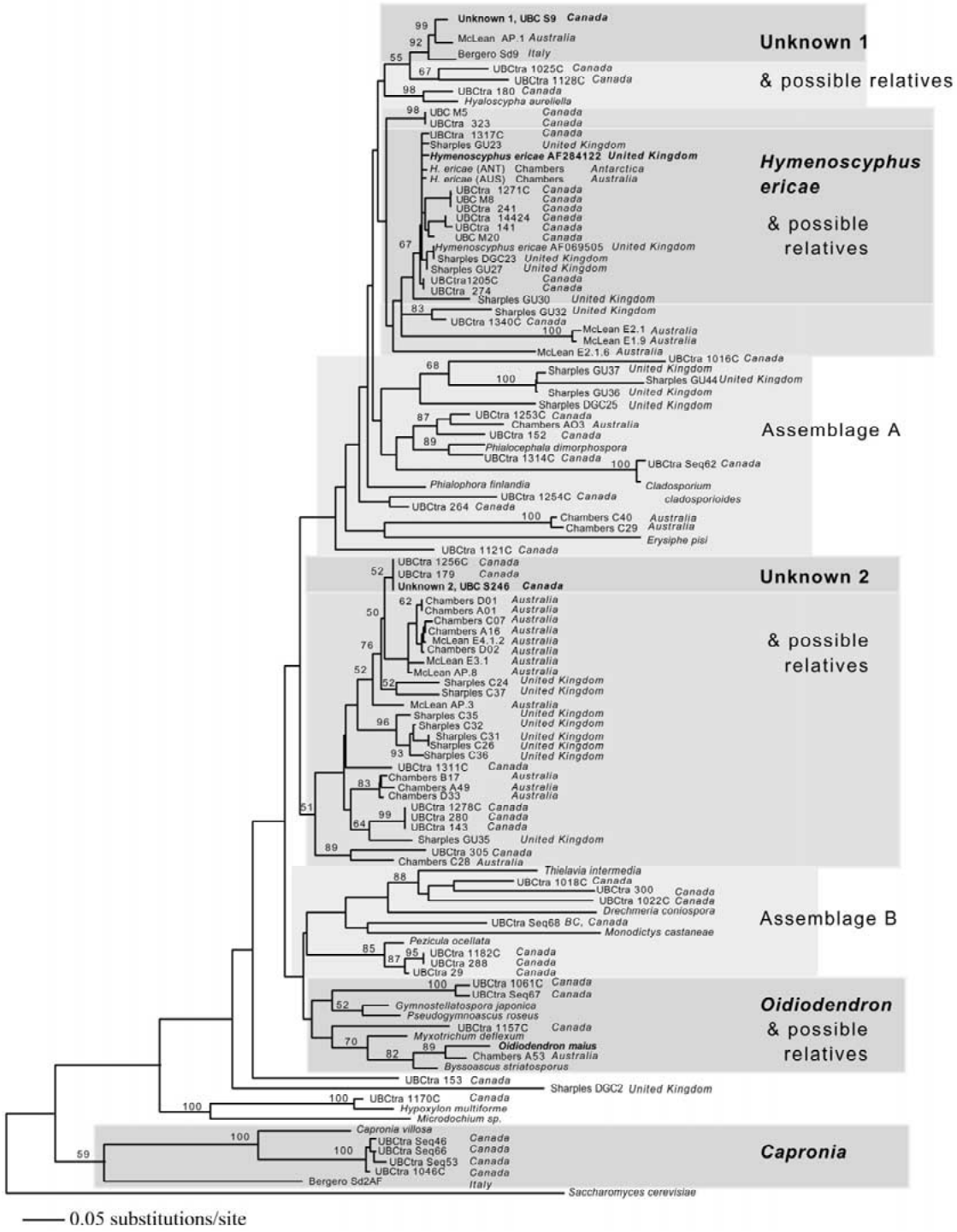

Figure 2. Tree comparing the relationships among ITS2 rDNA sequences of fungi in GenBank either from roots of Ericaceae and Epacridaceae or from other substrates but related to the ericoid mycorrhizal fungi. Tree illustrates main taxa and geographic origin of the sequences analysed. All isolates designated as originating from Canada are from a single research site on Vancouver Island, British Columbia. Distances for this neighbour-joining tree were calculated using a Jukes-Cantor correction and were based on an alignment of about 350 nucleotides of the 5.8S and ITS2 rDNA regions. Branch lengths are proportional to base pair differences. Percentages from 500 bootstrap replicates are reported (values <50% not shown).

liverwort, *Cephaloziella exiliflora,* in Australia and Antarctica (Chambers et al., 2000) (Figure 2).

Close relatives of the taxon originally recognized by Xiao (1994) as Unknown 1 from *Gaultheria shallon* in coastal British Columbia, Canada, have now also been reported from Epacridaceae in Australia (Chambers et al., 2000; McLean et al., 1999) and from ectomycorrhizae of *Quercus ilex* L. in Italy (Bergero et al., 2000) (Figure 2). These fungal isolates cluster in the Leotiales but their identity is still unknown and will remain so until isolates sporulate or more taxa are sequenced. Chambers et al. (2000) recognized that their isolate A03 grouped with *H. ericae* but sequence identity of A03 to *H. ericae* was low (<81%). Our analysis confirms this isolate, A03, clusters in Leotiales (as part of Assemblage A), but is not closely related to *H. ericae* (Figure 2).

Assemblage A consists of isolates known to form ericoid mycorrhizae with salal (e.g. UBCtra152) and sequences of DNA cloned directly from salal roots (e.g. UBCtra Seq62), a sequence from the roots of *Woollsia pungens* (Cav.) F. Muell. in Australia (Chamber et al., 2000), and sequences from *Calluna vulgaris* in Devon, UK (Sharples et al., 2000) (Figure 2). Assemblage A may be a paraphyletic or polyphyletic grouping of fungi, many of them root-inhabiting, that includes *Phialocephala dimorphospora* Kendrick, a potential root pathogen of black spruce (Yamasaki et al., 1998), and *Phialophora finlandia* which forms ectomycorrhizae on *Pinus strobus* L. (Ursic and Peterson, 1997).

Except for Canadian sequences from salal ericoid mycorrhizae, there are no ITS2 sequences identical to Unknown 2 in GenBank. However related fungal isolates have often been found in epacrid roots in Australia (Chambers et al., 2000, McLean et al., 1999), salal roots in BC, and *Calluna vulgaris* in the UK (Sharples et al., 2000) (Figure 2).

Outside of the Leotiales, three main groups have members known to form ericoid mycorrhizae. *Oidiodendron maius* and related taxa have now been reported to form mycorrhizae with *Gaultheria shallon* in British Columbia, Canada (Monreal, 1997; Xiao, 1994; this study), with *Vaccinium corymbosum* in Quebec, Canada (Dalpé, 1989; Hambleton et al., 1998), and with *Calluna vulgaris* in Devon, UK (Sharples et al., 2000). One isolate in the *Oidiodendron* group, A53, from Woollsia *pungens* in Australia did not form mycorrhizae after back inoculation on *Vaccinium macrocarpon* Ait. (Chambers et al., 2000).

A group of fungi related to *Capronia villosa*, Chaetothyriales, is now known to form ericoid mycorrhizae with *Gaultheria shallon* in British Columbia (this study) and with *Erica arborea* L. in Italy (Bergero et al., 2000). Fungi with very similar sequences have also been isolated from ectomycorrhizae of *Quercus ilex* L. (Figure 2) (Bergero et al., 2000).

Finally, Assemblage B may be a chance assemblage of unrelated taxa. It contains sequences from isolates shown to form ericoid mycorrhizae with *Gaultheria shallon* in British Columbia and sequences cloned directly from *G. shallon* roots, some of which may not come from mycorrhizal fungi. GenBank contains no other ericoid mycorrhizal fungus sequences belonging to this cluster of fungi. Another cluster in this assemblage groups with *Thielavia intermedia* Stchigel & Guarro in the Sordariales and *Drechmeria coniospora* (Drechsler) Gams & Jansson in the Hypocreales. A second cluster groups weakly with *Monodictys castaneae* (Wallr.) S. Hughes in the Pleosporales. The third groups with *Pezicula ocellata* (Pers.:Fr.) Seaver in the Leotiales.

Fungi detected by direct amplification and cloning of fungal DNA from root

Comparison of LSU sequences of representatives from each RFLP group found one group of basidiomycetes and several groups of ascomycetes (Figure 3; Appendix 2). In total, of 281 screened clones from 16 root segments, 193 had the DNA insert representing the basidiomycete and 88 had inserts from ascomycetes. The ascomycetes found in direct PCR amplification were also cultured from adjacent root segments, but the basidiomycetes were never obtained in cultures from the roots.

Discussion

Isolation of fungi from mycorrhizae of Ericaceae and Epacridaceae frequently produces sterile cultures that can be identified by ITS2 sequencing to belong to a relatively small number of groups. Of the 5 putative species and 2 assemblages with members forming ericoid mycorrhizae thus far reported in the literature and in GenBank, four are in the Leotiales (*Hymenoscyphus ericae*, Unknown 1, 2, and Assemblage A), one is in the Onygenales (*Oidiodendron*) and one is in the Chaetothyriales (*Capronia*). The seventh (Assemblage B) is polyphyletic with representatives from

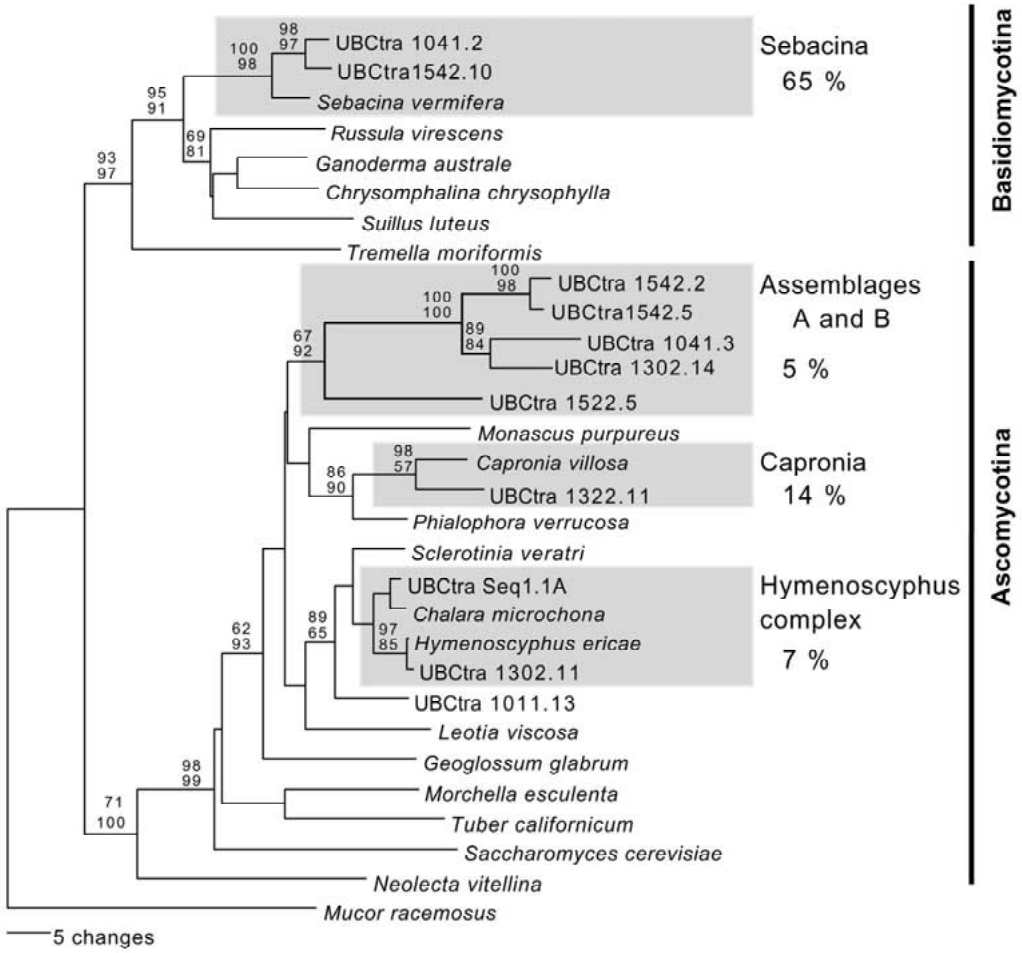

Figure 3. Tree comparing relationships among LSU rDNA sequences of fungi from salal roots at the SCHIRP installation on northern Vancouver Island illustrating the relative proportion of clones from each of the four main fungi detected from among a population of PCR amplified DNA fragments. Distances for this neighbour-joining tree were calculated using a Jukes-Cantor correction and were based on an alignment of LSU rDNA sequences that were about 500 nucleotides in length. Branch lengths are proportional to base pair differences. Percentages from 500 bootstrap replicates are shown with neighbour joining on top and fast parsimony without branch swapping on the bottom (values <50% not shown).

different orders. All but Assemblage B are widespread taxa, found now in three or four continents and forming mycorrhizae with various Ericaceae and Epacridaceae. Some of these fungi have also been reported from atypical habitats such as rhizoids of liverworts and ectomycorrhizae of oak.

However, we have determined that the success rate in isolating ericoid mycorrhizal fungi from *Gaultheria shallon* is quite low, in the range of 1 – 10%. We found this puzzling until we sequenced the LSU region and discovered that 65% of the mycorrhizal root segments of salal that we examined contained a basidiomycete that we could not culture. If this pattern is similar for other ericoid mycorrhizal plants, then it is possible that we are ignoring an important component of these fungus–plant systems when we concentrate on fungi that can be isolated and grown in culture. The strength of direct amplification and cloning of fungal DNA from root is that non-culturable root inhabitants can be detected; the weakness of course is that we cannot currently determine whether these fungi are really mycorrhiza formers.

Using phylogenetic reconstruction, we have determined that the basidiomycete found repeatedly in the fine roots of salal is related to *Sebacina vermifera* (Auriculariales), which has been reported to be an orchid and ectomycorrhizal fungus (Warcup, 1988; Weiss and Oberwinkler, 2001). Jelly fungi were previously detected in ericoid mycorrhizae of *Calluna vulgaris* based on ultrastructure of the hyphal septal

pore (Bonfante-Fasolo, 1980). The exact identity of our fungus and its role in *Gaultheria shallon* roots remain to be determined.

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Appendix 1. GenBank accession numbers for sequences of isolates included in ITS2 analysis

Accession number	Identity	Plant Source	Location	Mycorrhizal status	Source
	Unknown 1 and possible relatives				
AF081442	UBC S9, (Unknown 1)	*Gaultheria shallon* Pursh	Canada	+	GenBank
AF099089	AP-1	*Astroloma pinifolium* (R.Br.) Benth.	Australia	+	GenBank
AF269067	Sd9	*Quercus ilex* L.	Italy	+	GenBank
AF300741	UBCtra 1025C	*Gaultheria shallon*	Canada	+	Culture from root
AF300746	UBCtra 1128C	*Gaultheria shallon*	Canada	+	Culture from root
AF149071	UBCtra 180	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
U57495	*Hyaloscypha aureliella* (Nyl.) Huhtinen			N/A	GenBank
	Hymenoscyphus ericae and possible relatives				
AF081440	UBC M5	*Gaultheria shallon*	Canada	+	GenBank
AF149083	UBCtra 323	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF300748	UBCtra 1317C	*Gaultheria shallon*	Canada	+	Culture from root
AF252834	GU23	*Calluna vulgaris* (L.) Hull	UK	–	GenBank
AF284122	*H. ericae* (Read 100 5183)	*Calluna vulgaris*	UK	+	GenBank
AF069440	*H. ericae* (AUS)	*Cephaloziella exiliflora* (Tayl.) Steph.	Australia	Not tested	GenBank
AF069439	*H. ericae* (ANT)	*Cephaloziella exiliflora*	Antarctica	Not tested	GenBank
AF300750	UBCtra 1271C	*Gaultheria shallon*	Canada	+	Culture from root
AF081435	UBC M8	*Gaultheria shallon*	Canada	+	GenBank
AF149068	UBCtra 241	*Gaultheria shallon*	Canada	+	Culture from root
AF300751	UBCtra 14424	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF149067	UBCtra 141	*Gaultheria shallon*	Canada	+	Culture from root
AF081438	UBC M20	*Gaultheria shallon*	Canada	+	Culture from root
AF069505	*H .ericae* (Read 101 5183)	*Calluna vulgaris*	UK	+	GenBank
AF252833	DGC23	*Calluna vulgaris*	UK	+	GenBank
AF252835	GU27	*Calluna vulgaris*	UK	+	GenBank
AF300749	UBCtra 1205C	*Gaultheria shallon*	Canada	+	GenBank
AF149069	UBCtra 274	*Gaultheria shallon*	Canada	+	Culture from root
AF252836	GU30	*Calluna vulgaris*	UK	+	GenBank
AF252837	GU32	*Calluna vulgaris*	UK	+	GenBank
AF300752	UBCtra 1340C	*Gaultheria shallon*	Canada	–	Culture from root
AF148951	E2-1	*Epacris impressa* Labill.	Australia	–	GenBank
AF099978	E1-9	*Epacris impressa*	Australia	+	GenBank
AF098291	E2-1-6	*Epacris impressa*	Australia	+	GenBank
AF081439	**Scytalidium vaccinii* Dalpé, Sigler & Litten, UAMH5828	*Vaccinium angustifolium* Ait.	US	+	GenBank
AF081437	**H. ericae* Read 101	*Calluna vulgaris*	UK	+	GenBank
AF081436	**H. ericae* UAMH 6735	*Calluna vulgaris*	UK	+	GenBank
AF151089	**H. ericae* Read 100 5183	*Calluna vulgaris*	UK	+	GenBank
AF284124	*UBCtra 1302.11	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300726	*UBCtra 1011.13	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF284123	*UBCtra 1302.5	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF284127	*UBCtra 1.01	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300724	*UBCtra Seq1.1A	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300718	*UBCtra 1086.12	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300755	*UBCtra Seq68	*Gaultheria shallon*	Canada	N/A	PCR product from root
	Assemblage A				
AF300740	UBCtra 1016C	*Gaultheria shallon*	Canada	–	Culture from root
AF252843	GU37	*Calluna vulgaris*	UK	–	GenBank
AF252842	GU44	*Calluna vulgaris*	UK	+	GenBank
AF252840	GU36	*Calluna vulgaris*	UK	–	GenBank
AF252841	DGC25	*Calluna vulgaris*	UK	–	GenBank
AF300753	UBCtra 1253C	*Gaultheria shallon*	Canada	–	Culture from root
AF072291	A03	*Woollsia pungens* (Cav.) F.Muell.	Australia	+	GenBank
AF149072	UBCtra 152	*Gaultheria shallon*	Canada	+	Culture from root
AF081434	*Phialocephala dimorphospora* Kendrick			N/A	GenBank
AF300754	UBCtra 1314C	*Gaultheria shallon*	Canada	Ambiguous	Culture from root

Appendix 1. Continued

Accession number	Identity	Plant Source	Location	Mycorrhizal status	Source
AJ300335	*Cladosporium cladosporioides* (Fres.) de Vries			N/A	GenBank
AF300730	UBCtra Seq62	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF011327	*Phialophora finlandia* Wang & Wilcox			N/A	GenBank
AF300745	UBCtra 1254C	*Gaultheria shallon*	Canada	+	Culture from root
AF149070	UBCtra 264	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF072300	C40	*Woollsia pungens*	Australia	+	GenBank
AF072299	C29	*Woollsia pungens*	Australia	+	GenBank
AF073348	*Erysiphe pisi* DC			N/A	GenBank
AF300747	UBCtra 1121C	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
	Unknown 2 and possible relatives				
AF300737	UBCtra 1256C	*Gaultheria shallon*	Canada	+	Culture from root
AF149077	UBCtra 179	*Gaultheria shallon*	Canada	+	Culture from root
AF081443	UBC S246 (Unknown 2)	*Gaultheria shallon*	Canada	+	GenBank
AF072301	D01/C01	*Woollsia pungens*	Australia	+	GenBank
AF072296	*C01 /D01	*Woollsia pungens*	Australia	+	GenBank
AF072290	A01	*Woollsia pungens*	Australia	+	GenBank
AF072297	C07	*Woollsia pungens*	Australia	+	GenBank
AF072292	A16	*Woollsia pungens*	Australia	+	GenBank
AF097312	E4-1-2	*Epacris impressa*	Australia	+	GenBank
AF072302	D02	*Woollsia pungens*	Australia	+	GenBank
AF099979	E3-1	*Epacris impressa*	Australia	+	GenBank
AF099091	AP-8	*Astroloma pinifolium*	Australia	+	GenBank
AF252838	AC24	*Calluna vulgaris*	UK	+	GenBank
AF252850	AC37	*Calluna vulgaris*	UK	–	GenBank
AF099090	AP-3	*Astroloma pinifolium*	Australia	+	GenBank
AF252847	AC35	*Calluna vulgaris*	UK	–	GenBank
AF252846	AC32	*Calluna vulgaris*	UK	+	GenBank
AF252845	AC31	*Calluna vulgaris*	UK	+	GenBank
AF252844	AC26	*Calluna vulgaris*	Devon, UK	+	GenBank
AF252848	AC36	*Calluna vulgaris*	UK	–	GenBank
AF300739	UBCtra 1311C	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF072295	B17	*Woollsia pungens*	Australia	+	GenBank
AF072293	A49	*Woollsia pungens*	Australia	+	GenBank
AF072303	D33	*Woollsia pungens*	Australia	+	GenBank
AF300738	UBCtra 1278C	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF149073	UBCtra 280	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF149075	UBCtra 143	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF252839	GU35	*Calluna vulgaris*	UK	+	GenBank
AF149076	UBCtra 305	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF072298	C28	*Woollsia pungens*	Australia	+	
	Assemblage B				
AJ271588	*Thielavia intermedia* Stchigel & Guarro			N/A	GenBank
AF300727	UBCtra 1018C	*Gaultheria shallon*	Canada	+	Culture from root
AF149079	UBCtra 300	*Gaultheria shallon*	Canada	–	Culture from root
AF300728	UBCtra 1022C	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF106018	*Drechmeria coniospora* (Drechsler) Gams & Jansson			N/A	GenBank
AJ238678	*Monodictys castaneae* (Wallr.) S.J. Hughes			N/A	GenBank
AF141199	*Pezicula ocellata* (Pers.:Fr.) Seaver			N/A	GenBank
AF300744	UBCtra 1182C	*Gaultheria shallon*	Canada	–	Culture from root
AF149074	UBCtra 288	*Gaultheria shallon*	Canada	–	Culture from root
AF149087	UBCtra 29	*Gaultheria shallon*	Canada	–	Culture from root
	Oidiodendron and possible relatives				
AF300742	UBCtra 1061C	*Gaultheria shallon*	Canada	+	Culture from root
AF300743	UBCtra Seq67	*Gaultheria shallon*	Canada	N/A	PCR product from root

Appendix 1. Continued

Accession number	Identity	Plant Source	Location	Mycorrhizal status	Source
AF062818	*Gymnostellatospora japonica* Udagawa, Uchiyama & Kamiya			N/A	GenBank
AF062819	*Pseudogymnoascus roseus* Raillo			N/A	GenBank
AF300736	UBCtra 1157C	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF062814	*Myxotrichum deflexum* Berk.			N/A	GenBank
AF081424	*Oidiodendron maius* Barron, S4	*Gaultheria shallon*	Canada	+	Culture from root
AF062799	**Oidiodendron maius*	*Vaccinium corymbosum* L.	Canada	+	Culture from root
AF072294	A53	*Woollsia pungens*	Australia	–	GenBank
AF062817	*Byssoascus striatosporus* (Barron & Booth) von Arx			N/A	GenBank
	Other taxa				
AF149078	UBCtra 153	*Gaultheria shallon*	Canada	Ambiguous	Culture from root
AF252849	DGC26	*Calluna vulgaris*	Devon, UK	Not tested	GenBank
AF300729	UBCtra 1170C	*Gaultheria shallon*	Canada	+	Culture from root
AJ246219	*Hypoxylon multiforme* (Fr.:Fr.) Fr.			Not tested	GenBank
AJ279481	*Microdochium* sp.			Not tested	GenBank
	Capronia				
AF050261	*Capronia villosa* G.F. Samuels			N/A	GenBank
AF300731	UBCtra Seq46	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300732	UBCtra Seq66	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300733	UBCtra Seq53	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300734	UBCtra 1046C	*Gaultheria shallon*	Canada	+	Culture from root
AF284129	*UBCtra 1041.4	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF300725	*UBCtra 1086.11	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF284128	*UBCtra 1322.11	*Gaultheria shallon*	Canada	N/A	PCR product from root
AF269068	Sd2	*Quercus ilex*, *Erica arborea* L.	Italy	+	Culture from root
	Outgroup				
Z73326	*Saccharomyces cerevisiae* Meyen ex Hansen			N/A	GenBank

*Sequences have at least a 95% sequence similarity to previous sequence and were not included in phylogenetic trees.
N/A = not applicable, meaning that cultures were not available.

Appendix 2. GenBank accession numbers for sequences of isolates included in LSU analysis

Accession number	Identity	Source
	Basidiomycetes	
AF284135	UBCtra 1041.2	PCR product from root
AF284136	UBCtra 1542.10	PCR product from root
AF202728	*Sebacina vermifera* Oberw.	GenBank
AF041548	*Russula virescens* (Schff.) Fr.	GenBank
X78780	*Ganoderma australe* (Fr.:Fr.) Pat.	GenBank
U66430	*Chrysomphalina chrysophylla* (Fr.: Fr.) Clé.	GenBank
AF042622	*Suillus luteus* (Fr.) S. F. Gray	GenBank
AF075493	*Tremella moriformis* Smith: Berk.	GenBank
	Ascomycetes	
AF284131	UBCtra 1542.2	PCR product from root
AF284130	UBCtra 1542.5	PCR product from root
AF284132	UBCtra 1041.3	PCR product from root
AF284125	UBCtra 1302.14	PCR product from root
AF284133	UBCtra 1522.5	PCR product from root
AF033394	*Monascus purpureus* Went	GenBank
AF050261	*Capronia villosa* Samuels	GenBank
AF284128	UBCtra1322.11	PCR product from root
AF300719	*UBCtra 1322.2	PCR product from root
AF050283	*Phialophora verrucosa* Medlar	GenBank
AF113739	*Sclerotinia veratri* Cash & R.W. Davidson	GenBank
AF300724	UBCtra Seq1.1A	PCR product from root
AF222467	*Chalara microchona* W. Gams	GenBank
AF284122	*Hymenoscyphus ericae* (Read) Korf & Kernan	Culture UAMH
AF284124	UBCtra 1302.11	PCR product from root
AF300726	UBCtra 1011.13	PCR product from root
AF113737	*Leotia viscosa* Fr.	GenBank
AF113738	*Geoglossum glabrum* Pers.	GenBank
U42669	*Morchella esculenta* (L.) Pers.	GenBank
AF127120	*Tuber californicum* Harkn.	GenBank
Z73326	*Saccharomyces cerevisiae* Meyen ex Hansen	GenBank
U42695	*Neolecta vitellina* (Bres.) Korf & J.K. Rogers	GenBank
AJ271061	*Mucor racemosus* Fresen.	GenBank

*Sequences with at least a 95% sequence similarity to previous sequence were not included in phylogenetic trees.