THE ECTOMYCORRHIZAL ASSOCIATIONS OF *LARIX LARICINA* (DU ROI) (TAMARACK) K. KOCH AND *BETULA GLANDULOSA* MICHAUX (SCRUB BIRCH) SEEDLINGS IN PEATLANDS OF CENTRAL BRITISH COLUMBIA

by

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ABSTRACT

Peatlands are habitats where peat accumulation exceeds decomposition, resulting in poorly drained, nutrient-poor and acidic soils. Tamarack (*Larix laricina*, family Pinaceae), a deciduous conifer, and scrub birch (*Betula glandulosa*, family Betulaceae), a low-lying deciduous shrub, are two plant species well adapted to the cold climates and short growing seasons of central British Columbia and generally able to tolerate the wet, poorly drained soils of peatlands. Ectomycorrhizas are mutualistic associations formed between plant roots and symbiotic fungi; ectomycorrhizal fungi that facilitate nutrient acquisition and water uptake in exchange for host carbon, may play an important role in the survival of these species. This study characterized tamarack and scrub birch ectomycorrhizas in three different peatland habitats using morphological (light microscopy) and molecular analysis (PCR-RFLP) methods. Ectomycorrhizal morphotypes and corresponding genotypes (fragment patterns) are described and ideas of host and peatland site specificity are explored. Results suggest that ectomycorrhizal colonization in peatland habitats may be similar to that for other hosts in other habitat types. Both morphology and molecular results indicate a high potential for ectomycorrhizal fungal linkages between hosts. This study presents the first published information on ectomycorrhizal associations of scrub birch.

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INTRODUCTION

The wetland ecosystems of the interior of British Columbia present a challenging environment for many of the plants that occupy them. Efficient conservation and acquisition of nutrients, as well as tolerance of fluctuating water tables, may be advantageous traits for plant species in order to survive, grow, and reproduce in these often nutrient deficient, poorly drained environments. Peatlands, specifically bogs and fens, form in cool climate areas with stable, high water tables that promote peat formation and bryophyte cover (MacKenzie and Moran, 2003). Peat is derived from partially decomposed mosses (e.g. *Sphagnum* spp.) and sedges, resulting in an acidic environment. Peatlands occur in all biogeoclimatic zones in British Columbia, with the exception of the Bunchgrass/Ponderosa Pine (BG/PP) zone. They are especially common in the Boreal White and Black spruce/Spruce-Willow-Birch (BWBS/SWB), Interior Cedar-Hemlock (ICH), and Sub-Boreal Pine-Spruce/Sub-Boreal Spruce (SBPS/SBS) zones (Delong et al., 1991; Hope et al., 1991; Meidinger et al., 1991).

Wetlands can be sensitive to anthropogenic disturbance that can result in permanent conversion to a different wetland type or an upland ecosystem. Road construction can cause water run-off to be channeled into peatlands, or impede wetland drainage, thereby influencing the hydrodynamics of the system (MacKenzie and Moran, 2003). Browsing of vegetation by livestock, as well as selective cutting has altered the structure of forested wetlands in Sweden (Segerström, 1997). Harvesting of trees in forested wetlands can cause paludification, a rise in the water table due to conversion of mineral soil to peatland (Paavilainen and Paivanen, 1995), and make seedling

regeneration difficult. As a result, the subsequent drainage of these flooded areas, in order to increase soil aeration and create favorable conditions for tree establishment, has been investigated (Rothwell et al., 1996; Roy et al., 1999). It has also been suggested that continued harvesting activity could create new wetland types not indigenous to the regional area, or disrupt the successional stages required to produce the original wetland community (Gale et al., 1998).

Ectomycorrhizas are mutualistic associations between symbiotic fungi and plant roots. Ectomycorrhizal fungi facilitate nutrient acquisition and water uptake in exchange for carbon from the host plant. Mycelial networks of underground fungal hyphae can link different host plants that share common fungal symbionts (Björkman, 1960; Finlay and Read, 1986; Dahlberg and Stenlid, 1990; Simard et al., 1997b; McKendrick et al., 2000). The concept of mycelial networks is particularly relevant in regards to nutrient poor environments (e.g. fens and bogs) where carbon and nutrients can be exchanged across resource gradients (Tilman et al., 1996; Simard et al., 1997b). It has been established that many wetland plant species are mycorrhizal, however, the ecological role that symbiotic fungi play in wetland ecosystems has been relatively unexplored. Although the literature suggests that mycorrhizal fungi are important in nutrient poor, ground-water fed ecosystems (Turner et al., 2000) and that they may be an important mechanism in wetland rehabilitation following anthropogenic disturbance (Turner and Friese, 1998), more research into the mycorrhizal associations of wetland plants is still required to fully understand the relationship between these unique ecosystems and symbiotic fungi. The occurrence of mycelial networks, or shared mycorrhizal symbionts

between different host species, and their possible function in wetland environments, is largely unknown.

Tamarack is a unique deciduous conifer that is able to tolerate the conditions occurring in peatland environments. It is able to grow at a faster rate (Strong and LaRoi, 1983), conserve more foliar nutrients (Tyrell and Boerner, 1987), utilize a higher amount of available N (MacDonald and Lieffers, 1990), and be less affected by flooded conditions than its counterpart black spruce (Islam and MacDonald, 2003). One hypothesis for the success of tamarack in peatland ecosystems is attributed to its efficient genus-specific mutualistic ectomycorrhizal associations (Tyrell and Boerner, 1987).

Scrub birch is a low-lying shrub that is often found growing with tamarack in these environments. Even less is known about the associated ectomycorrhizal fungal symbionts of this peatland species. However, several studies have investigated the ectomycorrhizal relationships of the more northern swamp birch (*Betula nana* L.) (Miller, 1982), as well as upland *Betula* spp., such as paper birch (*Betula papyrifera* Marsh.) (Simard et al., 1997a and 1997b; Jones et al., 1997) and European white birch (*Betula pendula* Roth) (Miller, 1982; Feugy et al., 1999; Blaudez et al., 2001). It is possible that ectomycorrhizal fungi play an important role in the survival and growth of scrub birch, as well as tamarack, growing in these wetland ecosystems.

This project was established to examine the mycorrhizal associations of two plant species, tamarack and scrub birch, growing in three habitats i) scrub birch dominated, ii) tamarack-scrub birch, and iii) mixed tamarack-scrub birch-black spruce peatland site types. The specific objectives of this research project were to use a combination of

morphological (light microscopy) and molecular analysis (polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP)) to:

1. Characterize and identify the fungal symbionts that associate with tamarack and scrub birch host species in peatland sites,
2. Determine the abundance and diversity of the fungal symbionts associating with tamarack and scrub birch on the sites,
3. Assess differences in the ectomycorrhizal community occurring between the two host species as well as amongst the three different peatland site types,
4. Determine if the potential for fungal linkages exists between tamarack and scrub birch in these peatlands,
5. Determine if possible fungal linkages exist between these two host species and a third host, black spruce (*Picea mariana*), that co-occurs in the Mix peatland site type, using molecular data derived from a study by Robertson (2003).

LITERATURE REVIEW

Wetlands of British Columbia

Ecology and descriptions

Wetlands have been defined as "areas where soils are water-saturated for a sufficient length of time such that excess water and resulting low soil oxygen levels are principal determinants of vegetation and soil development" (MacKenzie and Moran, 2003). Many different types of ecosystems, such as fens, bogs, and swamps, are included in this definition. Water table attributes such as pH, annual fluctuation levels, and carbon concentration can influence the plant species distribution within these environments (Girardin et al., 2001). Composition of vegetation may also reflect regional geographic variations (Warner and Rubec, 1997). The high water table and poorly aerated soils of wetlands can make growing conditions difficult even for flood-tolerant vegetation. Poor growth rate and decreased rooting depth are characteristics of coniferous trees in wetland ecosystems (Lieffers and Rothwell, 1986). Peatland ecosystems, specifically fens and bogs, are of particular interest since they support the species under investigation: *Betula glandulosa* Michaux (=*B. nana*) (scrub birch) and *Larix laricina* (Du Roi) K. Koch (tamarack).

A fen, as described by Meidinger and Pojar (1991), is a non-tidal wetland that is fed water from belowground sources, and receives minerotrophic runoff from surrounding upland mineral soils. Fens are relatively higher in nutrients and lower in acidity, compared to the more acidic, nutrient-poor bog (Warner and Rubec, 1997). Moderately decomposed peat accumulates to more than 40 cm within the organic layer of

the Mesisol and Humisol soils, which maintain a high mineral content in the rooting zone (Meidinger and Pojar, 1991; MacKenzie and Moran, 2003). Fens are the most common wetland class in British Columbia, especially within the poorly drained basins of the Boreal Black and White Spruce (BWBS), Spruce Willow Birch (SWB), Interior Douglas-fir (IDF), Sub-Boreal Pine-Spruce (SBPS) and Sub-Boreal Spruce (SBS) biogeoclimatic zones. Associated non-ericaceous shrub and plant species include scrub birch, *Betula pumila* (swamp birch), *Carex* spp. (sedges), *Equisetum arvense* (common horsetail), and *Platanthera dilatata* (white bog-orchid). *Picea mariana* (black spruce), *P. glauca* (white spruce), and tamarack are the characteristic tree species within the BWBS biogeoclimatic zone (Meidinger and Pojar, 1991). However, a more recent wetland classification describes fen ecosystems as peatlands dominated by sedges and brown mosses (e.g. *Tomenthypnum*), with high water tables limiting the establishment of tall shrub and tree species (MacKenzie and Moran, 2003).

Bogs are nutrient-poor, acidic, *Sphagnum*-dominated ecosystems characterized by woody vegetation, such as conifers and ericaceous plants (MacKenzie and Moran, 2003). These wetlands are often raised or level with their immediate environment, which makes the minerotrophic run-off and nutrient-rich groundwater from the surrounding soils less available to the rooting zone (Meidinger and Pojar, 1991). Bogs are most common in the BWBS, SWB, SBPS, and SBS biogeoclimatic zones in British Columbia. Fibrisol, Mesisol, or Humisol soils, with upper layers of poorly decomposed peat moss, support slow-growing black spruce, tamarack, *Pinus contorta* (lodgepole pine), and scrub birch plant communities. The sparse dwarf shrub and herb layer consists of the ericaceous *Vaccinium oxycoccos* (bog-cranberry), *Andromeda polifolia* (bog-rosemary), and *Kalmia*

microphylla (western bog-laurel), as well as *Carex* spp. (sedges), *Drosera* spp. (sundews), and *Menyanthes trifoliata* (buckbean) (MacKenzie and Moran, 2003).

Mycorrhizal symbiosis

Definition and structure

Many plants and fungi form beneficial relationships that result in mutualistic symbioses which serve to increase both partner's fitness within their natural environment. The association between a fungus and the roots of a plant is termed 'mycorrhiza' (Smith and Read, 1997). There are seven different categories of mycorrhizal associations that are defined according to the morphological and anatomical characteristics that they exhibit, as well as to the plant and fungal partners involved in the relationships. The present study examines ectomycorrhizas; however, other categories of mycorrhizas that might be of interest in peatland ecosystems include arbuscular (AM), ericoid, and ectendomycorrhizas.

Ectomycorrhiza refers to the category commonly formed between basidiomycete or ascomycete fungi, and gymnosperm and angiosperm plant species, or more specifically, coniferous and deciduous trees. Ectomycorrhizal roots are typically colonized by fungi that form an outer mantle of fungal hyphae, as well as a Hartig net (an intercellular network of hyphae that surrounds the root cells up to the endodermis in gymnosperms, and up to the exodermis in angiosperms) (Molina et al., 1992). Arbuscular mycorrhizas are formed between many plant species (including the majority of angiosperm families), as well as some mosses and lycopods, and members of the order Glomales (zygomycete fungi). They are distinctly characterized by the presence of

highly branched arbuscules (formed within cortical root cells), and, in some species, intraradical vesicles (enlarged lipid-filled portions of hyphae formed within or between cortical cells) (Smith and Read, 1997; Peterson et al., in press). Typical wetland AM plants include members of the grasses (Poaceae), sedges (Cyperaceae), and willows (*Salix*) (Turner and Friese, 1998; Miller, 1999; Turner et al., 2000; Marshall and Pattullo, 1981). Ericoid mycorrhizas are named by the association with host plants involved in this symbiosis: the order Ericales, which includes many peatland plants such as Labrador tea (*Ledum groenlandicum*), bog cranberry (*Vaccinium oxycoccos*), and bog-rosemary (*Andromeda polifolia*). This category of mycorrhizas is characterized by the formation of narrow diameter "hair roots" by the host plant, whose root epidermal cells are colonized by fungi that produce unique, highly branched, hyphal complexes (Peterson et al., in press). Ectendomycorrhizas, a variant of ectomycorrhizas (Egger and Fortin, 1988), form primarily between *Pinus* and *Larix* host species, and E-strain (*Wilcoxina* spp.) ascomycete fungi (Yu et al., 2001). These mycorrhizas exhibit morphological characteristics similar to ectomycorrhizas, with the exception of intracellular hyphae that penetrate the cortical root cells (Laiho, 1965; Mikola, 1965; Yu et al., 2001). Ectomycorrhizas form the main type of symbiosis found on both tamarack and scrub birch tree species and are the main focus of this thesis.

Functions and benefits

It is well known that the fungal associates in mycorrhizal relationships facilitate the uptake of water (Dosskey et al., 1990; Bending and Read, 1995; Smith and Read, 1997) and nutrients to the host plant from soil (Harley and Smith, 1983; Perez-Moreno

and Read, 2000); however, mycorrhizas can also participate in the biological control against pathogenic root fungi and soil-borne diseases (Duchesne, 1994; Schelkle and Peterson, 1996; Ursic et al., 1997; Morin et al. 1999). Some mycorrhizal fungi can also degrade persistant organic soil pollutants (Meharg and Cairney, 2000; Meharg and Cairney, 2002), as well as withstand a range of environmental stresses (Anderson 1988; Kendrick, 1992; Colpaert and van Tichelen, 1994).

Mycorrhizal fungi have been shown to aid in nitrogen transformation from protein sources (Abuzinadah and Read, 1986; Li and Hung, 1987; Li et al., 1992), as well as from simple organic forms (reviewed in Leake and Read, 1997). Some mycorrhizal fungi can produce proteolytic enzymes that exploit N and P, which are important determinants of plant growth, from substrates in their natural environment (Read, 1991; Smith and Read, 1997; Read and Perez-Moreno, 2003). It was once thought that two distinctly separate groups of soil fungi existed: saprophytic decomposers that broke down organic substrates into usable forms, and mutualists, that associated with plant roots and absorbed mineral nutrient ions (Hibbett et al., 2000). However, molecular research has revealed that some inconspicuously fruiting ectomycorrhizal fungi can exhibit decomposer capabilities when also in the mycorrhizal state (Kõljalg et al., 2000). Genetic study of the phylogeny of ectomycorrhizal fungi has resulted in some uncertainty with respect to the distinction between these two fungal groups (Hibbett et al., 2000), as well as to our full understanding of the role of mycorrhizal fungi in this complex system.

Fungal mycelial networks

In addition to their impact on water and nutrient acquisition by the host plant, mycorrhizal fungi may also link different host plant species, or plants of the same species, via underground networks of fungal hyphae (Björkman, 1960; Finlay and Read, 1986; Dahlberg and Stenlid, 1990; Simard et al., 1997b; McKendrick et al., 2000). Plants that share common fungal symbionts may have the ability to tap into this functional pathway. Trees colonized by the same symbionts may have similar capabilities to capture soil nutrients, by connected mycelia, thereby possibly reducing competition for resources (Finlay, 1989; Horton and Bruns, 1998). Plant-to-plant nutrient transfer could be vital in nutrient poor or shaded environments where hyphal pathways may allow the transport of carbon and nutrients across resource gradients between host species (Tilman et al., 1996; Simard et al., 1997).

However, the structure and function of ectomycorrhizal communities, as well as the potential for interplant linkages in an ecosystem, is complex and not fully understood (Molina et al., 1992). The guild concept (Perry et al., 1989) describes the shared fungal linkages between ectomycorrhizal host species as strengthening ecosystem resiliency by contributing to its "mutual aid and the promotion of common interests". In terms of nutrient cycling within an ecosystem, it has been hypothesized that host species that share common symbionts may cycle nutrients among themselves, thereby excluding other host species that associate with different fungal partners (Newman, 1988). With respect to tamarack and scrub birch, the identity and linkage associations with mycorrhizal fungi have not yet been studied.

Mycorrhizas in wetland ecosystems

Plants growing in wetland ecosystems were once thought to be non-mycorrhizal (Powell, 1975). Instead of forming a mycorrhizal relationship, plants might increase root length in order to acquire more nutrients, a function possibly hampered in poorly aerated flooded soils (Powell, 1975; Coutts and Phillipson, 1978; Mosse et al., 1981). A more recent analysis by Turner and Friese (1998) stressed that it cannot be assumed that wetland plant species are non-mycorrhizal simply because their roots are submerged under water. Recent studies have shown that many wetland plant species are, in fact, mycorrhizal. Numerous species of aquatic grasses, sedges, and herbaceous plants growing in wetland environments often have AM associations (Stevens and Peterson, 1996; Turner and Friese, 1998; Miller, 1999; Turner et al., 2000). Marshall and Pattullo (1981) reported that willows were found to be ectomycorrhizal in a fen ecosystem. With respect to many shrub species and conifers, little is known about their mycorrhizal habits in wetland ecosystems.

It has been suggested that ectomycorrhizas associated with trees and woody shrubs in these wet environments may be able to exist, in part, due to soil aeration caused by seasonal fluctuations of the water table (Meyer, 1974); oxygen deficiency has been suggested as a limiting factor to mycorrhizal fungal formation (Stenström, 1991). Turner et al. (2000) suggest that mycorrhizas may have an important role in reduced nutrient and ground-water driven communities where colonized roots have been found to be more numerous.

Ectomycorrhizal diversity

Ectomycorrhizal community diversity can be simply defined as the measure of species richness, the number of different species found in the community, and community evenness, the relative abundance of each of those species within the community (Magurran, 1988). The belowground diversity of ectomycorrhizal fungi is thought to be directly influenced by the type of forest community, successional stages within a given forest community, as well as the distinctive microhabitats that encompass a forest landscape (Amaranthus, 1998).

Host receptivity refers to the range of fungal species with which a host plant associates (Molina et al., 1992). The diversity of mycorrhizal fungi associating with a given host can range from high (e.g. approximately 2,000 fungal species may associate with *Pseudotsuga menziesii* (Douglas-fir) (Trappe, 1977)), to low (e.g. *Alnus*, which has about 20 fungal associates) (Molina et al., 1992). Likewise, the level of specificity exhibited by ectomycorrhizal fungi in associating with a given host species can range from broad to narrow. For example, *Suillus grevillei* and *Boletinus cavipes* demonstrate a narrow specificity with members of the genus *Larix*, and appear to preferentially "choose" to associate with that genus (Finlay, 1989), whereas *Cenococcum* associates with most known ectomycorrhizal hosts (Molina et al., 1992). Given this specificity concept, maintaining plant host species diversity may be vital to supporting ectomycorrhizal fungi diversity, especially for fungi with apparently narrow host ranges (Massicotte et al., 1999).

Methods for measuring ectomycorrhizal diversity

Sporocarp surveys and seedling sampling

Most ectomycorrhizal fungi at some point in their life cycle produce reproductive fruiting bodies, known as sporocarps. Fruiting can occur aboveground (epigeous) or belowground (hypogeous) and is believed to be closely related to environmental conditions present at the site, such as soil temperature and moisture (Godbout and Fortin, 1990). Sporocarp surveys (hypogeous sporocarp collections may be included) have traditionally been used to assess ectomycorrhizal diversity; with this method, fruiting bodies may be identified to species using standard taxonomic approaches (Sakakibara et al., 2002). An important advantage of sporocarp surveys is that one can collect samples throughout several growing seasons. Sporocarp surveys allow for minimal interference within the study site, an important criterion for long-term monitoring projects. However, it is now widely accepted that the production of sporocarps is not always an accurate reflection of ectomycorrhizal species richness belowground (Mehmann et al., 1995; Gardes and Bruns, 1996; Dahlberg, 1997; Dahlberg, 2001). As well, not all sporocarps represent fungal species that are ectomycorrhizal; some may instead be saprophytic in nature. More recently, sporocarp surveys have been combined with other sampling methods in order to more accurately estimate fungal diversity (Bradbury et al., 1998). Ectomycorrhizal fungi can fruit sporadically at a specific site or remain as microscopic, undetected components in the soil, such as spores or sclerotia (Taylor, 2002). As well, sporocarp production varies both temporally and spatially, due to an array of different external factors (Watling, 1995). Some ectomycorrhizal fungi never appear to reproduce sexually and exist primarily in a vegetative state (e.g. *Cenococcum geophilum*), or the

sporocarps fruit belowground and are difficult to detect, or are resupinate in nature (Jonsson et al. 1999; Stendell et al. 1999; Taylor and Bruns 1999; Peter et al. 2001a). Some suggest the presence of a species is best assessed by its presence in its vegetative state (Luoma 1991; Horton 2002).

One of the most common ways used to assess ectomycorrhizal community diversity is by direct sampling (also referred to as field bioassays) of ectomycorrhizal root tips from planted or naturally regenerated seedlings. Entire seedlings can be removed with the surrounding soil in order to keep root systems relatively intact, and a sub-sample (or all) of the roots are examined for ectomycorrhizas. When whole seedling destructive sampling is not desirable, such as in regenerating clearcuts where stocking standards must be met, partial collection of lateral roots can also be conducted (Jones et al., 2002). In addition, root coring using cylindrical soil corers (Peter et al., 2001a) is often done in habitats where one host plant dominates, or when host roots can be easily identified (e.g. *Pinus* spp.), or when molecular analysis can be used to separate the different host species (Horton and Bruns, 1998). Compared to sporocarp surveys, which may repeatedly sample specimens over several seasons, seedling or root core sampling may occur only once or twice during a study, often due to time constraints or other determining factors (Horton and Bruns, 2001).

Microscopy and ectomycorrhiza characterization

Morphological classification of mycorrhizal root tips (morphotyping) using dissecting and compound microscopy is a common approach for family, genus and species identification. Although accurate characterization of ectomycorrhizas takes time

to learn (Dahlberg, 2001), macroscopic characteristics of ectomycorrhizas, such as shape, texture and colour, as well as microscopic features such as the presence of emanating hyphae, mantle, and rhizomorphs, can all aid in fungal identification (Agerer, 1987-2000; Ingleby et al., 1990; Goodman et al., 1996). Fungal diversity can be accurately assessed using detailed morphological descriptions and this assessment can provide a valuable basis for further molecular investigations (Horton, 2002). Nevertheless, morphotyping can sometimes be subjective and, if performed incorrectly, can lead to identification problems (Peter et al., 2001a). In some instances, it is not always possible to accurately group or distinguish all ectomycorrhizas whether from the same, or from different, fungal species (Sakakibara et al., 2002). To use morphotyping to its maximum benefit and to overcome some of the above limitations, morphological characterization of mycorrhizal root tips is often combined with molecular analysis techniques (Varga, 1998; Horton and Bruns, 1998; Hagerman et al., 1999; Jonsson et al., 1999; Mah et al., 2001; Robertson et al., 2003).

Molecular techniques

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), common molecular analysis techniques, have advanced the study of ectomycorrhizal community assessment through the identification of fungal symbionts and their genotypes. PCR is able to amplify specific regions of the fungal ribosomal genes and spacers through the development of universal and fungal specific primers (White et al., 1990; Cullings and Bruns, 1992; Gardes and Bruns, 1993; Egger, 1995). The target region of the nuclear encoded ribosomal DNA (rDNA) ranges from the 3' end

of the 18S small subunit, to the 5' end of the 28S large subunit, including both internal transcribed spacer (ITS1 and ITS2) regions. The ITS regions (moderately conserved regions) reveal species-specific variability allowing for the discrimination of closely related species, and the large and small subunit regions act as sites for primer design (Egger, 1995; Horton and Bruns, 2001). However, PCR analysis alone is not sufficient for the detection of genotypes or to distinguish between closely related species (Egger, 1995).

RFLP analysis, with the aid of restriction endonucleases, allows for the digestion of the amplified target region into fragments of variable sizes. Resulting fragment patterns reveal small size differences that enable the researcher to separate closely related fungal species (Egger, 1995; Mehmann et al., 1995; Gardes and Bruns, 1996; Horton and Bruns, 1998), and to identify these through comparison to established RFLP ectomycorrhizal root tip and sporocarp databases. This method is cost effective and useful for distinguishing between different ectomycorrhizal fungal species from root tip samples (Horton, 2002); however, identification is still not always possible for several reasons. RFLP databases tend to be primarily composed of commonly observed sporocarps, which may not account for the fungal species that do not fruit frequently or not at all (Jonsson et al. 1999; Stendell et al. 1999; Taylor and Bruns 1999; Horton and Bruns, 2001; Peter et al. 2001b). In addition, size estimates for fragment patterns, protocols, and restriction endonucleases can vary between research labs and may hinder comparisons; intraspecific variation within fungal species can also occur across large geographic scales (Kårén et al., 1997; Methven et al., 2000). In some cases, ITS-RFLP data offers limited taxonomic information for identification to the species or species

group level, information that DNA sequencing analysis, if used, might provide (Horton, 2002).

Measurements of ectomycorrhizal diversity

Numerous diversity indices are often used to assess the level of ecological complexity within and between communities (Magurran, 1988). Methods for calculating ectomycorrhizal diversity for host species or between sites can include measures of species richness, frequency of occurrence, and proportional abundance (percent); the resulting means and standard errors can be compared by analysis of variance (ANOVA) (Magurran, 1988). This study includes five indices that were selected to measure the ectomycorrhizal diversity of the fungal symbionts associated with tamarack and scrub birch: the Margalef (species richness), Shannon, Shannon Evenness, Simpson (species dominance), and Phi (molecular diversity) indices.

Species richness (a measure of the number of the species found) was calculated using the Margalef index. It is calculated as follows:

$$D_{mg} = (S\text{-}1)/\ln N$$

where S = number of species, and N = total number of individuals summed over all S species (Magurran, 1988).

The Shannon (H') and Shannon Evenness (E) diversity indices are based on proportional abundance of each species, as well as on species richness (the number of species). These indices place increased emphasis on species richness; with respect to mycorrhizas, this includes rare fungal species. The indices are calculated:

$$H' = -\sum p_i \ln p_i$$

$$E = H'/\ln S$$

where p_i = proportion of individuals found in the ith species, and where S = number of species. As the ectomycorrhizal diversity increases, the Shannon index values increase (values usually range from 1.5-3.4) as well. The Shannon Evenness index values range from 0-1.0, with 1.0 meaning all species are equally abundant. These indices assume that species are randomly sampled from an infinite population and that all species are represented. Failure to include all species is considered to be a common source of error when using these indices (Magurran, 1988).

The Simpson index (D) is also calculated using the proportional abundance of the each species, as well as the number of species identified. However, the index emphasizes the most abundant (dominant) species, and is often expressed as a reciprocal (1/D) value so that higher values represent increased diversity (Magurran, 1988). Since the index is weighted towards the more abundant species, it is less sensitive to species richness or rare species. The Simpson index is calculated as follows:--

$$D = \sum(n_i (n_i\text{-}1))/\ (N (N\text{-}1))$$

where n_i = number of individuals in the $_i$th species and N = the total number of individuals (Magurran, 1988).

The Phi index (Φ) was developed by Egger (Baldwin, 1999) to specifically assess molecular diversity within a community. This index uses pairwise distances (in contrast to proportional abundance data often used to calculate traditional diversity indices) for each sample, with more distantly related samples being separated by greater phylogenetic distance (Khetmalas et al., 2002). Dice's index matrices are calculated from RFLP fragment patterns to estimate the similarity between samples; then the average squared

distance is calculated for the entire data matrix. Values range from 0 (identical fragment patterns) to 1 (no fragments shared between any pairs) (Mah et al., 2001). The Phi index is calculated as follows:

$$\Phi = \frac{\sum_{j=1}^{n}\left[\frac{\sum_{i=1}^{n} d_{ij}^2}{n-1}\right]}{n}$$

For a data matrix with $i = j$ rows and columns, the pairwise distances (d) for each sample were squared, summed, then divided by n-1 to give an average squared distance for each column, where n equals the total number of samples in the matrix. As with all other indices, resulting mean Phi values can be compared using an ANOVA; an increase in the Phi value implies greater diversity.

Tamarack (*Larix laricina*)

Distribution and ecology

Uniquely characterized by deciduous needles, the genus *Larix* (family Pinaceae) is well adapted to the cold climates and short growing seasons typical of the boreal, montane and subalpine forests of the northern hemisphere (LePage, 1995). Three of the ten tree species in this genus are endemic to Canada and North America: *Larix occidentalis* Nutt. (western larch), *Larix lyallii* Parl. (alpine larch), and *Larix laricina* (Du Roi) K. Koch (tamarack) (Farrar, 1995). Tamarack, also known as eastern larch, is the widest ranging conifer species in North America; it occurs in every province and territory in Canada, as well as Alaska (Johnston, 1990). The species can generally tolerate most soil conditions, such as wet, organic *Sphagnum* peat found in lowland bogs, muskegs, or

fens, as well as well-drained, mineral soils found on upland northern slopes (Johnston, 1990; Farrar, 1995). In northern British Columbia, tamarack is commonly found within the BWBS biogeoclimatic zone (Delong et al., 1991), often occurring in mixed stands with black spruce and scrub birch in the wet, nutrient poor Sb-Tamarack site series association (Krestov et al., 2000). Tamarack is considered rare in the Sub Boreal Spruce (SBS) zone (Meidinger et al., 1991; Beaudry et al., 1999), but it can be locally common within the Tamarack – Water sedge – Fen moss (WB06) Bog Site Association (MacKenzie and Moran, 2003).

Tamarack exhibits several interesting physiological adaptations in response to its harsh growing conditions. High water tables, poor soil aeration, low nutrient availability and the cold substrate of fen and bog environments result in extremely slow growth rates (Payandeh, 1973; Lieffers and Rothwell, 1986, 1987; MacDonald and Lieffers, 1990), however, tamarack may still grow at a faster rate than black spruce (Strong and LaRoi, 1983). Tyrell and Boerner (1987) investigated how tamarack conserves foliar nutrients as a mechanism to persist in peatland environments without the evergreen habit that is exhibited by its counterpart, black spruce. They suggested that the efficient genus-specific mycorrhizal associations unique to tamarack may enable the tree to uptake a greater amount of nutrients than black spruce. This, when combined with a higher foliar nitrogen resorption, as well as a higher photosynthetic rate than black spruce, allows it to remain productive in bog environments. Further evidence of the benefits of this specific ectomycorrhizal relationship was demonstrated by Samson and Fortin (1986); they determined that the fungi previously identified as being *Larix*-specific (e.g. *Suillus grevillei*) in field conditions, showed faster and better mycorrhizal development (e.g.

extensive extramatrical hyphal networks) *in vitro*. As well, *Suillus grevillei* is consistently associated with tamarack in its full habitat distribution range, including wet, boggy areas (Samson and Fortin, 1986).

MacDonald and Lieffers (1990) also reported differences between tamarack and black spruce in their ability to utilize nitrogen; they found that tamarack was more effective in utilizing improved nutrient conditions following peatland drainage. Simulated flooding in a greenhouse caused reduced root hydraulic conductance, net assimilation rate, and stomatal conductance in both tamarack and black spruce seedlings; however, tamarack was less affected than black spruce in all measurements (Islam and MacDonald, 2003). It was also noted that tamarack showed no visible flooding damage symptoms, such as necrotic needles and electrolyte leakage as experienced by black spruce. Chakravarty and Chatarpaul (1990) reported that, in an *in vitro* tamarack study, inoculated seedlings with mycorrhizal fungi performed better than non-mycorrhizal seedlings in nutrient limited environments.

Identified fungal symbionts

Early studies describing the mycorrhizal associations for the genus *Larix* include those by McDougal (1914), Melin (1922), and Hammerlund (1923); these pioneer studies led others to attempt to identify the numerous fungal symbionts (Table 1.1). How (1940, 1941, 1942) completed detailed studies on *L. decidua*, including studies on its fungal associates and its specialized relationship with the fungus *Boletus elegans*. The fungal species *Suillus grevillei* and *S. cavipes* have been reported to be highly specific to *Larix* spp. as are several other fungal species that exhibit a narrow host preference (Molina et

al., 1992). Roots of tamarack sampled from the field have also indicated the possibility of an ectendomycorrhizal association, though the fungal species remained unidentified (Malloch and Malloch, 1981). Samson and Fortin (1986) also assessed fungal symbionts of tamarack by inoculating plantlets with different isolated fungi; they reported that 91 isolates belonging to 25 fungal species formed ectomycorrhizae with tamarack seedlings. Table 1.1 summarizes the reported mycorrhizal associations for three *Larix* species.

Scrub birch (*Betula glandulosa*)

Description and ecology

As its common name implies, scrub birch (*Betula glandulosa*) is a low lying spreading shrub that can reach two metres in height in both wetland and upland areas of British Columbia (Mackinnon et al., 1992). Within the northern half of the province, scrub birch is commonly found at low elevations in wetlands with black spruce, tamarack and lodgepole pine (*Pinus contorta* var. *latifolia*). Swamp birch (*Betula nana*) is a commonly misidentified species found in similar habitats but is a more northern and Eurasian species (Brayshaw, 1996). Some confusion can arise since swamp birch is also referred to as *Betula pumila* (dwarf birch) or *Betula glandulosa* var. *glandulifera* within various tree identification guides.

Table 1.1. Identified mycorrhizal symbionts of *Larix laricina*, *L. decidua*, and *L. occidentalis*.

Fungal associate	*L. laricina*	*L. decidua*	*L. occidentalis*
Amanita muscaria			●[6]
A. rubescens	●[1]		
Astraeus pteridis			●[6]
Boletus elegans		●[3,4]	
B. viscidus		●[4]	
B. edulis			●[6]
Cenococcum spp.	●[2]		●[6,9]
E-strain			●[9]
Fuscoboletinus aeruginascens	●[1]		●[6]
F. paluster	●[1]		
F. spectabilis	●[1]		
F. grisellus	●[1]		
F. glandulosus	●[1]		
F. ochraceoroseus	●[1]		
Hebeloma spp.		●[1,5]	
Laccaria laccata	●[1]		●[1]
L. amethystea		●[7]	
L. bicolor	●[1]		
Lactarius deliciosus			●[6]
Leccinum holopus var. *americanus*	●[1]		
Melanogaster intermedius			●[6]
Paxillus involutus	●[1]	●[4]	●[6]
Pisolithus tinctorius	●[1]		●[6]
Rhizopogon rubescens	●[1]		
R. vinicolor			●[6]
Scleroderma hypogaeum			●[6]
Sphaerosporella brunnea	●[8]		●[8]
Suillus grevillei	●[1]	●[6]	●[6]
S. cavipes	●[1]		●[6]
S. lakei			●[6]
Thelephora terrestris	●[1,5]		
Tricholoma pessundatum	●[1]		
T. vaccinum	●[1]		
T. flavovirens			●[6]
Truncocolumella citrina			●[6]

[1]Samson and Fortin (1986), [2]Malloch and Malloch (1981), [3]How (1940), [4]How (1942), [5]LeTacon and Bouchard (1986), [6]Molina and Trappe (1982), [7]Munzenberger et al. (1995), [8]Danielson (1984), [9]Laiho (1965), [10]Thormann et al. (1999).

Scrub birch is primarily found in the Spruce-Willow-Birch (SWB) biogeoclimatic zone, the most northerly subalpine zone of British Columbia (Pojar and Stewart, 1991). Within this zone, scrub birch grows on dry to wet, moderately well-drained upland soils in open forests and woodlands of the White spruce-Grey-leaved willow-Scrub birch site association, as well as in moderately rich, shrubby fens within the Barclay's willow-Scrub birch-Water sedge site association (Pojar and Stewart, 1991). The recently published guide to the wetland areas of interior British Columbia (Mackenzie and Moran, 2003) lists scrub birch as occurring mainly within the Scrub birch-Water sedge (WF02) and Scrub birch-Buckbean-Shore sedge (WF07) Fen Site Associations. A very wet, nutrient-medium Sb-Swamp birch site series association in the SBS (Sub-boreal Spruce) biogeoclimatic zone is tentatively identified by Krestov et al. (2000).

Identified fungal symbionts

The mycorrhizal associations of these small birches, scrub birch in particular, have been largely uninvestigated. However, one study in the subalpine tundra of Alaska examined swamp birch roots from the field and identified 12 species of ectomycorrhizal fungi (Miller, 1982). Numerous studies have recently explored the relationship between some of the larger *Betula* spp. and their fungal symbionts, including *Paxillus involutus* (Blaudez et al., 1998; Jordy et al., 1998; Feugy et al., 1999; Perez-Moreno and Read, 2000; Blaudez et al., 2001). It is important to note that most of these studies involve *Betula* spp. that grow in distinctly different (mostly well drained) habitats. Table 1.2 summarizes the ectomycorrhizal fungal symbionts of three *Betula* species.

Table 1.2. The identified mycorrhizal symbionts of *B. glandulosa*, *B. pendula* and *B. nana*.

Fungal Associate	*B. glandulosa*	*B. pendula*	*B. nana*
Amanita inaurata			•[1]
A. pantherina			•[1]
A. vaginata			•[1]
Boletus edulis			•[1]
Hebeloma pusillum			•[1]
H. cylindrosporum		•[3]	
Lactarius musteus			•[1]
L. uvidus			•[1]
Leccinum scabrum			•[1]
Hygrophorus chrysodon			•[1]
H. conicus			•[1]
Paxillus involutus		•[2]	•[1]
Russula emetica			•[1]
R. obscura			•[1]

[1]Miller, 1982; [2]Blaudez et al., 1998; [3]Feugy, 1999

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Morphological characterization of ectomycorrhizal associations of *Larix laricina* (Du Roi) (tamarack) K. Koch and *Betula glandulosa* Michaux (scrub birch) in peatlands of central British Columbia.

ABSTRACT

Peatland habitats accumulate peat in lowland areas, resulting in poorly drained, moderately acidic, and nutrient deficient soils. In these ecosystems, tamarack and scrub birch are often found growing in close proximity in central British Columbia. Morphological methods (light microscopy) were used to characterize the ectomycorrhizas of these two host species in three peatland site types (scrub birch-tamarack-black spruce (Mix), scrub birch-tamarack (BsLt), and scrub birch (Bs) only), and to determine differences in ectomycorrhizal community structure and diversity between hosts and peatland site types, as well as the potential for host-fungal linkages. A total of 30 morphotypes were described from 24 tamarack and 36 scrub birch seedlings; 17 common morphotypes were found on both hosts. MRA, Thelephoraceae 1 and *Tomentella*-like 2 found on scrub birch, and *Suillus* 2 and *Cenococcum* found on tamarack, were the most frequent morphotypes. *Lactarius* and *Suillus* also showed some host specificity. Some morphotypes exhibited site specificity (e.g. the three Thelephoraceae spp. (tamarack) in the Mix site, and cotton orange and *Tomentella*-like 1 (scrub birch) in the Bs and BsLt sites); many morphotypes were found in all site types. Although ectomycorrhizal abundance varied between hosts for some morphotypes, no overall difference in ectomycorrhizal diversity was seen between hosts. However, ectomycorrhizal diversity was highest in the Mix sites for both hosts compared to the BsLt sites (Margalef, Shannon, and Simpson indices) ($\alpha = 0.05$). Overall, ectomycorrhizal colonization of tamarack and scrub birch showed a high potential for fungal linkages in these peatland habitats.

INTRODUCTION

Peatlands form in cool climates where water input exceeds evaporation, and where deep formations of peat (poorly decomposed mosses and sedges) accumulate due to stagnant high water tables and slow decomposition rates (MacKenzie and Moran, 2003). In British Columbia, peatlands can be divided into two site classes: the Bog Wetland Class (Wb) which, with its highly acidic, nutrient and oxygen poor soils, supports ericaceous shrubs and coniferous trees, and the Fen Wetland Class (Wf) that is dominated by sedges and non-ericaceous shrubs (e.g. scrub birch (*Betula glandulosa*)) which grow in less acidic, minerotrophic soils (MacKenzie and Moran, 2003). In peatland ecosystems, growth of flood tolerant vegetation such as tamarack (*Larix laricina*) and black spruce (*Picea mariana*), is often stunted and slow (Payandeh, 1973; Lieffers and Rothwell, 1986 and 1987; MacDonald and Lieffers, 1990). Recent investigations into some of the mechanisms for the survival and growth of trees in these systems have shown that tamarack is more resistant to flooding damage (Islam and MacDonald, 2003), and conserves foliar nutrients more efficiently than black spruce (Tyrell and Boerner, 1987). However, few studies have explored the possible role of specialized plant-fungal relationships (ectomycorrhizas) in peatland environments.

Ectomycorrhizal fungi have developed a symbiotic relationship with plant roots; these symbioses facilitate the uptake of water and nutrients by the fungi in exchange for carbon from the host plant (Harley and Smith, 1983). Ectomycorrhizal fungi play an important role in forest communities where they provide protection to roots from soil pathogens and diseases (Duchesne, 1994; Schelkle and Peterson, 1996; Ursic et al., 1997; Morin et al., 1999), aid in nutrient cycling (Smith and Read, 1997), and can increase host

plant tolerance to environmental stress (Anderson 1988; Colpaert and Van Tichelen, 1994). Most interestingly, fungal symbionts can be shared by different plant species, as well as by neighboring plants of the same species, and these fungi can translocate nutrients between hosts, thereby linking hosts through underground mycelial networks of fungal hyphae (Björkman, 1960; Finlay and Read, 1986; Dahlberg and Stenlid, 1990; Simard et al., 1997b; McKendrick et al., 2000). These fungal linkages allow nutrients to be cycled between hosts, and such interactions may positively impact and reduce competition for soil resources (Newman, 1988; Finlay, 1989; Horton and Bruns, 1998).

Many wetland plants, such as woody plants (Thormann et al., 1999), willows (Marshall and Pattullo, 1981), and some aquatic grasses, sedges and herbaceous plants (Turner and Friese, 1998; Miller, 1999; Turner et al., 2000) are mycorrhizal. These symbiotic associations may be important to the trees and shrubs that occur in peatland ecosystems where wet, poorly aerated soils may impede plant growth and root formation (Lieffers and Rothwell, 1986). It has been hypothesized that genus-specific ectomycorrhizal fungi may enable tamarack roots to take up a greater amount of nutrients compared to other wetland species, thereby increasing the survival and growth rate of this species (Tyrell and Boerner, 1987). Mycelial networks could also allow the transport of carbon across resource gradients between host species in nutrient poor environments (Tilman et al., 1996; Simard et al., 1997b), such as fens and bogs. Jones et al. (1997) determined (through morphological investigation) that a high potential for carbon or nutrient transfer through hyphal linkages exists between paper birch (*Betula papyrifera*) and Douglas-fir (*Pseudotsuga menziesii*). However, the potential for, and the role of,

ectomycorrhizas and mycelial networks in peatland ecosystems has not been documented.

Tamarack and scrub birch are common plant species in certain fen and bog site associations in British Columbia. Several studies have attempted to identify some of the fungal symbionts associated with tamarack; these are mostly from *in vitro* inoculation trials (Samson and Fortin, 1986; Molina and Trappe, 1982; Danielson, 1984; LeTacon, 1986). Interestingly, the genus *Larix* has been found to associate with several genus-specific fungi (*Suillus grevillei* and *S. cavipes*) (Molina et al., 1992) and some *Larix* species have been shown to be ectendomycorrhizal with E-strain fungi (Laiho, 1965; Malloch and Malloch, 1981; Danielson, 1984). Less is known about the mycorrhizal associations of scrub birch; however, the fungal symbionts identified for swamp birch (*Betula nana*) growing in the subalpine tundra of Alaska included *Amanita*, *Lactarius*, *Russula* species, as well as several other genera (Miller, 1982). Little is known about the ectomycorrhizal communities associating with tamarack and scrub birch in natural peatland ecosystems in British Columbia.

A main objective of this study was to use morphological techniques to characterize the ectomycorrhizal associations of tamarack and scrub birch growing in three different peatland site types in the central interior of British Columbia. The three peatland site types included i) scrub birch dominated, ii) scrub birch-tamarack, and iii) mixed scrub birch-tamarack-black spruce site types. The second objective was to assess differences in the abundance and diversity of the ectomycorrhizal communities associating with the two host species, as well as between site types, and to determine the

potential for fungal linkages, through shared ectomycorrhizal symbionts, between tamarack and scrub birch.

METHODS

Site descriptions

Seedlings were sampled in three peatland areas within the dry, warm subzone variant of the Sub-boreal Spruce (SBSdw3) biogeoclimatic zone, specifically in the Norman Lake area (approximately 40 km west of Prince George) in central British Columbia, Canada (map of study area shown in Appendix I). Ranging in latitude from 51° 30' to 59° N, this zone is characterized by cold, snowy winters and warm, short summers (Meidinger et al., 1991). Scrub birch is found primarily in peatland systems within the SBS zone, most commonly within the Scrub birch – Water sedge (WF02) and Scrub birch - Buckbean - Shore sedge (WF07) Fen Site Associations (MacKenzie and Moran, 2003). Tamarack is considered rare within the SBS zone (Beaudry et al., 1999), but it can be locally common within the Tamarack – Water sedge – Fen moss (WB06) Bog Site Association (MacKenzie and Moran, 2003).

Three peatland site types were selected for study: scrub birch dominated (Bs), scrub birch and tamarack dominated (BsLt), and scrub birch, tamarack, and black spruce (Mix) (Figure 2.1). Two replicate sites were located for each peatland site type, for a total of six sampling sites. Boundaries of each site were determined by changes in the surrounding topography and vegetation. Sites were located a distance (>25 m) from

access roads to minimize airborne particulate matter, run-off, and other disturbance effects.

Table 2.1. Summary of replicate peatland sites and number of plants sampled for two hosts, tamarack and scrub birch.

Site type*	Tamarack	Scrub birch
Bs1	-	6
Bs2	-	6
BsLt1	6	6
BsLt2	6	6
Mix1	6	6
Mix2	6	6
Total	24	36

*Bs (scrub birch), BsLt (scrub birch and tamarack), Mix (scrub birch, tamarack, and black spruce). Note: All sites were located near the Norman Lake Road west of Prince George, access from Highway 16.

The Bs peatland site type was characterized by scrub birch and *Salix* spp. (willow) as the dominant shrub species, with sporadic and disparate *Pinus contorta* Dougl. Ex Loud. var. *latifolia* Engelm. (lodgepole pine) and occasional *Picea mariana* (Mill.) B.S.P. (black spruce) trees in < 1% of the site area. This site type also consisted of several dwarf shrubs, such as *Vaccinium oxycoccos* L. MacM. (bog cranberry), *Andromeda polifolia* L. (bog-rosemary), *Ledum groenlandicum* Oeder (Labrador tea), and *Rubus arcticus* L. (dwarf nagoonberry). Flowering herbaceous plants were absent from this site type, with the exception of *Potentilla palustris* (L.) Scop. (marsh cinquefoil). *Carex rostrata* Stokes and *C. interior* L.H. Bailey (beaked and inland sedge), as well as *Triglochin maritimum* L. (sea side arrow grass), were common sedge and grass species. The moss layer consisted of *Aulacomnium palustre* (Hedw.) (Schwaegr) (glow moss), *Sphagnum* spp. (peat moss), and *Tomenthypnum nitens* (Hedw.) Loeske (golden fuzzy fen moss).

The BsLt wetland site type was dominated by tamarack and scrub birch, as well as willow species; however, the only dwarf shrub present was Labrador tea. Sporadic lodgepole pine and black spruce trees occurred in < 1% of the site area. The presence of two orchid species, *Platanthera dilatata* (Pursh) Lindl. *ex* Beck and *P. hyperborea* (L.) Lindley (white and northern green bog orchids), were unique to this site type. Other flowering herbaceous plants included marsh cinquefoil, *Galium* spp. (bedstraw), and *Pyrola asarifolia* Michx. (pink wintergreen). All the sedge and grass species listed in the Bs site type were also found in the BsLt site type, with the addition of *Equisetum hyemale* L. (scouring rush). The moss layer consisted of glow moss, golden fuzzy fen moss and *Mnium* spp. (leafy moss), with a notable reduction in the amount of *Sphagnum* spp.

The third wetland site type, Mix, consisted of a dominant mixture of black spruce, tamarack and scrub birch. Dwarf shrubs included bog cranberry, bog-rosemary, *Kalmia microphylla* (bog-laurel), Labrador tea and dwarf nagoonberry. *Petasites sagittatus* (Banks x Pursh) A. Gray (arrow-leaved coltsfoot), *Menyanthes trifoliata* L. (buckbean), *Mitella nuda* L. (common mitrewort) and *Drosera rotundifolia* L. (round-leaved sundew) were unique herbaceous plants to this site type; Mix sites also contained bedstraw, white bog orchid, marsh cinquefoil, and pink wintergreen. Many of the common grass and sedge species on the other sites were also found here, such as beaked sedge, narrow-leaved cotton grass, scouring rush and sea-side arrow grass. Glow moss, peat moss and golden fuzzy fen moss were common in the moss layer, along with *Campylium stellatum* (Hedw.) Jens. (golden star moss). Figure 2.1 shows images of the three peatland site types, as well as plants and fungi found on those sites.

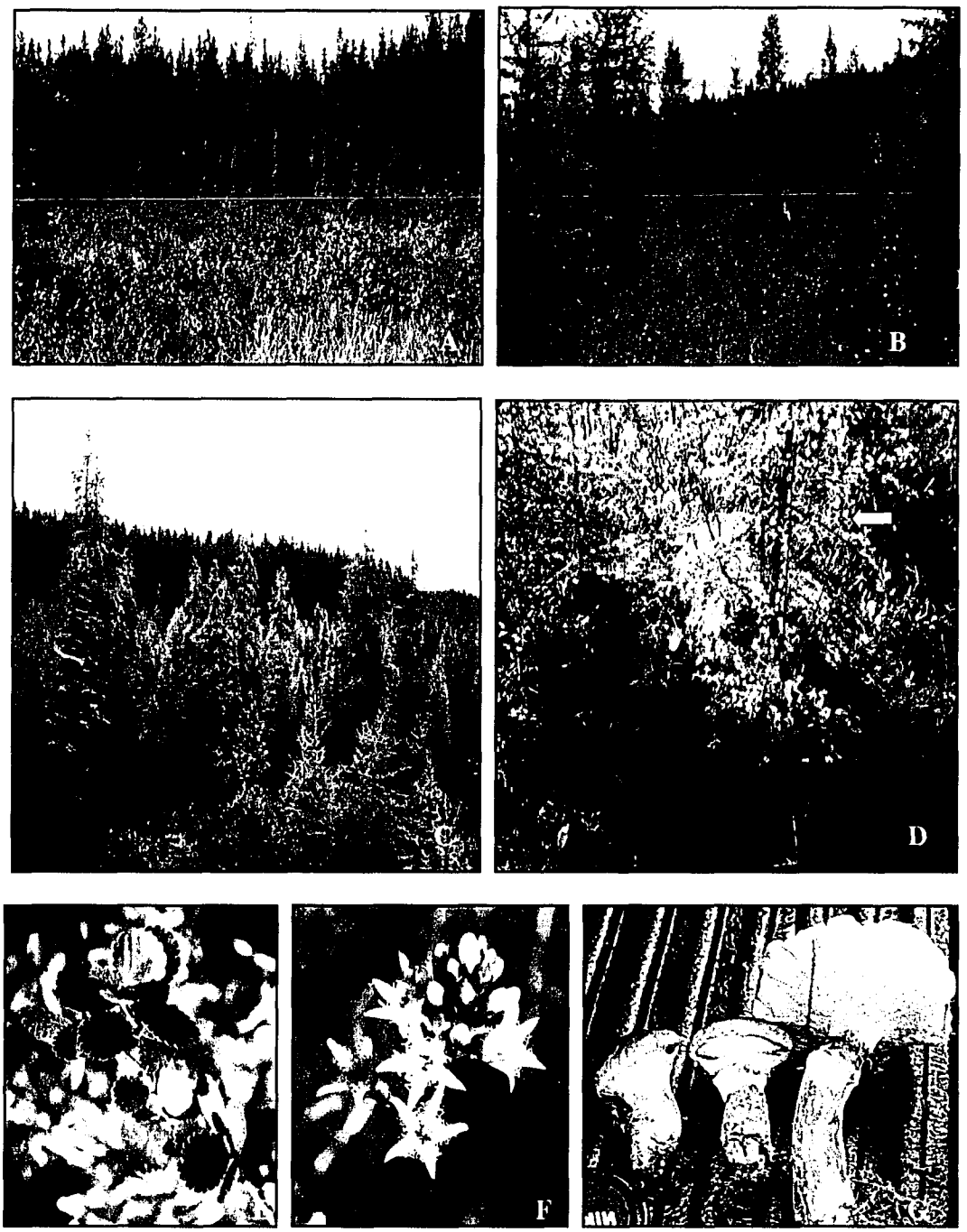

Figure 2.1. Photographs showing the three peatland site types in central BC selected for this study, local vegetation, and fungi. A) Bs peatland site type of scrub birch. (B) BsLt peatland site type of scrub birch and tamarack. (C) Mix peatland site type of scrub birch, tamarack and black spruce. (D) *Sphagnum* covered hummock in peatland with tamarack seedling. (E) scrub birch (*Betula glandulosa*). (F) buckbean (*Menyanthes trifoliata*). (G) larch suillus (*Suillus grevillei*).

Seedling sampling regime

Harvesting of entire plants with root systems occurred during the last week of July (2002) and the first week in August using a simple random sampling technique. In the interior of each site, a 50 x 50 m plot was established. In the BsLt and Mix sites, each tamarack seedling (between 15-30 cm in height) was flagged and numbered. Using a random number table, six tamarack seedlings were selected from each site. Due to the large number of birch plants present within all the sites, a 1 x 1 m grid sampling system was established in which each grid square was assigned a number. A birch plant (between 15-30 cm in height) was harvested if it was growing within a grid square (grid squares were chosen using a random number table). Six scrub birch plants were selected from each site. Tamarack plants that appeared to be layered or attached to older "parent" trees were eliminated from the selection process. Plants were harvested using a pruning saw (to cut through the peat moss and surrounding roots); organic matter was removed with each root system to minimize root disturbance. Plants were placed into 7 L plant pots, double bagged in plastic bags, and stored at 5°C until processing. During root assessment, several tamarack seedlings had few root tips and appeared to be layered seedlings. These seedlings were replaced in mid-September in an effort to assess only single seedlings.

Vegetation plot analysis and sporocarp sampling

To document vegetation growing on peatland sites, each site was divided into four quadrants and, within each, a representative 1 x 1 m vegetation plot was established. Bryophytes, herbaceous plants, shrubs and trees were identified and recorded (Appendix

II). All tree species that did not fall within the 1 m^2 plots were visually assessed throughout the entire site.

Epigeous sporocarps were collected during the summer months within all six sites. Sporocarp samples were collected throughout each entire site, placed in paper bags, and transported to the laboratory for identification. Sporocarp characteristics, such as shape, colour, size, and odour, as well as spore features, were described. Samples were identified to the closest family, genus, or species, which ever was possible. Sporocarp tissue (approximately 0.5 x 0.5 mm) was collected from the pileus and spore producing area and stored in sterile 1.5 ml microtubes at -20°C for later molecular analysis. Sporocarps were then dehydrated and kept as reference material.

Morphological characterization of ectomycorrhizas

All extraneous soil and organic matter (moss, herbaceous material, etc.) was gently removed from each root system through sequential soaking and rinsing with water. Shoots were removed and the remaining roots were cut into 2 cm lengths and placed on a numbered 1 cm^2 grid for random sampling. Two-hundred root tips were randomly selected for microscopic characterization (Massicotte et al., 1994; Durall et. al., 1999). A total of 60 plants were assessed; 24 tamarack and 36 scrub birch.

Ectomycorrhizal root tips were characterized using light microscopy following methods described by Ingleby et al. (1990), Massicotte et al. (1999), Agerer (1987-2000), Goodman et al. (1996), and Mah et al. (2001). Characteristics such as branching pattern, tip shape, colour, and texture, as well as inner and outer mantle patterns, depth of mantle and presence of a Hartig net were described. The presence and type of cystidia,

emanating hyphae, and rhizomorphs were determined. Each different type of ectomycorrhiza was tested for a reaction to 5% KOH. Representative permanent slides were made for some of the morphotypes. Characterized ectomycorrhizas were classified into morphotypes and given a family, genus or species name; if this was not possible, morphotypes were assigned a descriptive name based on their morphological features. To document certain morphotypes, photographs were taken with an automatic exposure camera (PM-10AK) attached to a compound (Olympus BX-50) or dissecting (Olympus SZ-40) microscope using Ektachrome 160T tungsten professional colour reversal film. The total number of morphotypes, as well as the number of root tips exhibiting each morphotype, was determined for each seedling.

Statistical analysis of morphological data

Morphotype descriptions were reviewed prior to data analysis; this resulted in merging several morphotypes that could not be separated by descriptive characteristics alone. The number of ectomycorrhizal morphotypes and their proportional abundance (percent of each morphotype) on the root system were calculated for each seedling. The seedling values were used to determine frequency of occurrence and morphotype mean abundance for each peatland site type. For each host, tamarack and scrub birch, a one-way ANOVA (Statistica version 6.1, 2002, StatSoft, Inc.) using morphotype abundance data, was used to assess differences between the peatland site types in which each host occurred ($\alpha = 0.05$). On the sites where the two hosts co-occurred, site type and host differences based on morphotype abundance were determined by a two-way ANOVA (α

= 0.05). The post hoc Fisher's Least Significant Difference (LSD) test (α = 0.05) was used to test mean comparisons.

To assess peatland site type diversity, the Margalef index (measure of species richness), the Shannon and Shannon evenness diversity index (considers both species richness and evenness), and the Simpson index (which places more weight on those morphotypes that are most abundant) were used (Magurran, 1988). Diversity values where calculated for each seedling based on the proportional abundance of each ectomycorrhizal morphotype and the number of morphotypes per seedling. These values were used to calculate diversity indices. For each host, a one-way ANOVA was used to determine peatland site types effects on diversity. On sites where the two hosts co-occurred, a two-way ANOVA was used to determine site type and host effects on diversity (α = 0.05). The Fisher's Least Significant Difference test (α = 0.05) was used to test mean comparisons.

RESULTS

Ectomycorrhiza morphotype richness, frequency and abundance

A total of 30 ectomycorrhizal morphotypes (excluding the lightly colonized category) were characterized from 11,600 root tips on 58 seedlings (Figure 2.2). Of these, 24 morphotypes were described from 34 scrub birch seedlings (two seedlings were eliminated due to very low root tip numbers), and 23 morphotypes were described from 24 tamarack seedlings. Seventeen of the 30 morphotypes were common on both host

species, seven were unique to scrub birch and six were unique to tamarack. Complete morphological descriptions of these morphotypes are presented in Appendix III and several images detailing distinct features are shown in Fig. 2.3.

The mean number of morphotypes for each host, within each peatland site type, is presented in Table 2.2. The number of morphotypes varied significantly between site types for scrub birch ($p = 0.051$), but not for tamarack ($p = 0.06$); for both host species, the greatest number of morphotypes occurred on seedlings from the Mix site (scrub birch, tamarack, and black spruce). The BsLt sites (scrub birch and tamarack) exhibited the lowest morphotype richness for both hosts.

Table 2.2. Mean number of ectomycorrhizal morphotypes (SE in parenthesis) for tamarack and scrub birch seedlings growing in three peatland site types: Bs (birch dominated), BsLt (scrub birch-tamarack), and Mix (scrub birch-tamarack-black spruce).

Host	F	P	BsLt	Mix	Bs
Tamarack	3.934	0.060	4.5 (0.4)	5.6 (0.4)	-
Scrub birch	3.288	**0.051**	3.6 (0.4)b	5.5 (0.7)a	4.2 (0.4)a

Morphotype richness values were tested using a one-way ANOVA for peatland site types ($\alpha = 0.05$) (tamarack df = 1,22) (birch df = 2,31). Note: Within rows, means followed by the same letter are not significantly different.

Lightly colonized root tips (those lacking distinguishable mantle features) represented 1.3% ($n = 85$) of all roots sampled for scrub birch; these occurred on seedlings more frequently in the Mix peatland site type (27.3% of seedlings), compared to the Bs (8.3%) and BsLt (0.0%) sites (Table 2.3). In contrast, 25.9% ($n = 1241$) of all tamarack roots were described as lightly colonized; these also occurred more frequently on seedlings in the Mix peatland site type (83.3% of seedlings), compared to the BsLt (50.0%) site (Table 2.4).

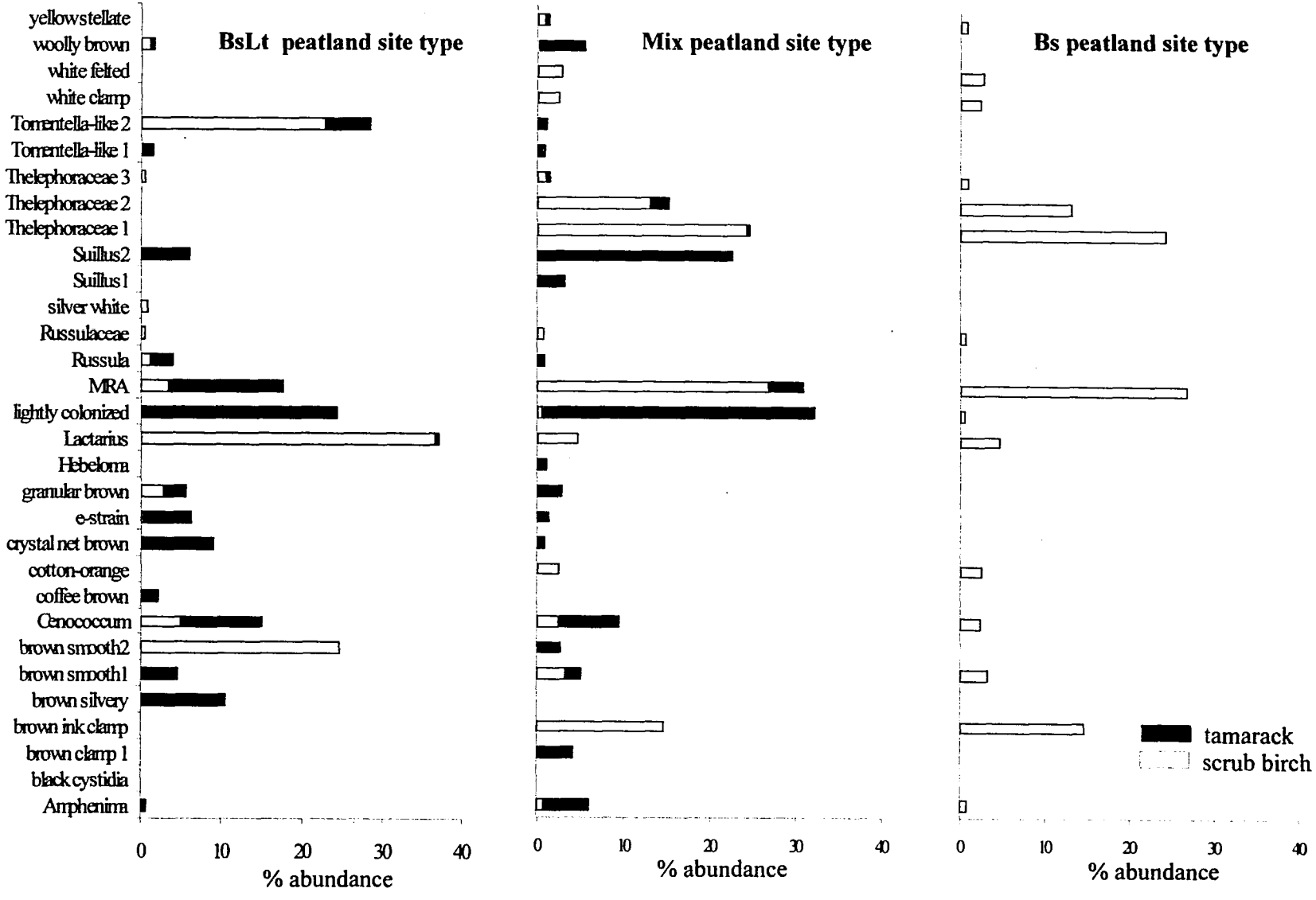

Figure 2.2. Comparison of ectomycorrhizal morphotype abundance of tamarack and scrub birch between the BsLt, Mix, and Bs peatland site types.

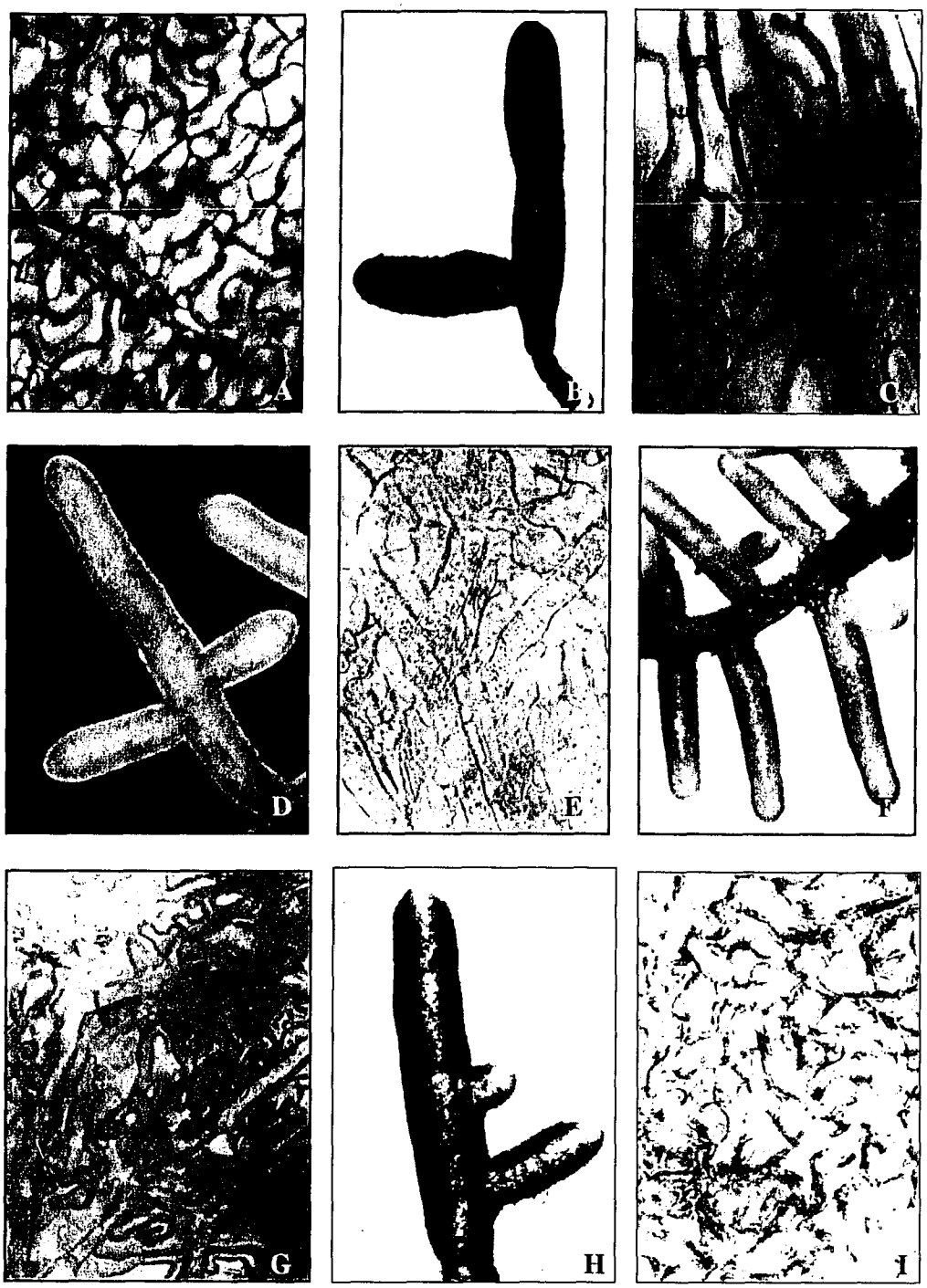

Figure 2.3. Photographs showing ectomycorrhizal morphotypes from tamarack and scrub birch. A, B, C, D, E, and F ectomycorrhizas on scrub birch, and G, H, and I ectomycorrhizas on tamarack. (A) *Tomentella*-like 2 outer mantle (OM). (B) *Tomentella*-like 2 ectomycorrhizal root tip. (C) E-strain OM with enlarged hyphal cells. (D) *Lactarius* ectomycorrhiza. (E) *Lactarius* OM with laticifers. (F) *Lactarius* root showing crystal-like deposits. (G) *Suillus* 2 OM (H) *Tomentella*-like 1 ectomycorrhizal root tip. (I) *Tomentella*-like 1 OM.

Scrub birch (*Betula glandulosa*) ectomycorrhizas

Of the 24 morphotypes characterized from scrub birch, 20 morphotypes were found in the Mix peatland site type, 14 morphotypes in the BsLt site type, and 16 morphotypes in the Bs site type. Seven morphotypes were common to all three peatland site types, and two morphotypes were unique to each of the site types.

The 13 most commonly occurring ectomycorrhizal morphotypes (found on four or more seedlings) belonged to the family Thelephoraceae, or to the genera *Lactarius*, *Tomentella*, *Cenococcum*, and *MRA* (Table 2.3). Four morphotypes (brown inky clamp, granular brown, brown smooth 2, and woolly brown) could not be assigned to a family. *Mycelium radicis atrovirens* was the most frequently occurring morphotype; it was found on 41.2% of all scrub birch seedlings and in all the site types. Other frequently occurring morphotypes, Thelephoraceae 2 (38.2% of seedlings) and brown inky clamp (29.5%) were absent from the BsLt sites; *Tomentella*-like 2 (38.2%), brown smooth 2 (32.4%) and granular brown (26.5%) were absent from the Bs sites. Interestingly, *Cenococcum* (20.6%), *Lactarius* (38.2%), Thelephoraceae 1 (29.5%), Thelephoraceae 3 (23.5%), and woolly brown (14.7%) were present in all site types (Table 2.3).

Table 2.3. Site effect, percent abundance (mean ±SE in parenthesis) and frequency of occurrence (%) of ectomycorrhizal morphotypes of scrub birch growing in three peatland site types.

			Mix (n = 11)		BsLt (n = 11)		Bs (n = 12)	
Morphotype	**F**	**P**	**Abundance**	**Freq**	**Abundance**	**Freq**	**Abundance**	**Freq**
Amphinema	1.116	0.340	1.4 (0.8)	27.3	0.0 (0.0)	0.0	0.7 (0.7)	8.3
black cystidia	1.049	0.363	1.0 (1.0)	9.1	0.0 (0.0)	0.0	0.0 (0.0)	0.0
brown inky clamp	1.845	0.175	16.4 (6.7)	45.5	0.0 (0.0)	0.0	14.6 (8.8)	41.7
brown smooth 1	1.572	0.224	0.0 (0.0)	0.0	0.0 (0.0)	0.0	3.2 (2.4)	16.7
brown smooth 2	4.290	**0.023**	10.6 (6.3)*ab*	36.4	24.6 (7.9)*a*	63.6	0.0 (0.0)*b*	0.0
Cenococcum	0.636	0.536	9.0 (5.4)	27.3	4.9 (4.6)	18.2	2.4 (2.1)	16.7
cotton orange	2.314	0.116	0.0 (0.0)	0.0	0.0 (0.0)	0.0	2.4 (1.5)	25.0
crystal net brown	1.049	0.363	0.2 (0.2)	9.1	0.0 (0.0)	0.0	0.0 (0.0)	0.0
E-strain	1.046	0.363	0.0 (0.0)	0.0	0.4 (0.4)	9.1	0.0 (0.0)	0.0
granular brown	2.653	0.086	5.7 (2.5)	45.5	2.8 (1.8)	36.4	0.0 (0.0)	0.0
Lactarius	5.251	**0.011**	8.4 (4.9)*b*	36.4	36.6 (12.4)*a*	54.5	4.6 (2.5)*b*	25.0
MRA	8.406	**0.001**	1.3 (1.0)*b*	27.3	3.5 (2.0)*b*	27.3	26.7 (7.8)*a*	66.7
Russula	0.716	0.496	0.6 (0.6)	9.1	1.2 (1.0)	18.2	0.0 (0.0)	0.0
Russulaceae	0.501	0.611	0.0 (0.0)	0.0	0.5 (0.5)	9.1	0.7 (0.7)	8.3
silver white	0.517	0.602	0.7 (0.5)	18.2	0.8 (0.8)	9.1	0.1 (0.1)	8.3
Thelephoraceae 1	11.26	**0.000***	3.9 (2.6)*b*	27.3	0.2 (0.2)*b*	9.1	24.2 (5.9)*a*	75.0
Thelephoraceae 2	3.070	0.061	7.3 (3.8)	45.5	0.0 (0.0)	0.0	13.1 (5.1)	41.7
Thelephoraceae 3	2.910	0.069	5.8 (2.9)	36.4	0.5 (0.5)	9.1	0.9 (0.7)	25.0
Tomentella-like 1	2.227	0.125	0.0 (0.0)	0.0	0.2 (0.2)	18.2	0.0 (0.0)	0.0
Tomentella-like 2	6.100	**0.006**	7.4 (3.4)*ab*	45.5	22.8 (7.7)*a*	72.7	0.0 (0.0)*b*	0.0
white clamp	0.779	0.467	8.6 (8.6)	9.1	0.0 (0.0)	0.0	2.4 (2.0)	16.7
white felted	0.609	0.550	1.6 (1.4)	18.2	0.0 (0.0)	0.0	2.8 (2.6)	16.7
woolly brown	3.068	0.061	6.6 (3.3)	36.4	1.0 (1.1)	9.1	0.2 (1.7)	8.3
yellow stellate	1.081	0.352	0.2 (0.2)	9.1	0.0 (0.0)	0.0	0.8 (0.6)	16.7
lightly colonized	2.574	0.092	3.4 (1.9)	27.3	0.0 (0.0)	0.0	0.5 (0.5)	8.3

* = 0.0001

Abundance values were assessed using a one-way ANOVA to test for site differences ($\alpha = 0.05$). Fisher's Least Significant Difference (LSD) test was used to test mean comparisons. Across each row, means followed be the same letter are not significantly different.

Significant differences in the abundance of some morphotypes occurred between the three peatland site types (Table 2.3). *Mycelium radicis atrovirens* ($p = 0.001$) and Thelephoraceae 1 ($p = 0.0001$) were most abundant in the Bs site type and least abundant

in the Mix and BsLt site types, respectively. In contrast, brown smooth 2 ($p = 0.023$), *Lactarius* ($p = 0.011$), and *Tomentella*-like 2 ($p = 0.006$) were most abundant in the BsLt site type, and least in the Bs site type. Several other morphotypes occurred in some peatland site types, but not in others. Granular brown was abundant in the Mix and BsLt site type, but absent in the Bs site type, and Thelephoraceae 2 and brown inky clamp were frequently identified in the Mix and Bs site types, and absent in the BsLt site type. The remaining less common or rarely occurring morphotypes (found on less than 4 seedlings) tended to be found in only one or two of the peatland site types.

Tamarack (*Larix laricina*) ectomycorrhizas

Of the 23 morphotypes characterized from tamarack, 21 morphotypes were found in the Mix peatland site type, and 16 morphotypes in the BsLt site type. The 13 most common morphotypes (occurring on four or more seedlings) on tamarack included ectomycorrhizas in the genera *Suillus*, *Amphinema*, *Tomentella*, MRA, and *Cenococcum* (Table 2.4), as well as several morphotypes that could not be assigned to a family or genus (brown silvery, woolly brown, brown smooth 1 and crystal net brown). All commonly occurring morphotypes were found in both the Mix peatland site type as well as the BsLt site type; however, *Suillus* 2 and *Cenococcum* were identified most frequently on all tamarack seedlings (58% and 38%, respectively). Crystal-net brown, brown silvery, *MRA*, and *Tomentella*-like 2 were more abundant (although not significant) in the BsLt site type, than the Mix site type. In contrast, *Suillus* 2 ($p = 0.041$), *Cenococcum*, woolly brown, and *Amphinema* were more abundant in the Mix site type compared to the

BsLt site type. The remaining less common, or rarely seen morphotypes were mostly described from the Mix site type (Table 2.4).

Table 2.4. Site effect, percent abundance (mean ±SE in parentheses) and frequency of occurrence (%) of ectomycorrhizal morphotypes of tamarack growing in two peatland site types.

			Mix (n = 12)		BsLt (n = 12)	
Morphotype	**F**	**P**	**Abundance**	**Freq**	**Abundance**	**Freq**
Amphinema	3.415	0.078	5.3 (2.4)	41.7	0.7 (0.66)	8.3
brown clamp	1.114	0.303	4.2 (4.0)	16.7	0.0 (0.0)	0.0
brown silvery	3.289	0.083	0.0 (0.0)	8.3	10.4 (5.7)	41.7
brown smooth 1	0.489	0.492	1.9 (1.9)	8.3	4.5 (3.1)	33.3
brown smooth 2	1.000	0.328	2.7 (2.7)	8.3	0.0 (0.0)	0.0
Cenococcum	0.153	0.699	7.1 (2.7)	50.0	10.2 (7.3)	25.0
coffee brown	1.000	0.328	0.0 (0.0)	0.0	2.1 (2.1)	8.3
crystal net-brown	2.202	0.152	0.8 (0.5)	16.7	8.9 (5.5)	41.7
E-strain	1.836	0.189	1.3 (0.9)	16.7	5.8 (3.2)	25.0
granular brown	0.000	0.989	2.8 (1.7)	25.0	2.8 (2.7)	16.7
Hebeloma-like	2.156	0.156	1.0 (0.6)	25.0	0.0 (0.0)	0.0
Lactarius	1.000	0.328	0.0 (0.0)	0.0	0.5 (0.5)	8.3
MRA	1.697	0.206	4.1 (2.7)	25.0	14.1 (7.1)	33.3
Russula	0.654	0.427	0.8 (0.6)	25.0	2.7 (2.3)	25.0
Suillus 1	1.231	0.298	3.1 (3.1)	8.3	0.0 (0.0)	0.0
Suillus 2	4.732	**0.041**	22.6 (7.3)*a*	66.7	6.0 (2.2)*b*	50.0
Thelephoraceae 1	3.211	0.087	0.4 (0.3)	25.0	0.0 (0.0)	0.0
Thelephoraceae 2	2.163	0.156	2.2 (1.5)	16.7	0.0 (0.0)	0.0
Thelephoraceae 3	3.564	0.072	0.5 (0.2)	25.0	0.0 (0.0)	0.0
Tomentella-like 1	0.216	0.647	0.8 (0.6)	25.0	1.2 (0.6)	33.3
Tomentella-like 2	1.904	0.181	1.0 (0.7)	16.7	5.5 (3.2)	41.7
woolly brown	2.805	0.108	5.2 (2.8)	33.3	0.5 (0.5)	8.3
yellow stellate	1.000	0.328	0.5 (0.5)	8.3	0.0 (0.0)	0.0
lightly colonized	0.375	0.546	31.7 (8.3)	83.3	24.3 (8.9)	50.0

Abundance values were assessed using a one-way ANOVA to test for site differences ($\alpha = 0.05$).

Amongst the 17 shared morphotypes between tamarack and scrub birch, five of these were identified as commonly occurring on both host species (i.e. *Cenococcum*, granular brown, MRA, *Tomentella*-like 2, and woolly brown). Four others were only

common on tamarack (*Amphinema*, brown smooth 1, crystal net brown, and *Tomentella*-like 1), and five were only common on scrub birch (*Lactarius*, Thelephoraceae 1, Thelephoraceae 2, Thelephoraceae 3, and brown smooth 2); three were uncommon, or rare, for both host species (*Russula*, E-strain, and yellow stellate). Interestingly, the most abundant morphotype found on tamarack, *Suillus* 2, was never found on any of the scrub birch seedlings. All shared morphotypes were present on both hosts in at least one of the two peatland site types in which they co-occurred (with the exception of brown smooth 1 that was found only on scrub birch in the Bs site type). The majority of shared morphotypes were found on tamarack and scrub birch in the Mix site type.

Table 2.5 shows the site, host, and interaction effects for the percent abundance of 15 shared morphotypes between tamarack and scrub birch. Several morphotypes had significant site and host differences. *Amphinema* ($p = 0.034$), Thelephoraceae 2 ($p = 0.025$), and woolly brown ($p = 0.025$) morphotypes were significantly more abundant in the Mix site type, than in the BsLt site type. Thelephoraceae 3 was also more abundant in the Mix site type, but the difference was not significant (Table 2.6). *Tomentella*-like 2 ($p = 0.044$) was the only shared morphotype significantly more abundant in the BsLt site type; *Lactarius* was also more abundant in this site, although not significant ($p = 0.069$) (Table 2.6).

Table 2.5. Two-way ANOVA showing site (BsLt and Mix), host (scrub birch and tamarack) and interaction effects based on mean percent abundance of 15 shared ectomycorrhizal morphotypes ($\alpha = 0.05$, df = 1, 42).

	Site Effect		Host Effect		Host*Site	
Morphotype	**F**	**P**	**F**	**P**	**F**	**P**
Amphinema	4.786	**0.034**	2.814	0.101	1.435	0.238
brown smooth 2	1.125	0.295	9.382	**0.004**	2.478	0.123
Cenococcum	0.010	0.923	0.103	0.750	0.451	0.505
crystal net brown	1.923	0.173	2.717	0.107	2.100	0.155
E-strain	2.816	0.101	2.136	0.152	1.729	0.196
granular brown	0.381	0.540	0.394	0.534	0.407	0.527
Lactarius	5.068	**0.030**	12.300	**0.001**	4.749	**0.035**
MRA	2.145	0.150	2.609	0.114	0.886	0.352
Russula	0.789	0.380	0.376	0.543	0.261	0.612
Thelephoraceae 1	2.816	0.101	2.136	0.152	1.729	0.196
Thelephoraceae 2	5.795	**0.021**	1.632	0.208	1.632	0.208
Thelephoraceae 3	4.101	**0.049**	4.299	**0.044**	2.914	0.095
Tomentella-like 1	0.481	0.492	3.699	0.061	0.033	0.857
Tomentella-like 2	5.194	**0.028**	7.332	**0.010**	1.553	0.220
woolly brown	5.220	**0.027**	0.191	0.665	0.032	0.859

Note: Mean percent abundance were tested using a 2-way ANOVA. Brown smooth 1 was not included in the analysis since the morphotype only occurred in the Bs peatland site type and yellow stellate was not included due to low abundance values.

With respect to host differences, brown smooth 2 ($p = 0.004$), *Lactarius* ($p = 0.002$), *Tomentella*-like 2 ($p = 0.013$), and Thelephoraceae 3 ($p = 0.055$) morphotypes were all significantly more abundant on scrub birch compared to tamarack when host abundance values were pooled for peatland sites types (Table 2.7). *Tomentella*-like 1 was also more abundant on tamarack ($p = 0.057$) compared to scrub birch. One interaction effect was observed for *Lactarius* ($p = 0.035$) (Table 2.5); this was possibly due to its dominance in the BsLt peatland site type and on scrub birch, since it was only detected on one tamarack seedling.

Table 2.6. One-way ANOVA showing site (BsLt and Mix) differences for percent abundance (mean ±SE) of 15 shared ectomycorrhizal morphotypes ($\alpha = 0.05$, df = 1, 44).

Morphotype	P	BsLt	Mix
Amphinema	**0.034**	0.340 (0.3)	3.430 (1.4)
brown smooth 2	0.374	11.758 (4.8)	6.498 (3.4)
Cenococcum	0.945	7.651 (4.4)	8.014 (2.9)
crystal net brown	0.167	4.633 (2.9)	0.475 (0.3)
E-strain	0.169	3.178 (1.7)	0.685 (0.5)
granular brown	0.552	2.836 (1.6)	4.164 (1.5)
Lactarius	0.069	17.731 (6.9)	4.034 (2.5)
MRA	0.145	9.015 (3.9)	2.778 (1.5)
Russula	0.360	1.938 (1.3)	0.698 (0.4)
Thelephoraceae 1	0.119	0.086 (0.1)	2.087 (1.3)
Thelephoraceae 2	**0.025**	0.000 (0.0)	4.648 (2.0)
Thelephoraceae 3	0.072	0.250 (0.3)	3.028 (1.5)
Tomentella-like 1	0.494	0.732 (0.4)	0.413 (0.3)
Tomentella-like 2	**0.044**	13.795 (4.4)	4.046 (1.8)
woolly brown	**0.025**	0.776 (0.6)	5.841 (2.1)

Note: Brown smooth 1 was not included in the analysis since this morphotype only occurred in the Bs peatland site type and yellow stellate was not included in analysis due to abundance values.

Table 2.7. One-way ANOVA showing host (tamarack and scrub birch) differences for percent abundance (mean ±SE) of 15 shared ectomycorrhizal morphotypes ($\alpha = 0.05$, df = 1, 44).

Morphotype	P	Scrub birch	Tamarack
Amphinema	0.117	0.682 (0.4)	2.992 (1.3)
brown smooth 2	**0.004**	17.600 (5.4)	1.361 (1.4)
Cenococcum	0.745	6.942 (3.5)	8.649 (3.8)
crystal net brown	0.115	0.088 (0.1)	4.815 (2.8)
E-strain	0.675	3.816 (0.2)	2.844 (1.7)
granular brown	0.528	4.237 (1.6)	2.825 (1.6)
Lactarius	**0.002**	22.503 (7.2)	0.229 (0.2)
MRA	0.118	2.406 (1.1)	9.096 (3.9)
Russula	0.539	0.882 (0.6)	1.718 (1.2)
Thelephoraceae 1	0.162	2.027 (1.3)	0.224 (0.1)
Thelephoraceae 2	0.233	3.642 (2.0)	1.117 (0.8)
Thelephoraceae 3	**0.055**	3.179 (1.6)	0.226 (0.1)
Tomentella-like 1	0.057	0.116 (0.1)	0.991 (0.4)
Tomentella-like 2	**0.013**	15.111 (4.5)	3.246 (1.7)
woolly brown	0.675	3.816 (1.8)	2.844 (1.5)

Note: Brown smooth 1 was not included in the analysis since this morphotype only occurred in the Bs peatland site type and yellow stellate was not included in analysis due to abundance values.

Ectomycorrhizal community diversity

According to all diversity indices, ectomycorrhizal community diversity was highest in the Mix sites for both host species. For scrub birch, ectomycorrhizal diversity decreased from the Mix, to the Bs sites, with the lowest diversity occurring in the BsLt peatland site type (Table 2.8). The Simpson index showed significant differences ($p = 0.020$) between the peatland site types for this host species; the Shannon index also showed strong differences, although these were not significant. No significant differences in ectomycorrhizal diversity were detected between peatland site types for

tamarack, although all indices suggested that diversity was greater in the Mix compared to the BsLt peatland site types (Table 2.9).

Table 2.8. One-way ANOVA for diversity indices (Margalef, Shannon Evenness, Shannon, and Simpson) comparing peatland site types for scrub birch ($\alpha = 0.05$, df = 2, 31).

Diversity Index	F	P	Bs	BsLt	Mix
Margalef	2.301	0.117	0.602 (0.085)	0.489 (0.057)	0.777 (0.129)
Shannon Evenness	0.403	0.672	0.718 (0.075)	0.644 (0.077)	0.737 (0.077)
Shannon	3.108	0.059	1.046 (0.120)	0.764 (0.094)	1.209 (0.157)
Simpson	4.446	**0.020**	2.774 (0.285)*ab*	1.932 (0.185)*b*	3.265 (0.431)*a*

Diversity values are means (±SE in parentheses). Fisher's Least Significant Difference (LSD) test was used to determine where significant differences between means occurred. Means followed be the same letter are not significantly different.

Table 2.9. One-way ANOVA for diversity indices (Margalef, Shannon Evenness, Shannon, and Simpson) comparing peatland site types for tamarack ($\alpha = 0.05$, df = 1, 22).

Diversity Index	F	P	BsLt	Mix
Margalef	2.235	0.166	0.594 (0.088)	0.813 (0.115)
Shannon Evenness	1.829	0.206	0.635 (0.087)	0.698 (0.071)
Shannon	0.208	0.658	0.896 (0.137)	1.138 (0.136)
Simpson	0.157	0.700	2.396 (0.330)	3.497 (0.633)

Diversity values are means (±SE in parentheses).

When ectomycorrhizal diversity indices were assessed for scrub birch and tamarack on sites where they co-occurred, ANOVA showed significant site effects (Table 2.10). Species richness (Margalef Index, $p = 0.029$), as well as the Shannon ($p = 0.021$) and Simpson Indices ($p = 0.011$), indicated greater diversity in the Mix compared to the BsLt peatland site type (Table 2.11). Shannon Evenness values were similar between site types. No significant host or interaction effects were detected (Table 2.10).

Table 2.10. Two-way ANOVA for diversity indices (Margalef, Shannon Evenness, Shannon, and Simpson) showing comparison between peatland site types (BsLt and Mix), host (tamarack and scrub birch), and interaction effects ($\alpha = 0.05$, df = 1, 42).

	Site Effect		Host effect		Host*Site	
Diversity Index	**F**	**P**	**F**	**P**	**F**	**P**
Margalef	6.802	**0.013**	1.719	0.197	0.186	0.668
Shannon Evenness	0.780	0.382	0.401	0.530	0.315	0.577
Shannon	7.119	**0.011**	1.040	0.314	1.168	0.286
Simpson	7.272	**0.010**	1.037	0.314	0.156	0.695

Table 2.11. One-way ANOVA for diversity indices (Margalef, Shannon Evenness, Shannon, and Simpson) for combined host species showing comparison between two peatland site types. ($\alpha = 0.05$, df = 1, 44)

Diversity Index	**F**	**P**	**BsLt**	**Mix**
Margalef	5.128	**0.029**	0.544 (0.053)	0.769 (0.084)
Shannon Evenness	1.217	0.276	0.639 (0.057)	0.724 (0.051)
Shannon	5.769	**0.021**	0.832 (0.084)	1.149 (0.102)
Simpson	7.015	**0.011**	2.174 (0.196)	3.308 (0.381)

Diversity values are means (±SE in parentheses) and include values for both tamarack and scrub birch.

DISCUSSION

Ectomycorrhizal morphotype frequency and abundance

This study presents some of the first information available on ectomycorrhizal colonization for scrub birch in peatland ecosystems. It also extends our knowledge on tamarack mycorrhizal associations, as well as on potential fungal linkages in peatland sites. Overall, 30 ectomycorrhizal morphotypes were characterized from the two host species, with 23 and 24 morphotypes found on tamarack and scrub birch, respectively. In similar studies investigating multiple host species, it appears that ectomycorrhizal

species richness can vary considerably. For example, Kranabetter et al. (1999) examined three different conifer seedling species (lodgepole pine (*Pinus contorta* var. *latifolia*), white spruce (*Picea glauca*) and subalpine fir (*Abies lasiocarpa*)) planted on the edges of forest gaps, and found 74 morphotypes, with an average of 52 morphotypes per host species. In contrast, an investigation into the fungal symbionts of Douglas-fir (*Pseudotsuga menziesii*) and paper birch (*Betula papyrifera*) revealed only 11 morphotypes for those two hosts, with seven morphotypes found on both Douglas-fir and paper birch (Simard et al., 1997a). Jones et al. (1997), also studying Douglas-fir and paper birch, identified 43 morphotypes on the two host species three years after outplanting; 26 ectomycorrhizal morphotypes were described on paper birch seedlings, and 32 morphotypes on Douglas-fir seedlings. Interestingly, the number of morphotypes described by Jones et al. (1999) for paper birch is similar to the number found on scrub birch in our study.

Studies investigating single ectomycorrhizal host species also show variation in the number of morphotypes identified. Robertson (2003) described 33 morphotypes on naturally regenerating black spruce (*Picea mariana*) seedlings growing in both peatland and upland habitats. Mah et al. (2001) reported similar species richness, with 24 morphotypes occurring on naturally regenerating and planted hybrid spruce (*Picea glauca* x *engelmannii*) seedlings in disturbed (cut and burned), as well as undisturbed, Sub-boreal Spruce habitats. When non-mycorrhizal hybrid spruce seedlings were out-planted onto a cut block, 15 distinct morphotypes were identified within one year of planting (Hagerman et al., 1999). Japanese larch (*Larix kaempferi*) seedlings that were harvested from a naturally regenerated volcano in Japan exhibited 12 different

ectomycorrhizal morphotypes (Yang et al., 1998). Interestingly, prior to the catastrophic eruption disturbance, the volcano was dominated by an Erman birch (*Betula ermanii*) forest. Reasons for the differences in ectomycorrhizal richness amongst studies could be due to the differences in seedling age, in sample size or intensity, and in host receptivity to fungal species, as well as variation in environmental conditions across the sampling sites (Robertson, 2003). Numbers of characterized ectomycorrhizas and species richness values presented in this study for tamarack or scrub birch growing in peatland environments generally agree with those described by Robertson (2003), Mah et al. (2001) and Jones et al. (1997) for other host species growing in a variety of different habitats in British Columbia.

One of the most abundant and frequently occurring groups of ectomycorrhizal roots was the lightly colonized; some lightly colonized roots occurred on 67% of all tamarack seedlings, but only 15% of scrub birch seedlings. This was especially so for tamarack seedlings in both peatland site types. This group represented a large portion of the ectomycorrhizal community, especially for tamarack, that could not be identified. Many of these roots most likely were weakly colonized examples of the already identified morphotypes, but they could not be distinguished morphologically. Some roots may have been colonized by ectomycorrhizal fungi that were not identified in this study. Robertson (2003) also reported a large portion (66.7%) of black spruce seedlings, harvested from wetland and upland sites, to have some level of non-mycorrhizal or lightly colonized roots. Mah et al. (2001) found lightly colonized roots on almost all hybrid spruce seedlings growing in disturbed and mature forest sites, with approximately 18% of all root tips sampled to be poorly colonized.

Tamarack (*Larix laricina*) morphotype frequency and abundance

Many of the 23 ectomycorrhizal morphotypes described on tamarack might be described as intermediate to broad host ranging fungi (Molina et al., 1991). They included such genera as *Amphinema*, *Cenococcum*, E-strain, members of the Russulaceae (*Russula*), and Thelephoraceae (including *Tomentella*). Some of these fungi were often relatively abundant on tamarack and many have been described on other host species. Robertson (2003) and Mah et al. (2001) identified ectomycorrhizas in these fungal genera/families on black spruce seedlings growing in wetland and upland sites, as well as on hybrid spruce seedlings in disturbed and mature sites, respectively. Jones et al. (1997), in a greenhouse and field bioassay study, and Simard et al. (1997a), in a soil bioassay greenhouse study, also described many of these fungi on paper birch growing in single species monoculture, or in mixed species dual culture, with Douglas-fir. These intermediate or broad host ranging fungal species have the potential to not only contribute substantially to ectomycorrhizal functioning, but also to linkages within forest ecosystems (Massicotte et al., 1999).

Other studies have investigated the ectomycorrhizal fungal symbionts of tamarack, as well as other *Larix* spp., growing in different habitat types. *Cenococcum*, E-strain, *Hebeloma*, *Suillus*, and *Thelephora* were all reported to occur on tamarack, European larch (*Larix decidua*), and/or western larch (*Larix occidentalis*) (Laiho, 1965; Malloch and Malloch, 1981; Molina and Trappe, 1982; LeTacon and Bouchard, 1986; Samson and Fortin, 1986; Thormann et al., 1999). These genera (or closely related members) were identified on tamarack roots from our study.

The most abundant and frequently occurring morphotype for tamarack was *Suillus* 2, followed by *Cenococcum*. *Suillus* 2 was identified on 66.7% of all tamarack seedlings, and this rhizomorphic morphotype represented 22.6% of the entire ectomycorrhizal community for tamarack in the Mix peatland site type. Although this morphotype was also found on many seedlings in the BsLt site type, it was never as abundant. The genus *Suillus* is known to have a narrower host range, and prefers to associate with members of the Pinaceae, including *Pinus* and *Pseudotsuga*, as well as *Larix* spp. (Molina et al, 1992). For example, *Suillus grevillei* was found to be highly specific to western larch (*Larix occidentalis*) (Melin, 1922; Molina and Trappe, 1982), whereas *S. cavipes* often associates with European larch (*Larix eurolepis*) (Finlay, 1989). *Suillus* 2 was a dominant ectomycorrhizal fungi in the Mix peatland site type, which included black spruce as an ectomycorrhizal host species. Even though *Suillus* primarily associates with *Pinus* and *Larix* spp., it has been documented that black spruce can form ectomycorrhizas with some species, such as *S. granulatus* (Browning and Whitney, 1991) and *S. cavipes* (Stein et al., 1990) following inoculation. However, *Suillus* was not identified on black spruce in these Mix sites (Robertson, 2003), nor on scrub birch seedlings in any of the peatland site types. In addition, the literature does not report this genus on any other birch species. Black spruce may play a greater role in the abundance and frequency of other ectomycorrhizal fungal species occurring in these site types.

Another frequently occurring morphotype unique to tamarack was brown silvery; this ectomycorrhiza was almost exclusively retrieved from the BsLt peatland site type. The identity of this ectomycorrhiza remains unknown and, although it had no clamps and no rhizomorphs, we cannot exclude it from the Basidiomycetes. Brown clamp, coffee

brown, and *Hebeloma*-like ectomycorrhiza were also primarily found to associate with tamarack and were often found on only one site type. Even though some of these morphotypes could not be identified to the family or genus level, and were only found in small numbers, they still contributed to the ectomycorrhizal species richness for the peatland site types.

Scrub birch (*Betula glandulosa*) morphotype frequency and abundance

This study was able to characterize 24 ectomycorrhizal morphotypes for scrub birch; many could also be considered to have intermediate to broad host specificity and included such fungi as *Amphinema*, *Cenococcum*, E-strain, MRA, *Lactarius*, numerous species in the Thelephoraceae (including *Tomentella*), as well as several Russulaceae (including *Russula*). Many of these also occurred on tamarack, and most occurred in all or two of the peatland site types. Robertson (2003) described many of these fungi as also occurring on black spruce seedlings growing in wetland and upland habitats.

However, there were seven morphotypes that were unique to scrub birch (black cystidia, cotton orange, silver white, white clamp, white felted, brown inky clamp, and a Russulaceae), as well as a *Lactarius* (one exception on tamarack). Although most of these morphotypes were infrequent, two types (brown inky clamp and *Lactarius*) occurred both frequently and abundantly. Brown inky clamp shared some morphological features with *Lactarius*, but laticifers were never observed and emanating hyphae were wider and clamped. The genus *Lactarius* is generally considered to have a narrow to intermediate host range, with approximately a quarter of the species associating with a broad array of ectomycorrhizal hosts; these include members of the Pinaceae (i.e. *Picea*,

Pinus and *Larix*) and Betulaceae (i.e. *Betula* and *Alnus*) (Molina et al., 1992). Interestingly, very few tamarack roots were colonized by *Lactarius* in all the peatland site types, even though the host genus is known to associate with these fungi.

There were also some similarities between ectomycorrhizal fungal species identified on other *Betula* spp. and fungi identified on scrub birch. Jones et al. (1997) and Simard et al. (1997a) reported numerous fungal genera (*Amphinema* (only identified by Jones et al. (1997)), *Cenococcum*, E-strain, *Hebeloma*, *Lactarius*, MRA, *Russula* (only identified by Jones et al. (1997)), and *Thelephora*) on paper birch seedlings; fungi in all of these genera were also identified on scrub birch from our study. Miller (1982) investigated the ectomycorrhizal symbionts associated with swamp birch (*Betula nana*) growing in the sub-alpine tundra of Alaska; three of the fungal genera (*Hebeloma*, *Lactarius*, *Russula*) identified in his study were found to associate with scrub birch as well. Although these two *Betula* species share similar growth forms (i.e. low-lying shrub) and habitat requirements (i.e. wetlands such as fens and bogs), only three out of the five fungal genera identified as associating with swamp birch were found on scrub birch in our study. The differences may be partly attributed to the fact that Miller (1982) identified ectomycorrhizal fungi from sporocarps fruiting near the host and assumed these to be ectomycorrhizal with swamp birch. However, characterization was not performed on the birch roots and sporocarp occurrence is not always an accurate measure of ectomycorrhizal species richness belowground (Mehmann et al., 1995; Gardes and Bruns, 1996; Dahlberg, 1997; Dahlberg, 2001).

Scrub birch seedlings in the BsLt and Bs peatland site types had fewer ectomycorrhizal morphotypes, but several species dominated each of the two peatland

site types. Three morphotypes dominated scrub birch seedlings in the BsLt site type, *Lactarius*, brown smooth 2, and *Tomentella*-like 2. Interestingly, Robertson (2003) identified two *Lactarius* morphotypes on black spruce growing in the same Mix peatland sites with tamarack and scrub birch. *Lactarius* was also present on scrub birch in the Mix and Bs site types, but it did not dominate those sites. The *Lactarius* morphotype identified on scrub birch was the most abundant species in the BsLt site, suggesting a high level of host specificity on this site. In the Bs peatland site type, two other morphotypes, MRA and Thelephoraceae 1, dominated scrub birch. No single morphotype appeared to dominate scrub birch or tamarack in all three peatland site types in which each host occurred. Robertson (2003) found that many Thelephoraceae and *Tomentella* morphotypes on black spruce were predominantly identified in the wetland compared to the upland habitats. In addition, MRA was found on one third of all her wetland black spruce seedlings.

Fewer potential ectomycorrhizal host species were present in the BsLt site (scrub birch and tamarack), and the Bs site was solely composed of birch (with a negligible component of black spruce, but generally coniferous species were absent). The observed decrease in the number of ectomycorrhizal morphotypes (species richness) on these two sites may be closely associated with the reduction of host species. As well, the frequency and abundance of several morphotypes seemed to greatly increase when fewer fungal species were present on the scrub birch seedling root systems. This may account for a small decrease in evenness in these sites when compared to the Mix site type. Jones et al. (1997) found that when paper birch and Douglas-fir were planted in mixtures, evenness values for the ectomycorrhizal types present on the roots of the two hosts, increased.

Morphotype frequency and abundance by peatland site type

Tamarack and scrub birch both exhibited the highest number of morphotypes in the Mix peatland site type, when compared to the other site types in which they occurred. Host species planted in mixture have been reported to influence the frequency, abundance, and the proportion of ectomycorrhizas associating with the co-occurring species (Simard et al., 1997a; Massicotte et al., 1999). Jones et al. (1997) determined that when paper birch and Douglas-fir were planted together, an increase occurred in the abundance of the minor morphotypes on Douglas-fir. It is possible that with the increase of ectomycorrhizal host species (i.e. black spruce) in the Mix site, tamarack and scrub birch had the potential to associate with a wider array of fungal species that may not have been present in the sites with fewer hosts. Robertson (2003) found the ectomycorrhizal species diversity was greater (though not significant) on black spruce growing in the Mix site type than in pure black spruce wetland habitats.

Potential for shared ectomycorrhizal fungal symbionts

Over half of the morphotypes (53.3%) characterized in this study were found on both tamarack and scrub birch, and have the potential for forming fungal linkages for carbon transfer between host species. The majority of morphotypes that were shared between the hosts were found on seedlings growing in both the Mix (scrub birch-tamarack-black spruce) peatland site type and the BsLt (scrub birch-tamarack) site type, suggesting that these peatland sites have a good possibility of supporting fungal linkages between the two hosts.

A number of studies have investigated the potential for shared ectomycorrhizal fungal species between different host species using isotope tracers and morphological characterization techniques. Most notably, Simard et al. (1997b) used gaseous, pulse labeled, C^{13} and C^{14}, to demonstrate the bi-directional carbon transfer between paper birch and shaded Douglas-fir seedlings via shared fungal symbionts. This study and others (Björkman, 1960; Finlay and Read, 1986; Finlay, 1989; Dahlberg and Stenlid, 1990; McKendrick et al., 2000) provide additional evidence to support the hypothesis that common mycorrhizal symbionts associated with different host species can form hyphal linkages, or mycelial networks, for the transport of carbon between plants.

Ectomycorrhiza characterization is a commonly used indirect method for establishing the potential for mycelial networks between host species; although it may not provide as conclusive evidence as the tracer technique, morphotype characterization can determine if two or more hosts are able to form mycorrhizal associations with the same fungal species. Prior to the use of isotope tracers, Simard et al. (1997) characterized seven morphotypes, out of a total of 11 identified fungal species, shared between paper birch and Douglas-fir (Simard et al., 1997). Jones et al. (1997) reported that five of the six most common morphotypes found on out-planted paper birch and Douglas-fir seedlings were shared between the hosts. In our study, six morphotypes belonged to those that occurred frequently on both hosts. Nine others, although shared, were often disproportionately more abundant (or occurred more often) on one of the two host. For example, brown smooth 2, *Lactarius*, and *Tomentella*-like 2 had much higher abundance values on scrub birch compared to tamarack. Kranabetter et al. (1999) investigated multiple host species (lodgepole pine, white spruce, and subalpine fir) seedlings planted

on mature-forest edges, and determined that 47% of the ectomycorrhizal community colonized all three conifer species. In a bioassay study examining the ectomycorrhizas from plants grown in mixed-pot cultures, Massicotte et al. (1999) reported that 14 morphotypes, from a total of 18 identified, were found to associate with two or more host species; hosts included grand fir (*Abies grandis*), tanoak (*Lithocarpus densiflora*), ponderosa pine (*Pinus ponderosa*), Douglas-fir, and madrone (*Arbutus menziesii*). The present study, and those cited, all suggest that shared mycorrhizal fungi may be the normal situation, rather than the exception, in many forest ecosystems, including peatlands.

Ectomycorrhizal diversity

Overall, for the peatland site types examined in this study, ectomycorrhizal diversity was always greatest in the Mix sites, compared to the BsLt and Bs peatland site types for tamarack and scrub birch. This difference was significant for diversity indices (except the Shannon evenness) when values for host species were pooled. For separate hosts, the Mix site type was also the most diverse, but differences were only significant for scrub birch (Simpson Index). With respect to scrub birch, ectomycorrhizal diversity decreased from the Mix to the Bs, with the BsLt peatland site type having the lowest. The Bs and BsLt sites were similar, in that they both had fewer fungal species, with several that appeared to dominate each of these habitats.

In the bioassay study by Massicotte et al. (1999), similar numbers of morphotypes were retrieved from both monoculture treatments (host species growing in single culture), as well as mixed (four hosts per pot) species cultures; however, in most cases, more

morphotypes were identified from the mature stands (more potential ectomycorrhizal hosts) compared to clearcut sites. Results for ectomycorrhizal community diversity on black spruce support the findings in this study; Robertson (2003) found higher ectomycorrhizal diversity in the tamarack-black spruce mix wetland habitat compared to the black spruce dominated wetland sites. Since the peatland site types generally did not differ in soil or moisture regimes, differences in fungal species richness are most likely due to variations in the vegetation and ectomycorrhizal host species composition. Dwarf shrub and grass species varied across the three peatland site types. The BsLt site type contained one ericaceous plant species, compared to five species in the other two site types. Although Poaceae spp. were observed in all the peatland site types, grasses were particularly common in the Bs sites. Perhaps the absence or presence of AM grasses and/or ericoid shrubs has also influenced the level of ectomycorrhizal diversity within the peatland site types.

Neighboring plants have been reported to influence the frequency of occurrence and abundance of mycorrhizal development (Simard et al., 1997a; Jones et al., 1997). More ectomycorrhizal host species were available for colonization in the Mix site, which could account for the higher number of fungal species; or perhaps a greater fungal inoculum potential existed in this site type and allowed for the establishment of more host species. Van der Heijden et al. (1998) suggested that AM fungi species composition and diversity below-ground, may have the potential to determine plant biodiversity above-ground, in a natural ecosystem. However, given that black spruce exhibited approximately 19 morphotypes in this site type, it is more likely that the additional host species contributed more potential fungal symbionts for tamarack and scrub birch. The

addition of black spruce to the mixture of tamarack and scrub birch appears to have increased the possibility of potential linkages via shared fungi between these two hosts; this supports the concept of companion plants influencing the ability of ectomycorrhizal fungi to colonize neighboring plants (Molina et al., 1992; Massicotte et al., 1994).

Although site type appeared to have a significant effect on the ectomycorrhizal diversity between the Mix and BsLt peatland site types, diversity between the two hosts did not appear to differ. Similar numbers of morphotypes were identified for both tamarack and scrub birch and, for both hosts, these showed a decrease from the Mix to the BsLt habitats (Bs sites having intermediate values for scrub birch).

Morphological analysis of tamarack and scrub birch ectomycorrhizas resulted in the characterization of 30 morphotypes. Some morphotypes were found on both hosts, suggesting a high potential for shared fungal linkages, whereas others were unique to either tamarack or scrub birch. Both hosts appear to be equally receptive to a wide range of ectomycorrhizal fungi. In addition, several morphotypes were site-specific, as well as more abundant in certain peatland site types. Ectomycorrhizal diversity was highest in the Mix peatland site type for both hosts; however, for scrub birch, the Bs sites were more diverse compared to the BsLt site type. Our results indicate that these peatland environments appear to be similar to upland terrestrial forest ecosystems in regards to ectomycorrhizal abundance, frequency and diversity.

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Molecular analysis of ectomycorrhizal associations of *Larix laricina* (Du Roi) (tamarack) K. Koch and *Betula glandulosa* Michaux (scrub birch) in peatlands of central British Columbia.

ABSTRACT

Tamarack and scrub birch are ectomycorrhizal hosts often found growing in the wet, nutrient poor, peatland ecosystems of British Columbia. Fungal linkages can allow for carbon and nutrient transfer between hosts that share the same symbionts. Molecular analysis (PCR-RFLP) of 326 tamarack and 360 scrub birch root tips was used to assess genetic diversity of ectomycorrhizal fungi associating with tamarack and scrub birch in three peatland site types (scrub birch-tamarack-black spruce (Mix), scrub birch-tamarack (BsLt), and scrub birch (Bs) only) in central BC, and to determine the potential for fungal linkages between the two hosts. Twenty-six of 30 described morphotypes (plus the lightly colonized) generated fragment patterns that were classified into 69 distinct genotypes (38 for tamarack, and 43 for scrub birch). *Suillus* 2 on tamarack and *Lactarius* on scrub birch appeared host specific and each contained five genotypes; many morphotypes had two or more genotypes. Twelve genotypes from 10 morphotypes were shared between the hosts. One genotype each, belonging to silver white, *Suillus* 2, and *Lactarius* (plus brown silvery and yellow stellate) matched sporocarp fragment patterns for *Cortinarius*, *Hebeloma*, and *Hygrocybe*, respectively. More genotypes were on both hosts in the Mix compared to the BsLt sites; BsLt and Bs sites contained similar numbers for scrub birch. However, site differences in molecular diversity were not significant as measured by the Phi index. Similarities between scrub birch and tamarack genotypes and several sporocarps, suggest a high probability for fungal linkages in these peatland ecosystems.

INTRODUCTION

Ectomycorrhizal fungi are an integral part of a forest ecosystem; they serve as symbiotic partners in mutualistic relationships with the roots of many gymnosperm and angiosperm species (Smith and Read, 1997; Amaranthus, 1998). These fungi provide nutrients, such as nitrogen and phosphorous, and water to host plants in exchange for fixed carbon. The resultant underground network of hyphae can serve as linkages for the movement of nutrients and carbon between the same or different host plant species that share the same fungal symbionts (Björkman, 1960; Finlay and Read, 1986; Dahlberg and Stenlid, 1990; Simard et al., 1997b; McKendrick et al., 2000). Considering the possibility that emanating hyphae from numerous different ectomycorrhizal fungi can travel through the rhizosphere, and contact and colonize roots from neighboring trees and shrubs (Read, 1987), it has been hypothesized that plant-to-plant nutrient transfer could be a common occurrence in ecosystems (Newman, 1988).

Although many questions still remain concerning the relationship between ectomycorrhizal fungi and plant community structure, advances in molecular research have provided a crucial step towards the identification of the fungal species involved in these complex systems (Egger, 1995; Horton and Bruns, 2001). The amplification of minute quantities of ribosomal DNA from colonized roots, using the polymerase chain reaction (PCR) technique, followed by restriction fragment length polymorphism (RFLP) analysis, allows for species identification through comparison of restriction fragment patterns to those existing in reference databases (Egger, 1995; Horton and Bruns, 2001; Mah et al., 2001; Bruns and Bidartondo, 2002; Robertson, 2003). When combined with

morphological characterization of ectomycorrhizas, molecular analysis of fungal DNA can be a powerful tool to separate fungal taxa, as well as to determine genotypic variation within taxa (Horton and Bruns, 2002; Sakakibara et al., 2002).

Several recent studies that have used molecular methods to describe the ectomycorrhizal composition and diversity of seedlings growing under different treatment regimes include Hagerman et al. (1999), Baldwin (1999), Mah et al. (2001), and Robertson (2003). Horton and Bruns (1998) investigated ectomycorrhizal fungi of Douglas-fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*) and used molecular methods to determine potential hyphal linkages between the two host species. They concluded that since the two hosts were found to associate with a majority of the same fungal symbionts in the study site, that these two host species may have been connected by fungal mycelia and perhaps shared similar capabilities for resource acquisition.

Many plants growing in nutrient-poor and water-saturated peatland ecosystems seem able to withstand a wide range of environmental and physiological stresses (MacKenzie and Moran, 2003). Tamarack (*Larix laricina*) and scrub birch (*Betula glandulosa*), two ectomycorrhizal hosts that often occur together, also appear to be able to tolerate the conditions of peatland environments. The extent of the ectomycorrhizal colonization in peatlands for these two hosts, and whether this facilitates adaptation to such environments, is largely unknown. With respect to the genetic composition of the ectomycorrhizal fungal community associated with these tamarack and scrub birch in peatlands, this has not been investigated at the molecular level. Recently however, Robertson (2003) used molecular techniques to determine the genotypic variation of the

fungal symbionts of black spruce (*Picea mariana*), another commonly occurring peatland species. The study described the number of fungal genotypes associated with black spruce growing in both peatland and upland sites, and provided insight into the ectomycorrhizal community in these environments.

The first objective of this study was to use PCR-RFLP analysis to describe and compare the molecular diversity and genotypic variation of the ectomycorrhizal fungi associating with tamarack and scrub birch growing in three different peatland site types in central British Columbia. The three peatland site types included i) scrub birch dominated, ii) mixed scrub birch-tamarack, and iii) mixed scrub birch-tamarack-black spruce. The second objective was to determine, using the genotypic information, the potential for fungal linkages between tamarack and scrub birch in these peatland ecosystems. In addition, my goal was to compare the fungal genotypes identified for these two hosts with the results from a previous study on black spruce that were sampled from the same mixed scrub birch-tamarack-black spruce peatland sites, to determine the potential for further linkages.

METHODS

Ectomycorrhizal root selection and DNA extraction

From the 200 roots tips per seedling sampled for morphological assessment, a proportional number of tips (10%) of each mycorrhizal morphotype (a total of 20 tips per seedling) were selected for molecular analysis (Mah et al., 2001; Robertson, 2003). Individual root tips were stored in 1.5 ml microtubes at –20°C until processed. A modified Zolan and Pukkila (1986) hexadecyltrimethyl ammonium bromide (CTAB)

protocol was used to extract the fungal DNA from the mycorrhizal root tips, as well as from sporocarp samples (Baldwin and Egger, 1996). Using glass micromortars and micropestles, individual frozen root tips were crushed cold (–20°C) in 350 ml CTAB extraction buffer (5 M NaCl (Sigma), 1 M Tris-HCL (pH 8) (Invitrogen)), 0.5 M ethylenediaminetetraacetic acid (EDTA) (Invitrogen), 10% CTAB, and 0.2% β-mercaptoethanol (Sigma), transferred into sterile 1.5 ml microtubes, and incubated at 60°C in a water bath heat block (VWR Scientific) for 45-60 min. Tubes were removed from the heat block, 350 µL of a chloroform (BDH): isoamyl alcohol (24:1) solution (Fisher Chemicals) was added to each, and briefly vortexed. Tubes were then centrifuged (Hermle, Mandel Scientific Co. Ltd.) at 13000 x *g* for 10 min at room temperature. The top aqueous layer was transferred to a new sterile microtube and 350 µL of cold (–10°C) absolute isopropanol (BDH) was added. The solutions were mixed by inverting the microtubes several times over 1 min, and then placed in a -10°C freezer overnight. Prior to a second centrifugation at 13000 x *g* for 10 min, the tubes were again inverted several times. The aqueous phase was poured off and the remaining DNA pellet was washed twice with 175 µL of cold (–10°C) 70% ethanol then centrifuged at 13000 x *g* for 3 min. The tubes were left to dehydrate overnight in a dessicator, and then the dried pellet was resuspended in 50 µL of Tris-EDTA buffer and stored at –20°C.

DNA amplification and restriction endonuclease digestion

The extracted DNA samples were subjected to the polymerase chain reaction (PCR) in order to amplify an approximate 1,100 bp fragment of nuclear-encoded ribosomal DNA (rDNA) gene repeat. The fungal specific primer, NL6Bmun (CAA GCG

TTT CCC TTT CAA CA) (Egger, 1995), and the universal primer, ITS1 (TCC GTA GGT GAA CCT GCG G) (White et al., 1990), were used to amplify the target region (3′ end of the 18S small subunit to the 5′ end of the 28S large subunit rDNA gene, including both internal transcribed spacer (ITS1 and ITS2) regions). A single PCR reaction master mix consisted of 16.5 μL millipore H_20, 2.9 μL 10X PCR buffer (Invitrogen), 2.9 μL dNTP (Invitrogen) mixture (containing equal amounts of 100 μM dATP, dCTP, dGTP and dTTP), 2.3 μL $MgCl_2$ (25 μM) (Invitrogen), 1.2 μL of each primer (10 μM) (Gibco BRL), and 3.0 μL Taq DNA polymerase (Gibco BRL). While working on ice, 27 μL of PCR master mix was added to a 0.2 ml microtube containing 3 μL of either a 1:10 dilution of ectomycorrhizal DNA or a 1:50 dilution of sporocarp DNA. Tubes were placed in a PTC-100™ Programmable Thermal Controller (MJ Research, Inc.) and underwent the following program: denaturation at 94°C for 30 s and 93°C for 35 s, annealing at 50-52°C for 53 s and extension at 72°C for 5 min. Following amplification, 5 μL of PCR product was mixed with 1.8 μL of 10X xylene cyanole loading buffer (Sigma). A 150 ml 0.7% agarose gel (0.7 g agarose in 100 ml of Tris-borate (TBE) buffer) (Gibco BRL), containing 11 μL of ethidium bromide (fluorescent stain for DNA visualization), was submerged in a gel box of TBE buffer. In the first well of the agarose gel, 5 μL of *Hind* III DNA ladder (Invitrogen) was loaded; 4 μL of each DNA sample were loaded in subsequent wells. Gels were run at approximately 90-110 mV for 35-45 min. Once complete, gels were visualized under UV light using a Gel Print 2000i photodocumentation system (Bio/Can Scientific), photographed, and printed on Mitsubishi thermal paper (K65H Mitsubishi Electronic Corp.). Samples that did not amplify (or produced faint bands) were re-amplified in an attempt to improve resolution.

Samples that appeared to contain DNA from more than one fungal species (double bands) were eliminated from the analysis.

The resulting PCR product was cleaved at specific sites using three restriction endonucleases for restriction fragment length polymorphism (RFLP) analysis: *Alu*I (AGCT), *Hin*fI (GANTC), and *Rsa*I (GTAC) (Invitrogen). While working on ice, 6.3 μL millipore H_2O, 0.8 μL of either 10x React® 1 or React® 2 assay buffer (Invitrogen), 0.5 μL of one of the three restriction enzymes, and 7 μL of PCR product was added to a 0.2 mL microtube. This procedure was repeated for each restriction enzyme. Tubes were incubated in a 37°C oven for 5 h or overnight. Following incubation, a 2.5% L.M.P (low melting point) (Invitrogen) agarose gel (1 g agarose and 1.5 g L.M.P. agarose in 100 ml 10X TBE) containing 11 μL of ethidium bromide (10mg/mL) was submerged in a gel box containing TBE. To each digestion microtube, 4 μL of loading buffer (bromophenol blue and glycerol) (Sigma) was added. The 1st, 15th, and 30th wells contained 5 μL of 1kb ladder (Invitrogen); remaining wells contained the digestion samples. Gels ran at 90-100 mV for 2.5 to 3 h, and were then visualized under UV light, photographed, and images saved to disk using the BioPhotonics Gel Print 2000i system. Partial and incomplete digests were removed from the data set and were not re-digested.

Molecular analysis

RFLP gel images were imported into Gene Profiler, Version 4.05 (Scanalytics, Inc.), a genotyping and DNA fragment analysis software. Individual restriction fragments were selected and their bp size calibrated against the 1kb ladder standards (1018, 514, 356, 344, 298, 220, 201, 154, 134, and 75 bp fragments) using the Desmile

calibration method with log piecewise linear curve-fitting. Fragments of 75 bp or less were not included in the analysis. Once all fragments were marked, fragment patterns for individual samples (keeping the two hosts separated) were imported and sorted into both seedling and morphotype databases created in Database Manager, Version 4.05 (Scanalytics, Inc.). Sporocarp fragment patterns were compiled into a separate fungal database. Pairwise comparisons of all band patterns were made for each database; a 5% match tolerance was set to obtain fragment pattern similarity values for every sample pair and for each restriction enzyme. The neighbor-joining/unweighted pair-group method with arithmetic means (UPGMA) option in PHYLIP (Phylogeny Inference Package), Version 3.573c, (Felsenstein, J., University of Washington) was used to perform UPGMA cluster analysis on the resulting similarity matrix.

To examine host species and site ectomycorrhizal community structure, individual ectomycorrhizal morphotype databases were merged to create an all-inclusive morphotype database for each host species. The sporocarp database was merged with each tamarack and birch all-inclusive ectomycorrhizal morphotype database to determine if sporocarp fragment patterns matched with ectomycorrhizal fragment patterns. Resulting phylograms were viewed in TreeView, Version Win 3.2 (1998, Roderick DM Page). The Dice's index (Dice, 1945) was used to match pairs of ectomycorrhizal root tip band patterns and to create a distance matrix for each pair of samples in order to calculate Phi Index values for an estimation of genetic diversity within each ectomycorrhizal morphotype, and between peatland site types for each host species (Mah et al., 2001; Khetmalas et al., 2002; Robertson, 2003).

The fragment pattern base pair sizes were imported into a Microsoft® Excel 2000, Version 9.0, spreadsheet to aid in the classification of genotypes, as well as possible identification of lightly colonized morphotypes. Fragment patterns were sorted by morphotype, and then grouped into genotypes based on their molecular weights (5% tolerance of similarity), as well as their position in the neighbor-joining phylogram. Matching fragment patterns were averaged for each genotype for each host species; patterns for both hosts were compared to determine shared genotypes. Fragment patterns within morphotypes that did not match any of the determined genotypes, as well as those within the lightly colonized morphotype group, were compared to all other fragment patterns to determine their placement and possible identification. Morphotype RFLP databases, and all related matrices, were modified according to the above changes.

To determine genetic diversity, Phi index values were calculated from fragment patterns from individual seedlings for the commonly occurring ectomycorrhizal morphotypes, as well as for those morphotypes shared by both host species, and for each peatland site type. The index values range from 0-1, where a higher Phi value implies greater diversity. For the four BsLt and Mix sites where the two hosts co-occurred, a two-way ANOVA was used to test effects of peatland site type and host species on genetic diversity ($\alpha = 0.05$). A one-way ANOVA (Statistica version 6.1, 2002, StatSoft, Inc.) was used to compare the genetic diversity between site types in which each host occurred ($\alpha = 0.05$). Mean comparisons were tested using Fisher's Least Significant Difference (LSD) test ($\alpha = 0.05$).

RESULTS

Amplification and digestion success rates

From a total of 1048 ectomycorrhizal root tips, 686 (65%) were successfully amplified and digested (Table 3.1). This represented 326 (76%) of all tamarack root tips and 360 (58%) of all scrub birch root tips. The morphotypes brown smooth 1 and brown smooth 2 on tamarack, and woolly brown and lightly colonized tips on scrub birch roots exhibited the lowest amplification success rates (Table 3.1). In addition, E-strain (one tip) and Russulaceae (two tips) on scrub birch did not amplify. In contrast, the woolly brown and lightly colonized roots on tamarack had a high amplification success rate even though the lightly colonized root tips lacked a developed mantle. Table 3.1 shows a summary of the root tip sample size for each morphotype and the success rate for DNA amplification and digestion.

Phylogenetic analysis of ectomycorrhizal root tips

Successful rDNA amplification and digestion of tamarack and scrub birch ectomycorrhizal root tips yielded fragment patterns that were used to determine differences between and within morphotypes, and to identify genotypes. In total, 69 distinct genotypes were generated from 26 of the 30 morphotypes (plus the lightly colonized group) that were described (Chapter 2) for tamarack and scrub birch.

Table 3.1. Summary of ectomycorrhizal root tip DNA amplification (PCR) and digestion (RFLP) success rates (%) from tamarack and scrub birch seedlings.

	Tamarack			Scrub birch		
Morphotype	**(n)**	**Amplification rate (%)****	**Digestion rate (%)**	**(n)**	**Amplification rate (%)****	**Digestion rate (%)**
Amphinema	14	92.9	92.9	5	80.0	80.0
black cystidia	-	-	-	3	66.7	66.7
brown clamp	11	63.6	63.6	-	-	-
brown inky clamp	-	-	-	51	78.4	49.0
brown silvery	26	88.5	84.6	-	-	-
brown smooth 1	13	38.5	23.1	7	42.9	0.0
brown smooth 2	7	42.9	42.9	68	82.4	63.2
Cenococcum	21	85.7	71.4	35	85.7	65.7
coffee brown	6	83.3	83.3	-	-	-
cotton orange	-	-	-	8	75.0	37.5
crystal net brown	12	100.0	100.0	15	100.0	66.7
E-strain	14	64.3	64.3	1	0.0	0.0
granular brown	14	100.0	100.0	15	86.7	86.7
Hebeloma-like	3	100.0	100.0	-	-	-
Lactarius	1	100.0	100.0	85	84.7	56.5
MRA	34	61.8	47.1	52	65.4	61.5
Russula	9	100.0	100.0	2	100.0	100.0
Russulaceae	-	-	-	2	0.0	0.0
silver white	-	-	-	5	100.0	100.0
Suillus 1	8	87.5	75.0	-	-	-
Suillus 2	69	84.1	82.6	-	-	-
Thelephoraceae 1	4	75.0	25.0	59	69.5	62.7
Thelephoraceae 2	5	100.0	100.0	51	74.5	58.8
Thelephoraceae 3	3	66.7	66.7	21	71.4	57.1
Tomentella-like 1	7	100.0	85.7	4	50.0	50.0
Tomentella-like 2	30	80.0	70.0	69	69.6	49.3
white clamp	-	-	-	27	85.2	66.7
white felted	-	-	-	11	90.9	90.9
woolly brown	13	92.3	84.6	14	21.4	21.4
yellow stellate	2	100.0	100.0	2	100.0	100.0
lightly colonized	101	90.1	82.2	9	22.2	22.2
Total/mean*	427	83.2*	76.9*	621	68.1*	56.5*

**includes ectomycorrhizal root tips which exhibited weakly amplified bands and excludes ectomycorrhizal root tips that exhibited double bands.

From the 23 morphotypes (plus the lightly colonized group) characterized for tamarack, 38 genotypes (fragment patterns) were generated. Six uncommon morphotypes occurring on tamarack produced poor fragment patterns that could not be used in the analysis. These included brown smooth 1, brown smooth 2, coffee brown, *Lactarius*, Thelephoraceae 1, and *Hebeloma*-like. One to five patterns were identified within each morphotype and those generally varied in one or more restriction endonucleases (Table 3.2). In some cases, such as *Amphinema*, variation only occurred in one or two fragments. Five morphotypes (excluding the lightly colonized category) were unique to tamarack: *Suillus* 2 (five genotypes), brown silvery (three genotypes), E-strain (two genotypes), *Suillus* 1 (one genotype) and brown clamp (one genotype). It should be noted that one sample of E-strain did occur on scrub birch, but it did not amplify. The remaining morphotypes that were found on tamarack each contained one or more genotypes that were shared with scrub birch; however, not all fragment patterns in these morphotypes were common to both hosts.

Fragment patterns for the lightly colonized root tips (84 tips of those successfully amplified and digested for tamarack) were compared to established genotypes. Of these, 35 matched patterns for *Suillus* 2, ten were placed with crystal net brown, five were placed in *Tomentella*-like 2, and three were placed with other morphotypes. The remaining 16 were sorted into the three lightly colonized genotypes; 15 could not be placed and remained as unknowns.

Twenty-one of the 24 scrub birch morphotypes produced 43 genotypes. Within each morphotype, fragment patterns varied from one to five, with the most genotypes occurring in *Lactarius* (five) (Table 3.2). Interestingly, the morphotypes *Suillus* 2 and

Lactarius, unique to tamarack and scrub birch, respectively (with the exception of *Lactarius* one root tip on tamarack that did not produce a fragment pattern), had the most genotypes and were the most dominant morphotypes found on the two hosts. Six morphotypes that were only found on scrub birch produced the following numbers of genotypes: black cystidia (one), brown inky clamp (three), cotton orange (one), silver white (one), white felted (two) and white clamp (one). Three other morphotypes that were also found on tamarack in small numbers only produced fragment patterns for scrub birch: brown smooth 2 (three genotypes), *Lactarius* (five genotypes), and Thelephoraceae 1 (two genotypes). The remaining scrub birch morphotypes generated patterns of which some, for each morphotype, were shared with tamarack.

When genotypes were compared at the 5% tolerance level, some that belonged to different morphotypes had very similar fragment patterns (Table 3.2). For example, brown inky clamp (genotype 2) matched white clamp (genotype 1), E-strain (genotype 1) matched MRA (genotype 1), *Tomentella*-like 2 (genotype 1), yellow stellate (genotype 1), and Thelephoraceae 2 (genotype 3) shared similar fragment patterns, as did brown silvery (genotype 2), *Lactarius* (genotype 2), and yellow stellate (genotype 3). No attempt was made to merge or re-assign these genotypes (Table 3.2).

Phylogenetic trees based on the restriction fragment patterns from ectomycorrhizal root tips were created for each host species; these aided in the classification of genotypes (Appendix IV and V). With the exception of *Suillus* 2, groups within the tamarack phylogenetic tree were not well defined, since the branch clusters often contained more than one morphotype (Appendix IV). *Suillus* 2 consisted of five genotypes, four of which formed distinct groups in the first half of the tree. *Suillus* 1,

Russula, brown stringy and woolly brown did not share their branches with any other morphotype. Some clusters included genotypes from several different morphotypes, whereas others contained only one morphotype. For scrub birch, Thelephoraceae 2 formed several very tight branches that separated from the rest of the samples (Appendix V). Neither MRA nor *Cenococcum* grouped with other morphotypes compared to crystal net brown, white clamp and brown inky clamp genotypes that clustered together on the same branches. Interestingly, several Thelephoraceae 2 samples grouped with *Lactarius*.

Genotype distribution within peatland site types

With respect to genotypes that were successfully generated from tamarack root tips, 27 occurred within the Mix (scrub birch-tamarack-black spruce) peatland site type compared to 22 in the BsLt (scrub birch-tamarack) site type; 11 genotypes were present in both peatland site types (Table 3.2). Within ectomycorrhizal morphotypes that were found in both peatland site types, the genotypic distribution sometimes varied. Almost one third of the morphotypes (*Suillus* 2, *Cenococcum*, granular brown, *Tomentella*-like 1, *Tomentella*-like 2, and woolly brown) exhibited one or two genotypes that occurred in both the BsLt and Mix site types. In some cases, one or two additional genotypes within these morphotypes were site specific (Table 3.2). Three morphotypes (MRA, crystal net brown, and E-strain), although found in both site types, produced genotypes that were site-specific, that is to say, each genotype was only identified from one or the other site type, never both. Although 12 genotypes belonging to eight morphotypes (brown silvery, *Russula*, *Amphinema*, brown clamp, Thelephoraceae 2, Thelephoraceae 3, *Suillus* 1, and yellow stellate) appeared to show some specificity to one or the other peatland site type,

these often belonged to morphotypes that were either only found in one site type, or to morphotypes for which root tips on the corresponding site type failed to produce fragment patterns (Table 3.2).

With respect to scrub birch genotypes, almost twice as many fragment patterns were identified in the Mix site type (33), compared to the BsLt (18) or Bs (17) sites (Table 3.2). Only four genotypes were present in all three peatland site types: *Cenococcum* (genotype 1), *Lactarius* (genotype 5), and *Tomentella*-like 2 (genotype 1 and 2). Although five morphotypes (*Cenococcum*, *Lactarius*, Thelephoraceae 2, *Tomentella*-like 2, and yellow stellate) occurred in all peatland site types, they produced genotypes that occurred mostly in one, or in a combination of two of the site types. Ten morphotypes generated genotypes that only occurred in two of the three peatland types; some of these genotypes were found in both site types and others in only one of the two site types. The remaining six morphotypes, and their genotypes, were site-specific, only occurring within one peatland site type. As with tamarack, some genotype-peatland specificity was due to morphotypes occurring only in one site type, or to a loss of fragment patterns during PCR/RFLP analysis (Table 3.2).

Table 3.2. Approximate fragment sizes of the amplified ITS region for ectomycorrhizas from tamarack (Lt) and scrub birch (Bs) seedlings occurring in three peatland site types (scrub birch dominated (B), scrub birch and tamarack (L), and scrub birch, tamarack, and black spruce (M)).

Morphotypes	Host			Undigested	Approximate Fragment Sizes (bp)													
and Genotypes	Lt	Bs	(n)	Size (bp)	*Alu*I					*Hin*fI					*Rsa*I			
Amphinema																		
genotype 1	M	M,B	13	840	585	190	110			330	280	165	150		825	210		
genotype 2	M		4	845	350	190	110			325	290	155			780	180		
black cystidia																		
genotype 1		M	2	855	410	190	80			320	220	155	130		980			
brown clamp																		
genotype 1	M		2	915	670	420				350	300				1000			
brown inky clamp																		
genotype 1		M	8	830	355	190	150	130	115	285	180	165	105		430	385		
[a]genotype 2		M,B	9	925	400	245	190	115		295	220	165	150		1000			
genotype 3		B	4	765	245	175	150	90		415	240	180			600	200	165	130
brown silvery																		
genotype 1	L		8	815	300	225	185	130	115	335	165	145	100		395	350		
[b]genotype 2	L		7	820	355	185	150			345	255	165	105		440	200	160	105
genotype 3	L		3	890	390	200	185	140		340	165	150	125		865	110		
brown smooth 2																		
genotype 1		M,L	11	975	390	225	190	115		305	215	165	150		1020			
genotype 2		L	10	955	700	190	135	115		350	250	160	150		565	250	170	110
genotype 3		L	3	750	560	185	150	115		315	180	165	100		410			

Morphotypes and Genotypes	Host Lt	Host Bs	(n)	Undigested Size (bp)	Approximate Fragment Sizes (bp) *AluI*					*HinfI*					*RsaI*			
Cenococcum																		
genotype 1	M,L	M,L, B	19	770	410	150	110			280	165	125	95	85	890			
genotype 2		M	5	750	450	160	120			285	170	130			940			
genotype 3		M,L	6	755	405	150	115			345	240	145			870			
genotype 4	M,L		3	950	380	185	135	110		335	205	170	155	135	1030			
cotton orange																		
genotype 1		B	4	950	605	185	130			345	220	170	150		865	175		
crystal net brown																		
genotype 1	M	M	21	885	405	245	185	110		295	220	165	150		885			
genotype 2	L		3	940	355	185	150	125	105	1005					420	200	160	105
genotype 3	L		6	985	540	210	165	110		300	195	150	115	90	420	365	295	
granular brown																		
genotype 1		M	3	1020	400	215	165	110	90	350	310	175	155	120	985			
genotype 2		M	4	780	410	190	90			220	175	150	130		980			
genotype 3	M,L	M,L	15	915	395	190	130	115		340	280	170	155		975			
genotype 4	M		4	985	805	190	115			295	240	165	150		1000			
E-strain																		
[c]genotype 1	M		3	935	355	260	185	115		515	175	155	135		735	180		
genotype 2	L		5	930	450	190	150	130	115	300	270	205	125		345	270	240	

Morphotypes and Genotypes	Host		Undigested		Approximate Fragment Sizes (bp)													
	Lt	Bs	(n)	Size (bp)	*Alu*I					*Hin*fI					*Rsa*I			
Lactarius																		
genotype 1		L	5	1010	345	230	160	115		475	275	195	195		800	235		
[b]genotype 2		M,L	17	900	355	185	150	120	95	345	260	170	100		435	205	160	115
genotype 3		M,L	10	800	350	185	150	130	110	285	175	160	100		410	355		
genotype 4		M	3	990	430	275	185	115		355	320	170	155		965			
genotype 5		M,L, B	32	1070	525	280	185	110		390	350	170	150		1045			
lightly colonized																		
genotype 1	L		10	940	550	250	185			340	195	165	130	85	920			
genotype 2	M		3	750	655	130	115			440	215	165			355	180		
genotype 3	M		3	965	660	350	115			415	275	185			710	190		
MRA																		
[c]genotype 1	M		3	975	360	265	190	115	85	510	160	150	135		775	185		
genotype 2	L		9	1035	725	185	115			335	240	125	115		825	445		
genotype 3		B	20	880	575	180	110			415	295	165			450	250	195	
genotype 4		B,M	6	855	575	135	115			655	340				480	265		
Russula																		
genotype 1	L	M,L	8	910	415	180	145	130	110	290	270	210	130		315	290	255	
silver white																		
genotype 1		M	3	935	400	190	145	110	85	330	285	165	155		765	175		
Suillus 1																		
genotype 1	M		6	940	795	190	115			220	195	170	105		1030			

Morphotypes and Genotypes	Host Lt	Host Bs	(n)	Undigested Size (bp)	Approximate Fragment Sizes (bp) *Alu*I					*Hin*fI					*Rsa*I	
Suillus 2																
genotype 1	M		10	1050	350	250	185	145	115	415	285	230	165		1005	
genotype 2	L,M		8	920	345	250	185	160	110	410	255	165	145		960	
genotype 3	L,M		6	905	350	250	150	110		270	230	180	165		1015	
genotype 4	L,M		36	910	350	245	185	110		415	165	145			935	185
genotype 5	L,M		30	875	515	185	110			240	205	165	135	100	795	180
Thelephoraceae 1																
genotype 1		B	3	885	395	190	155	110	95	320	220	150			845	180
[c]genotype 2		B,M	26	1045	495	285	190	110		365	345	165	150		1025	
Thelephoraceae 2																
[c]genotype 1		B	10	1055	490	285	190	115		360	345	165	150		1065	
genotype 2	M	M,B	16	930	385	190	150	115	95	325	220	160	140	115	890	
[d]genotype 3		M,L	5	1010	405	195	155	110	95	350	330	170	160		890	175
genotype 4		M	4	900	465	225	190	110		310	160	145	90		1005	
genotype 5	M		2	1080	650	350				715	285	260			980	
Thelephoraceae 3																
genotype 1	M	M,L	8	935	385	185	120	110		350	320	165	150		1015	
genotype 2		M	5	870	350	190	120	110		350	325	170	155		845	
Tomentella-like 1																
genotype 1	M,L	L	7	820	365	185				345	315	170	155		930	

Morphotypes and Genotypes	Host Lt	Host Bs	(n)	Undigested Size (bp)	Approximate Fragment Sizes (bp) *Alu*I					*Hin*fI					*Rsa*I			
Tomentella-like 2																		
[d]genotype 1	M,L	M,L, B	25	885	395	185	120	95		335	210	165	150		1005			
genotype 2	M,L	M,L, B	8	780	400	175	135			235	185	160	140		905			
genotype 3	M	M,L	6	930	395	190	140	85		350	300	155	135		860	175		
genotype 4	L		3	785	580	190	155	115		320	185	170	120	90	435			
genotype 5	L		5	855	770	195	120			325	225	130	115		690			
white felted																		
genotype 1		M,B	3	875	365	185	130	115		350	295	165	150		425	340		
genotype 2		M	3	720	530	210	145	115		220	205	170	95		200	160	105	
white clamp																		
[a]genotype 1		M,B	15	930	405	245	190	110		300	220	170	155		990			
woolly brown																		
genotype 1	M,L	M	13	795	280	250	190			350	180	150			990			
yellow stellate																		
[d]genotype 1		L,B	4	1040	395	190	155	115	95	325	225	170	155		1020	275		
genotype 2		M	2	855	585	205	190			355	335	165	150		585			
[b]genotype 3	M		2	880	355	185	150	125	110	335	245	160	105		425	190	155	95

Genotypes that shared similar fragment patterns between morphotypes are indicated by the same letter (a,b,c,d, or e).

Ectomycorrhizal fragment pattern comparison between tamarack and scrub birch

In addition to genotypes that were similar within (or amongst) morphotypes, some fragment patterns of tamarack and scrub birch ectomycorrhizal genotypes, when compared at an approximate 5% tolerance level, were also similar. In total, 12 genotypes belonging to 10 morphotypes appeared to occur on both host species. Restriction fragment patterns for genotypes that occurred on both hosts were averaged and the resultant fragment patterns appear in Table 3.2. They include: *Amphinema* (genotype 1), *Cenococcum* (genotype 1), crystal net brown (genotype 1), granular brown (genotype 3), *Russula* (genotype 1), Thelephoraceae 2 (genotype 2), Thelephoraceae 3 (genotype 1), woolly brown (genotype 1), *Tomentella*-like 1 (genotype 1), and *Tomentella*-like 2 (genotypes 1, 2, and 3).

Ectomycorrhizal fragment pattern comparisons with black spruce (*Picea mariana*)

A study by Robertson (2003) examined black spruce ectomycorrhizas in two of the same peatland sites as the present study ("T" black spruce-tamarack wetland sites (Robertson, 2003) = "Mix" scrub birch-tamarack-black spruce peatland sites). By assessing the database information on ectomycorrhizal fragment patterns from her study, black spruce genotypes were compared to those for tamarack and scrub birch. Interestingly, scrub birch shared eight genotypes with black spruce in these sites, whereas tamarack shared only two fragment patterns (Table 3.3). Only one of these genotypes was found on all three hosts (woolly brown, from tamarack and scrub birch, matched an *Amphinema* identified on black spruce). Some of these fragment patterns contain one or two fragments that varied between host species; however, given that individual fragment

pattern selection can be subjective, and that standards can vary between users and between experiments, these genotypes were considered to be very similar.

Table 3.3. Approximate fragment sizes of the amplified ITS region of ectomycorrhizas that were potentially shared between hosts (scrub birch (Bs), tamarack (Lt), and black spruce (Sb)).

Morphotype	Host Species	Shared Site†	Undigested Size (bp)	Approximate Fragment Sizes (bp)														
				*Alu*I					*Hin*fI					*Rsa*I				
Cenococcum[1*]	Sb	+	790	440	150	110	80		275	165	130	100	920					
Cenococcum[2]	Bs	+	750	450	160	120			285	170	130		940					
Amphinema[6]	Sb	+	920	275	240	185	175	110	370	170	155		1085					
woolly brown[1]	Bs/Lt	+	795	280	250	190			350	180	150		990					
Tomentella-like 3[1]	Sb	+	875	415	185	120	110	90	220	190	165	150	980					
granular brown[2]	Bs	+	780	410	190	90			220	175	150	130	980					
Thelephoraceae-like 1[1]	Sb	+	950	420	185	150	110	95	320	225	165	150	855	175				
Thelephoraceae 1[1]	Bs		885	395	190	155	110	95	320	220	150		845	180				
Amphinema[3]	Sb		940	365	235	150	125	100	335	285	165	110	775	175				
silver white[1]	Bs	+	935	400	190	145	110	85	330	285	165	155	765	175				
Amphinema[1]	Sb		940	365	190	140	110		325	295	165	155	780	175				
white felted[1]	Bs	+	875	365	185	130	115		350	295	165	150	425	340				
Russulaceae 2[1]	Sb		950	690	190	110			335	290	165	150	555	195	175			
brown smooth 2[2]	Bs	+	955	700	190	135	115		350	250	160	150	565	250	170	110		

Morphotype	Host Species	Shared Site	Undigested Size (bp)	Approximate Fragment Sizes (bp)											
				AluI				*HinfI*					*RsaI*		
Russulaceae 2[7]	Sb		860	370	195	110		320	290	165	150	980			
Amphinema[2]	Lt	+	845	350	190	110		325	290	155		780	180		
Lactarius 2[2]	Sb	+	980	520	190	115	85	340	320	165	155	1055			
Lactarius[5]	Bs	+	1070	525	280	185	110	390	350	170	150	1045			

* = denotes genotype, † = indicates ectomycorrhiza(s) came from a host in the shared Mix peatland site type (no + means the root tips originated from another site type. Note: The first morphotype in each pair is always from the study on black spruce, Robertson (2003) with permission. The second morphotype is from the present study.

Phylogenetic analysis of sporocarps

Results from successful amplification and digestion of sporocarp samples are presented in Table 3.4. Species in the same genus, whose identity was uncertain, were given a "group" number. Approximately twice as many sporocarps/genotypes were identified for the Mix peatland site type (13), compared to the BsLt (6) and Bs (6) site types. In total, 19 restriction patterns were generated from 13 genera/families (n = 35) (Table 3.4). Although this likely represents only a small sub-sample of the potential sporocarps for these sites, when ectomycorrhiza and sporocarp fragment patterns were compared, several genotypes were determined to be very similar (Table 3.5). Matching patterns included silver white (genotype 1) on scrub birch and the fungus *Cortinarius* (group 1), as well as *Suillus* 2 (genotype 4) on tamarack and the fungus *Hebeloma* (group 1). In addition, a larger group of three morphotypes (*Lactarius* (genotype 2) on scrub birch, brown silvery (genotype 2) and yellow stellate (genotype 3) both on tamarack were all similar to the fungus *Hygrocybe* (group 1).

Table 3.4. Approximate fragment sizes (bp) of the amplified ITS region for sporocarps collected in Mix, BsLt and Bs peatland site types.

	Site Type				Undigested	Approximate Fragment Sizes (bp)											
Sporocarp	**Bs**	**BsLt**	**Mix**	**(n)**	**Size (bp)**			***Alu*I**				***Hin*fI**				***Rsa*I**	
Amanita vaginata	+			1	745	395	200	140	120		295	160			925		
Chroogomphus vinicolor			+	1	900	365	320	185	110		355	165	110		915	175	
Cortinarius spp.																	
group 1		+	+	8	845	365	190	145	120	95	340	165	150		795	175	
group 2		+	+	5	940	645	185	150	110		350	170	155		875	175	
Entolomataceae	+		+	2	935	290	190	145	100		390	345	165	150	855	175	
Fuscoboletinus spectabilis			+	1	970	525	195	125			630	235	195		1010		
Hebeloma sp.		+		1	915	335	245	185	170	110	345	165	150		870	175	
Hygrocybe spp.																	
group 1			+	1	825	350	195	140	105		345	270	165		430	305	175
group 2	+			1	985	375	280	190	110		340	305	205	165	805	175	
group 3			+	1	1025	540	285	190			355	345	165	150	1045		
Laccaria laccata		+		1	830	530	185	145	110		345	170	150		685	175	
Lactarius spp.																	
group 1			+	1	1030	630	210	150	125		580	370	160		870		
group 2	+		+	2	1025	605	205	140	125		595	335	170		905		
Leccinum sp.	+			1	1065	550	230	175	125		680	405			800	230	
Russula emetica 1		+	+	2	960	440	275	190	120		315	235	165	145	875	115	
Russula emetica 2	+		+	2	975	470	285	195	125		325	255	160	145	535	430	

Sporocarp	Site Type			(n)	Undigested Size (bp)	Approximate Fragment Sizes (bp)									
	Bs	BsLt	Mix			*Alu*I			*Hin*fI					*Rsa*I	
Scutellinia scutellata		+		1	980	715	190	120	480	335	160			940	
Suillus spp.															
group 1			+	2	965	785	190	120	290	235	165	105		1010	
group 2			+	1	1030	785	195	125	605	215	165			1005	

+, indicates that sporocarps were collected from that site type.

Table 3.5. Approximate fragment sizes (bp) of the amplified ITS region for sporocarps and for closest ectomycorrhiza match. Samples originated from the Mix, BsLt, and Bs peatland site types.

Morphotype		Site Type‡			Undigested	Approximate Fragment Sizes (bp)											
and Sporocarp	Host†	Bs	BsLt	Mix	Size (bp)			*Alu*I					*Hin*fI			*Rsa*I	
silver white[1*]	Bs	+			935	400	190	145	110	85	330	285	165	155	765	175	
Cortinarius spp.[1]			+	+		365	190	145	120	95	340	165	152		795	175	
Suillus 2[4]	Lt		+	+	910	350	245	185	110		415	165	145		935	185	
Hebeloma[1]			+		916	335	245	185	170	110	345	165	150		870	175	
Lactarius[2]	Bs		+	+	900	355	185	150	120	95	345	260	170	100	435	205	160
yellow stellate[3]	Lt			+	880	355	185	150	125	110	335	245	160	105	425	190	155
brown silvery[2]	Lt		+		820	355	185	150			345	255	165	105	440	200	160
Hygrocybe spp.[1]				+	827	350	195	140	105		345	270	165		430	305	175

*, superscript number denotes genotype (morphotype) or group (sporocarp) number, † = indicates host from which ectomycorrhizas originated (Lt = tamarack, Bs = scrub birch), ‡ = indicates on which site type ectomycorrhiza(s)/sporocarp(s) were found.

Molecular diversity within ectomycorrhizal morphotypes

Phi diversity values derived from the restriction fragment patterns for 17 commonly occurring and/or shared (found on both host species) ectomycorrhizal morphotypes are presented in Table 3.6. Values ranged between 0.002 (low intraspecific diversity) to 0.550 (high intraspecific diversity) and were not always similar for the same morphotype on the two hosts. On tamarack, *Russula*, *Tomentella*-like 1, and Thelephoraceae 3 morphotypes each had only one genotype and exhibited the lowest Phi diversity values. Thelephoraceae 2 had the highest diversity value although it had only two genotypes and represented a small sample size. The next highest values were for crystal net brown, followed by *Tomentella*-like 2, each of which had three genotypes. Interestingly, *Suillus* 2, which had five genotypes, had an intermediate diversity value when compared to all other morphotypes.

For scrub birch, *Russula* and *Tomentella*-like 1 morphotypes also had the lowest diversity values, as well as crystal net brown, each with one genotype. *Lactarius* (with five genotypes) had the highest Phi diversity values for this host, followed by the brown inky clamp (three genotypes) and MRA (two genotypes) morphotypes. When Phi diversity values were pooled for the shared morphotypes, *Russula*, *Tomentella*-like 1, and *Amphinema* had the lowest value, compared to MRA, Thelephoraceae 2, and crystal net brown that exhibited the highest diversity.

Table 3.6. Phi diversity values for commonly occurring and shared (those found on both host species) ectomycorrhizal morphotypes on tamarack and scrub birch.

	Tamarack			Birch			Shared†		
Morphotype	**(n)**	**genotypes***	**Phi**	**(n)**	**genotypes**	**Phi**	**(n)**	**genotypes**	**Phi**
Amphinema	13	2	0.131	4	1	0.157	17	2	0.137
brown inky clamp	-	-	-	19	3	0.358	-	-	-
brown silvery	18	3	0.220	-	-	-	-	-	-
brown smooth 2	-	-	-	19	3	0.309	-	-	-
Cenococcum	12	2	0.278	23	3	0.208	35	4	0.237
crystal net brown	20	3	0.355	10	1	0.010	30	3	0.291
E-strain	7	2	0.332	-	-	-	-	-	-
granular brown	15	2	0.182	11	3	0.207	26	4	0.199
Lactarius	-	-	-	67	5	0.361	-	-	-
MRA	12	2	0.153	26	2	0.311	38	4	0.515
Russula	5	1	0.002	3	1	0.002	8	1	0.016
Suillus 2	84	5	0.225	-	-	-	-	-	-
Thelephoraceae 1	-	-	-	30	2	0.138	-	-	-
Thelephoraceae 2	5	2	0.550	30	4	0.276	35	5	0.331
Thelephoraceae 3	2	1	0.137	12	2	0.294	14	2	0.282
Tomentella-like 1	5	1	0.093	2	1	0.028	7	1	0.131
Tomentella-like 2	20	4	0.341	28	3	0.190	48	5	0.286

† = pooled Phi values for those morphotypes on both tamarack and scrub birch, n = number of root tips successfully amplified and used to calculate Phi value, * = number of genotypes identified for each ectomycorrhizal morphotype
Note: lower Phi values suggest lower intraspecific diversity in that morphotype

Peatland site type effects on ectomycorrhizal diversity

In terms of genotypic diversity as measured by the Phi index, a two-way ANOVA showed no significant differences between peatland site types or between the two host species (Table 3.7). However, for both tamarack and scrub birch, mean Phi values were higher (although not significant) in the BsLt peatland site types, compared to the Mix site type with the highest diversity values for scrub birch within the Bs site type (Table 3.8).

Table 3.7. Two-way ANOVA showing site (BsLt and Mix), host (tamarack and scrub birch), and interaction effects based on Phi values for ectomycorrhizal genotypes (α = 0.05).

	Site Effect		Host Effect		Host*Site	
Diversity index	**F**	**P**	**F**	**P**	**F**	**P**
Phi	1.857	0.245	0.274	0.628	0.0003	0.986

Table 3.8. One-way ANOVA showing Phi diversity values (mean ±SE) for ectomycorrhizal genotypes originating from tamarack and scrub birch from three peatland site types (α = 0.05).

				Site	
Host Species	**F**	**P**	**Bs**	**BsLt**	**Mix**
Tamarack	3.583	0.199	-	0.460 (0.043)	0.376 (0.013)
Birch	0.727	0.553	0.474 (0.069)	0.428 (0.059)	0.341 (0.102)

DISCUSSION

Ectomycorrhizal genotypes, host specificity, and site distribution

Overall, 69 distinct genotypes were identified from 26 successfully amplified morphotypes in this study for tamarack and scrub birch. This included 38 genotypes from 17 ectomycorrhizas on tamarack, and 43 genotypes from the 21 ectomycorrhizas on scrub birch. The number of genotypes identified are similar to those reported by Robertson (2003) and Sakakibara et al. (2002), who identified 65 genotypes from 29 ectomycorrhizal morphotypes on black spruce, and 26 genotypes form 11 morphotypes characterized on Douglas-fir, respectively. Mah et al. (2001) characterized 46 genotypes from 24 ectomycorrhizal morphotypes on hybrid spruce. Some genotypes within a

morphotype showed variation in only one of the restriction endonucleases, while other genotypes varied in two or more; genotypes of *Lactarius* show examples of both occurrences. Horton (2002) and Sakakibara et al. (2002) also report a similar range in genetic variation within their identified ectomycorrhizal morphotypes. Differences in the amount of genotypic variation in our study compare favourably to those by Mah et al. (2001) and Robertson (2003), but it is perhaps higher than that found in other studies, such as the one by Hagerman et al. (1999). Reported differences could be due to the number of seedlings studied or to the number of root tips analyzed in each of the studies. For example, our sample size resulted in 34 scrub birch compared to 24 tamarack seedlings, and the study by Robertson (2003) examined 45 black spruce seedlings. The number of root tips successfully digested for molecular analysis by Hagerman et al. (1999) was 38 compared to 686 in the present study, and to approximately 1276 by Mah et al. (2001).

Some of the fungal genotypes belonging to morphotypes in our study appeared to be host and/or site specific. For example, three tamarack morphotypes (MRA, E-strain, and crystal net brown) produced genotypes that were found on both the BsLt and Mix site types, but individual genotypes were specific to one or the other peatland site type. Eleven tamarack genotypes were found on both site types; these included most *Suillus* 2, all *Cenococcum*, and several *Tomentella* genotypes. The remaining 26 genotypes for tamarack were found in only one of the two peatland site types; genotypes showing the greatest specificity belonged to brown silvery (only in the BsLt sites) and some Thelephoraceae (Mix sites). With respect to scrub birch, some genotypes within five morphotypes were found in all three of the peatland site types; at least one fragment

pattern in many of the remaining morphotypes occurred in two of the three peatland site types. A few genotypes mostly from rarely found morphotypes were often recorded from only one site (e.g. black cystidia and silver white). Robertson (2003) found that 54 of the 65 genotypes were retrieved from only one of three sites; however, some genotypes from *Cenococcum*, MRA, Russulaceae, Cortinariaceae, and E-strain ectomycorrhizas occurred on all three site types. Studies by Gehring et al. (1998), Jonsson et al. (1999), Mah et al. (2001), Sakakibara et al. (2002) also support this trend.

The distribution of the numbers of genotypes in these sites for both tamarack and scrub birch was always highest in the Mix sites, followed by the BsLt site type, and finally the Bs site type (for scrub birch). In total, 50 genotypes were identified from the Mix peatland site type, compared to 34 from the BsLt sites. Only 17 genotypes were described from the Bs sites; this lower number might be due in part to one host instead of two being examined on this site. Although Robertson (2003) also described similar patterns of an uneven distribution of genotypes across sites (i.e. genotypes occurring in all sites vs in two sites vs only one), she reported equal numbers of genotypes (approximately 30) in each of the three habitats. Interestingly, Robertson (2003) identified numerous genotypes belonging to fungi in the family Thelephoraceae, and in the genera *Tomentella* and *Lactarius*, from her two wetland sites. These were also genotypes that often occurred in the present study.

The decrease in genotypic variation on scrub birch in the BsLt (absence of black spruce) and Bs (absence of black spruce and tamarack) peatland site types could be due to fewer woody host species occurring on these sites. Although one might also expect to see a difference between the BsLt and Bs sites, but this was not observed.

When we examined the pooled results for fragment patterns for both hosts, the number of genotypes on the Mix and BsLt site types reflected the number of successfully amplified morphotypes. Twenty-four morphotypes (not including the lightly colonized group) generated 48 genotypes on the Mix site, decreasing to 16 morphotypes and 33 genotypes, respectively, for the BsLt site type. The Bs site had the least number of morphotypes (12) and, compared to the other two site types, had proportionately fewer genotypes (only 17). This decrease in genotypes may indicate that a lower ectomycorrhizal host diversity or a change in the plant community may be influencing the level of intraspecific variation expressed in the ITS region, resulting in a decrease in the number of genotypes exhibited by a given number of fungal species. Robertson (2003) suggested that genotype differences within and between sites might be due to localized heterogeneity in soil characteristics, site features, and vegetation composition in the peatland environments.

Despite numerous examples of genotypic variation described between hosts and sites, when the genetic diversity between peatland site types was compared using the Phi index, no significant differences in diversity were detected. In fact, the Bs and BsLt peatland site types resulted in higher Phi values than the Mix site type. Mah et al. (2001) and Robertson (2003) also did not find significant differences when genotypic diversity was compared between sites using the Phi index. However, Robertson (2003) did find that Phi values were highest for genotypic diversity within the Mix peatland site type compared to the black spruce dominated wetland sites.

Ectomycorrhizas: Intraspecific variation

Intraspecific variation for the 26 morphotypes that generated fragment patterns varied between one and five genotypes. Combining results for both hosts (tamarack and scrub birch), 17 morphotypes plus the lightly colonized each had more than one genotype, and seven of these contained four or more genotypes. *Suillus* 2 and *Lactarius* ectomycorrhizas showed the most intraspecific variation expressed on a single host species, with five genotypes each. Thelephoraceae 2 (five), MRA (four), *Tomentella*-like 2 (four), granular brown (four) and *Cenococcum* (four) had the most genotypes expressed present on both host species. In most cases, an ectomycorrhizal morphotype had one or two dominant genotypes (representing higher numbers of ectomycorrhizal roots), with the remaining genotypes containing fewer samples in number and being more evenly distributed. Sakakibara et al. (2002) and Mah et al. (2002) also noted that some of the morphotypes that exhibited more than one fragment pattern tended to have a dominant pattern and other less frequently occurring patterns. The remaining nine morphotypes in our study expressed little variation, with only one genotype each. Some of these morphotypes were considered to be rare types, and were usually only represented by a few ectomycorrhizal root tip samples. The majority of morphotypes identified by Hagerman et al. (1999) exhibited only one RFLP pattern; however, this low genetic variability may have been due to the small sample size (38 roots representing 10 morphotypes) collected for molecular analysis.

Genotypic variation in ectomycorrhizal species has been investigated in other studies. Robertson (2003) detected several morphotypes with large intraspecific variation (6-7 genotypes), including *Amphinema*, Cortinariaceae, and Russulaceae species. Mah et

al. (2001) identified multiple genotypes in the morphotypes of *Amphinema* and MRA, and Horton (2002) yielded multiple RFLP fragment patterns in *Laccaria*, *Tricholoma*, and *Lactarius* species. Even though our study sampled fewer seedlings for each host species compared to the above-mentioned studies, more morphotypes containing four or more genotypes were detected. Several reasons may partially explain differences in genotypic numbers for morphotypes. Fragment selection during RFLP analysis is somewhat subjective and selection protocols may vary among laboratories (e.g. fragment patterns can be manually marked by hand (Sakakibara et al., 2002), or one can utilize the software such as GeneProfiler for fragment pattern selection (Mah et al., 2001; Robertson, 2003). High intraspecific variation within the ITS region of some ectomycorrhizal morphotypes does exist; Horton (2002), Gardes et al. (1991) and Kårén et al. (1997) have attributed multiple fragment patterns to intraspecific RFLP polymorphisms within ectomycorrhizal fungi. In addition, several studies have suggested that some variation in RFLP fragment patterns may be due to differences in morphological characterization and the selection of ectomycorrhizal root tips; misidentification or selection might lead to genotypes within a morphotype that were perhaps actually from a different fungal species.

Molecular diversity within ectomycorrhizal morphotypes, for each host species as measured by the Phi Index, indicated that Thelephoraceae 2 on tamarack, and *Lactarius* and brown inky clamp on scrub birch, had the most intraspecific diversity. Interestingly, Thelephoraceae 2 had only two genotypes and a Phi value of 0.550, compared to *Suillus* 2 that had five genotypes and a Phi value of 0.225. This provides an example of how molecular diversity, as measured by the Phi Index, does not necessarily increase for a

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morphotype with an increase in the number of genotypes. This is because the Phi Index does not calculate diversity based on proportional abundance; instead, it uses pairwise distances between ectomycorrhizal samples. A morphotype, such as *Suillus* 2, that consists of five genotypes that have very similar fragments patterns can have a low Phi index value. Genotypes that share few fragments, such as in Thelephoraceae 2, even though they may comprise fewer distinct genotypes, can have a higher Phi index value. The Phi index measures the average squared distance in the data matrix, not the proportional abundance of the different genotypes.

The morphotypes with only one genotype (e.g. *Russula* and *Tomentella*-like 1 on both host species) had low within morphotype diversity (low Phi values), suggesting a high level of similarity between the samples that comprised the morphotype. When Phi values of morphotypes that were shared by both tamarack and scrub birch were combined, molecular diversity within each morphotype was highest for MRA, Thelephoraceae 2, and crystal net brown, compared to low diversity within *Tomentella*-like 1 and *Russula*. Robertson (2003) reported high within morphotype diversity for the black spruce morphotypes *Tomentella*-like 1 (not necessarily the same morphotype as described in the present study), Thelephoraceae 4, and MRA 1, and low Phi values for *Piloderma* and cottony halo. Compared to our results, Mah et al. (2001) had relatively low within morphotype diversity for all commonly occurring morphotypes found on hybrid spruce; however, the greatest intraspecific diversity according to the Phi index was also for an MRA morphotype.

Potential linkages between tamarack, scrub birch and black spruce ectomycorrhizas

The results from this study suggest that there is a very high potential for fungal linkages between tamarack and scrub birch in these peatland site types. Twelve fragment patterns (almost one fifth of all genotypes), representing 10 morphotypes (33%), were identified on both tamarack and scrub birch. This included genotypes for *Amphinema*, *Cenococcum*, *Russula*, several Thelephoraceae spp. and *Tomentella* spp., as well as crystal net brown, granular brown and woolly brown morphotypes. In a molecular study investigating Douglas-fir and bishop pine, Horton and Bruns (1998) reported that 12 out of 16 (75%) fungal species were shared between the two hosts. Some of the commonly shared fungi in their study, which took place in a mixed forest ecosystem along the California coastline, included *Tomentella*, *Russula*, *Amanita*, and *Cenococcum* spp.

Of the 12 genotypes that were shared between tamarack and scrub birch in our study, 10 were found on both hosts in the Mix peatland site type, six were found on both hosts in the BsLt site type, and four were found in both hosts in both the Mix and BsLt sites. Several genotypes also occurred on scrub birch in the Bs site type. Greater numbers of shared genotypes occurred in the Mix peatland site type; this suggests that an increase in host species in the Mix sites, with three potential ectomycorrhizal hosts compared to two in the BsLt, and one in the Bs sites, may play an important role in the establishment of potential linkages. It is interesting to note that several of these genotypes were not frequently observed, and only represented by a few samples. This may mean that less frequent genotypes may also be important in forming fungal linkages, or it that these genotypes were simply under-sampled. The genetic variation that we

identified as representing the potential for fungal linkages could actually be greater had we been able to increase the seedling and/or root tip sample size. Time constraints in processing larger sample sizes precluded this in this study.

When tamarack and scrub birch ectomycorrhizal fragment patterns were compared to the black spruce fragment patterns identified by Robertson (2003), nine genotypes were identified as being highly similar between the host species. Interestingly, scrub birch and black spruce shared more fragment patterns compared to tamarack which matched only two out of nine black spruce genotypes. The genotypes included the ectomycorrhizal morphotypes *Cenococcum*, *Lactarius*, and *Amphinema*, as well as several members of the Thelephoraceae 1 and Russulaceae. Genotypes also belonged to the unidentified ectomycorrhizal morphotypes granular brown, white felted, silver white, brown smooth 2, and woolly brown, some of which had morphological features similar to these identified ectomycorrhizas. Since both tamarack and black spruce shared more fungal symbionts with scrub birch than with each other, scrub birch may be the major common link between host species in these peatland ecosystems. In the Mix site type, where three hosts occurred, more genotypes were identified on scrub birch than for tamarack or for the other peatland site types. Robertson (2003) successfully identified 30 genotypes for black spruce on the same Mix peatland site type. Of the nine genotypes found on black spruce that were considered to be shared fragment patterns, four come from sites others than the Mix site type.

Sporocarp and ectomycorrhiza genotype comparison

Of the 19 fragment patterns identified from sporocarp samples, four genotypes were similar to ectomycorrhizal fragment patterns. Although not a large sample, it is

interesting considering that only a small percentage of ectomycorrhizal fungi produce sporocarps (Gardes and Bruns, 1996), and that sporocarp sampling only occurred over one summer season. In addition, several studies suggest that there is a poor correlation between sporocarp and ectomycorrhizas occurrence (Gardes and Bruns, 1996; Kårén et al., 1997; Robertson, 2003). Nevertheless, Dahlberg et al. (1997) reported that several ectomycorrhizal species characterized on Norway spruce roots in a Swedish old-growth forest were identified using a sporocarp RFLP database composed of fungal species found within the study site. Horton and Bruns (1998) also successfully identified over half of their ectomycorrhizal fungal species using RFLP fragment patterns from voucher sporocarp specimens.

In two cases in the present study, the fungal sporocarp and ectomycorrhizal morphotype were present in the same peatland site type. *Lactarius*, brown silvery and yellow stellate ectomycorrhizas all shared one genotype with a *Hygrocybe* species, suggesting that these genotypes might belong to the same fungal genus. Fragment patterns of one genotype belonging to *Suillus* 2 were similar to the fungus *Hebeloma*, even though the morphological characteristics between the ectomycorrhiza and fungi varied. Two bands differed between the fragment patterns and it remains unclear whether this *Suillus* genotype was actually a *Hebeloma* species. Although the morphotype silver white (one genotype) and the fungus *Cortinarius* did not co-occur on the same site type, their shared morphological features, as well as their similar fragment patterns increase the likelihood that this morphotype could be a species of *Cortinarius*.

Challenges with genotype classification

Lightly colonized root tips represented a large portion (25.7%) of all tamarack roots characterized. However, most (63.1%) of these were successfully placed in several of the established genotypes. Mah et al. (2001) matched five lightly colonized genotypes with other morphotypes identified in that study. Root tips characterized as lightly colonized were often brown with weakly developed mantles. The remaining roots (36.9%) that could not be placed with an established genotype formed three distinct genotypes referred to as lightly colonized and remain as unidentified morphotypes.

Five genotypes that belonged to different morphotypes had matching fragment patterns. Jonsson et al. (1999) also found that some RFLP-taxa (genotypes or fragment patterns) were detected in more than one morphotype, and Mah et al. (2001), identified several identical genotypes from different morphotypes. In this study, some of these matching genotypes belonged to the different host species. These genotypes most likely represent mis-characterized samples of ectomycorrhizal root tips that were sorted into the wrong morphotype during initial classification. The question remains as to which of the two (or three) morphotypes these samples belong. No attempt was made to re-classify these genotypes.

The molecular analysis of tamarack and scrub birch ectomycorrhizas identified numerous genotypes, some of which exhibited both host and peatland site type preferences. In addition, intraspecific variation was observed within most morphotypes, with up to five genotypes being expressed for several commonly occurring ectomycorrhizas. Most importantly, this study has provided strong evidence for the

existence of potential fungal linkages between both tamarack and scrub birch, as well as with black spruce, in these peatland sites.

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CONCLUSION

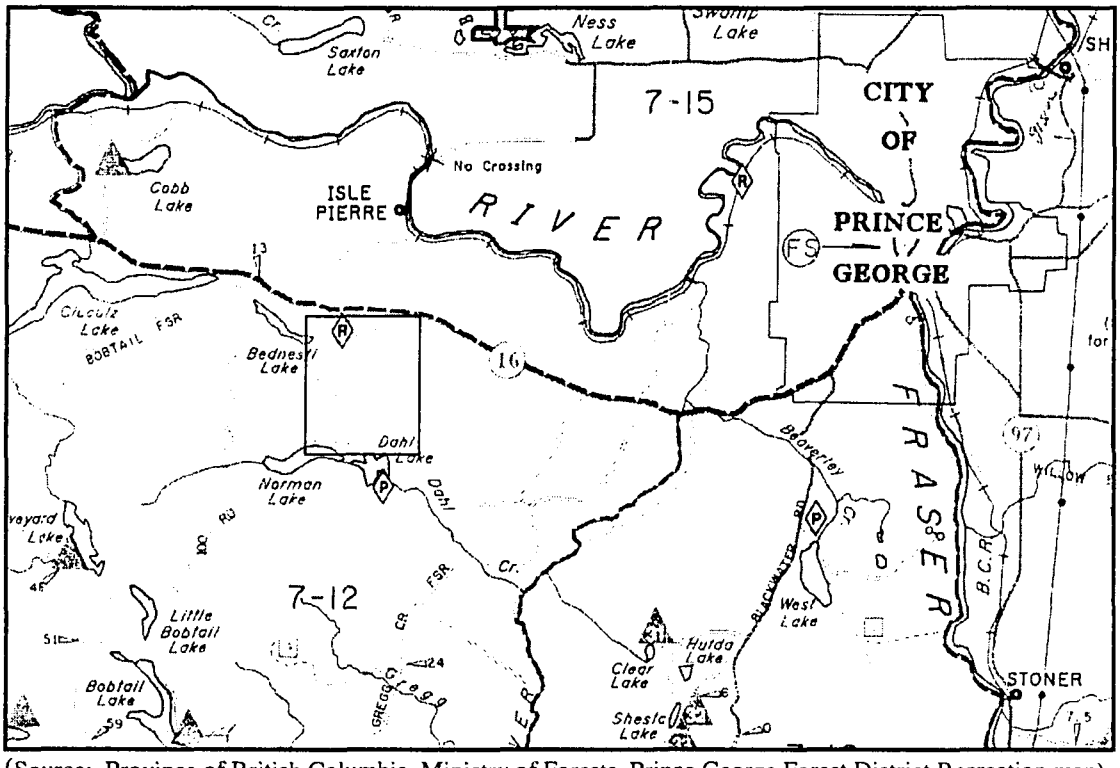
Peatlands, also referred to as bogs or fens, are unique ecosystems in British Columbia. Although we have extensive information on the plant communities associated with peatlands, we known little about the ectomycorrhizal status, in particular for tamarack and scrub birch in these habitats. This study investigated the ectomycorrhizal associations of these two hosts growing in peatland environments of central British Columbia, and addressed several questions concerning below-ground, ectomycorrhizal communities in peatland habitats. Through morphological and molecular analysis, we determined that the ectomycorrhizal abundance and diversity, even though peatlands are often described as poorly drained, nutrient-poor environments, did not appear to differ noticeably compared to the literature for upland forest ecosystems. More importantly, it appears that there is a high potential for fungal linkages between tamarack, scrub birch, and black spruce in these systems.

Morphological characterization described 30 ectomycorrhizas on tamarack and scrub birch roots, some of which exhibited host and/or site specificity. Ectomycorrhizal diversity was highest (as measured by the Margalef, Shannon, and Simpson indices) in the peatland site type that contained three potential host species (scrub birch, tamarack, and black spruce) compared to the sites that consisted of only one or two ectomycorrhizal hosts. Molecular analysis of the ectomycorrhizas identified numerous genotypes that reflected high intraspecific variation within some morphotypes, especially for those morphotypes which occurred in high abundance. Although more fungal genotypes were found in sites with three ectomycorrhizal hosts, compared to two or one host sites,

molecular diversity, according to the Phi index, was highest in the sites with only one potential ectomycorrhizal host, and lowest in the site type with three host species. This difference was not significant and most likely reflects the fact that genotypes on those sites, although fewer in numbers compared to the Mix site type, may have been separated by greater branch distance on the phylogenetic tree.

Both morphological and molecular analyses determined that numerous ectomycorrhizal fungi were found on both host species. However, the molecular investigation into the genetic composition of these ectomycorrhizas provided strong supporting evidence for fungal linkages in these environments. Shared ectomycorrhizal fungi between tamarack and scrub birch, as well as with black spruce, may be part of a complex underground system of mycelial networks. The transfer of carbon or nutrients between different host species, facilitated by symbiotic fungi, especially in these wet, nutrient-poor habitats, may be vital for the survival and growth of many peatland plant species.

Appendix I. Map of study area showing approximate locations (indicated by rectangle) of the six peatland sites in the Prince George Forest District in central British Columbia.



(Source: Province of British Columbia, Ministry of Forests, Prince George Forest District Recreation map)

Appendix II. Plant species list of vegetation growing within four 1 m x 1 m plots in each of the Mix (scrub birch-tamarack-black spruce), BsLt (scrub birch-tamarack), and Bs (scrub birch) peatland site types.

		Site Type					
Latin name	**Common name**	**Bs1**	**Bs2**	**BsLt1**	**BsLt2**	**Mix1**	**Mix2**
trees/shrubs							
Picea mariana	black spruce					•	•
Larix laricina	tamarack			•	•	•	•
Pinus contorta	lodgepole pine	•	•				
Betula glandulosa	scrub birch	•	•	•	•	•	•
Salix spp.	willow	•	•	•	•	•	
dwarf shrubs							
Vaccinium oxycoccos	bog cranberry	•	•			•	
Andromeda polifolia	bog-rosemary	•	•			•	
Rubus pubescens	trailing raspberry	•	•				
Kalmia microphylla	bog-laurel					•	•
Ledum groenlandicum	labrador tea	•	•		•	•	•
Rubus arcticus	dwarf nagoonberry		•				•
wildflowers							
Petasites sagittatus	arrow-leaved coltsfoot						•
Platanthera dilatata	white bog orchid			•		•	
Platanthera hyperborea	northern green bog orchid			•			
Mitella nuda	common mitrewort						•
Potentilla palustris	marsh cinquefoil	•	•		•	•	•
Galium spp.	bedstraw			•	•	•	
Menyanthes trifoliata	buckbean					•	
Pyrola asarifolia	pink wintergreen				•		•
Drosera rotundifolia	round-leaved sundew					•	
sedges/others							
Carex rostrata	beaked sedge	•	•	•	•	•	•
Carex interior	inland sedge	•	•	•	•		
Eriophorum angustifolium	narrow-leaved cotton grass	•	•	•	•	•	
Equisetum spp.	horsetail			•	•	•	•
Triglochin maritimum	sea-side arrow grass				•	•	
Poaceae spp.	grass	•	•	•	•		•
mosses/lichens							
Aulacomnium palustre	glow moss	•		•	•	•	•
Sphagnum spp.	peat moss	•	•			•	•
Mnium spp.	leafy moss				•		
Tomenthypnum nitens	golden fuzzy fen moss	•	•	•	•	•	•

Surveys were conducted in four 1 x 1 m plots within each site replicate. Presence of vegetation is indicated by '•'.

Appendix III. Descriptions of tamarack (Lt) and scrub birch (Bs) ectomycorrhizal morphotypes from Mix (scrub birch-tamarack-black spruce), BsLt (scrub birch-tamarack), and Bs (scrub birch) peatland site types.

Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
Amphinema (Lt and Bs)	orange-brown, cottony, unbranched straight tips (0.2 mm wide x 0.4 mm long)	outer mantle (OM)/inner mantle (IM) net synenchyma to non-interlocking irregular synenchyma, mantle ~20 µm thick	yellow emanating hyphae (EH), highly branched, ornamented, 3-3.5 µm wide; clamps	yellow, loose undifferentiated; hyphae finely verrucose with clamps
black cystidia (Bs)	black, short spiny, straight tips with monopodial pinnate branching (0.25 mm wide x 1 mm long)	OM regular synenchyma, IM net synenchyma (cells 5-10 µm wide), mantle 40-50 µm thick	brown cystidia, bottle-shaped, bent neck, 3-5 µm wide x 10-30 µm long; few septa with no clamps	not observed
brown clamp (Lt)	brown, smooth, unbranched straight tips (0.25 mm wide x 1.5 mm long)	OM net prosenchyma (cells 3-5 µm wide), IM net synenchyma (cells 1-2 µm wide), mantle ~20 µm thick	EH yellow-orange, smooth, up to 2 µm wide; clamps	not observed
brown inky clamp (Bs)	white with brown net-like overlay, smooth, straight tips with monopodial pinnate branching (0.25 mm wide x 1.5 mm long)	OM net prosenchyma (cells 3-4.5 µm wide), IM net synenchyma (cells 2-3 µm wide), stains rust in KOH, mantle 15-30 µm thick	hyaline EH, smooth, 3-4 µm wide; clamps	white, loose, undifferentiated, up to 150 µm wide; hyphae 5-6 µm wide with rounded clamps

Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
brown smooth 1 (Lt and Bs)	brown, smooth, unbranched, straight tips	OM net prosenchyma to interlocking irregular synenchyma (cells 2-3 µm wide), IM net synenchyma (cells 2-2.5 µm wide), mantle ~30 µm thick	not observed	not observed
brown smooth 2 (Lt and Bs)	mottled yellow brown, smooth, unbranched beaded tips	OM net synenchyma (cells 1.5-2 µm wide), mantle 15-20 µm thick	hyaline EH, smooth, ~1 µm wide; no clamps observed	not observed
brown silvery (Lt)	brown, silvery, unbranched straight tips (0.5 mm wide x 0.5-1 mm long)	OM felt prosenchyma (cells ~1 µm wide), IM net synenchyma, mantle ~20 µm thick	hyaline EH, smooth, 0.5-2 µm wide; no clamps observed	not observed
Cenococcum (Lt and Bs)	black, woolly, unbranched straight to beaded tips (0.5 mm wide x 0.5 mm long)	OM net synenchyma with typical stellate pattern (cells 2-5 µm wide)	dark brown EH, thick walled, mostly smooth, 2-5 µm wide; no clamps observed	not observed
coffee brown (Lt)	dark brown, shiny, straight tips with monopodial pinnate branching	OM net prosenchyma (cells 4-5 µm wide), IM net synenchyma (cells 1-2 µm wide)	yellow EH, smooth, 2-3 µm wide; clamps	not observed

Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
cotton orange (Bs)	orange-brown, cottony, unbranched straight tips (0.5 mm wide x 0.75 mm long)	OM obscured by EH, IM interlocking irregular synenchyma, mantle ~20 µm thick	EH pale yellow, highly branched, smooth, thin walled, 5 µm wide; clamps	not observed
crystal net brown (Lt and Bs)	brown, smooth to felty, unbranched straight tips (0.25 mm wide x 1.5 mm long)	OM net synenchyma (cells 1-2 µm wide), mantle ~30 µm thick	yellow EH, highly branched, net-like in appearance, verrucose, 2-3 µm wide; no clamps observed	not observed
E-strain (Lt and Bs)	brown to dark brown, smooth, shiny, unbranched straight tips (0.5 mm wide x 2 mm long)	OM net prosenchyma (cells 5-10 µm wide), IM net synenchyma (cells somewhat angular in appearance), mantle ~20 µm thick	not observed	not observed
granular brown (Lt and Bs)	yellow to dark brown, grainy appearance, unbranched straight tips	OM regular synenchyma (cells 5-7 µm wide)	EH yellow to dark brown, sometimes verrucose, thick walled, 5-7 µm wide; clamps occasionally observed	not observed

Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
Hebeloma-like (Lt)	brown often with white at base, silvery, cottony, unbranched straight tips	OM/IM obscured by EH	hyaline EH, smooth to verrucose, 5-5.5 µm wide; clamps	white, undifferentiated; hyphae smooth to verrucose; clamps
Lactarius (Lt and Bs)	yellow to light brown, smooth, straight to beaded tips with monopodial pinnate branching (0.5 mm wide x 2 mm long)	OM net synenchyma (cells 4-5 µm wide) with possible crystals, laticifers (4-6 µm wide, 40-100 µm long), producing rust colour when squashed, mantle ~20 µm thick	pale yellow EH, smooth, ~1 µm wide; fine septa with no clamps observed	yellow, slightly differentiated, hyphae 3-8 µm wide; no clamps observed
MRA (Lt and Bs)	brown-black, grainy, shiny, unbranched straight tips (0.2 mm wide x 0.2 mm long)	OM net prosenchyma (cells 3-4 µm wide), IM non-interlocking irregular synenchyma (4-5 µm wide), mantle 10-25 µm thick	dark yellow EH, smooth, 3-4.5 µm wide; no clamps observed	not observed
Russula (Lt and Bs)	brown, spiny, unbranched, straight tips (0.5 mm wide x 4 mm long)	OM interlocking irregular synenchyma (cells 2-7 µm wide), IM net synenchyma (cells 2-3 µm wide), mantle 20-40 µm thick	cystidia hyaline to pale yellow, smooth, two types a) awl-shaped (2-5 µm wide x 110-150 µm long) and b) flask shaped with apical knob (3-4 µm wide x 100-130 µm long); no clamps observed	not observed

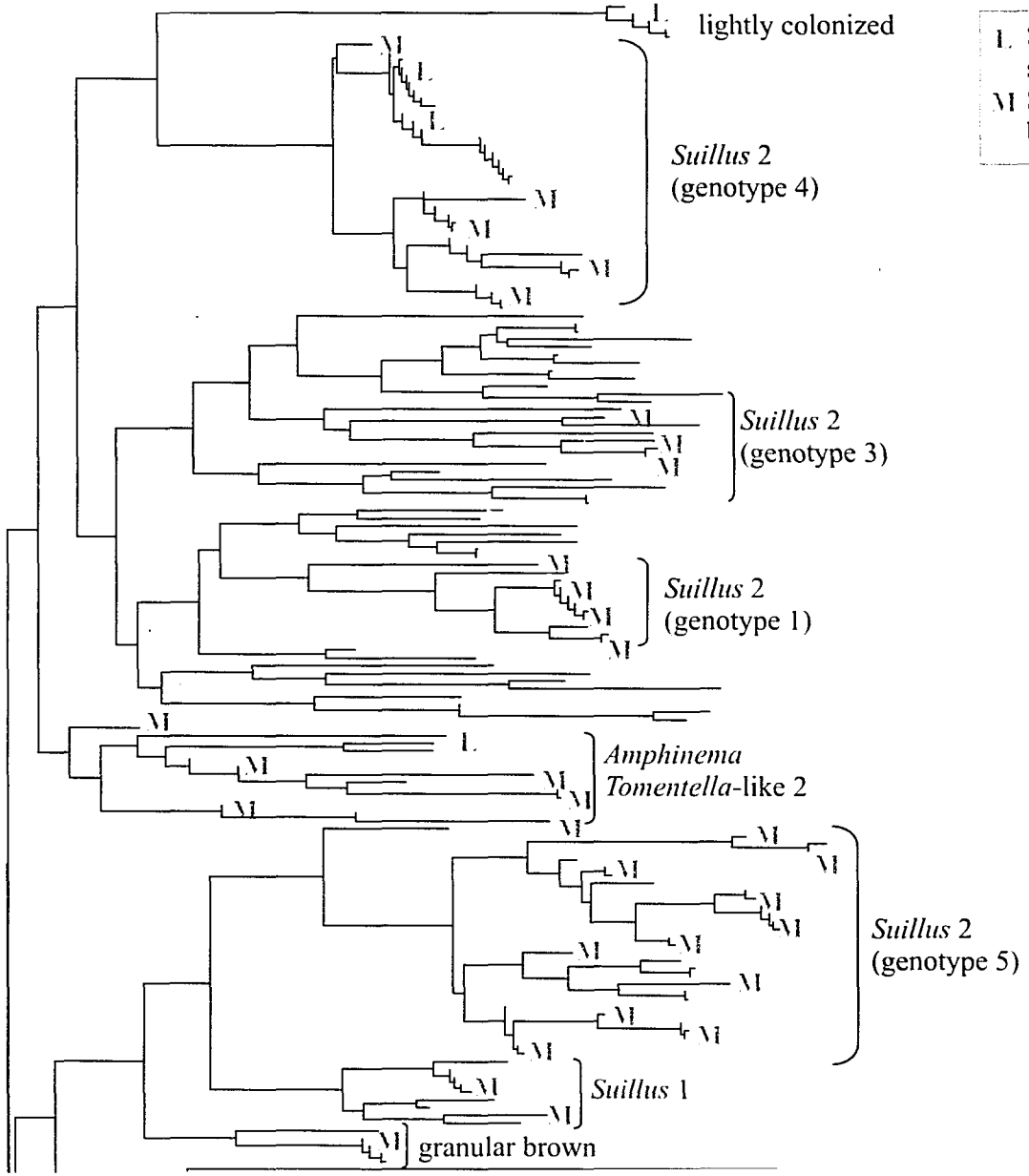
Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
Russulaceae (Bs)	light brown, smooth, unbranched straight tips (0.2 mm wide x 1.5 mm long)	OM non-interlocking irregular synenchyma, IM net synenchyma (cells 3-5 µm wide), produces an orange colour when squashed	hyaline EH, smooth, 2-3 µm wide; no clamps observed; EH not observed on all tips	not observed
silver white (Bs)	white to yellow, cottony, unbranched straight tips (0.25 mm wide x 1 mm long)	OM elongated interlocking irregular synenchyma, mantle 15-20 µm thick	hyaline EH, highly branched, finely verrucose, ~3 µm wide; clamps	not observed
Suillus 1 (Lt)	patchy yellow and white, silvery, stringy, straight tips with monopodial pinnate branching (0.5 mm wide x 1 mm long)	OM net prosenchyma (cells 2-3 µm wide), yellow crystals (20-25 µm wide) deposited on mantle, mantle ~30 µm thick	hyaline EH, 3-4 µm wide; no clamps observed; reddish purple amorphous crystals ornament EH	yellow, differentiated (central core), up to 40 µm wide, hyphae 2-10 µm wide, ornamented with reddish violet crystals
Suillus 2 (Lt)	brown, felt-like, straight tips with monopodial pinnate branching (0.5 mm wide x 4 mm long)	OM felt to net prosenchyma (cells 2.5-3 µm wide), IM net synenchyma (spiral-shaped cells 1-2.5 µm wide), mantle 15-20 µm thick	EH dark yellow to olive, verrucose, 2-4 µm wide; no clamps observed	rust, undifferentiated to slightly differentiated, compact, 70-90 µm wide; hyphae ornamented with rusty amorphous crystals (~2 µm wide x 15 µm long)

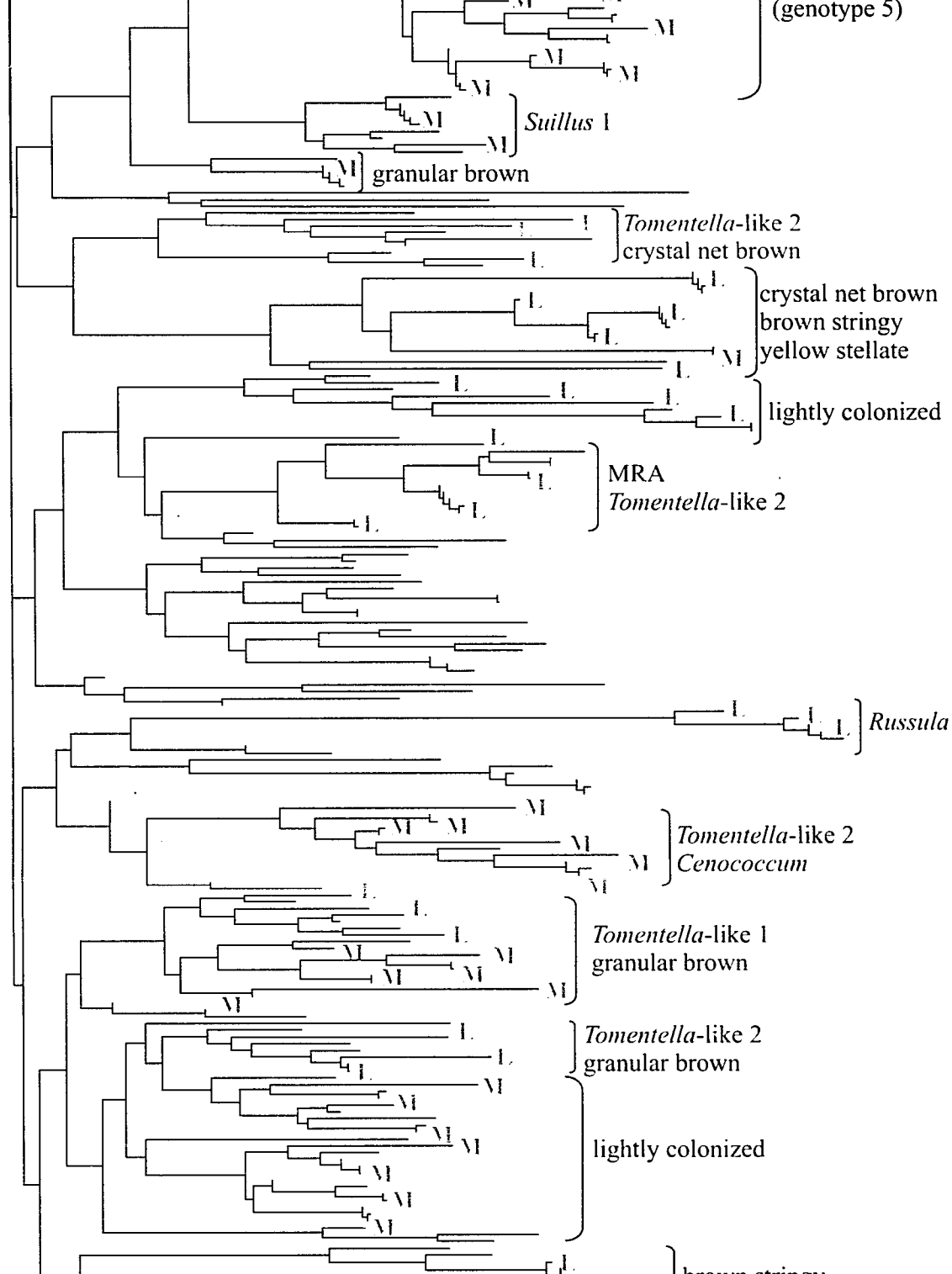
Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
Thelephoraceae 1 (Lt and Bs)	white to beige, smooth, straight tips with monopodial pinnate branching (0.25 mm wide x 1.5 mm long)	OM interlocking irregular synenchyma (cells 4-10 µm wide), mantle ~20 µm thick	not observed	not observed
Thelephoraceae 2 (Lt and Bs)	black with reflective metallic bronze colour, grainy, straight to beaded tips (0.25 mm wide x 1 mm long)	OM interlocking irregular synenchyma (cells 1.5-2 µm wide); stains blue-green in KOH, mantle ~50 µm thick	EH dark brown, thick walled (~2 µm), 3-5 µm wide, smooth, no clamps observed	not observed
Thelephoraceae 3 (Lt and Bs)	olive yellow, grainy, unbranched straight tips (0.5 mm wide x 0.75 mm long)	OM regular synenchyma (cells 5-10 µm wide), IM net synenchyma (cells 2-3 µm wide), mantle ~30 µm thick	yellow EH, smooth, 2-2.5 µm wide; clamps	yellow, loose undifferentiated, 30-40 µm wide; hyphae with clamps
Tomentella-like 1 (Lt and Bs)	yellow-brown, sparsely spiny, unbranched straight tips (0.5 mm wide x 0.75 mm long)	OM rounded non-interlocking irregular synenchyma (cells 5-10 µm wide), mantle ~20 µm thick	cystidia yellow, smooth, 2-5 µm wide x ~50 µm long, awl-like, thick-walled, clamped at base	yellow, undifferentiated, up to 30 µm wide, hyphae verrucose; clamps

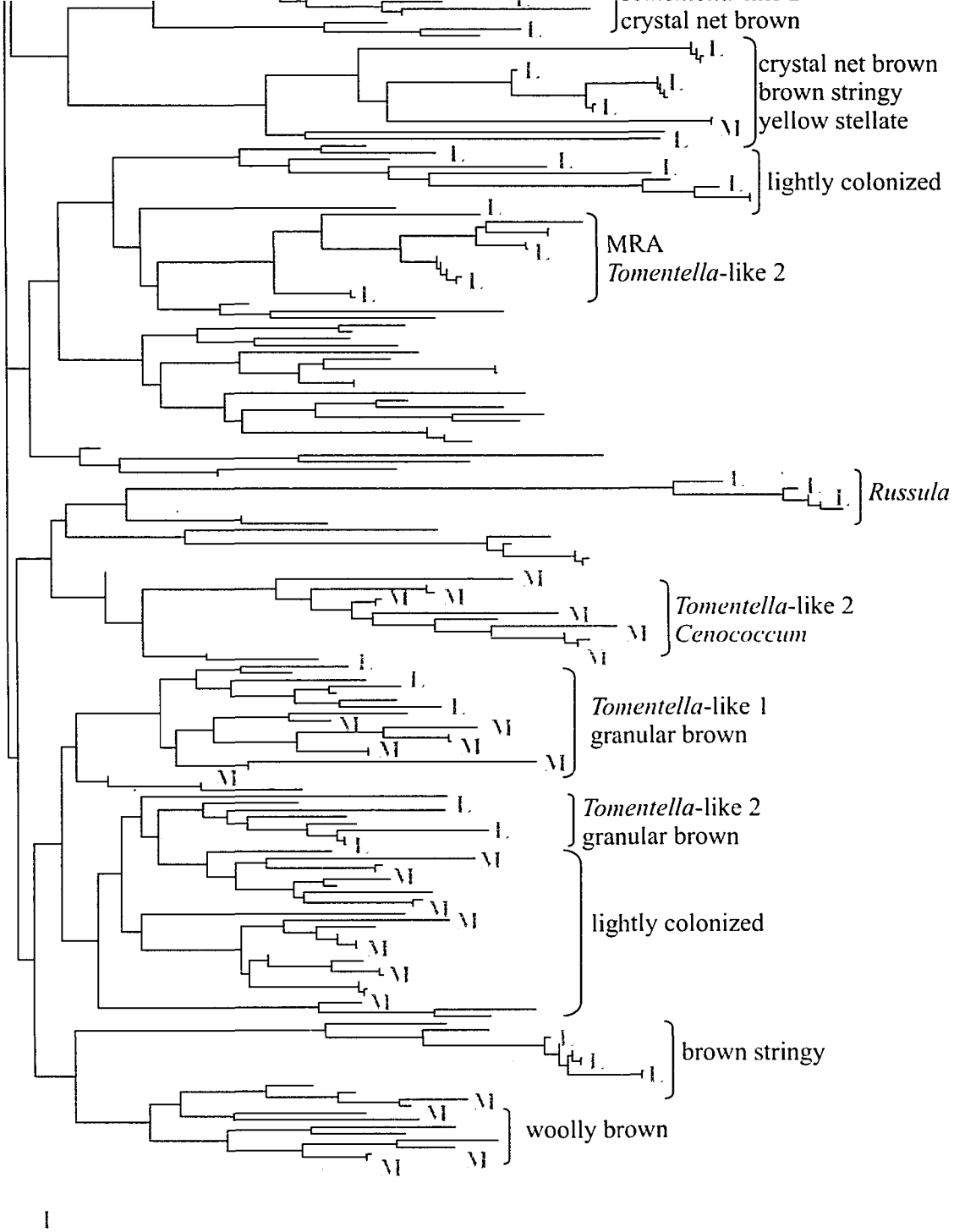
Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
Tomentella-like 2 (Lt and Bs)	black, grainy to rough, unbranched straight tips (0.25 mm wide x 1 mm long)	OM interlocking to non-interlocking irregular synenchyma, no KOH reaction, mantle 20-30 µm thick	dark brown EH, thick walled (up to 1 µm), ~5 µm wide; clamps occasionally observed	not observed
white clamp (Bs)	white with black net-like appearance, smooth, straight tips with monopodial pinnate branching (0.2 mm wide x 0.3 mm long)	OM net prosenchyma (cells 4-5 µm wide), yellow ornaments on surface noticeable when squashed, mantle ~20 µm thick	hyaline EH, smooth, short, ~2 µm wide x 10 µm long; clamps	not observed
white felted (Bs)	white, felt-like, unbranched, straight tips (0.25 mm wide x 1 mm long)	OM felt prosenchyma (cells 1-1.5 µm wide), IM net synenchyma (cells 2-3 µm wide), mantle ~20 µm thick	hyaline EH, verrucose, 1-1.5 µm wide; no clamps observed	not observed
woolly brown (Lt and Bs)	brown, woolly to cottony, unbranched straight tips (~0.25 mm wide x 0.5-1 mm long)	OM elongated interlocking irregular net synenchyma (cells ~3 µm wide), IM net synenchyma (cells 4-5 µm wide), stains rust-yellow in KOH, mantle ~20 µm thick	EH yellow-orange, smooth, 4-5 µm wide, forming a hyphal fan; clamps	yellow, loose undifferentiated, strands 30-35 µm wide; hyphae with clamps

Morphotype (Host)	Macroscopic Features	Microscopic Features	Emanating Hyphae	Rhizomorphs
yellow stellate (Lt and Bs)	dark yellow, smooth, unbranched tortuous tips (0.5 mm wide x 3 mm long)	OM net synenchyma with stellate pattern (cells 1-2 µm wide), IM net synenchyma (cells ~1 µm wide)	hyaline EH, smooth, fine septa, 1-2 µm wide; no clamps observed	not observed

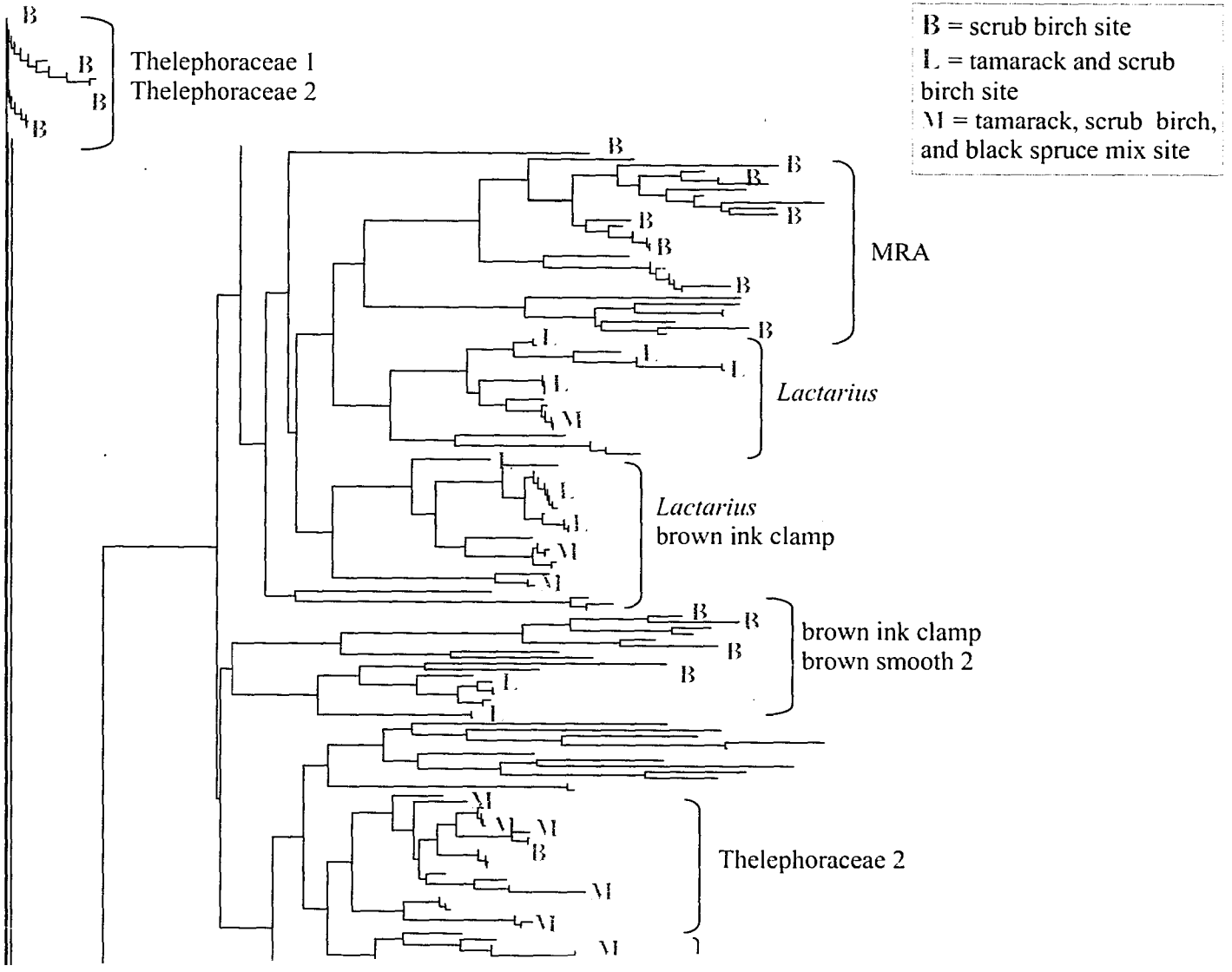
Appendix IV. Unrooted phylogram generated from restriction fragment patterns of tamarack ectomycorrhizal morphotypes. Phylogram shows the relationship between morphotypes and peatland site types.

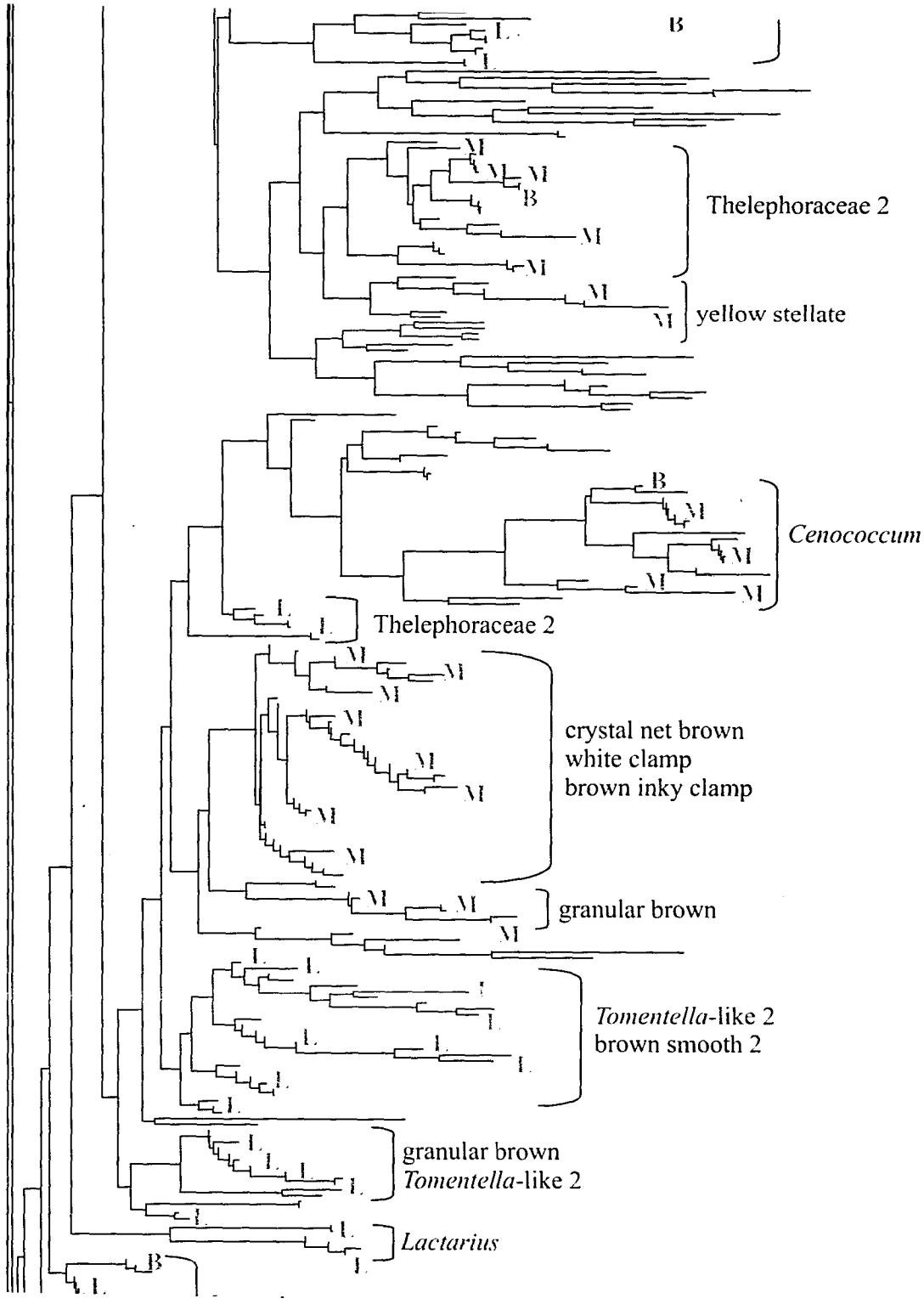


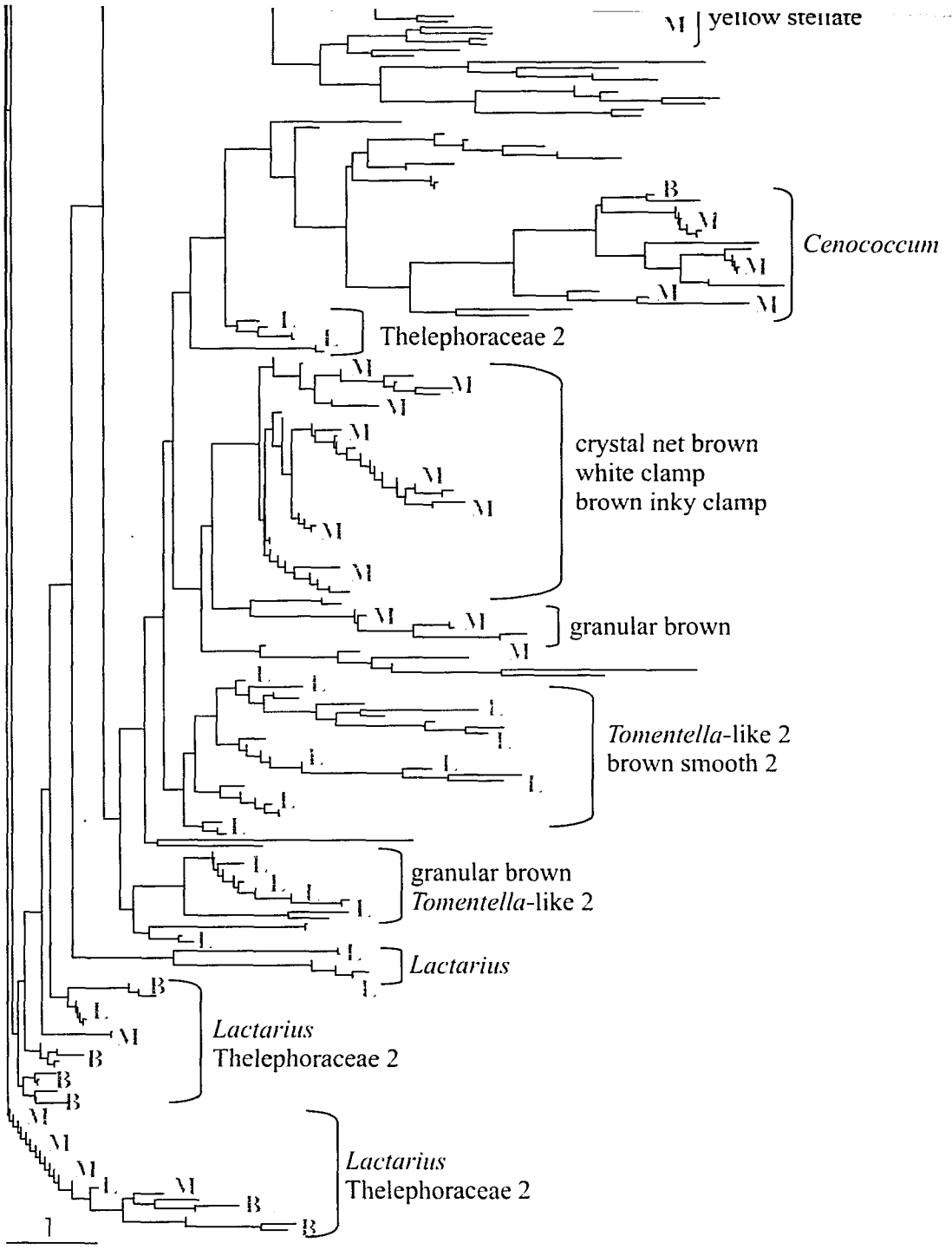

(genotype 5)
Suillus 1
granular brown
Tomentella-like 2
crystal net brown
crystal net brown
brown stringy
yellow stellate
lightly colonized
MRA
Tomentella-like 2
Russula
Tomentella-like 2
Cenococcum
Tomentella-like 1
granular brown
Tomentella-like 2
granular brown
lightly colonized


crystal net brown
crystal net brown
brown stringy
yellow stellate
lightly colonized
MRA
Tomentella-like 2
Russula
Tomentella-like 2
Cenococcum
Tomentella-like 1
granular brown
Tomentella-like 2
granular brown
lightly colonized
brown stringy
woolly brown
1

Appendix V. Unrooted phylogram generated from restriction fragment patterns of scrub birch ectomycorrhizal morphotypes. Phylogram shows the relationship between morphotypes and peatland site types.




Thelephoraceae 2
yellow stellate
Cenococcum
Thelephoraceae 2
crystal net brown
white clamp
brown inky clamp
granular brown
Tomentella-like 2
brown smooth 2
granular brown
Tomentella-like 2
Lactarius


yellow stellate
Cenococcum
Thelephoraceae 2
crystal net brown
white clamp
brown inky clamp
granular brown
Tomentella-like 2
brown smooth 2
granular brown
Tomentella-like 2
Lactarius
Lactarius
Thelephoraceae 2
Lactarius
Thelephoraceae 2