



Systematics of Rocky Mountain alpine *Laccaria* (basidiomycota, agaricales, tricholomataceae) and ecology of Beartooth Plateau alpine macromycetes  
by Todd William Osmundson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Sciences and Plant Pathology  
Montana State University  
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Abstract:

The alpine zone is comprised of habitats at elevations above treeline. Macromycetes (fungi that produce mushrooms) play important ecological roles as decomposers and mycorrhizal symbionts here as elsewhere. This research examined alpine macromycetes from the Rocky Mountains over 3 years, and includes: 1) a morphological taxonomic study of alpine *Laccaria* species, 2) a molecular phylogenetic study of alpine *Laccaria* using ribosomal DNA internal transcribed spacer (rDNA-ITS) sequences, and 3) a plot-based synecological study of macromycetes on the Beartooth Plateau (Montana/Wyoming, USA). The genus *Laccaria* is an important group of ectomycorrhizal (EM) basidiomycetes widely used in experimental and applied research on EM fungi. Five taxa are recognized in the Rocky Mountain alpine using macro- and micromorphological and culture data. All occur in Colorado, and are: *Laccaria bicolor*, *L. laccata* var. *pallidifolia*, *L. pumila*, *L. montana* and *L. sp.* (a new taxon similar to *L. montana*, with more elliptical, finely echinulate basidiospores). Only *L. pumila* and *L. montana* occur on the Beartooth Plateau. All are associated with species of *Salix*, and *L. laccata* also with *Dryas octopetala* and *Betula glandulosa*. Maximum-parsimony phylogenetic analysis of rDNA-ITS sequences for 16 alpine *Laccaria* collections provided strong support for morphological species delineations. *Laccaria laccata* var. *pallidifolia* is highly divergent relative to other taxa. *Laccaria pumila* and *L. montana* are supported as distinct species, along with a putative new taxon related to both. All taxa are supported by molecular synapomorphies except *L. pumila*, which exhibits a unique combination of insertion-deletions and single nucleotide polymorphisms. Alpine *L. bicolor* often lacks a violet basal tomentum, but differs from *L. laccata* by a robust, striate stipe and finely fibrillose pileus, characters supported by phylogenetic results. Interspecific ITS variation ranges from 1.6-7.3 %, and intraspecific variation from 0-1% in analyzed collections. Fifteen plots on the Beartooth Plateau, most containing a single EM host, were sampled multiple times per season. A total of 33 species (48% of estimated Beartooth Plateau total) were recorded. Small sampling plots focused on EM hosts were effective in representing most common EM fungal species; however, the EM host range of most species was not fully represented.

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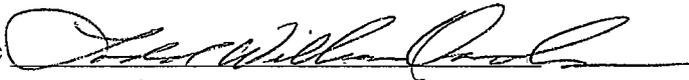


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## ABSTRACT

The alpine zone is comprised of habitats at elevations above treeline. Macromycetes (fungi that produce mushrooms) play important ecological roles as decomposers and mycorrhizal symbionts here as elsewhere. This research examined alpine macromycetes from the Rocky Mountains over 3 years, and includes: 1) a morphological taxonomic study of alpine *Laccaria* species, 2) a molecular phylogenetic study of alpine *Laccaria* using ribosomal DNA internal transcribed spacer (rDNA-ITS) sequences, and 3) a plot-based synecological study of macromycetes on the Beartooth Plateau (Montana/Wyoming, USA). The genus *Laccaria* is an important group of ectomycorrhizal (EM) basidiomycetes widely used in experimental and applied research on EM fungi. Five taxa are recognized in the Rocky Mountain alpine using macro- and micromorphological and culture data. All occur in Colorado, and are: *Laccaria bicolor*, *L. laccata* var. *pallidifolia*, *L. pumila*, *L. montana* and *L. sp.* (a new taxon similar to *L. montana*, with more elliptical, finely echinulate basidiospores). Only *L. pumila* and *L. montana* occur on the Beartooth Plateau. All are associated with species of *Salix*, and *L. laccata* also with *Dryas octopetala* and *Betula glandulosa*. Maximum-parsimony phylogenetic analysis of rDNA-ITS sequences for 16 alpine *Laccaria* collections provided strong support for morphological species delineations. *Laccaria laccata* var. *pallidifolia* is highly divergent relative to other taxa. *Laccaria pumila* and *L. montana* are supported as distinct species, along with a putative new taxon related to both. All taxa are supported by molecular synapomorphies except *L. pumila*, which exhibits a unique combination of insertion-deletions and single nucleotide polymorphisms. Alpine *L. bicolor* often lacks a violet basal tomentum, but differs from *L. laccata* by a robust, striate stipe and finely fibrillose pileus, characters supported by phylogenetic results. Interspecific ITS variation ranges from 1.6-7.3 %, and intraspecific variation from 0-1% in analyzed collections. Fifteen plots on the Beartooth Plateau, most containing a single EM host, were sampled multiple times per season. A total of 33 species (48% of estimated Beartooth Plateau total) were recorded. Small sampling plots focused on EM hosts were effective in representing most common EM fungal species; however, the EM host range of most species was not fully represented.

## CHAPTER 1

### LITERATURE REVIEW AND PROJECT OVERVIEW

#### Introduction

Alpine organisms live close to life's fringes, surviving under nearly constant conditions of environmental stress. While the climate, faunas, and vascular plant floras of alpine regions have been well (though by no means comprehensively) studied, fungi and other soil microorganisms, though of critical importance to ecosystem functioning, are relatively poorly known. This study is an attempt to make progress toward filling this gap in our understanding. The research described comprises two major components: a systematic study of Rocky Mountain alpine *Laccaria* species (Order Agaricales) using morphological and molecular data, and a plot-based synecological study of alpine macrofungi (Phylum Basidiomycota, Order Agaricales) on the Beartooth Plateau in southern Montana and northern Wyoming.

The term "alpine zone" refers to high mountain habitats situated at elevations above the climatic treeline, on an elevational gradient between the subalpine and nival (permanent snow cover) zones (Fig. 1). Treeline, the elevational limit for growth of the tree form in vascular plants, is situated at altitudes above which trees are physiologically unable to ripen shoots quickly enough to withstand adverse environmental conditions. Wardle (1974) comments that "timberline is therefore one of the most significant boundaries in biological nature, separating two fundamentally different ecosystems."

The alpine zone comprises approximately 4 million square kilometers, or 3% of the earth's land surface (Körner, 1999). North temperate alpine habitats are similar in terms of climate and vegetation to the Arctic, i.e., habitats situated at latitudes beyond treeline, and the two habitats are often considered collectively as comprising the "tundra," or arctic-alpine, biome that covers approximately 8 percent of the Earth's land surface (Chapin and Körner, 1995).

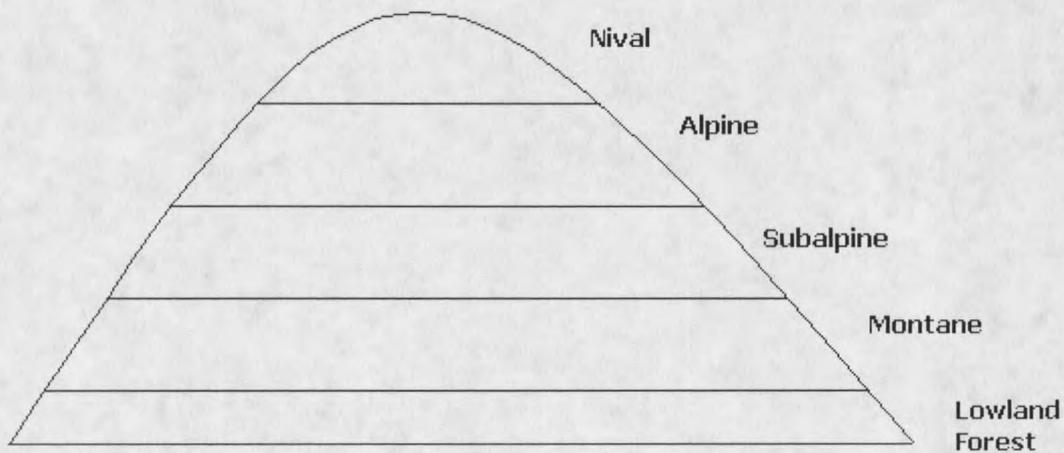


Figure 1. Schematic diagram showing elevational gradient of mountain vegetation zones.

Although most north-temperate alpine regions are geographically distant from the Arctic, similarities in climate-imposed physiological limits in addition to geologic events have shaped species distribution patterns and resulted in a high number of species having both arctic and alpine distributions. The following sections on patterns and origins of arctic-alpine biodiversity draw upon the literature pertaining to vascular plants. In

contrast to arctic-alpine vascular plant floras, arctic-alpine mycotas ("fungal floras" or fungal species assemblages) are poorly known in many parts of the world, precluding the ability to discern any large-scale patterns of species distribution. There are two primary reasons why a discussion of arctic-alpine vascular plant distributions may be relevant here. First, it is plausible that environmental factors have acted similarly in influencing distributions of both plants and fungi. Secondly, the presence of close associations between plants and both saprobic and mycorrhizal fungi predicts that fungal species distributions may follow those of particular plant species (or vice versa). For these reasons, the biogeographic patterns observed in arctic-alpine vascular plants provide an initial hypothesis for studies of such patterns in arctic-alpine macrofungi.

#### General Patterns of Arctic-Alpine Biodiversity

Although arctic and alpine habitats share a large number of plant species, the two physiographic regions differ in two main ways in regard to patterns of species diversity. First, the contribution of forest populations to overall species distributions, being a function of distance, is likely to be of greater importance in alpine than in arctic habitats (Gardes and Dahlberg, 1996). In addition, species diversity tends to be higher in alpine areas than in the arctic, both as a result of the proximity of dissimilar habitats (due to large altitudinal changes over a short distance) and the occurrence of a larger number of distinct, favorable microclimates (Billings, 1973, 1974a; Chapin & Körner, 1995; Müller & Magnuson, 1987). In general, species diversity tends to decrease with increases in altitude and latitude (Chapin & Körner, 1995).

### Origins of Alpine and Arctic Floras

The present north-temperate alpine vascular plant flora is hypothesized to have a complex evolutionary history resulting from a number of factors, including adaptive radiations in older species relocated and isolated by tectonic processes, migration of arctic species during periods of glaciation, adaptations in lower elevation montane species, and ice age survival of species in glacial refugia (Körner, 1995; Löve & Löve, 1974; Murray, 1995). The floras of tropical alpine regions are vastly different from those of temperate regions, and are hypothesized to be composed largely of evolutionary lineages derived from lower elevation plants. Due to these differences in species composition and evolutionary history, tropical alpine floras will not be further discussed in this study. However, one exceptional element of these tropical floras seems worthy of mention here in relationship to the floras of north temperate alpine ecosystems: a Tertiary "remnant of an old flora of the mountain chain north of the Tethys Sea" that makes up part of the flora of both the Tibetan alpine zone and the southern Rocky Mountains (Löve & Löve, 1974).

### Early Western North American Alpine Floras

Although the lack of early Mesozoic alpine plant fossils makes the early evolutionary history of alpine plants difficult to trace, evidence indicates that plants had colonized North American alpine zones at least as early as the late Cretaceous period (Billings, 1974b). Species distribution patterns and the occurrence of endemic species provide evidence that upward migration of lower elevation species and subsequent

radiation of new evolutionary lineages, as well as long-distance events or "alpine island-hopping," were important forces contributing to the present alpine flora (Billings, 1974b). Billings (1974b) recognizes a "strong endemic element" in the Rocky Mountain flora, and notes that the flora of the Beartooth Plateau of southern Montana and northern Wyoming consists of approximately 50% arctic-alpine and 50% western North American endemic species. The percentage of arctic-alpine species exhibits a southward decrease, with only about 25% of southern Rocky Mountain species having arctic-alpine distributions; a majority of species are related either to central Asian alpine species, southwest desert species, or represent endemic taxa.

#### Development of the Early Arctic Flora

Fossil evidence indicates that no representatives of current arctic-alpine plant genera were present in the northern plains during the early Tertiary period (Löve & Löve, 1974). Rather, it appears that by the early Eocene, the vegetation of the northern latitudes was dominated by elements of the Tertiary nemoral flora as the result of northward continental drift. Evolution of elements of the present arctic-alpine flora from these Tertiary species appears to have occurred in latitudes north of the conifer zone by the Late Miocene, and an early arctic flora of approximately 1,500 species had achieved a circumpolar distribution prior to the beginning of the Pliocene glaciation events (Löve & Löve, 1974).

### Effects of Pliocene/Pleistocene Glaciation

The glacial events of the Pliocene and Pleistocene periods profoundly influenced the distribution of arctic-alpine floras through the combined effects of climate change, physical disruption of species ranges, migration barriers, and occurrence of glacial and interglacial refugia. During periods of glaciation, ice cover resulted in the physical disruption of formerly circumpolar species distributions, and climatic cooling resulted in the southward displacement of species ranges. It is these southward displacements, and subsequent upward altitudinal migration of species, that is hypothesized to result in the observed patterns of shared species between arctic and alpine habitats (Löve & Löve, 1974). Of those species that did not extend southward, some, it can be presumed, suffered extinction. Some species, however, survived in northern regions in glacial refugia such as coastlines, islands, or nunataks (unglaciated mountain peaks surrounded by glacial ice) that escaped glaciation. These species, in addition to lower-latitude alpine species that experienced northward range extensions during interglacial periods, then colonized or recolonized arctic habitats during late Pleistocene interglacial periods (Abbott et al., 2000; Billings, 1974b; Ives, 1974b; Müller & Magnuson, 1987). The observation that most alpine populations of arctic-alpine species differ at the ecotype level from their arctic counterparts underscores the effect of barriers to gene flow during glacial periods, and suggests that populations surviving in glacial refugia may have been more important than northward-expanding populations in serving as interglacial source populations (Billings, 1974b; Löve & Löve, 1974). Billings (1974b) stresses the

importance of interglacial as well as glacial refugia in affecting the evolution of alpine species. In the former, alpine species were able to survive during upward elevational forest migrations during periods of deglaciation when climates became warmer. In addition to displacing ranges southward and isolating populations in refugia, glaciation events led to accelerated paces of evolution by fragmenting once-circumpolar species into isolated, independently evolving lineages (Löve & Löve, 1974).

An alternate hypothesis states that a widely distributed Northern Hemisphere arctic-alpine flora (in contrast to the solely Arctic flora mentioned above) was present prior to Pleistocene glaciation events, and that Pleistocene glaciation resulted in dissecting this arctic-alpine flora rather than forcing southward migrations of species. The presence of plants having Rocky Mountain – central Asian disjunct distributions seems to provide evidence supporting this hypothesis (Blair, 1996).

In regard to contemporary forces, work by Riebesell (1982) suggests that plant migration and extinction are ongoing processes in the alpine, comparable to island communities, and that alpine plant populations are dynamic rather than representing strictly relict populations. In summary, alpine vascular plant floras consist of elements having a complex mixture of historical origins: endemic species evolved from adapted populations of lower elevation species coexisting with local populations of more widely distributed species with distribution patterns resulting from a number of geological phenomena.

### Arctic-Alpine Mycotas

The biogeographic and evolutionary patterns of alpine macrofungi are much more poorly understood than are those of vascular plants; for instance, the level of endemism in western North American alpine species cannot yet be determined due to the lack of any previous large-scale studies of the mycota of this region, and a lack of baseline biogeographic data makes overall evolutionary patterns difficult to discern. Watling (1987) posed the question of whether the distributions of macrofungi are directly related to the migration of plant species, mirror patterns of plant distributions on the basis of similar physiological tolerance zones rather than on directly following plant migrations, or are, in fact, not similar to patterns of arctic-alpine plant distributions. An addition to these questions is whether saprobic fungal lineages follow the same evolutionary patterns as symbiotic or parasitic lineages. The biogeographic and evolutionary patterns observed for vascular plants can serve as a template for investigations of the evolution of alpine fungal taxa; however, more baseline biodiversity data are necessary before patterns for fungi can be reliably interpreted:

As mentioned earlier, the presence of mycorrhizal associations may predict that the distribution patterns of mycorrhizal macrofungi follow that of their plant hosts (or vice versa). Mycorrhizae are mutualistic associations between certain species of fungi and the roots of certain species of vascular plants. Ectomycorrhizae are a type of mycorrhizae in which the fungal hyphae form a sheath around the fine root tips of the plant. Nutrient exchange takes place in a netlike-structure (Hartig net) that surrounds the

plant epidermal cells. Ectomycorrhizae are generally formed by macrofungi in the Basidiomycota, and occasionally Ascomycota. Other types of mycorrhizal fungi are not considered in the present study.

Most mycorrhizal fungi are obligant symbionts, able to complete their life cycles only when associated with a suitable host plant. Ectomycorrhizal (EM) fungi exhibit various degrees of host specificity, with most having a wide host range while others (e.g. *Suillus* spp.) are restricted to a single host genus or species within a genus (Smith & Read, 1997). Watling (1992) notes that most EM macrofungi associated with willows (*Salix* spp.) in Great Britain are species having broad host ranges; however, he also notes that willow-dominated regions in Great Britain share many EM fungal species with willow-dominated regions in continental Europe. This finding suggests that the distributions of EM fungi are influenced by, or at least mirror, those of EM host plants.

Previous arctic-alpine fungal inventories indicate that between 28% and 60% of these mycotas comprise EM fungi (Cripps et al., 2001; Gulden, 1996); these fungi are associated with a limited number of host plant species (Table 1). While historical factors may explain broader patterns of species distributions, localized distributions of EM host plants, especially *Salix* spp. (the most common EM host plants in the North American alpine zone), may be the most important factor affecting Rocky Mountain alpine EM fungal distributions (C. Cripps, personal communication).

Because saprobic fungi appear to exhibit a degree of substrate specificity, it is likely that their distributions are influenced by the distribution of host plants as well. Preliminary evidence suggests that some saprobic macrofungi are host specific. A study

Table 1. Ectomycorrhizal host plants documented in arctic alpine habitats. Adapted from Gardes & Dahlberg (1996) with additions by T. Osmundson.

Species	References
<i>Arctostaphylos alpina</i>	Michelsen et al. 1996
<i>Arctostaphylos rubra</i>	Miller et al. 1982
<i>Betula glandulosa</i>	Eddington & Cripps (manuscript in preparation), Lange 1957
<i>Betula nana</i>	Michelsen et al. 1996, Miller et al. 1982, Treu et al 1996, Väre et al. 1997
<i>Cassiope tetragona</i>	Miller 1982, Miller & Laursen 1974, Kohn & Stasovski 1990, Stutz 1972
<i>Crepis aurea</i>	Read & Haselwandter 1981
<i>Daphne striata</i>	Read & Haselwandter 1981
<i>Dryas integrifolia</i>	Bledsoe et al. 1990, Kohn & Stasovski 1990, Miller & Laursen 1974, Read & Haselwandter 1981, Stutz 1972, Väre et al. 1992
<i>Dryas octopetala</i>	Bledsoe et al. 1990, Debaud et al. 1981, Haselwandter & Read 1980, Lesica & Antibus 1986, Miller 1982, Read & Haselwandter 1981, Treu et al. 1996, Väre et al. 1992
<i>Festuca rubra</i>	Read & Haselwandter 1981
<i>Helianthemum oelandicum</i>	Read & Haselwandter 1981
<i>Homogyne alpina</i>	Read & Haselwandter 1981
<i>Kobresia bellardii</i> (= <i>K. myosuroides</i> )	Haselwandter & Read 1980, Kohn & Stasovski 1990, Massicotte et al. 1998, Read & Haselwandter 1981
<i>Pedicularis capitata</i>	Kohn & Stasovski 1990
<i>Pedicularis dasyantha</i>	Väre et al. 1992
<i>Pedicularis hirsuta</i>	Stutz 1972
<i>Polygonum viviparum</i>	Haselwandter & Read 1980, Lesica & Antibus 1986, Massicotte et al. 1998, Michelsen et al. 1996, Read & Haselwandter 1981, Treu et al 1996
<i>Potentilla hyparctica</i>	Bledsoe et al. 1990, Haselwandter & Read 1980
<i>Potentilla reptans</i>	Read & Haselwandter 1981
<i>Potentilla stricta</i>	Read & Haselwandter 1981
<i>Pyrola grandiflora</i>	Kohn & Stasovski 1990
<i>Salix</i> spp.	Bledsoe et al. 1990, Dhillion 1994, Haselwandter & Read 1980, Kohn & Stasovski 1990, Laursen & Chmielewski 1982, Linkins & Antibus 1982, Michelsen et al. 1996, Miller 1982, Read & Haselwandter 1981, Stutz 1972, Treu et al. 1996, Väre et al. 1992, 1997
<i>Saxifraga oppositifolia</i>	Kohn & Stasovski 1990, Stutz 1972
<i>Saxifraga paniculata</i>	Read & Haselwandter 1981
<i>Silene acaulis</i>	Read & Haselwandter 1981
<i>Vaccinium uliginosum</i>	Stutz 1972

by Horak and Miller (1992) indicates that species of *Galerina*, a genus of moss-decomposing fungi, are restricted to certain mosses. In addition, the saprobic species *Marasmius epidryas* is reported only on dead leaves of *Dryas* spp.

In addition to historical and biotic factors, abiotic factors such as soil type and microclimate may influence macrofungal distributions. Lesica and Antibus (1986) found that levels of root colonization by arbuscular mycorrhizal fungi were significantly higher on calcareous than on acidic soils, speculating that this phenomenon is due to the fact that phosphorous availability can be limiting at pH levels higher than 7 and that mycorrhizal associations may therefore be particularly advantageous for plant growth on calcareous soils. It has not been established whether a similar pattern exists for EM fungi.

#### Alpine Climate

Perhaps the most common characterization of the temperate alpine zone is as a cold-dominated region: the effects of low average temperatures are in fact widespread, including short growing seasons, high incidence of water stress, and low soil nutrient availability due to reduced rates of weathering and mineralization processes, low biomass production, low decomposition rates and high levels of aeolian erosion (Gardes & Dahlberg, 1996; Körner, 1999; Lesica & Antibus, 1986). However, other factors such as high maximum solar radiation and reduced atmospheric pressure may be more common among alpine regions on a global scale; therefore, it can be said that cold temperatures alone do not account for patterns of adaptation exhibited by alpine plants (Körner, 1999).

In the Rocky Mountain temperate alpine zone, distribution of plant (and presumably fungal) species may be further influenced by low water availability due to high winds that remove snow cover in winter and increase evapotranspiration in plants during the growing season (Lesica & Antibus, 1986), and by low annual precipitation due to the influences of a continental climate type.

Perhaps the most critical determinant of species diversity levels and local distribution patterns is the occurrence of numerous, diverse, topologically influenced microclimates, or "topoclimates" (Barry and Van Wie, 1974; Billings, 1974a; Körner, 1999). Local differences in solar radiation, slope, and exposure (in turn influenced by wind speed, air temperature and soil type) influence soil temperatures, snow drift and cover patterns and melt rates, depth of soil thaw, effects of wind and local differences in plant canopy structure, and may in fact be more important than the more generalized "alpine climatic conditions" in determining species distributions (Billings, 1974a; Billings & Bliss, 1959; Körner, 1999). Billings (1974a) draws a sharp contrast between arctic-alpine and forested habitats in terms of the relationship between vegetation and the physical environment, stating that, "unlike the situation within a forest, the modification of microclimate by vegetation is minimal and the physical environment dominates the vegetation." Distinct microclimates can result from the location of a number of physical features, including rocks, solifluction terraces, soil polygons, or, for soil organisms or small-statured plants, from shelter offered by shrubs or other plants (Billings, 1974a).

### Adaptations in Arctic-Alpine Plants and Fungi

The origin of alpine life forms required adaptations to the unique climatic challenges associated with altitudinal extremes. In plants, these adaptations include avoidance of low temperature extremes through genetically determined patterns of growth form (e.g., cushion, prostrate shrub and rosette morphologies), phenological and life history patterns (e.g., dormancy, responses to temperature and photoperiod, predominance of perennial over annual species) and microhabitat selection (Körner, 1999). While compiling data on arctic-alpine fungal adaptations is outside the scope of the present research, a brief discussion of hypothesized adaptations is presented here because a better understanding of these mechanisms will ultimately enhance the understanding of evolutionary patterns in arctic-alpine fungi.

In terms of temperature adaptation, fungi must be able to endure freezing temperatures even during the growing season and resume metabolism without having to undergo an extended dormancy phase (Savile, 1982). Putative fungal adaptations to arctic-alpine and cold-dominated environments include physiological adaptations allowing growth at low temperatures, biochemical adaptations such as the production of cryoprotectant compounds, spore germination requiring incubation at sub-freezing temperatures, the ability of basidiocarps to resume spore production following freezing, production of dormant spores, thick and/or pigmented cell and spore walls, spores having a gelatinous outer coating, and self-compatible mating systems (Aragno, 1981; Gardes & Dahlberg, 1986; Ingold, 1982; Müller & Magnuson, 1987; Robinson, 2001). Research on

cold tolerance in fungi indicates that cold-adapted ecotypes exist within species (Cline et al., 1987; Tibbett et al., 1998), a phenomenon well known in vascular plant species (e.g., Billings & Mooney, 1968; McGraw, 1985); whether this ecotypic variation in fungi is the result of phenotypic plasticity or genetic drift is a question open to further research.

The aforementioned occurrence of self-compatible mating systems may be of particular importance in certain ectomycorrhizal basidiomycetes. The occurrence of fungi that produce basidia having a two-sterigmate condition (two spores borne on each basidium), instead of the typical four-sterigmate condition (resulting from meiosis and subsequent migration of one nucleus into each spore), is hypothesized to occur more frequently in arctic-alpine than in temperate species (Gardes and Dahlberg, 1996). This state occurs in genera such as *Laccaria* and *Inocybe* that are commonly found in disturbed and primary successional habitats as well as in arctic-alpine regions. In the two-sterigmate condition, basidiospores normally contain twice the normal number of nuclei (Mueller et al., 1993) and may therefore be secondarily homothallic, i.e., contain both mating type alleles and therefore be self-fertile. Secondary homothallism may allow rapid colonization of disturbed or extreme habitats, since mycelia from two spores of opposite mating types do not have to find each other for successful mating to occur.

The association of fungi in symbioses may represent an additional means of adaptation to conditions (in arctic-alpine or non-arctic-alpine habitats) of water stress or high ultraviolet radiation. The occurrence of Ascomycete and (very few) Basidiomycete fungi with algae or cyanobacteria in lichen symbioses is plentiful in arctic-alpine habitats, suggesting a potentially adaptive situation (Billings & Mooney, 1968; Larcher & Bauer,

1981; Redhead & Kuyper, 1987). Basidiolichens are comparatively abundant in arctic-alpine habitats, and represent a recently evolved symbiosis compared to ascolichens (Lutzoni & Vilgalys, 1995).

Studies of roots of arctic-alpine plants indicate that the incidence of arbuscular mycorrhizal colonization decreases with altitude (Körner, 1999). However, formation of ectomycorrhizae with woody dwarf and shrub plants (e.g., *Salix* spp., *Betula* spp., *Dryas* spp.) is nearly ubiquitous in arctic-alpine habitats (Eddington and Cripps, manuscript in preparation; Gardes & Dahlberg, 1996). This finding concurs with the observation by Moser (1966) that conifer species at treeline are almost universally ectomycorrhizal. These observations suggest that the formation of ectomycorrhizae may be critical for survival of woody plant species at high altitudes and latitudes. Gardes and Dahlberg (1996) suggest that greater knowledge of the population structures of arctic and alpine ectomycorrhizal fungi would be valuable in understanding these and other possibly advantageous adaptations.

#### What is an Alpine Species?

In discussing distribution patterns in alpine plant species, Körner (1999) notes that species observed in the alpine zone can represent: 1) Species with ranges centered at low elevations but extending above treeline, 2) Species with ranges centered in the montane zone and extending both to lower elevations and into the alpine zone, 3) Species with ranges predominantly in the alpine zone but extending into lower altitudes, and

4) Species restricted to the alpine zone. Previous studies indicate that these patterns apply to distributions of macrofungi as well (Gardes & Dahlberg, 1996; Moser, 1982, 2002; Singer, 1954). Of these categories, Körner considers only the last two to represent "true" arctic-alpine species. However, from a standpoint of identifying cold-adapted ecotypes, inferring evolutionary and biogeographic patterns, and identifying the physiological and host ranges of species, the study of species potentially belonging to any of these categories is important. In addition to these reasons, study of species occurring in the first two categories is of importance because these species may represent a significant component of the alpine mycota. To cite an example from the Arctic, a study of the saprobic fungal genus *Galerina* on the arctic island of Svalbard by Gulden (1987) reported the occurrence of 12 species: three having predominantly boreal distributions extending into the Arctic, four with wide (temperate to arctic) distributions, and five with predominantly arctic-alpine distributions. Of these latter five species, only two appear to be restricted to arctic-alpine habitats.

Distinguishing true arctic-alpine species may be complicated by the occurrence of environmentally - influenced morphological modifications (Bendiksen et al., 1993; Gardes and Dahlberg, 1996) that result from phenotypic plasticity rather than speciation events. Recognizing true arctic-alpine species from ecotypes of more widely distributed species can be approached with more confidence using molecular identification and/or mating studies. The present project attempts to take this approach toward better elucidating species identities in Rocky Mountain alpine species of *Laccaria* in hopes of

leading to a better understanding of the evolution of arctic-alpine mycorrhizal fungi in general.

### Arctic-Alpine Macromycete Studies

The study of arctic and alpine macromycetes (fungi, particularly Basidiomycetes but including Ascomycetes, forming conspicuous fruiting structures) is a relatively immature field of study compared to arctic-alpine studies involving vascular plants. While several small-scale examinations of arctic and alpine collections were conducted in the late nineteenth century, the first major works on arctic (Greenland: Lange, 1948-1957) and alpine (Swiss Alps: Favre, 1955) macromycetes were not carried out until the mid-twentieth century (Gulden, 1996, Horak et al., 2002). More recently, arctic-alpine mycotas have been documented for Europe, Iceland, Svalbard, and the Canadian Arctic but relatively little work has been done in North America or in the tropical alpine zones. Of macrofungi in general, Billings (1974b) states that "little is known of their ecology in alpine ecosystems." More recent studies have begun to elucidate the community structures and host relationships of alpine ectomycorrhizal basidiomycetes, but clearly more work is needed in this regard (see Gardes & Dahlberg, 1996 for review and discussion).

There are a number of reasons to explain this discrepancy between the states of knowledge pertaining to arctic-alpine fungi and vascular plants. Studies of macromycete systematics and biodiversity has historically lagged far behind that of vascular plants in general. In addition, relatively few mycological studies have been conducted in arctic-

alpine areas, most likely due to the relative inaccessibility of these habitats and their geographic distance from most centers of academic research.

A number of challenges are encountered in studying macromycetes in arctic-alpine habitats. First, the fruiting of macromycetes occurs during a short window of time and can be unpredictable due to the occurrence of summer freezing and potentially dry conditions. In a 3-year study of macrofungi in Rocky Mountain subalpine forests, Keck (2001) noted that 65% of the species recorded fruited only in a single year with above-average precipitation. Second, the basidiocarps of arctic-alpine macromycetes tend to be small and often hidden under shrubs or other vegetation, making collecting difficult. Third, the presence of diverse topoclimates results in patchy patterns of plant species distribution, resulting in small areas of species establishment and making placement of large research plots for quantitative studies difficult. However, the existence of diverse topoclimates probably serves to increase overall species diversity (Körner, 1999), and discrete vegetation units can be beneficial for inferring mycorrhizal host-symbiont associations and choosing appropriate comparative sites using small sampling plots. Two general types of approaches have been used in previous arctic-alpine macromycete studies to account for habitat patchiness: wide-scale sampling over diverse topoclimates (e.g., Lange, 1957), and focused sampling in specific habitat types of high fungal diversity, e.g. snowbed (Graf, 1994; Senn-Irlet, 1988) or mire (Senn-Irlet, 1993) communities.

In addition to fostering a better understanding of the ecology of cold-adapted organisms, further studies of arctic-alpine mycorrhizal fungi may have implications for

the overall understanding of mycorrhizal symbioses. As stated by Gardes and Dahlberg (1996), "cold-dominated environments provide extreme conditions for the establishment and functioning of mycorrhizal associations. Therefore, such systems are simple models to address the ecology and evolution of mycorrhizal symbioses."

The present review covers the body of arctic-alpine literature on two general types of studies: biotic inventories and mycosociological studies. As previously mentioned, a number of arctic-alpine regions have been the subject of macrofungal biotic inventories. Table 2 provides a summary of species numbers (fleshy Basidiomycetes only) reported in these biotic inventories; studies restricted to a single fungal genus are numerous and are not included here. As evidenced by these figures, many of the fungal inventories involving cold-adapted species has been conducted in Arctic ecosystems. Data suggest that macrofungal species diversity is greater in arctic than in alpine regions, in contrast with patterns observed in vascular plants. Whether this difference represents a true pattern resulting from historic or climatic factors or is simply an artifact of sampling effort will only become clear with further intensive surveys in alpine ecosystems.

While biotic inventories are primarily concerned with documenting the biodiversity of fungi within a study region, mycosociological studies are more concerned with elucidating aspects of community structure and ecological interactions between fungal species, between fungi and other organisms, and between fungi and environmental factors. Pertaining to the arctic-alpine biome, perhaps the most well-known study in this regard is that of Lange (1957), who documented distributions of Greenland macrofungi in relationship to plant community types.

Table 2. Summary of arctic-alpine macrofungal biotic inventories. Species numbers include fleshy Basidiomycetes (Agaricales, Boletales, Russulales, Gasteromycetes) only. Compilations include checklists or reviews not referring to single studies.

Study Location	Arctic/Alpine	Study Duration	Number of Species	Reference
Alaska, USA	Arctic	1 yr.	53	Kobayasi et al. 1967
Alaska, USA	subarctic tundra/taiga	10 yrs.	28	Miller 1982b
Alaska, USA	Arctic	10 yrs	22	Miller et al 1982, Gillman & Miller 1977
Baffin Island	Arctic	1 yr	18	Parmelee 1969
Greenland	Arctic	1 yr.	28	Watling 1977
Greenland	Arctic	Compilation	25	Watling 1983
Greenland	Arctic	1 yr.	44	Kobayasi et al. 1971
Greenland	Arctic	Compilation	560	Borgen et al. 2000
Greenland	Arctic	9 yrs.	218	Lange 1948-57
Godhavn area, W Greenland	Arctic	5 yrs	150	Lamoure et al. 1982
Iceland	Arctic	1 yr.	140	Christiansen 1941
Iceland	Arctic	11 yrs.	60*	Hallgrímsson 1981
Iceland	Arctic	Compilation	466	Hallgrímsson 1998
Iceland	Arctic	Compilation	13	Watling 1983
Northern Norway	Arctic	Compilation	212	Lange & Skifte 1967
Norway	Arctic	Compilation	15	Watling 1983
Svalbard	Arctic	Compilation	155	Ohenoja 1971, Gulden & Torkelsen 1996
Svalbard	Arctic	1 yr.	28	Kobayasi et al. 1968
Svalbard	Arctic	Compilation	19	Watling 1983
Fennoscandia	Arctic, Alpine	compilation	406	Bendiksen & Ohenoja, unpublished
Alberta, Canadian Rockies	Alpine	5 yrs.	13 **	Kernaghan & Currah 1998
Fiera di Primiero, Italian Alps	Alpine	Single foray	22	Bon 1987
Rhaetian Alps	Alpine	Single foray	22 **	Senn-Irlet 1992
Swiss Alps	Alpine	3 yrs	69	Graf 1994
Swiss National Park, Swiss Alps	Alpine	13 yrs.	213	Favre 1955
Southern and Central Rocky Mountains, USA	Alpine	4 yrs	150+	Cripps et al 2002

\* Family Tricholomataceae only.

\*\* Study included subalpine as well as alpine areas; only alpine species are included here.

The ecological aspects of these studies are reviewed in Chapter 4 (Beartooth plot studies); only the inventory data related to these studies are summarized here (Table 3).

In contrast to the body of fungal inventories, the majority of mycosociological studies have been conducted in alpine areas, especially the European Alps.

Table 3. Summary of species inventory data generated during arctic-alpine mycosociological and ecological studies. Species numbers include fleshy Basidiomycetes (Agaricales, Boletales, Russulales, Gasteromycetes) only.

Study Location	Arctic/Alpine	Study Duration	Number of Species	Reference
Alps	Alpine	2 yrs.	39	Eynard 1977
Swiss Alps	Alpine	3 yrs	69	Graf 1994
Swiss Alps	Alpine	3-5 yrs.	88	Senn-Irlet 1988
Swiss Alps	Alpine	5 yrs	25	Senn-Irlet 1987
Western Italian Alps	Alpine	5 yrs	99	Lo Bue et al 1994
Eagle Summit, Alaska, USA	Arctic	Not listed	16 (EM only)	Miller 1982
Godhavn area, W. Greenland	Arctic	3 yrs	65	Petersen 1977
Greenland	Arctic	9 yrs.	218	Lange 1948-57
NW Finnish Lapland	Arctic	8 yrs.	56	Metsanheimo 1987

Further research in two areas is suggested by the studies cited here: additional inventories in previously under-investigated regions, and further research into the ecology of arctic-alpine fungi. Many of the alpine fungal inventories have been focused on the Alps; investigations of mountain ranges in regions such as North America and temperate Asia are necessary for better understanding biogeographic patterns. Lack of knowledge of the latter region could represent language biases in the literature rather than true gaps in research. Among agaric fungi, ectomycorrhizal fungi are dominant in arctic-alpine habitats in terms of species richness, comprising between 28 and 60 percent of macromycete species observed (Cripps et al., 2001; Gulden, 1996). Further investigations on mycorrhizal community structure, host associations, and factors affecting species composition and diversity will help elucidate this important aspect of arctic-alpine ecology.

### The Rocky Mountain Alpine Mycota Project

As mentioned above, the arctic-alpine mycota has been the subject of biotic inventories in several areas, but that of the North American alpine is virtually unknown. The present study was conducted as a part of the Rocky Mountain Alpine Mycota project, a National Science Foundation Biotic Surveys and Inventories Program-sponsored project dedicated to conducting the first large-scale survey of macrofungal biodiversity in the North American alpine zone (Cripps & Horak, 1999; Cripps et al., 2002).

The importance of better understanding species biodiversity in the Rocky Mountain and other alpine regions is underscored by the function of these areas as repositories for winter precipitation (of particular importance to the arid regions of western North America), as potential indicator biomes responding to climate change events, and as understudied physiographic regions.

Because of their high latitudes/altitudes and areas of perennial snow cover, arctic-alpine areas are likely to be particularly sensitive to the effects of large-scale climate change events (Grabherr et al., 1995; Smaglik, 2000). Such events could have pronounced effects on arctic-alpine habitats by changing snowmelt timing and/or patterns, affecting organismal physiology through higher levels of ultraviolet radiation, allowing upward elevational shifts in treeline, causing melting of permafrost layers (either directly through higher mean air temperatures or indirectly through modifications to plant and soil community composition), changing the abundance of shrubby vegetation and altering levels of net CO<sub>2</sub> emissions. Warmer mean annual temperatures could affect

glacial formation and melt rates, with subsequent effects on the uses of glaciers as irrigation sources, archives of past climatic conditions, scenic and recreational sources, and future sources of drinking water (Ives, 1974a; Nelson et al., 2001; Oechel et al., 2000; Østrem, 1974; Sturm et al., 2001). Ecological consequences of climate change already in evidence include changes in organismal phenologies, range shifts, changes in community composition, invasions of nonnative species, and changes in species recruitment and trophic interactions (Walther et al., 2002). Hypothesized effects of changes to soil community composition underscore the fact that we know little about the community structure of fungi and other soil microorganisms in arctic-alpine habitats.

As understudied physiographic regions, arctic-alpine habitats may hold important keys to understanding larger evolutionary patterns. Müller & Magnuson (1987) suggest that the survey of additional areas is necessary in order to better determine the origins of particular arctic-alpine species, and that incomplete data on species distributions hampers our ability to arrive at conclusions regarding the biogeography and evolution of taxa. Additionally, Moncalvo et al. (2000) suggest that addressing questions regarding the higher-level phylogenetic relationships among the agaricoid fungi is restricted by gaps created by yet-undiscovered taxa. Biodiversity studies in under-studied biomes may help to more quickly fill in these gaps, contributing to studies in the areas of systematics, evolutionary biology, biogeography, and ecology.

### Applied Aspects of Fungi in Arctic-Alpine Habitats

For most of the period of human existence, the inhospitable environment and inaccessibility of the alpine zone have prevented severe human-mediated disturbance of these regions. The advent of all-terrain vehicle technology and increasing population growth and industrial production present severe challenges for these ecosystems. Disturbance of alpine habitats by human activities such as snowmobiling, off-road motorized recreation, livestock grazing, ski area development, mining and air pollution have increased over recent decades (Graf, 1997; Ives, 1974c; Körner, 1999). As a result, the importance of research into alpine ecosystem functioning and reclamation have increased. One aspect of this research is to better understand the functional importance and potential applications of ectomycorrhizal fungi in alpine environments. Species such as *Laccaria* spp. may be particularly useful for such applications because of their ability to grow in pure culture and form ectomycorrhizae under laboratory conditions, and have a wide distribution throughout the alpine zone (Graf & Brunner, 1995).

In applied studies focused on developing appropriate systems for reclamation in alpine areas (particularly ski slopes), Graf (1997) synthesized *Laccaria bicolor* (Maire) Orton and *L. montana* Singer mycorrhizae with the host plants *Dryas octopetala* L. and *Salix herbacea* L. Mycorrhizal fungi added to soil significantly increased soil aggregation, reduced erosion in simulated rain treatments, and increased retention of small grain-sized soil particles on slopes. Ectomycorrhizal fungi used to inoculate

seedlings for the revegetation of a high-elevation subalpine Colorado mine site resulted in increased seedling growth rates (Grossnickle & Reid, 1982, 1983).

In addition to applications involving direct synthesis of ectomycorrhizae with host plants, ectomycorrhizal fungi could have additional applied uses. For example, because the hyphae that comprise the thallus of an ectomycorrhizal fungus can absorb nutrients over large geographic areas, basidiocarps may potentially be used as sensitive bioindicators for the accumulation of pollutants such as heavy metals (Peintner, 1998). Better understanding the ecology (i.e. host association), distribution, and physiology (fruiting frequency and periodicity, abundance) of cold-adapted mycorrhizal fungi will have important implications for applied research by facilitating selection of appropriate experimental systems.

#### Description and Goals of Present Research

The present research consists of the following three studies:

- 1.) A detailed taxonomic study of alpine species in the genus *Laccaria* (Phylum Basidiomycota, Order Agaricales, Family Tricholomataceae).
- 2.) A molecular phylogenetic study of Rocky Mountain alpine *Laccaria* species using nuclear ribosomal internal transcribed spacer (ITS) DNA sequences.
- 3.) A plot-based synecological study of alpine fungi on the Beartooth Plateau, a large alpine region located in southern Montana and northern Wyoming.

### Taxonomic Study of Rocky Mountain Alpine *Laccaria*

As part of this project, a detailed systematic study of Rocky Mountain alpine *Laccaria* species was conducted. The cosmopolitan genus *Laccaria* Berkeley & Broome comprises a group of agaric (mushroom-forming) fungi (Phylum Basidiomycota, Class Hymenomycetes, Order Agaricales) classified in the family Tricholomataceae on the basis of having a white spore print, attached lamellae, non-divergent lamellar trama, and lack of both an annulus and volva (Singer, 1986). *Laccaria* species are frequently collected and are documented as ectomycorrhizal symbionts of numerous plant species, suggesting an important ecological role. Some species are found in disturbed or primary successional habitats such as recently deglaciated soils (Jumpponen et al., 1999), smelter sites (Cripps, 2001), recently reclaimed mine sites (Tommerup et al., 1991) and young forests (Dighton et al., 1986; Tommerup et al., 1991), indicating a role as pioneer species following ecological disturbance events. Because a number of *Laccaria* species can grow in culture and form mycorrhizae with host plants under laboratory conditions, they have been frequently used in applied studies on mycorrhizal fungi (Mueller, 1992). A better understanding of species limits, infrageneric and higher-order relationships, ecology and host associations in *Laccaria* could provide useful insight into character evolution and the evolution of host associations in ectomycorrhizal fungi. *Laccaria* is a commonly encountered genus demonstrated to be important in soil aggregation, plant nutrient acquisition, soil nutrient cycling, and primary succession in disturbed habitats. A better understanding of host-symbiont relationships in alpine species promises to have

additional implications toward facilitating selection of appropriate experimental systems for applied high-altitude ecological and reclamation research.

The genus *Laccaria* is supported as monophyletic by at least one synapomorphic character, the presence of spores with echinulae formed by microtubules oriented perpendicular to the epispore layer (Mueller, 1992). This character results in a distinctive echinulate, or “spiny,” basidiospore morphology. *Laccaria* species are recognized in the field by having orange-brown, red-brown or violet basidiocarps with moderately thick lamellae. In a monograph of North American species, Mueller (1992) recognized 19 *Laccaria* species in North America north of Mexico, with an additional 17 species provisionally recognized from other regions of the world. Recent studies using molecular evidence reveal uncertainty in the higher-order phylogenetic position of *Laccaria*. Ribosomal and mitochondrial DNA-based phylogenetic studies suggest that *Laccaria* may be more closely related to the brown-spored genus *Cortinarius* (Family Cortinariaceae) than to other genera in the Tricholomataceae (Binder & Hibbett, 2002; Hibbett et al., 2001; Moncalvo et al., 2000). While such a relationship seems reasonable due to shared ecology (ectomycorrhizal mutualists), phylogenetic clades including both *Laccaria* and *Cortinarius* received low bootstrap support in these studies, and a later study by Moncalvo et al. (2002) showed the position of *Laccaria* to be unresolved. Phenetic clustering analysis of ITS-RFLP data by Kernaghan (2001) placed the analyzed *Laccaria* species in a clade with *Tricholoma* spp.; however, in such analyses, homoplasy of fragment sizes could cause unrelated taxa to cluster together. Though the genus *Laccaria* is well supported as monophyletic by morphological data, the conflicting results

of these molecular studies underscore the level of uncertainty regarding the correct higher-level phylogenetic position of *Laccaria*.

Several authors have suggested that the arctic and alpine species of *Laccaria* are particularly in need of circumscription (Gulden, 1982; Lamoure et al., 1982). A number of taxonomic problems, as well as the lack of a detailed taxonomic treatment of arctic-alpine taxa, have resulted in confusion regarding the identification of a number of taxa. The genus *Laccaria* lacks clear morphological variation between some taxa, and in some cases exhibits a larger degree of variability within than between taxa. A number of characters commonly used in species identification in other genera (e.g., basidiocarp coloration, odor, and taste, and microscopic characters such as pleurocystidia and cheilocystidia) are either too variable within taxa or not variable enough between taxa, so that these characters may be taxonomically useful in identifying some *Laccaria* species but not others. Recourse to infrequently used characters such as somatic culture mat morphology is necessary for circumscription of some taxa (Mueller, 1985; Fries & Mueller, 1984).

As mentioned above, some *Laccaria* species display a high level of phenotypic plasticity; in some cases, this plasticity results in morphological continua that do not allow for the natural segregation of species and infraspecific taxa (Fries & Mueller, 1984; Mueller & Vellinga, 1986a). Perhaps these problems are most evident in *Laccaria laccata* (Scop.: Fr.) Cooke, the most widely reported species in this genus. On one hand, these factors have resulted in a proliferation of infraspecific taxa that probably overrepresents the true number of infraspecific varieties worthy of formal taxonomic

recognition (Mueller, 1992; Mueller & Vellinga, 1986a). On the other hand, the difficulty of identifying *Laccaria* species has resulted in widespread misidentification of taxa, and the name *Laccaria laccata* has been applied in a loose sense (*sensu lato*) to most *Laccaria* species lacking violet basidiocarp pigments, therefore underestimating true species diversity. Nomenclatural synonymy, missing or poor quality type collections, and literature descriptions conflicting with extant type specimens have caused further confusion in species identifications.

For the reasons provided above, the identities and distributions of arctic-alpine species of *Laccaria* are not known with certainty for most areas and collections from these habitats remain in need of further study. Ectomycorrhizal host associations of arctic and alpine *Laccaria* species, especially in North America, remain largely unknown with a few exceptions. Lange (1957) noted occurrence of *Laccaria laccata* in plots dominated by *Betula glandulosa* Michx., *Betula nana* L., and *Salix herbacea* L. in Greenland. However, as described above, collections documented under the name *Laccaria laccata* may be referable to other taxa in light of more recent work in circumscribing species in the *L. laccata* complex (Mueller, 1985, 1991a; Mueller & Vellinga, 1986a; Fries & Mueller, 1984). Several species have been reported to be associated with *Salix herbacea*, including *Laccaria altaica* Singer (= *L. pumila* Fayod), *L. bicolor* (Maire) Orton, *L. farinacea* (Hudson) Singer (= *L. laccata* (Scop.: Fr.) Cke), *L. laccata* (Scop.: Fr.) Cooke, *L. montana* Singer, *L. proxima* (Boud.) Pat., *L. proximella* Singer (= *L. proxima* (Boud.) Pat. in arctic-alpine habitats), *L. pumila* Fayod, and *L. tetraspora* Singer (= *L. ohienensis* (Mont.) Singer, though *L. tetraspora* is more likely a misapplied name for *L. montana*

Singer in the arctic-alpine literature) (summarized in Graf, 1994; synonyms according to Mueller, 1992).

Two species, *L. montana* and *L. pumila*, are recognized by Mueller (1992) as being restricted to arctic, alpine and boreal habitats. *Laccaria* species are documented under 16 names in the arctic-alpine literature, although synonymy and probable instances of misapplication of names most likely cause this number to be an overrepresentation of true species diversity (See Chapter 2). Arctic-alpine *Laccaria* collections are recorded from the Alps, Greenland, Scotland, Iceland, Kamchatka, Finland, Norway, Svalbard, Alaska, Alberta and Baffin Island.

In the Rocky Mountains, subalpine *Laccaria* records include *L. amethysteo-occidentalis* G.M. Mueller, *L. bicolor* (Maire) Orton, *L. laccata* var. *pallidifolia* (Peck) Peck, *L. montana* Singer, *L. nobilis* G.M. Mueller, *L. proxima* (Boud.) Pat., and *L. pumila* Fayod (Cripps, 2001; Mueller, 1992; Osmundson, unpublished data). However, no published arctic-alpine *Laccaria* records were encountered during the course of the literature review, indicating that the Rocky Mountain alpine species are largely unknown. Ectomycorrhizal host associations are also not documented for *Laccaria* in the Rocky Mountain alpine zone. The degree of host specificity and the distributions of host plants may be expected to have a large effect on distributions of *Laccaria* species.

According to observed biogeographic patterns, some plant species found in the Rocky Mountain alpine are part of the more widely-distributed Rocky Mountain and temperate arctic-alpine floras, with the Southern Rocky Mountains, and the San Juan Range in particular, having some endemic taxa recorded neither from the Arctic nor from

the Central and Northern Rocky Mountains (Johnson & Billings, 1962; Löve & Löve, 1974; Weber & Wittmann, 2001). It could be hypothesized that similar patterns apply to macrofungi, and may either be directly related to EM plant distributions or attributable to historical factors. At present, insufficient data exist to either support or refute this hypothesis.

As previously mentioned, alpine habitats are observed to be more species-rich in vascular plants than arctic areas due to a greater number of diverse topoclimates and closer proximity to subalpine source populations. Whether this pattern applies to ectomycorrhizal fungi in general is outside the scope of the present project; however, whether it applies to *Laccaria* in particular, and the degree to which distributions of *Laccaria* spp. follow host plant distributions, will be evaluated for the Rocky Mountain alpine zone on the basis of field data and a review of the arctic-alpine literature.

The objectives of the taxonomic study of Rocky Mountain *Laccaria* species are to: 1) Identify *Laccaria* species occurring in the Rocky Mountain alpine zone and provide a taxonomic treatment for North American alpine taxa using macro- and micromorphological data, 2) Document EM hosts associated with *Laccaria* spp. in Rocky Mountain alpine habitats, and 3) Delineate Rocky Mountain alpine geographic distributions for *Laccaria* species, comparing those for the Beartooth Plateau and Colorado field sites, and between the Rocky Mountains and other arctic-alpine areas.

Broader goals of this study are to produce a classification based on a large number of collections that can serve as a basis for further studies of arctic-alpine *Laccaria* taxa worldwide, and to address taxonomic and ecological questions about arctic-alpine

*Laccaria* as a means of exploring the use of this genus as a model system in studying the evolution of arctic-alpine ectomycorrhizal fungi. Specimens were collected over a period of 3 years at alpine field sites in Colorado, Montana, and Wyoming and examined using macroscopic, microscopic, and occasionally cultural (somatic morphology of laboratory cultures) characters.

#### Molecular Systematics of Rocky Mountain Alpine *Laccaria*

As previously mentioned, the taxonomy of *Laccaria* species has been complicated by a scarcity of systematically informative macro- and micromorphological characters and a high degree of phenotypic plasticity among some species, especially for *Laccaria laccata*. Considering the ecological importance and applied potential of *Laccaria* species, generating a better understanding of species limits and identifying systematically informative characteristics could have important implications for further research. Nucleotide sequence data can provide additional characters useful for stabilizing taxon groupings and infrageneric classifications. As part of the biosystematic study of Rocky Mountain alpine *Laccaria* species, a phylogenetic analysis of ribosomal DNA internal transcribed spacer (ITS) sequences was employed as a means of determining species-level groups, defining unique suites of morphological character states useful for identifying taxa, and assessing the systematic usefulness of various morphological characters.

Sequence data have previously been used for *Laccaria* at the levels of ordinal and population studies, but molecular studies at the infrageneric level are presently lacking.

As previously mentioned, recent molecular phylogenetic studies including the order Agaricales have underscored the level of uncertainty regarding the correct higher-level phylogenetic position of *Laccaria* by suggesting that the genus may be more closely related to the brown-spored genus *Cortinarius* (Family Cortinariaceae) than to the other genera currently classified in the family Tricholomataceae (Binder & Hibbett, 2002; Moncalvo et al., 2000). At the population level, molecular data have been used to identify strains (Albee et al., 1996) and to examine the persistence of individual genotypes on host roots, the effect of genotype on fruiting phenology, and the spatial distribution of genets (de la Bastide et al., 1994; Gherbi et al., 1999; Selosse et al., 2001).

Species concepts used in the delimitation of fungal species include morphological species (species delimited on the basis of differences in morphological characteristics), biological species (species delimited on the basis of mating compatibility of single-spore isolates) and phylogenetic species (species delineated on the basis of evolutionary, usually molecular genetic data). To-date, morphological and biological species concepts have served as the basis for species delimitations in *Laccaria*.

Studies in infrageneric relationships in *Laccaria* have, in a sense, served as a model system for the taxonomic study of ectomycorrhizal basidiomycetes. Because many *Laccaria* species can be grown under laboratory conditions and possess spores that can germinate in culture, characters such as culture morphology and mating intersterility have been used in addition to cladistic analyses of basidiocarp macro- and micromorphological characters to delimit species (Fries & Mueller, 1984; Mueller, 1985, 1987b, 1991a, 1992; Mueller & Gardes, 1991). Such a multifaceted approach to the

taxonomic study of ectomycorrhizal basidiomycetes (or even agaric fungi in general) is uncommon. However, with the exception of studies using RFLP patterns to distinguish putative species (Gardes et al., 1990, 1991; Mueller & Gardes, 1991), use of molecular data in *Laccaria* systematics has not previously been reported.

Rigorous taxonomic studies involving *L. montana* and *L. pumila*, two of the most commonly reported arctic-alpine-boreal taxa, have been hampered by the recalcitrance of these species to spore germination and/or pairing of isolates in the laboratory. The two species are morphologically nearly identical, with the exception of the number of spores borne on each basidium (two on *L. pumila*, and four on *L. montana*). Although it has been suggested that the two taxa are conspecific (Singer, 1977), Mueller (1992) retains them as distinct species based on the observation that the number of spores borne on each basidium is constant, not exhibiting both basidial types in single basidiocarp collections in contrast to some other species (e.g., some *Inocybe* species). Determination of whether or not these taxa are conspecific could lead to a better understanding of their ecological roles, as they are often reported from similar habitats. In particular, future studies should assess the importance of secondary homothallism, or self-fertility, that is hypothesized to occur in bisporic basidiomycetes and could represent an ecological advantage under difficult environmental conditions. Populations of both taxa appear to occur sympatrically on the Beartooth Plateau. Better understanding their species-level relationships, as well as their distributions and host associations, could lead to a better understanding of their ecologies. Molecular data could provide evidence either

supporting or refuting the existence of gene flow between sympatric populations of *L. pumila* and *L. montana*.

The objectives of this study are to: 1) Use genetic data to assess morphological species groupings for Rocky Mountain taxa, 2) Assess the systematic utility of various morphological characters, determining whether taxon groups inferred using molecular data can be defined by either morphological synapomorphies or unique combinations of morphological character states, 3) Evaluate the utility of the ITS region for further phylogenetic studies in *Laccaria*, 4) Provide a basic characterization of the internal transcribed spacer region in the *Laccaria* species studied, determining levels of sequence variation between species and examining whether molecular divergence between geographically separated populations within species can be detected using the ITS region, 5) Conduct a preliminary molecular test of the hypothesis of genetic isolation between *Laccaria pumila* and *L. montana*, and 6) Create a preliminary phylogenetic backbone for further molecular phylogenetic studies in *Laccaria*.

Future studies could seek to examine whether there is evidence of molecular divergence between alpine and subalpine populations within species, and between alpine populations associated with different ectomycorrhizal host plants, and to test the hypothesis that the arctic-alpine *Laccaria* species form a monophyletic group by examining whether there is evidence that these species evolved from different lineages within *Laccaria*. In terms of arctic-alpine macromycetes in general, Gulden (1996) states that some species "are clearly closely related to 'lowland' species. Originally many of these were described as varieties, subspecies, or forms of 'lowland' species. There has

been a trend to raise these infraspecific taxa to specific level. We need, however, better criteria to support such opinions. Modern molecular methods will probably be very helpful in deciding this.”

As part of the present study, an ITS sequence dataset was generated using 17 *Laccaria* collections from alpine field sites in Colorado, Montana and Wyoming and analyzed using a maximum parsimony method to produce a phylogenetically-based classification of the collections. While the scope of the project precluded expansion of the dataset to include reference specimens examined in Mueller’s (1992) monographic study of North American *Laccaria* species, reference specimens were examined morphologically and may be included in a future molecular analysis. This project is the first study to employ a phylogenetic analysis using ribosomal ITS sequence data for examining species-level relationships in *Laccaria*.

#### Plot-Based Synecological Study of Beartooth Plateau Macrofungi

In studies seeking to describe the synecology of organisms, it is rarely possible to survey the entire ecosystem of interest. This fact necessitates the use of sampling plots from which relationships can be inferred for the larger ecosystem using data collected in smaller, representative areas. Plot-based studies have several additional benefits, allowing: 1) More accurate measurement of temporal effects on community composition by pinpointing precise areas for repeated sampling, 2) Selection and comparison of areas differing in terms of one or more biotic or abiotic factors (e.g. stand age, plant community type, EM host plants, soil composition, microclimate, etc.), 3) Quantification

of community parameters such as species diversity and evenness, species abundance and species richness using sporocarps (Schmit et al., 1999; Senn-Irlet et al., 1999) or mycorrhizal root tips from soil cores (Kernaghan, 2001), and 4) Estimation of sampling effectiveness using species-effort or species-area curves.

A number of potential problems are inherent in plot studies, including the presence of confounding factors and the possibility of failure to detect heterogeneity that would be uncovered if the whole study area were surveyed (Braun-Blanquet, 1932; Cripps & Miller, 1993). In most macrofungal studies, the presence of above-ground sporocarps in a plot is used to infer the presence of fungal mycelia or mycorrhizae in the soil. The short-lived nature and unpredictable (or at least poorly understood) production of sporocarps, as well as the observation that species composition inferred from sporocarp data appears to differ from that inferred from molecular analysis of mycorrhizal root tips, further complicates drawing conclusions about macrofungal community structure (Gardes & Bruns, 1996; Schmit et al., 1999).

In addition to these problems, plot studies in alpine habitats must also consider the effect of patchy plant distributions resulting from the vast diversity of microclimates that characterize these ecosystems. On the positive side, such distributions can facilitate the selection of sampling plots containing a single ectomycorrhizal host plant or other parameter of interest. However, the lack of continuous stands of host plants makes small plot sizes necessary and reduces the probability of environmental homogeneity between plots, so a number of possible confounding factors not directly related to host plant

species must be considered in association with observed patterns of macrofungal community composition.

Only a small number of plot-based fungal ecology studies have been conducted in arctic-alpine habitats. Lange (1957) used sampling plots to describe fungal species occurrence in relation to arctic plant community types in Greenland. Graf (1994) examined the community structure of macromycetes associated with a single host plant (*Salix herbacea*) in the Swiss Alps, documenting sporocarp abundance, frequency, and periodicity, and relating these characteristics for selected species to microclimate, snowmelt patterns, and soil characteristics. Similarly, Eynard (1977) focused exclusively on the ecology of macromycetes in *Salix herbacea* snowbed communities in the French Alps. Petersen (1977) used sampling plots to conduct repeated sampling in identical locations in Greenland in order to examine the phenology of macromycete sporocarp production in relation to climatic and soil factors. Senn-Irlet (1988) used repeated plot sampling to compare macromycete communities in silicious and calcareous alpine snowbed communities in the Swiss Alps.

The objectives of the present study are to: 1) Document mycorrhizal host-fungal associations using plots containing a single mycorrhizal host plant, 2) Measure the degree of similarity in mycorrhizal fungal communities within and between host plant species in a quantitative manner using Simpson's index of similarity, 3) Provide a preliminary assessment of plant community composition in relation to fungal species assemblages, 4) Assess methods for alpine plot studies in a continental climate, where patchy plant distributions and lack of moisture can complicate assessment of fungal biodiversity using

sporocarps, and 5) More rigorously document host-symbiont associations as part of the study of Rocky Mountain alpine *Laccaria* species.

The present study incorporates a study design using 15 small (12.5 m<sup>2</sup> each) permanent plots situated on the Beartooth Plateau (Montana/Wyoming, USA) that were sampled multiple (2-4) times per season during the three years of the study. Plots were selected to contain a single putative ectomycorrhizal (EM) host plant whenever possible, and included the host plants *Betula glandulosa*, *Dryas octopetala*, *Salix planifolia*, *S. glauca*, *S. arctica*, and *S. reticulata*, comprising all known ectomycorrhizal plants on the Beartooth Plateau with the exception of *Polygonum viviparum*. This design (using single hosts whenever possible) allowed the ability to link mycorrhizal fungi to hosts with more confidence, to examine host specificity phenomena, to produce preliminary assessments of species abundance, and to quantitatively compare sampled areas using similarity indices based on species presence/absence data. In addition, repeated sampling during each season and over a number of seasons allowed observation of annual differences in species composition and fruiting phenology. The present study represents the first plot-based synecological study of macromycetes in the south/central Rocky Mountain alpine zone.

## CHAPTER 2

TAXONOMIC STUDY OF ROCKY MOUNTAIN ALPINE *LACCARIA*Introduction

The genus *Laccaria* Berkeley & Broome is a commonly encountered group of ectomycorrhizal (EM) fungi (Phylum Basidiomycota, Class Hymenomycetes, Order Agaricales) that produce gilled mushrooms. The genus is defined by basidiocarps having orange-brown to red-brown or violaceous pigmentation, lamellae (gills) that are attached, subdistant to distant, thick and waxy-looking, and echinulate (spiny) basidiospores that are white to cream or violaceous-white in mass. *Laccaria* is placed in the family Tricholomataceae by Singer (1986), Moser (1983) and most other authors primarily on the basis of having a white spore print, attached gills, and by the absence of a volva. However, Kühner (1984) placed *Laccaria* and the sequestrate genus *Hydnangium* in a separate family, Hydnangiaceae, on the basis of having multinucleate, echinulate basidiospores with echinulae formed by microtubules perpendicular to the episporium, a unique trait in the family Tricholomataceae sensu Singer. Recent molecular phylogenetic studies suggest that *Laccaria* could be more closely related to the brown-spored genus *Cortinarius* (Family Cortinariaceae) than to other genera in the Tricholomataceae (Binder & Hibbett, 2002; Moncalvo et al., 2000); however, these groupings received low bootstrap support in the phylogenetic analyses, and additional analyses by Moncalvo et al. (2002) left the higher-order position of *Laccaria* unresolved. Additionally, RFLP data by Kernaghan (2001) place *Laccaria* closer to *Tricholoma* (Family Tricholomataceae)

than to *Cortinarius*. As *Laccaria* is one of the few EM genera currently placed in the family Tricholomataceae, elucidating the higher-order phylogenetic relationships of this genus has implications for better understanding evolutionary patterns related to the EM symbiosis; however, such studies are outside the scope of the present project.

*Laccaria* species are frequently documented as mycorrhizal symbionts of numerous plant species, suggesting an important ecological role. Some species are found in disturbed or primary successional habitats such as recently deglaciated soils (Jumpponen et al., 1999), smelter sites (Cripps, 1995, 2001), recently reclaimed mine sites (Tommerup et al., 1991) and young forests (Dighton et al., 1986; Tommerup et al., 1991), indicating a role as pioneer species following ecological disturbance events. The ability of some *Laccaria* species to grow in culture and form mycorrhizae with host plants under laboratory conditions has allowed their use in examining phenomena such as predation of invertebrates by ectomycorrhizal fungi (Klironomos & Hart, 2001), cellular interactions / gene expression during mycorrhiza formation (Lei et al., 1991; Podila et al., 2002), and as sources of mycorrhizal inocula for tree plantations (Buschena et al., 1991; Selosse et al., 2001). *Laccaria* species have been used as experimental systems for genetic transformation of ectomycorrhizal fungi (Bills et al., 1999) and as model systems for studying genetic mechanisms and population biology of EM basidiomycetes (de la Bastide et al., 1994, 1995, 1995b; Gherbi et al., 1999; Kropp, 1997; Selosse et al., 1996, 2001). A better understanding of species limits, infrageneric and higher-order relationships, ecology and host associations in *Laccaria* could provide useful insight into character evolution and evolution of host associations in EM fungi, as well as facilitate

selection of appropriate experimental systems for applied ecological and reclamation research.

Several authors have suggested that the arctic and alpine species of *Laccaria* are particularly in need of circumscription (Gulden, 1982; Lamoure et al., 1982). A number of taxonomic problems, as well as the lack of a detailed taxonomic treatment of arctic-alpine taxa, have resulted in confusion regarding taxon identification. In addition, arctic-alpine macrofungi in the Rocky Mountains have not been studied extensively; none of the collections in Mueller's (1992) monographic study of North American *Laccaria* were collected in alpine habitats, and Mueller (personal communication) suggests that patterns of EM host association for alpine taxa are relatively unknown. The present project was conducted as part of the Rocky Mountain Alpine Mycota project (C. Cripps, NSF grant 9971210), the first intensive study of alpine macrofungi in this region. The goals of the present research are to present a taxonomic treatment of alpine species of *Laccaria* in the Rocky Mountains, and to examine distributions, host associations, and ecology of alpine *Laccaria* species in the Southern and Central Rocky Mountains. A taxonomic classification based on a large number of regional alpine collections could serve as a basis for further studies of arctic-alpine *Laccaria* species worldwide. The present study represents the first report on North American alpine *Laccaria* species, based on an intensive study of 86 Rocky Mountain collections. This report identifies morphological species, provides data on EM host associations, and examines geographical distributions for Rocky Mountain alpine taxa. Five *Laccaria* species, *L. bicolor* (Maire) Orton, *L. laccata* var. *pallidifolia* (Peck) Peck, *L. montana* Singer, *L. pumila* Fayod and a putative

new taxon are described. Pertinent ecological and distributional data are provided for each taxon.

### Infrageneric Classification in *Laccaria*

While the genus *Laccaria* as a whole is well delineated and presumably monophyletic, *Laccaria* species lack a number of the distinctive morphological characters commonly used to distinguish species in other agaric genera, resulting in a small number of taxonomically informative characters. There is little variation in basidiocarp coloration between most species, and useful microscopic features such as pleurocystidia and cheilocystidia are rare, often varying between basidiocarps within a single collection. Recourse to infrequently used characters such as somatic culture mat morphology is necessary for circumscription of some taxa (Fries & Mueller, 1984; Mueller, 1985). In addition, some species display a high level of phenotypic plasticity, resulting in morphological continua that do not allow for the natural segregation of species and infraspecific taxa (Fries & Mueller, 1984; Mueller & Vellinga, 1986a). These problems are perhaps most evident in *Laccaria laccata* (Scopoli : Fries) Cooke, the most widely reported species in the genus. On one hand, the difficulty of identifying *Laccaria* species has resulted in widespread application of the name *Laccaria laccata* in a loose sense (*sensu latu*) to most *Laccaria* species lacking violet basidiocarp pigments, therefore underestimating true diversity and adding to misidentifications of species in both alpine and non-alpine habitats. On the other hand, these factors have resulted in a proliferation of infraspecific varieties that probably over-represents the true number of

taxa worthy of formal recognition (Mueller, 1992; Mueller & Vellinga, 1986a). Nomenclatural synonymy, missing or poor quality type collections, and literature descriptions conflicting with extant type specimens have caused further confusion in species identifications (Mueller, 1987, 1992, 1997).

Studies (Fries & Mueller, 1984; Gardes et al., 1990, 1991; Mueller, 1984, 1985, 1991a) leading to a monographic treatment of North American *Laccaria* species by Mueller (1992) resolved a number of taxonomic questions by combining morphological data with cultural, mating, and molecular RFLP analyses. Mueller's monograph recognized 19 species of *Laccaria*, and two varieties within *L. laccata*, for North America north of Mexico (Table 1) and 36 species, with 3 varieties in *L. laccata*, and 2 varieties in each of *L. masonii* and *L. ohiensis*, worldwide (Table 2). The cladistic analysis of morphological characters performed as part of Mueller's study separated *Laccaria* into two subgroups: Metasection *Amethystina*, consisting of species having violet basidiocarp pigments, and Metasection *Laccaria*, consisting of species lacking violet pigmentation. The one notable exception in this classification is the inclusion of *L. oblongospora* G.M. Mueller in Metasection *Laccaria*; although this species possesses a violaceous basal tomentum and somatic culture mat on PDA and MMN media, other characteristics, such as finely ornamented and ellipsoid basidiospores, suggest a phylogenetic affinity to *L. proxima* (Boudier) Patouillard and some collections of *L. laccata*. Because of the inclusion of *L. oblongospora* with the *Laccaria* species lacking violet pigmentation in the cladistic analysis, no synapomorphies support the monophyly of either subgroup.

Table 4. North American *Laccaria* species recognized by Mueller (1992). Western North America boreal distributions listed.

<u>Species</u>	<u>Ecology &amp; Distribution</u>	<u>Mycorrhizal Host Plants</u>	<u>W. N. Am. Boreal Distribution</u>
(Metasection <i>Laccaria</i> ) <i>L. proxima</i> (Boudier) Patouillard	reforested areas, boreal; N America, Europe	Pinaceae, <i>Populus tremuloides</i>	AK, CO, ID, OR, WA, BC
<i>L. oblongospora</i> G.M. Mueller	sandy soil; Gulf coast	<i>Pinus palustris</i>	none
<i>L. laccata</i> var. <i>laccata</i> (Scopoli Fries) Cooke	Europe, N. America; uncommon	unknown	none
<i>L. laccata</i> var. <i>pallidifolia</i> (Peck) Peck	cosmopolitan	Pinaceae, Fagaceae, Betulaceae	AK, CO, ID, OR, WA, WY, BC
<i>L. longipes</i> G.M. Mueller	mosses; Great Lakes region and New York	<i>Picea mariana</i> , <i>Larix laricina</i> , <i>Alnus rugosa</i>	none
<i>L. fraterna</i> (Cooke & Massée:Saccardo) Pegler	Australia; worldwide (introduced)	<i>Eucalyptus</i>	none
<i>L. montana</i> Singer	arctic, alpine, boreal; poor soil, humus, moss	Pinaceae, <i>Betula</i> , <i>Salix</i>	AK, CO, ID, MT, WY, WA, BC
<i>L. pumila</i> Fayod	arctic, alpine, boreal; poor soil, humus, moss	Pinaceae, <i>Betula</i> , <i>Salix</i>	AK, CO, WA, WY
<i>L. striatula</i> (Peck) Peck	mosses; eastern North America	<i>Tsuga canadensis</i> , Pinaceae, Fagaceae	none
<i>L. ohiensis</i> (Montagne) Singer	mostly subtropical, tropical, south temperate	unknown	none
<i>L. tortilis</i> (Bolton) Cooke	bare soil; cosmopolitan, uncommon	Pinaceae, Fagaceae	OR, WA, WY
(Metasection <i>Amethystina</i> ) <i>L. trichodermophora</i> G.M. Mueller	SE N America; temperate conifer or mixed forests	<i>Pinus</i>	none
<i>L. bicolor</i> (Maire) Orton	soil, mosses; western N. America east to Michigan, Ontario	unknown	AK, ID, OR, WA, BC
<i>L. nobilis</i> Smith <i>apud</i> G.M. Mueller	western N America, Great Lakes region	Pinaceae	CO, ID, WA
<i>L. trullisata</i> (Ellis) Peck	sand dunes or very sandy soil, eastern and midwestern N. America	<i>Pinus</i>	none
<i>L. maritima</i> (Teodorowicz) Singer <i>ex</i> Huhtinen	sand; w/ or w/o mycorrhizal assoc, eastern Canada, northern Europe	unknown	none

Table 4, continued.

<i>L. ochropurpurea</i> (Berkeley) Peck	temperate deciduous forests; eastern N. America	<i>Quercus, Fagus</i>	none
<i>L. amethysteo-occidentalis</i> G.M. Mueller	conifer forests; western N. America	<i>Pseudotsuga menziesii</i>	OR, WA, BC
<i>L. amethystina</i> Cooke	temperate deciduous forests; eastern N. America, Europe, Central/South America	<i>Quercus, Fagus</i>	CO
<i>L. vinaceobrunnea</i> G.M. Mueller	sandy soil, Gulf coast	<i>Quercus virginiana</i>	none

Table 5. Tentative list of world species of *Laccaria* recognized by Mueller (1992).

<u>Species</u>	<u>Distribution</u>
<i>L. amethysteo-occidentalis</i> G.M. Mueller	Western North America
<i>L. amethystina</i> Cooke	Eastern North America, Europe, Central and South America
<i>L. bicolor</i> (Maire) Orton	Western North America, Great Lakes region, Europe
<i>L. bullulifera</i> Singer	Mexico
<i>L. canaliculata</i> (Cooke & Masee) Pegler	under native Australasian trees
<i>L. chibiensis</i> Michal	USSR
<i>L. fibrillosa</i> McNabb	New Zealand
<i>L. fraterna</i> (Cooke & Masee) Pegler	Australia, widely distributed where <i>Eucalyptus</i> introduced
<i>L. galerinoides</i> Singer	South America
<i>L. gomezu</i> Singer & G.M. Mueller	Costa Rica, Colombia
<i>L. grabripes</i> McNabb	New Zealand
<i>L. impolita</i> Vellinga & G.M. Mueller	under native European trees
<i>L. laccata</i> (Scop..Fr.) Cooke var <i>laccata</i>	Europe, North America; rare
<i>L. laccata</i> var <i>moelleri</i> Singer	Europe
<i>L. laccata</i> var. <i>pallidifolia</i> (Peck) Peck	North America, Europe
<i>L. lilacina</i> Stevenson	New Zealand
<i>L. longipes</i> G.M. Mueller	North America (Great Lakes region)
<i>L. maritima</i> (Teod.) Singer ex Huhtinen	Eastern North America (rare), Northern Europe
<i>L. masonii</i> var. <i>brevispinosa</i> McNabb	New Zealand
<i>L. masonu</i> var <i>masonu</i> Stevenson	New Zealand
<i>L. montana</i> Singer	cosmopolitan in arctic, alpine, boreal habitats
<i>L. murina</i> Imai	Japan
<i>L. nana</i> Masee	England
<i>L. nigra</i> Hongo	Japan
<i>L. nobilis</i> G.M. Mueller	Western North America, Great Lakes region
<i>L. oblongospora</i> G M Mueller	North America (Gulf Coast states)
<i>L. ochropurpurea</i> (Berk.) Peck	Eastern North America
<i>L. ohioensis</i> (Mont.) Singer	Eastern North America, Central and South America, Europe
<i>L. ohioensis</i> var. <i>paraphysata</i> McNabb	New Zealand
<i>L. proxima</i> (Boud ) Pat	cosmopolitan
<i>L. proximella</i> Singer	Southern Argentina, Chile
<i>L. pumila</i> Fayod	cosmopolitan in arctic, alpine, boreal habitats
<i>L. purpureobadia</i> Reid	Western Europe
<i>L. striatula</i> (Peck) Peck	Eastern North America
<i>L. tortilis</i> (Bolt.) Cooke	North and South America, Europe
<i>L. trichodermophora</i> G M. Mueller	SE North America, Mexico, Central America
<i>L. trullissata</i> (Ellis) Peck	Eastern North America
<i>L. vinaceoavellanea</i> Hongo	Japan
<i>L. vinaceobrunnea</i> G.M. Mueller	USA (Gulf Coast states)
<i>L. violaceoniger</i> Stevenson	New Zealand

Therefore, the major subgroups are treated as Metasections rather than true Sections (Mueller, 1992). Current species limits in *Laccaria* are based primarily on macro- and micromorphological characters, with support from mating and restriction fragment length polymorphism (RFLP) data. The recent development of techniques for generating and analyzing DNA sequence data allows the use of additional characters for phylogenetic reconstruction. Molecular phylogenetic analysis could therefore represent a means of evaluating the robustness of Mueller's (1992) proposed infrageneric classification for *Laccaria*.

#### *Laccaria* in Arctic Alpine Habitats

*Laccaria* species are commonly collected in arctic and alpine habitats in the Northern hemisphere, and appear to be ecologically important (Gardes & Dahlberg, 1996). However, for the reasons presented above, the identities of arctic-alpine species of *Laccaria* are not known with certainty for most areas, and collections from these habitats remain in need of further study. In addition, ectomycorrhizal host associations of arctic and alpine *Laccaria* species, especially in North America, remain largely unknown. Gardes and Dahlberg (1996), in a review of mycorrhizal fungi in alpine habitats and drawing on information from Gulden & Jenssen (1988), report 3 arctic-alpine species (not specified by name), with *Betula*, *Dryas*, *Salix*, and *Saxifraga* reported as host genera. Lange (1957) noted the occurrence of *Laccaria laccata* in plots dominated by *Betula glandulosa* Michx., *Betula nana* L., and *Salix herbacea* L. in Greenland. However, as described above, collections of *Laccaria laccata* may be referable to other taxa in light of

more recent work in circumscribing species in the *L. laccata* complex (Fries & Mueller, 1984; Mueller, 1985, 1991a; Mueller & Vellinga, 1986a). Numerous species have been reported in association with *Salix herbacea* in alpine habitats (summarized in Graf, 1994; synonyms according to Mueller, 1992), including: *Laccaria altaica* Singer (= *L. pumila* Fayod), *L. bicolor* (Maire) Orton, *L. farinacea* (Hudson) Singer (= *L. trichodermophora* G.M. Mueller, probably referable to *L. bicolor* (Maire) Orton in arctic-alpine habitats), *L. laccata* (Scop.: Fr.) Cooke, *L. montana* Singer, *L. proxima* (Boud.) Pat., *L. proximella* Singer (= *L. proxima* (Boud.) Pat.), *L. pumila* Fayod, and *L. tetraspora* Singer (= *L. ohiensis* (Mont.) Singer); *L. tetraspora* may be a misapplied name for *L. montana* Singer in the arctic-alpine literature. Mueller (1992) recognizes two species, *L. montana* and *L. pumila*, as being restricted to arctic, alpine and boreal habitats. *Laccaria* species are documented under 16 names in the arctic-alpine literature (Table 6). In light of the nomenclatural confusion and problems of misapplied names within *Laccaria*, it is probable that the 16 taxa reported for arctic-alpine habitats are an over-representation of true species diversity. Drawing on concepts of synonymy and nomenclatural misapplication proposed by Gulden and Torkelsen (1996) and Mueller (1992), a conservative list of arctic-alpine taxa from the literature is presented (Table 7); concepts of synonymy and nomenclatural misapplication are discussed in the section "Discussion of Species Records in the Arctic-Alpine Literature" below. When these "concepts" are applied, the number of probable species in arctic-alpine habitats is reduced to seven: *L. avachaensis* Kalamees, *L. bicolor* (Maire) Orton, *L. laccata* (Scop.: Fr.) Cooke (mostly var. *pallidifolia*, although var. *moelleri* and var. *laccata* may be present), *L. maritima*

(Theodor) Singer, *L. montana* Singer, *L. proxima* (Boud.) Pat., and *L. pumila* Fayod.

Arctic-alpine *Laccaria* collections are recorded from the Alps, Greenland, Scotland, Iceland, Kamchatka, Finland, Norway, Svalbard, Alaska, Alberta and Baffin Island.

Given the possible over-representation and misidentification of taxa in the arctic-alpine literature, cataloging accurate patterns of global geographical distribution will be difficult until herbarium specimens corresponding to literature records can be examined.

Table 6. Previous reports of *Laccaria* species in arctic-alpine habitats.

Taxon	Location	References
<i>L. altaica</i> Singer	Western Alps Altai Mountains, USSR Svalbard Northwest Territories, Canada Baffin Island, Canada	Kühner & Lamoure 1986, Trimbach 1978 Singer 1967 Skifte 1979 Lahaie 1981 Lahaie 1981
<i>L. avachaensis</i> Kalamees	Rodygino, Kamchatka	Kalamees & Vaasma 1993
<i>L. bicolor</i> (Maire) Orton	Kronok Nature Res., Kamchatka Radont Valley, Switzerland Godhavn area, W. Greenland Scotland	Kalamees & Vaasma 1993 Graf 1994 Lamoure et al. 1982 Watling 1987
<i>L. laccata</i> (Scop.: Fr.) Cooke	Greenland Uzon Caldera, Kamchatka [ss. Mueller and Vellinga] Western Italian Alps Western Alps French Alps Swiss Alps Iceland Norway Alaska Svalbard	Lange 1957, Lamoure et al. 1982, Wathng 1983 Kalamees & Vaasma 1993 Lo Bue et al. 1994 Kühner & Lamoure 1986 Eynard 1977 Senn-Irlet 1992, 1993 Hallgrímsson 1981, 1998, Watling 1983 Lange & Skifte 1967 Linkins & Antibus 1982 Gulden & Torkelsen 1996, Oñenoja 1971, Väre et al. 1992
<i>L. laccata</i> var. <i>subalpina</i> Singer	Scotland	Watling 1987
<i>L. maritima</i> (Theodor) Singer	Iceland Greenland North Sea coastal	Hallgrímsson 1998 Lange 1955 (see <i>L. trullisata</i> var. <i>rugulospora</i> ) Redhead 1989

Taxon	Location	References
<i>L. montana</i> Singer	Fiera di Primiero, Italy Uzon Caldera, Kamchatka Mt. Rae, Alberta, Canada Northwest Territories, Canada Ellesmere Island, Canada Baffin Island, Canada Svalbard  Iceland Radont Valley, Switzerland Eastern Swiss Alps Central Swiss Alps	Bon 1987 Kalamees & Vaasma 1993 Kernaghan & Currah 1998 Lahaie 1981 Lahaie 1981 Lahaie 1981 Gulden & Torkelsen 1996, Väre et al. 1992, Osmundson (unpublished) Hallgrímsson 1998 Graf 1994 Favre 1955 Senn-Irlet 1987
<i>L. ohuensis</i> (Mont.) Singer	Greenland Svalbard	Lamoure et al. 1982, Watling 1977 Watling & Watling 1988
<i>L. proxima</i> (Boud.) Pat.	Radont Valley, Switzerland Eastern Swiss Alps Godhavn area, W. Greenland Western Italian Alps Umat, Alaska Finnish Lapland Norway	Graf 1994 Favre 1955 Lamoure et al. 1982 Lo Bue et al. 1994 Kobayasi et al. 1967 Kallio & Kankainen 1964 Lange & Skifte 1967
<i>L. proximella</i> Singer	Scotland	Watling 1987
<i>L. pumila</i> Fayod	Svalbard  Western Italian Alps Eastern Swiss Alps Iceland Switzerland Alaska	Gulden & Torkelsen 1996, Väre et al. 1992, Osmundson (unpublished) Lo Bue et al. 1994 Favre 1955 Hallgrímsson 1998 Senn-Irlet 1988, 1992 Mueller 1992
<i>L. striatula</i> (Peck) Peck	Alaska  Svalbard	Laursen & Ammirati 1982, Laursen & Chmielewski 1982, Linkins & Antibus 1982 Reid 1979
<i>L. tetraspora</i> Singer	Switzerland Baffin Island Alaska  Svalbard Greenland	Senn-Irlet 1988 Parmalee 1969 Kobayasi et al. 1967, Miller et al. 1982 Ohenoja 1971 Lange 1957
<i>L. tortilis</i> (Bolton) Cooke	Greenland Western Italian Alps Alaska  Svalbard Iceland Kamchatka	Lamoure et al. 1982, Lange 1957 Lo Bue et al. 1994 Kobayasi et al. 1967, Laursen & Chmielewski 1982, Miller et al. 1982 Ohenoja 1971 Hallgrímsson 1981, 1998 Kalamees & Vaasma 1993
<i>L. trullisata</i> (Ellis) Peck f. <i>rugulospora</i> M. Lange	Greenland	Lamoure et al. 1982, Lange 1957

Table 7. *Laccaria* records from the arctic-alpine literature after accounting for probable synonyms and misapplied names. Application of synonyms and nomenclature follows Gulden and Torkelsen (1996) and Mueller (1992).

*L. avachaensis* Kalamees

*L. bicolor* (Maire) Orton

*L. laccata* (Scop.: Fr.) Cooke (mostly var. *pallidifolia*, although var. *moelleri* and var. *laccata* may be present).

*L. maritima* (Theodor) Singer (reported from arctic, but not alpine, habitats)

*L. montana* Singer

*L. proxima* (Boud.) Pat.

*L. pumila* Fayod

#### Discussion of Species Records in the Arctic-Alpine Literature

*L. altaica* Singer

Mueller (1992; Mueller & Vellinga, 1986) considers *L. altaica* to be a later synonym of *L. pumila*. However, Sivertsen (1993) considers *L. altaica* to be a distinct species, differing slightly from *L. pumila* in spore shape and echinulae density. Due to the level of morphological similarity between collections referable to each taxon and in the absence of genetic data distinguishing the two taxa, *L. altaica* will be considered a synonym of *L. pumila* in this study.

*L. avachaensis* Kalamees

*L. avachaensis* is reported to be most closely related to *L. bicolor*, differing in having a densely fibrillose-squamulose pileus, more reddish-brown coloration, and larger, more finely echinulate basidiospores (Kalamees & Vaasma, 1993). The type specimen was collected on volcanic slag and ash fields in a sparsely vegetated high mountainous region of Kamchatka (host plant not reported). *L. avachaensis* has presently been reported only from the type locality.

*L. bicolor* (Maire) Orton

Considered to be associated with conifers in North America (Mueller 1992), *L. bicolor* is reported in the arctic-alpine literature to be associated with *S. herbacea* in Greenland, Scotland and Switzerland, and with *Betula ermanii* Cham. and *Larix dahurica* Turcz. ex Trantv. shrubs at treeline in Kamchatka.

*L. laccata* (Scop.: Fr.) Cooke

*L. laccata* is commonly reported in the arctic-alpine literature. No information is reported on distinctions at the subspecies level, and given the level of macromorphological similarity and number of systematic questions within the *L. laccata* complex, herbarium collections referred to as *L. laccata* should be more closely examined. Reported in association with *Betula nana* L., *B. glandulosa* Michx., *Salix glauca* L., *S. arctophila* Cock. ex Heller, *S. herbacea* L., *S. retusa* L., *S. rotundifolia* Trautv., and *Polygonum viviparum* L.

*L. laccata* var. *subalpina* Singer

Mueller (1992) treats this taxon as a synonym of *L. laccata* var. *pallidifolia*.

*L. maritima* (Theodor) Singer

Reported only from Iceland and Greenland (as *L. trullisata* f. *rugulospora*) in the arctic-alpine literature, this species is associated with sandy soils and sand dunes.

*L. montana* Singer

This taxon is one of the most widely collected *Laccaria* species in arctic-alpine habitats. Reported in association with *Salix* spp. and *Dryas* spp. See additional comments under *L. tetraspora*.

*L. ohiensis* (Mont.) Singer

Considerable taxonomic confusion is associated with this taxon. The holotype of *L. ohiensis* is a quadristerigmate species; however, the name *L. ohiensis* was later used in some cases to describe a bisterigmate species. At least some records of *L. ohiensis* from arctic-alpine areas probably represent collections of *L. pumila* (Gulden and Torkelsen, 1996).

*L. proxima* (Boud.) Pat.

This species has been reported from the Alps and Greenland, in association with *Salix herbacea* and *Polygonum viviparum*. See comments under *L. proximella*.

*L. proximella* Singer

Like *L. proxima*, *L. proximella* is characterized by having ellipsoid spores with low ornamentation. *L. proximella* was reported by Watling (1987) as the "alpine equivalent" of *L. proxima*, differing in terms of having smaller stature, a lilaceous tinge in the lamellae and a less distinctly course fibrillose pileus and stipe, and lacking a raphanoid odor. Mueller (1992) considers *L. proximella* to differ in terms of having violet mycelium at the base of the stipe and exhibiting a preference for poor, rocky soil. However, he reports examining a large number of collections referable to *L. proxima* from arctic-alpine habitats, and therefore considers *L. proximella* to be a species described only from South America and arctic-alpine records of *L. proximella* to be *L. proxima* (Mueller 1992).

*L. pumila* Fayod

This taxon is one of the most commonly collected and widely distributed *Laccaria* in arctic-alpine habitats (Gulden & Jenssen, 1988), occasionally appearing in the arctic-alpine literature under the synonyms *L. laccata* var. *pumila* (Fayod) Favre and *L. altaica* Singer. In addition, the epithets *L. tortilis* (Bolt.) Cke. ss. Kobayasi et al 1968, M. Lange 1955, Miller et al. 1982 and Ohenoja 1971, *L. altaica* sensu Skifte (1979), *L. striatula* (Pk.) Pk. sensu Reid 1979 and Singer 1943, and possibly *L. ohiensis* (Mont.) Sing. sensu Watling & Watling 1988 have been applied to collections referable to *L. pumila* (Gulden & Jenssen 1988, Gulden & Torkelsen, 1996). See comments under *L. altaica* regarding synonymy. Reported in the literature to be associated with *Polygonum viviparum*, *Salix herbacea*, and *Dryas octopetala*.

*L. striatula* (Peck) Peck

This species was reported in association with *S. rotundifolia* by Linkins and Antibus (1982) in Alaska. Mueller (1991) and Gulden and Torkelsen (1996) report that the name *L. striatula* is occasionally misapplied to collections of *L. pumila*.

*L. tetraspora* Singer

Mueller (1992) treats the name *L. tetraspora* Singer as a synonym of *L. ohiensis* (Mont.) Singer. However, he states that at least some of the collections of *L. tetraspora* reported from arctic-alpine areas appear to be referable to *L. montana* Singer. *L. montana* is distinguished from *L. ohiensis* by having broadly ellipsoidal (rather than globose) spores with narrower echinulae.

*L. tortilis* (Bolton) Cooke

This species has been reported in association with *Salix glauca* and *Polygonum viviparum*. Mueller (1991, 1992) and Gulden and Torkelsen (1996) indicate that at least some of the collections reported under the name *L. tortilis* from arctic regions (e.g., Kobayashi, 1967) are actually *L. pumila* Fayod. Both species have bisterigmate basidia, but *L. tortilis* is characterized by larger, globose basidiospores.

*L. trullisata* (Ellis) Peck f. *rugulospora* M. Lange

This species was described by M. Lange (1957) in association with *Salix glauca* in sand dunes in Greenland. Høiland (1976) considers this taxon to be a synonym of *L. maritima*.

### *Laccaria* in the Rocky Mountains

In the Rocky Mountains, subalpine *Laccaria* records include *L. amethysteo-occidentalis*, *L. bicolor*, *L. laccata* var. *pallidifolia*, *L. montana*, *L. nobilis*, *L. proxima*, and *L. pumila* (Cripps, 2001; Evenson, 1993; Mueller, 1992; Osmundson, unpublished).

However, no arctic-alpine *Laccaria* records were encountered during the course of the literature review, indicating that the Rocky Mountain alpine species are largely unknown. Based on biogeographic patterns observed in Rocky Mountain alpine plants (Johnson & Billings, 1962; Löve & Löve, 1974; Weber & Wittmann, 2001) it can be hypothesized that some species found in the Rocky Mountain alpine should be part of the more widely-distributed Rocky Mountain and temperate arctic-alpine mycofloras, but that the Southern Rocky Mountains, and the San Juan Range in particular, could harbor endemic taxa recorded from neither the Arctic nor from the Central and Northern Rocky Mountains. Based also on patterns observed for vascular plants, it can be hypothesized that alpine areas will be more species-rich than arctic areas due to a greater number of diverse topoclimates and closer proximity to subalpine source populations (Körner, 1999). Whether this pattern applies to ectomycorrhizal fungi in general is outside the scope of the present project; however, whether it applies to *Laccaria* in particular will be evaluated on the basis of field data and a review of the arctic-alpine literature.

The level of genetic relatedness between alpine populations and nearby subalpine populations within morphological species has not been assessed for ectomycorrhizal basidiomycetes. A study on biological and morphological species in the saprobic genus *Psilocybe* (Boekhout et al., 2002) observed interfertility between an ex-type strain of the alpine species *P. chionophila* and a morphologically similar subalpine isolate, showing that, at least in saprobic species, conspecific alpine and subalpine populations exist. The study did not assess whether alpine isolates from various regions are more closely related to each other than to nearby subalpine populations, a question with implications for better

elucidating evolutionary patterns in arctic-alpine fungi. An assessment of genetic relatedness for ectomycorrhizal species would provide evidence for addressing the question of whether alpine populations are derived from lower elevation populations (or vice versa), or are more closely related to alpine populations in other locations.

The relationship between the distributions of ectomycorrhizal (EM) basidiomycetes and their mycorrhizal host plants in arctic-alpine habitats was posed by Watling (1987), who suggested that EM fungi may have either followed their hosts, mirrored host distributions due to similar physiological optima, or have different (e.g., more restricted) distributions. Host specificity patterns in relation to EM fungal distributions, as well as whether host shifts between subalpine and alpine populations have occurred, represent questions in need of further study. Alpine *Laccaria* species in North America are mycorrhizal symbionts of dwarf and some shrubby *Salix* spp, *Betula* spp., and *Dryas* spp. The distributions of these host plants may be important in influencing the distributions of *Laccaria* spp.

Among the existing problems pertaining to arctic-alpine *Laccaria* are: 1) stabilization of nomenclature for taxa, 2) gaining a better understanding of species distributions, host associations, and patterns of host specificity, 3) examining associations with subalpine taxa in terms of assessing genetic evidence for conspecificity, studying the population biology of alpine and subalpine populations, identifying morphological adaptations to alpine environmental factors, and identifying host shifts between subalpine and alpine populations, 4) examining evidence to support or reject the treatment of *Laccaria altaica* and *L. pumila* as synonyms, and 5) examining evidence to diagnose *L.*

*pumila* and *L. montana* as distinct species or as variants (bisterigmate and quadristerigmate, respectively) within a single species. The difference between bisterigmate and quadristerigmate species may be fundamental in an ecological sense. Cytological studies by Tommerup et al. (1991) and Mueller et al. (1993) determined that bisporic basidia usually produce spores having 4 nuclei, and quadrisporic basidia usually produce spores having 2 nuclei. Most spores from bisporic basidia contained nuclei of compatible mating types, as evidenced by most single spores producing clamped, dikaryotic hyphae (Tommerup et al., 1991), an occurrence not noted in quadrisporic species (Doudrick & Anderson, 1989; Fries & Mueller, 1984). The fact that these spores can form dikaryotic hyphae without need of joining with the mycelium of a compatible mating type (i.e., "secondarily homothallic") may represent a means for rapid colonization of disturbed and primary successional habitats (Tommerup et al., 1991), or potentially for survival under adverse environmental conditions.

Better understanding species limits, infrageneric and higher-order relationships, ecology and host associations in *Laccaria* could provide useful insight into character evolution, evolution of host associations, and morphological adaptations to cold climates. In addition, identifying host-fungus mycorrhizal partnerships may be important toward the development of systems for reclamation of high-altitude disturbed sites and improving the understanding of mycorrhizal host specificity patterns as they relate to alpine habitats. The present research addresses several (but not all) of the problems listed above by documenting *Laccaria* spp. with Rocky Mountain alpine distributions, presenting data on mycorrhizal host associations, and examining collections and host

associations of Rocky Mountain subalpine *Laccaria* species collected near alpine sites. Analysis of ribosomal DNA internal transcribed spacer sequences (Chapter 3) was conducted in order to: 1) evaluate the morphological species concepts presented in this chapter, 2) evaluate the delimitation of *L. montana* and *L. pumila* as distinct species using molecular data, and 3) evaluate morphological features useful for species delineation.

#### Systematically Informative Characters in *Laccaria* Classification

As previously discussed, species delimitation in *Laccaria* is complicated by a lack of systematically informative macromorphological traits and by a high degree of phenotypic plasticity among some species (particularly *L. laccata*). The use of a combination of macromorphological, micromorphological, cultural and molecular characters, in addition to mating studies, has aided in the delimitation and nomenclatural stabilization of some taxa (Mueller, 1992).

#### Macromorphological Characters

Although macromorphology in *Laccaria* species can be highly variable, traits such as basidiocarp color, size, and texture can be useful features in identifying taxa. Basidiocarp color in *Laccaria* ranges from violet to orange-brown, red-brown, or gray, and can be a useful field characteristic in distinguishing violet-pigmented taxa such as *L. amethystina* and *L. amethysteo-occidentalis* or gray-pigmented taxa such as *L. murina* from taxa having orange-brown or red-brown basidiocarps (Imai, 1938; Mueller, 1992). However, basidiocarp colors often change with age or weathering, and can be highly variable within species. Lamellae color can be systematically informative, for example,

providing a distinguishing feature between *L. bicolor*, having pale violet to vinaceous lamellae, and members of the *L. laccata* complex, having pinkish-orange to pale orange-brown lamellae. Like basidiocarp color, lamellae color often fades in older specimens. The color of the mycelium at the base of the stipe appears to be a systematically informative character, with species in Metasection *Amethystina* having violet basal mycelium and those in Metasection *Laccaria* (excepting *L. oblongospora*) having white basal mycelia. However, basal mycelium color is sensitive to age and environment; as a result, field collections of species in Metasection *Amethystina* will often have a white, rather than violet, basal mycelium (Mueller, 1992; Osmundson & Cripps, unpublished data).

Basidiocarp size in many *Laccaria* species is highly variable, and thus represents an unreliable systematic character in most cases. However, size appears to be systematically informative in distinguishing the large, robust species *L. nobilis* from the smaller-statured *L. bicolor* and *L. trichodermophora*, as well as from members of the *L. laccata* complex (Mueller, 1984, 1992). The small stature of *L. montana*, *L. pumila*, *L. tortilis*, and *L. ohiensis* is distinctive for these taxa; however, use of this character for identification requires caution, as small basidiocarps of other taxa within the *L. laccata* complex could be mistaken for these taxa and can be distinguished only on the basis of micromorphological characters. Additionally, the long, narrow stipe stature of *L. striatula* and the long stipes of *L. longipes* can be useful in distinguishing these species (Mueller, 1991a, 1992).

Like basidiocarp color and size, basidiocarp texture is often highly variable, but can be diagnostic for species in some cases. The scaly pileus and stipe of *L. nobilis*, in addition to large size and robust stature, can often be used to distinguish this species from other species in the *L. bicolor* complex and species in the *L. laccata* complex (Mueller, 1984, 1992). The presence of a strongly translucent-striate pileus margin can be used to distinguish *L. pumila*, *L. montana*, and *L. tortilis* from *L. laccata* in many, but not all, cases, since *L. laccata* basidiocarps are occasionally striate and this character can be highly variable in *L. pumila*, *L. montana* and *L. tortilis* (Mueller, 1992); basidiocarp texture is therefore not a systematically informative characteristic in delimiting these species.

#### Micromorphological Characters

Macromorphological characters in *Laccaria* can be highly variable within taxa. In addition, many characters used in classifications within other agaric genera (lamellar attachment, pileus and stipe context morphology, odor, and taste) are not consistently systematically informative in *Laccaria* (Mueller, 1992). For these reasons, micromorphological characters are usually required to differentiate species.

The number of sterigmata (i.e., number of spores produced) on each basidium in *Laccaria* appears to be highly consistent within taxa, and therefore systematically informative. *Laccaria fraterna*, *L. pumila*, and *L. tortilis* are characterized by 2 (-3) sterigmata per basidium; all other *Laccaria* species have (3-) 4 sterigmata per basidium (Mueller, 1992).

Although the frequency, size, and shape of cheilocystidia (sterile cells borne on the edges of lamellae) can be highly variable within taxa, the presence of large, abundant, clavate cheilocystidia in *L. amethystina*, *L. amethysteo-occidentalis*, and *L. vinaceobrunnea* appears to be consistent and distinctive and can be used in combination with other characters to distinguish these species (Mueller, 1992).

The arrangement of hyphae in the pileipellis (outermost cell layer of the pileus) is distinctive in some *Laccaria* species. Most species are characterized by having interwoven hyphae with scattered fascicles of perpendicular, aerial hyphae. However, *L. trichodermophora* is usually characterized by having hyphae arranged in a trichodermium (layer of uniform hyphae oriented perpendicular to the pileal surface), and *L. vinaceobrunnea* (and occasionally *L. amethystina*) by having a pileipellis of interwoven hyphae with occasional long, singular, perpendicular aerial hyphae (Mueller, 1992).

Spore characteristics are highly important in distinguishing *Laccaria* species. Spore shape in *Laccaria* ranges from globose (e.g., *L. amethystina*, *L. ochropurpurea*, *L. tortilis*) to subglobose or broadly ellipsoid (e.g. *L. amethysteo-occidentalis*, *L. nobilis*, *L. bicolor*, *L. pumila*, *L. montana*, *L. laccata* var. *pallidifolia*), ellipsoid (e.g., *L. proxima*), oblong (e.g. *L. oblongospora*, *L. maritima*), or subfusiform (e.g. *L. trullisata*). The length and width of the echinulae (spines) on the basidiospores are also characteristics of high systematic importance. Echinulae length can be used to distinguish species, and ranges from finely roughened (e.g., *L. trullissata*) to finely echinulate ( $< 1 \mu\text{m}$ , e.g., *L. maritima*, *L. proxima*, *L. oblongospora*), moderately echinulate (1-1.5  $\mu\text{m}$ , e.g., *L. bicolor*, *L. laccata*, *L. montana*, *L. pumila*), or strongly echinulate ( $>1.5 \mu\text{m}$ , e.g., *L. tortilis*, *L.*

*striatula*, *L. ohiensis*). The width of echinulae at the base is additionally important for distinguishing *L. ohiensis* and *L. striatula*, having broad ( $>1.2 \mu\text{m}$ ) echinulae bases, from *L. laccata* var. *pallidifolia* (Mueller, 1991a). Spore thickness is useful for distinguishing *L. trullissata* (thin-walled) from the similar *L. maritima* (thick-walled). Average spore size can be useful in distinguishing members of the *L. bicolor* complex, having smaller spores, from members of the *L. laccata* complex; however, the range of spore sizes can overlap between species in these complexes (Mueller, 1992; Osmundson, unpublished data).

#### Cultural Characters

Because a number of *Laccaria* species can be grown in culture, observation of somatic culture mat morphology can be used to provide additional characters for delimiting species. Culture mat color can be used to distinguish species in Metasection *Amethystina*, with violet culture mats on PDA and MMN media, from species in Metasection *Laccata* (with the exception of *L. oblongospora*), having white culture mats on PDA and MMN (Mueller, 1992). Culture mat morphology, especially the distribution of zones of thicker growth can additionally distinguish taxa in the *L. bicolor* complex. Additional cultural characteristics such as average linear growth rate, micromorphology of early hypha, and appearance of zones of denser mycelial growth support species designations based on morphological, molecular, and mating compatibility data (Mueller, 1985, 1992; Fries & Mueller, 1984).

### Molecular Characters

Molecular data provide additional characters for systematic analysis and their use can be a powerful tool for inferring species limits and phylogenetic classifications, especially when used in addition to morphological and mating compatibility data. Thus far, the use of molecular data in *Laccaria* systematics has been limited to restriction fragment length polymorphisms (RFLPs) of ribosomal and mitochondrial DNA as data to support species delimitations based on mating compatibility and morphological data. Restriction fragment patterns of nuclear ribosomal DNA (rDNA) distinguished the species *L. amethystina*, *L. bicolor*, *L. laccata* and *L. proxima*, and distinguished biological species within the *L. laccata* complex and populations within species between North America and Europe (Gardes et al. 1990). Restriction fragment patterns of mitochondrial DNA (mtDNA) produced similar results to those of rDNA, although higher variability was detected between *L. bicolor* populations, and evidence for mtDNA divergence between North American and European *L. bicolor* populations was not found (Gardes et al., 1991). Gardes and Mueller (1991) determined that RFLP patterns for rDNA and mtDNA were congruent with morphological and mating compatibility data in distinguishing three species (*L. bicolor* sensu stricto, *L. nobilis*, and *L. trichodermophora*) within the *L. bicolor* species complex. While RFLP data represent an additional, powerful set of characters to support species recognitions, problems in determining homology of observed banding patterns precludes the use of RFLPs in phylogenetic reconstruction. The use of sequence data in the present study (Chapter 3) provides a preliminary assessment of the utility of ribosomal internal transcribed spacer (rDNA-ITS)

sequences for recognizing species, and produces a framework for future studies toward generating a robust phylogeny for the genus *Laccaria*.

### Cytological Characters

*Laccaria* species are characterized by having multinucleate spores (usually 2 nuclei per spore in quadristerigmate and 4 nuclei per spore in bisterigmate species; Tommerup et al., 1991), a rare condition in the family Tricholomataceae sensu Singer (1986). This characteristic was used by Kühner (1984) to support the placement of *Laccaria* and the sequestrate genus *Hydnangium* in a separate family, Hydnangiaceae. An extensive survey of the nuclear condition of *Laccaria* and *Hydnangium* species and an extensive literature review of nuclear condition in other Agaricales by Mueller and Ammirati (1993) confirmed that the multinucleate condition is ubiquitous in *Laccaria* and *Hydnangium*, but determined that it is not restricted to these genera. In other words, the presence of multinucleate basidiospores supports the monophyly of *Laccaria* and *Hydnangium*, but is not systematically informative at the family level due to homoplasy within the order Agaricales.

### Mating Studies

The biological species concept, as applied to the Agaricales, assesses the potential conspecificity of isolates on the basis of the ability of homokaryotic mycelia derived from single spore isolates to mate, forming dikaryotic mycelia, in culture. The application of this species concept has increased in recent years as a means of assessing

and refining species delimitations based on morphological characters (Petersen, 1995a, 1995b). Because the spores of many *Laccaria* species germinate in culture, spore mating studies have been used to elucidate species limits, and the studies of Fries and Mueller (1984), Mueller (1991a, 1991c) and Mueller and Gardes (1991) serve as a model for the integration of mating and morphological data for systematic studies in ectomycorrhizal basidiomycetes.

Early single-spore intercollection pairing experiments between Swedish isolates of *L. laccata sensu lato* resulted in the determination of four intersterility groups (ISGs), Groups I, II, III, and IV (Fries, 1983). Correlation of these isolates with taxonomic data resulted in assigning these groups to *L. proxima* (Group I), *L. bicolor* (Group II), and *L. laccata sensu stricto* (Groups III and IV); mating of additional isolates resulted in delimiting the two additional ISGs Group V, corresponding to *L. amethystina*, and Group VI, corresponding to *L. altaica* (= *L. pumila*) (Fries & Mueller, 1984). In each case, biological species (i.e., ISGs) were found to correspond to morphological species; the presence of two ISGs referable to *L. laccata* indicated the presence of cryptic species within a single morphological species.

Studies on restriction fragment length polymorphisms in selected *Laccaria* species (Gardes et al., 1990, 1991a) employed a revised nomenclature for ISGs, referring to ISGs within *L. laccata* as Biological Species 1, 2, 3, and 4, with groups 1 and 2 consisting of North American isolates and groups 3 and 4 consisting of Swedish isolates. Correlation of mating data with morphometric analyses and RFLP data resulted in assignment of these intersterility groups to North American *Laccaria laccata* var.

*pallidifolia* (Biological Species 1), *L. longipes* (Biological Species 2), Swedish *L. laccata* var. *pallidifolia* and *L. laccata* var. *moelleri* (Biological Species 3), and *L. ohiensis* (Biological Species 4), and delimited an additional group (Biological Species 5) corresponding to *L. striatula* (Mueller, 1991a, 1991c, 1992). Similarly, intercollection pairing reactions combined with RFLP data and a phenetic analysis of morphological characteristics resulted in delimiting three species within the *Laccaria bicolor* complex: *L. bicolor sensu stricto*, *L. nobilis*, and *L. trichodermophora* (Mueller & Gardes, 1991).

The results of these studies in the *L. laccata* and *L. bicolor* species complexes illustrate the utility of mating data for addressing taxonomic problems. Mating data can provide evidence supporting the presence of genetic isolation between populations, thereby supporting or refuting character selection and weighting schemes used in systematic analyses and encouraging the rigorous evaluation of morphological differences between putative taxa, e.g., spore size between *L. longipes* vs. *L. laccata* var. *moelleri*, echinulae basal width between *L. ohiensis* and *L. laccata*, stipe length, texture, and color between *L. striatula* and *L. ohiensis*, and basidiocarp size, texture and color between *L. bicolor*, *L. nobilis* and *L. trichodermophora* (Mueller, 1991a, 1991c; Mueller & Gardes, 1991).

Several weaknesses are inherent in applying the biological species concept to Agaricales, including the inability to examine species whose spores do not germinate in culture, the possibility that the ability to mate in culture is retained even though morphological divergence or the presence of barriers to reproduction in nature exist (due to mating compatibility being a plesiomorphic trait), and the fact that there is a

significant distinction that must be made between the ability to form dikaryotic mycelia and the ability to produce viable offspring (Petersen, 1995a, 1995b). In addition, the presence of compatible mating type nuclei in many spores of bisterigmate species precludes their use in mating studies unless they have been dikaryotized first (Fries & Mueller, 1984). Mating studies to test the hypothesized genetic isolation of *L. montana* and *L. pumila* have been unsuccessful due to the inability to reliably obtain homokaryotic isolates of either species (Mueller, 1991). Due to these biological limitations, in addition to methodological limitations imposed by conducting a study with multiple researchers collecting over a large geographic region and broad range of taxa, obtaining homokaryotic cultures and conducting mating studies was beyond the scope of the present project. Mating data, or alternatively, the incorporation in molecular phylogenetic analyses of reference specimens previously used in mating studies, could be employed in future research to evaluate the species delimitations made in the present project.

#### Materials and Methods

The present study consists of a detailed taxonomic examination of Rocky Mountain alpine *Laccaria* species using material collected over a 5-year period at alpine field sites in Colorado, Montana, and Wyoming, USA. Collections were analyzed using macromorphological and micromorphological characters, and cultural characters whenever possible.

### Specimen Collection

Specimens were collected at field sites above treeline in the southern and central Rocky Mountains between 1997 and 2002. Southern Rockies field sites, all located in Colorado, included Independence Pass (elevation 3600-3700 m), Linkins Lake Valley (elev. 3600 m), Cumberland Pass (elev. 3662 m) and Cottonwood Pass (3700 m) in the Sawatch Range; Cinnamon Pass (elev. 3700-3850 m), Black Bear Pass (elev. 3760 m), Mineral Basin (3900 m), U.S. Basin (elev. 3660 m) and Horseshoe Lake (elev. 3810 m) in the San Juan Mountains; Loveland Pass (elev. 3700 m) and Haggeman's Pass (elev. 3600 m) in the Front Range, and Blue Lake Dam (elev. 3300 m) in the Tenmile Range. North-central Rocky Mountain sites were located on the Beartooth Plateau in southern Montana and northern Wyoming (elev. 2950-3264 m). Ectomycorrhizal host plants in close proximity to basidiocarps were noted for each collection. In addition to general collecting, a more systematic analysis on the Beartooth Plateau was conducted using fifteen 1-m<sup>2</sup> sampling plots in order to better assess mycorrhizal host plant associations for macromycetes including, but not limited to, *Laccaria* spp. (Chapter 4). Spore deposits and/or tissue cultures were attempted when feasible. Cultures were maintained on potato dextrose (PDA) or modified Melin-Norkrans (MMN; Marx, 1969) media. Basidiocarps were preserved by warm air drying on an electric dryer and deposited in the Montana State University – Bozeman herbarium (MONT), Fungal Section. *Laccaria* specimens collected during the course of this study are listed by taxon in Tables 8-12

following species descriptions (Results section). Specimens are designated with C.L. Cripps (CLC) or T.W. Osmundson (TWO) collection numbers.

### Morphological Descriptions

Macromorphological descriptions of basidiocarps were made from fresh specimens. Descriptive terms follow Largent (1986). Color designations correspond to Kornerup and Wanscher (1967), and are noted as combinations of plate number, column, and row (e.g., 8A5). Preparation of hand sections for observation of microscopic characters and use of descriptive terms follow Largent et al. (1977). Sections were mounted in 3% KOH for measurement of spores and microscopic features, and in Melzer's reagent (0.5 g iodine, 1.5 g potassium iodide, 20 mL dH<sub>2</sub>O, 20 mL chloral hydrate) to test for amyloid reactions. Spore measurements were made with apiculus and ornamentation excluded. Length-width ratios ( $Q$ ) were calculated for each spore, and a mean calculated for each collection ( $Q^m$ ). Average length-width ratios in the species descriptions are presented as the range of values encountered for collections examined. Basidiospore measurements were made from hymenial tissue in order to maintain consistency between collections, and in order to maintain consistency of methods with those used in Mueller's (1992) monographic treatment of North American *Laccaria* species. Measurements were made from a random sampling of 10-20 spores and 5-20 basidia and cystidia for each collection, using multiple basidiocarps wherever possible in order to capture variation within collections. Width measurements of basidia and cystidia were made at the widest point. Drawings of microscopic features were prepared using a

drawing tube attached to a Leica research microscope. Culture morphology was observed, noting color, relative growth rate, and growth form on PDA and/or MMN media. Some taxa, including *L. montana*, did not grow in culture, and successful isolation of *L. pumila* was rare.

#### Scanning Electron Microscopy

Lamellar fragments (approximately 1 mm<sup>2</sup>) were removed from dried basidiocarps, attached to aluminum mounts using double-stick tape, and gold-palladium sputter coated at a nominal coating thickness of 15 nm using a Hummer VII sputtering system (Anatech Ltd., Alexandria, VA). Basidiospores were examined at 15 kV using a JEOL JSM-6100 scanning electron microscope.

#### Results

Five species of *Laccaria* were collected in Rocky Mountain alpine habitats: *L. bicolor* (Maire) Orton, *L. laccata* var. *pallidifolia* (Peck) Peck, *L. montana* Singer, *L. pumila* Fayod, and *Laccaria* sp., putatively representing a new species. Rocky Mountain distributions for these taxa are discussed below.

#### Key to Rocky Mountain Alpine *Laccaria* Species

A key to *Laccaria* species collected in the Rocky Mountain alpine zone is presented. Though not found in alpine habitats during the course of the present study, *L. proxima* (Boud.) Pat. is included both because this species was found in Beartooth

subalpine areas in mixed EM plant communities that included *Salix* shrubs, and because its apparent affinity for disturbed sites and mine tailings makes it likely to be found in such sites above treeline.

Key to Rocky Mountain alpine *Laccaria* species:

1. Basidia bisterigmate; basidiocarps small (pileus  $\leq 3$  (-3.5) cm in diameter), pale orange-brown to nearly red-brown; basidiospores (8-) 9-13.5 x (6.8-) 7.5-10.5 (-12)  $\mu\text{m}$ ; subglobose to broadly ellipsoidal ( $Q^m = 1.08-1.18$ ), with moderately coarse echinulae ( $\leq 1.5$  (-2)  $\mu\text{m}$  in length, 0.3-1 (-1.25)  $\mu\text{m}$  wide at base) .....  
.....*Laccaria pumila* Fayod
1. Basidia tetrasterigmate.....2
2. Basidiocarps small to medium-sized (pileus 0.5-3.5 cm in diameter); pileus translucent-striate or nonstriate, glabrous to indistinctly fibrillose, pale orange to dark orange-brown or red-brown; stipe +/- equal, glabrous to minutely fibrillose.....3
2. Basidiocarps medium to large in size (pileus 1.5-7 cm in diameter); pileus distinctly finely fibrillose, pale pinkish-orange to dark orange-brown or red-brown; stipe robust, basally enlarged to clavate, distinctly fibrous-striate.....5
3. Basidiocarps small to medium (pileus 1-3.5 cm in diameter, stipe 1.5-5 cm long), glabrous, indistinctly striate to nonstriate; pale orange; basidiospores globose to subglobose ( $Q^m = 1-1.07$ ) with moderately coarse echinulae ( $\leq 1.5$  (-2)  $\mu\text{m}$  in length, 0.3-1.1  $\mu\text{m}$  wide at base) .....*Laccaria laccata* var. *pallidifolia* (Peck) Peck
3. Basidiocarps small (pileus 0.5-2 (-3.5) cm in diameter, stipe generally  $\leq 3$  cm long), usually translucent-striate; orange brown to red-brown; basidiospores subglobose to broadly ellipsoidal ( $Q^m = 1.04-1.18$ ), finely to moderately echinulate.....4
4. Basidiospores subglobose to broadly ellipsoidal ( $Q^m = 1.04-1.16$ ), moderately large ( $x = 9.1-10.1 \times 8.7-9.1 \mu\text{m}$ ) with moderately coarse echinulae ( $\leq 1.7$  (-2.5)  $\mu\text{m}$  in length, 0.3-1.1  $\mu\text{m}$  wide at base).....*Laccaria montana* Singer
4. Basidiospores generally more ellipsoidal than above ( $Q^m = 1.16-1.18$ ), sometimes smaller ( $x = 7.9-9.7 \times 6.8-8.3 \mu\text{m}$ ), having finer echinulae than above ( $\leq 1$  (-1.7 at spore apex)  $\mu\text{m}$  in length, 0.3-0.6  $\mu\text{m}$  wide at base)..... *Laccaria* sp.
5. Basidiocarps pale pinkish-orange to dark orange-brown, medium to large (pileus in mature specimens 2-7 cm in diameter); basal tomentum white to violet; colonies on

- PDA bright violet, fading to red-brown, pale violet or nearly white; basidiospores subglobose to broadly ellipsoidal ( $Q^m = 1.06-1.15$ ) with moderately coarse echinulae ( $\leq 1.5 (-2.5) \mu\text{m}$  in length,  $0.4-1.2 \mu\text{m}$  wide at base), associated with shrub *Salix* species. .... *Laccaria bicolor* (Maire) Orton  
 (NOTE: Large specimens of *L. laccata* var. *pallidifolia* (especially when somewhat dry) may key out here due to pileus diameter, and can be distinguished by having less clavate, glabrous to indistinctly fibrillose stipes and white cultures on PDA).
5. Basidiocarps dark orange-brown to red-brown, medium to large (pileus in mature specimens 1.5-5.5 cm in diameter); basal tomentum white; colonies on PDA white; basidiospores ellipsoidal ( $Q^m = 1.36-1.51$ ) and finely echinulate (echinulae  $\leq 1 \mu\text{m}$  in length with occasional long echinulae (1-1.8  $\mu\text{m}$  long) at apex; echinulae 0.2-0.75  $\mu\text{m}$  wide at base); subalpine; commonly on mine tailings and disturbed sites .....  
 ..... *Laccaria proxima* (Boud.) Pat.

#### Taxonomy of Rocky Mountain Alpine *Laccaria*

##### *Laccaria bicolor* (Maire) Orton

Figures 2, 3

Maire, 1937. Publ. Inst. Bot. Barcelona 3: 84 (as *L. laccata* var. *bicolor*)

Macromorphology: Pileus (1-) 2-5 (-7) cm in diameter, convex, occasionally becoming nearly plane with shallow central depression in age; pale pinkish orange to dark orange, occasionally darker at margin, hygrophanous, drying to pale orange; nearly glabrous when young, becoming minutely scaly with concentrically-arranged fine scales in age; not striate. Margin involute becoming decurved to uplifted, entire to undulating slightly, occasionally rimulose in age. Context thick, white to pale orange, pinkish-white or pale violet. Lamellae adnate to adnexed, broad, moderately thick, subdistant, pink; edges entire; lamellulae present. Stipe 2-5 x 0.3-0.8 cm, equal or more frequently gradually basally enlarged to clavate, robust, long, tough, solid becoming hollow at least in some collections; concolorous to paler than pileus, pale whitish orange to pale orange-

brown; apex pink in some young specimens. Surface longitudinally striate, rough-fibrous. Basal tomentum generally white under field conditions, but noted as violet in one collection (CLC 1482). Odor and taste mild.

Micromorphology: Pileipellis of interwoven, inamyloid, cylindrical, mostly repent hyphae with widely scattered fascicles of hyphae oriented nearly perpendicular to pileal surface. Hyphae hyaline or having intracellular pigment appearing pale orange-brown in 3% KOH. Clamp connections abundant. Stipitipellis of parallel, cylindrical, repent, inamyloid hyphae. Terminal elements in one collection (CLC 1469) swollen. Clamp connections present. Caulocystidia absent. Lamellar trama of subparallel to interwoven, cylindrical, hyaline, inamyloid hyphae. Clamp connections present. Subhymenium undifferentiated. Pleurocystidia absent. Cheilocystidia filiform, cylindrical to irregular, hyaline, 29-44 x 1-4  $\mu\text{m}$ ; absent to abundant. Basidia clavate, hyaline, 26-44 x 8-11  $\mu\text{m}$ , tetrasterigmate; sterigmata  $\leq 7$   $\mu\text{m}$  in length. Basidiospores 5.4-8 (-9.5) x (4.1-) 5.5-7.7 (-8.5)  $\mu\text{m}$  (mean = 6.2-8 x 5.5-7  $\mu\text{m}$ ),  $Q = 1-1.32$  ( $Q^m = 1.06 - 1.15$ ), subglobose to broadly ellipsoidal, hyaline, echinulate; echinulae  $\leq 1.5$  (-2.5)  $\mu\text{m}$  in length, (0.2-) 0.4-1.2  $\mu\text{m}$  wide at base.

Culture morphology: Dikaryotic cultures on PDA and MMN moderately fast-growing; colonies white, becoming bright violet then fading to red-brown, pale violet or nearly white.

Rocky Mountain alpine habitat and distribution: Solitary to scattered, occurring in alpine habitats in the Front Range, Sawatch Range and San Juan Mountains in Colorado.

Associated with dwarf willows including *Salix arctica* and *S. reticulata*, and shrub willows including *Salix planifolia* and *S. glauca*.

Comments: *L. bicolor* is distinguished by having medium to large-sized, robust, minutely scaly basidiomata, subglobose to broadly ellipsoidal basidiospores borne on tetrasterigmate basidia and violet mycelial growth on PDA. The presence of a violet basal tomentum in one collection (CLC 1482) was noted. While this character can be useful in identifying *L. bicolor* in the field, it was rarely present in Colorado alpine collections. *Laccaria bicolor* and *L. laccata* var. *pallidifolia* are the two medium- to large-sized taxa collected at alpine field sites during the course of this study. Both have tetrasterigmate basidia and globose to subglobose basidiospores; *L. laccata* var. *pallidifolia* generally has larger spores, but the present study failed to separate the two species based on spore characteristics alone (i.e., division based on spore characteristics conflicts with those based on macromorphological and molecular data); because of these overlaps in spore size and shape, *L. bicolor* can be difficult to distinguish from *L. laccata* var. *pallidifolia* in the absence of a violet basal tomentum and/or mycelial culture. In the present study, basidiocarp stature and texture were found to be useful characters for distinguishing the two species: *L. bicolor* basidiocarps were consistently more robust than *L. laccata* var. *pallidifolia*, with minutely scaly pilei and basally enlarged to nearly clavate, rough fibrous-striate stipes. Distinctions based on these characters were supported by molecular data (Chapter 3). *Laccaria bicolor* was collected at alpine field sites in Colorado, but was not collected on the Beartooth Plateau. Examination of the collection TENN 42529 (Appendix A), the Representative Specimen (c.f. neotype) of *L. bicolor*, showed

micromorphological features corresponding to those of the smaller-spored Rocky Mountain alpine collections examined.

Material examined: USA. COLORADO. Summit/Clear Creek Co.: Front Range, Loveland Pass, 7 August 1999, *CLC 1304* (MONT); San Juan Co.: San Juan Mountains, Black Bear Basin, 3 August 2000, *CLC 1445* (MONT), Mineral Basin, 7 August 2001, *CLC 1672* (MONT), Stony Pass, 28 July 2002, *CLC 1825* (MONT), Horseshoe Lake, 6 August 2001, *CLC 1656* (MONT), Cinnamon Pass, 10 August 2001, *CLC 1709* (MONT); Pitkin/Chaffee Co.: Sawatch Range, Independence Pass, 11 August 1999, *CLC 1347* (MONT), 13 August 1999, *CLC 1365* (MONT), 6 August 2000, *CLC 1469* (MONT), 13 August 2001, *CLC 1742* (MONT); Pitkin Co.: Sawatch Range, Linkins Lake Valley, 8 August 2000, *CLC 1482* (MONT). Collections of *Laccaria bicolor* examined, with collection data and EM host plants, are shown in Table 8.

Table 8. Rocky Mountain alpine *L. bicolor* (Maire) Orton collections examined, showing collection data and ectomycorrhizal host associates.

<u>ID</u>	<u>Location</u>	<u>Range</u>	<u>State</u>	<u>Plant Associations</u>	<u>Date</u>
CLC 1304	Loveland Pass	Front	CO	<i>Salix</i> shrub	8/7/99
CLC 1445	Black Bear Basin	San Juan	CO	<i>Salix arctica</i>	8/3/00
CLC 1709	Cinnamon Pass	San Juan	CO	<i>Salix arctica</i>	8/10/01
CLC 1672	Mineral Basin	San Juan	CO	<i>Salix reticulata</i>	8/7/01
CLC 1656	Horseshoe Lake	San Juan	CO	<i>Salix reticulata</i>	8/6/01
CLC 1825	Stony Pass	San Juan	CO	<i>Salix planifolia</i>	7/28/02
CLC 1469	Independence Pass	Sawatch	CO	<i>Salix planifolia</i>	8/6/00
CLC 1482	Linkins Lake Valley	Sawatch	CO	<i>Salix planifolia</i>	8/8/00
CLC 1742	Independence Pass	Sawatch	CO	<i>Salix planifolia</i>	8/13/01
CLC 1347	Independence Pass	Sawatch	CO	<i>Salix planifolia</i> , <i>S. glauca</i>	8/11/99
CLC 1365	Independence Pass	Sawatch	CO	<i>Salix</i> shrub	8/13/99

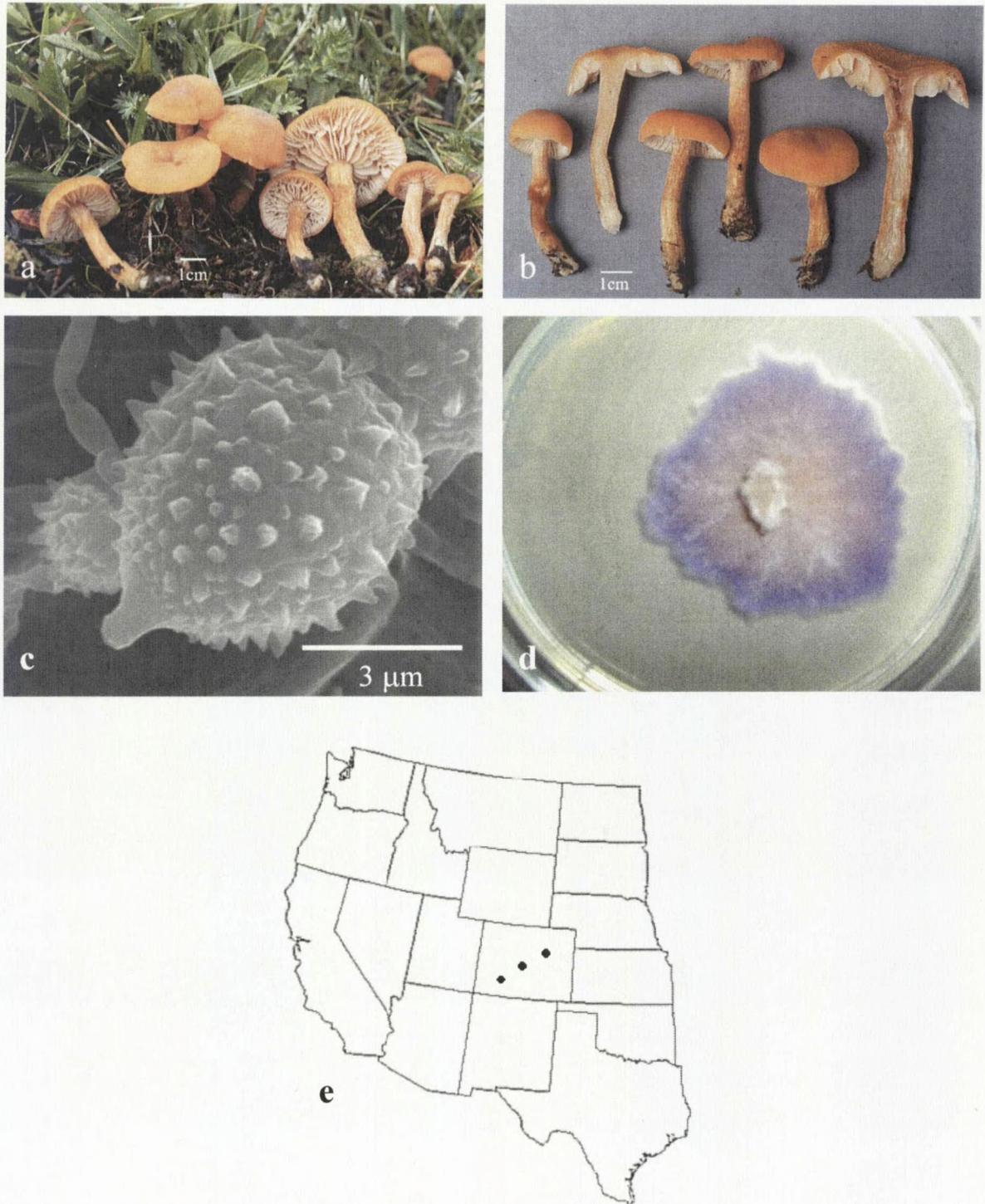


Figure 2. *Laccaria bicolor* (Maire) Orton **a, b**. Basidiocarps (CLC 1672). **c**. Scanning electron micrograph of basidiospores (CLC 1482). **d**. Culture morphology on PDA medium (CLC 1825). **e**. Rocky Mountain alpine distribution map.

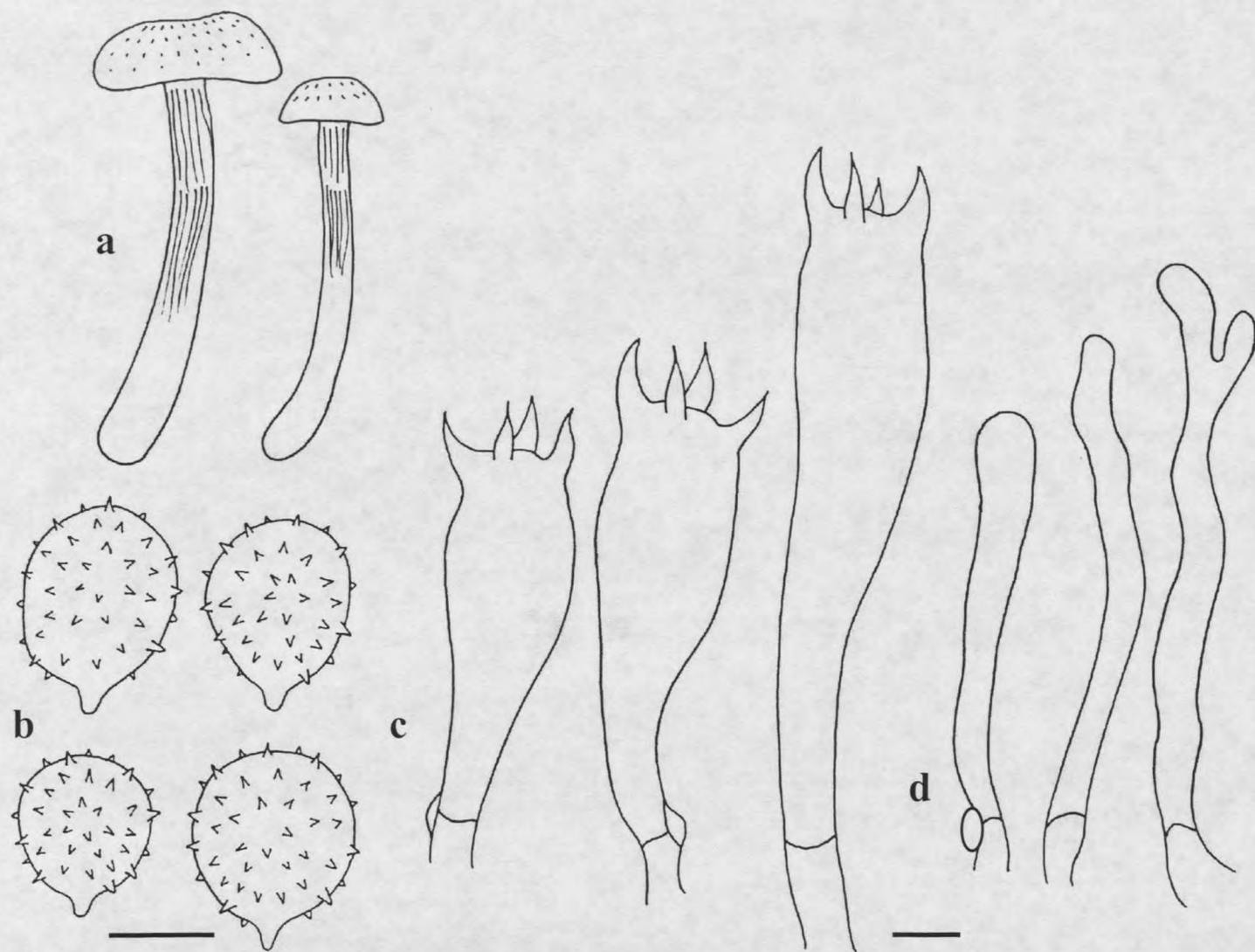


Figure 3. *Laccaria bicolor* (Maire) Orton, CLC 1445. a. Basidiomata, actual size. b. Basidiospores. c. Basidia. d. Cheilocystidia. Scale bars = 10  $\mu$ m.

*Laccaria laccata* var. *pallidifolia* (Peck) Peck

Figures 4, 5

Peck, 1890. Annual Rep. New York State Botanist 43: 274 (as *Clitocybe laccata* var. *pallidifolia*)

Macromorphology: Pileus (0.5) 1-2 (-3) cm in diameter, convex to shallow convex or nearly omphaloid; indistinctly translucent-striate to nonstriate, pale orange (5A8 to darker), hygrophanous, drying to paler orange (5B6), glabrous, occasionally lubricous. Margin equal to uneven or crenulate; rimulose in age. Context white to pale orange. Lamellae adnate to subdecurrent or rarely decurrent, broadly attached, adnate, thick, pale orange to pink, subdistant. Edges entire; lamellulae present. Stipe 1.5-5 x 0.2 x 0.5 cm, equal to slightly enlarged toward base, generally with long, thin appearance; straight to undulating, pale orange, glabrous to minutely fibrillose, fibrous-striate; occasionally tough, rubbery. Basal tomentum white. Odor mild.

Culture morphology: Cultures not obtained.

Micromorphology: Pileipellis of interwoven, inamyloid, cylindrical, mostly repent hyphae with widely scattered fascicles of hyphae oriented nearly perpendicular to pileal surface. Hyphae hyaline or having intracellular pigment appearing pale orange-brown in 3% KOH. Clamp connections abundant. Stipitipellis of parallel, cylindrical, repent, inamyloid hyphae. Clamp connections present. Caulocystidia absent. Lamellar trama of subparallel to interwoven, cylindrical, hyaline, inamyloid hyphae. Clamp connections present. Subhymenium undifferentiated. Pleurocystidia absent. Cheilocystidia filiform, cylindrical, hyaline, 32-44 (-50) x 3-5  $\mu\text{m}$ ; rare to absent. Basidia clavate, hyaline, 26-50 x 9-12  $\mu\text{m}$ , tetrasterigmate; sterigmata  $\leq 8$  (-11)  $\mu\text{m}$  in length. Basidiospores (5-) 6.2-10

(-10.9) x (4.1-) 5.6-9.8 (-10.8)  $\mu\text{m}$  (mean = 6.2-8.8 x 5.5-8.8  $\mu\text{m}$ ),  $Q = 1-1.22$  (-1.32) ( $Q^m = 1-1.07$  (-1.18)), subglobose to broadly ellipsoidal, hyaline, echinulate; echinulae  $\leq 1.5$  (-2)  $\mu\text{m}$  in length, 0.3-1.1  $\mu\text{m}$  wide at base.

Rocky Mountain alpine habitat and distribution: Solitary to scattered, occurring in alpine habitats in the Sawatch Range, 10-mile Range, Front Range and San Juan Mountains in Colorado. Associated with *Dryas octopetala*, *Betula glandulosa*, *Salix reticulata*, *Salix glauca* and in mixed habitats with *D. octopetala* + unidentified dwarf *Salix* and *Betula glandulosa* + *Salix cf. reticulata*.

Comments: *Laccaria laccata* var. *pallidifolia* exhibits a wide range of phenotypic variability (Mueller, 1992), and can therefore be difficult to distinguish from a number of other *Laccaria* species (see comments under other species descriptions). As such, reports of this species in the literature must be viewed cautiously. Widely reported from arctic-alpine habitats, *L. laccata* var. *pallidifolia* is reported from Colorado field sites but was not collected on the Beartooth Plateau during the course of this study. Host overlap with *L. bicolor* occurs in terms of associations with dwarf willows; however, *L. laccata* is reported here in association with the additional hosts *Dryas octopetala* and *Betula glandulosa*, and is reported only once with shrubby *Salix* species. *Laccaria laccata* var. *pallidifolia* is a cosmopolitan, commonly collected species. Rocky Mountain subalpine collections are reported from Colorado and Idaho (Mueller, 1992). Host plant families are reported as Pinaceae, Fagaceae and Betulaceae (Mueller, 1992). Examination of the reference collection TENN 43090 (Appendix A) showed micromorphological features corresponding to those noted for the Rocky Mountain alpine collections examined.

Material examined: U.S.A. COLORADO. Pitkin/Lake Co.: Sawatch Range, Independence Pass, 14 August 1999, *CLC 1370* (MONT); Summit Co.: 10-mile Range, Blue Lake Dam, near Breckenridge, 2 August 2001, *CLC 1603* (MONT), 3 August 2001, *CLC 1633* (MONT); San Juan Co.: San Juan Mountains, Horseshoe Lake, 6 August 2001, *CLC 1655* (MONT); Gunnison/Chaffee Co.: San Juan Mountains, Cottonwood Pass, 12 August 2001, *CLC 1724* (MONT). Collections of *Laccaria laccata* var. *pallidifolia* examined, with collection data and EM host plants, are shown in Table 9.

Table 9. Rocky Mountain alpine *L. laccata* var. *pallidifolia* (Peck) Peck collections examined, showing collection data and ectomycorrhizal host associates.

<u>ID</u>	<u>Location</u>	<u>Range</u>	<u>State</u>	<u>Plant Associations</u>	<u>Date</u>
CLC1603	Blue Lake Dam	Tenmile	CO	<i>Salix reticulata</i> , <i>Betula glandulosa</i>	8/2/01
CLC1633	Blue Lake Dam	Tenmile	CO	<i>Betula glandulosa</i>	8/3/01
CLC1308	Loveland Pass	Front	CO	?	8/8/99
CLC1655	Horseshoe Lake	San Juan	CO	<i>Salix reticulata</i>	8/6/01
CLC1648	Cottonwood Pass	San Juan	CO	<i>Salix glauca</i>	8/4/01
CLC1724	Cottonwood Pass	San Juan	CO	<i>Dryas octopetala</i>	8/12/01
CLC1238b	Independence Pass	Sawatch	CO	<i>Salix reticulata</i> , <i>Salix arctica</i> ?	8/13/98
CLC1370	Independence Pass	Sawatch	CO	dwarf <i>Salix</i> , <i>Dryas octopetala</i>	8/14/99
CLC1653	Cumberland Pass	Sawatch	CO	<i>Betula glandulosa</i>	8/4/01

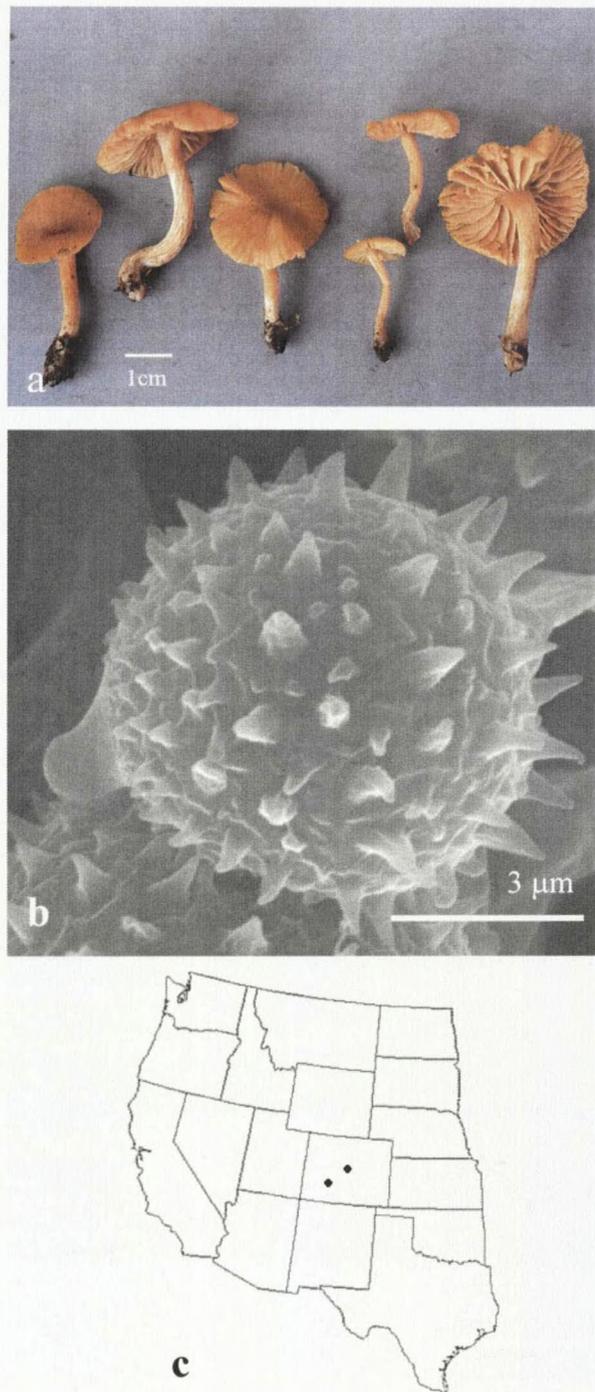


Figure 4. *Laccaria laccata* var. *pallidifolia* (Peck) Peck. **a.** basidiocarps (CLC1655). **b.** Scanning electron micrograph of basidiospores (CLC 1633). **c.** Rocky Mountain alpine distribution map.

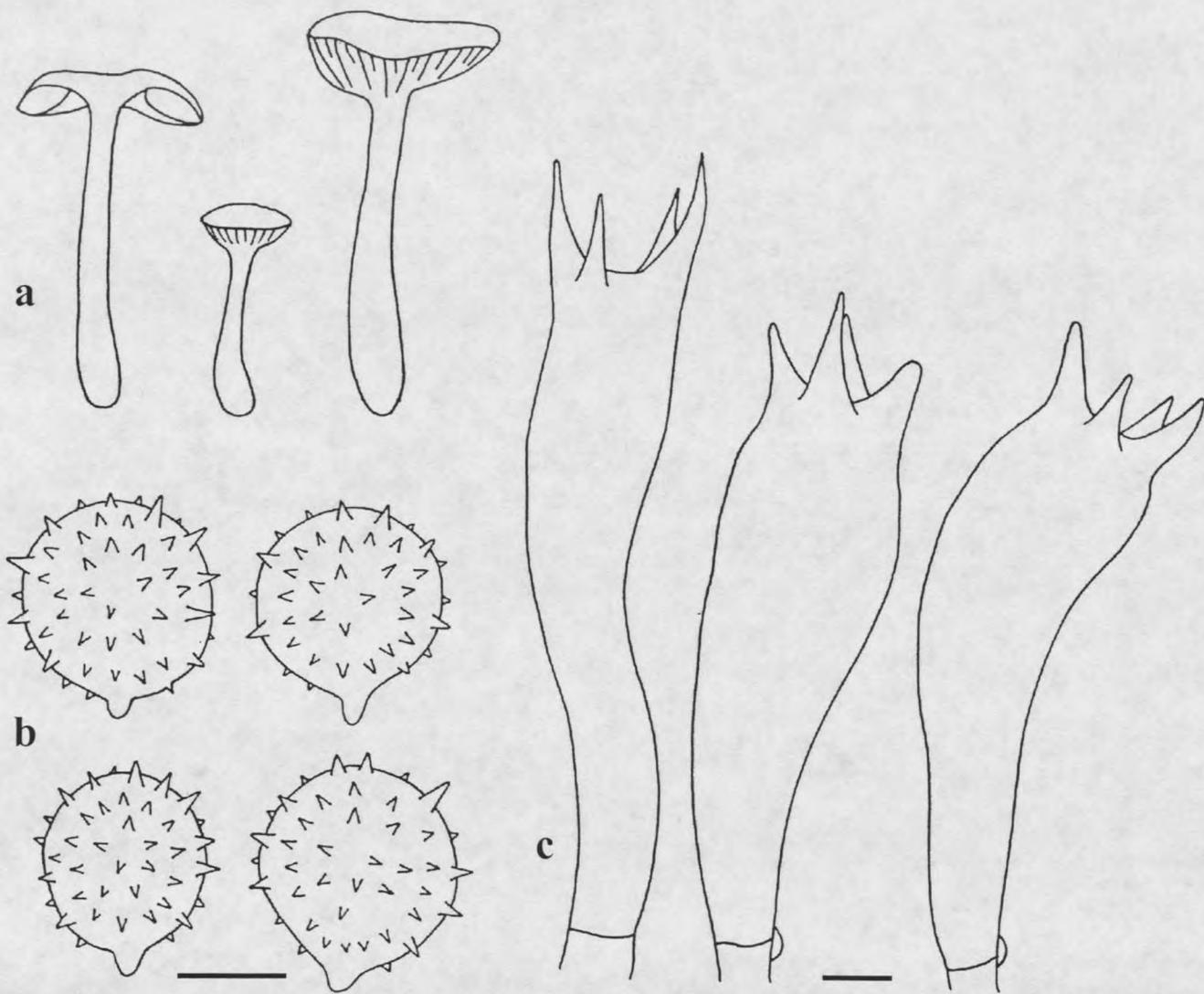


Figure 5. *Laccaria laccata* var. *pallidifolia* Peck (Peck), CLC 1370. a. Basidiomata, actual size. b. Basidiospores. c. Basidia. Scale bars = 10  $\mu$ m.

*Laccaria montana* Singer

Figures 6, 7

Singer, 1973. Sydowia 7: 89.

Macromorphology: Pileus 0.5 – 2.5 (-3.5) cm in diameter, convex becoming plane / or occasionally uplifted, with shallow central depression or rarely a low broad umbo; orange brown (“raw Sienna,” 6D7 to “caramel,” 6B5-6C6) to red brown or brick red (7D6 – 7E8), glabrous to minutely scaly, especially when dry; translucent-striate to plicate-sulcate, mildly to strongly hygrophanous, drying to pale orange buff (“Pompeian yellow,” 5C6). Margin involute to decurved, often uplifted-undulating in age; entire or occasionally crenate, occasionally splitting in age. Context thin, white to pale orange-brown. Lamellae adnate or rarely short decurrent, moderately thick, broad (occasionally narrow or ventricose), subdistant, greyish orange (5B5-7B5) to pinkish orange-brown (6C5-6C7). Edges entire; lamellulae present. Stipe 1.0 – 2.8 (-4.7) x (0.1-) 0.2-0.3 (-0.4) cm,  $\pm$  equal, solid becoming hollow in age, glabrous to minutely fibrillose,  $\pm$  concolorous with pileus, brownish-orange to red-brown (5C5-7D6). Basal tomentum white, scant to moderately dense. Odor and taste mild.

Micromorphology: Pileipellis of interwoven, inamyloid, cylindrical, mostly repent hyphae with widely scattered fascicles of hyphae oriented nearly perpendicular to pileal surface. Hyphae hyaline or having intracellular pigment appearing pale orange-brown in 3% KOH. Clamp connections abundant. Terminal elements in one collection (TWO 561) inflated, subclavate. Stipitipellis of parallel, cylindrical, repent, inamyloid, hyaline hyphae. Clamp connections abundant. Caulocystidia absent. Lamellar trama of subparallel to interwoven, hyaline, inamyloid hyphae; cells barrel-shaped. Clamp

connections present. Subhymenium undifferentiated. Pleurocystidia absent.

Cheilocystidia filiform, cylindrical to irregular, hyaline, 31-55 (-64) x 3-6  $\mu\text{m}$ ; absent in some collections while scattered to abundant in others. Basidia clavate, hyaline, 32-51 (-65) x (9-) 11-14 (-17)  $\mu\text{m}$ , tetrasterigmate; sterigmata  $\leq$  10 (-12)  $\mu\text{m}$  in length.

Basidiospores (8-) 9-11 (-12) x (7-) 7.9-9.5 (-10.5)  $\mu\text{m}$  (mean = 9.1-10.1 x 8.7-9.1  $\mu\text{m}$ ),  $Q = 1-1.29$  ( $Q^m = 1.04 - 1.16$ ), subglobose to broadly ellipsoidal or occasionally globose, hyaline, echinulate; echinulae  $\leq$  1.7 (-2.5)  $\mu\text{m}$  in length, 0.3-1.1 (-1.3)  $\mu\text{m}$  wide at base.

Culture morphology: Culture not obtained.

Rocky Mountain alpine habitat and distribution: Scattered to gregarious, rarely solitary; usually among mosses; occurring in alpine habitats on the Beartooth Plateau in Montana/Wyoming. Only one collection observed from alpine field sites in Colorado, collected in the San Juan mountains. Associated primarily with the shrub willows *Salix planifolia* and *S. glauca*; also found in association with the dwarf willow *Salix arctica* in Wyoming.

Comments: *Laccaria montana* appears similar in the field to *L. pumila*, *L. tortilis* and small, striate forms of *L. laccata* var. *pallidifolia* (Mueller, 1992). *Laccaria laccata* var. *pallidifolia* is characterized by having smaller, globose to subglobose basidiospores. *Laccaria tortilis* and *L. pumila* are characterized by having bisterigmate basidia. See additional comments under *L. pumila*. *Laccaria montana* was observed only once in alpine habitats in Colorado during the present study. A specimen (*A.H. Smith 87400* (MICH)) identified as *L. montana* from Independence Pass, Colorado was examined by Lahaie (1981), who reported finding both bisterigmate and tetrasterigmate basidia;

therefore, this record cannot be confirmed without further examination. Mueller (1992) states that at least some previous arctic records of *L. tetraspora* (e.g., Kobayasi et al., 1967; Miller et al., 1982) are *L. montana*. Lahaie (1981) reports *L. montana* to have been collected near *Betula nana* and *Arctostaphylos alpina* from an arctic site on the Tuktoyaktuk Peninsula, Northwest Territories, Canada. *Laccaria montana* is reported in Rocky Mountain subalpine habitats in Colorado, Idaho, Montana and Wyoming, in association with *Betula* spp., *Salix* spp. and species in the Pinaceae. Examination of the reference collection TENN 42880 (Appendix A) showed micromorphological features corresponding to those of the Rocky Mountain alpine collections examined.

Material examined: U.S.A. COLORADO. San Juan County: San Juan Mountains, Mineral Basin, 30 July 2002, *CLC 1853* (MONT). MONTANA. Carbon Co.: Beartooth Plateau, near source of Quad Creek, 10 August 1999, *TWO 264* (MONT), 28 July 2001, *TWO 441* (MONT); Carbon Co. at Wyoming State Line, Beartooth Plateau, Highline Trailhead, 1 August 2000, *TWO 319* (MONT), 14 July 2001, *TWO 369* (MONT), 3 August 2001, *TWO 504* (MONT), 3 August 2001, *TWO 505* (MONT), 16 August 2001, *TWO 540* (MONT), 19 August 2001, *TWO 559* (MONT), 19 August 2001, *TWO 561* (MONT), 18 July 2002, *TWO 613* (MONT), 15 August 2002, *TWO 710* (MONT). WYOMING. Park Co.: Beartooth Plateau, north of Frozen Lake, 31 July 2001, *TWO 477* (MONT), 4 August 2001, *TWO 512* (MONT), 17 August 2001, *TWO 553* (MONT), 1 September 2001, *TWO 591* (MONT). Collections of *Laccaria montana* examined, with collection data and EM host plants, are shown in Table 10.

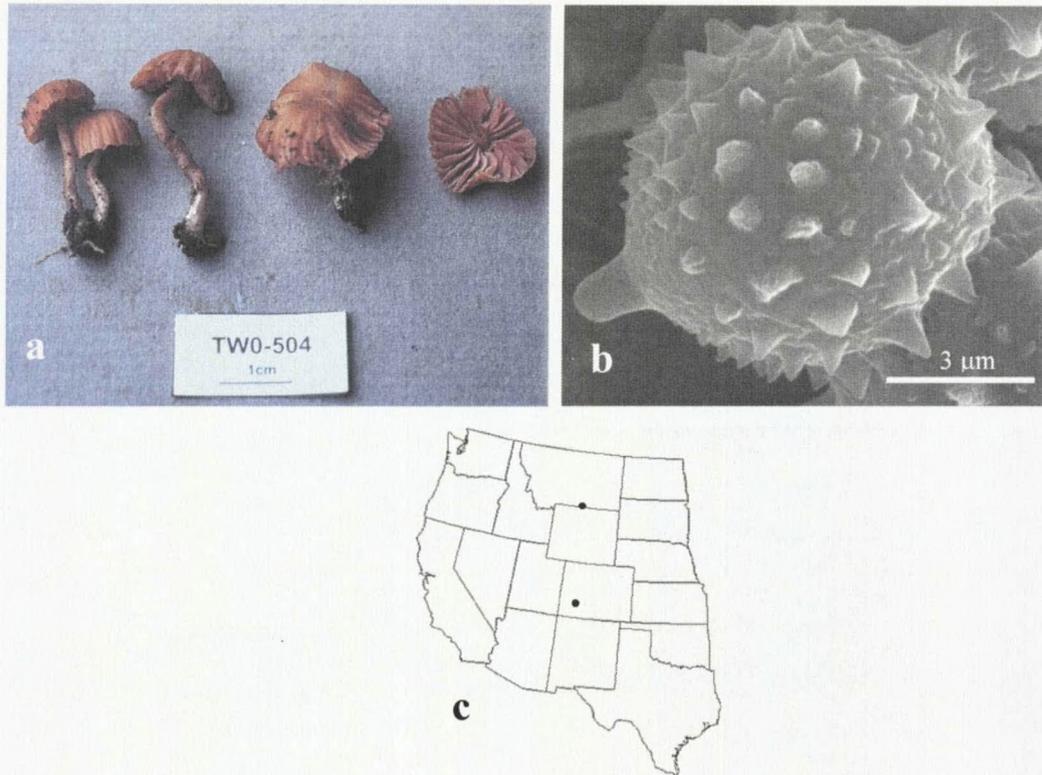


Figure 6. *Laccaria montana* Singer. **a.** Basidiocarps (TWO 504). **b.** Scanning electron micrograph of basidiospores (TWO 613). **c.** Rocky Mountain alpine distribution map.

Table 10. Rocky Mountain alpine *Laccaria montana* Singer collections examined, showing collection data and ectomycorrhizal host associates.

<u>ID</u>	<u>Location</u>	<u>Range</u>	<u>State</u>	<u>Plant Associations</u>	<u>Date</u>
TWO264	Quad Creek	Beartooth	MT	<i>Salix planifolia</i>	8/10/99
TWO441	Quad Creek	Beartooth	MT	<i>Salix planifolia</i>	7/28/01
TWO369	Highline Trailhead	Beartooth	MT/WY	<i>Salix planifolia</i>	7/14/01
TWO540	Highline Trailhead	Beartooth	MT/WY	<i>Salix planifolia</i>	8/16/01
TWO613	Highline Trailhead	Beartooth	MT/WY	<i>Salix planifolia</i>	7/18/02
TWO504	Highline Trailhead	Beartooth	MT/WY	<i>Salix glauca</i>	8/3/01
TWO505	Highline Trailhead	Beartooth	MT/WY	<i>Salix glauca</i>	8/3/01
TWO559	Highline Trailhead	Beartooth	MT/WY	<i>Salix glauca</i>	8/19/01
TWO561	Highline Trailhead	Beartooth	MT/WY	<i>Salix glauca</i>	8/19/01
TWO319	Highline Trailhead	Beartooth	MT/WY	?	8/1/00
TWO710	Highline Trailhead	Beartooth	MT/WY	?	8/15/02
TWO477	Frozen Lake	Beartooth	WY	<i>Salix arctica</i>	7/31/01
TWO553	Frozen Lake	Beartooth	WY	<i>Salix arctica</i>	8/17/01
TWO512	Frozen Lake	Beartooth	WY	<i>Salix planifolia</i>	8/4/01
TWO591	Frozen Lake	Beartooth	WY	<i>Salix planifolia</i>	9/1/01
CLC1853	Mineral Basin	San Juan	CO	<i>Salix arctica</i>	7/30/02

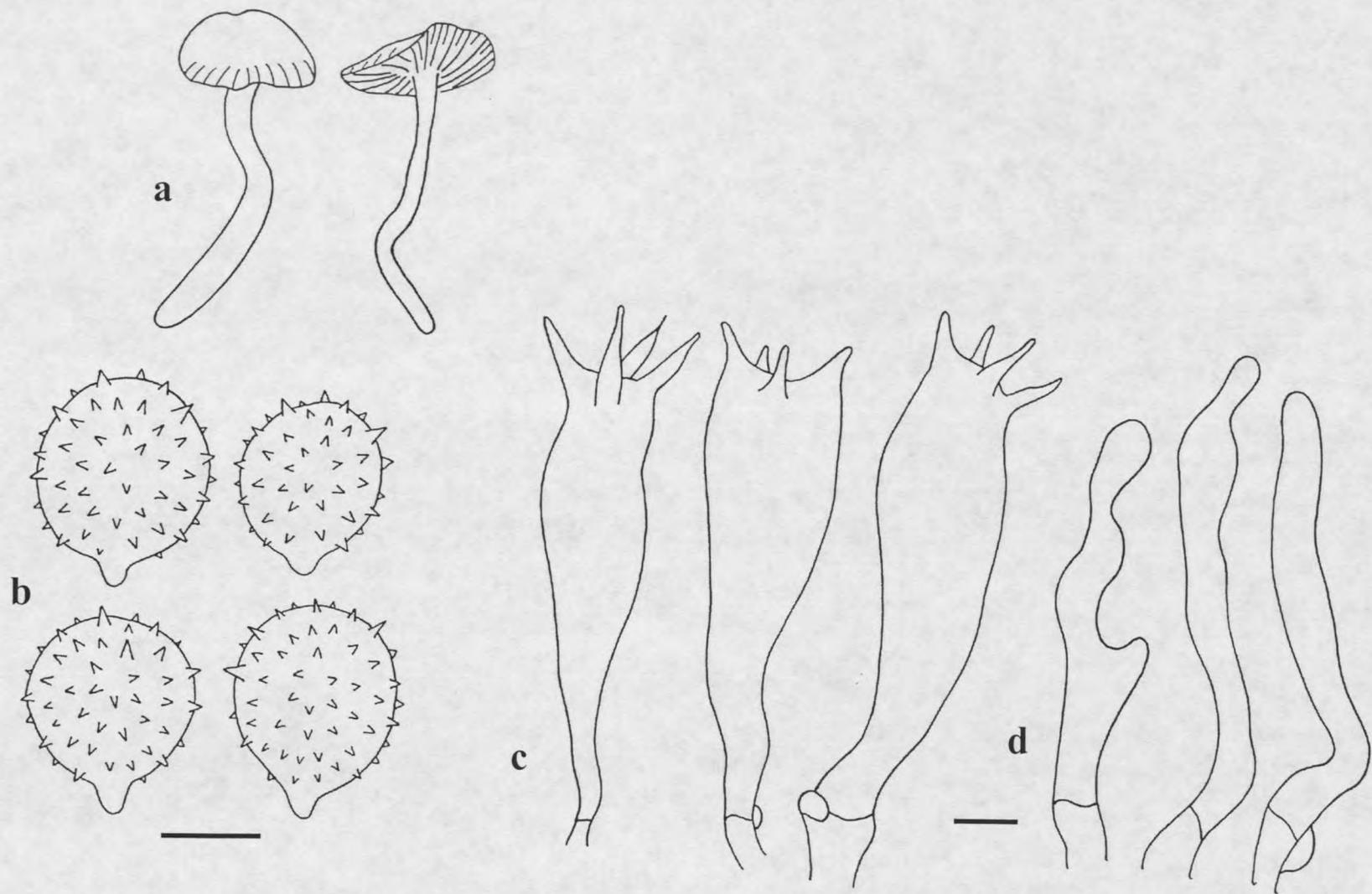


Figure 7. *Laccaria montana* Singer a. Basidiomata, actual size (TWO 441, left; TWO 561, right). b. Basidiospores (TWO 540). c. Basidia (TWO 540). d. Cheilocystidia (TWO 540). Scale bars = 10  $\mu$ m.

*Laccaria pumila* Fayod

Figures 8, 9

Fayod, 1893. Annali Accad. Agric. Torino 35: 91.

Macromorphology: Pileus 0.5-1.5 (-3.5) cm in diameter, convex to plane to nearly omphaloid, often with slight central depression, usually translucent-striate, glabrous to minutely fibrillose, occasionally lubricous; strongly hygrophanous, pale orange-brown (6D8) to nearly red-brown. Margin decurved, often becoming uplifted in age; entire to irregular or slightly eroded. Context thin, white to pale orange. Lamellae adnate to short decurrent, narrow to moderately broad, moderately thick to thick, subdistant, greyish-orange to pinkish orange (6B5-6B6), occasionally forked; edges entire; lamellulae present. Stipe (0.6-) 1.5-3.5 (-5) x 0.1-0.4 cm, solid, often tough,  $\pm$  equal, glabrous to minutely fibrillose, pale pinkish brown to pinkish orange or dark orange-red ("Raw sienna," 6D7); base of stipe often opaque, whitish. Basal tomentum white, moderately dense to lacking entirely. Odor and taste mild.

Micromorphology: Pileipellis of interwoven, inamyloid, cylindrical, mostly repent hyphae with widely scattered fascicles of hyphae oriented nearly perpendicular to pileal surface. Hyphae hyaline or having intracellular pigment appearing pale orange-brown in 3% KOH. Clamp connections abundant. Stipitipellis of parallel, cylindrical, repent, inamyloid hyphae 3-8  $\mu$ m in width. Clamp connections abundant. Caulocystidia absent. Lamellar trama of subparallel to interwoven, cylindrical, hyaline, inamyloid hyphae. Clamp connections present. Subhymenium undifferentiated. Pleurocystidia absent. Cheilocystidia filiform, hyaline, 28-35 (-46) x 3-5  $\mu$ m; uncommon. Basidia clavate, hyaline, (28-) 32-44 (-50) x 8-15  $\mu$ m, bisterigmate; sterigmata  $\leq$  13  $\mu$ m in length.

Basidiospores (8-) 9-13.5 (-15) x (6.8-) 7.5-10.5 (-14.5)  $\mu\text{m}$  (mean = 9.5-11.4 x 8.1-9.5 (-10.5)  $\mu\text{m}$ ),  $Q = 1-1.3$  ( $Q^m = 1.08 - 1.18$ ), subglobose to broadly ellipsoidal or occasionally globose, hyaline, echinulate; echinulae  $\leq 1.5$  (-2)  $\mu\text{m}$  in length, 0.3-1 (-1.25)  $\mu\text{m}$  wide at base.

Culture morphology: Dikaryotic cultures on PDA and MMN slow-growing; colonies white.

Rocky Mountain alpine habitat and distribution: Solitary to scattered or gregarious, usually among mosses; occurring in alpine habitats in the Sawatch Range and San Juan Mountains in Colorado and the Beartooth Plateau in Montana/Wyoming. Primarily associated with dwarf willows in Colorado, and with the shrub willows *Salix planifolia* and *S. glauca* in Montana and Wyoming.

Comments: In Rocky Mountain alpine habitats, *Laccaria pumila* appears to be indistinguishable from *L. montana* on the basis of macromorphological characteristics alone; however, *L. pumila* is easily distinguished from *L. montana* by having bisterigmate basidia and slightly larger basidiospores. The two species are distributed sympatrically on the Beartooth Plateau. *Laccaria pumila* is reported by Mueller (1992) as appearing similar to *L. tortilis* and to small, striate forms of *L. laccata* var. *pallidifolia*. *Laccaria pumila* can be distinguished from *L. laccata* var. *pallidifolia* by its larger, more broadly ellipsoidal spores and bisterigmate basidia, and from *L. tortilis*, which has globose basidiospores with longer, wider echinulae. *Laccaria tortilis* has been reported from subalpine habitats in the western United States (O.K. Miller Jr., unpublished data; Mueller, 1992), but was not observed in alpine habitats during the present study. Mueller

(1992) states that previous arctic records of *L. tortilis* (Kobayasi et al., 1967; Lange, 1955) are most likely *L. pumila*, and that *L. altaica*, commonly reported from arctic-alpine habitats, is a synonym of *L. pumila*. Sivertsen (1993) considers *L. pumila* and *L. altaica* to be distinct species differing in spore shape and echinulae density. Examination of Kobayashi's (1967) basidiospore illustrations during the present study confirms Mueller's identification of this taxon as *L. pumila*. In addition to alpine habitats, *L. pumila* is found in subalpine habitats in Montana in association with *Salix* spp. and *Populus tremuloides* (Cripps, unpublished; Osmundson, unpublished). Mueller (1992) reports collections from Rocky Mountain subalpine habitats in Wyoming and Colorado, and reports *L. pumila* to be associated with *Salix* spp, *Betula* spp., and species in the Pinaceae. Examination of the reference collection TENN 42553 (Appendix A) showed micromorphological features corresponding to those of the Rocky Mountain alpine collections examined.

Material examined: U.S.A. COLORADO. San Juan County: San Juan Mountains, Black Bear Basin, 3 August 2000, *CLC 1446* (MONT), Cinnamon Pass, 1 August 2000, *CLC 1435* (MONT), 10 August 2001, *CLC 1699* (MONT), Mineral Basin, 30 July 2002, *CLC 1850* (MONT), *CLC 1851* (MONT), Stony Pass, 28 July 2002, *CLC 1819* (MONT), Emma Lake, 31 July 2002, *CLC 1872* (MONT); Ouray Co.: San Juan Mountains, Imogene Pass, 29 July 2002, *CLC 1835* (MONT), *CLC 1837* (MONT); Lake Co.: Sawatch Range, Haggeman's Pass, 14 August 1998, *CLC 1252* (MONT). MONTANA. Carbon Co.: Beartooth Plateau, near source of Quad Creek, 10 August 1999, *TWO 265* (MONT), *TWO 268* (MONT), 31 July 2000, *TWO 314* (MONT), 21

August 2000, *TWO 335* (MONT), *TWO 337* (MONT), *TWO 348* (MONT), 28 July 2001, *TWO 442* (MONT), 30 July 2001, *TWO 465* (MONT), 5 August, 2001, *TWO 520* (MONT), 27 August 2002, *TWO 716* (MONT), *TWO 717* (MONT), *TWO 718* (MONT), At Wyoming state line, Beartooth Plateau, Highline Trailhead, 7 August 1998, *CLC 1201* (MONT), 19 August 2001, *TWO 560* (MONT), *TWO 562* (MONT), 15 August 2002, *TWO 709* (MONT), 28 August 2002, *TWO 726* (MONT), *TWO 730* (MONT), Clark Fork Picnic Area (subalpine, with *Salix* shrubs and possibly conifers), 12 July 2001, *TWO 362* (MONT); 19 July 2001, *TWO 374* (MONT), McLaren mine tailings (subalpine, with conifers and *Salix* shrubs), 21 July 2001, *TWO 411* (MONT). WYOMING. Park Co.: Beartooth Plateau, north of Frozen Lake, 29 July 1997, *CLC 1104* (MONT), 21 August 1999, *CLC 1404* (MONT), 3 August 2001, *TWO 501* (MONT), 21 August 2001, *CLC 1777* (MONT), 1 September 2001, *TWO 589* (MONT), North of Gardner Headwall, 31 July 2002, *TWO 663* (MONT), Beartooth Highway, near Top of the World Store (subalpine, with *Salix* shrubs), 21 July 2001, *TWO 409* (MONT). Collections of *Laccaria pumila* examined, with collection data and EM host plants, are shown in Table 11.

Table 11. Rocky Mountain alpine *Laccaria pumila* Fayod collections examined, showing collection data and ectomycorrhizal host associates.

<u>ID</u>	<u>Location</u>	<u>Range</u>	<u>State</u>	<u>Plant Associations</u>	<u>Date</u>
CLC1435	Cinnamon Pass	San Juan	CO	<i>Salix reticulata</i>	8/1/00
CLC1699	Cinnamon Pass	San Juan	CO	<i>Salix reticulata</i>	8/10/01
CLC1850	Mineral Basin	San Juan	CO	<i>Salix arctica</i>	7/30/02
CLC1851	Mineral Basin	San Juan	CO	<i>Salix arctica</i>	7/30/02
CLC1819	Stony Pass	San Juan	CO	<i>Salix arctica</i>	7/28/02
CLC1835	Imogene Pass	San Juan	CO	<i>Salix arctica</i>	7/29/02
CLC1837	Imogene Pass	San Juan	CO	<i>Salix arctica</i>	7/29/02
CLC1446	Black Bear Basin	San Juan	CO	<i>Salix arctica</i> , <i>S. planifolia</i>	8/3/00
CLC1872	Emma Lake	San Juan	CO	<i>Salix planifolia</i>	7/31/02
CLC1252	Haggeman's Pass	Sawatch	CO	<i>Salix planifolia</i>	8/14/98
TWO268	Quad Creek	Beartooth	MT	<i>Salix planifolia</i>	8/10/99
TWO314	Quad Creek	Beartooth	MT	<i>Salix planifolia</i>	7/31/00
TWO337	Quad Creek	Beartooth	MT	<i>Salix planifolia</i>	8/21/00
TWO716	Quad Creek	Beartooth	MT	<i>Salix planifolia</i>	8/27/02
TWO442	Quad Creek	Beartooth	MT	<i>Salix glauca</i>	7/28/01
TWO465	Quad Creek	Beartooth	MT	<i>Salix glauca</i>	7/30/01
TWO520	Quad Creek	Beartooth	MT	<i>Salix glauca</i>	8/5/01
TWO717	Quad Creek	Beartooth	MT	<i>Salix glauca</i>	8/27/02
TWO348	Quad Creek	Beartooth	MT	<i>Salix shrub</i>	8/21/00
TWO265	Quad Creek	Beartooth	MT	<i>Salix shrub</i>	8/10/99
TWO718	Quad Creek	Beartooth	MT	<i>Salix shrub</i>	8/27/02
TWO335	Quad Creek	Beartooth	MT	?	8/21/00
TWO726	Highline Trailhead	Beartooth	MT/WY	<i>Salix planifolia</i>	8/28/02
TWO560	Highline Trailhead	Beartooth	MT/WY	<i>Salix glauca</i>	8/19/01
TWO562	Highline Trailhead	Beartooth	MT/WY	<i>Salix glauca</i>	8/19/01
TWO730	Highline Trailhead	Beartooth	MT/WY	<i>Salix glauca</i>	8/28/02
TWO709	Highline Trailhead	Beartooth	MT/WY	<i>Salix shrub</i>	8/15/02
CLC1201	Highline Trailhead	Beartooth	MT/WY	<i>Salix</i> spp.	8/7/98
CLC1104	Frozen Lake	Beartooth	WY	dwarf <i>Salix</i>	7/29/97
CLC1404	Frozen Lake	Beartooth	WY	<i>Salix arctica</i>	8/21/99
TWO501	Frozen Lake	Beartooth	WY	<i>Salix planifolia</i>	8/3/01
CLC1777	Frozen Lake	Beartooth	WY	<i>Salix planifolia</i>	8/21/01
TWO589	Frozen Lake	Beartooth	WY	?	9/1/01
TWO663	Gardner Headwall	Beartooth	WY	<i>Salix arctica</i>	7/31/02
TWO362	Clark Fork Picnic Area *	Beartooth	MT	<i>Salix shrub</i> , <i>Pinus contorta</i> , <i>Picea</i> sp.	7/12/01
TWO374	Clark Fork Picnic Area *	Beartooth	MT	<i>Salix shrub</i>	7/19/01
TWO411	McLaren Mine Tailings *	Beartooth	MT	<i>Salix shrub</i>	7/21/01
TWO409	Top of the World Store *	Beartooth	WY	<i>Salix shrub</i>	7/21/01

\* Denotes subalpine collecting site

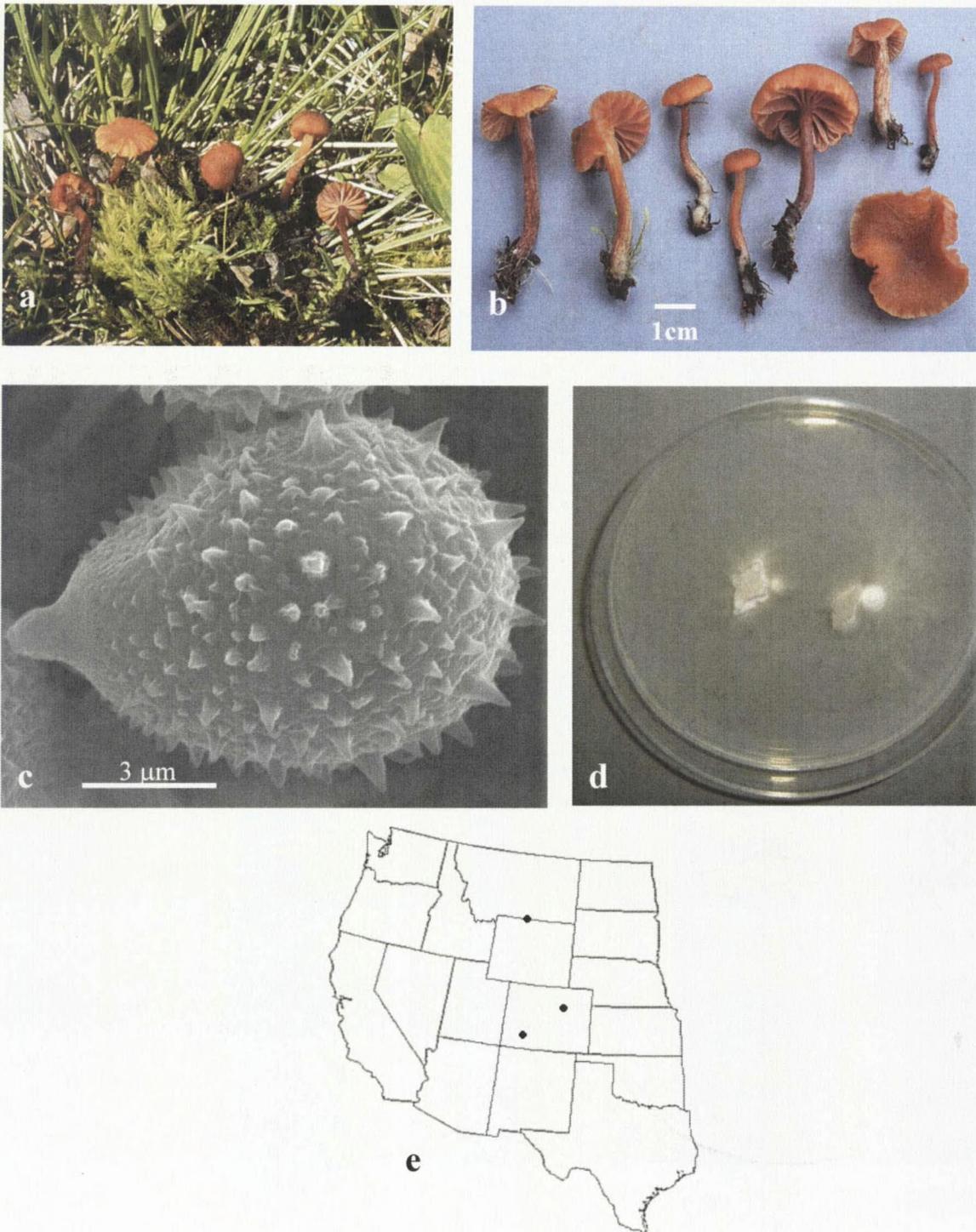


Figure 8. *Laccaria pumila* Fayod. **a.** Basidiocarps (TWO 442). **b.** Basidiocarps (CLC 1446). **c.** Scanning electron micrograph of basidiospores (TWO 501). **d.** Culture morphology on PDA medium (TWO 362). **e.** Rocky Mountain alpine distribution map.

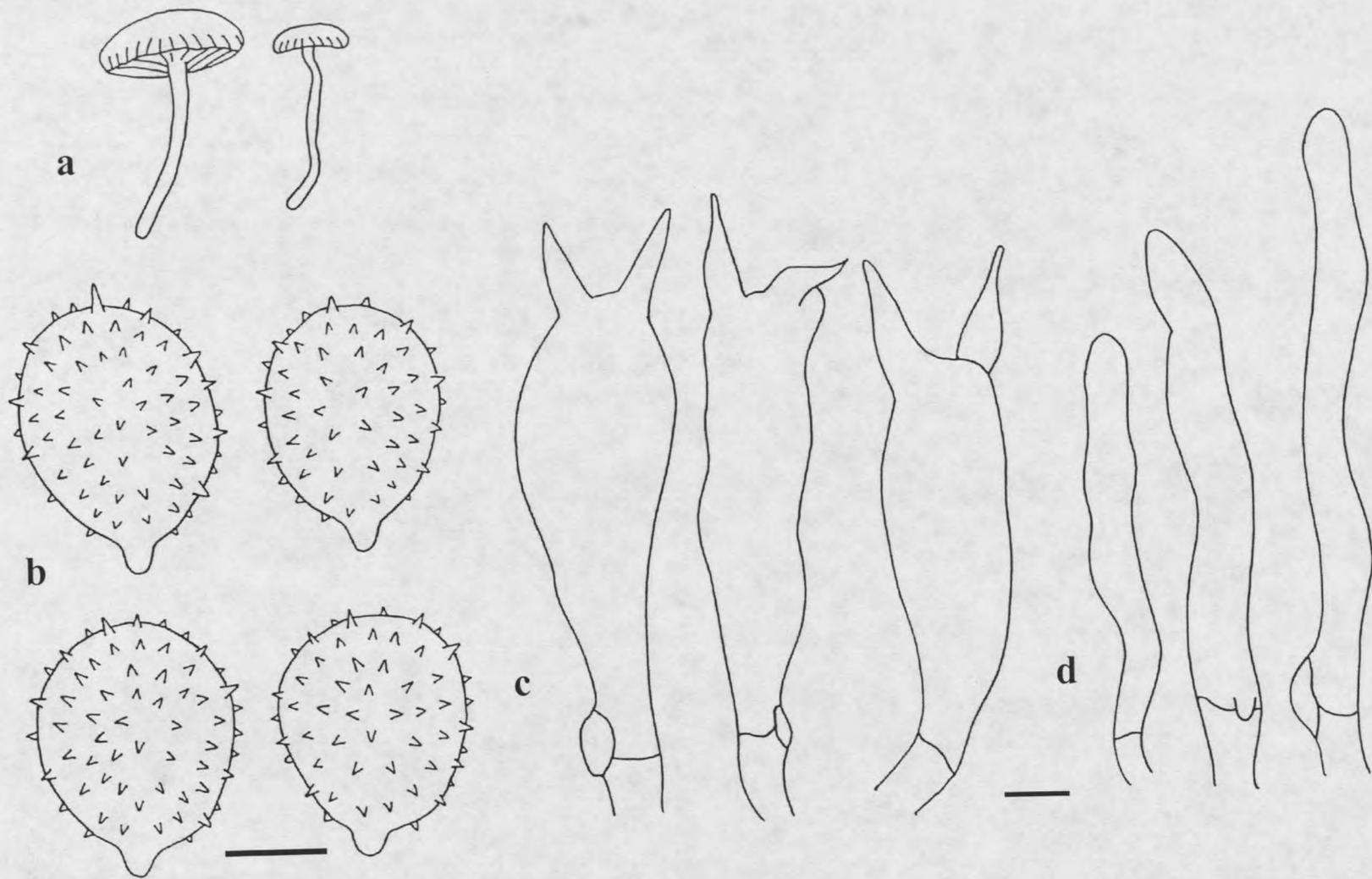


Figure 9. *Laccaria pumila* Fayod (CLC 1435). a. Basidiomata, actual size. b. Basidiospores. c. Basidia. d. Cheilocystidia. Scale bars = 10  $\mu$ m.

*Laccaria* sp.

Figures 10, 11

Macromorphology: Pileus 0.4-1 (-1.8) cm in diameter, convex to plane, glabrous, slightly striate, hygrophanous, dark orange to red-brown. Margin decurved, entire to crenate. Context thin. Lamellae adnate to subdecurrent, thick, subdistant, pink; edges entire; lamellulae present. Stipe 1-1.5 x 0.1-0.2 cm, equal, glabrous to fibrous-striate, dark orange-brown to red-brown.

Micromorphology: Pileipellis of interwoven, inamyloid, cylindrical, mostly repent hyphae with widely scattered fascicles of hyphae oriented nearly perpendicular to pileal surface. Hyphae hyaline or having intracellular pigment appearing pale orange-brown in 3% KOH. Clamp connections abundant. Stipitipellis of parallel, cylindrical, repent, inamyloid, hyaline hyphae. Clamp connections abundant. Caulocystidia absent. Lamellar trama of subparallel to interwoven, hyaline, inamyloid hyphae. Clamp connections present. Subhymenium undifferentiated. Pleurocystidia absent. Cheilocystidia absent. Basidia clavate, hyaline, 32.5-37.5 x 10-12.5  $\mu\text{m}$ , tetrasterigmate; sterigmata  $\leq 8$   $\mu\text{m}$  in length. Basidiospores (6.5-) 8.5-10.8 x (6-) 7.5-9.5  $\mu\text{m}$  (mean = 7.9-9.7 x 6.8-8.3  $\mu\text{m}$ ),  $Q = 1.04-1.39$  ( $Q^m = 1.16 - 1.18$ ), subglobose to broadly ellipsoidal, hyaline, echinulate; echinulae  $\leq 1$  (-1.7)  $\mu\text{m}$  in length, 0.3-0.6  $\mu\text{m}$  wide at base.

Culture morphology: Culture not obtained.

Rocky Mountain alpine habitat and distribution: Scattered, usually among mosses; occurring in alpine habitats in the 10-mile Range and San Juan Mountains in

Colorado. Associated with *Salix glauca*, an unidentified shrubby *Salix* sp., and in a mixed stand of *Salix planifolia* and *Betula glandulosa*. Not reported from the Beartooth Plateau.

Comments: *Laccaria* sp. is distinguished by having small, dark orange to red-brown basidiocarps, tetrasterigmate basidia, and broadly ellipsoidal, finely echinulate basidiospores. *Laccaria* sp. closely resembles *L. montana* in both macro- and micromorphology. The presence of a distinct taxon was first revealed by phylogenetic analysis of ribosomal DNA internal transcribed spacer (rDNA-ITS) sequence data (Chapter 3). Further morphological examination revealed the presence of morphological differences between the two taxa. *Laccaria* sp. is characterized by having basidiospores with shorter and narrower echinulae and a slightly more ellipsoidal shape, and often having smaller basidiocarps than *L. montana*.

Collections of *Laccaria* sp. form a distinct, well-supported clade in phylogenetic analyses of rDNA-ITS sequences, and are supported by exhibiting four synapomorphic single nucleotide polymorphisms relative to the other taxa included in the present study.

The present study included two subalpine and one alpine herbarium collections previously identified as *L. montana* and collected in Colorado. The two subalpine collections are included in the monographic study by Mueller (1992), and were used as nomenclatural reference specimens to represent *L. montana* for the present study. Examinations of these collections indicate that one subalpine collection (TENN 42877, Appendix A) corresponds to Beartooth Plateau *L. montana* collections in terms of spore shape and echinulae dimensions. The other subalpine reference collection (TENN 42880, Appendix A) and the alpine reference collection (DBG 20424, Appendix A) appear more

similar to *Laccaria* sp. than to the Beartooth Plateau *L. montana* collections. Examination of the type specimen of *L. montana* (Singer M5464, F!, holotype) by Mueller (1992) showed basidiospores having a globose to broadly ellipsoidal shape ( $Q = 1-1.11 (-1.26)$ ) and echinulae 1.5 – 1.8 mm long and uncrowded, similar to the spore characteristics exhibited in the Beartooth Plateau *L. montana* collections. The reference specimens were not included in the molecular phylogenetic portion of the present study; further morphological and molecular analyses including these and other collections of *L. montana sensu lato* will be necessary to determine whether there is a sufficient basis for describing *Laccaria* sp. as a new species.

*Laccaria bicolor* differs from *Laccaria* sp. by having larger, robust basidiocarps with distinctly fibrillose pilei and rough fibrous-striate, basally enlarged stipes. Basidiospores of *L. bicolor* are generally smaller, less ellipsoidal and more coarsely echinulate than those of *Laccaria* sp.

*Laccaria* sp. can be distinguished from small *L. laccata* var. *pallidifolia* basidiocarps by its more broadly ellipsoidal, more finely echinulate basidiospores and generally darker basidiocarp coloration, and from *L. pumila* by having tetrasterigmate basidia and smaller, more finely echinulate basidiospores.

*Laccaria* sp. is associated with shrubby willows (*Salix* spp.) in Colorado, and is not reported from the Beartooth Plateau.

Material examined: U.S.A. COLORADO. Summit Co.: 10-mile Range, Blue Lake Dam, near Breckenridge, 23 August 2001, *CLC 1625* (MONT); Pitkin/Lake Co.: San Juan Mountains, Independence Pass, 15 August 2001, *CLC 1771* (MONT); San Juan

Co.: San Juan Mountains, U.S. Basin, 8 August 2001, *CLC 1682* (MONT). Collections of *Laccaria* sp. examined, with collection data and EM hosts, are shown in Table 12.

Table 13 provides a summary of systematically informative morphological characters for distinguishing the 5 Rocky Mountain alpine *Laccaria* species described above.

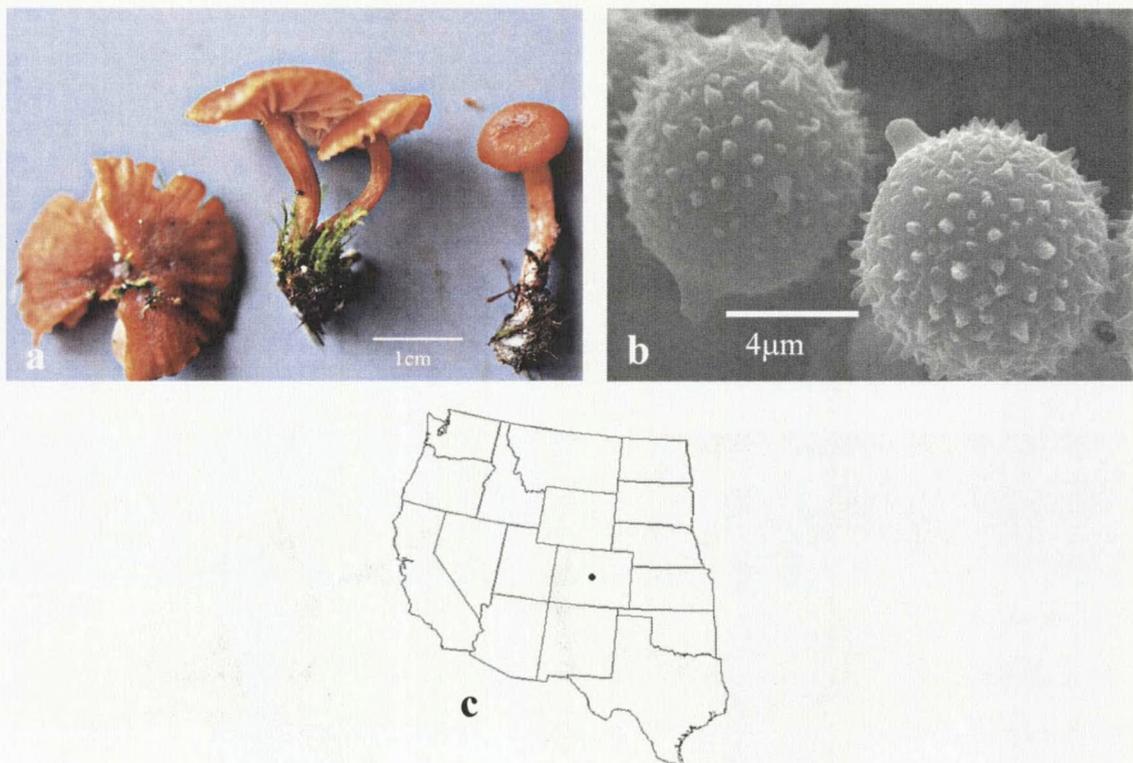


Figure 10. *Laccaria* sp. **a.** Basidiocarps (CLC 1625). **b.** Scanning electron micrograph of basidiospores (magnification?) (CLC ----). **c.** Rocky Mountain alpine distribution map.

Table 12. Rocky Mountain alpine *Laccaria* sp. collections examined, showing collection data and ectomycorrhizal host associates.

<u>ID</u>	<u>Location</u>	<u>Range</u>	<u>State</u>	<u>Plant Associations</u>	<u>Date</u>
CLC1625	Blue Lake Dam	Tenmile	CO	<i>Salix planifolia</i> , <i>Betula glandulosa</i>	8/23/01
CLC1771	Independence Pass	San Juan	CO	<i>Salix</i> shrub	8/15/01
CLC1682	U.S. Basin	San Juan	CO	<i>Salix glauca</i>	8/8/01

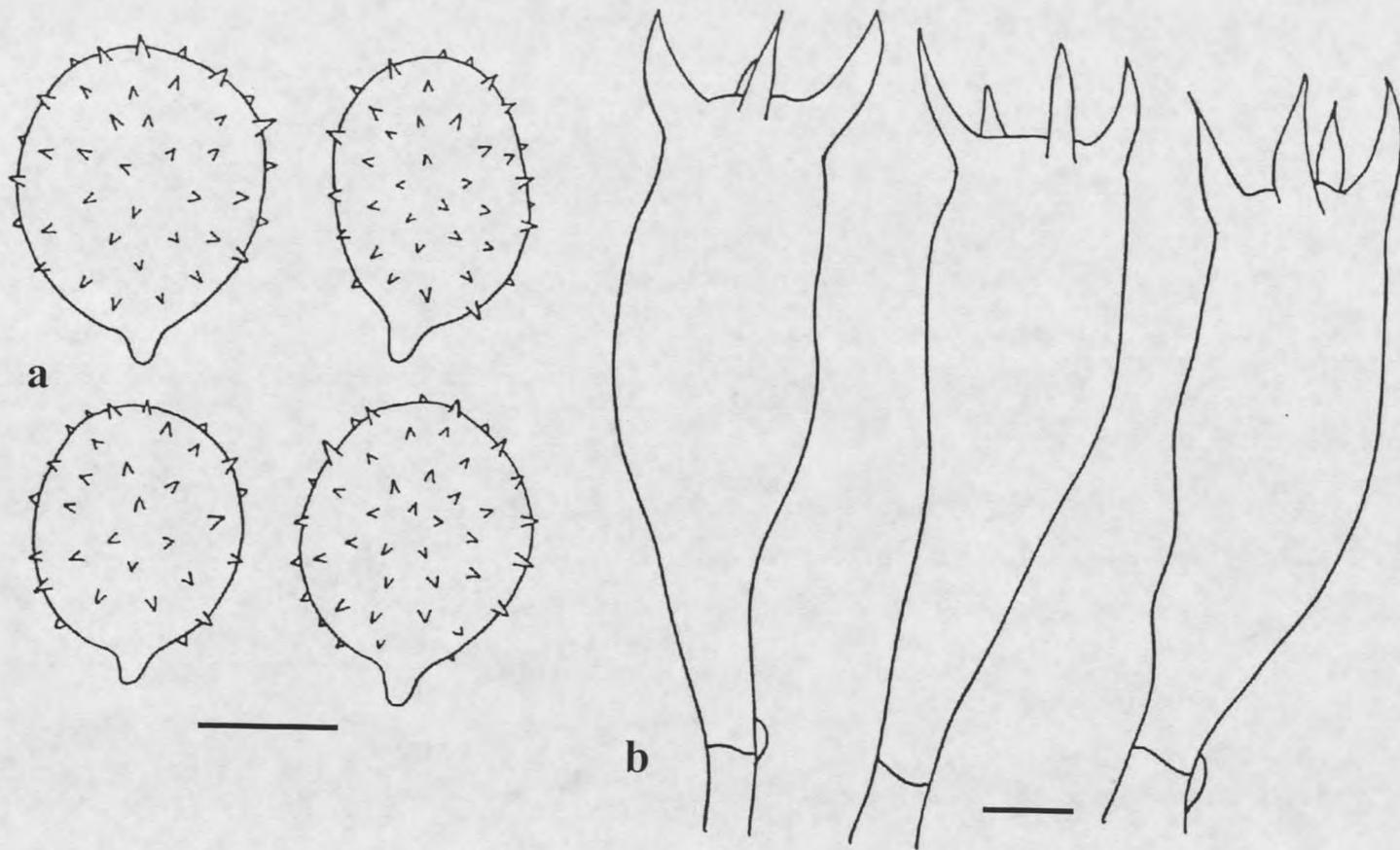


Figure 11. *Laccaria* sp. (CLC 1625). a. Basidiospores. b. Basidia. Scale bars = 10  $\mu$ m.

Table 13. Comparison of systematically informative characters in Rocky Mountain alpine *Laccaria* species.

Character Taxon	Pileus	Stipe	Basal tomentum	Spore Size	Spore shape (Q)	Spore Echinulae	spore #	Culture color
<i>L. bicolor</i>	1-7 cm, convex, pale pink orange to dark orange; minutely fibrillose-scaly, nonstriate	2-5 x 0.3-0.8 cm, concolorous to paler than pileus; basally enlarged to clavate; robust, striate, rough-fibrous	violet, fading to white	5.4-8 (-9.5) x (4.1-) 5.5-7.7 (-8.5) $\mu\text{m}$ (x=6.2-8 x 5.5-7 $\mu\text{m}$ )	Q=1.06-1.15, subglobose to broadly elliptical	$\leq 1.5$ (-2.5) $\mu\text{m}$ long; (0.2-) 0.4-1.2 $\mu\text{m}$ wide at base	4	violet
<i>L. laccata</i> var. <i>pallidifolia</i>	0.5-3 cm, convex to omphaloid, pale orange, glabrous, indistinctly translucent-striate to nonstriate	1.5-5 x 0.2-0.5 cm, pale orange, +/- equal, glabrous to minutely fibrillose	white	(5-) 6.2-10 (-10.9) x (4.1-) 5.6-9.8 (-10.8) $\mu\text{m}$ (x=6.2-8.8 x 5.5-8.8 $\mu\text{m}$ )	Q=1.107 (-1.18); subglobose to broadly elliptical	$\leq 1.5$ (-2) $\mu\text{m}$ long, 0.3-1.1 $\mu\text{m}$ wide at base	4	N/A
<i>L. montana</i>	0.5-3.5 cm, convex to plane or occasionally uplifted; orange-brown to red-brown, usually translucent-striate	1-4.7 x 0.1-0.4 cm, concolorous with pileus, equal, glabrous to minutely fibrillose	white, usually sparse	(8-) 9-11 (-12) x (7-) 7.9-9.5 (-10.5) $\mu\text{m}$ (x=9.1-10.1 x 8.7-9.1 $\mu\text{m}$ )	Q=1.04-1.16; subglobose to broadly elliptical	$\leq 1.7$ (-2.5) $\mu\text{m}$ long; 0.3-1.1 (-1.3) $\mu\text{m}$ wide at base	4	N/A
<i>L. pumila</i>	0.5-3.5 cm, convex to plane or nearly omphaloid; pale orange-brown to red-brown; usually translucent striate	0.6-5 x 0.1-0.4 cm, concolorous with pileus, +/- equal, glabrous to minutely fibrillose	white, usually sparse	(8-) 9-13.5 x (6.8-) 7.5-10.5 (-12) $\mu\text{m}$ (x=9.5-11.4 x 8.1-9.5 (-10.5) $\mu\text{m}$ )	Q=1.08-1.18, subglobose to broadly elliptical	$\leq 1.5$ (-2) $\mu\text{m}$ long; 0.3-1 (-1.25) $\mu\text{m}$ wide at base	2	white
<i>Laccaria</i> sp.	0.4-1.8 cm, convex to plane, dark orange to red-brown, glabrous, slightly striate	1-1.5 x 0.1-0.2 cm, dark orange-brown to red brown, equal, glabrous to fibrous-striate	N/A	(6.5-) 8.5-10.8 x (6-) 7.5-9.5 $\mu\text{m}$ (x=7.9-9.7 x 6.8-8.3 $\mu\text{m}$ )	Q=1.16-1.18; broadly elliptical	$\leq 1$ (-1.7) $\mu\text{m}$ long, 0.3-0.6 $\mu\text{m}$ wide at base	4	N/A

### Discussion

The present study represents the first report on North American alpine *Laccaria* species based on an intensive study of Rocky Mountain collections. Objectives of this study were to identify morphological species, determine mycorrhizal host plant associations, and examine geographical distributions for *Laccaria* collections from alpine field sites in the southern and central Rocky Mountains. Four species and a potentially new taxon are reported from the Rocky Mountain alpine zone: *L. bicolor* (Maire) Orton, *L. laccata* var. *pallidifolia* (Peck) Peck, *L. montana* Singer, *L. pumila* Fayod, and *Laccaria* sp.

The five taxa described in this study can be distinguished on the basis of macro- and micromorphological characters. *Laccaria pumila*, *L. montana* and *Laccaria* sp. are distinguished by having small basidiocarps, often with translucent-striate pilei, and having relatively large, subglobose to broadly ellipsoidal basidiospores. *Laccaria pumila* is distinguished from *L. montana* and *Laccaria* sp. by having bisterigmate basidia and larger basidiospores. *Laccaria* sp. is distinguished from *L. montana* by having slightly more ellipsoidal, more finely echinulate basidiospores. Although two of the *Laccaria* sp. collections examined had small (0.5-1.5 cm), red-brown basidiocarps, there appears to be overlap in terms of macromorphology between *Laccaria* sp. and *L. montana*, so macromorphological traits are probably not consistently reliable in distinguishing the two species. Examination of three herbarium collections previously identified as *L. montana* from Colorado revealed that two of these collections (one alpine and one subalpine) have spore morphologies similar to *Laccaria* sp., whereas the third (a subalpine) collection

appears more similar to Beartooth Plateau *L. montana* collections and to the type specimen of *L. montana* as described in the monographic study by Mueller (1992). Morphological examination and analysis of DNA sequence data for additional collections of *L. montana* will be useful in establishing species limits for *L. montana* and *Laccaria* sp., and thereby determining whether *Laccaria* sp. should be described as a distinct species. This taxonomic information will hopefully in turn lead to a better understanding of the geographic distributions and ecology of these taxa.

*Laccaria bicolor* and *L. laccata* var. *pallidifolia* in the Rocky Mountain alpine zone are characterized by producing larger basidiocarps (longer stipes and generally broader pilei) than the other species described in the present study. *Laccaria bicolor* can be distinguished from *L. laccata* var. *pallidifolia* in Rocky Mountain alpine habitats by having more robust basidiocarps with distinctly fibrillose pilei and basally-enlarged to clavate, rough fibrous-striate stipes, and producing violet colonies when cultured on potato dextrose agar (PDA).

Species described on the basis of morphological characters are supported by phylogenetic analysis of ribosomal DNA internal transcribed spacer sequences (Chapter 3). Although macromorphological characters are generally considered to be variable and unreliable for identification in *Laccaria*, molecular analysis supports the distinction between *L. bicolor* and *L. laccata* var. *pallidifolia* on the basis of basidiocarp stature and texture. Violet pigmentation of the basal tomentum in *L. bicolor*, which can be an extremely useful field characteristic, is generally lacking in Rocky Mountain alpine collections and was encountered in only one of the alpine collections examined in the

present study. The two species can sometimes be distinguished by differences in spore size, with *L. bicolor* generally having smaller basidiospores. However, overlaps in spore shape and size were encountered between collections of the two taxa used in this study, so that identification required macromorphological characters in addition to spore morphology. Species descriptions of *L. laccata* var. *pallidifolia* and *L. bicolor* in Mueller's (1992) monograph of North American species are consistent with the observation of overlaps in spore size and length-width ratio. In the case of the Rocky Mountain alpine collections used in the present study, the macromorphological appearance of basidiocarps (the more robust stature, scaly pilei and densely fibrillose stipes of *L. bicolor* in particular) was consistently reliable for distinguishing species. When used, culture morphologies were consistent with those reported by Mueller (1992) and can aid in distinguishing the two species. It was observed in subalpine *L. bicolor* collections lacking a violet basal tomentum that storing fresh specimens in a covered plastic container under refrigeration for several days resulted in new growth of violet mycelium at the base of the stipe (Osmundson, unpublished). This method may represent a simpler alternative to obtaining tissue cultures for observing mycelial coloration.

It is possible that, due to environmental differences, the morphological differences such as basidiocarp size used to delimit species in the present study do not hold outside of the Rocky Mountain region (E. Vellinga, personal communication). Examining arctic-alpine collections worldwide, as well as corroborating morphological traits for worldwide collections with DNA sequence data, is necessary to evaluate this possibility and represents a possible avenue for further research.

The two taxa characterized by more robust basidiocarps (*L. laccata* var. *pallidifolia* and *L. bicolor*) and *Laccaria* sp., are reported only from the Colorado field sites. *Laccaria pumila* occurs in both Colorado and Montana/Wyoming. *Laccaria montana* is regularly collected on the Beartooth Plateau, but was only collected once in Colorado during the course of this study. Both *L. laccata* var. *pallidifolia* and *L. bicolor* are reported from subalpine forests in Montana and Wyoming in association with conifers, aspen and possibly Betulaceae (in the case of *L. laccata*) as in Colorado, suggesting that differences in environmental or soil conditions may result in the absence of these species in Montana/Wyoming alpine habitats. Additionally, the data from this study suggest that ectomycorrhizal host shifts have occurred from subalpine to alpine populations of these species, so historical factors may result in the observed distributional patterns.

*Laccaria bicolor* is associated with the EM host plants *Salix planifolia*, *Salix glauca* (both shrubby species), *S. arctica*, and *S. reticulata* (both dwarf species). *Laccaria laccata* var. *pallidifolia* is associated with *Dryas octopetala*, *Betula glandulosa* and *Salix reticulata*, and was collected only once near a shrubby *Salix* species (*S. glauca*). *Laccaria pumila* is associated with both dwarf and shrubby *Salix* species. *Laccaria montana* is associated predominantly with the shrub willows *S. planifolia* and *S. glauca*. *Laccaria* sp. is associated with *Salix* shrubs and was encountered in a mixed stand of *Salix* shrubs and *Betula glandulosa*. The three smaller-statured species are almost always encountered in moss-covered areas in proximity to the ectomycorrhizal host plant. The occurrence of sympatric populations of *L. pumila* and *L. montana* on the

Beartooth Plateau allows the opportunity to observe differences in small-scale distributional and ecological patterns between the two species. Although *L. pumila* is associated primarily with dwarf *Salix* species and *L. montana* primarily with shrubby *Salix* species, the observation that *L. pumila* is occasionally found with *Salix* shrubs suggests that the ecological functions of the two species are not defined by host specificity patterns alone. It is possible that biological differences between bisterigmate and tetrasterigmate species may contribute to ecological niche occupation. Tommerup et al. (1990) suggest that secondary homothallism (i.e., production of single basidiospores containing both mating type nuclei required for formation of dikaryotic mycelium) may represent an advantage in primary successional or disturbed sites. The stability of sterigmata number within *Laccaria* basidiocarps (Lahaie, 1981; Mueller, 1992), along with the presence of sympatric populations of *L. pumila* and *L. montana* on the Beartooth Plateau, suggests that sterigmata number in these species is under genetic rather than environmental control. The hypothesis of genetic isolation between *L. pumila* and *L. montana* suggested by Mueller (1992) was evaluated in the present study (Chapter 3) using rDNA-ITS sequence data. These data indicate that no recombination in the ITS genetic region occurs between the two morphological species; however, the analysis included only 2 isolates from each morphological species, therefore this result should be considered preliminary.

*Laccaria bicolor*, reported in the present study in association with *Salix* spp., is reported in association with conifer species in subalpine habitats and therefore suggests that a mycorrhizal host shift has occurred in arctic-alpine populations of this taxon. A

similar host shift from gymnosperm (*Pinus*) to angiosperm (*Quercus costaricensis*) hosts has been documented for the closely related species *L. trichodermophora* (Mueller & Strack, 1992). Results of an analysis of rDNA-ITS sequence data (Chapter 3) incorporating alpine (Colorado) and subalpine (Montana) *L. bicolor* collections indicate that a significant amount of genetic divergence has occurred between these populations; further analyses including additional isolates over a larger geographic area would be useful in better clarifying the relationship between alpine and subalpine populations.

The presence of ectomycorrhiza-forming plants in the genera *Salix*, *Betula*, and *Dryas* appears necessary for the occurrence of *Laccaria* spp. in Rocky Mountain arctic-alpine habitats. However, the observation that *L. bicolor*, *L. laccata* var. *pallidifolia* and *Laccaria* sp. (species reported from alpine habitats in Colorado but lacking on the Beartooth Plateau) are associated with EM host plants that occur in both regions suggests that host plant distribution alone does not account for observed distribution patterns of *Laccaria* species. It is therefore probable that environmental and/or historical factors as well as EM host distributions influence the distributions of these fungi. One factor that may help to account for the observed differences in species composition is variability in soil types. The Beartooth Plateau is characterized by a relatively homogeneous soil type derived from Precambrian crystalline rocks that form the core of the Beartooth uplift (Johnson & Billings, 1962), a factor that may account for the lower diversity of macromycete species observed in comparison to the southern Rocky Mountains (C. Cripps, personal communication). Differences in host abundance may also help to account for the observed distributional patterns. *Salix glauca*, for example, is more

common in Colorado alpine sites than on the Beartooth Plateau (C. Cripps, personal communication), and may influence the relative abundance of associated species such as *L. bicolor* in Colorado. Successional patterns, host specificities and species-specific ecological roles are poorly understood for ectomycorrhizal basidiomycetes, particularly in arctic-alpine habitats, and these subjects offer important avenues for further research.

All species found in the present study are reported in the literature to occur in other arctic-alpine localities with the exception of the newly described taxon *Laccaria* sp., which may be found to be more widely distributed after additional collections previously identified as *Laccaria montana* have been examined. This result suggests a lack of endemism in Rocky Mountain alpine *Laccaria*. This lack is in contrast with the occurrence of endemic vascular plant species in the Rocky Mountain alpine zone, but concordant with the observation that none of the ectomycorrhizal host plants associated with *Laccaria* spp. in this region represent Rocky Mountain endemic species.

Preliminary world distribution maps for the *Laccaria* species reported from arctic-alpine habitats (based on Table 7) are provided in Figure 12, with Rocky Mountain distributions from the present study included. Collections identified under different names and representing either likely synonyms or misapplied names appear on these maps under the most likely correct identification, determined as previously described in the section of this chapter pertaining to literature records of *Laccaria* in arctic-alpine habitats.

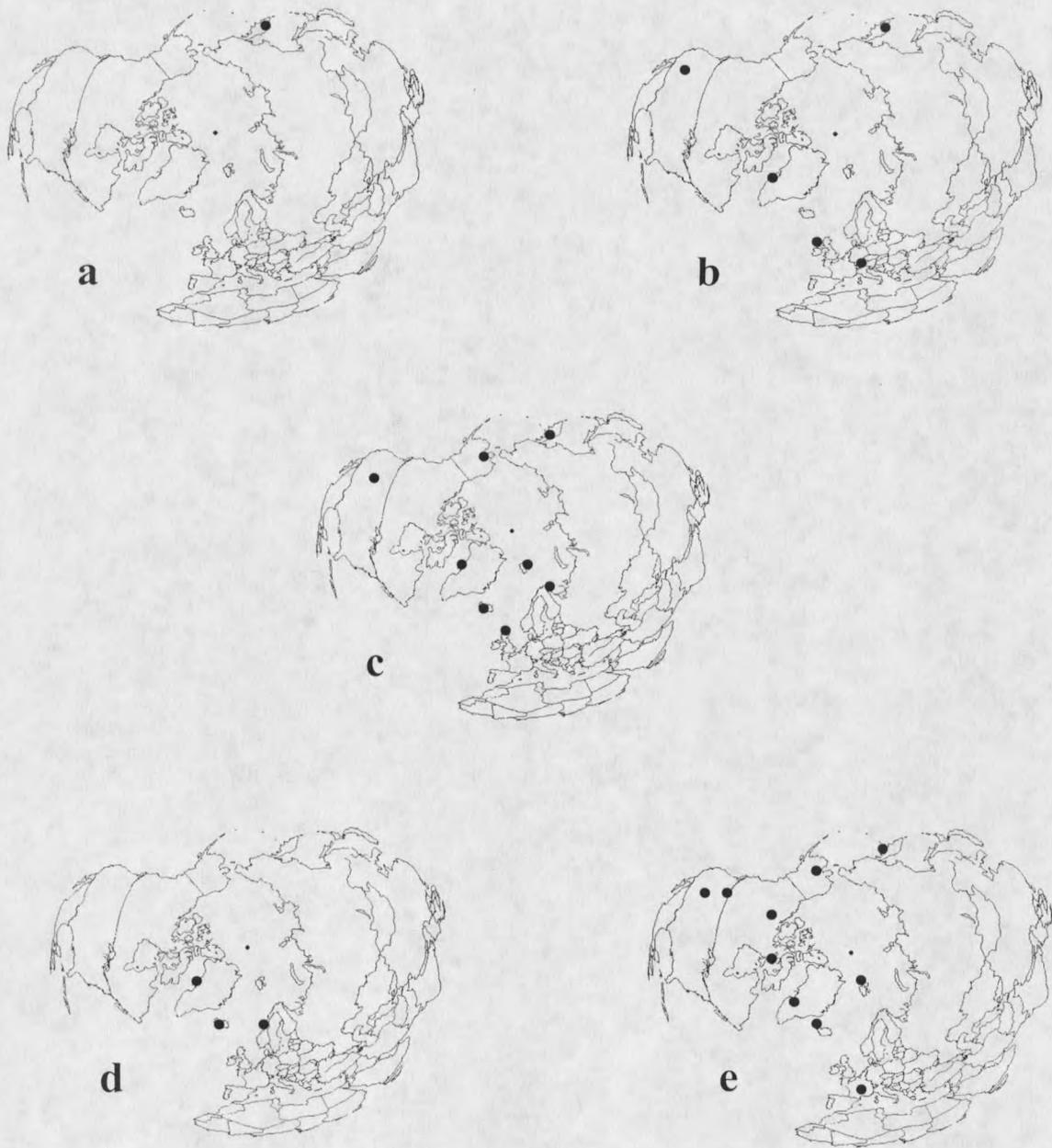


Figure 12. Distribution maps for arctic-alpine *Laccaria* species. Mapped records refer to one or more collections. Probable synonyms and misapplied names are taken into account as described in the section on *Laccaria* in arctic-alpine habitats. **A.** *L. avachensis* Kalamees & Vaasma, **B.** *L. bicolor* (Maire) Orton, **C.** *L. laccata* (Scop:Fr) Cke., **D.** *L. maritima* (Teod.) Sing. ex Huhtinen, **E.** *L. montana* Singer, **F.** *L. proxima* (Boud.) Pat., **G.** *L. pumila* Fayod, **H.** *Laccaria* sp. (current study).

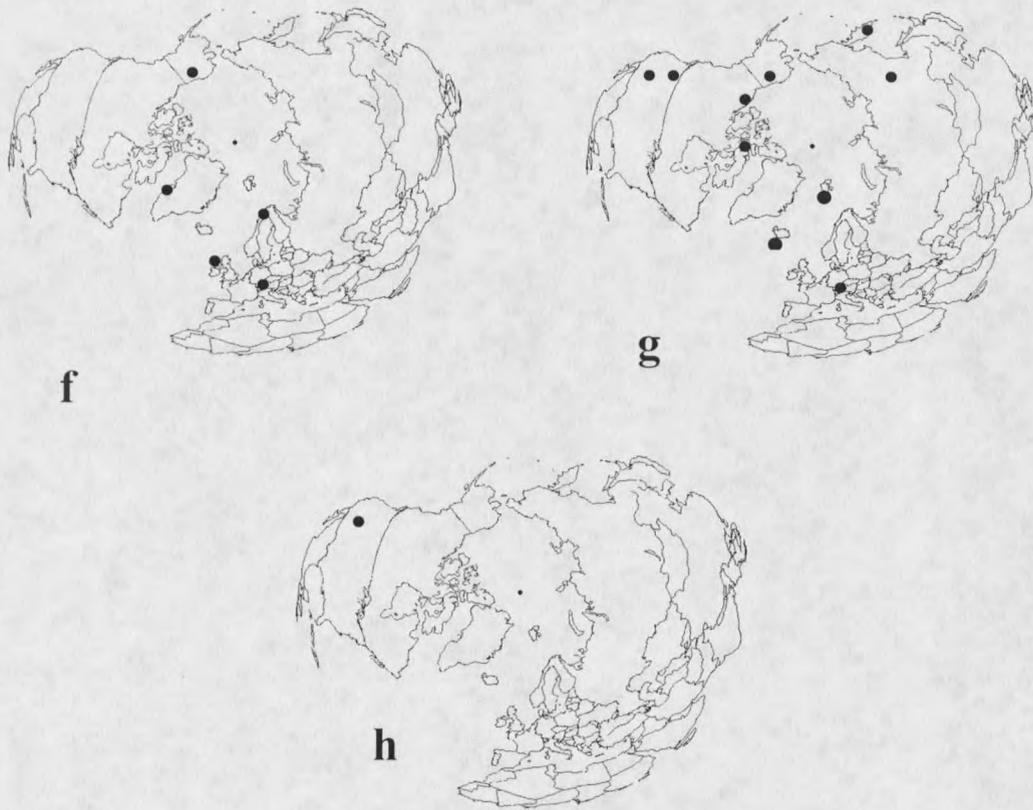


Figure 12 – Continued.

The ability of many *Laccaria* species to be grown and manipulated under laboratory conditions has made *Laccaria* an important genus for applied and experimental work with ectomycorrhizal fungi. Better understanding species limits, geographic distributions, host associations, and ecological roles of alpine species can provide a foundation for the development of plant-fungal systems for reclamation applications in high altitude habitats. In addition, the wide distribution of the genus in arctic-alpine habitats may facilitate the use of *Laccaria* as a model genus for evolutionary studies in arctic-alpine mycorrhizal macromycetes; a robust taxonomic classification of

arctic-alpine taxa is a necessary foundation for such studies. Avenues for further research toward such a classification include examination of arctic-alpine *Laccaria* collections worldwide, clarification of the relationship between the taxa *L. pumila* and *L. altaica* (Sivertsen, 1993), and conducting mating studies between arctic-alpine isolates from various geographic regions and between alpine and subalpine populations within species.

## CHAPTER 3

## MOLECULAR SYSTEMATICS OF ROCKY MOUNTAIN

ALPINE *LACCARIA*Introduction

Traditionally, species delimitations and inferences of evolutionary relatedness in the Agaricales have been based largely upon morphological characteristics of the basidiomata. In the late 1980s, the development of the Polymerase Chain Reaction (PCR), a technique allowing exponential amplification of DNA from small amounts of source material (Mullis et al., 1986; Mullis & Faloona, 1987; Saiki et al., 1988), made it possible to amplify genes of interest for use in molecular phylogenetic studies. The present study incorporates an analysis of ribosomal DNA internal transcribed spacer sequence data as a means of evaluating the morphological species concepts for Rocky Mountain alpine *Laccaria* species presented in Chapter 2. The following introduction is comprised of a literature review concerning species concepts in fungi, use of ribosomal DNA sequence data for phylogenetic inference in fungi, and systematic studies and molecular characterization of *Laccaria* species.

Classification of fungal species is generally based on one or more of the three major species concepts: morphological species, biological species, and phylogenetic species. The morphological species concept delimits species on the basis of differences in macroscopic and microscopic morphological characteristics, the biological species concept delimits species on the basis of mating compatibility of single-spore isolates, and

the phylogenetic species concept delimits species on the basis of evolutionary, often molecular genetic, data. An additional species concept, the ecological species concept (delimitation of species based on differences in habitat or host preference), merits mention here. Although the use of this concept is most often associated with plant pathogenic fungi, it has been used to some extent (usually in combination with morphological characters and/or mating data) in agaric genera such as *Pleurotus* (*P. populinus*/*P. pulmonarius*; Vilgalys et al., 1993) and *Armillaria* (Banik et al., 1996; Volk et al., 1996).

Early fungal taxonomic work beginning with Elias Fries (1794-1878) classified the gilled fungi predominantly on spore print color (the color of the spores deposited in mass, prepared by removing the mushroom cap (pileus) and placing gill-side down on a sheet of paper for several hours) and basidiocarp stature. Basidiocarp stature comprises a number of features, including pileus diameter and shape, stipe length, width, shape and attachment, mode of attachment of the lamellae (gills), shape of the pileal margin, and the presence/absence of an annulus (ring around the stipe) and/or volva (cuplike tissue around the base of the stipe). Additional macromorphological characteristics include basidiocarp color, odor, taste, lamellar thickness and spacing, and pileus and stipe texture (e.g., scales, fibrils, etc.). Biochemical characters and ecological observations such as trophic status (e.g., substrate preference, nematophagy, mycorrhizal or saprotrophic mode) have been used, often in combination with morphological and/or phylogenetic data, to delimit species or species groups (e.g., Besl & Bresinsky, 1997; Thorn et al., 2000).

Microscopic characteristics of the basidiomata are also widely used as important morphological characters for delimiting species. Microscopic characters of particular systematic importance include spore size and shape, spore ornamentation, spore wall thickness, chemical reactions of spores, tissues and individual cell types (e.g., basidia), morphology of the outermost cell layers of the pileus and stipe (pileipellis and stipitipellis, respectively), morphology of the basidia (size, shape and number of sterigmata, i.e., number of spores borne on each basidium), presence/absence and morphology (size, shape) of sterile cells on the edge or face of the lamellae (cheilocystidia and pleurocystidia, respectively), and cellular organization of the lamellar trama.

The biological species concept, as applied to the Agaricales, assesses the potential conspecificity of isolates on the basis of the ability of homokaryotic mycelia derived from single spore isolates to mate, forming dikaryotic mycelia, in culture. The application of this species concept has been used primarily as a means of assessing and refining species delimitations originally based on morphological characters, and as a means of identifying cryptic species within broader morphospecies. Examples of application of the biological species concept to the Agaricales include studies in the saprobic *Collybia dryophila* complex (Vilgalys, 1991), *Melanotus* (Sime & Petersen, 1999), *Pleurotus* (Petersen, 1995a, b; Vilgalys et al., 1993), *Lentinula* (Shimomura et al., 1992) and *Armillaria* (Banik & Burdsall, 1998; Banik et al., 1996, Volk et al., 1996). Because of the inability of the spores of most ectomycorrhizal (EM) basidiomycetes to germinate in culture, application of the biological species concept to EM species is

comparatively rare. Because the spores of many *Laccaria* species do germinate in culture, mating studies in this genus (Fries & Mueller, 1984; Mueller, 1991a) serve as models for determining biological species in EM fungi.

The phylogenetic species concept delimits species on the basis of inferred differences in evolutionary history. Groups (clades) at a given taxonomic level are defined on the basis of monophyly, i.e., containing a hypothetical ancestor and all of its descendants. Phylogenetic analyses can be performed using morphological and/or molecular data, usually employing computer algorithms to analyze morphological data matrices and DNA, RNA, or protein sequence data. These analyses provide a measure of evolutionary relatedness that can be used to infer species limits.

Molecular phylogenetic data have been used in fungi to examine relationships between fungal phyla (Bruns et al., 1992), to infer divergence times for major fungal groups and produce time estimates for the origins of major morphological adaptations (Berbee & Taylor, 1993, 1995), examine higher-level relationships in the Agaricales (Binder & Hibbett, 2002; Hibbett et al., 2000; Moncalvo et al., 2000b, 2002), examine the relationships of sequestrate or hypogeous taxa (Gasteromycetes) to species traditionally belonging to the Agaricales (Baura et al., 1992; Hibbett et al., 1997; Peintner et al., 2001), and in numerous studies of infrageneric relationships in the Agaricales.

Present species limits in *Laccaria* are based primarily on morphological species concepts, with the limits of some species supported or revised on the basis of mating and/or restriction fragment length polymorphism (RFLP) data. While nuclear and mitochondrial rDNA sequences for selected *Laccaria* species have been used in higher-

order phylogenetic studies (Binder & Hibbett, 2002; Hibbett et al., 2000; Moncalvo et al., 2000b, 2002), phylogenetic analyses using DNA sequence data to examine infrageneric relationships, infer phylogenetic species and evaluate morphological characters used in delimiting species are lacking for *Laccaria*.

Mueller (1992) conducted a cladistic analysis of *Laccaria* species based on a morphological data matrix to examine relationships between morphological species; the results of this analysis led to the proposal of Metasections *Amethystina* and *Laccaria* as major subdivisions within the genus *Laccaria*, and produced a hypothesis for evolutionary relationships between species within the genus. Thus far, the use of molecular data in *Laccaria* infrageneric systematics has been limited to restriction fragment length polymorphisms (RFLPs) of ribosomal and mitochondrial DNA as data to support species delimitations based on mating compatibility and morphological data.

Additionally, molecular data have been used at the population level to identify *Laccaria* strains (Albee et al., 1996), and in field studies to examine the persistence of individual genotypes on host roots, the effect of genotype on fruiting phenology and the spatial distribution of genets (de la Bastide et al., 1994; Gherbi et al., 1999; Selosse et al., 2001).

#### Ribosomal DNA: Description and Use in Fungal Phylogenetics

The present study uses a portion of the nuclear ribosomal DNA (nrDNA) repeat unit in order to examine molecular sequence-level characters in Rocky Mountain alpine *Laccaria* collections. The nrDNA unit is a tandemly repeated cluster comprising three

genes encoding the 18S (small subunit, or SSU), 28S (large subunit, or LSU) and 5.8S ribosomal DNA subunits separated by two internal transcribed spacer (ITS) regions, designated ITS1 and ITS2 (Figure 13). Each repeat is separated by an intergenic spacer (IGS) and external transcribed spacer (ETS) region.

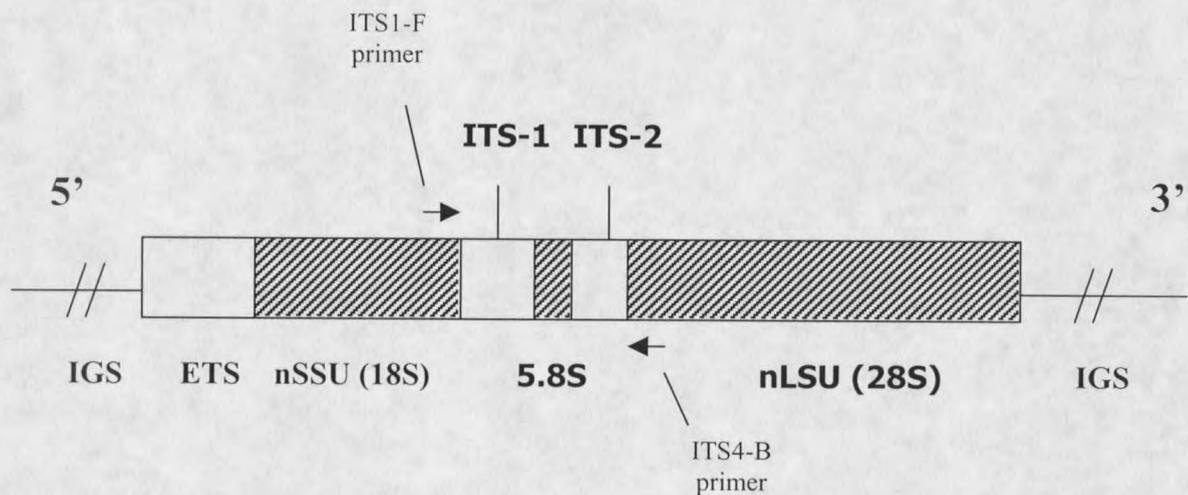


Figure 13. Schematic diagram of the structure of the eukaryotic nuclear ribosomal repeat unit showing gene arrangement and location of PCR primer binding sites. Adapted from Hillis & Dixon (1991) and White et al. (1990). IGS = Intergenic Spacer, ETS = External Transcribed Spacer (=NTS or Nontranscribed Spacer), nSSU = nuclear Small Subunit, ITS = Internal Transcribed Spacer, nLSU = nuclear Large Subunit.

The nrDNA repeat unit is widely used for fungal molecular phylogenetic studies (Hibbett, 1992). Several important characteristics make this genetic region particularly useful for phylogenetic studies. The ribosomal gene cluster is present in multiple copies, facilitating PCR amplification. Ribosomal DNA occurs in all organisms and is therefore useful for phylogenetic studies at a variety of taxonomic levels. This high conservation

of some regions has allowed the design of universal PCR primers useful for a variety of species (White et al., 1990).

The nrDNA cluster has been shown to undergo concerted evolution, reducing the occurrence of multiple sequences that could be problematic in studies using multigene families that include pseudogenes or sequence variation between repeated units (Hillis & Dixon, 1991). In addition, the ribosomal repeat unit consists of both highly conserved and highly variable regions, enabling phylogenetic studies at a variety of taxonomic levels.

The highly conserved coding regions (18S, 28S, 5.8S) are phylogenetically useful at higher taxonomic levels including the family, phylum, and kingdom levels. The IGS region is highly variable, and therefore useful at the population or occasionally species levels in fungi (Bruns et al., 1991).

#### Use of nrDNA and ITS Sequence Data in Fungal Phylogenetics

The nuclear ribosomal DNA repeat has been widely used in phylogenetic studies of fungi at all taxonomic levels. The utility of rDNA for fungal phylogenetics is reviewed in Hibbett (1992) and Bruns et al. (1991). The present review does not seek to provide exhaustive coverage of the vast amount of literature on rDNA-based fungal systematics, but rather to highlight important studies and examples (especially in the Agaricales) illustrating the utility of rDNA for phylogenetic studies and providing the methodological basis for the present research.

As previously mentioned, the rDNA repeat region has been used in phylogenetic studies at all taxonomic levels. Bruns et al. (1992) and Berbee and Taylor (1993, 1995) used nrDNA 18S sequence data to examine phylogenetic relationships among the true fungi. The presence of three major lineages within the Basidiomycota was examined using 18S rDNA (Swann & Taylor, 1993). Nuclear and mitochondrial large and small subunit genes have been used to examine relationships among the Agaricales (gilled mushrooms) and relatives (Binder & Hibbett, 2002; Hibbett et al., 2000; Moncalvo et al., 2000b, 2002), and nrDNA large subunit sequences were used in inferring coevolutionary patterns in basidiolichen-forming *Omphalina* species (Lutzoni & Vilgalys, 1995). The evolution of sequestrate growth forms has been examined using mitochondrial LSU sequences in *Suillus* and relatives (Bruns et al. 1989), ribosomal SSU and ITS sequences in the Lycoperdales (Krueger et al., 2001) and ITS sequences for *Cortinarius* and relatives (Peintner et al., 2001). Ribosomal IGS sequences have been used at the species and population levels in a number of genera, for example *Armillaria* (Coetzee et al., 2000, 2001).

The ribosomal ITS region, used in the present study, has been used in numerous phylogenetic studies of species relationships within genera in the Agaricales. Examples of genera examined include *Dermocybe* and *Cortinarius* (Høiland & Holst-Jensen, 2000; Liu et al., 1997; Seidl, 2000), *Lentinula* (Hibbett et al., 1995), *Hebeloma* (Aanen et al., 2000), *Lepiota* s.l. (Johnson, 1999), *Agaricus* (Mitchell & Bresinsky, 1999), the *Galerina marginata* species complex (Gulden et al., 2001), *Chroogomphus* and *Gomphidius* (Miller & Aime, 2001) and *Armillaria* (Piercey-Normore et al., 1998). In most cases,

ITS sequences appear to yield well-resolved infrageneric phylogenies; however, in the genus *Inocybe*, ITS sequences do not appear to provide sufficient resolution of branches and better-supported results are obtained using other loci such as the RNA polymerase II large subunit gene, RPB1 (Matheny et al., 2002).

Molecular rDNA-ITS phylogenies have been correlated with morphological or cultural characters for a number of genera including *Collybia* s.str. (Hughes et al., 2001); similar studies have been carried out for *Lyophyllum* section *Difformia* (Moncalvo et al., 1993) and the Pleurotaceae (Thorn et al., 2000) using nLSU sequences. In terms of biogeography, ITS sequences have been used in establishing the presence of geographically-correlated clades and inferring historical biogeographical patterns in Shiitake (*Lentinula edodes* and relatives) (Hibbett, 2001; Hibbett et al., 1995), examining biogeographical relationships between eastern North American and eastern Asian macrofungi (Mueller et al., 2001), examining patterns of dispersal and disjunction in *Flammulina* (Methven et al., 2000) and *Pleurotopsis* (Hughes et al., 1998), and in tracing the causative species and probable origin of *Armillaria* root rots in South Africa (Coetzee et al., 2001). Henkel et al. (2000) used ITS sequence data to compare DNA from mycorrhizal root tips and basidiocarps of pleurotoid Russulaceae, establishing an ectomycorrhizal trophic mode for two species previously described as lignicolous. Studies of genetic mechanisms in Agaricales using ITS sequence data include characterizing apparent recombination or gene conversion in a *Flammulina* hybrid (Hughes & Petersen, 2001) and identifying geographically-based evolutionary lineages

and providing evidence of a mechanism for patterns of concerted evolution of ribosomal repeats in *Schizophyllum commune* (James et al., 2001).

In addition to strict phylogenetic inference, rDNA-ITS sequence and RFLP data are probably the most widely used means of molecular identification for fungi, frequently used for strain typing, identification of fungal species from mycorrhizae on roots or in soil isolates, and correlation of genetic with morphological and/or ecological data (Bruns, 2001; Gardes & Bruns, 1993, 1996a, 1996b; Gardes et al., 1991a; Henrion et al., 1992; Horton & Bruns, 2001; Kernaghan, 2001; Moncalvo & Vilgalys, 2002). In addition, species-specific ITS PCR primer pairs can be developed for identifying the presence of particular species of interest, as demonstrated for the ectomycorrhizal ascomycete genus *Tuber* (Amicucci et al., 1998).

#### Molecular Characterization in *Laccaria*

Previous molecular characterizations of *Laccaria* spp. include molecular strain typing, RFLP analysis, and rDNA sequencing. Because of the importance of *Laccaria* as an ectomycorrhizal inoculant in forestry applications, a number of studies have used molecular strain typing methods in order to characterize population level characteristics such as spatial distribution, fruiting patterns, and mycelial persistence of *Laccaria* genets in field settings. Weber et al. (2002) developed a set of sequence-characterized amplified region (SCAR) markers in order to selectively identify a particular *L. bicolor* strain on mycorrhizal roots and demonstrate long-term persistence of this individual genotype in an inoculated Douglas Fir plantation. Similarly, randomly amplified

polymorphic DNA (RAPD) markers demonstrated persistence of *L. bicolor* genets for at least 3 years under natural conditions on Norway spruce roots (de la Bastide et al., 1994). Basidiocarp studies on *L. amethystina* in forest plots have demonstrated infraspecific variability in RAPD and randomly amplified microsatellite (RAMS) banding patterns, showing that even closely located genets exhibit genotypic variation (Fiore-Donno & Martin, 2001; Gherbi et al., 1999). While these molecular markers can be used to demonstrate variability within species and identify individual strains, they are less robust than sequence data in phylogenetic analyses due to the difficulty of establishing homology between bands of similar length.

RFLP analysis has been used in a number of studies in identifying *Laccaria* species. Gardes et al. (1990) determined that RFLPs using the entire nrDNA repeat could distinguish the four species *L. laccata*, *L. bicolor*, *L. proxima* and *L. amethystina*, as well as distinguish biological species in the *L. laccata* complex and distinguish European from North American isolates in *L. bicolor* and *L. amethystina*. However, RFLP patterns using only the ITS region were unable to unambiguously distinguish these four species (Gardes et al., 1991b), suggesting that direct sequence comparison is necessary to better characterize interspecific variation patterns in the ITS region. RFLP patterns in mitochondrial DNA (mtDNA) revealed high variability both within and between species, and provided preliminary evidence for length (insertion/deletion) as well as point mutations in *Laccaria* mtDNA (Gardes et al., 1991a). However, mtDNA RFLP patterns could not distinguish between European and North American populations in *L. bicolor*, suggesting that these populations have not been separated long enough for all ancestral

mtDNA genotypes to have become extinct. Mitochondrial DNA RFLP polymorphism supported the existence of distinct biological species in both *L. bicolor* and *L. laccata* that were supported by mating data, and mtDNA and nrDNA RFLP data combined with mating studies and phenetic analysis of basidiocarp and basidiospore morphology were used to delimit three species within the *L. bicolor* complex: *L. nobilis*, *L. bicolor* sensu stricto, and *L. trichodermophora* (Mueller & Gardes, 1991). While RFLP data represent an additional set of characters for use in inferring genetic divergence and supporting species recognitions, problems in determining homology of observed banding patterns precludes the use of RFLPs in phylogenetic reconstruction.

In addition to taxonomic applications at the species level, RFLPs can be useful in molecular typing at the strain level. Using the variable nrDNA IGS2 region, RFLP patterns can be used to distinguish *L. proxima* strains even from similar localities (Albee et al., 1996). Similarly, length polymorphisms of PCR-amplified genes can be useful in species and subspecies identification. While the length of the ITS region appears to be nearly constant (approximately 700 b.p. including ITS1, 5.8S, and ITS2) in *Laccaria* (Gardes et al., 1991b), the IGS region has been shown to exhibit length variation even in closely separated genets of *L. amethystina* in forest plots (Fiore-Donno & Martin, 2001).

Previous DNA sequence analysis in *Laccaria* is limited in comparison to RFLP-based studies. Gardes et al. (1991b) characterized a short fragment (168 b.p.) from the 3' end of the rDNA-ITS region for *L. laccata*, *L. proxima* and 3 strains of *L. bicolor*, determining levels of sequence difference of 1-2% between *L. bicolor* strains, approximately 3.5% between *L. bicolor* strains and *L. laccata*, and up to 5% between *L.*

*bicolor* strains and *L. proxima*. While these findings establish an estimate of variability in the ITS region between *Laccaria* species, this estimate should be considered as preliminary due to the short length of the fragment and limited number of isolates used.

Several higher-order molecular phylogenetic studies have included *Laccaria* species: *L. pumila* and *L. amethystina* using nrDNA SSU and LSU and mtrDNA SSU and LSU sequences (Hibbett et al., 2000; Binder & Hibbett, 2002), *L. bicolor* using nrDNA LSU sequences (Moncalvo et al., 2000) and *L. bicolor*, *L. vulcanica* and *L. ochropurpurea* using nrDNA LSU sequences (Moncalvo et al., 2002). Each of these studies, with the exception of Moncalvo et al. (2002), suggested a relationship between *Laccaria* and *Cortinarius* (Family Cortinariaceae); however this relationship was not well-supported (bootstrap values  $\leq 53\%$ ). The study of Moncalvo et al. (2002) provided strong support for the monophyly of *Laccaria*; however, it did not resolve the higher-order relationships of the genus. Phenetic clustering of ITS RFLP data by Kernaghan (2001) suggests a close relationship between *Laccaria* and *Tricholoma*, both currently placed in the Family Tricholomataceae by most authors. While RFLP phenetic clustering data must be interpreted cautiously due to problems in determining homology of RFLP fragments, this relationship receives preliminary support in the present study due to the ability to unambiguously align published ITS sequences for *Tricholoma* but not *Cortinarius* with the *Laccaria* sequences obtained in this study.

### Study Objectives

As discussed in Chapter 2, identification of arctic-alpine *Laccaria* species has been complicated by problems including a scarcity of systematically informative morphological characters, nomenclatural synonymy, missing or poor-quality type collections for some species and literature descriptions conflicting with extant type specimens, and that a detailed taxonomic treatment of arctic-alpine taxa is currently lacking. In addition, rigorous taxonomic studies involving *L. montana* and *L. pumila*, two of the most commonly reported arctic-alpine-boreal taxa, have been hampered by the recalcitrance of these species to spore germination and/or pairing of isolates in the laboratory. The two species are morphologically nearly identical, with the exception of spore size and the number of spores borne on each basidium (two on *L. pumila*, and four on *L. montana*). Although it has been suggested by Singer (1977) that the two taxa may be conspecific, Mueller (1992) retains them as distinct species based on the observation that the number of spores borne on each basidium is constant, not exhibiting both character states as is the case in some genera, i.e., *Inocybe* and *Hebeloma* (Cripps, 1997; Smith et al., 1983). The inability to conduct mating studies between *L. montana* and *L. pumila* precludes the application of the biological species concept to determining the conspecificity of these species; therefore a molecular approach will be useful in addressing this longstanding taxonomic question. Determining whether or not these two taxa are conspecific could lead to a better understanding of their particular ecological roles, as they are often reported from similar habitats. In particular, future studies should assess the importance of secondary homothallism, or self-fertility, that is hypothesized to

occur in bisporic basidiomycetes and could represent an ecological advantage under difficult environmental conditions. Populations of both taxa appear to occur sympatrically on the Beartooth Plateau. Better understanding their species-level relationships, as well as their distributions and host associations, can lead to a better understanding of their ecologies.

The objectives of the present study are to: 1) Use genetic data to assess morphological species groupings for Rocky Mountain taxa, 2) Assess the systematic usefulness of various morphological characters by determining whether taxon groups inferred using molecular data can be defined by either morphological synapomorphies or unique combinations of morphological character states, 3) Evaluate the utility of the ITS region for further phylogenetic studies in *Laccaria*; 4) Provide a basic characterization of the internal transcribed spacer region in the *Laccaria* species studied, determine levels of sequence variation between species and examine whether molecular divergence between geographically separated populations within species can be detected using the ITS region, 5) Conduct a preliminary molecular test of the hypothesis of genetic isolation between *Laccaria pumila* and *L. montana*; and 6) Create a preliminary phylogenetic backbone for further molecular phylogenetic studies in *Laccaria*.

The present study consists of a phylogenetic analysis of rDNA-ITS sequences from 16 Rocky Mountain alpine *Laccaria* collections representing the 5 morphological species described in the previous chapter, as well as a Rocky Mountain subalpine, conifer-associated *L. bicolor* collection included as a reference specimen for that taxon.

## Materials and Methods

### Specimen Collection

Specimens were collected during the years 1997-2001 at alpine field sites in Colorado, Montana, and Wyoming as described in Chapter 2. Specimens were preserved by warm air drying on an electric dryer and deposited in the Montana State University Herbarium (MONT), Fungal Section. Dried basidiocarp collections used for DNA extraction and phylogenetic analysis are listed in Table 13.

### DNA Extraction

DNA was extracted from dried basidiocarps using a procedure modified from Edwards et al. (1991) and Weiss et al. (1998). Preliminary experiments showed that treatment of dried tissue by  $-80^{\circ}\text{C}$  or liquid nitrogen freezing did not noticeably increase DNA yield (data not shown). Dried tissue was ground and suspended in 500  $\mu\text{l}$  extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS), vortexed 5 sec, and incubated 1 h in a  $65^{\circ}\text{C}$  heating block. Sample was then centrifuged 5 minutes at 14,000 rpm. The supernatant was transferred to a new tube, then treated with 1  $\mu\text{l}$  ribonuclease A (RNase A, 1  $\mu\text{g}/\mu\text{l}$ ) and allowed to sit for 15 min. DNA was precipitated by adding 1000  $\mu\text{l}$  ice-cold 95% ethanol and 50  $\mu\text{l}$  3M sodium acetate, pH 5.2 and mixed by inverting. The sample was frozen at  $-20^{\circ}\text{C}$  for 30-45 minutes, then centrifuged 10 minutes at 14,000 rpm. The supernatant was discarded, and the pellet was washed with 1000  $\mu\text{l}$  70% ethanol and centrifuged 10 minutes at 14,000 rpm. The

supernatant was discarded, and DNA dried in a vacuum oven 15 min at 50°C. DNA was resuspended in 25 µl 1X TE (Tris-EDTA) buffer, pH 8.0 and stored at -20 °C until use. Extraction products were visualized on 0.8% agarose gels containing 0.003 % ethidium bromide.

Table 14. Rocky Mountain alpine *Laccaria* collections used in molecular analysis. Dried basidiocarp material was used as DNA source for all collections. All collections are housed at MONT herbarium.

Taxon	Geographical Origin	EM Host Species	Collector and Collection Number
<i>L. bicolor</i>	CO, Front Range, Loveland Pass	<i>Salix</i> sp. (shrub)	C Cripps* 1304
	CO, San Juan Mts , Black Bear Pass	unknown	C Cripps 1445
	CO, Sawatch Range, Independence Pass	<i>Salix planifolia</i>	C Cripps 1469
	CO, Sawatch Range, Lmkins Lake Valley	<i>Salix planifolia</i>	C Cripps 1482
	CO, San Juan Mts , Mineral Basin	<i>Salix arctica</i>	C Cripps 1672
<i>L. laccata</i> var <i>pallidifolia</i>	CO, Sawatch Range, Independence Pass	<i>Salix</i> sp. (dwarf), <i>Dryas octopetala</i>	C Cripps 1370
	CO, 10-mile Range, Blue Lake Dam	<i>Salix reticulata</i> , <i>Betula glandulosa</i>	C Cripps 1603
	CO, 10-mile Range, Blue Lake Dam	<i>Betula glandulosa</i>	C Cripps 1633
	CO, San Juan Mts , Horseshoe Lake	<i>Salix reticulata</i>	C. Cripps 1655
	CO, Sawatch Range, Cottonwood Pass	<i>Dryas</i> sp.	C. Cripps 1724
<i>L. montana</i>	MT/WY, Beartooth Plateau, Highline Tr.	unknown	T. Osmundson* 319
	WY, Beartooth Plateau, Frozen Lake	<i>Salix planifolia</i>	T Osmundson 591
<i>L. pumila</i>	CO, Front Range, Haggeman's Pass	<i>Salix planifolia</i>	C Cripps 1252
	WY, Beartooth Plateau, Frozen Lake	<i>Salix planifolia</i>	T Osmundson 501
<i>Laccaria</i> sp	CO, 10-mile Range, Blue Lake Dam	<i>Betula glandulosa</i> , <i>Salix planifolia</i>	C. Cripps 1625
	CO, Sawatch Range, Independence Pass	<i>Salix</i> sp (shrub)	C Cripps 1771

#### Additional Sequences Used

*Tricholoma unifactum*, GenBank Accession Number AF241514 (Outgroup taxon)

*Tricholoma portentosum*, GenBank Accession Number AF349686 (Outgroup taxon)

*Laccaria bicolor*, Gallatin Co , MT, USA, T Osmundson 752 (Subalpine comparative specimen)

\*For collection designations throughout chapter, C Cripps = CLC and T. Osmundson = TWO

Preliminary experiments showed that *Laccaria* genomic DNA crude extractions failed to PCR amplify; therefore, genomic DNA was further purified using a phenol:chloroform extraction according to Sambrook et al. (1989). DNA was diluted to a volume of 100  $\mu$ l in a 1.5 ml microcentrifuge tube, and 100  $\mu$ l phenol / chloroform / isoamyl alcohol (25:24:1) was added to the sample and mixed to form an emulsion. Tubes were centrifuged for 30 sec at 12,000 rpm. The aqueous phase was transferred to a new tube, and the organic phase "back-extracted" by the addition of an equal volume of TE buffer, pH 7.8 and centrifugation for 30 sec at 12,000 rpm; the aqueous phase from the back-extraction was added to the first aqueous phase. An equal volume of chloroform : isoamyl alcohol (24:1) was added to the aqueous phase and mixed, then centrifuged for 30 sec at 12,000 rpm. The aqueous phase from this step was transferred to a new tube, and DNA was precipitated by adding a 1/10 volume of 3M sodium acetate, pH 5.2, and 2 volumes of ice cold 95% ethanol and incubated at  $-20^{\circ}\text{C}$  for 60 min. The mixture was centrifuged for 10-15 min at 14,000 rpm and the supernatant discarded. The DNA pellet was washed with 500  $\mu$ l 75% ethanol and centrifuged for 10 min at 14,000 rpm. The supernatant was discarded and the DNA dried in a vacuum oven at  $55^{\circ}\text{C}$  for 15 min or until all traces of ethanol were removed. The pellet was then resuspended in 25  $\mu$ l TE buffer and stored at  $-20^{\circ}\text{C}$ . An aliquot of the DNA solution was diluted 1:25 to 1:125 (determined empirically, depending on sample) with sterile ddH<sub>2</sub>O prior to PCR amplification.

### PCR Amplification

Ribosomal ITS gene products were amplified using the polymerase chain reaction (Mullis et al., 1986; Saiki et al., 1988). Amplifications used the basidiomycete-specific primers ITS-1F and ITS-4B (Gardes & Bruns, 1993). Primer sequences are as follows: ITS-1F (CTTGGTCATTTAGAGGAAGTAA); ITS-4B CAGGAGACTTGTACACGGTCCAG) (Sigma-Genosys, Woodlands, TX, USA). Reactions were carried out in 40  $\mu$ l mixtures (2 per sample) consisting of 8  $\mu$ l template DNA (approximately 50-100 ng), 8  $\mu$ l each of forward and reverse primers (20ng /  $\mu$ l), 4  $\mu$ l 10X PCR buffer (Fisher Buffer A containing 1.5mM MgCl<sub>2</sub>, Fisher Scientific, Pittsburgh, PA, USA), 3.2  $\mu$ l dNTP mixture (2.5mM each dNTP; Promega Corp., Madison, WI, USA), 0.3  $\mu$ l Eppendorf MasterTaq DNA polymerase (Brinkmann Instruments, Westbury, NY, USA), and 8.5  $\mu$ l sterile ddH<sub>2</sub>O. Reactions were performed using an Eppendorf Mastercycler Gradient thermocycler (Brinkmann Instruments, Westbury, NY, USA) using the following conditions: initial denaturation 94°C 2 min; 30 cycles of denaturation 94°C 30 sec, annealing 55°C 1 min, and extension 72°C 1 min; final extension 72°C for 5 min. Lid temperature was set at 105°C, and temperature ramping was set at 1°C / sec. PCR products were visualized using 1.5% agarose gels containing 0.003 % ethidium bromide. PCR products were purified prior to sequencing using the QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA, USA) according to manufacturer's instructions, with an additional wash in 0.75 mL 35% guanidine hydrochloride as recommended by the manufacturer to reduce primer-dimers in the final eluate, and with final elution in 30  $\mu$ l

elution buffer. DNA was concentrated by ethanol precipitation as described in the section on phenol-chloroform extraction, with the amount of ice cold 95% ethanol added in the first step modified to 3 volumes. The dried DNA product was resuspended in 7  $\mu$ l autoclaved ddH<sub>2</sub>O.

### DNA Sequencing

DNA sequencing reactions were performed using the ABI Prism BigDye Terminator v.2.0 cycle sequencing kit (Applied Biosystems, Foster City, CA). Sequences were generated in both directions using the primers ITS-1F or ITS-4B in the following sequence reaction mixture: 2  $\mu$ l BigDye, 0.5  $\mu$ l forward or reverse primer (20 ng /  $\mu$ l), 3.5  $\mu$ l DNA, overlaid with mineral oil. The sequencing reaction was performed on a Perkin-Elmer GeneAmp 9600 thermocycler (Perkin-Elmer Corp., Norwalk, CT, USA) for 45 cycles using the following parameters: denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec, and elongation at 60°C for 4 min. The fluorescently labeled product was cleaned by removing mineral oil, adding 30  $\mu$ l autoclaved ddH<sub>2</sub>O, and separated from residual mineral oil on a paraffin film. DNA was precipitated as described for ethanol precipitation prior to the sequencing reaction, and resuspended in 0.8 Blue Dextran dye. DNA was denatured by placing for 2 min in a boiling water bath then placing on ice immediately prior to loading samples on the sequencing gel. Sequencing products were analyzed using an ABI PRISM 377 automated DNA sequencer (Applied Biosystems, Foster City, CA).

Sequence chromatographs were examined and reverse-complements of the reverse (ITS-4B-primed) sequences produced using Chromas version 1.45 software (copyright Conor McCarthy, Griffith University, Southport, Queensland, Australia; available as freeware at <http://www.technelysium.com.au/chromas.html>). Forward and reverse-complemented reverse sequences were aligned and edited to create a consensus contig using the BioEdit Sequence Alignment Editor (copyright Tom Hall, North Carolina State University; available as freeware at <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Regions of poor sequence quality at the 5' and 3' ends were deleted. Sequence identity was confirmed as belonging to *Laccaria* spp. by means of a BLAST search ([www.ncbi.nih.org/blast](http://www.ncbi.nih.org/blast)). Multiple sequence alignments were performed using ClustalX software (Jeanmougin et al., 1998) and edited manually.

#### Phylogenetic Analysis

The final multiple sequence alignment was formatted as a Nexus file and analyzed using PAUP\* 4.0b10 software (Swofford, 2001) on a Dell microcomputer running under Microsoft Windows 98. Maximum parsimony was used as the optimality criterion. Characters were specified as unordered and equally weighted, with gaps treated as missing data. Insertion/deletion events (indels) were coded as additional, binary characters denoting presence/absence. Sensitivity analyses omitting these additional characters, as well as analyses incorporating each outgroup sequence separately were conducted to examine the effects of these perturbations on tree topology. Exact searches were conducted using the branch-and-bound algorithm with the addition sequence set to

“furthest,” initial MAXTREES set to 100, initial upper bound computed heuristically, MULTREES option in effect, and branches collapsed if maximum branch length equaled zero. Strict consensus trees were generated for each analysis. The partition homogeneity test was employed to test for phylogenetic congruence between ITS1, 5.8S, and ITS2 sequences. Branch robustness was assessed using 1000 bootstrap replicates (Felsenstein, 1985) using a branch-and-bound search. The neighbor-joining method in PAUP\* was employed to produce a genetic distance matrix.

Outgroup taxa chosen were *Tricholoma unifactum* (GenBank accession number AF241514) and *Tricholoma portentosum* (GenBank accession number AF349686). Outgroups were chosen based on shared family classification (Family Tricholomataceae in Singer's (1986) classification) and trophic type (ectomycorrhizal) with *Laccaria*. Although recent large-scale molecular phylogenetic studies by Moncalvo et al. (2000), Hibbett et al. (2000) and Binder and Hibbett (2002) suggest that *Laccaria* may be more closely related to the dark-spored genus *Cortinarius* than to *Tricholoma*, the clades containing *Laccaria* grouped with *Cortinarius* received low bootstrap support in these studies and a relationship between *Laccaria* and *Tricholoma* was supported by phenetic clustering of RFLP data by Kernaghan (2001). In addition, *Laccaria* ribosomal ITS sequences could not be aligned unambiguously to those of *Cortinarius* spp. deposited in GenBank.

## Results

Phylogenetic congruence between ITS1, 5.8S and ITS2 regions was supported by the partition homogeneity test ( $p=0.33$ ). The dataset consisted of 696 characters for analyses with indels coded as binary characters, and 669 characters for analyses with binary characters omitted.

The primary analysis included both *Tricholoma* outgroup sequences and included indel presence/absence coded as binary characters. Of 696 characters, 524 were constant, 65 variable but parsimony uninformative, and 107 parsimony informative. This analysis yielded 4 most parsimonious trees of length 229 steps, consistency index (CI) = 0.8253, retention index (RI) = 0.8910, and rescaled consistency index (RC) = 0.7354. The strict consensus tree for this analysis is shown in Figure 14. This tree reveals the presence of well-supported clades corresponding to the morphological species (previously determined, see Chapter 2) *L. bicolor* (97% bootstrap), *L. laccata* var. *pallidifolia* (100%), *L. montana* (100%), and *L. pumila* (89%). An additional, well-supported (99% bootstrap) clade was discovered that corresponded to a species appearing similar to *L. pumila* and *L. montana* under field conditions. This unidentified species, *Laccaria* sp., is supported by differences in macro- and micromorphology as described in Chapter 2. A sister group relationship between this taxon and *L. pumila* and *L. montana* received moderate (73%) bootstrap support. An additional clade within *L. bicolor*, comprising collections CLC 1672 and CLC 1304, received 83% bootstrap support in this analysis; however, this clade was found to be sensitive to omitting binary indel codes in subsequent sensitivity analyses (see below). The relationship between alpine collections

of *L. bicolor* and the subalpine *L. bicolor* reference specimen TWO 752 received only moderate support (bootstrap 75%); however, this relationship was repeatedly supported by sensitivity analyses.

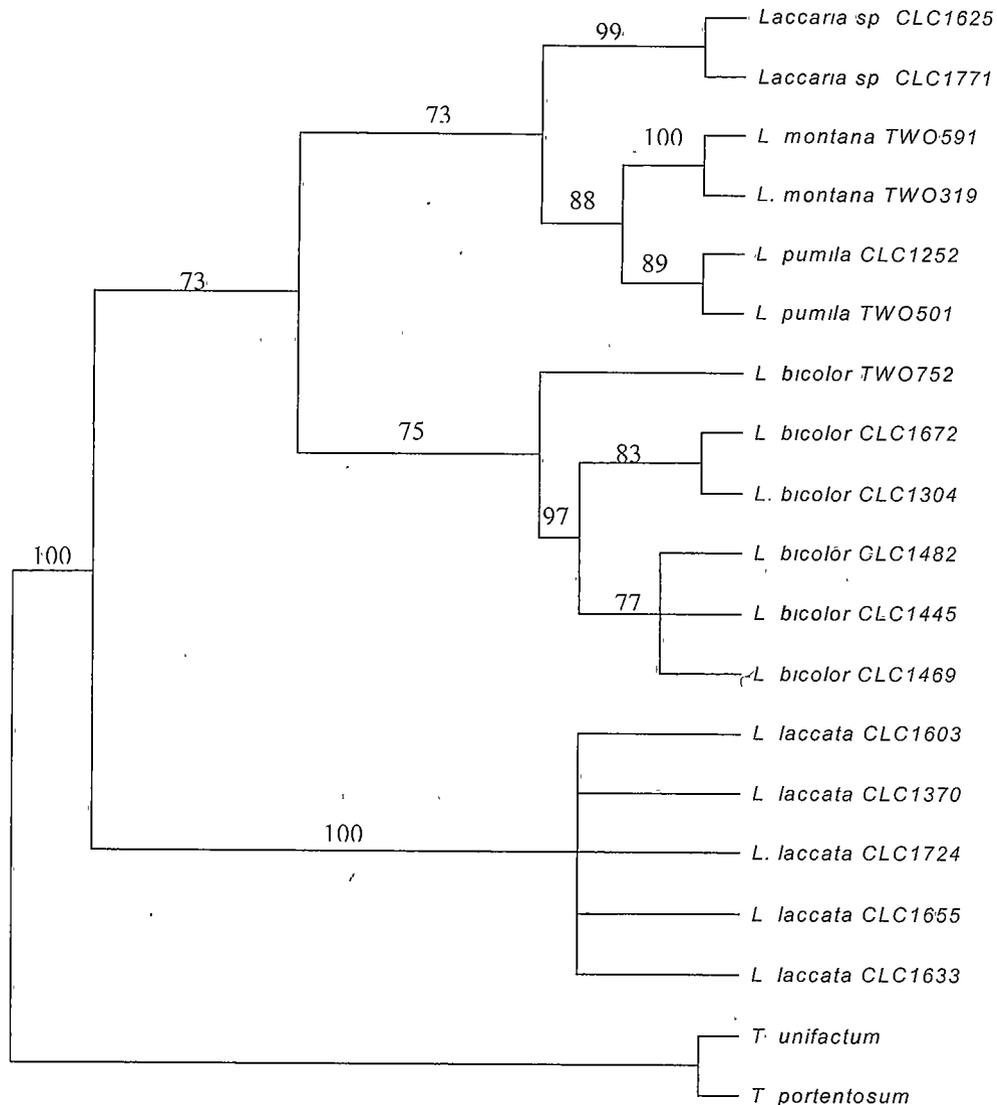


Figure 14. Strict consensus of 4 most-parsimonious trees for the primary *Laccaria* rDNA ITS phylogenetic analysis. Presence or absence of insertion-deletion events ("indels") were coded as an additional set of binary characters. *Tricholoma unifactum* (GenBank accession AF241514) and *Tricholoma portentosum* (GenBank accession AF349686) were used as outgroup taxa. Bootstrap values (>50%) are shown above branches.

### Sensitivity Analyses

Sensitivity analyses were conducted to test the effects on tree topology of removing outgroup taxa and/or omitting binary codes. The analysis including both *Tricholoma* outgroups and omitting indel codes consisted of 669 characters (524 constant, 62 variable but parsimony uninformative, and 83 parsimony informative) and produced 9 most-parsimonious trees of length 188 steps, consistency index (CI) = 0.8617, retention index (RI) = 0.9048, and rescaled consistency index (RC) = 0.7796. The strict consensus tree for this analysis is shown in Figure 15. Although the grouping of collections corresponding morphologically to *L. bicolor* is still strongly supported as monophyletic (95% bootstrap support), the arrangement of subclades within *L. bicolor* is rearranged slightly with only collection CLC1672 forming a subclade distinct from other alpine collections and all other alpine collections forming a polytomy.

Analyses removing either one of the *Tricholoma* outgroup taxa and maintaining binary gap codes did not result in changes in tree topology (Figs. 16, 17). The only notable difference between these results and those of the primary analysis is reduced bootstrap support (59% vs. 73-77%) for the branch joining *Laccaria* sp. with *L. montana* and *L. pumila* when the *T. portentosum* outgroup was removed (Fig. 17).



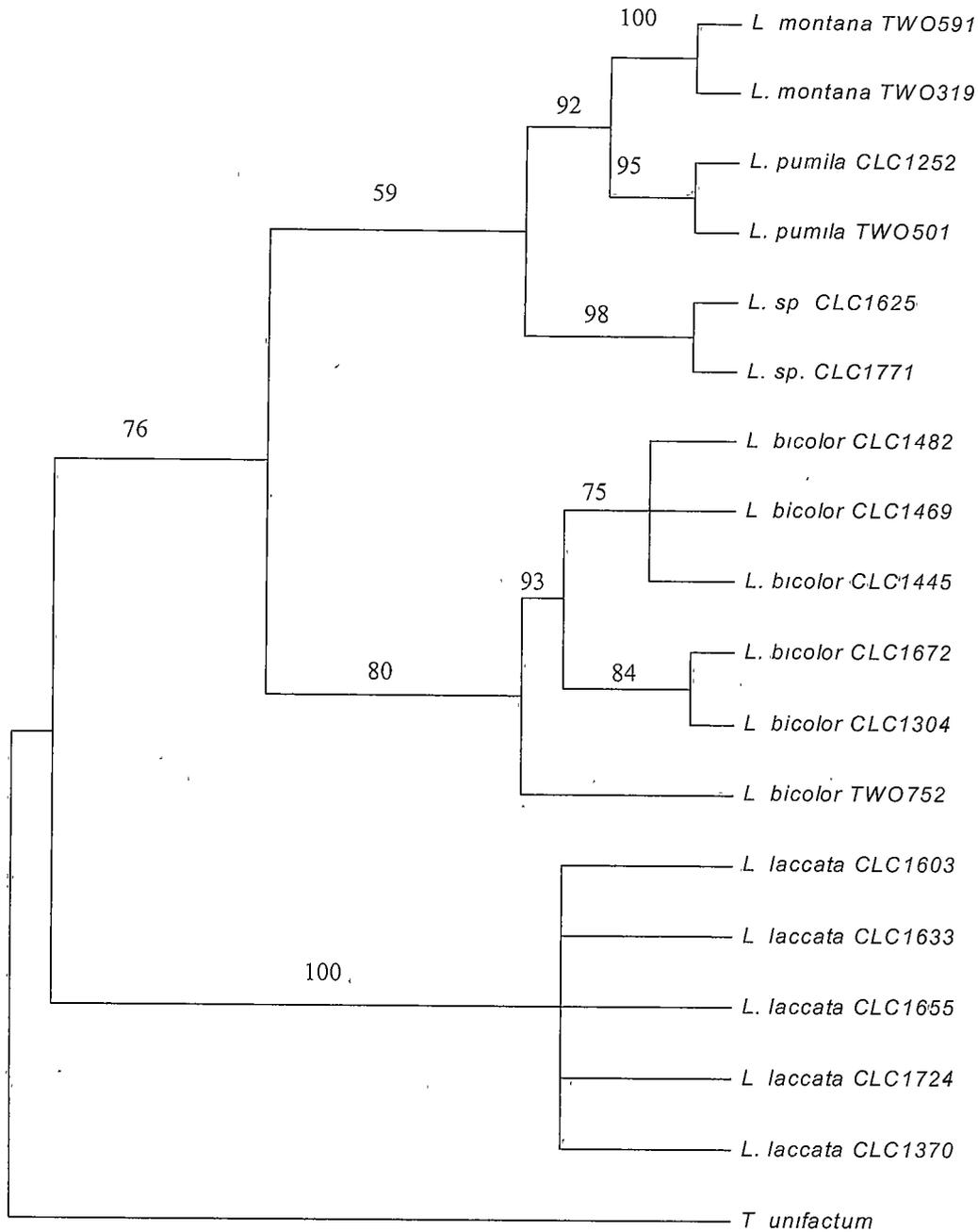


Figure 16. Strict consensus of 4 most-parsimonious trees for the sensitivity analysis including binary indel codes and using only *T. unifactum* as the outgroup taxon. Bootstrap values (>50%) are shown above branches. Each minimal length tree had length = 184 steps, CI = 0.8424, RI = 0.9085, RC = 0.7653.

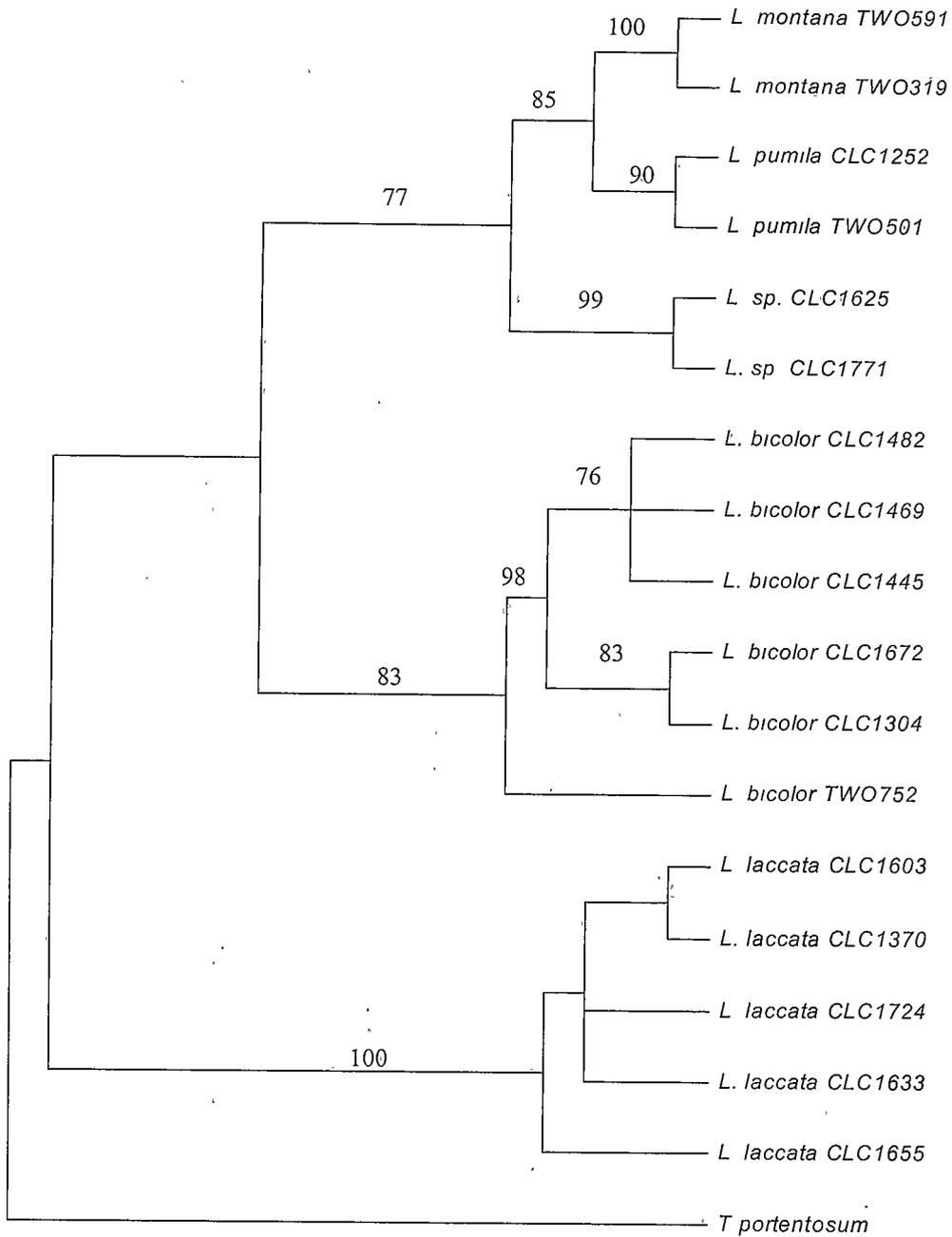


Figure 17. Single most-parsimonious tree for the sensitivity analysis including binary indel codes and using only *T. portentosum* as the outgroup taxon. Bootstrap values (>50%) are shown above branches. Tree length = 188 steps, CI = 0.8404, RI = 0.9080, RC = 0.7631.

Analyses removing either one of the outgroup taxa and omitting gap codes resulted in the same tree topology as the analyses omitting gap codes and retaining both outgroup taxa (Figs. 18, 19), with the only notable difference being increased bootstrap support (75% vs. 63-65%) for the branch connecting *L. pumila* and *L. montana* when the *T. portentosum* outgroup was removed (Fig.18).

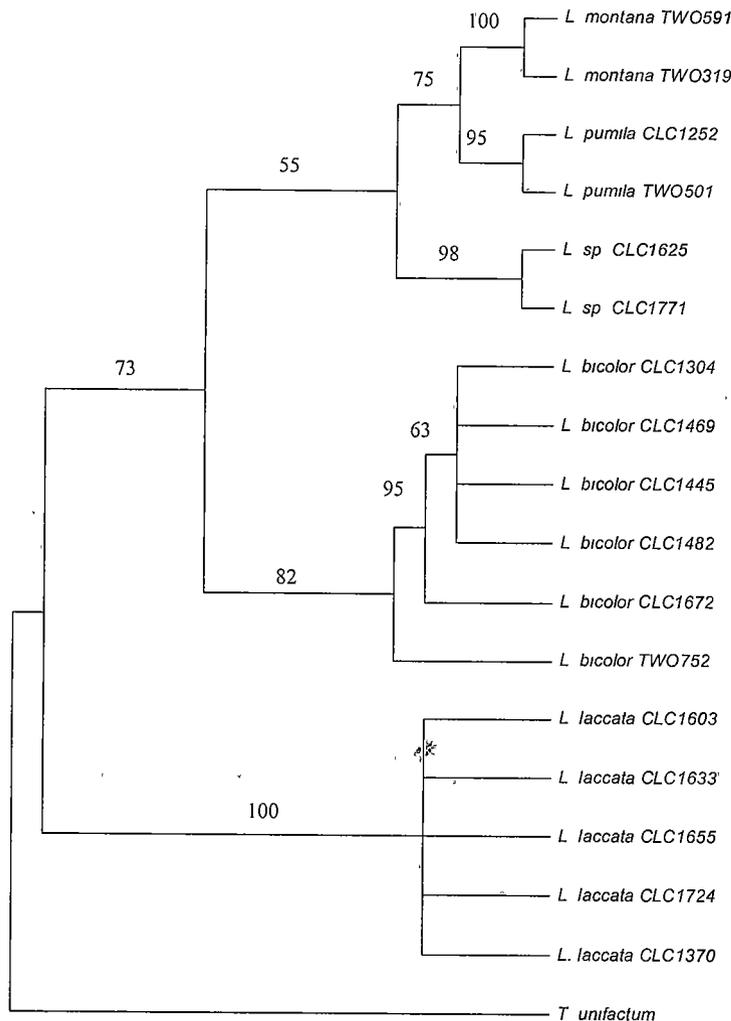


Figure 18. Strict consensus of 9 most-parsimonious trees for the sensitivity analysis excluding binary indel codes and using only *T. unifactum* as the outgroup taxon. Bootstrap values (>50%) are shown above branches. Each minimal length tree had length = 145 steps, CI = 0.8828, RI = 0.9254, RC = 0.8169.

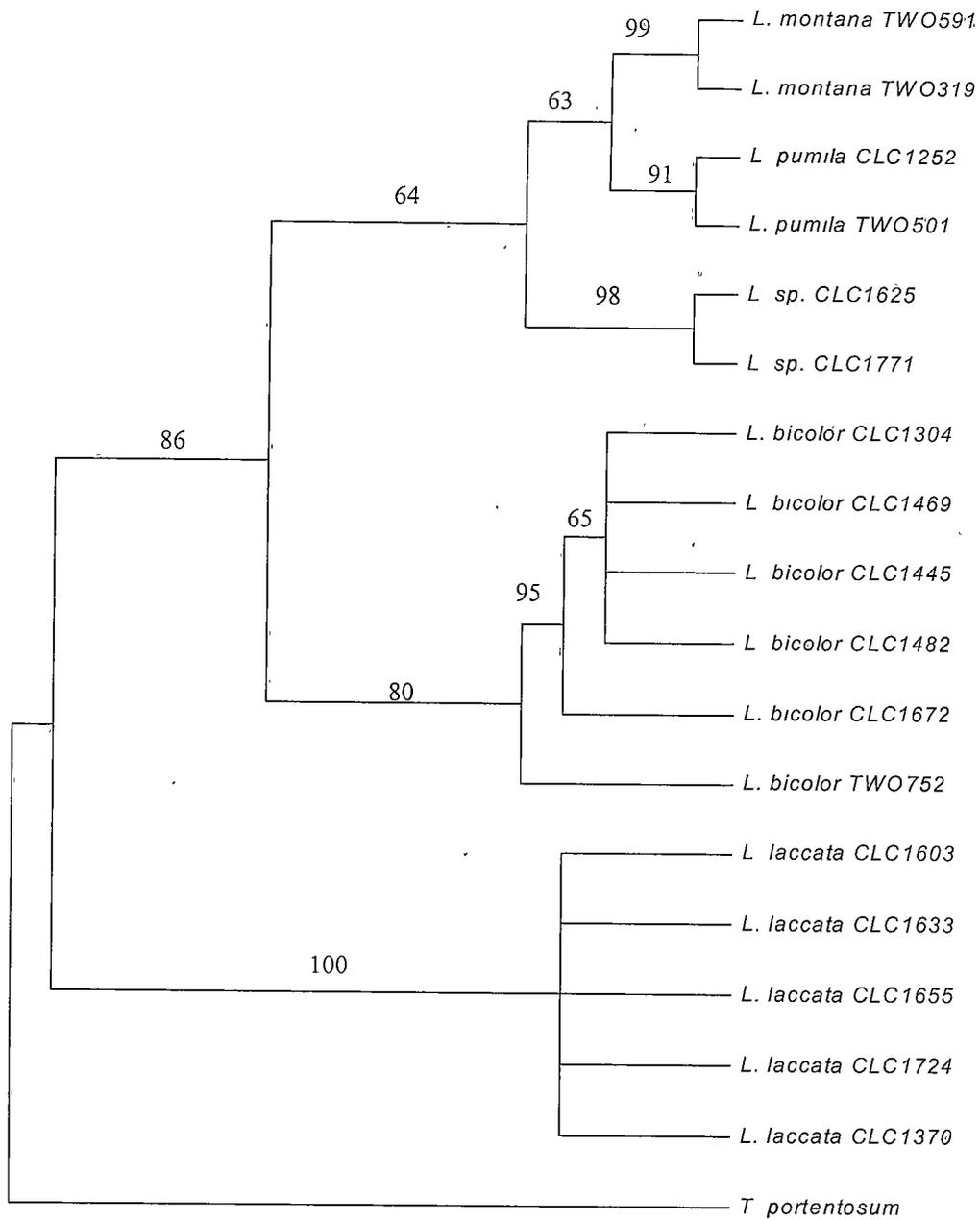


Figure 19. Strict consensus of 4 most-parsimonious trees for the sensitivity analysis excluding binary indel codes and using only *T. portentosum* as the outgroup taxon. Bootstrap values (>50%) are shown above branches. Each minimal tree had length = 150 steps, CI = 0.8733, RI = 0.9195, RC = 0.8030.

Molecular Characterization of Rocky Mountain Alpine *Laccaria* spp.

Intraspecific and Interspecific Genetic Variation

The neighbor joining method in PAUP\* was used to generate a genetic distance matrix for the combined ITS1 / 5.8S / ITS2 dataset (Figure 20). Intraspecific variation was found to be extremely low: 0-0.3% between alpine *L. bicolor* collections, 0.2-1% between *L. laccata* var. *pallidifolia* collections, 0.2% in *L. montana*, 0.3% in *Laccaria* sp., and 0% in *L. pumila* (Table 14). Intraspecific variation in *L. bicolor* between alpine collections from Colorado and subalpine collections from Montana was higher, ranging from 2.1-2.4%. Interspecific variation ranged from 1.6-1.8 % between *L. montana* and *L. pumila* to 6-7% between *L. laccata* var. *pallidifolia* and *Laccaria bicolor* (7.9-8.4% when subalpine *L. bicolor* collection TWO 752 was included; Table 15). The most divergent clade was found to be *L. laccata* var. *pallidifolia*, ranging from 6-8.4% difference from other taxa included in this study.

Table 15. Intraspecific genetic distance for Rocky Mountain alpine *Laccaria* species, computed using neighbor-joining method in PAUP version 4.0b10.

Taxon	% Intraspecific Variation
<i>L. bicolor</i>	0-0.3 (2.1-2.4)*
<i>L. laccata</i> var. <i>pallidifolia</i>	0.2-1.0
<i>L. montana</i>	0.2
<i>L. pumila</i>	0
<i>L. sp.</i>	0.3

\* Range when subalpine *L. bicolor* collection TWO 752 included.

Species	1625	1603	1672	1304	1482	1445	591	1771	1370	1252	319	501	1724	1655	1469	1633
L.sp.1625																
L.lac.1603	0.063															
L.bic.1672	0.026	0.060														
L.bic.1304	0.027	0.063	0.002													
L.bic.1482	0.027	0.063	0.002	0.000												
L.bic.1445	0.029	0.065	0.003	0.002	0.002											
L.mont.591	0.026	0.066	0.033	0.034	0.034	0.036										
L.sp.1771	0.003	0.065	0.029	0.030	0.030	0.032	0.026									
L.lac.1370	0.065	0.004	0.059	0.063	0.063	0.065	0.066	0.066								
L.pum.1252	0.019	0.063	0.029	0.030	0.030	0.032	0.016	0.019	0.062							
L.mont.319	0.028	0.068	0.034	0.036	0.036	0.037	0.002	0.028	0.068	0.018						
L.pum.501	0.019	0.063	0.029	0.031	0.030	0.032	0.016	0.019	0.063	0.000	0.018					
L.lac.1724	0.068	0.005	0.065	0.068	0.068	0.070	0.071	0.070	0.005	0.068	0.073	0.068				
L.lac.1655	0.068	0.009	0.065	0.069	0.068	0.070	0.071	0.070	0.009	0.068	0.073	0.068	0.011			
L.bic.1469	0.027	0.063	0.002	0.000	0.000	0.002	0.034	0.030	0.063	0.030	0.036	0.030	0.068	0.068		
L.lac.1633	0.064	0.002	0.061	0.065	0.064	0.066	0.068	0.066	0.002	0.064	0.070	0.064	0.004	0.007	0.064	
L.bic.752	0.035	0.079	0.021	0.023	0.023	0.024	0.037	0.039	0.079	0.031	0.039	0.031	0.084	0.084	0.023	0.080

Figure 20. Neighbor-joining uncorrected ("p") genetic distance matrix for *Laccaria* species rDNA-ITS1/ 5.8S / ITS2 region.

Table 16. Interspecific ITS1 / 5.8S / ITS2 genetic distance for Rocky Mountain alpine *Laccaria* species, computed using neighbor joining method in PAUP version 4.0b10.

Taxon Pairs	% Interspecific Variation
<i>L. bicolor</i> vs. <i>L. laccata</i>	6.0-7.0 (7.9-8.4)*
<i>L. bicolor</i> vs. <i>L. montana</i>	3.3-3.7 (3.7-3.9)*
<i>L. bicolor</i> vs. <i>L. pumila</i>	2.9-3.2 (3.1)
<i>L. bicolor</i> vs. <i>L. sp.</i>	2.6-3.2 (-3.9)*
<i>L. laccata</i> vs. <i>L. montana</i>	6.6-7.3
<i>L. laccata</i> vs. <i>L. pumila</i>	6.2-6.8
<i>L. laccata</i> vs. <i>L. sp.</i>	6.0-7.0
<i>L. montana</i> vs. <i>L. pumila</i>	1.6-1.8
<i>L. montana</i> vs. <i>L. sp.</i>	2.6-2.8
<i>L. pumila</i> vs. <i>L. sp.</i>	1.9

\* Range when subalpine *L. bicolor* collection TWO 752 included.

#### Characterization of Variable Nucleotide Positions

In addition to characterizing the degree of genetic variation within and between species, an objective of the present study was to characterize the occurrence of insertion-deletion events (indels) and single nucleotide polymorphisms (SNPs) corresponding to morphological species groups. Table 16 provides a comparison of variable sequence regions by taxon. The clade corresponding to *L. laccata* var. *pallidifolia* represents the most divergent taxon in the analysis, and is characterized by a number of synapomorphic indels and SNPs. Sequence gaps occur for this species at alignment positions 116-142, 153-173, 205, 442-444, 452-455, 591-599, 602-604, 628, 630 and 635. Apparent insertions occur at positions 255-256 and 491-494. Synapomorphic SNPs occur at positions 15, 105, 145, 149, 181, 212, 257, 261, 325, 436, 447, 451, 460, 483, 495, 497, 541, 563, 564, 572, 624, 638; 639 and 652.

Table 17. Comparison of variable nucleotide positions by collection. Synapomorphic single-nucleotide polymorphisms appear in bold type.

		<u>Alignment Position</u>									
		6	15	19	29	43-49	64	75-80	97	105	116-142
<u>Taxon</u>											
<i>L. bicolor</i>	CLC1304	C	A	-	A	TT-CGAA	A	---ATC	-	T	CCTCTCGAGG <b>AA</b> ACTCGGATTTG-AGG
<i>L. bicolor</i>	CLC1445	C	A	A	A	TTTCGAA	A	T--ATC	T	T	CCTCTCGAGG <b>AA</b> ACTCGGATTTG-AGG
<i>L. bicolor</i>	CLC1469	C	A	A	A	TTTCGAA	A	T--ATC	T	T	CCTCTCGAGG <b>AA</b> ACTCGGATTTG-AGG
<i>L. bicolor</i>	CLC1482	C	A	A	A	TTTCGAA	A	T--ATC	T	T	CCTCTCGAGG <b>AA</b> ACTCGGATTTG-AGG
<i>L. bicolor</i>	CLC1672	C	A	-	A	TT-CGAA	A	---ATC	-	T	CCTCTCGAGG <b>AA</b> ACTCGGATTTG-AGG
<i>L. laccata</i>	CLC1370*	C	G	-	G	T----AG	A	TATATC	T	A	-----
<i>L. laccata</i>	CLC1603	C	G	-	G	-----A	A	TATATA	T	A	-----
<i>L. laccata</i>	CLC1633	C	G	A	G	T----AG	A	TATATA	T	A	-----
<i>L. laccata</i>	CLC1655	C	G	A	G	T----AG	A	TATATA	T	A	-----
<i>L. laccata</i>	CLC1724	C	G	A	G	T----AG	A	TATATA	T	A	-----
<i>L. montana</i>	TWO319	C	A	-	G	TTTCGAA	G	TA-ATC	T	T	<b>A</b> CTCTCGAGG <b>CA</b> ACTCGGATTTT-AGG
<i>L. montana</i>	TWO591	C	A	A	G	TTTCGAA	G	TA-ATC	T	T	<b>A</b> CTCTCGAGG <b>CA</b> ACTCGGATTTT-AGG
<i>L. pumila</i>	CLC1252	C	A	A	A	TT-CGAA	G	TA-ATC	T	T	CCTCTCGAGG <b>CA</b> ACTCGGATTTT-AGG
<i>L. pumila</i>	TWO501	-	A	-	A	TTTCGAA	G	TA-ATC	T	T	CCTCTCGAGG <b>CA</b> ACTCGGATTTT-AGG
<i>Laccaria</i> sp.	CLC1625	C	A	A	G	TTTC <b>AAA</b>	G	TA-AT <b>T</b>	T	T	CCTCTCGAGG <b>CA</b> ACTCGGATTTT-AGG
<i>Laccaria</i> sp.	CLC1771	C	A	A	G	TTTC <b>AAA</b>	G	TA-AT <b>T</b>	T	T	CCTCTCGAGG <b>CA</b> ACTCGGATTTT-AGG

\* All *L. laccata* specimens belong to *L. laccata* var. *pallidifolia*

Table 17, continued.

		<u>Alignment Position</u>											
<u>Taxon</u>		145	148	149	153-173	181	196-198	205	212	220	233	237	255-256
<i>L. bicolor</i>	CLC1304	C	C	G	CTGTACAAGTCGGCTTTTCTT	C	T--	A	T	T	C	G	--
<i>L. bicolor</i>	CLC1445	C	C	G	CTGTACAAGTCGGCTTTTCTT	C	T--	A	T	T	C	G	--
<i>L. bicolor</i>	CLC1469	C	C	G	CTGTACAAGTCGGCTTTTCTT	C	T--	A	T	T	C	G	--
<i>L. bicolor</i>	CLC1482	C	C	G	CTGTACAAGTCGGCTTTTCTT	C	T--	A	T	T	C	G	--
<i>L. bicolor</i>	CLC1672	C	C	G	CTGTACAAGTCGGCTTTTCTT	-	T--	A	T	T	C	G	--
<i>L. laccata</i>	CLC1370	G	T	C	-----	A	-CA	-	C	A	C	A	TA
<i>L. laccata</i>	CLC1603	G	T	C	-----	A	TCA	-	C	A	C	A	TA
<i>L. laccata</i>	CLC1633	G	T	C	-----	A	TCA	-	C	A	C	A	TA
<i>L. laccata</i>	CLC1655	G	T	C	-----	A	TCA	-	C	A	C	A	TA
<i>L. laccata</i>	CLC1724	G	T	C	-----	A	TCA	-	C	A	C	A	TA
<i>L. montana</i>	TWO319	C	C	G	CT-----GCTTTCCTT	C	T--	A	T	A	G	G	--
<i>L. montana</i>	TWO591	C	C	G	CT-----GCTTTCCTT	C	T--	A	T	A	G	G	--
<i>L. pumila</i>	CLC1252	C	T	G	CTGTAAAAGTCAGCTTTCCTC	C	T--	A	T	A	C	A	--
<i>L. pumila</i>	TWO501	C	T	G	CTGTAAAAGTCAGCTTTCCTC	C	T--	A	T	A	C	A	--
<i>Laccaria</i> sp.	CLC1625	C	C	G	CTGTAAAAGTCAGCTTTCCTC	C	-C-	A	T	A	C	G	--
<i>Laccaria</i> sp.	CLC1771	C	C	G	CTGTAAAAGTCAGCTTTCCTC	C	-C-	A	T	A	C	G	--

Table 17, continued.

Taxon		Alignment Position															
		257	261	315	325	387	436	442-444	447	451	452-455	460	465	482	483	491-494	495
<i>L. bicolor</i>	CLC1304	A	T	G	A	T	T	ACT	T	A	GCTT	A	T	C	G	----	T
<i>L. bicolor</i>	CLC1445	A	T	G	A	T	T	ACT	T	A	GCTT	A	T	C	G	----	T
<i>L. bicolor</i>	CLC1469	A	T	G	A	T	T	ACT	T	A	GCTT	A	T	C	G	----	T
<i>L. bicolor</i>	CLC1482	A	T	G	A	T	T	ACT	T	A	GCTT	A	T	C	G	----	T
<i>L. bicolor</i>	CLC1672	A	T	G	A	T	T	ACT	T	A	GCTT	A	T	C	G	----	T
<i>L. laccata</i>	CLC1370	G	C	G	T	T	A	---	A	T	----	T	T	C	A	TTTG	A
<i>L. laccata</i>	CLC1603	G	C	G	T	T	A	---	A	T	----	T	T	C	A	TTTG	A
<i>L. laccata</i>	CLC1633	G	C	G	T	T	A	---	A	T	----	T	T	C	A	TTTG	A
<i>L. laccata</i>	CLC1655	G	C	G	T	T	A	---	A	T	----	T	G	C	A	TTTG	A
<i>L. laccata</i>	CLC1724	G	C	G	T	T	A	---	A	T	----	T	T	C	A	TTTG	A
<i>L. montana</i>	TWO319	A	T	G	A	C	T	GCT	T	G	GCTT	A	T	T	G	----	T
<i>L. montana</i>	TWO591	A	T	G	A	T	T	GCT	T	G	GCTT	A	T	T	G	----	T
<i>L. pumila</i>	CLC1252	A	T	G	A	T	T	GCT	T	A	GCTT	A	T	T	G	----	T
<i>L. pumila</i>	TWO501	A	T	G	A	T	T	GCT	T	A	GCTT	A	T	T	G	----	T
<i>Laccaria</i> sp.	CLC1625	A	T	G	A	T	T	ACT	T	A	GCTT	A	T	C	G	----	T
<i>Laccaria</i> sp.	CLC1771	A	T	C	A	T	T	GCT	T	A	GCTT	A	T	C	G	----	T

Table 17, continued.

<u>Taxon</u>	<u>Alignment Position</u>																	
	496	497	498	500	506	526	527	541	547	563	564	572	574	579	581	589	590	
<i>L. bicolor</i> CLC1304	C	A	C	G	G	G	T	G	A	A	T	G	C	G	G	A	G	
<i>L. bicolor</i> CLC1445	C	A	C	G	G	G	T	G	A	A	T	G	C	G	G	A	G	
<i>L. bicolor</i> CLC1469	C	A	C	G	G	G	T	G	A	A	T	G	C	G	G	A	G	
<i>L. bicolor</i> CLC1482	C	A	C	G	G	G	T	G	A	A	T	G	C	G	G	A	G	
<i>L. bicolor</i> CLC1672	C	A	A	G	G	G	T	G	A	A	T	G	C	G	G	A	G	
<i>L. laccata</i> CLC1370	C	T	A	G	A	G	T	T	A	T	C	A	T	A	G	G	G	
<i>L. laccata</i> CLC1603	C	T	A	G	A	G	T	T	A	T	C	A	T	A	G	G	G	
<i>L. laccata</i> CLC1633	C	T	A	G	A	G	T	T	A	T	C	A	T	A	G	G	G	
<i>L. laccata</i> CLC1655	C	T	A	A	A	C	T	T	A	T	C	A	T	A	G	A	G	
<i>L. laccata</i> CLC1724	C	T	A	G	A	G	T	T	A	T	C	A	T	A	G	G	G	
<i>L. montana</i> TWO319	T	A	A	G	A	G	C	G	G	A	T	G	T	A	G	A	T	
<i>L. montana</i> TWO591	T	A	A	G	A	G	C	G	G	A	T	G	T	A	G	A	T	
<i>L. pumila</i> CLC1252	T	A	A	G	A	G	C	G	G	A	T	G	C	A	G	A	G	
<i>L. pumila</i> TWO501	T	A	A	G	A	G	C	G	G	A	T	G	C	A	G	A	G	
<i>Laccaria</i> sp. CLC1625	C	A	A	G	G	G	C	G	G	A	T	G	C	A	T	A	G	
<i>Laccaria</i> sp. CLC1771	C	A	A	G	G	G	C	G	G	A	T	G	C	A	T	A	G	

Table 17, continued.

		<u>Alignment Position</u>															
		591-599	602-604	608	611	617	624	627	628	630	631	635	638	639	640-641	652	658
<u>Taxon</u>																	
<i>L. bicolor</i> CLC1304	CT----TTA	AAG	T	T	C	C	T	G	C	T	G	A	A	--	A	A	
<i>L. bicolor</i> CLC1445	CT----TTA	AAG	T	T	C	C	T	G	C	T	G	A	A	--	A	C	
<i>L. bicolor</i> CLC1469	CT----TTA	AAG	T	T	C	C	T	G	C	T	G	A	A	--	A	A	
<i>L. bicolor</i> CLC1482	CT----TTA	AAG	T	T	C	C	T	G	C	T	G	A	A	--	A	A	
<i>L. bicolor</i> CLC1672	CT----TTA	AAG	T	T	C	C	T	G	C	T	G	A	A	--	A	A	
<i>L. laccata</i> CLC1370	-----	---	A	T	C	T	T	-	-	T	-	T	T	AC	C	A	
<i>L. laccata</i> CLC1603	-----	---	A	T	C	T	T	-	-	T	-	T	T	AC	C	A	
<i>L. laccata</i> CLC1633	-----	---	A	T	C	T	T	-	-	T	-	T	T	AC	C	A	
<i>L. laccata</i> CLC1655	-----	---	A	T	C	T	T	-	-	T	-	T	T	AC	C	A	
<i>L. laccata</i> CLC1724	-----	---	A	T	C	T	C	-	-	G	-	T	T	AC	C	A	
<i>L. montana</i> TWO319	AT----TTA	AAG	A	T	T	C	T	G	C	T	G	A	A	-T	A	A	
<i>L. montana</i> TWO591	AT----TTA	AAG	A	T	T	C	T	G	C	T	G	A	A	-T	A	A	
<i>L. pumila</i> CLC1252	CT----TTA	AAG	A	T	T	C	T	G	C	T	G	A	A	-T	A	A	
<i>L. pumila</i> TWO501	CT----TTA	AAG	A	T	T	C	T	G	C	T	G	A	A	-T	A	A	
<i>Laccaria</i> sp. CLC1625	CT----TTA	AAG	A	C	C	C	T	G	C	T	G	A	A	--	A	A	
<i>Laccaria</i> sp. CLC1771	CT----TTA	AAG	A	C	C	C	T	G	C	T	G	A	A	--	A	A	

*Laccaria bicolor* exhibits no synapomorphic indels, but exhibits synapomorphic SNPs at positions 126, 138, 158, 164, 170, 220, 579 and 608. *Laccaria* sp. is also not characterized by any synapomorphic indels, but exhibits synapomorphic SNPs at alignment positions 47, 80, 581, and 611. *Laccaria montana* exhibits one synapomorphic indel at positions 155-164, and synapomorphic SNPs at positions 116, 233, 451, 590, and 591. *Laccaria pumila* is not characterized by any synapomorphic indels or SNPs, although it possesses a unique pattern of shared nucleotide variation.

Results of the present study are summarized as follows:

- 1) Molecular phylogenetic analysis of rDNA ITS1 / 5.8S / ITS2 sequences supports the distinction of the morphological species *L. bicolor*, *L. laccata* var. *pallidifolia*, *L. montana* and *L. pumila* as delimited by morphological features (Chapter 2). These groupings receive strong bootstrap support.
- 2) In addition, sequence data strongly support the distinction of an unidentified species (*Laccaria* sp.), described in Chapter 2.
- 3) Sensitivity analyses show that tree topology is robust to the deletion of outgroup taxa and exclusion of binary codes indicating presence / absence of indel regions.
- 4) Molecular characterization of the ITS region of the species studied reveals intraspecific genetic variability of  $\leq 1\%$  among alpine collections, with up to 2.4% divergence observed between Colorado alpine and Montana subalpine collections of *L. bicolor*. Interspecific variability ranges from 1.6% (*L. montana* vs. *L. pumila*) to 8.4% (*L. laccata* var. *pallidifolia* vs. subalpine *L. bicolor*).

## Discussion

The present study examined evidence for either supporting or refuting delimitation of species as set forth in the previous chapter by determining whether taxon groups inferred using molecular data could be defined by either morphological synapomorphies or unique combinations of morphological character states. Maximum parsimony phylogenetic analysis of the rDNA ITS region provided strong support for recognition of the morphological species described in Chapter 2. Clades corresponding to the morphological species *L. bicolor*, *L. laccata* var. *pallidifolia*, *L. montana*, *L. pumila*, and *Laccaria* sp. were supported by high bootstrap values and were robust to analytical perturbations related to outgroup selection and recoding or omitting indel regions. Each morphological species group exhibits a number of synapomorphic indels and / or single nucleotide polymorphisms with the exception of *L. pumila*, which is characterized by having a unique combination of variable nucleotide positions but has no unique individual indels or SNPs.

Furthermore, the molecular phylogenetic study assessed morphological traits having apparent systematic importance that can be used to reliably identify *Laccaria* species in the Rocky Mountain alpine zone. The results of this study supported a number of observations made during the morphological taxonomic study.

Distinguishing *L. bicolor* and *L. laccata* var. *pallidifolia* can be difficult due to similar morphology and phenotypic plasticity. *Laccaria bicolor* has generally been distinguished by having violaceous lamellae and a violet tomentum at the base of the stipe (Mueller, 1992). Additionally, the two species can often be distinguished on the

basis of basidiospore morphology, with *L. bicolor* generally having smaller, more broadly ellipsoid (as opposed to subglobose in *L. laccata* var. *pallidifolia*) spores; however, the ranges of both basidiospore size and shape overlap between the two species (Mueller, 1992), and overlap strongly between alpine collections.

The results of the present study indicate that *L. bicolor* can be distinguished from *L. laccata* var. *pallidifolia*, at least in the North American alpine zone, on the basis of basidiocarp macromorphology. *Laccaria bicolor* has basidiocarps with minutely scaly pilei and more robust, rough fibrous-striate, basally enlarged to subclavate stipes. Violet coloration in the lamellae and basal tomentum was only found in one collection (CLC 1482); however, this collection was shown to be closely related to otherwise morphologically similar collections that lacked violet coloration, including the collection CLC 1469 that exhibited no ITS sequence divergence from CLC 1482. These findings indicate that violet pigmentation in the basal tomentum, while useful for identification, may be rare under field conditions in the Rocky Mountain alpine zone and that the lack of this character in *L. bicolor* collections has likely lead to identifying *L. bicolor* specimens as *L. laccata* var. *pallidifolia* (e.g., Lahaie, 1981). *Laccaria bicolor* can be distinguished by production of violet mycelial mats on PDA media, underscoring the value of attempting to obtain tissue cultures from field specimens to facilitate identification. In addition, it has been observed in subalpine collections of *L. bicolor* lacking violet basal mycelia that storing fresh basidiocarps in a plastic covered container under refrigeration often results in new growth of violet mycelia at the base of the stipe (Osmundson, unpublished).

An additional morphotype having small, dark orange to red-brown basidiomata was collected during the course of field studies in Colorado alpine habitats. Originally thought to represent a small form of *L. laccata* var. *pallidifolia* or *L. montana*, these specimens were found to have more broadly ellipsoid and generally more finely echinulate basidiospores than *L. laccata* var. *pallidifolia* and *L. bicolor*, to have smaller and more finely echinulate basidiospores than *L. montana*, and much smaller, less robust basidiocarps than *L. bicolor*. This species is provisionally referred to as *Laccaria* sp. in the previous chapter. This species is supported by high bootstrap values and several synapomorphic single nucleotide polymorphisms as comprising a distinct phylogenetic clade. It appears likely that *Laccaria* sp. represents a distinct and possibly undescribed taxon; however, additional material is needed in order to better characterize this species morphologically and ecologically.

*Laccaria laccata* var. *pallidifolia* is supported in this analysis as a distinct but morphologically variable taxon including both convex and nearly omphaloid pileal forms and exhibiting a wide range in basidiospore size. *Laccaria laccata* var. *pallidifolia* appears to be highly divergent at the molecular level compared to the other species studied, and is supported by numerous synapomorphic indels and SNPs in the ITS region. Morphologically, this species is generally more robust and often (though not always) has a less striate pileus than *L. montana* and *L. pumila*. In addition, *L. laccata* var. *pallidifolia* has basidiospores that are smaller and more subglobose than *L. montana* and *L. pumila*. Though strongly supported as distinct species genetically, *L. laccata* var. *pallidifolia* and *L. bicolor* exhibit a large degree of overlap in basidiospore shape and size. These two

species are distinguishable, at least in the Rocky Mountain alpine zone, by macromorphological differences as previously discussed.

The present study provides strong preliminary support for retaining *L. montana* and *L. pumila* as distinct species as suggested by Mueller (1992), rather than as varieties of a single species varying in number of sterigmata per basidium (4 vs. 2, respectively) (Singer, 1977). Both clades are supported by high bootstrap percentages, and *L. montana* is supported by a synapomorphic indel and several synapomorphic SNPs. While *L. pumila* is not supported in the present analysis by any synapomorphic molecular traits, this taxon exhibits a unique combination of indels and SNPs (each individual indel / SNP is shared by at least one other taxon) when compared to the other species in this study. No variation was discovered between *L. pumila* collections from the southern and central Rocky Mountains. This lack of intraspecific variation as well as the lack of synapomorphic molecular characters might suggest that *L. pumila* is relatively recently evolved; however, the nearly cosmopolitan arctic-alpine-boreal distribution of this species suggests either a long evolutionary history or a rapid dispersal time. It is difficult to come to any firm conclusions in this regard based on the small number of samples analyzed here; future studies using larger sample sizes may uncover variation that was not observed in the present study. The lack of variation between Colorado and Montana populations, presence of synapomorphic indels and SNPs in *L. montana*, and the well-supported clustering of *L. pumila* collections distinct from *L. montana* provide evidence that *L. pumila* and *L. montana* are distinct species rather than 2- and 4-spored variants, respectively, of a single species.

The relative position of clades was found to be robust to changes in outgroups and/or coding of indel regions. However, due to the limited number of taxa included, the present study should be interpreted as providing only an extremely preliminary assessment of species relationships in *Laccaria*. A robust molecular phylogeny of *Laccaria* with wide taxon sampling is necessary in order to better assess infrageneric relationships and discern evolutionary and biogeographic trends.

Levels of interspecific ITS sequence variability were found in this study to be in the range of those observed by Gardes et al. (1991b), although a higher level of divergence between *L. bicolor* and *L. laccata* was observed in the present study (up to 5% in Gardes et al., 1991b, and up to 8.4% in the present study with the inclusion of the subalpine *L. bicolor* collection TWO 752). The level of intraspecific variation in *L. bicolor* in the present study appears to be much lower (0.2%) than the 1-2% observed by Gardes et al. (1991b) when only Colorado alpine collections are considered; however, addition of the Montana subalpine collection TWO 752 results in an observed level of 2.1-2.4 % intraspecific variability. It appears that the levels of interspecific ITS sequence variation observed in the present study are sufficient for resolving species groups in *Laccaria*; however, it is as yet unclear whether robust hypotheses of interspecific relationships can be produced using ITS sequences due to the restricted taxon and geographic sampling employed here. The low level of intraspecific variability observed suggests that population groups may not be discriminated by phylogenetic analyses using ITS sequences. It should be kept in mind, however, that the present study includes only a limited number of samples collected within a relatively restricted geographic and

physiographic region. The genetic variability observed between alpine *Salix*-associated and the subalpine conifer-associated *L. bicolor* collection over a limited geographic area (Colorado to Montana) suggests that the lack of variability may be restricted only to limited geographic and/or physiographic regions. The high resolution of clades observed in the present study indicates that the rDNA-ITS region is a good candidate for use in a molecular phylogenetic study of the entire genus *Laccaria* incorporating broad geographical sampling.

Avenues of future research include the addition of reference specimens, preferably supported by data from mating studies, in order to stabilize the nomenclature assigned to the species groups in the present study, addition of subalpine collections to better assess Rocky Mountain biogeographical patterns, and the use of additional Rocky Mountain alpine collections to further characterize intraspecific genetic variation, assess host associations and further examine the relationship between *L. montana* and *L. pumila*.

## CHAPTER 4

## SYNECOLOGICAL STUDY OF BEARTOOTH PLATEAU MACROMYCETES

Introduction

Although numerous studies have gathered biotic data for macromycetes in arctic and alpine habitats, aspects of the ecology of these arctic-alpine fungi are poorly known. Only a handful of synecological studies have been conducted, and ectomycorrhizal (EM) host associations, which have been the subject of study in forest fungi (e.g., Trappe, 1962), are comparatively little known for arctic-alpine fungi. Documenting links between EM host and fungal species is a first step toward better understanding mycorrhizal specificity phenomena and specific ecosystem functions of fungal species in arctic-alpine habitats.

The use of sampling plots allows the inference of biotic or ecological patterns for a larger area using data collected in smaller, presumably representative, areas. Compared to random sampling, plot-based studies have several benefits, allowing: 1) more accurate measurement of temporal aspects of community composition by pinpointing precise areas for repeated sampling, 2) selection and comparison of areas differing in terms of one or more biotic or abiotic factors (e.g. stand age, plant community type, soil composition, microclimate, etc.), 3) quantification of community parameters such as species diversity and evenness, species abundance and species richness using sporocarps (Schmit et al., 1999; Senn-Irlet & Bieri, 1999) or mycorrhizal root tips from soil cores (Kernaghan,

2001), and 4) estimation of sampling effectiveness using species-effort or species-area curves.

Macromycete ecological studies are usually conducted using sporocarp (i.e., mushroom) surveys. It is important to note that a sporocarp represents only the reproductive structure of an organism that exists primarily obscured from sight in the soil or other substrate; therefore, a number of issues unique to fungi necessitate sampling methods different from those used for plants or lichens. The ephemeral nature of sporocarps, combined with species-specific seasonal periodicity in sporocarp production, makes it necessary to survey plots regularly (Arnolds, 1992; Straatsma et al., 2001). Annual fluctuations in fruiting due to genetic or environmental factors necessitate studies conducted over several years, and some infrequently-fruiting species may be recorded only in years where environmental factors (e.g., abundant rainfall) result in years of uncommonly high sporocarp production (Keck, 2001; Straatsma et al., 2001). The use of molecular identification techniques allows sampling of fungi in the absence of sporocarps, and studies of EM fungal communities indicate that there is poor correspondence between above-ground and below-ground species composition and abundance (e.g., Gardes & Bruns 1996b). Nonetheless, sporocarp surveys hold some distinct advantages for synecological research. Sporocarps are generally conspicuous to the unaided eye, and therefore provide an efficient means of surveying a large area. Additionally, taxonomic classifications for macrofungi are based largely on sporocarp morphology, so sporocarp material can be used to link taxonomic and ecological

information as well as to identify species that are not yet represented in DNA sequence databases.

In addition to these problems, plot studies in alpine habitats must also consider the effect of patchy plant distributions resulting from the vast diversity of microclimates that characterize these ecosystems. On the positive side, such distributions can facilitate the selection of sampling plots containing a single ectomycorrhizal host plant or other parameter of interest. However, the lack of continuous stands of host plants makes small plot sizes necessary and reduces the probability of environmental homogeneity between plots so that a number of possible confounding factors not directly related to host plant species must be considered in association with observed patterns of macrofungal community composition.

Only a small number of plot-based fungal ecology studies have been conducted in arctic-alpine habitats. Lange (1957) used sampling plots to describe fungal species occurrence in relation to arctic plant community types in Greenland. Graf (1994) examined the community structure of macromycetes associated with a single host plant (*Salix herbacea*) in the Swiss Alps, documenting sporocarp abundance, frequency, and periodicity, and relating these characteristics for selected species to microclimate, snowmelt patterns, and soil characteristics. Similarly, Eynard (1977) focused exclusively on the ecology of macromycetes in *Salix herbacea* snowbed communities in the French Alps. Petersen (1977) used sampling plots to conduct repeated sampling in identical locations in Greenland in order to examine the phenology of macromycete sporocarp production in relation to climatic and soil factors. Senn-Irlet (1988) used repeated plot

sampling to compare macromycete communities in silicious and calcareous alpine snowbed communities in the Swiss Alps. LoBue et al. (1994) determined fungal community compositions for vegetation types defined by cluster analysis of plant relevés. Kernaghan and Harper (2001) compared fungal species across an alpine-subalpine ecotone using small plots along transects for collecting soil cores to be used for molecular identification of mycorrhizal species.

The present study differs from those mentioned above by using plots constructed around particular ectomycorrhizal host plants in order to directly compare fungal assemblages between host species. The study of Lange (1957) differs from the present study in that, while the plot design considered ectomycorrhizal host plant species in characterizing vegetation types, it did not attempt to restrict plots to containing a single host species; in addition, most plots in Lange's study were not surveyed more than once due to time constraints. Studies by Graf (1994) and Eynard (1977) differ from the present study in characterizing fungal communities in the context of a single ectomycorrhizal host (*Salix herbacea*), and Senn-Irlet (1988) in characterizing fungal communities within a single community type (*Salix*-rich snowbeds).

Primary objectives of the present study are to 1) document mycorrhizal host-fungal associations, 2) assess the degree of fungal species similarity between host plants in a quantitative manner using a coefficient of overlap, 3) assess methods for alpine plot studies in a continental climate, where patchy plant distributions and lack of moisture can complicate assessment of fungal biodiversity using sporocarp studies, and 4) more

rigorously assess host-symbiont associations as part of the study of Rocky Mountain alpine *Laccaria* species.

Ectomycorrhizal fungi in arctic-alpine habitats are associated with a number of shrubs, dwarf woody plants and herbaceous species (see Chapter 1). On the Beartooth Plateau, major host species include the shrub willows *Salix glauca* and *S. planifolia*, dwarf willows *S. arctica* and *S. reticulata*, the recumbant mat plant *Dryas octopetala*, and the bog birch *Betula glandulosa*. The sampling design used in the present study (using single hosts whenever possible) allowed the ability to link mycorrhizal fungi to hosts with more confidence and to quantitatively compare sampled areas using similarity indices based on species presence/absence data. In addition, repeated sampling during each season and over a number of seasons allowed observation of annual differences in species composition and fruiting phenology.

The present study also seeks to assess methods for alpine plot studies in a continental climate, where patchy plant distributions and lack of moisture can complicate assessment of fungal biodiversity using sporocarp studies. In particular, the size, number and location of sampling plots are considered in terms of their ability to estimate ectomycorrhizal species diversity and EM host ranges when compared to species totals derived from the combination of plot-based and general (non-plot-based) sampling over the course of the study, as well as compared to results from other arctic-alpine plot studies.

The plot data collected in this study were used to perform a more rigorous assessment of host-symbiont associations of Rocky Mountain alpine *Laccaria* species in

order to contribute to the better understanding of biogeographic and ecological patterns in this important EM genus, and to facilitate selection of host-fungus systems for potential use in alpine restoration projects. Host associations have not been previously assessed in combination with a detailed biosystematic study of the arctic-alpine species of *Laccaria*.

## Materials and Methods

### Description of Collecting Sites

Collection of macromycete sporocarps was conducted at five primary sites on the Beartooth Plateau (Figure 21): Site 1, near the source of Quad Creek, located in Carbon County, Montana; Site 2, Highline Trailhead on the Montana / Wyoming border; Site 3, near Frozen Lake, located in Park County, Wyoming; Site 4, north of Gardner Headwall, located in Park County, Wyoming, and Site 5, consisting of a group of solifluction terraces located to the east of Site 4 in Park County, Wyoming. Collection plots were constructed at Sites 1-4, with collecting at Site 5 restricted to general collecting.

### Plot Selection and Specimen Collection

Sixteen 12.6 m<sup>2</sup> (2 m diameter) circular sampling plots were distributed among Sites 1-4 (Table 18). Plots were selected to contain particular ectomycorrhizal (EM) host plants, isolated from other EM plants wherever possible. In several cases, a particular EM host could not be located on a given site except in proximity to other EM plants. In these cases, additional EM plant species on the plots are noted. Selection of species as probable host plants was based on previous studies that examined roots for mycorrhizae



Table 18. Location and description of Beartooth Plateau alpine sampling plots.

<u>Site</u>	<u>Plot</u>	<u>EM Plant Species</u>	<u>Elevation</u>	<u>GPS Coordinates</u>
Quad Creek Carbon Co , MT	1	<i>Dryas octopetala</i>	3016 m	45° 01.444 N, 109° 24 486 W
	2	<i>Salix planifolia</i>	3004 m	45° 01.392 N, 109° 24.526 W
	3	<i>Salix glauca</i>	3002 m	45° 01.333 N, 109° 24.551 W
	4	<i>Betula glandulosa</i> , <i>Salix planifolia</i> , <i>S reticulata</i>	2994 m	45° 01.427 N, 109° 24.597 W
Highline Trailhead Carbon Co., MT/Park Co., WY	1	<i>Salix glauca</i>	3089 m	45° 00.716 N, 109° 23 724 W
	2	<i>Salix glauca</i>	3093 m	45° 00.356 N, 109° 24 387 W
	3	<i>Salix reticulata</i>	3062 m	45° 00.131 N, 109° 24.377 W
	4	<i>Dryas octopetala</i> , <i>Salix reticulata</i>	3063 m	45° 00.129 N, 109° 24.344 W
	5	<i>Salix planifolia</i>	3064 m	45° 00.135 N, 109° 24.380 W
	6	<i>Dryas octopetala</i> , <i>Salix reticulata</i>	3061 m	45° 00.129 N, 109° 24 338 W
	7	<i>Salix reticulata</i>	3097 m	44° 59.994 N, 109° 24.564 W
Frozen Lake Park Co , WY	1	<i>Salix arctica</i>	3194 m	44° 57.929 N, 109° 29.057 W
	2	<i>Salix reticulata</i>	3195 m	44° 57.932 N, 109° 28.998 W
	3	<i>Salix arctica</i>	3193 m	44° 57 852 N, 109° 28.857 W
Gardner Headwall Park Co , WY	1	<i>Salix arctica</i>	3228 m	44° 58.532 N, 109° 27.326 W
	2	<i>Salix arctica</i>	3171 m	44° 58 685 N, 109° 27.230 W

(See Chapter 1). Most of the putative EM plants on the Beartooth Plateau were subsequently examined for the presence of ectomycorrhizae; all putative hosts examined were confirmed to be ectomycorrhizal (Eddington & Cripps, manuscript in preparation). Host plants selected were *Salix reticulata*, *S. arctica*, *S. planifolia*, *S. glauca*, *Dryas octopetala*, and *Betula glandulosa* (Fig. 22). Other plants (forbs only) in addition to the EM host plant were documented for each plot (Appendix D). Plots were surveyed every 1-3 weeks during the field season (mid-July to early September) between the years 1999-2002 (Appendix E). Sporocarps collected from plots were photographed, described, then preserved by warm air drying on an electric food dehydrator. Specimens were deposited in the Montana State University, Bozeman, herbarium (MONT), Fungal Section. Specimens collected on plots are listed in Appendix F.



Figure 22. Beartooth Plateau EM host plants. a. *Salix reticulata*; b. *S. arctica*; c. *S. glauca*; d. *Dryas octopetala*; e. *Betula glandulosa*.

#### Characterization of Sampling Plots By Site

Four sampling plots were constructed at Site 1, located near the source of Quad Creek in Carbon County, Montana (Figure 23). Plot 1 contains the prostrate, mat-forming shrub *Dryas octopetala* L. isolated from other EM plants. Plots 2 and 3 contain the shrub willows *Salix planifolia* Pursh and *S. glauca* L. (= *S. brachycarpa* Nutt. in this study), respectively. Plot 4 contains the bog birch *Betula glandulosa* Michx., the only birch shrub found on the Beartooth Plateau during the course of the present study. Due to the rarity of *B. glandulosa* on the Beartooth Plateau, a plot was constructed to include this

individual even though it occurs directly adjacent to *Salix planifolia* and the dwarf willow *S. reticulata* L. All Site 1 plots were constructed in July, 1999 prior to macromycete fruiting.

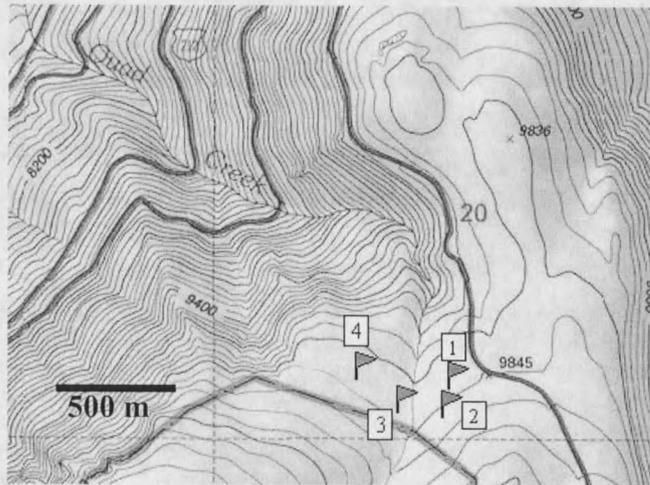


Figure 23. Site 1 ("Quad Creek") detail map showing locations of sampling plots.

Seven plots were constructed at Site 2, located near the Highline Trailhead on the Montana / Wyoming state line (Figure 24). Plots 1 and 2 contain *S. glauca* and Plot 3 contains *S. reticulata*. Plots 4 and 6 were selected for the presence of *D. octopetala*, but also include *S. reticulata*. Plot 5 is located in a moist area adjacent to Wyoming Creek, and contains *S. planifolia*. Plot 7 contains the EM host *S. reticulata*. Plots 1-3 were constructed in 1999, and 4-7 constructed in 2000.



Figure 24. Site 2 ("Highline Trailhead") detail map showing locations of sampling plots.

Three plots were constructed at Site 3, located near Frozen Lake in Park County, Wyoming (Figure 25). Plots 1 and 3 contain the dwarf willow *Salix arctica* Pall.; Plot 2 contains the dwarf willow *S. reticulata*. All Site 3 plots were constructed in July, 1999, prior to macromycete fruiting.

Two plots were constructed at Site 4, located near Gardner Headwall in Park County, Wyoming (Figure 25). Both plots contain *S. arctica* as the EM host, and were constructed in 2000.

Site 5 consists of a series of solifluction terraces located slightly east of Site 4 in Park County, Wyoming. No plots were constructed at this site; however, general collecting was conducted on a regular basis.

In addition to employing plot-based collection for documenting and comparing host-fungal associations, the present study includes specimens from general collecting outside of plots in order to provide a means of evaluating the ability of the

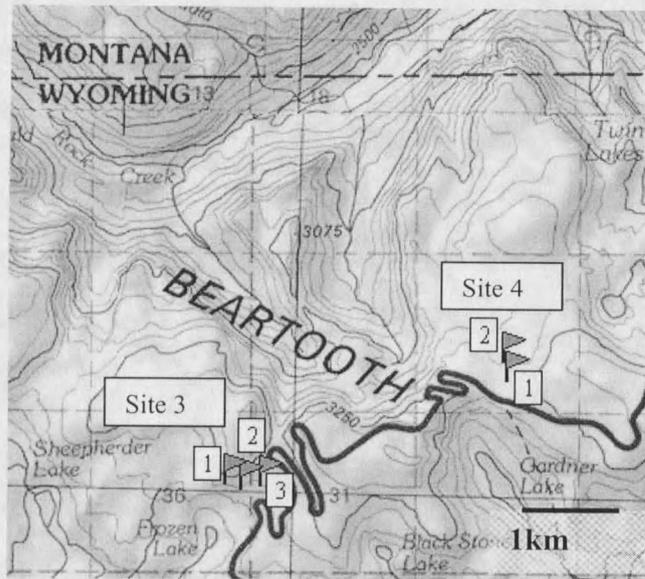


Figure 25. Site 3 (“Frozen Lake”) and 4 (“Gardner Headwall”) detail map showing locations of sampling plots.

present number and size of plots to adequately represent the fungal communities associated with particular EM host plants and adequately represent the host ranges of particular fungal species. Ectomycorrhizal host plants in close proximity were documented for specimens obtained through general collection; in addition, each Beartooth Plateau collection was assigned a location code to represent the certainty that the collection occurred with a single host plant. Location code 1 was assigned to collections occurring within a sampling plot; location code 2 was assigned to collections occurring outside of plots but in a pure stand of an EM host plant; location code 3 was assigned to collections occurring outside of plots and in a mixed EM host stand. Only collections having location codes 1 and 2 are included in the present study.

### Fungal Community Characterization and Similarity Comparison

Macrofungal species were tabulated by host plant and plot number. In order to compare fungal community composition across hosts, the present study uses the overlap coefficient  $a / a + b + c$ , where  $a$  is the number of species shared between two samples,  $b$  is the number of species in the first sample, and  $c$  is the number of species in the second sample (Rossman et al., 1998). In the present study, the samples compared consisted of the number of species occurring with a particular EM host plant (i.e., the pooled species lists for the individual plots representing each host species). An additional list of species having location code 2, along with the EM host plant, was prepared and used to evaluate the ability of the plot design (i.e., size and number) to accurately represent fungal communities associated with particular host plants and host ranges for particular fungal species. Fungal collections were recorded by date collected in order to examine temporal trends in macromycete fruiting.

### Results

#### Fungal Species Recorded in Sampling Plots

A total of 31 basidiomycete and 2 ascomycete species were collected on sampling plots (Table 19). Of the basidiomycete species, 21 are ectomycorrhizal species and 10 saprobic.

Table 19. Species collected on Beartooth Plateau sampling plots, with EM host plant listed. Additional host plants associated with specimens not collected on sampling plots (i.e., location code 2) are provided where applicable. \* Plot also contains *S. planifolia* and *S. reticulata*. † Non-mycorrhizal species.

<u>Species</u>	<u>EM Hosts on Plot</u>	<u>Additional Hosts</u>
<u>Amanitaceae:</u>		
<i>Amanita absarokensis</i> Cripps & Miller (unpub.)	<i>S. reticulata</i> , <i>S. glauca</i>	<i>S. arctica</i> , <i>S. planifolia</i>
<u>Cortinariaceae:</u>		
<i>Cortinarius absarokensis</i> Moser & McKnight	<i>S. glauca</i>	<i>S. planifolia</i>
<i>Cortinarius</i> cf. <i>anomalous</i> Fr.	<i>S. glauca</i>	
<i>Cortinarius anomoli</i> group (aff. <i>epsomiensis</i> P D Orton)	<i>S. glauca</i>	
<i>Cortinarius favrei</i> Moser ex Henderson	<i>S. reticulata</i>	<i>S. arctica</i>
<i>Cortinarius tenebricus</i> Favre	<i>D. octopetala</i>	
<i>Cortinarius</i> sp. #1 (Telamonia)	<i>B. glandulosa</i> *	
<i>Cortinarius</i> sp. #2 (Telamonia)	<i>S. glauca</i> , <i>B. glandulosa</i> *	
<i>Cortinarius</i> sp. #3 (Telamonia)	<i>S. reticulata</i>	
<i>Cortinarius</i> sp. #4 (Telamonia)	<i>S. glauca</i>	
<i>Dermocybe cinnamomeolutea</i> (Orton) Moser	<i>S. glauca</i> , <i>B. glandulosa</i> *	<i>S. planifolia</i>
<i>Hebeloma</i> cf. <i>mesophaeum</i> (Pers.:Fr.) Quel.	<i>S. planifolia</i> , <i>S. glauca</i>	
<i>Inocybe giacomii</i> (group) Favre	<i>S. reticulata</i> , <i>B. glandulosa</i> *	
<i>Inocybe</i> aff. <i>salicis</i> Kuehn	<i>S. reticulata</i>	
<u>Entolomataceae:</u>		
<i>Entoloma</i> sp. #1 †	<i>S. planifolia</i>	
<i>Entoloma</i> sp. #2 †	<i>S. planifolia</i>	
<i>Entoloma</i> sp. #3 †	<i>B. glandulosa</i> *	
<u>Tricholomataceae:</u>		
<i>Clitocybe</i> sp. #1 †	<i>B. glandulosa</i> *	
<i>Clitocybe</i> sp. #2 †	<i>D. octopetala</i>	
<i>Laccaria montana</i> Singer	<i>S. arctica</i> , <i>S. planifolia</i>	<i>S. glauca</i>
<i>Laccaria pumila</i> Fayod	<i>S. arctica</i> , <i>S. glauca</i>	<i>S. planifolia</i>
<i>Lepista</i> aff. <i>irina</i> (Fr.) Bigelow †	<i>S. reticulata</i>	
<i>Mycena citrinomarginata</i> Gill †	<i>S. planifolia</i>	
<i>Mycena</i> sp. †	<i>S. glauca</i>	
<i>Omphalina rivulicola</i> (Favre) Lamoure †	<i>S. arctica</i>	
<i>Rickenella</i> cf. <i>fibula</i> (Bull.:Fr.) Raithehl †	<i>B. glandulosa</i> *	
<u>Russulales:</u>		
<i>Lactarius glyciosmus</i> (Fr.:Fr.) Fr.	<i>B. glandulosa</i> *	
<i>Lactarius</i> cf. <i>nanus</i> Favre	<i>S. arctica</i>	
<i>Russula pascua</i> (Moll & Schaff.) Kuehn.	<i>S. arctica</i> , <i>S. planifolia</i>	
<i>Russula norvegica</i> D.A. Reid	<i>S. glauca</i> , <i>B. glandulosa</i> *	<i>S. planifolia</i>
<u>Boletales:</u>		
<i>Leccinum</i> cf. <i>rotundifoliae</i> (Sing.) Smith, Theirs & Watl	<i>B. glandulosa</i> *	
<u>Ascomycetes</u>		
<i>Bryoglossum gracile</i> (Karst.) Redhead †	<i>S. glauca</i>	
Unid. Ascomycete (pale yellow, stalked cup)	<i>S. planifolia</i>	

Table 20 shows species of macrofungi listed by plot. Numbers of species per plot ranged from zero (Site 1 - Plot 1, 4-1, 4-2) to 11 (Site 1- Plot 3). Because basidiomycetes, and EM basidiomycetes in particular, were the focus of the present study, only these species were used for further analysis. Basidiomycete species collected on sampling plots are presented in a relevé table showing EM host plant, site-plot designation, and number of collections made (Table 21). A frequency histogram constructed using this data shows that the majority of species (17 of 31) were only collected once on plots during the four years of the study (Fig. 26), with frequency ranging from one to thirteen collections.

Table 20. Beartooth Plateau alpine macrofungi listed by plot. Ectomycorrhizal host species listed. \* Denotes non-mycorrhizal species.

<u>Site</u>	<u>Plot</u>	<u>EM Host</u>	<u>Fungal Species</u>
1	1	<i>Dryas octopetala</i>	(none)
1	2	<i>Salix planifolia</i>	<i>Entoloma</i> sp. #2* <i>Laccaria montana</i> <i>Russula pascua</i>
1	3	<i>Salix glauca</i>	<i>Cortinarius absarokensis</i> <i>Dermocybe cinnamomeolutea</i> <i>Cortinarius</i> sp. #4 <i>Cortinarius anomali</i> group (aff. <i>epsomienensis</i> Orton) <i>Hebeloma</i> cf. <i>mesophaeum</i> <i>Cortinarius</i> cf. <i>anomolous</i> <i>Laccaria pumila</i> <i>Bryoglossum gracile</i> * <i>Mycena</i> sp. (small, gray)* <i>Russula norvegica</i> <i>Cortinarius</i> sp. #2
1	4	<i>Betula glandulosa</i> , <i>Salix reticulata</i> , <i>S. planifolia</i>	<i>Clitocybe</i> sp #1*  <i>Cortinarius</i> sp. #2 <i>Dermocybe cinnamomeolutea</i>

Table 20, continued

<u>Site</u>	<u>Plot</u>	<u>EM Host</u>	<u>Fungal Species</u>
1	4	<i>Betula glandulosa</i> , <i>Salix reticulata</i> , <i>S. planifolia</i> (continued)	<i>Inocybe giacomii</i> group  <i>Entoloma</i> sp #3* <i>Lactarius glyciosmus</i> <i>Leccinum</i> cf. <i>rotundifoliae</i> <i>Rickenella</i> cf. <i>fibula</i> * <i>Russula norvegica</i> <i>Cortinarius</i> sp. #1
2	1	<i>Salix glauca</i>	<i>Amanita absarokensis</i>
2	2	<i>Salix glauca</i>	<i>Amanita absarokensis</i> <i>Cortinarius absarokensis</i>
2	3	<i>Salix reticulata</i>	<i>Amanita absarokensis</i> <i>Cortinarius favrei</i> <i>Inocybe giacomii</i> group
2	4	<i>Dryas octopetala</i> , <i>Salix reticulata</i>	<i>Cortinarius tenebricus</i>
2	5	<i>Salix planifolia</i>	Ascomycete (pale yellow, stalked cup)* <i>Entoloma</i> sp #1* <i>Hebeloma</i> cf. <i>mesophaeum</i> <i>Mycena citrinomarginata</i> *
2	6	<i>Dryas octopetala</i> , <i>Salix reticulata</i>	<i>Clitocybe</i> sp. #2*
2	7	<i>Salix reticulata</i>	<i>Amanita absarokensis</i>
3	1	<i>Salix arctica</i>	<i>Laccaria montana</i> <i>Russula pascua</i>
3	2	<i>Salix reticulata</i>	<i>Cortinarius favrei</i> <i>Cortinarius</i> sp. #3 <i>Inocybe</i> aff. <i>salicis</i> <i>Lepista</i> aff. <i>Irina</i> *
3	3	<i>Salix arctica</i>	<i>Laccaria montana</i> <i>Laccaria pumila</i> <i>Lactarius</i> cf. <i>namus</i> <i>Omphalina rivulicola</i> *
4	1	<i>Salix arctica</i>	(none)
4	2	<i>Salix arctica</i>	(none)

Table 21. Releve table for Beartooth Plateau alpine macrofungi showing number of times collected for each fungal species, arranged by ectomycorrhizal host species and site-plot designation.

Fungal Species	EM Host Species:																
	Site-Plot:	<i>Salix reticulata</i>			<i>Salix arctica</i>				<i>Salix planifolia</i>		<i>Salix glauca</i>			<i>Betula glandulosa</i>	<i>Dryas octopetala</i>		
		2-3	2-7	3-2	3-1	3-3	4-1	4-2	1-2	2-5	2-1	2-2	1-3	1-4**	1-1	2-4*	2-6*
<i>Inocybe giacomii</i> group	1												1				
<i>Cortinarius favrei</i>	1		2														
<i>Cortinarius</i> sp. #3			1														
<i>Inocybe</i> aff. <i>salicis</i>			1														
<i>Lepista</i> aff. <i>irina</i> ***			1														
<i>Lactarius</i> cf. <i>nanus</i>					1												
<i>Omphalina rivulicola</i> ***					1												
<i>Laccaria montana</i>				1	1			2									
<i>Russula pascua</i>				1				4									
<i>Laccaria pumila</i>					1							6					
<i>Amanita absarokensis</i>	3	1								3	1						
<i>Hebeloma</i> cf. <i>mesophaeum</i>									1			2					
<i>Entoloma</i> cf. <i>sericeum</i>								1									
<i>Entoloma</i> sp.									1								
<i>Mycena citrinomarginata</i> ***									1								
<i>Cortinarius</i> sp. #4												1					
<i>Cortinarius</i> aff. <i>epsomiensis</i>												1					
<i>Cortinarius</i> cf. <i>anomolus</i>												1					
<i>Mycena</i> sp.***												1					
<i>Cortinarius absarokensis</i>											1	3					
<i>Dermocybe cinnamomeolutea</i>												12	1				
<i>Russula norvegica</i>												3	1				
<i>Cortinarius</i> sp. #2												1	1				
<i>Clitocybe</i> sp. #1***													2				
<i>Entoloma</i> sp. ( <i>sericeum</i> ?)***													3				
<i>Lactarius glyciosmus</i>													9				
<i>Leccinum</i> cf. <i>rotundifoliae</i>													1				

Table 21., continued

	EM Host Species:																
	Site-Plot:	<i>Salix reticulata</i>			<i>Salix arctica</i>				<i>Salix planifolia</i>		<i>Salix glauca</i>			<i>Betula glandulosa</i>	<i>Dryas octopetala</i>		
		2-3	2-7	3-2	3-1	3-3	4-1	4-2	1-2	2-5	2-1	2-2	1-3	1-4**	1-1	2-4*	2-6*
<i>Rickenella cf. fibula</i> ***														1			
<i>Cortinarius</i> sp. #1														1			
<i>Cortinarius tenebricus</i>																1	
<i>Clitocybe</i> sp. #2***																	1

\* Mixed plot including *Salix reticulata*

\*\* Mixed plot including *Salix reticulata* and *Salix planifolia*

\*\*\* Non-mycorrhizal species. All other species are ectomycorrhizal.

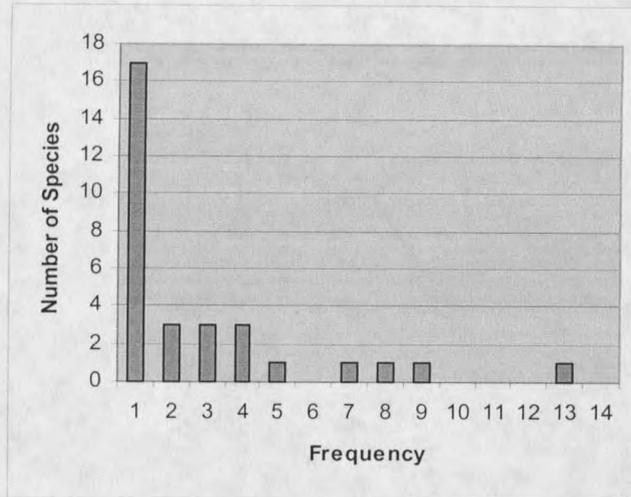


Figure 26. Collection frequency histogram for Beartooth Plateau alpine sampling plots for the years 1999-2002.

#### Species Overlap Calculation

Percent species overlap was calculated for EM fungi for all pairwise combinations of the EM hosts *Salix arctica*, *S. reticulata*, *S. planifolia*, *S. glauca*, *Dryas octopetala* and *Betula glandulosa* (Table 22). Total species numbers for each host species were as follows (number of EM species in parentheses): *S. arctica*, 5 (4); *S. reticulata*, 6 (5); *S. planifolia*, 6 (3); *S. glauca*, 11 (10); *D. octopetala*, 2 (1); *B. glandulosa*, 10 (7).

The two dwarf willows, *S. arctica* and *S. reticulata*, shared 0 species and therefore had a calculated similarity coefficient of 0%. *S. arctica* exhibited 40% similarity with *S. planifolia*, 8% with *S. glauca* and 0% with *B. glandulosa*. *S. reticulata* exhibited 0% similarity with *S. planifolia*, 7% with *S. glauca*, and 9% with *B. glandulosa*.

The two shrub willows, *S. glauca* and *S. planifolia*, shared 1 species and therefore had a calculated similarity coefficient of 33.3%. *S. glauca* exhibited 0% (0/3)

Table 22. Species overlap matrix for Beartooth Plateau sampling plots. Similarity values were calculated for ectomycorrhizal fungi only, using the overlap coefficient  $a/a+b+c$  (Rossman et al., 1998). Coefficients are expressed as percent overlap.

	<i>S. reticulata</i>	<i>S. arctica</i>	<i>S. planifolia</i>	<i>S. glauca</i>	<i>B. glandulosa</i> *	<i>D. octopetala</i>
<i>S. reticulata</i>	---	---	---	---	---	---
<i>S. arctica</i>	0	---	---	---	---	---
<i>S. planifolia</i>	0	40	---	---	---	---
<i>S. glauca</i>	7	8	8	---	---	---
<i>B. glandulosa</i> *	9	0	0	21	---	---
<i>D. octopetala</i>	0	0	0	0	0	---

\* Plot also includes *S. reticulata* and *S. planifolia*

similarity with *B. glandulosa*. *S. planifolia* exhibited 42.9 (3/7) similarity with *B. glandulosa*. *Dryas octopetala* shared 0 species with all other hosts, and therefore had a calculated similarity coefficient of 0% in all pairwise comparisons.

#### Addition of Non-Plot Collections

The inclusion of specimens obtained by general collecting outside of sampling plots and denoted with location code 2 (i.e., in pure stands of an EM host plant) resulted in the addition of seven species not recorded in sampling plots (Table 23). Inclusion of non-plot collections corresponding to species represented in sampling plots resulted in the expansion of host ranges for the EM species *Amanita absarokensis*, *Cortinarius favrei*, *Dermocybe cinnamomeolutea*, and *Russula norvegica* (see Table 19, column 3).

Table 23. Additional Beartooth Plateau alpine macromycete species collected with single EM hosts but outside of sampling plots.

<u>Species</u>	<u>EM Host</u>	<u>Collection</u>
<i>Inocybe praetervisa</i> Quelet	<i>S. reticulata</i>	TWO 567
<i>Inocybe</i> cf. <i>bulboissima</i> (Kuhner) Bon	<i>S. reticulata</i>	TWO 426
<i>Inocybe dulcamara</i> (Alb. & Schw.) Kummer (group)	<i>Salix</i> shrub	TWO 651
<i>Inocybe leiocephala</i> Stuntz	<i>S. arctica</i> , <i>D. octopetala</i> / <i>S. reticulata</i>	TWO 571,407
<i>Inocybe</i> sp	<i>S. planifolia</i>	TWO 351
<i>Hebeloma</i> aff. <i>alpinum</i> (Favre) Bruchet	<i>S. planifolia</i>	TWO 406
<i>Russula nana</i> Fr.	<i>S. reticulata</i>	TWO 514
<i>Entoloma alpicola</i>	<i>S. reticulata</i> , <i>S. arctica</i>	TWO 437, 568, 569

### Fruiting Phenology

Temporal trends in basidiocarp production were examined by recording collection dates for all specimens collected. The number of collections obtained on sampling plots is tabulated by week in Table 24. Annual patterns were examined by plotting the number of collections for each collecting date (Figure 27).

Table 24. Number of macromycete collections obtained on Beartooth Plateau alpine sampling plots per week, field seasons 1999-2002.

Week	Number of Collections				Total
	1999	2000	2001	2002	
July 1-7					0
July 8-14			1		1
July 15-21			5	1	6
July 22-28			5		5
July 29-Aug. 4		6	5	11	22
Aug. 5-11	8		7		15
Aug. 12-18			11	11	22
Aug. 19-25	9	4			13
Aug. 26-Sept. 1			3	6	9
Sept. 2-8					0
Sept 9-15					0
Sept. 16-22					0

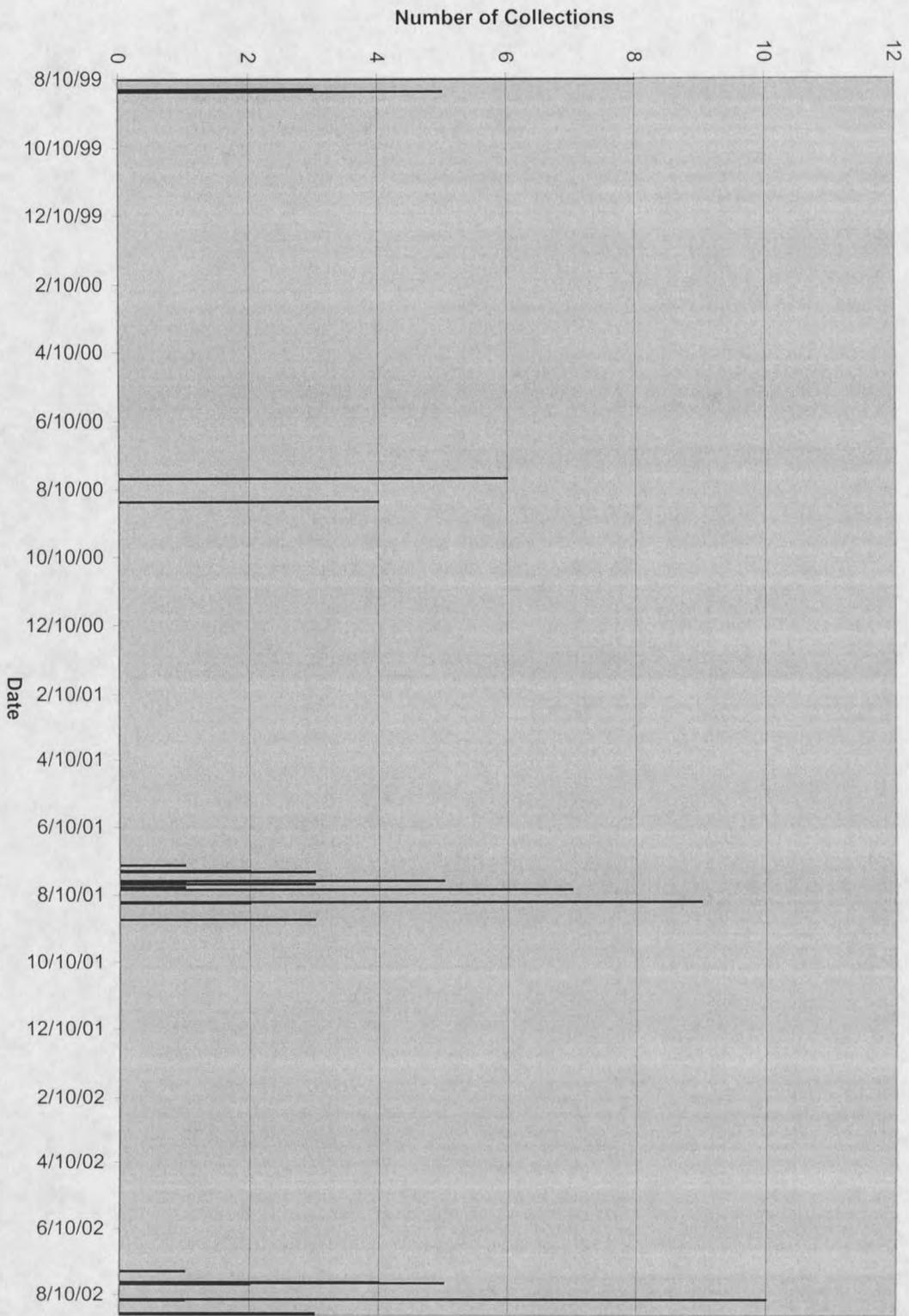


Figure 27. Graph showing number of macromycete collections by date obtained from Beartooth Plateau alpine sampling plots, field seasons 1999-2002.

For the two years (2001 and 2002) for which there was a higher frequency of collecting excursions, data show that macromycete fruiting on the Beartooth Plateau occurs primarily from mid-July to early September, with peak fruiting occurring in mid-August.

Fruiting phenology was examined for 3 selected species that were collected greater than 5 times on a particular plot (Table 25). *Dermocybe cinnamomeolutea* on Site 1, Plot 3 fruited in all four years of the study (1999-2002), and appears to produce basidiocarps in all but the earliest and latest parts of the fruiting season. *Lactarius glyciosmus* on Site 1, Plot 4 fruited in all years except 2000, producing basidiocarps from late July to early September. Additional collection data show that *L. glyciosmus* was also collected in the same location in 1997 and 1998 (Cripps, unpublished data). *Laccaria pumila* on Site 1, Plot 3 fruited in all four years of the study, though with lower frequency than *D. cinnamomeolutea* and *L. glyciosmus*. Fruiting was generally observed in the middle part of the fruiting season.

#### Host Association Patterns in Rocky Mountain Alpine *Laccaria*

Plot collection data were used to confirm host associations for *Laccaria* species occurring on the Beartooth Plateau. These data confirmed associations of *L. pumila* with *S. arctica* (Plot 3-3) and *S. glauca* (Plot 1-3), and *L. montana* with *S. arctica* (Plots 3-1 and 3-3) and *S. planifolia* (Plot 1-2). Data from the taxonomic study of Rocky Mountain alpine *Laccaria* species (Chapter 2) were used to construct a table showing host

associations by state (Table 26). These data provide evidence for additional associations of *L. pumila* with *S. reticulata* and *S. planifolia*, and *L. montana* with *S. glauca*.

Table 25. Fruiting phenology of selected Beartooth Plateau macromycetes, *Dermocybe cinnamomeolutea*, *Lactarius glyciosmus*, and *Laccaria pumila*. Species were selected that were collected at least five times within the same sampling plot during the course of the study.

Species	Site-Plot	1999		2000			2001						
		8/10	8/19	7/17	7/31	8/21	7/13	7/19	7/23	7/28	7/31	8/5	8/16
<i>D. cinnamomeolutea</i>	1-3	X	X		X	X		X		X		X	X
<i>L. glyciosmus</i>	1-4	X	X						X	X	X	X	X
<i>L. pumila</i>	1-3	X				X			X	X	X		

Species	Site-Plot	2001		2002			
		8/20	9/1	7/17	7/29	8/14	8/27
<i>D. cinnamomeolutea</i>	1-3			X	X	X	
<i>L. glyciosmus</i>	1-4		X	X	X		
<i>L. pumila</i>	1-3						X

## Discussion

The plot design in the present study was used to simultaneously allow a quantitative approach to examining ectomycorrhizal (EM) host-fungus associations while addressing the issue of conducting plot-based studies in a habitat characterized by patchy distributions of host plants. The results generated by this study are intended to provide confirmation of specific host-fungal associations, provide a preliminary comparison of EM fungal communities between different host species, and assess the capacity of a set of small sampling plots to accurately represent species diversity and EM host ranges for alpine macrofungi in a continental climate.

Table 26. Host associations for Rocky Mountain alpine *Laccaria* species, arranged by state. Numbers of collections obtained with each host are listed for Colorado and Beartooth Plateau (Montana/Wyoming) alpine field sites. Only collections found with a single host plant are listed.

Species	EM Host Species:																
	<i>Salix reticulata</i>		<i>Salix arctica</i>		<i>Salix planifolia</i>		<i>Salix glauca</i>		<i>Salix</i> sp. (shrub)		<i>Salix</i> sp. (dwarf)		<i>Betula glandulosa</i>		<i>Dryas octopetala</i>		
	State:	CO	MT/WY	CO	MT/WY	CO	MT/WY	CO	MT/WY	CO	MT/WY	CO	MT/WY	CO	MT/WY		
<i>Laccaria bicolor</i>		2	0	2	0	4	0	0	0	2	0	0	0	0	0	0	
<i>Laccaria laccata</i>		1	0	0	0	0	0	1	0	0	0	0	0	2	0	1	0
<i>Laccaria montana</i>		0	0	1	2	0	7	0	4	0	0	0	0	0	0	0	0
<i>Laccaria pumila</i>		2	0	5	2	2	7	0	7	0	4	0	1	0	0	0	0
<i>Laccaria</i> sp.		0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0

A total of 33 species were collected in sampling plots: 2 Ascomycota and 31 Basidiomycota. Of the basidiomycete species, 21 (68%) were EM and 10 (32%) saprobic species. A preliminary list of Beartooth Plateau species by Cripps et al. (2002), combined with additional species records generated during the present study, documents 65 species: 38 EM (58%) and 27 saprobic (42%) species are documented. Plots were therefore effective in representing 48% of the total species (31/65), 55% of the EM species (21/38) and 37% of the saprobic species (10/27). Considering the small number and size of plots used in the present study, the design used appears to be an effective and efficient method for sample collection.

The plot design in this study was particularly effective for the collection of EM species: 68% of the species collected on plots were EM taxa; EM species represent 58% of the estimated Beartooth species total. This result confirms the initial hypothesis that constructing sampling plots centered on EM host plants is an effective strategy for collecting EM fungi in a habitat characterized by patchy host distributions. Species found on plots represented most of the common EM taxa, with the notable exceptions of the commonly collected species *Russula nana* and *Entoloma alpicola*. The plot design was less effective in representing saprobic species: 32% of the species collected on plots are saprobes, compared to 42% saprobic species in the estimated Beartooth Plateau species total. Several genera -- *Mycena*, *Clitocybe*, and *Entoloma* -- are represented. However, a number of important saprobic genera are not represented on plots; these genera include *Agaricus*, *Agrocybe*, *Calvatia*, *Galerina*, *Gymnopus* and *Omphalina*. This result is likely to be a function of habitat preference and sampling design. *Clitocybe*, *Mycena*, and

*Entoloma*, although saprobic, often fruit near *Salix* or *Betula* shrubs. In contrast, *Agaricus*, *Agrocybe*, *Calvatia*, and *Gymnopus* exhibit a preference for open grassy areas and were therefore selected against by the design of the plot study (plots sited on EM host plants). Saprobic and basidiolichen *Omphalina* species are also often collected in more open habitats (with adequate moss and moisture for the saprobic species). *Galerina* species share habitat preferences with *Mycena* species and *Bryoglossum gracile*, i.e., mossy areas with shrub cover, that were collected on plots; it is therefore likely that the lack of *Galerina* records from the sampling plots is more a function of the small size and/or number of sampling plots rather than a matter of habitat sampling. In addition to the genera mentioned above, saprobic species requiring specialized substrates (e.g., animal dung) would be difficult to represent on sampling plots, and basidiomycete wood decomposers are rare in arctic-alpine habitats.

Evidence for several specific host-fungal associations was generated in this study and supported by data for collections obtained by general (non-plot) collecting: *Lactarius glyciosmus* with *Betula glandulosa*, *Leccinum cf. rotundifoliae* with *Betula glandulosa*, *Cortinarius tenebricus* with *Dryas octopetala*, and *Dermocybe cinnamomeolutea* with *Salix* shrubs. The relationships between *Cortinarius favrei* and dwarf *Salix*, and *Cortinarius absarokensis* and *Salix* shrubs, that appear in this study represent a circular argument, as host association was used as a criterion in distinguishing these nearly morphologically-identical species. Moser and McKnight (1987) described *C. absarokensis* (with the type collection originating from the Beartooth Plateau) as microscopically very similar to *C. favrei*, and distinguished the two species on

basidiocarp size (*C. absarokensis* being significantly larger) and host preference, with *C. favrei* associated with dwarf willows and *C. absarokensis* associated with shrub willows. Similarly, in Colorado collections, the two species appear to be distinguishable by size, with *C. favrei* basidiocarps mostly having pilei under 3.5 cm in diameter, and *C. absarokensis* having pilei greater than 3.5 cm in diameter (C. Cripps, unpublished data). On the Beartooth Plateau, collections distinguished by host association could in many cases not be distinguished by basidiocarp size; therefore, the host associations reported here might not be an accurate assessment. Furthermore, additional examination of the taxonomic relationship between these taxa, particularly using molecular data, seems warranted (Peintner et al., 2002). Few species were documented with *Dryas octopetala*. Examination of *D. octopetala* roots from Beartooth collections revealed a high incidence of mycorrhizal colonization by *Cenococcum geophilum* (E. Campbell and C. Cripps, unpublished), an ascomycete that does not produce sporocarps; this condition is possibly a function of the dry conditions encountered during the period of this study, as *Cenococcum* colonization has been demonstrated to increase under drought conditions (Worley & Hacksaylo, 1959), or might reflect a particular *Dryas* ecotype occurring on the Beartooth Plateau.

A majority of species (17 of 31 basidiomycetes) were found only once on the sampling plots. Similarly, Senn-Irlet (1988) reported that two-thirds of species in *Salicetum retuso-reticulatae* calciphilous snowbed communities fruited only once during a five-year study, and Keck (2001) reported that 65% of macromycete species collected in Rocky Mountain subalpine field plots fruited only in the wettest year of the study.

Yearly fluctuations in fruiting as well as the occurrence of transient or unique (appearing only one year) species are widely reported in macromycete sporocarp studies (Arnolds, 1992; Straatsma et al., 2001). The length of the present study was too restricted to favor any particular explanation for this finding, and the high percentage of species occurring in the frequency class of one collection underscores the need for sporocarp studies to be conducted over several years (Arnolds, 1992). The years comprising the present study were characterized by below average rainfall, which would be expected to result in underestimating species diversity and host ranges.

Overlap coefficients between host species ranged from 0% between *Salix reticulata* and *S. arctica*, *S. reticulata* and *S. planifolia*, *S. planifolia* and *Betula glandulosa*, *S. arctica* and *Betula glandulosa*, and between *Dryas octopetala* and all other species, to 21% between *Salix glauca* and *Betula glandulosa*, and 40% between *Salix planifolia* and *S. arctica*. This overlap between *S. planifolia* and *S. arctica* results from the presence of *Laccaria montana* and *Russula pasqua*. Additional Rocky Mountain records indicate that both of these species are also associated with *Salix reticulata* and *S. glauca*, and that the overlap exhibited between *S. planifolia* and *S. arctica* is therefore the result of EM fungal species that are more general *Salix* associates. Additional collection data for *Dermocybe cinnamomeolutea* and *Russula norvegica*, the two identified overlapping taxa between *S. glauca* and *B. glandulosa*, document the common occurrence of these species with *Salix* hosts; therefore, it is quite likely that the high species overlap between *S. glauca* and *B. glandulosa* is a result of the presence of *Salix planifolia* and *S. reticulata* in the *Betula* plot. However, the results of the present study

cannot preclude the possibility that the overlapping species are associated with *Betula*; examination of mycorrhizal *Betula* roots would aid in clarifying this relationship.

Addition of species obtained by general (i.e., non-plot) collecting have a large effect on coefficient values. Addition of collections found outside of sampling plots but in pure stands of an EM host plant (i.e., location code 2) both increases the total number of species and the host ranges of species that also occur within the sampling plots. Addition of expanded host range has a tendency to increase overlap coefficients; for example, *Salix* pairs exhibiting 0% overlap in the present study would show significant increases if this additional host information were to be included. However, addition of unique non-plot species records would have the opposite effect of reducing overlap coefficients. A more accurate picture of species overlap between hosts would require incorporating both plot and non-plot data. The change in overlap that occurs when non-plot collections, especially in terms of host range, are considered indicates that the number and/or size of sampling plots used in the present study produced insufficient data to accurately interpret patterns of host specificity.

Addition of non-plot, location code 2 collections results in a 20% increase in the number of EM species with unambiguous host association data, including the commonly collected species *Entoloma alpicola* and *Russula nana*. Underrepresented genera on the Beartooth plots include *Inocybe* (5 spp. having location code 2), and presumably *Cortinarius* (numerous location code 2 collections have insufficient data for positive identification).

Data on fruiting phenology show that the fruiting season on the Beartooth Plateau is largely concentrated into a period from mid-July to early September. The species *Dermocybe cinnamomeolutea*, *Laccaria pumila*, and *Lactarius glyciosmus* were frequently collected and fruited every year of the study (every year except 2000 for *L. glyciosmus*), and may represent species that are reproductively active during dry seasons; in addition, all were collected under shrubs that tend to retain soil moisture. The study occurred during an El Niño cycle and resultant four-year drought in the central Rocky Mountain region. Sporocarp studies in a continental climate can be strongly influenced by precipitation patterns. In a study of macromycetes in subalpine forests by Keck (2001), 92 % (including 65% of the species total represented by species unique to a single year) of the total species reported were found to occur in one year (1997) with above average precipitation, compared to 18-31% in drier years. Because there were no wet years during the course of the present study, it is likely that the species numbers reported here underestimate true species richness. In addition, the length of the present study, while comparable to most previous arctic-alpine plot studies (summarized below), is of a short duration for a sporocarp-based plot study. In a 25-year study of a Swiss forest plot, Straatsma et al. (2001) noted unique species each year, underscoring an important temporal component that plot studies must take into account.

A comparison of the present study with previous arctic-alpine plot studies indicates that the number of taxa per unit plot area is comparable to that of other alpine studies, whereas the number of taxa per unit plot area is much lower overall in alpine than in arctic studies (Table 27). The study of Senn-Irlet (1988) reports a much higher

species per unit area value compared to the other studies; this may reflect the location of this study in snow-bed communities, known to be rich in macromycete species diversity, or possibly underestimation of plot size given in Table 27. Given this similarity, it seems reasonable that increasing the number or area of plots would be effective in increasing the number of taxa represented. The patchy distribution of EM host species in alpine areas precludes the approach of increasing plot size if maintaining single-host plots is a goal of the research design, so increasing the number of small plots would be a better approach; however, an increase in plot number would require more personnel, time and collecting resources than were available in the present study.

Table 27. Comparison of the present study with previous arctic-alpine plot studies.

Location	Plots	Total m <sup>2</sup>	Years	Excursions	Taxa	Taxa/m <sup>2</sup>	Reference
<b>Arctic</b>							
Greenland	30	30	3	51	25	0.83	Petersen (1977)
Greenland	84	282	1	1-2	128	0.45	Lange (1957)
<b>Alpine</b>							
Alps	7	230	1	13	39	0.17	Eynard (1977)
Alps	6	182*	3-5	10-16	79	0.43	Senn-Irlet (1988)
Alps	6	596	3	47	69	0.12	Graf (1994)
Beartooth Plateau	16	202	4	18	33	0.16	present study

\*Minimum total plot size Plot size listed in publication as 30-100 m<sup>2</sup>, with two specific plots listed as 30 and 32 m<sup>2</sup>, therefore, total plot area may be up to 462 m<sup>2</sup>, in which taxa/m<sup>2</sup> would equal 0.17.

The goals of using the plot study in conjunction with the taxonomic study of Rocky Mountain *Laccaria* species was twofold: to confirm EM host associations for Beartooth Plateau taxa, and to use the taxonomic and host data from the systematic study (Chapters 2-3) as a check on whether the plot data reflects the true range of host

associations for the Beartooth Plateau taxa. The plot study confirmed associations of *L. pumila* with *S. arctica* and *S. glauca*, and *L. montana* with *S. arctica* and *S. planifolia*. However, additional data show *L. pumila* associated with *S. reticulata* and *S. planifolia*, and *L. montana* with *S. glauca*; this provides further evidence that plot size/number was insufficient to represent true host ranges.

In summary, the use of a small number of small plots centered on EM host species was effective for representing species diversity; an increase in the number of plots as well as constructing plots in open, grass- or moss-covered areas would result in better representation. The plot surveys were useful for confirming a number of host-fungus associations and indicated that most of the species collected are not restricted to a single EM host species. However, the plot data obtained underrepresent the observed host range of the EM fungi collected, therefore making similarity comparisons unreliable. Presumably, increased replication of host-specific plots would help to rectify this situation.

This is the first reported plot-based study of alpine macromycetes in the Rocky Mountains, and the data reported here may serve as a baseline for research on the ecology of alpine macromycetes in a continental climate.

LITERATURE CITED

- Aanen, D.K., T.W. Kuyper, T. Boekhout, and R.F. Hoekstra, 2000. Phylogenetic relationships in the genus *Hebeloma* based on ITS1 and 2 sequences, with special emphasis on the *Hebeloma crustuliniforme* complex. *Mycologia* 92 (2): 269-281.
- Abbott, R.J., L.C. Smith, R.I. Milne, R.M.M. Crawford, K. Wolff, and J. Balfour, 2000. Molecular analysis of plant migration and refugia in the arctic. *Science* 289: 1343-1346.
- Albee, S.R., G.M. Mueller, and B.R. Kropp, 1996. Polymorphisms in the large intergenic spacer of the nuclear ribosomal repeat identify *Laccaria proxima* strains. *Mycologia* 88 (6): 970-976.
- Amicucci, A., A. Zambonelli, G. Giomaro, L. Potenza, and V. Stocchi, 1998. Identification of ectomycorrhizal fungi of the genus *Tuber* by species-specific ITS primers. *Molecular Ecology* 7: 273-277.
- Aragno, M., 1981. Responses of microorganisms to temperature. In: A. Pirson and M.H. Zimmermann, eds. *Physiological Plant Ecology I: Responses to the Physical Environment*. *Encyclopedia of Plant Physiology* n.s. vol. 12A. Berlin: Springer-Verlag. pp. 339-369.
- Arnolds, E. 1992. The analysis and classification of fungal communities with special reference to macrofungi. In: W. Winterhoff, ed. *Fungi in Vegetation Science. Handbook of Vegetation Science* 19(1). Dordrecht, Netherlands: Kluwer Academic Publishers. pp. 7-47.
- Banik, M.T. and H.H. Burdsall Jr., 1998. Assessment of compatibility among *Armillaria cepistipes*, *A. sinapina*, and North American biological species X and XI, using culture morphology and molecular biology. *Mycologia* 90(5): 798-805.
- Banik, M.T., T.J. Volk, and H.H. Burdsall Jr., 1996. *Armillaria* species of the Olympic Peninsula of Washington State, including confirmation of North American biological species XI. *Mycologia* 88(3): 492-496.
- Barry, R.G. and C.C. Van Wie, 1974. Topo- and microclimatology in alpine areas. In J.D. Ives and R.G. Barry, eds. *Arctic and Alpine Environments*. London: Methuen & Co. pp. 73-83.
- Baura, G., T.M. Szaro, and T.D. Bruns, 1992. *Gastrospilus laricinus* is a recent derivative of *Suillus grevillei*: molecular evidence. *Mycologia* 84: 592-597.
- Bendiksen, E., T.E. Brandrud, K. Bendiksen, and H. Lindström, 1993. A study of the *Cortinarius helobius* complex, with special emphasis on arctic-alpine material. In: O. Petrini and G.A. Laursen, eds. *Arctic and Alpine Mycology* vols. 3-4, Berlin: J. Cramer. pp. 3-15.

- Bendiksen, K. and E. Ohenoja, 2002. A preliminary list of basidiomycetes (excl. wood-inhabiting Aphyllophorales) in the studied areas in the arctic-alpine zones of Fennoscandia. 7<sup>th</sup> International Mycological Congress, Oslo, Norway, August, 2002.
- Berbee, M.L. and J.W. Taylor, 1993. Dating the evolutionary radiations of the true fungi. *Canadian Journal of Botany* 71: 1114-1127.
- Berbee, M.L. and J.W. Taylor, 1995. From 18S ribosomal sequence data to evolution of morphology among the fungi. *Canadian Journal of Botany* 73 (Supplement 1): S677-S683.
- Besl, H. and A. Bresinsky, 1997. Chemosystematics of Suillaceae and Gomphidiaceae (suborder Suillineae). *Plant Systematics and Evolution* 206: 223-242.
- Billings, W.D., 1973. Arctic and alpine vegetations: similarities, differences, and susceptibility to disturbance. *BioScience* 23 (12): 697-704.
- Billings, W.D., 1974a. Arctic and alpine vegetation: plant adaptations to cold summer climates. *In*: J.D. Ives and R.G. Barry, eds. *Arctic and Alpine Environments*. London: Methuen & Co. pp. 403-443.
- Billings, W.D., 1974b. Adaptations and origins of alpine plants. *Arctic and Alpine Research* 6 (2): 129-142.
- Billings, W.D. and L.C. Bliss, 1959. An alpine snowbank environment and its effects on vegetation, plant development, and productivity. *Ecology* 40 (3): 388-397.
- Billings, W.D. and H.A. Mooney, 1968. The ecology of arctic and alpine plants. *Cambridge Philosophical Society Biological Reviews* 43: 481-529.
- Bills, S., G.K. Podila, and S. Hiremath, 1999. Genetic engineering of an ectomycorrhizal fungus *Laccaria bicolor* for use as a biological control agent. *Mycologia* 91 (2): 237-242.
- Binder, M. and D.S. Hibbett, 2002. Higher-level phylogenetic relationships of homobasidiomycetes (mushroom-forming fungi) inferred from four rDNA regions. *Molecular Phylogenetics and Evolution* 22 (1): 76-90.
- Blair, R., ed., 1996. *The Western San Juan Mountains, their geology, ecology, and human history*. Boulder: University of Colorado Press. 416 pp.
- Bledsoe, C., P. Klein, and L.C. Bliss, 1990. A survey of mycorrhizal plants on Truelove Lowland, Devon Island, Northwest Territories, Canada. *Canadian Journal of Botany* 68: 1848-1856.

- Boekhout, T., J. Stalpers, S.J.W. Verduin, J. Rademaker and M. E. Noordeloos, 2002. Experimental taxonomic studies in *Psilocybe* sect. *Psilocybe*. *Mycological Research* 106(11): 1251-1261.
- Bon, M. 1987. Quelques récoltes mycologiques de la zone alpine au 7ème convegnò di micologia – Fiera di Primiero (Italie). *Micologia Italiana* 17(3): 267-270.
- Borgen, T., S.A. Elborne and H. Knudsen, 2000. A preliminary checklist of the basidiomycota of Greenland. Draft presented at 6<sup>th</sup> International Symposium on Arctic-Alpine Mycology, Kangerlussuaq & Sisimiut, Greenland, August 11-21, 2000.
- Braun-Blanquet, J., 1932. *Plant Sociology*. G.D. Fuller and H.S. Conard, trans. New York: McGraw-Hill. 439 pp.
- Bruns, T.D., 2001. ITS reality. *Mycological Society of America Inoculum* 52 (6): 2-3.
- Bruns, T.D., R. Fogel, T.J. White, and J.D. Palmer, 1989. Accelerated evolution of a false-truffle from a mushroom ancestor. *Nature* 339: 140-142.
- Bruns, T.D., T. White, and J. Taylor, 1991. Fungal molecular systematics. *Annual Review of Ecology and Systematics* 22: 525-564.
- Bruns, T.D., R. Vilgalys, S.M. Barns, D. Gonzalez, D.S. Hibbett, D.J. Lane, L. Simon, S. Stickel, T.M. Szaro, W.G. Weisburg, and M.L. Sogin, 1992. Evolutionary relationships within the fungi: analysis of nuclear small subunit rRNA sequences. *Molecular Phylogenetics and Evolution* 1: 231-241.
- Buschena, C.A., R.L. Doudrick, and N.A. Anderson, 1992. Persistence of *Laccaria* spp. as ectomycorrhizal symbionts of container-grown black spruce. *Canadian Journal of Forestry Research* 22: 1883-1887.
- Chapin III, F.S. and C. Körner, 1995. Patterns, causes, changes and consequences of biodiversity in arctic and alpine ecosystems. *In*: F.S. Chapin III and C. Körner, eds. *Arctic and Alpine Biodiversity: Patterns, Causes, and Ecosystem Consequences*. Berlin: Springer-Verlag, pp. 313-320.
- Christiansen, M.P., 1941. Studies in the larger fungi of Iceland. *The Botany of Iceland*, 3(2): 191-227
- Cline, M.L., R.C. France, and C.P.P. Reid, 1987. Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures. *Canadian Journal of Botany* 65: 869-875.

- Coetzee, M.P.A., B.D. Wingfield, T.A. Coutinho and M.J. Wingfield, 2000. Identification of the causal agent of *Armillaria* root rot of *Pinus* species in South Africa. *Mycologia* 92(4): 777-785.
- Coetzee, M.P.A., B.D. Wingfield, T.C. Harrington, J. Steimel, T.A. Coutinho, and M.J. Wingfield, 2001. The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* 10 (2): 387-396.
- Cripps, C.L., 1995. Ecological and taxonomic studies of mycorrhizal fungi associated with aspen. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA. 224 pp.
- Cripps, C. L., 1997. The genus *Inocybe* in Montana aspen stands. *Mycologia* 89(4): 670-688.
- Cripps, C. L., 2001. Mycorrhizae of Aspen Forests: ecology and potential application. Sustaining Aspen in Western Landscapes: Proceedings of the Symposium on Western Aspen Forests, Grand Junction, CO, June 2000. pp. 285-298.
- Cripps, C.L., 2002. Alpine macrofungi of North America (Rocky Mountains). 7<sup>th</sup> International Mycological Congress, Oslo, Norway, August 11-17, 2002 (abstract).
- Cripps, C.L. and E. Horak, 1999. Arctic and alpine mycota, Rocky Mountains, USA: a preliminary report. 16<sup>th</sup> International Botanical Congress. St. Louis, MO, USA, August 1-7, 1999 (abstract).
- Cripps, C.L. and O.K. Miller Jr., 1993. Ectomycorrhizal fungi associated with aspen on three sites in the north-central Rocky Mountains. *Canadian Journal of Botany* 71: 1414-1420.
- Cripps, C.L., E. Horak, and T. Osmundson, 2001. Rocky Mountain alpine project: documenting agarics above treeline. Mycological Society of America annual meeting, Salt Lake City, UT, August 25-29, 2001 (abstract).
- Cripps, C.L., E. Horak, and T. Osmundson, 2002. Arctic alpine mycota (Agaricales), Rocky Mountain tundra, USA: cold climate mushrooms above treeline. Mycological Society of America annual meeting, Corvallis, OR, June 23-26, 2002 (abstract).
- Debaud, J.C., R. Pepin, and G. Bruchet, 1981. Etude des ectomycorhizes de *Dryas octopetala*. Obtention de synthèses mycorhiziennes et de carpophores d'*Hebeloma alpinum* et *H. marginatum*. *Canadian Journal of Botany* 59: 1014-1020.
- de la Bastide, P.Y., B.R. Kropp, and Y. Piché, 1994. Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. *New Phytologist* 127: 547-556.

de la Bastide, P.Y., B.R. Kropp, and Y. Piché, 1995a. Population structure and mycelial phenotypic variability of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) Orton. *Mycorrhiza* 5: 371-379.

de la Bastide, P.Y., B.R. Kropp, and Y. Piché, 1995b. Mechanisms for the development of genetically variable mycorrhizal mycelia in the ectomycorrhizal fungus *Laccaria bicolor*. *Applied and Environmental Microbiology* 61(10): 3609-3616.

Dhillon, S.S., 1994. Ectomycorrhizae, arbuscular mycorrhizae, and *Rhizoctonia* sp. of alpine and boreal *Salix* spp. in Norway. *Arctic and Alpine Research* 26 (3): 304-307.

Dighton, J., J.M. Poskitt, and D.M. Howard, 1986. Changes in occurrence of basidiomycete fruit bodies during forest stand development: with specific reference to mycorrhizal species. *Transactions of the British Mycological Society* 87 (1): 163-171.

Doudrick, R.L. and N.A. Anderson, 1989. Incompatibility factors and mating competence of two *Laccaria* spp. (Agaricales) associated with black spruce in northern Minnesota. *Phytopathology* 79: 694-700.

Eddington, L.H. and C.L. Cripps, 2003. Mycorrhizal status of selected alpine plants on the Beartooth Plateau along the Montana-Wyoming border: a contribution on mycorrhizae in arctic-alpine habitats. Manuscript in preparation.

Edwards, K., C. Johnstone, and C. Thompson, 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research* 19(6): 1349.

Evenson, V.S., 1997. *Mushrooms of Colorado and the Southern Rocky Mountains*. Englewood, Colorado: Westcliffe Publishers. 224 pp.

Eynard, M., 1977. Contribution a l'etude ecologique des Agaricales des groupements a *Salix herbacea*. These d'Etat, Lyon. 202 pp. as cited in Graf, 1994.

Favre, J., 1955. Les champignons superieurs de la zone alpine du Parc National Suisse. *Ergebn. Wissensch. Unters. Schweiz. Nat.* 5 (N.F.) 33: 1-212.

Fiore-Donno, A.-M. and F. Martin, 2001. Populations of ectomycorrhizal *Laccaria amethystina* and *Xerocomus* spp. show contrasting colonization patterns in a mixed forest. *New Phytologist* 152: 533-542.

Fries, N., 1983. Spore germination, homing reaction, and intersterility groups in *Laccaria laccata* (Agaricales). *Mycologia* 75: 221-227.

Fries, N. and G.M. Mueller, 1984. Incompatibility systems, cultural features and species circumscriptions in the ectomycorrhizal genus *Laccaria* (Agaricales). *Mycologia* 76 (4): 633-642.

Gardes, M. and T. Bruns, 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118.

Gardes, M. and T. Bruns, 1996a. ITS-RFLP matching for identification of fungi. In: J.P. Clapp, ed. *Methods in Molecular Biology, Vol. 50: Species Diagnostics Protocols: PCR and Other Nucleic Acid Methods*. Totowa, NJ: Humana Press, Inc. pp. 177-186.

Gardes, M. and T. Bruns, 1996b. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Canadian Journal of Botany* 74: 1572-1583.

Gardes, M. and A. Dahlberg, 1996. Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytologist* 133: 147-157.

Gardes, M., J.A. Fortin, G.M. Mueller, and B.R. Kropp, 1990. Restriction fragment length polymorphisms in the nuclear ribosomal DNA of four *Laccaria* spp.: *L. bicolor*, *L. laccata*, *L. proxima*, and *L. amethystina*. *Phytopathology* 80: 1312-1317.

Gardes, M., G.M. Mueller, J.A. Fortin, and B.R. Kropp, 1991a. Mitochondrial DNA polymorphisms in *Laccaria bicolor*, *L. laccata*, *L. proxima*, and *L. amethystina*. *Mycological Research* 95 (2): 206-216.

Gardes, M., T.J. White, J.A. Fortin, T.D. Bruns, and J.W. Taylor, 1991b. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Canadian Journal of Botany* 69: 180-190.

Gherbi, H., C. Delaruelle, M.-A. Selosse, and F. Martin, 1999. High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150-year-old beech forest. *Molecular Ecology* 8: 2003-2013.

Gillman, L.S. and O.K. Miller, Jr., 1977. A study of the boreal, alpine, and arctic species of *Melanoleuca*. *Mycologia* 69: 927-951.

Grabherr, G., M. Gottfried, A. Gruber, and H. Pauli, 1995. Patterns and current changes in alpine plant diversity. In F.S. Chapin & C. Korner, eds. *Arctic and Alpine Biodiversity*. Ecological Studies, Vol. 113. Berlin: Springer-Verlag. pp. 167-182.

Graf, F., 1994. Ecology and sociology of macromycetes in snow-beds with *Salix herbacea* L. in the alpine Valley of Radont (Grisons, Switzerland). *Dissertationes Botanicae* 235. Berlin-Stuttgart: J. Cramer. 242 p.

- Graf, F., 1997. Ectomycorrhiza in alpine soil bioengineering. Actes du Deuxieme Colloque Ecologie et Biogeographie Alpines, La Thuile, Italy, pp. 335-342.
- Graf, F. and I. Brunner, 1995. Alpine dwarf willow and its ectomycorrhizal partners: a potential system for alpine ski slope restoration? Proceedings of the 11<sup>th</sup> High Altitude Revegetation Workshop. Fort Collins: Colorado State University, pp. 214-223.
- Grossnickle, S.C. and C.P.P. Reid, 1982. The use of Ectomycorrhizal conifer seedlings in the revegetation of a high-elevation mine site. Canadian Journal of Forest Research 12: 354-361.
- Grossnickle, S.C. and C.P.P. Reid, 1983. Ectomycorrhiza formation and root development patterns of conifer seedlings on a high-elevation mine site. Canadian Journal of Forest Research 13: 1145-1158.
- Gulden, G., 1987. The genus *Galerina* on Svalbard. In: G.A. Laursen, J.F. Ammirati and S.A. Redhead, eds. Arctic and Alpine Mycology II. New York: Plenum Press. pp. 177-204.
- Gulden, G., 1996. Fungal life on the arctic archipelago of Svalbard. *McIlvainea* 12 (2): 4-20.
- Gulden, G. and K.M. Jenssen, 1988. Arctic and alpine fungi 2. Oslo: Soppkonsulentent. 58pp.
- Gulden, G. and A.-E. Torkelsen, 1996. Part 3: Fungi I. Basidiomycota: Agaricales, Gasteromycetales, Aphyllophorales, Exobasidiales, Dacrymycetales and Tremellales. In: A. Elvebakk and P. Prestrud, eds. A catalogue of Svalbard plants, fungi, algae and cyanobacteria. Norsk Polarinstitutt Skrifter 198. pp. 173-206.
- Gulden, G., S. Dunham, and J. Stockman, 2001. DNA studies in the *Galerina marginata* complex. *Mycological Research* 105 (4): 432-440.
- Hallgrímsson, H. 1981. Preliminary account of the Icelandic species of Tricholomataceae. *Acta Bot. Isl.* 6: 29-41.
- Hallgrímsson, H. 1998. Checklist of Icelandic fungi V: Agarics. Náttúrufræ istofnun Íslands.
- Haselwandter, K. and D.J. Read, 1980. Fungal associations of roots of dominant and sub-dominant plants in high-alpine vegetation systems with special reference to mycorrhiza. *Oecologia (Berl.)* 45: 57-62.

Henkel, T.W., M.C. Aime, and S.L. Miller, 2000. Systematics of pleurotoid Russulaceae from Guyana and Japan, with notes on their ectomycorrhizal status. *Mycologia* 92(6): 1119-1132.

Henrion, B., F. Le Tacon, and F. Martin, 1992. Rapid identification of genetic variation of ectomycorrhizal fungi by amplification of ribosomal RNA genes. *New Phytologist* 122: 289-298.

Hibbett, D.S., 1992. Ribosomal RNA and fungal systematics. *Transactions of the Mycological Society of Japan* 33: 533-556.

Hibbett, D.S., 2001. Shiitake mushrooms and molecular clocks: historical biogeography of *Lentinula*. *Journal of Biogeography* 28 (2): 231-241.

Hibbett, D.S., Y. Fukumasa-Nakai, A. Tsuneda, and M.J. Donoghue, 1995. Phylogenetic diversity in shiitake inferred from nuclear ribosomal DNA sequences. *Mycologia* 87 (5): 618-638.

Hibbett, D.S., E.M. Pine, E. Langer, G. Langer, and M.J. Donoghue, 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proceedings of the National Academy of Sciences of the United States of America* 94(22): 12002-12006.

Hibbett, D.S., L.-B. Gilbert, and M.J. Donoghue, 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* 407: 506-508.

Hillis, D.M. and M.T. Dixon, 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *The Quarterly Review of Biology* 66 (4), 411-453.

Høiland, K., 1976. A comparison of two sand-dwelling *Laccaria*, *L. maritima* and *L. trullisata*. *Norwegian Journal of Botany* 23: 79-82.

Høiland, K. and A. Holst-Jensen, 2000. *Cortinarius* phylogeny and possible taxonomic implications of ITS rDNA sequences. *Mycologia* 92 (4): 694-710.

Horak, E. and O.K. Miller Jr., 1992. *Phaeogalera* and *Galerina* in arctic-subarctic Alaska (USA) and the Yukon territory (Canada). *Canadian Journal of Botany* 70: 414-433.

Horak, E., O.K. Miller Jr., and C.L. Cripps, 2002. Arctic-alpine agarics and boletes (Basidiomycota) past, present and future. 7<sup>th</sup> International Mycological Congress, Oslo, Norway, August 11-17, 2002. (abstract).

Horton, T.R. and T.D. Bruns, 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10: 1855-1871.

- Hughes, K.W. and R.H. Petersen, 2001. Apparent recombination or gene conversion in the ribosomal ITS region of a *Flammulina* (Fungi, Agaricales) hybrid. *Molecular Biology and Evolution* 18 (1): 94-96.
- Hughes, K.W., T.L. Toyohara, and R.H. Petersen, 1998. DNA sequence and RFLP analysis of *Pleurotopsis longinqua* from three disjunct populations. *Mycologia* 90(4): 595-600.
- Hughes, K.W., R.H. Petersen, J.E. Johnson, J.-M. Moncalvo, R. Vilgalys, S.A. Redhead, T. Thomas, and L.L. McGhee, 2001. Infragenic phylogeny of *Collybia s.str.* based on sequences of ribosomal ITS and LSU regions. *Mycological Research* 105 (2): 164-172.
- Imai, S., 1938. Studies on the Agaricaceae of Hokkaido I. *Journal of the Faculty of Agriculture of the Hokkaido Imperial University Sapporo* 43 (1): 89-92.
- Ingold, C.T., 1982. Resistance of certain basidiomycetes to freezing. *Transactions of the British Mycological Society* 79 (3): 554-556.
- Ives, J.D., 1974a. Permafrost. In *Arctic and Alpine Environments*. J.D. Ives and R.G. Barry, eds. London: Methuen & Co. pp. 159-194.
- Ives, J.D., 1974b. Biological refugia and the nunatak hypothesis. In *Arctic and Alpine Environments*. J.D. Ives and R.G. Barry, eds. London: Methuen & Co. pp. 605-636.
- Ives, J.D., 1974c. Small-scale examples (I): the impact of motor vehicles on the tundra environments. In *Arctic and Alpine Environments*. J.D. Ives and R.G. Barry, eds. London: Methuen & Co. pp. 907-924.
- James, T.Y., J.-M. Moncalvo, S. Li, and R. Vilgalys, 2001. Polymorphism at the ribosomal DNA spacers and its relation to breeding structure of the widespread mushroom *Schizophyllum commune*. *Genetics* 157: 149-161.
- Jeanmougin, F., J.D. Thompson, M. Gouy, D.G. Higgins and T.J. Gibson, 1998. Multiple sequence alignment with Clustal X. *Trends in Biochemical Sciences* 23: 403-5.
- Johnson, J., 1999. Phylogenetic relationships within *Lepiota* sensu lato based on morphological and molecular data. *Mycologia* 91(3): 443-458.
- Johnson, P.L. and W.D. Billings, 1962. The alpine vegetation of the Beartooth Plateau in relation to cryopedogenic processes and patterns. *Ecological Monographs* 32(2): 105-135.
- Jumpponen, A., J.M. Trappe, and E. Cázares, 1999. Ectomycorrhizal fungi in Lyman Lake Basin: a comparison between primary and secondary successional sites. *Mycologia* 91 (4): 575-582.

Kalamees, K. and M. Vaasma, 1993. Mycobiota of alpine and subalpine sites of Kamchatka. *In*: O. Petrini and G.A. Laursen, eds. Arctic and Alpine Mycology vols. 3-4, Berlin: J. Cramer. pp.121-131.

Kallio, P. and E. Kankainen, 1964. Notes on the macromycetes of Finnish Lapland and adjacent Finnmark. *Ann. Univ. Turku A*, II:32. (Rep. Kevo Subarctic Sta. 1): 178-235.

Keck, J.H., 2001. Macrofungi of the altitudinal gradient, northern Rocky Mountains. M.S. Thesis, Montana State University, Bozeman. 73pp.

Kerhaghan, G., 2001. Ectomycorrhizal fungi at tree line in the Canadian Rockies II. Identification of ectomycorrhizae by anatomy and PCR. *Mycorrhiza* 10: 217-229.

Kernaghan, G. and R.S. Currah, 1998. Ectomycorrhizal fungi at tree line in the Canadian Rockies. *Mycotaxon* 69: 39-80.

Kernaghan, G. and K.A. Harper, 2001. Community structure of ectomycorrhizal fungi across an alpine/subalpine ecotone. *Ecography* 24: 181-188.

Klironomos, J.N. and M.M. Hart, 2001. Animal nitrogen swap for plant carbon. *Nature* 410: 651-652.

Kobayasi, Y., N. Hiratsuka, R.P. Korf, K. Tubaki, K. Aoshima, M. Soneda, and J. Sugiyama, 1967. Mycological studies of the Alaskan arctic. Annual Report of the Institute for Fermentation, Osaka 3: 1-138.

Kobayasi, Y., K. Tubaki and M. Soneda, 1968. Enumeration of the higher fungi, moulds and yeasts of Spitsbergen. *Bulletin of the Natural Science Museum Tokyo* 11(1): 33-80.

Kobayasi, Y., N. Hiratsuka, Y. Otani, K. Tubaki, S. Udagawa, J. Sugiyama and K. Konno, 1971. Mycological studies of the Angmagssalik region of Greenland. *Bulletin of the Natural Science Museum Tokyo* 14: 1-96.

Kohn, L. and E. Stasovski, 1990. The mycorrhizal status of plants at Alexandra Fiord, Ellesmere Island, Canada, a high arctic site. *Mycologia* 82 (1): 23-35.

Körner, C., 1995. Alpine plant diversity: a global survey and functional interpretations. *In*: F.S. Chapin & C. Körner, eds. Arctic and Alpine Biodiversity. Ecological Studies, Vol. 113. Berlin: Springer-Verlag. pp. 45-62.

Körner, C., 1999. Alpine plant life: functional plant ecology of high mountain ecosystems. Berlin: Springer-Verlag. 338 pp.

Kornerup, A. and J.H. Wanscher, 1967. Methuen Handbook of Colour, 2nd edition. London: Methuen Co 243 pp + 30 plates.

Kretzer, A., Y. Li, T. Szaro, and T.D. Bruns, 1996. Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: phylogenetic and taxonomic implications. *Mycologia* 88 (5): 776-785.

Kropp, B.R., 1997. Inheritance of the ability for ectomycorrhizal colonization of *Pinus strobus* by *Laccaria bicolor*. *Mycologia* 89(4): 578-585.

Krueger, D., M. Binder, M. Fischer, and H. Kreisel, 2001. The Lycoperdales: a molecular approach to the systematics of some gasteroid mushrooms. *Mycologia* 93 (5): 947-957.

Kühner, R., 1984. Some mainlines of classification in the gill fungi. *Mycologia* 76: 1059-1074.

Kühner, R. & D. Lamoure, 1986. Catalogue des Agaricales (Basidiomycetes) de la zone alpine du Parc Nationale de la Vanoise et des régions limitrophes. *Trav. Sci. Parc Nat. Vanoise* 15: 103-187.

Lahaie, D.G., 1981. The genus *Laccaria* in the boreal forest of eastern Canada. M.S. Thesis, University of Toronto. 211 pp.

Lamoure, D., M. Lange and P. Milan Petersen, 1982. Agaricales found in the Godhavn area, W. Greenland. *Nordic Journal of Botany* 2: 85-90.

Lange, M., 1955. Macromycetes II: Greenland Agaricales. *Meddelelser om Grønland* 147: 1-69.

Lange, M., 1957. Macromycetes III: Greenland Agaricales. *Meddelelser om Grønland* 148(2): 1-125.

Lange, M. and O. Skifte, 1967. Notes on the macromycetes of northern Norway. *Acta Borealia* 23. 51 pp.

Larcher, W. and H. Bauer, 1981. Ecological significance of resistance to low temperature. *In*: A. Pirson and M.H. Zimmermann, eds. *Physiological Plant Ecology I: Responses to the Physical Environment*. *Encyclopedia of Plant Physiology* n.s. vol. 12A. Berlin: Springer-Verlag. pp. 403-437.

Largent, D.L., 1986. How to identify mushrooms to genus I: macroscopic features. Eureka, CA: Mad River Press. 166 pp.

- Largent, D.L., D. Johnson, and R. Watling, 1977. How to identify mushrooms to genus III: microscopic features. Eureka, CA: Mad River Press. 148 pp.
- Laursen, G.A. and J.F. Ammirati, 1982. The FISAM [First International Symposium on Arcto-Alpine Mycology] in retrospect. *In*: G.A. Laursen and J.F. Ammirati, eds. Arctic and Alpine Mycology. Seattle: University of Washington Press. pp. 532-544.
- Laursen, G.A. and M.A. Chmielewski, 1982. The ecological significance of soil fungi in arctic tundra. *In*: G.A. Laursen and J.F. Ammirati, eds. Arctic and Alpine Mycology. Seattle: University of Washington Press. pp. 432-488.
- Lei, J.-Y., K.K.Y. Wong, and Y. Piché, 1991. Extracellular concanavalin A-binding sites during early interactions between *Pinus banksiana* and two closely related genotypes of the ectomycorrhizal basidiomycete *Laccaria bicolor*. *Mycological Research* 95 (3): 357-363.
- Lesica, P. and R.K. Antibus, 1985. Mycorrhizae of alpine fell-field communities on soils derived from crystalline and calcareous parent materials. *Canadian Journal of Botany* 64: 1691-1697.
- Linkins, A. and B. Antibus, 1982. Mycorrhizae of *Salix rotundifolia* in coastal arctic tundra. *In*: G.A. Laursen and J.F. Ammirati, eds. Arctic and Alpine Mycology. Seattle: University of Washington Press. pp. 509-525.
- Liu, Y.J., S.O. Rogers, and J.F. Ammirati, 1997. Phylogenetic relationships in *Dermocybe* and related *Cortinarius* taxa based on nuclear ribosomal DNA internal transcribed spacers. *Canadian Journal of Botany* 75: 519-532.
- Lo Bue, G., F. Montacchini, and A. Ceruti, 1994. Macromycetes of the alpine belt: mycocoenological investigations in the western Italian Alps by multivariate methods. *Coenoses* 9(3): 103-153.
- Löve, A. and D. Löve, 1974. Origin and evolution of the arctic and alpine floras. *In*: J.D. Ives and R.G. Barry, eds. Arctic and Alpine Environments. London: Methuen & Co. pp. 571-603.
- Lutzoni, F. and R. Vilgalys, 1995. *Omphalina* (Basidiomycota, Agaricales) as a model system for the study of coevolution in lichens. *Cryptogamic Botany* 5 (1): 71-81.
- Marx, D.H., 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* 59: 153-163.

- Massicotte, H.B., L.H. Melville, R.L. Peterson, and D.L. Luoma, 1998. Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardii* (Cyperaceae). *Mycorrhiza* 7: 287-292.
- Matheny, P.B., Y.J. Liu, J.F. Ammirati, and B.D. Hall, 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). *American Journal of Botany* 89 (4): 688-698.
- McGraw, J.B., 1985. Experimental ecology of *Dryas octopetala* ecotypes. III. Environmental factors and plant growth. *Arctic and Alpine Research* 17 (3): 229-239.
- Methven, A., K.W. Hughes, and R.H. Petersen, 2000. *Flammulina* RFLP patterns identify species and show biogeographical patterns within species. *Mycologia* 92(6): 1064-1070.
- Metsänheimo, K., 1987. Sociology and ecology of larger fungi in the subarctic and oroarctic zones in northwest Finnish Lapland. *In*: G.A. Laursen, J.F. Ammirati, and S.A. Redhead, eds. *Arctic and Alpine Mycology II*. New York: Plenum Press. pp. 61-70.
- Michelsen, A., I.K. Schmidt, S. Jonasson, C. Quarmby and D. Sleep, 1996. Leaf <sup>15</sup>N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. *Oecologia* 105: 53-63.
- Miller, O.K., Jr., 1982a. Mycorrhizae, mycorrhizal fungi and fungal biomass in subalpine tundra at Eagle Summit, Alaska. *Holarctic Ecology* 5: 125-134.
- Miller, O.K., Jr., 1982b. Higher fungi in Alaskan subarctic tundra and taiga plant communities. *In*: G.A. Laursen and J.F. Ammirati, eds. *Arctic and Alpine Mycology*. Seattle: University of Washington Press. pp. 123-149.
- Miller, O.K. Jr. and M.C. Aime, 2001. Systematics, ecology and world distribution in the genus *Chroogomphus* (Gomphidiaceae). *In*: J.K. Misra and B.W. Horn, eds. *Trichomycetes and other fungal groups: Robert W. Lichtwardt Commemoration Volume*. Enfield, New Hampshire: Science Publishers Inc. pp. 315-333.
- Miller, O.K., Jr., G.A. Laursen, and D.F. Farr, 1982. Notes on Agaricales from arctic tundra in Alaska. *Mycologia* 74(4): 576-591.
- Mitchell, A.D. and A. Bresinsky, 1999. Phylogenetic relationships of *Agaricus* species based on ITS-2 and 28S ribosomal DNA sequences. *Mycologia* 91 (5): 811-819.
- Moncalvo, J.-M., S.A. Rehner, and R. Vilgalys, 1993. Systematics of *Lyophyllum* section *Difformia* based on evidence from culture studies and ribosomal DNA sequences. *Mycologia* 85 (5): 788-794.

- Moncalvo, J.-M., F.M. Lutzoni, S.A. Rehner, J. Johnson, and R. Vilgalys, 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* 49 (2): 278-305.
- Moncalvo, J.-M., R. Vilgalys, S.A. Redhead, J.E. Johnson, T.Y. James, M.C. Aime, V. Hofstetter, S.J.W. Verduin, E. L arsson, T.J. Baroni, R.G. Thorn, S. Jacobsson, H. Cl emen on, and O.K. Miller Jr., 2002. One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution* 23: 357-400.
- Moser, M., 1966. Die ektotrophe Ern ahrungsweise an der Waldgrenze. *Allgemeine Forstzeitung* 77: 120-127.
- Moser, M., 1983. Keys to Agarics and Boleti (Polyporales, Boletales, Agaricales, Russulales). London: Roger Phillips. 535 pp.
- Moser, M., 2002. How alpine are "alpine" fungi? 7<sup>th</sup> International Mycological Congress, Oslo, Norway, August 11-17, 2002 (abstract).
- Moser, M. and K.H. McKnight, 1987. Fungi (Agaricales, Russulales) from the alpine zone of Yellowstone National Park and the Beartooth Mountains with special emphasis on *Cortinarius*. In: G.A. Laursen, J.F. Ammirati and S.A. Redhead, eds. *Arctic and Alpine Mycology II*. New York: Plenum Press. pp. 299-317.
- Mueller, G.J., G.M. Mueller, L.-H. Shih, and J.F. Ammirati, 1993. Cytological studies in *Laccaria* (Agaricales). I. Meiosis and postmeiotic mitosis. *American Journal of Botany* 80 (3): 316-321.
- Mueller, G.M., 1984. New North American species of *Laccaria* (Agaricales). *Mycotaxon* 20 (1): 101-116.
- Mueller, G.M., 1985. Numerical taxonomic analyses on *Laccaria* (Agaricales). *Mycologia* 77 (1): 121-129.
- Mueller, G.M., 1987a. Designation of type collections for *Laccaria proxima*, *L. tortilis*, and *L. trullissata*. *Mycotaxon* 28 (2): 303-311.
- Mueller, G.M., 1987b. How does a taxonomist approach a problem like *Laccaria*? *McIlvainea* 8: 52-58.
- Mueller, G.M., 1991a. *Laccaria laccata* complex in North America and Sweden: intercollection pairing and morphometric analysis. *Mycologia* 83 (5): 578-594.
- Mueller, G.M., 1991b. The Swedish taxa of *Laccaria* (Tricholomataceae) with notes on their distribution. *Nordic Journal of Botany* 10: 665-680.

Mueller, G.M., 1991c. *Laccaria longipes*, a new North American species of the *Laccaria laccata* complex. *Mycotaxon* 40: 145-150.

Mueller, G.M., 1992. Systematics of *Laccaria* (Agaricales) in the continental United States and Canada, with discussions on extralimital taxa and descriptions of extant types. *Fieldiana, Botany, New Series* 30: 1-158.

Mueller, G.M., 1997. Designation of epitypes for *Laccaria proxima* and *Laccaria tortilis* (Agaricales). *Mycotaxon* 61: 205-207.

Mueller, G.M. and J.F. Ammirati, 1993. Cytological studies in *Laccaria* (Agaricales). II. Assessing phylogenetic relationships among *Laccaria*, *Hydnangium*, and other Agaricales. *American Journal of Botany* 80 (3): 322-329.

Mueller, G.M. and N. Fries, 1985. Identifying isolates of *Laccaria* using cultural and mating studies. *In: Proceedings of the 6th North American Conference on Mycorrhizae*, R. Molina, ed. Corvallis, OR: Forest Research Laboratory. p. 435.

Mueller, G.M. and M. Gardes, 1991. Intra- and interspecific relations within *Laccaria bicolor sensu lato*. *Mycological Research* 95 (5): 592-601.

Mueller, G.M. and B.A. Strack, 1992. Evidence for a mycorrhizal host shift during migration of *Laccaria trichodermophora* and other agarics into neotropical oak forests. *Mycotaxon* 65: 249-256.

Mueller, G.M. and E.C. Vellinga, 1986. Taxonomic and nomenclatural notes on *Laccaria* B. & Br.: *Laccaria amethystea*, *L. fraterna*, *L. laccata*, *L. pumila*, and their synonyms. *Persoonia* 13 (1): 27-43.

Mueller, G.M., Q.-X. Wu, Y.-Q. Huang, S.-Y. Guo, R. Aldana-Gomez, and R. Vilgalys, 2001. Assessing biogeographic relationships between North American and Chinese macrofungi. *Journal of Biogeography* 28: 271-281.

Müller, E. and J.A. Magnuson, 1987. On the origin and ecology of alpine plant parasitic fungi. *In: G.A. Laursen, J.F. Ammirati, and S.A. Redhead, eds. Arctic and Alpine Mycology II*. New York: Plenum Press. pp. 3-16.

Mullis, K.B. and F.A. Faloona, 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155: 335-350.

Mullis, K.B., F.A. Faloona, S.J. Scharf, R.K. Saiki, G.T. Horn, and H.A. Erlich, 1986. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology, Vol. LL*: 263-273.

- Murray, D.F., 1995. Causes of Arctic plant diversity: origin and evolution. *In*: F.S. Chapin & C. Korner, eds. Arctic and Alpine Biodiversity. Ecological Studies, Vol. 113. Berlin: Springer-Verlag. pp. 21-32.
- Nelson, F.E., O.A. Anisimov, and N.I. Shiklomanov, 2001. Subsidence risk from thawing permafrost. *Nature* 410: 889.
- O'Brien, H.E., J.A. Jackson, J.E. Johnson, J.L. Parrent, J.-M. Moncalvo and R. Vilgalys, 2002. Fungal community analysis using environmental genomics. 7<sup>th</sup> International Mycological Congress, Oslo, Norway, August 11-17, 2002 (abstract).
- Oechel, W.C., G.L. Vourlitis, S.J. Hastings, R.C. Zulueta, L. Hinzman, and D. Kane, 2000. Acclimation of ecosystem CO<sub>2</sub> exchange in the Alaskan Arctic in response to decadal climate warming. *Nature* 406: 978-981.
- Ohenoja, E., 1971. The larger fungi of Svalbard and their ecology. *Rep. Kevo Subarctic Res. Stat.* 8: 122-147.
- Østrem, G., 1974. Present alpine ice cover. *In*: Arctic and Alpine Environments. J.D. Ives and R.G. Barry, eds. London: Methuen & Co. pp. 159-194.
- Parmalee, J.A., 1969. Fungi of central Baffin Island. *The Canadian Field Naturalist* 83: 48-53.
- Peintner, U., N.L. Bougher, M.A. Castellano, J.-M. Moncalvo, M.M. Moser, J.M. Trappe, and R. Vilgalys., 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortinariaceae). *American Journal of Botany* 88 (12): 2168-2179.
- Peintner, U., M. Moser, E. Horak, and R. Vilgalys, 2002. *Cortinarius favrei*: an example for phylogenetic, morphological, and ecological species concepts in alpine fungi. 7<sup>th</sup> International Mycological Congress, Oslo, Norway, August 11-17, 2002 (abstract).
- Petersen, P.M., 1977. Investigations on the ecology and phenology of the macromycetes in the Arctic. *Meddelelser om Grønland* 199: 1-72.
- Petersen, R.H., 1995a. Contributions of mating studies to mushroom systematics. *Canadian Journal of Botany* 73 (Supplement 1): S831-S842.
- Petersen, R.H., 1995b. There's more to a mushroom than meets the eye: mating studies in the Agaricales. *Mycologia* 87 (1): 1-17.
- Piercey-Normore, M.D., K.N. Egger, and J.A. Berube, 1998. Molecular phylogeny and evolutionary divergence of North American biological species of *Armillaria*. *Molecular Phylogenetics and Evolution* 10(1): 49-66.

Podila, G.K., J. Zheng, S. Balasubramanian, S. Sundaram, S. Hiremath, J.H. Brand and M.J. Hymes, 2002. Fungal gene expression in early symbiotic interactions between *Laccaria bicolor* and red pine. *Plant and Soil* 244: 117-128.

Read, D.J. and K. Haselwandter, 1981. Observations on the mycorrhizal status of some alpine plant communities. *New Phytologist* 88: 341-352.

Redhead, S.A., 1989. A biogeographical overview of the Canadian mushroom flora. *Canadian Journal of Botany* 67: 3003-3062.

Redhead, S.A. and T.W. Kuyper, 1987. Lichenized agarics: taxonomic and nomenclatural riddles. *In*: G.A. Laursen, J.F. Ammirati and S.A. Redhead, eds. *Arctic and Alpine Mycology II*. New York: Plenum Press. pp. 319-348.

Reid, D.A., 1979. Some fungi from Spitsbergen. *Reports of the Kevo Subarctic Research Station* 15: 41-47.

Riebesell, J.F., 1982. Arctic-alpine plants on mountaintops: agreement with island biogeography theory. *The American Naturalist* 119 (5): 657-674.

Robinson, C., 2001. Cold adaptation in Arctic and Antarctic fungi. *New Phytologist* 151: 341-353.

Rossmann, A.Y., R.E. Tulloss, T.E. O'Dell, and R.G. Thorn, 1998. Protocols for an all taxa biodiversity inventory of fungi in a Costa Rican conservation area. Boone, NC: Parkway Publishers, Inc. 195 pp.

Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, and H.A. Erlich, 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487-491.

Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989. *Molecular cloning: a laboratory manual*, 2<sup>nd</sup> edition. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory. 3 vol.

Savile, D.B.O., 1982. Adaptations of fungi to arctic and subarctic conditions. *In*: G.A. Laursen and J.F. Ammirati, eds. *Arctic and Alpine Mycology*. Seattle: University of Washington Press. pp. 357-362.

Schmit, J.P., J.F. Murphy, and G.M. Mueller, 1999. Macrofungal diversity of a temperate oak forest: a test of species richness estimators. *Canadian Journal of Botany* 77: 1014-1027.

Seidl, M.T., 2000. Phylogenetic relationships within *Cortinarius* subgenus *Myxacium*, sections *Defibulati* and *Myxacium*. *Mycologia* 92 (6): 1091-1102.

- Selosse, M.-A., G. Costa, C. Di Battista, F. Le Tacon, and F. Martin, 1996. Meiotic segregation and recombination of the intergenic spacer of the ribosomal DNA in the ectomycorrhizal basidiomycete *Laccaria bicolor*. *Current Genetics* 30: 332-337.
- Selosse, M.-A., F. Martin, and Le Tacon, F., 2001. Intraspecific variation in fruiting phenology in an ectomycorrhizal *Laccaria* population under Douglas fir. *Mycological Research* 105 (5): 524-531.
- Senn-Irlet, B., 1987. Oekologie, soziologie und taxonomie alpiner makromyceten (Agaricales, Basidiomycetes) der Schweizer Zentralalpen. Dissertation, Universitat Bern. 310 pp. as cited in Graf, 1994.
- Senn-Irlet, B., 1988. Macromycetes in alpine snow-bed communities – mycocoenological investigations. *Acta Bot. Neerl.* 37(2): 251-263.
- Senn-Irlet, B., 1992. Botanischer reichthum am weg von Davos über die Bergüner Furgga zum Albula: sommerexcursion 1991 im Anklang an die erste excursion der Schweizerischen Botanischen Gesellschaft 1890. 6. Makromyzeten (Basidiomycota, Agaricales, Aphyllophorales). *Bot. Helv.* 102: 49-59.
- Senn-Irlet, B., 1993. The mycoflora of alpine mire communities rich in *Salix*. In Petrini, O. and G.A. Laursen, eds. *Arctic and Alpine Mycology* 3. *Bibl. Mycol.* 150: 235-249. Berlin-Stuttgart: J. Cramer.
- Senn-Irlet, B. and G. Bieri, 1999. Sporocarp succession of soil-inhabiting macrofungi in an autochthonous subalpine Norway spruce forest of Switzerland. *Forest Ecology and Management* 124: 169-175.
- Shimomura, N., K. Hasebe, Y. Nakai-Fukumasa and M. Komathu, 1992. Intercompatibility between geographically distant strains of Shiitake. *Reports of the Tottori Mycological Institute* 26-29.
- Sime, A.D. and R.H. Petersen, 1999. Intercontinental interrelationships among disjunct populations of *Melanotus* (Strophariaceae, Agaricales). *Mycotaxon* 71: 481-492.
- Singer, R., 1943. Type studies on basidiomycetes. II. *Mycologia* 35: 142-163.
- Singer, R., 1954. The cryptogamic flora of the arctic: VI. fungi. *Botanical Review* 20: 451-462.
- Singer, R., 1967. Notes sur le genre *Laccaria*. *Bulletin Trimestriel de la Société Mycologique de France* 83: 104-123.

- Singer, R., 1977. Die gruppe der *Laccaria laccata* (Agaricales). Plant Systematics and Evolution 126: 347-370.
- Singer, R., 1986. The Agaricales in Modern taxonomy, 4<sup>th</sup> edition. Koenigstein, Germany: Koeltz Scientific Books. 981 pp.
- Sivertsen, S., 1993. *Laccaria pumila* Fayod and *L. altaica* Sing. – are they really synonyms? Polarflokken 17 (2): 331-338.
- Skifte, O., 1979. Storsopp på Svalbard. Ottar 110-112, 29-39, as cited in Gulden & Torkelsen, 1996.
- Smaglik, P. 2000. US climate report underlines local impacts of warming. Nature 405: 725.
- Smith, A.H., V.S. Evenson, and D. H. Mitchel, 1983. The veiled species of *Hebeloma* in the Western United States. Ann Arbor: The University of Michigan Press. 219 pp.
- Smith, S.E. and D.J. Read, 1997. Mycorrhizal Symbiosis, 2<sup>nd</sup> edition. San Diego: Academic Press. 605 pp.
- Straatsma, G., F. Ayer, and S. Egli, 2001. Species richness, abundance, and phenology of fungal fruit bodies over 21 years in a Swiss forest plot. Mycological Research 105 (5): 515-523.
- Sturm, M., C. Racine, and K. Tape, 2001. Increasing shrub abundance in the Arctic. Nature 411: 546-547.
- Stutz, R.C., 1972. Survey of mycorrhizal plants. In: L.C. Bliss, ed. Devon Island IPB Project Report. Edmonton: University of Alberta. pp. 214-216.
- Swann, E.C. and J.W. Taylor, 1993. Higher taxa of basidiomycetes: an 18S rRNA gene perspective. Mycologia 85: 923-936.
- Swofford, D.L., 2001. PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b command reference. Sunderland, Massachusetts: Sinauer Associates, Inc. 142 pp.
- Thorn, R.G., J.-M. Moncalvo, C.A. Reddy, and R. Vilgalys, 2000. Phylogenetic analyses and the distribution of nematophagy support a monophyletic Pleurotaceae within the polyphyletic pleurotoid-lentinoid fungi. Mycologia 92 (2): 241-252.
- Tibbett, M., F.E. Sanders, and J.W.G. Cairney, 1998. The effect of temperature and inorganic phosphorus supply on growth and acid phosphatase production in arctic and temperate strains of ectomycorrhizal *Hebeloma* spp. in axenic culture. Mycological Research 102: 129-135.

- Tommerup, I.C., N.L. Bougher, and N. Malajczuk, 1990. *Laccaria* species ectomycorrhizal with Eucalypts: why does the ecology of bisporic species differ from quadrisporic species? Proceedings of the 8<sup>th</sup> North American Conference on Mycorrhizae. Jackson, WY, USA, September 5-9, 1990 (abstract).
- Tommerup, I.C., N.L. Bougher, and N. Malajczuk, 1991. *Laccaria fraterna*, a common ectomycorrhizal fungus with mono- and bi-sporic basidia and multinucleate spores: comparison with the quadristerigmate, binucleate spored *L. laccata* and the hypogeous relative *Hydnangium carneum*. *Mycological Research* 95 (6): 689-698.
- Trappe, JM 1962. Fungus associates of ectotrophic mycorrhizae. *Botanical Review* 28: 538-606.
- Treu, R., G.A. Laursen, S.L. Stephenson, J.C. Landolt, and R. Densmore, 1996. Mycorrhizae from Denali National Park and Preserve, Alaska. *Mycorrhiza* 6: 21-29.
- Trimbach, J., 1978. Matériel pour une "check-list" des Alpes Maritimes. *Doc. Mycol.* VII, 29: 39-53.
- Väre, H., M. Vestberg, and S. Euroala 1992. Mycorrhiza and root-associated fungi in Spitsbergen. *Mycorrhiza*. 93-104.
- Väre, H., M. Vestberg, and R. Ohtonen, 1997. Shifts in mycorrhiza and microbial activity along an Oroarctic altitudinal gradient in Northern Fennoscandia. *Arctic and Alpine Research* 29 (1): 93-104.
- Vellinga, E.C. and G.M. Mueller, 1987. Taxonomic and nomenclatural notes on *Laccaria* B. & Br. - II: *Laccaria bicolor*, *L. fraterna*, *L. laccata* var. *pallidifolia*. *Persoonia* 13 (3): 383-385.
- Vilgalys, R., 1991. Speciation and species concepts in the *Collybia dryophila* complex. *Mycologia* 83(6): 758-773.
- Vilgalys, R., A. Smith, B.L. Sun, and O.K. Miller Jr., 1993. Intersterility groups in the *Pleurotus ostreatus* complex from the continental United States and adjacent Canada. *Canadian Journal of Botany* 71: 113-128.
- Volk, T.J., H.H. Burdsall Jr., and M.T. Banik, 1996. *Armillaria nabsnona*, a new species from western North America. *Mycologia* 88(3): 484-491.
- Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T.J.C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein, 2002. Ecological responses to recent climate change. *Nature* 416: 389-395.

- Wardle, P., 1974. Alpine treelines. *In*: J.D. Ives and R.G. Barry, eds. Arctic and Alpine Environments. London: Methuen & Co. pp. 371-402.
- Watling, R. 1977. Larger fungi from Greenland. *Astarte* 10: 61-71.
- Watling, R. 1983. Larger cold-climate fungi. *Sydowia, Ann. Mycol. Ser. II*, 36: 308-325.
- Watling, R. 1987. Larger arctic-alpine fungi in Scotland. *In*: G.A. Laursen, J.F. Ammirati, and S.A. Redhead, eds. Arctic and Alpine Mycology II. New York: Plenum Press. pp.17-45.
- Watling, R., 1992. Macrofungi associated with British willows. *Proceedings of the Royal Society of Edinburgh* 98B: 135-147.
- Watling, C. and R. Watling, 1988. Svalbard fungi. *British Schools' Exploration Soc. Rep.* 1987-1988, as cited in Gulden and Torkelsen, 1996.
- Weber, J., J. Díez, M.-A. Selosse, D. Tagu, and F. Le Tacon, 2002. SCAR markers to detect mycorrhizas of an American *Laccaria bicolor* strain inoculated in European Douglas-fir plantations. *Mycorrhiza* 12: 19-27.
- Weber, W.A. and R.C. Wittmann, 2001. *Colorado Flora: Western Slope*. 3<sup>rd</sup> edition. Boulder: University Press of Colorado. 488 pp.
- Weiss, M., Z.-L. Yang, and F. Oberwinkler, 1998. Molecular phylogenetic studies in the genus *Amanita*. *Canadian Journal of Botany* 76: 1170-1179.
- White, T.J., T. Bruns, S. Lee, and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds. *PCR Protocols: a Guide to Methods and Applications*. Academic Press, Inc. pp. 315-322.
- Worley, J.F. and E. Hackskaylo, 1959. The effect of available soil moisture on the mycorrhizal association of Virginia pine. *Forest Science* 5: 267-268.

APPENDICES

APPENDIX A

ROCKY MOUNTIAN ALPINE AND REFERENCE *LACCARIA*

SPECIMENS EXAMINED

Appendix A. Rocky Mountain alpine and reference *Laccaria* included in molecular phylogenetic analysis.

Coll.	ID#	Genus	Species	Day	Mo.	Year	State	Country	Range	Specific Locations	Elevation (m)	Plant associations	Plot	Notes
CLC	1104	<i>Laccaria</i>	<i>pumila</i>	27	7	1997	WY	USA	Beartooth	Frozen Lakes	3111-3233	Sdw		
CLC	1201	<i>Laccaria</i>	<i>pumila</i>	7	8	1998	WY	USA	Beartooth	Highline Trailhead	3050-3263.5	<i>Salix</i> spp.		
CLC	1238b	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	13	8	1998	CO	USA	Sawatch	Independence Pass	3660	Sr, Si?		
CLC	1252*	<i>Laccaria</i>	<i>pumila</i>	14	8	1998	CO	USA	Front	Haggeman's Pass	3600	Sn		
CLC	1304*	<i>Laccaria</i>	<i>bicolor</i>	6	8	1999	CO	USA	Front	Loveland Pass	3050	Sbu		
CLC	1308	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	8	8	1999	CO	USA	Front	Loveland Pass	3050	?		omphaloid
CLC	1347	<i>Laccaria</i>	<i>bicolor</i>	11	8	1999	CO	USA	Sawatch	Independence Pass	3660	Sbu		
CLC	1365	<i>Laccaria</i>	<i>bicolor</i>	13	8	1999	CO	USA	Sawatch	Independence Pass	3660	Sbu		large
CLC	1370*	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	14	8	1999	CO	USA	Sawatch	Independence Pass	3660	Do, Sdw		omphaloid
CLC	1404	<i>Laccaria</i>	<i>pumila</i>	21	8	1999	WY	USA	Beartooth	Frozen Lakes	3111-3233	Si	3	
CLC	1435	<i>Laccaria</i>	<i>pumila</i>	01	08	2000	CO	USA	San Juan	Cinnamon Pass	3700	Sr		
CLC	1445*	<i>Laccaria</i>	<i>bicolor</i>	02	08	2000	CO	USA	San Juan	Black Bear	3761	?		
CLC	1446	<i>Laccaria</i>	<i>pumila</i>	02	08	2000	CO	USA	San Juan	Black Bear	3761	Sn, Si		
CLC	1469*	<i>Laccaria</i>	<i>bicolor</i>	06	08	2000	CO	USA	Sawatch	Independence Pass	3660	Sn		
CLC	1482*	<i>Laccaria</i>	<i>bicolor</i>	08	08	2000	CO	USA	Sawatch	Linkins Lake Valley	3597	Sn (alpine/subalpine)		violet
CLC	1595	<i>Laccaria</i>	<i>proxima</i>	21	07	2001	WY	USA	Beartooth	McLaren Mine Tailings	(subalpine)	<i>Salix, Picea, Pinus</i>	0	

Coll.	ID#	Genus	Species	Day	Mo.	Year	State	Country	Range	Specific Locations	Elevation (m)	Plant associations	Plot	Notes
CLC	1603*	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	02	08	2001	CO	USA	10-mile Range	Blue Lake Dam, Breckenridge	3300	Sr, Bg	0	
CLC	1625*	<i>Laccaria</i>	<i>sp. (provisional)</i>	03	08	2001	CO	USA	10-mile Range	Blue Lake Dam, Breckenridge	3300	Bg, Sn	0	
CLC	1633*	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	03	08	2001	CO	USA	10-mile Range	Blue Lake Dam, Breckenridge	3300	Bg	0	
CLC	1648	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	04	08	2001	CO	USA	Sawatch	Cumberland Pass	3662	Sg	0	
CLC	1653	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	04	08	2001	CO	USA	Sawatch	Quartz Creek	(subalpine)	<i>Betula</i>	0	
CLC	1655*	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	06	08	2001	CO	USA	San Juan	Horseshoe Lake	3810	Sr	0	
CLC	1656	<i>Laccaria</i>	<i>bicolor</i>	06	08	2001	CO	USA	San Juan	Horseshoe Lake	3810	Sr	0	
CLC	1672*	<i>Laccaria</i>	<i>bicolor</i>	07	08	2001	CO	USA	San Juan	Mineral Basin	3900	Si	0	
CLC	1682	<i>Laccaria</i>	<i>sp. (provisional)</i>	08	08	2001	CO	USA	San Juan	U.S. Basin	3658	Sg	0	
CLC	1699	<i>Laccaria</i>	<i>pumila</i>	10	08	2001	CO	USA	San Juan	Cinnamon Pass	3840	Si	0	
CLC	1709	<i>Laccaria</i>	<i>bicolor</i>	10	08	2001	CO	USA	San Juan	Cinnamon Pass	3840	Si	0	
CLC	1724*	<i>Laccaria</i>	<i>laccata</i> var. <i>pallidifolia</i>	12	08	2001	CO	USA	Sawatch	Cottonwood Pass	3696	<i>Dryas</i>	0	
CLC	1742	<i>Laccaria</i>	<i>bicolor</i>	13	08	2001	CO	USA	Sawatch	Independence Pass	3660-3687	Sn	0	
CLC	1771*	<i>Laccaria</i>	<i>sp. (provisional)</i>	15	08	2001	CO	USA	Sawatch	Independence Pass	3660-3687	Sbu	0	
CLC	1777	<i>Laccaria</i>	<i>pumila</i>	21	08	2001	WY	USA	Beartooth	Frozen Lake	3100-3250	Sn	0	
CLC	1819	<i>Laccaria</i>	<i>pumila</i>	28	7	2002	CO	USA		Stony Pass	3840			
CLC	1825	<i>Laccaria</i>	<i>bicolor</i>	28	7	2002	CO	USA		Stony Pass	3840			
CLC	1835	<i>Laccaria</i>	<i>pumila</i>	29	7	2002	CO	USA		Imogene	3850			
CLC	1837	<i>Laccaria</i>	<i>pumila</i>	29	7	2002	CO	USA		Imogene	3850			
CLC	1850	<i>Laccaria</i>	<i>pumila</i>	30	7	2002	CO	USA		Mineral Basin	3822-3850			
CLC	1851	<i>Laccaria</i>	<i>pumila</i>	30	7	2002	CO	USA		Mineral Basin	3822-3850			
CLC	1853	<i>Laccaria</i>	<i>montana</i>	30	7	2002	CO	USA		Mineral Basin	3822-3850			
CLC	1872	<i>Laccaria</i>	<i>pumila</i>	31	7	2002	CO	USA		Emma Lake	3688			

Coll.	ID#	Genus	Species	Day	Mo.	Year	State	Country	Range	Specific Locations	Elevation (m)	Plant associations	Plot	Notes
TWO	264	<i>Laccaria</i>	<i>montana</i>	10	08	1999	MT	USA	Beartooth	Birch Site	2958.5-3050	Sn	2	
TWO	265	<i>Laccaria</i>	<i>pumila</i>	10	08	1999	MT	USA	Beartooth	Birch Site	2958.5-3050	Sbu	3	
TWO	268	<i>Laccaria</i>	<i>pumila</i>	10	08	1999	MT	USA	Beartooth	Birch Site	2958.5-3050	Sn	0	
TWO	314	<i>Laccaria</i>	<i>pumila</i>	31	07	2000	MT	USA	Beartooth	Birch Site	2958.5-3050	Sn	0	
TWO	319*	<i>Laccaria</i>	<i>montana</i>	01	08	2000	T	USA	Beartooth	Highline Trail	3050-3263.5	moss	0	
TWO	335	<i>Laccaria</i>	<i>pumila</i>	21	08	2000	MT	USA	Beartooth	Birch Site	2958.5-3050	moss	0	
TWO	337	<i>Laccaria</i>	<i>pumila</i>	21	08	2000	MT	USA	Beartooth	Birch Site	2958.5-3050	Sn	0	
TWO	348	<i>Laccaria</i>	<i>pumila</i>	21	08	2000	MT	USA	Beartooth	Birch Site	2958.5-3050	Sbu	3	
TWO	362	<i>Laccaria</i>	<i>pumila</i>	12	07	2001	MT	USA	Beartooth	Clark Fork Picnic Area	(subalpine)	Sbu, <i>Picea</i> , <i>Pinus contorta</i>	0	
TWO	369	<i>Laccaria</i>	<i>montana</i>	14	07	2001	T	USA	Beartooth	Highline Trail	3050-3263.5	Sn	0	
TWO	374	<i>Laccaria</i>	<i>pumila</i>	19	07	2001	MT	USA	Beartooth	Clark Fork Picnic Area	(subalpine)	moss, Sbu	0	
TWO	408	<i>Laccaria</i>	<i>proxima</i>	21	07	2001	MT	USA	Beartooth	McLaren Mine Tailings	(subalpine)	<i>Pinus contorta</i> , <i>Picea engelmannii</i> , Sbu	0	
TWO	409	<i>Laccaria</i>	<i>pumila</i>	21	07	2001	WY	USA	Beartooth	Top of the World Store	(subalpine)	moss, Sbu	0	
TWO	411	<i>Laccaria</i>	<i>pumila</i>	21	07	2001	MT	USA	Beartooth	McLaren Mine Tailings	(subalpine)	Sbu	0	
TWO	441	<i>Laccaria</i>	<i>montana</i>	28	07	2001	MT	USA	Beartooth	Birch Site	2958.5-3050	Sn	2	
TWO	442	<i>Laccaria</i>	<i>pumila</i>	28	07	2001	MT	USA	Beartooth	Birch Site	2958.5-3050	Sg	3	
TWO	465	<i>Laccaria</i>	<i>pumila</i>	30	07	2001	MT	USA	Beartooth	Birch Site	2958.5-3050	Sg	3	
TWO	477	<i>Laccaria</i>	<i>montana</i>	31	07	2001	WY	USA	Beartooth	Frozen Lake	3111-3233	Si	3	

Coll.	ID#	Genus	Species	Day	Mo.	Year	State	Country	Range	Specific Locations	Elevation (m)	Plant associations	Plot	Notes
TWO	495	<i>Laccaria</i>	<i>proxima</i>	2	08	2001	MT	USA	Beartooth	McLaren Mine Tailings	(subalpine)	Sbu, <i>Pinus</i> (2-needed)	0	
TWO	498	<i>Laccaria</i>	<i>proxima</i>	2	08	2001	MT	USA	Beartooth	McLaren Mine Tailings	(subalpine)	Sbu, <i>Pinus</i> (2-needed)	0	
TWO	501*	<i>Laccaria</i>	<i>pumila</i>	3	08	2001	WY	USA	Beartooth	Frozen Lake	3111-3233	Sn	0	
TWO	504	<i>Laccaria</i>	<i>montana</i>	3	08	2001	T	USA	Beartooth	Highline Trail	3050-3263.5	Sg	0	
TWO	505	<i>Laccaria</i>	<i>montana</i>	3	08	2001	T	USA	Beartooth	Highline Trail	3050-3263.5	Sg	0	
TWO	512	<i>Laccaria</i>	<i>montana</i>	4	08	2001	WY	USA	Beartooth	Frozen Lake	3111-3233	Sn	0	
TWO	520	<i>Laccaria</i>	<i>pumila</i>	5	08	2001	MT	USA	Beartooth	Birch Site	2958.5-3050	Sg	3	
TWO	540	<i>Laccaria</i>	<i>montana</i>	16	08	2001	WY/M	USA	Beartooth	Highline Trail	3050-3263.5	Sn	0	
TWO	553	<i>Laccaria</i>	<i>montana</i>	17	08	2001	WY	USA	Beartooth	Frozen Lake	3111-3233	Si	1	
TWO	559	<i>Laccaria</i>	<i>montana</i>	19	08	2001	WY/M	USA	Beartooth	Highline Trail	3050-3263.5	<i>Salix cf. glauca</i>	0	
TWO	560	<i>Laccaria</i>	<i>pumila</i>	19	08	2001	T	USA	Beartooth	Highline Trail	3050-3263.5	<i>Salix cf. glauca</i>	0	
TWO	561	<i>Laccaria</i>	<i>montana</i>	19	08	2001	WY/M	USA	Beartooth	Highline Trail	3050-3263.5	<i>Salix cf. glauca</i>	0	
TWO	562	<i>Laccaria</i>	<i>pumila</i>	19	08	2001	T	USA	Beartooth	Highline Trail	3050-3263.5	<i>Salix cf. glauca</i>	0	
TWO	589	<i>Laccaria</i>	<i>pumila</i>	01	09	2001	WY	USA	Beartooth	Frozen Lake	3111-3233		0	
TWO	591*	<i>Laccaria</i>	<i>montana</i>	01	09	2001	WY	USA	Beartooth	Frozen Lake	3111-3233	Sn	0	
TWO	613	<i>Laccaria</i>	<i>montana</i>	18	07	2002	T	USA	Beartooth	Highline Trail	3050-3263.5	Sn	0	
TWO	663	<i>Laccaria</i>	<i>pumila</i>	31	7	2002	WY	USA	Beartooth	Solifluction Terraces		Si, moss	0	
TWO	709	<i>Laccaria</i>	<i>pumila</i>	15	8	2002	WY	USA	Beartooth	Highline Trail	3050-3263.5	Sbu, moss	0	
TWO	710	<i>Laccaria</i>	<i>montana</i>	15	08	2002	T	USA	Beartooth	Highline Trail	3050-3263.5	<i>Salix</i> sp.	0	
TWO	716	<i>Laccaria</i>	<i>pumila</i>	27	08	2002	MT	USA	Beartooth	Birch Site	2958.5-3050	Sn	0	
TWO	717	<i>Laccaria</i>	<i>pumila</i>	27	08	2002	MT	USA	Beartooth	Birch Site	2958.5-3050	Sg	3	
TWO	718	<i>Laccaria</i>	<i>pumila</i>	27	08	2002	MT	USA	Beartooth	Birch Site	2958.5-3050	Sbu	0	

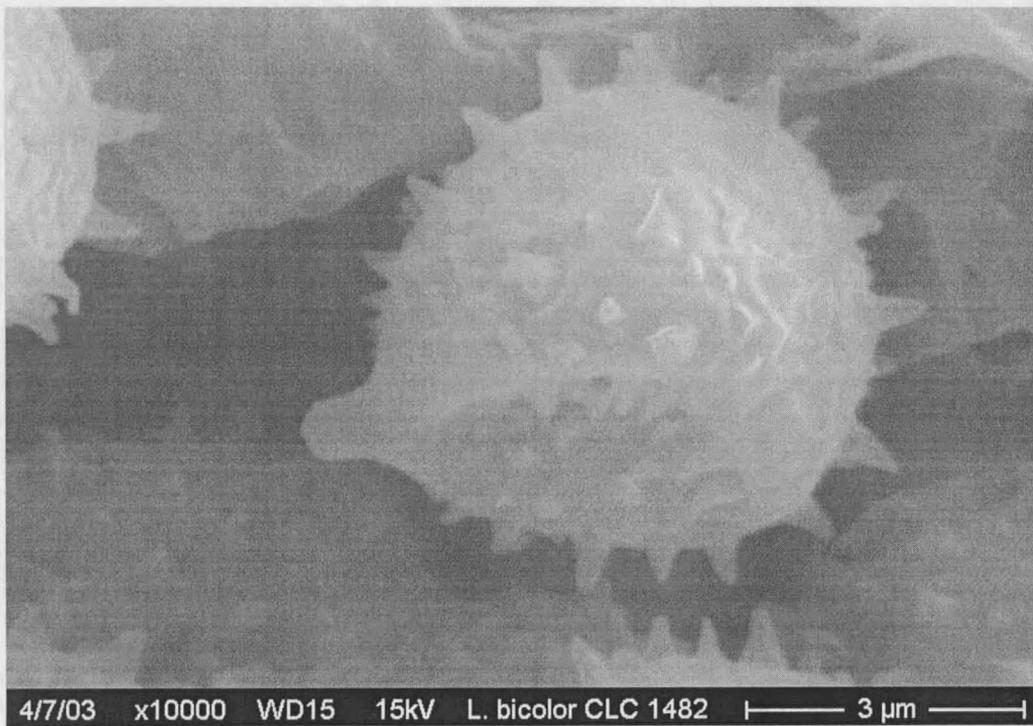
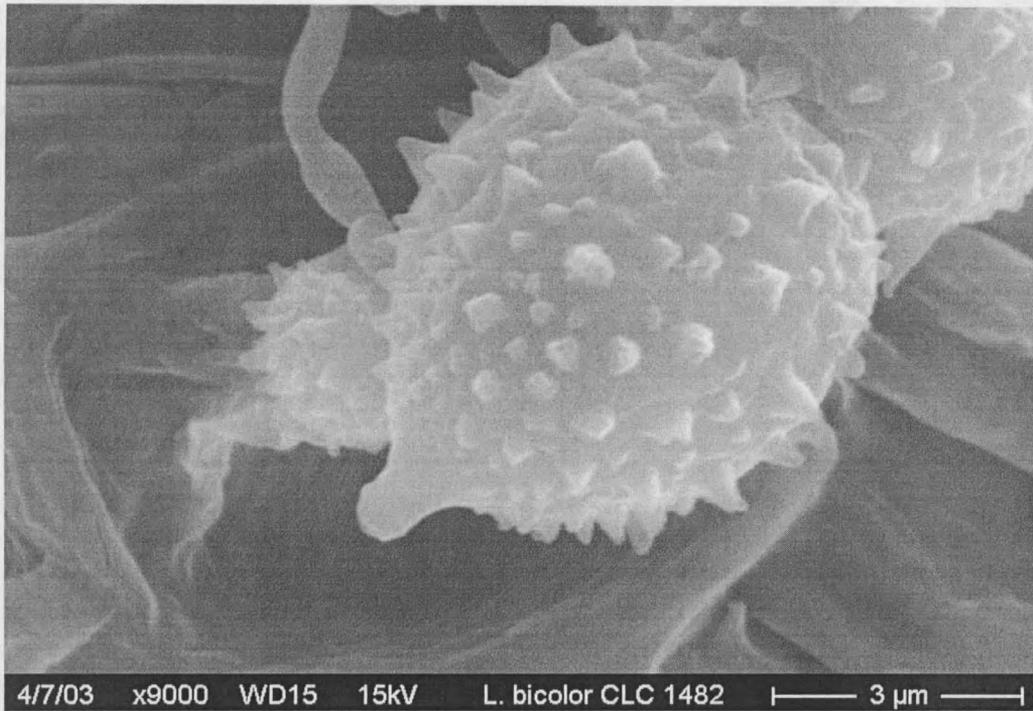
Coll.	ID#	Genus	Species	Day	Mo.	Year	State	Country	Range	Specific Locations	Elevation (m)	Plant associations	Plot	Notes
TWO	726	<i>Laccaria</i>	<i>pumila</i>	28	08	2002	T	USA	Beartooth	Highline Trail	3050-3263.5	Sn	0	
TWO	730	<i>Laccaria</i>	<i>pumila</i>	28	08	2002	WY/M T	USA	Beartooth	Highline Trail	3050-3263.5	Sbu (probably Sg)	0	
TWO	752	<i>Laccaria</i>	<i>cf. bicolor</i>	8	9	2002	MT	USA		Gallatin Co., Hyalite Canyon	(subalpine)	conifers		
TENN	42877	<i>Laccaria</i>	<i>montana</i>	13	9	1981	CO	USA		Blue Lake Trail, Larimer Co.	3048+			(GMM 1194)
TENN	42880	<i>Laccaria</i>	<i>montana</i>	15	9	1981	CO	USA		Cameron Pass, Jackson Co.	3300			(GMM 1214)
DBGH	20424	<i>Laccaria</i>	<i>montana</i>	20	8	1999	CO	USA		Loveland Pass Lk., Arapaho NF, Summit Co.	3620	<i>Salix</i> , moss		

EM Hosts: Si = *Salix arctica*; Sr = *S. reticulata*; Sg = *S. glauca*; Sn = *S. planifolia*; Sbu = *Salix* shrub; Sdw = dwarf *Salix*; Do = *Dryas octopetala*; Bg = *Betula glandulosa*

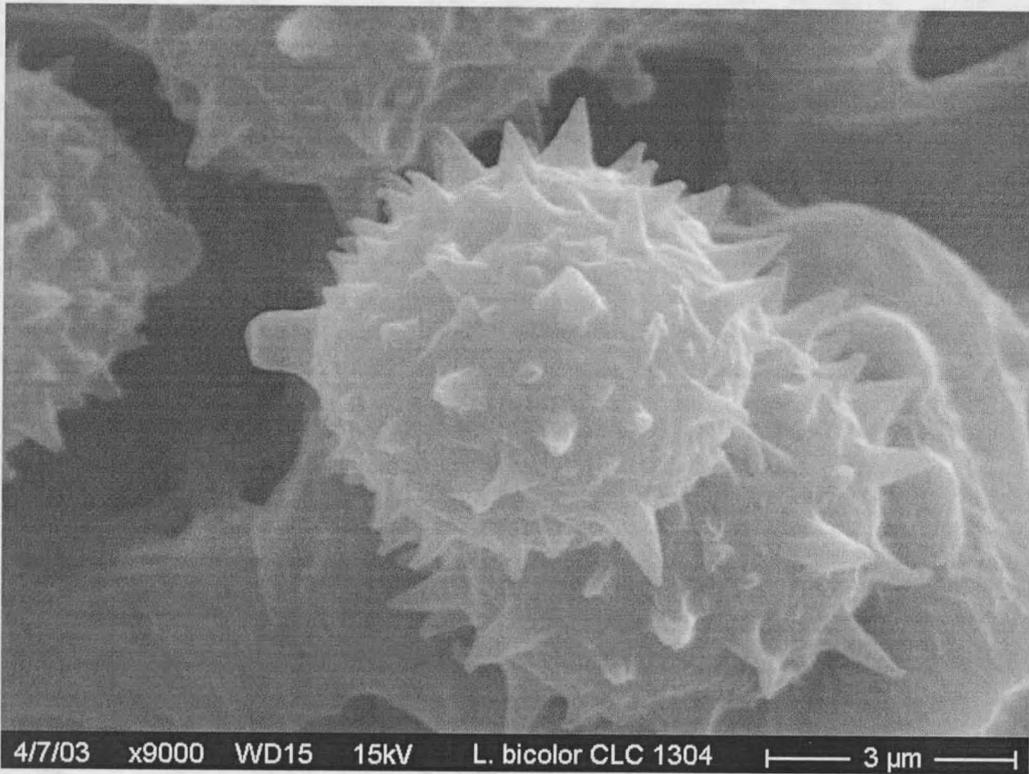
APPENDIX B

SCANNING ELECTRON MICROGRAPHS OF

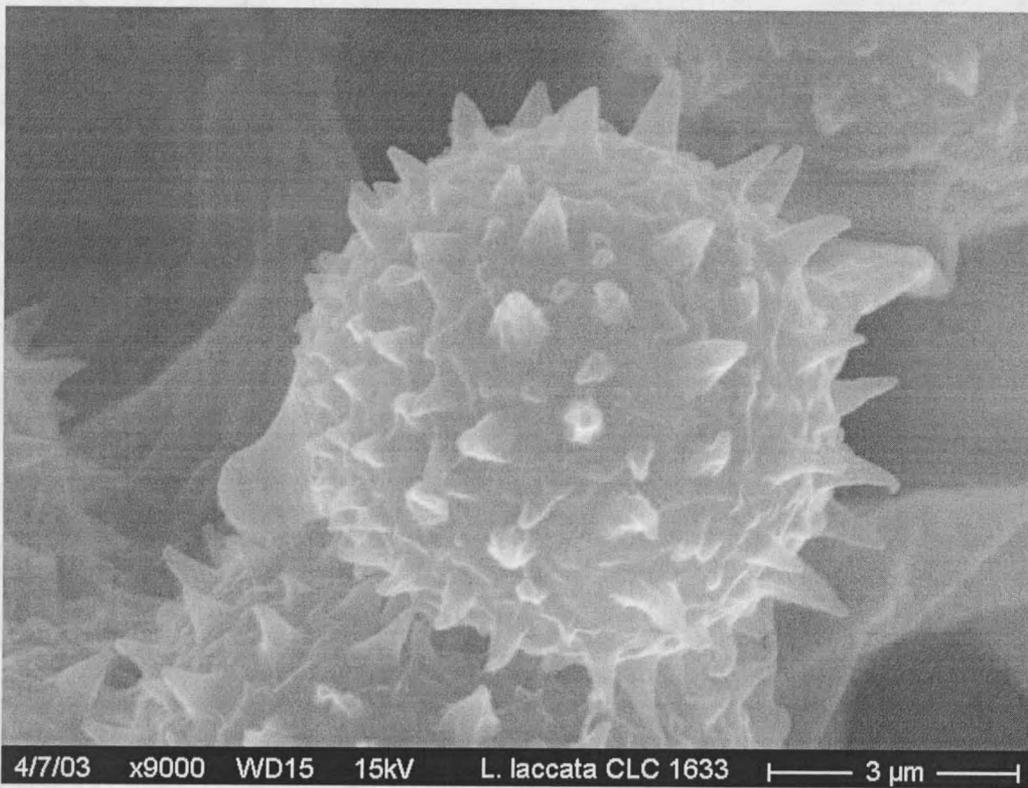
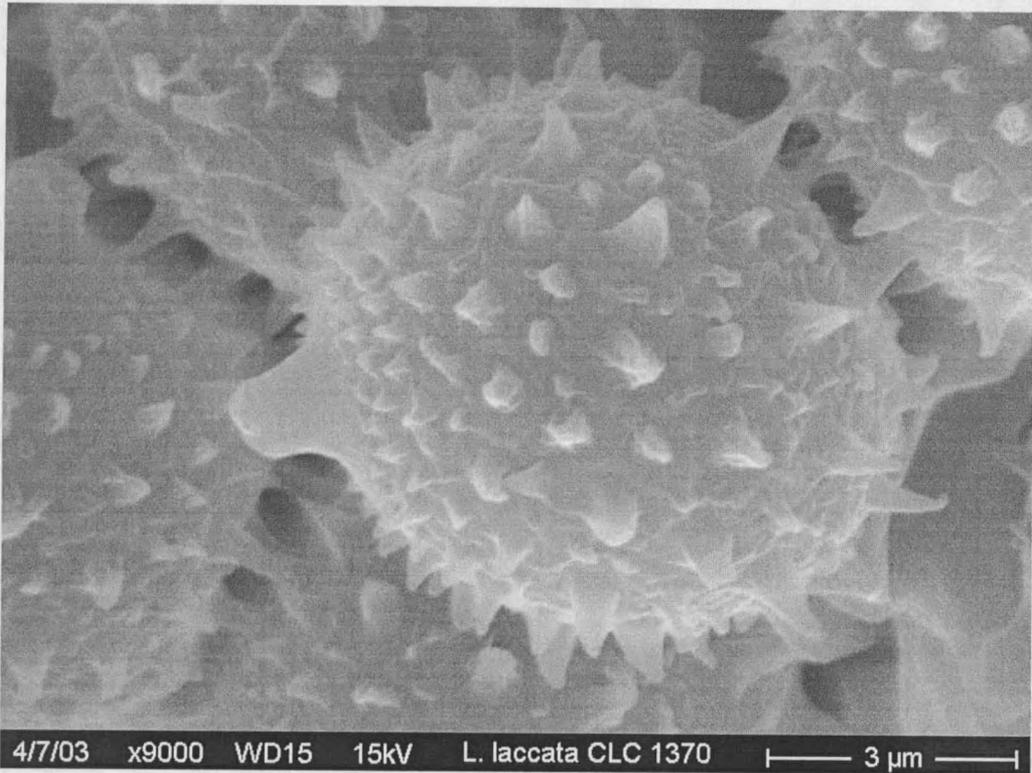
*LACCARIA* BASIDIOSPORES



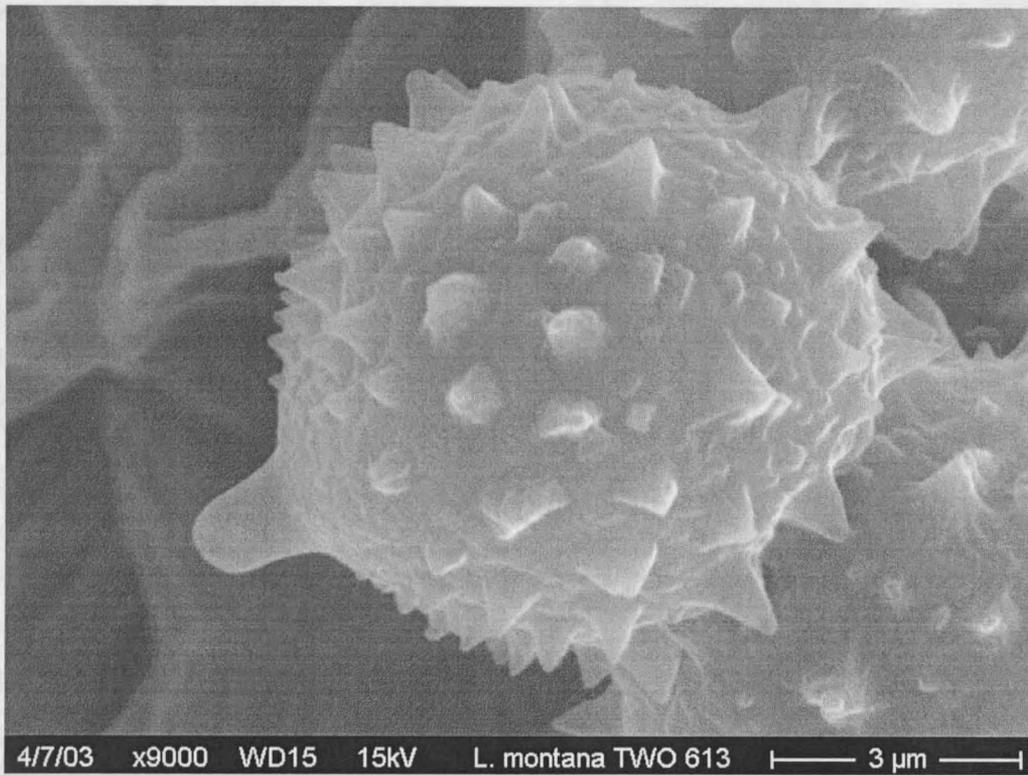
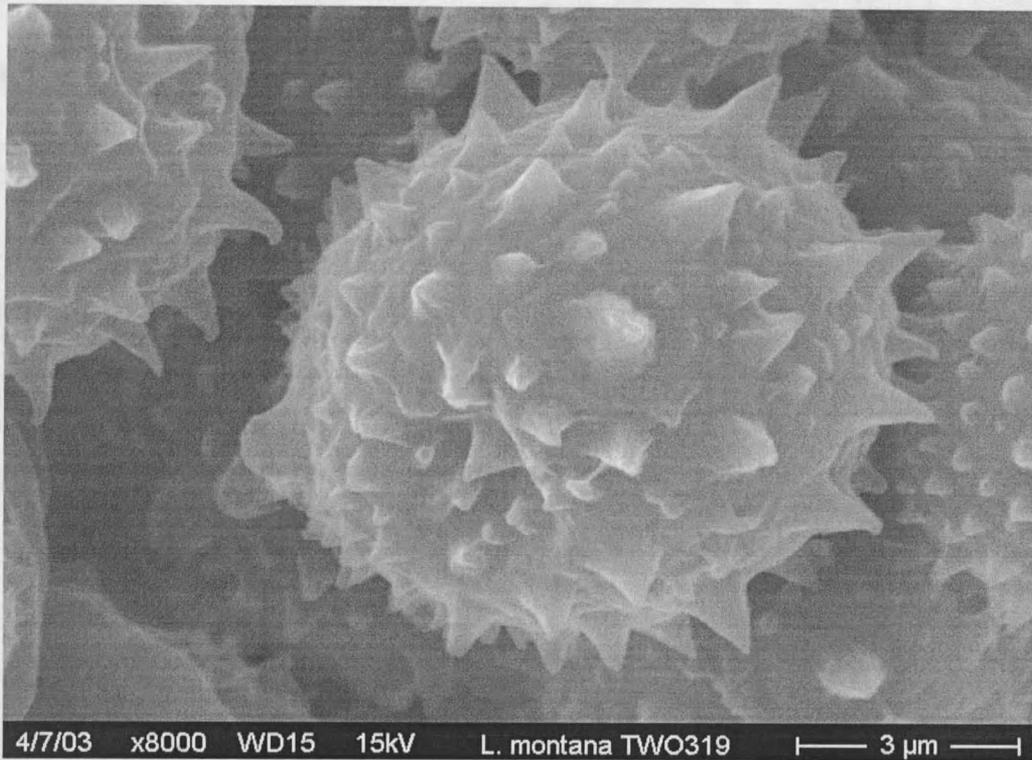
*Laccaria bicolor*: Scanning electron micrographs of basidiospores.



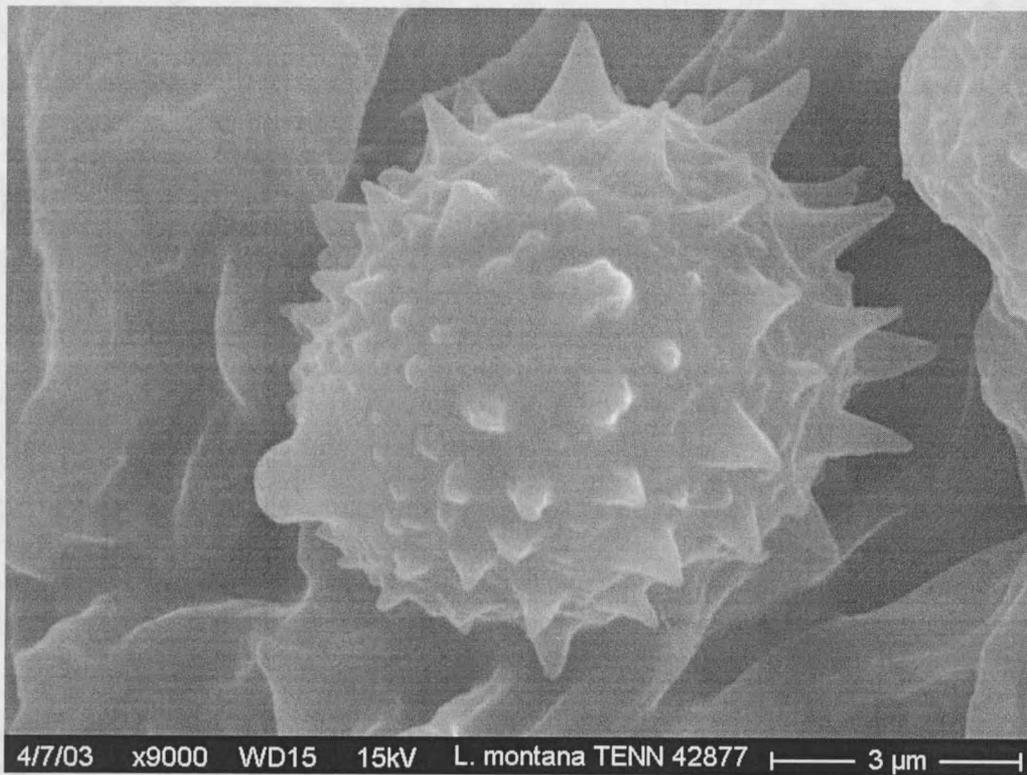
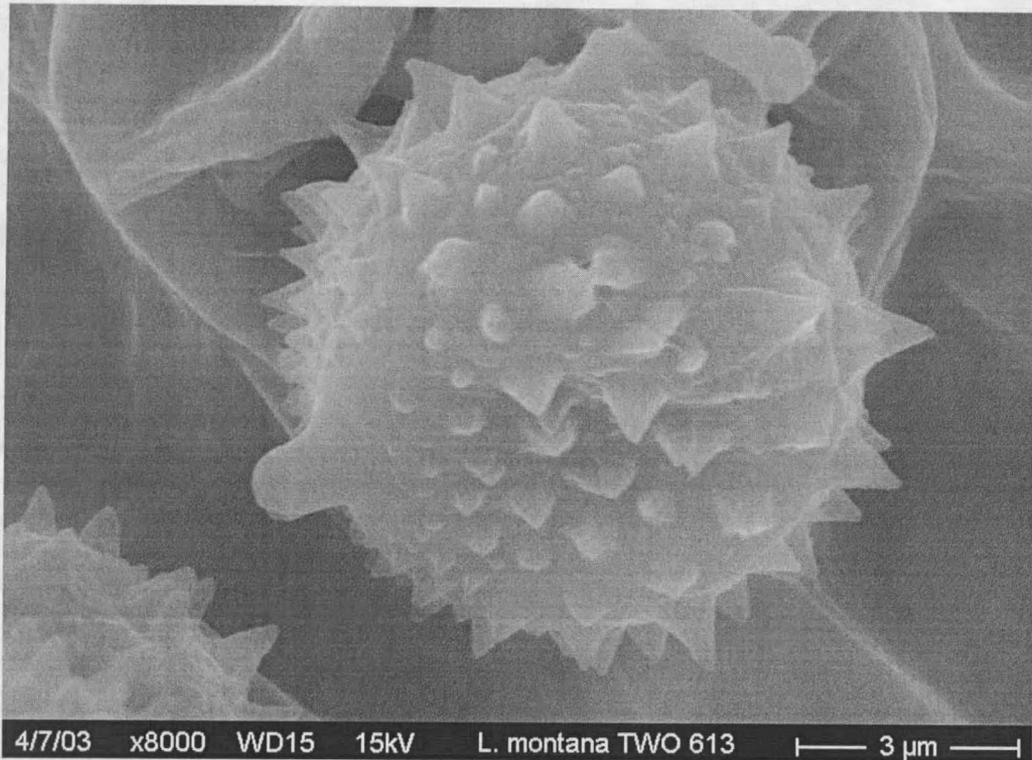
*Laccaria bicolor*: Scanning electron micrograph of basidiospores.



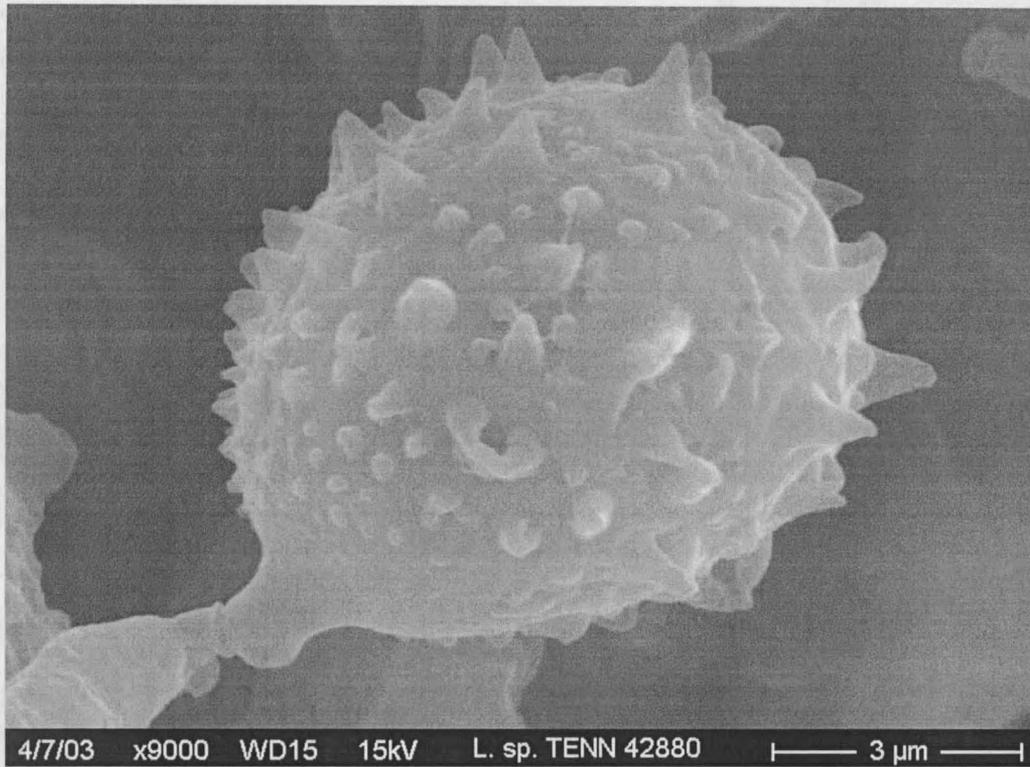
*Laccaria laccata* var. *pallidifolia*: Scanning electron micrographs of basidiospores.



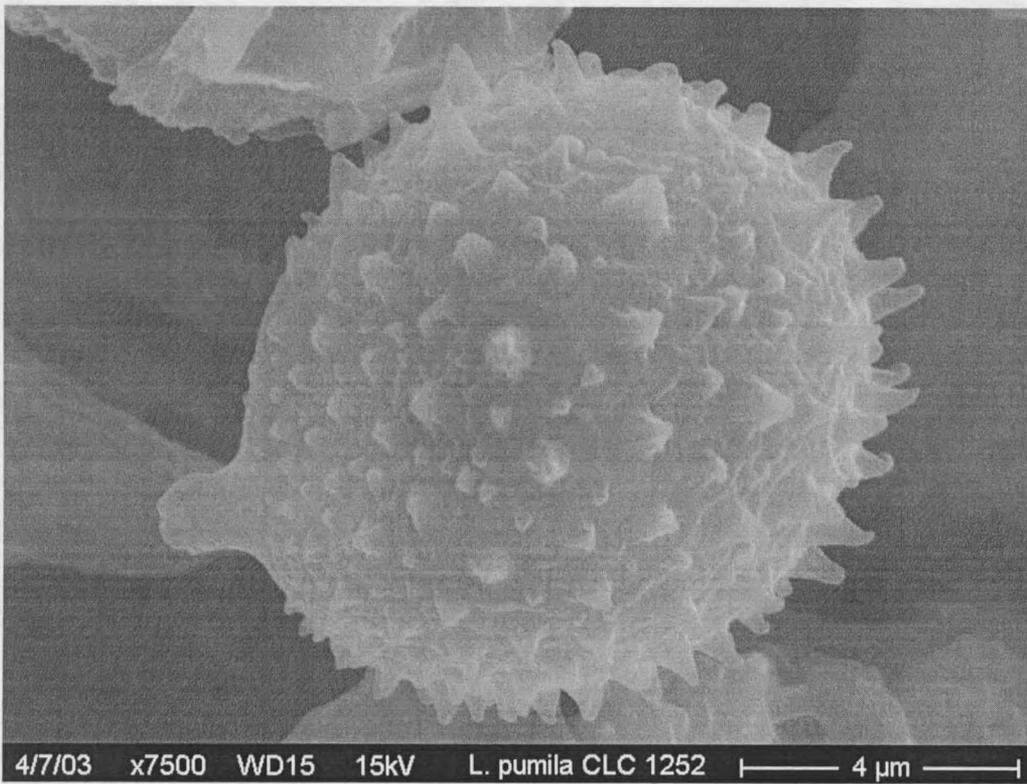
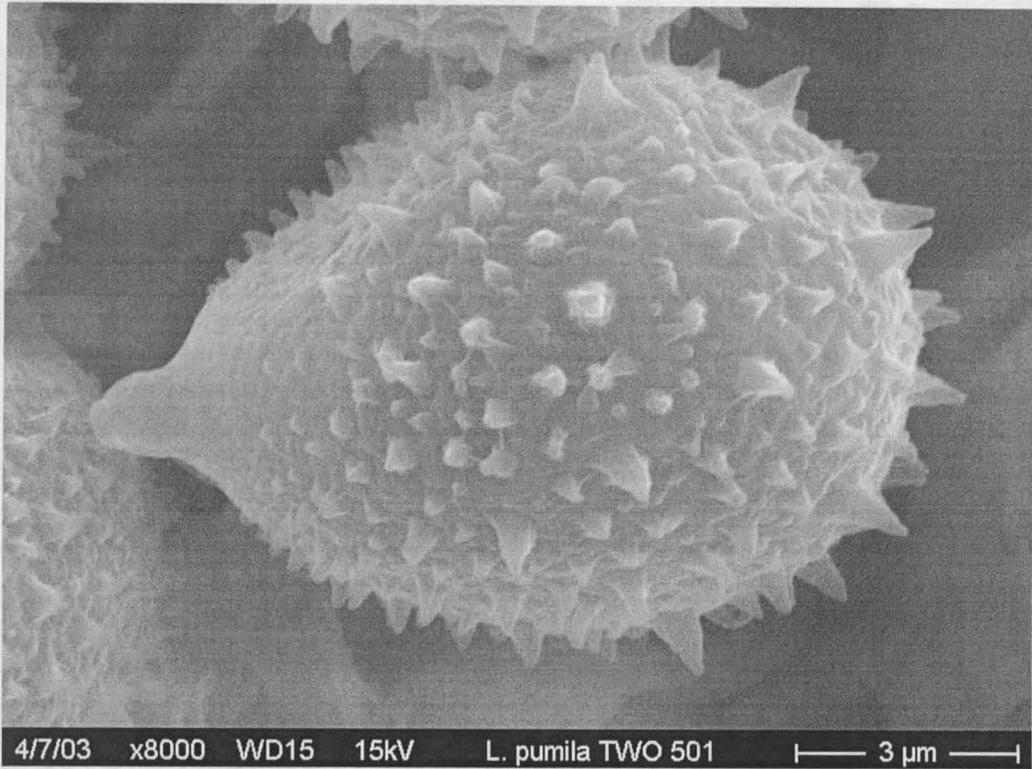
*Laccaria montana*: Scanning electron micrographs of basidiospores.



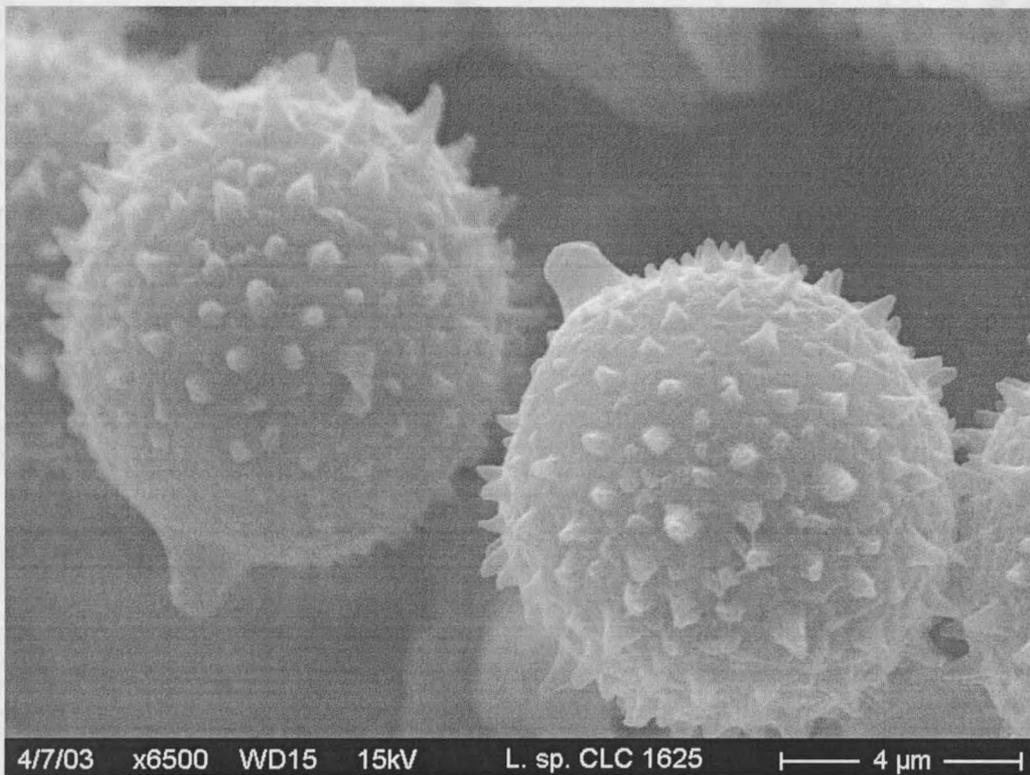
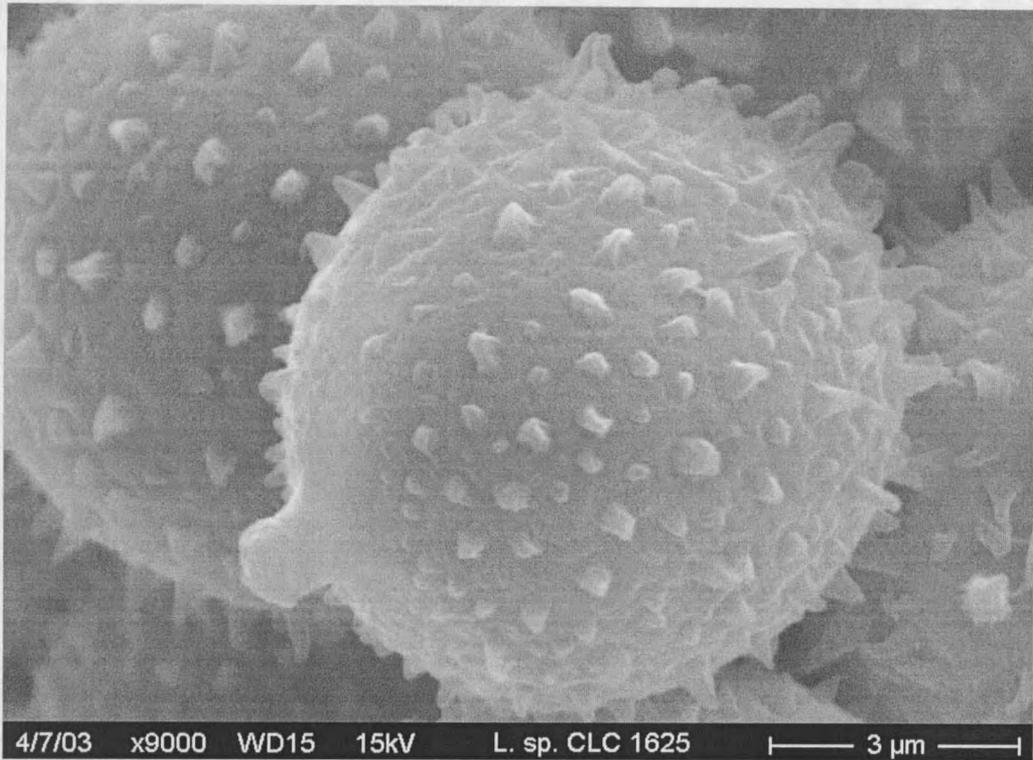
*Laccaria montana*: Scanning electron micrographs of basidiospores.



*Laccaria montana*: Scanning electron micrographs of basidiospores.



*Laccaria pumila*: Scanning electron micrographs of basidiospores.



*Laccaria* sp.: Scanning electron micrographs of basidiospores.

APPENDIX C

DNA SEQUENCE ALIGNMENT USED FOR PHYLOGENETIC ANALYSIS

Appendix C. Sequence alignment of rDNA complete ITS1/5.8S/ITS2 region used for phylogenetic analysis of Rocky Mountain alpine *Laccaria* species (see Chapter 3). Alignment includes a consensus sequence for all alpine ingroup taxa (i.e., all ingroup taxa with the exception of the subalpine collection *L. bicolor* TWO752). Variable nucleotide positions denoted with an asterisk in the consensus sequence. Gaps denoted by “-”.

	5	15	25	35	45	55	65	75
L.sp.1625	AGGATCATT	TTGAATAAAC	CTGATGTGGC	TGTTAGCTGG	CTTTTCAAAG	CATGTGCTCG	TCCGTCATC-	--TTTA-ATT
L.laccatal603	AGGATCATT	TTGAGTAA-C	CTGATGTGGC	TGTTAGCTGG	CT-----AG	CATGTGCTCG	TCCATCATC-	--TTTATATA
L.bicolor1672	AGGATCATT	TTGAATAA-C	CTGATGTGAC	TGTTAGCTGG	CTTT-CGAAG	CATGTGCTCG	TCCATCATC-	--TT---ATC
L.bicolor1304	AGGATCATT	TTGAATAA-C	CTGATGTGAC	TGTTAGCTGG	CTTT-CGAAG	CATGTGCTCG	TCCATCATC-	--TT---ATC
L.bicolor1482	AGGATCATT	TTGAATAAAC	CTGATGTGAC	TGTTAGCTGG	CTTTTCGAAG	CATGTGCTCG	TCCATCATC-	--TTT--ATC
L.bicolor1445	AGGATCATT	TTGAATAAAC	CTGATGTGAC	TGTTAGCTGG	CTTTTCGAAG	CATGTGCTCG	TCCATCATC-	--TTT--ATC
L.montana591	AGGATCATT	TTGAATAAAC	CTGATGTGGC	TGTTAGCTGG	CTTTTCGAAG	CATGTGCTCG	TCCGTCATC-	--TTTA-ATC
L.sp.1771	AGGATCATT	TTGAATAAAC	CTGATGTGGC	TGTTAGCTGG	CTTTTCAAAG	CATGTGCTCG	TCCGTCATC-	--TTTA-ATT
L.laccatal370	AGGATCATT	TTGAGTAA-C	CTGATGTGGC	TGTTAGCTGG	CTT---AGG	CATGTGCTCG	TCCATCATC-	--TTTATATC
L.pumila1252	AGGATCATT	TTGAATAAAC	CTGATGTGAC	TGTTAGCTGG	CTTT-CGAAG	CATGTGCTCG	TCCGTCATC-	--TTTA-ATC
L.montana319	AGGATCATT	TTGAATAA-C	CTGATGTGGC	TGTTAGCTGG	CTTTTCGAAG	CATGTGCTCG	TCCGTCATC-	--TTTA-ATC
L.pumila501	AGGAT-ATTA	TTGAATAA-C	CTGATGTGAC	TGTTAGCTGG	CTTTTCGAAG	CATGTGCTCG	TCCGTCATC-	--TTTA-ATC
L.laccatal724	AGGATCATT	TTGAGTAAAC	CTGATGTGGC	TGTTAGCTGG	CTT---AGG	CATGTGCTCG	TCCATCATC-	--TTTATATA
L.laccatal655	AGGATCATT	TTGAGTAAAC	CTGATGTGGC	TGTTAGCTGG	CTT---AGG	CATGTGCTCG	TCCATCATC-	--TTTATATA
L.bicolor1469	AGGATCATT	TTGAATAAAC	CTGATGTGAC	TGTTAGCTGG	CTTTTCGAAG	CATGTGCTCG	TCCATCATC-	--TTT--ATC
L.laccatal633	AGGATCATT	TTGAGTAAAC	CTGATGTGGC	TGTTAGCTGG	CTT---AGG	CATGTGCTCG	TCCATCATC-	--TTTATATA
L.bicolor752	AGGATCATT	TTGAATAAAC	CTGATGTGAT	TGTTAGCTGG	CTTTTCGAAG	CATGTGCTCA	TCCGTCATC-	--TTTAT--C
T.unifactum	AGGATCATT	TTGAATAAAC	TTGTTTGGGT	TGTT-GCTGG	CTCTTAGGAG	CATGTGCACG	CCTGACA-CA	--TTT-----
T.portentosum	AGGATCATT	TTGAATAAGC	TTGGTTAGGT	TGTT-GCTGG	CTCTTCGGGG	CATGTGCACA	CCTGACACCA	ACTTT---TC
Ingrp.Consensus	AGGAT*ATTA	TTGA*TAA*C	CTGATGTG**	TGTTAGCTGG	CT*****G	CATGTGCTC*	TCC*TCATC-	--TT*****

	85	95	105	115	125	135	145	155
L. sp.1625	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGCAACT	CGGATTTT-A	GGATCGCCG-	TGCTGTAAAA
L.laccatal603	TCTCCACCTG	TGCACATTTT	GTAGACTT-G	G-ATA-----	-----	-----	--ATGGCTC-	TG-----
L.bicolor1672	TCTCCACCTG	TGCACA-TTT	GTAGTCTT-G	G-ATACCTCT	CGAGGAAACT	CGGATTT-GA	GGATCGCCG-	TGCTGTACAA
L.bicolor1304	TCTCCACCTG	TGCACA-TTT	GTAGTCTT-G	G-ATACCTCT	CGAGGAAACT	CGGATTT-GA	GGATCGCCG-	TGCTGTACAA
L.bicolor1482	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGAAACT	CGGATTT-GA	GGATCGCCG-	TGCTGTACAA
L.bicolor1445	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGAAACT	CGGATTT-GA	GGATCGCCG-	TGCTGTACAA
L.montana591	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATAACTCT	CGAGGCAACT	CGGATTTT-A	GGATCGCCG-	TGCT-----
L.sp.1771	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGCAACT	CGGATTTT-A	GGATCGCCG-	TGCTGTAAAA
L.laccatal370	TCTCCACCTG	TGCACATTTT	GTAGACTT-G	G-ATA-----	-----	-----	--ATGGCTC-	TG-----
L.pumila1252	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGCAACT	CGGATTTT-A	GGATCGCTG-	TGCTGTAAAA
L.montana319	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATAACTCT	CGAGGCAACT	CGGATTTT-A	GGATCGCCG-	TGCT-----
L.pumila501	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGCAACT	CGGATTTT-A	GGATCGCTG-	TGCTGTAAAA
L.laccatal724	TCTCCACCTG	TGCACATTTT	GTAGACTT-G	G-ATA-----	-----	-----	--ATGGCTC-	TG-----
L.laccatal655	TCTCCACCTG	TGCACATTTT	GTAGACTT-G	G-ATA-----	-----	-----	--ATGGCTC-	TG-----
L.bicolor1469	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGAAACT	CGGATTT-GA	GGATCGCCG-	TGCTGTACAA
L.laccatal633	TCTCCACCTG	TGCACATTTT	GTAGACTT-G	G-ATA-----	-----	-----	--ATGGCTC-	TG-----
L.bicolor752	TCTCCACCTG	TGCACATTTT	GTAGTCTT-G	G-ATACCTCT	CGAGGAAACT	CGGATTT---	GAATCGCTG-	TGCTGTACAA
T.unifactum	TTACCACCTG	TGCACCCTTT	GTAGTCCT-G	GAACACCTCT	CGAGGCAACT	CGG-TTT-GA	GGATTGCCGC	TGCTGT-TAA
T.portentosum	TTACCACCTG	TGCACTTTT	GTAGACTTTG	GAATACCTCT	CGAGGAAACT	CGG-TTTTGA	GGACTGCTG-	TGC-G--CAA
Ingrp.Consensus	TCTCCACCTG	TGCACA*TTT	GTAG*CTT-G	G-ATA*****	*****	*****	**AT*GC**-	TG*****

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	165	175	185	195	205	215	225	235
L. sp.1625	GTCAGCTTTC	CTCTCATTT-	CCAAGACTAT	GTTTT-C-AT	ATACACCAA	GTATGTTTAA	AGAATGTCA-	-TCAATGGGA
L.laccata1603	-----	---TCATTT-	ACAAGACTAT	GTTTTTCAAT	ATAC-CCAAA	GCATGTTTAA	AGAATGTCA-	-TCAATAGGA
L.bicolor1672	GTCGGCTTTT	CTTTCATTT-	-CAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAT	AGAATGTCA-	-TCAATGGGA
L.bicolor1304	GTCGGCTTTT	CTTTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAT	AGAATGTCA-	-TCAATGGGA
L.bicolor1482	GTCGGCTTTT	CTTTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAT	AGAATGTCA-	-TCAATGGGA
L.bicolor1445	GTCGGCTTTT	CTTTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAT	AGAATGTCA-	-TCAATGGGA
L.montana591	----GCTTTC	CTTTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAA	AGAATGTCA-	-TGAATGGGA
L. sp.1771	GTCAGCTTTC	CTCTCATTT-	CCAAGACTAT	GTTTT-C-AT	ATACACCAA	GTATGTTTAA	AGAATGTCA-	-TCAATGGGA
L.laccata1370	-----	---TCATTT-	ACAAGACTAT	GTTTT-CAAT	ATAC-CCAAA	GCATGTTTAA	AGAATGTCA-	-TCAATAGGA
L.pumila1252	GTCAGCTTTC	CTCTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAA	AGAATGTCA-	-TCAATAGGA
L.montana319	----GCTTTC	CTTTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAA	AGAATGTCA-	-TGAATGGGA
L.pumila501	GTCAGCTTTC	CTCTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAA	AGAATGTCA-	-TCAATAGGA
L.laccata1724	-----	---TCATTT-	ACAAGACTAT	GTTTTTCAAT	ATAC-CCAAA	GCATGTTTAA	AGAATGTCA-	-TCAATAGGA
L.laccata1655	-----	---TCATTT-	ACAAGACTAT	GTTTTTCAAT	ATAC-CCAAA	GCATGTTTAA	AGAATGTCA-	-TCAATAGGA
L.bicolor1469	GTCGGCTTTT	CTTTCATTT-	CCAAGACTAT	GTTTTT--AT	ATACACCAA	GTATGTTTAT	AGAATGTCA-	-TCAATGGGA
L.laccata1633	-----	---TCATTT-	ACAAGACTAT	GTTTTTCAAT	ATAC-CCAAA	GCATGTTTAA	AGAATGTCA-	-TCAATAGGA
L.bicolor752	GTCGGCTTTT	CTTTCATTA-	CCAAGACTAT	GTTTTTATAT	ACAC--CAA	GTATGTTTAT	AGAATGTCA-	-TCAATGGGA
T.unifactum	GCCGGCTTTC	CTTGCGTT-C	CC-GCTCTAT	GTCTTT--AT	ATACCCCAT-	GAATGT-AAC	TGAATGTCT-	-TTAATGGG-
T.portentosum	GCCGGCTTTC	CTTACATTTT	CC-GGTCTAT	GTTTTTCTAT	ATACCCTATA	GTATGT-CAC	AGAATGTCT-	-TTAATGGG-
Ingrp.Consensus	*****	***TCATT*-	*CAAGACTAT	GTTTT***AT	A*AC**CAA	G*ATGTTTA*	AGAATGTCA-	-T*AAT*GGA

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	245	255	265	275	285	295	305	315
L.sp.1625	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.laccata1603	ACTT-GTTTC	CTATTAGAAA	CTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.bicolor1672	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.bicolor1304	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.bicolor1482	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.bicolor1445	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.montana591	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.sp.1771	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.laccata1370	ACTT-GTTTC	CTATTAGAAA	CTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.pumila1252	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.montana319	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.pumila501	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.laccata1724	ACTT-GTTTC	CTATTAGAAA	CTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.laccata1655	ACTT-GTTTC	CTATTAGAAA	CTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.bicolor1469	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.laccata1633	ACTT-GTTTC	CTATTAGAAA	CTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
L.bicolor752	ACTT-GTTTC	CTAT--AAAA	TTA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
T.unifactum	CCTTAGT-GC	CTTT--AAAT	CAAATACAAC	TTTCAACAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
T.portentosum	-CTTAATTGC	CTTT--AAAC	CTA-TACAAC	TTTCAACAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCAGCGAAA
Ingrp.Consensus	ACTT-GTTTC	CTAT**AAA	*TA-TACAAC	TTTCAGCAAC	GGATCTCTTG	GCTCTCGCAT	CGATGAAGAA	CGCA*CGAAA

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	325	335	345	355	365	375	385	395
L. sp.1625	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. laccatal1603	TGCGTTAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. bicolor1672	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. bicolor1304	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. bicolor1482	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. bicolor1445	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. montana591	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. sp.1771	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. laccatal1370	TGCGTTAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. pumila1252	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. montana319	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. pumila501	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. laccatal1724	TGCGTTAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. laccatal1655	TGCGTTAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. bicolor1469	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. laccatal1633	TGCGTTAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
L. bicolor752	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
T. unifactum	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
T. portentosum	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCCTTGG	TATTCCGAGG
Ingrp. Consensus	TGCG*TAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACCTTG	CGCTCC*TGG	TATTCCGAGG

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	405	415	425	435	445	455	465	475
L. sp.1625	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AACTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. laccatal603	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCAT-CC	A---TTAATT	T---GGTTT	GGCTTGGATG	TGGGGGTT--
L. bicolor1672	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AACTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. bicolor1304	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AACTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. bicolor1482	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AACTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. bicolor1445	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AACTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. montana591	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AGCTTTTATT	GGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. sp.1771	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AGCTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. laccatal370	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCAT-CC	A---TTAATT	T---GGTTT	GGCTTGGATG	TGGGGGTT--
L. pumila1252	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AGCTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. montana319	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AGCTTTTATT	GGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. pumila501	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AGCTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. laccatal724	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCAT-CC	A---TTAATT	T---GGTTT	GGCTTGGATG	TGGGGGTT--
L. laccatal655	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCAT-CC	A---TTAATT	T---GGTTT	GGCTTGGATG	TGGGGGTT--
L. bicolor1469	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AACTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
L. laccatal633	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCAT-CC	A---TTAATT	T---GGTTT	GGCTTGGATG	TGGGGGTT--
L. bicolor752	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTT-CC	AACTTTTATT	AGCTTGGTTA	GGCTTGGATG	TGGGGGTT--
T. unifactum	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTTCC	AGCTTTTATT	AGCTTGGTCA	GGCTTGGATG	TGGGGGTT--
T. portentosum	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACCTTATC	AGCTTTTATT	GGCT-GATTA	GGCTTGGATG	TGGGAGTTTT
Ingrp. Consensus	AGCATGCCTG	TTTGAGTGTC	ATTAAATTCT	CAACC*T-CC	A***TT*ATT	****GGTT*	GGCT*GGATG	TGGGGGTT--

	485	495	505	515	525	535	545	555
L.sp.1625	GCGGGCTTCA	----TCAATG	AGGTCGGCTC	TCCTTAAATG	CATTAGCGGA	AC-TTTT-GT	GGAC-CGTCT	ATTGGTGTGA
L.laccatal1603	GCAGGCTTCA	TTTGACTATG	AGGTCAGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	TGAC-CATCT	ATTGGTGTGA
L.bicolor1672	GCGGGCTTCA	----TCAATG	AGGTCGGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	GGAC-CATCT	ATTGGTGTGA
L.bicolor1304	GCGGGCTTCA	----TCACTG	AGGTCGGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	GGAC-CATCT	ATTGGTGTGA
L.bicolor1482	GCGGGCTTCA	----TCACTG	AGGTCGGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	GGAC-CATCT	ATTGGTGTGA
L.bicolor1445	GCGGGCTTCA	----TCACTG	AGGTCGGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	GGAC-CATCT	ATTGGTGTGA
L.montana591	GTGGGCTTCA	----TTAATG	AGGTCAGCTC	TCCTTAAATG	CATTAGCGGA	AC-TTTT-GT	GGAC-CGTCT	ATTGGTGTGA
E.sp.1771	GCGGGCTTCA	----TCAATG	AGGTCGGCTC	TCCTTAAATG	CATTAGCGGA	AC-TTTT-GT	GGAC-CGTCT	ATTGGTGTGA
L.laccatal1370	GCAGGCTTCA	TTTGACTATG	AGGTCAGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	TGAC-CATCT	ATTGGTGTGA
L.pumila1252	GTGGGCTTCA	----TTAATG	AGGTCAGCTC	TCCTTAAATG	CATTAGCGGA	AC-TTTT-GT	GGAC-CGTCT	ATTGGTGTGA
L.montana319	GTGGGCTTCA	----TTAATG	AGGTCAGCTC	TCCTTAAATG	CATTAGCGGA	AC-TTTT-GT	GGAC-CGTCT	ATTGGTGTGA
L.pumila501	GTGGGCTTCA	----TTAATG	AGGTCAGCTC	TCCTTAAATG	CATTAGCGGA	AC-TTTT-GT	GGAC-CGTCT	ATTGGTGTGA
L.laccatal1724	GCAGGCTTCA	TTTGACTATG	AGGTCAGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	TGAC-CATCT	ATTGGTGTGA
L.laccatal1655	GCAGGCTTCA	TTTGACTATA	AGGTCAGCTC	TCCTTAAATG	CATTACTGGA	AC-TTTT-GT	TGAC-CATCT	ATTGGTGTGA
L.bicolor1469	GCGGGCTTCA	----TCACTG	AGGTCGGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	GGAC-CATCT	ATTGGTGTGA
L.laccatal1633	GCAGGCTTCA	TTTGACTATG	AGGTCAGCTC	TCCTTAAATG	CATTAGTGGA	AC-TTTT-GT	TGAC-CATCT	ATTGGTGTGA
L.bicolor752	GCGGGCTTCA	----TTAATG	AGGTCGGCTC	TCCTTAAATG	CATTAGCGGA	AC-TTTT-GT	GGAC-CGTCT	ATTGGTGTGA
T.unifactum	GCGGGCTTC-	-----TCAG	AAGTCGGCTC	TCCTTAAATG	CATTAGCGGA	ACCTTT--GT	TGAC-CAGCT	-CTGGTGTGA
T.portentosum	GCGGGCTTT-	-----TAAG	AAGTCGGCTC	TCCTTAAATG	CATTAGCGGG	ACCTTTTGGT	TGCCTCAGCT	ACTGGTGTGA
Ingrp.Consensus	G**GGCTTCA	*****T*	AGGTC*GCTC	TCCTTAAATG	CATTA**GGA	AC-TTTT-GT	*GAC-C*TCT	ATTGGTGTGA

	565	575	585	595	605	615	625	635
L. sp.1625	TAATTATCTA	CGCCGTGGAT	T-TGAAGCAG	CT----TTAT	GAAGTTCAGC	CTCTAACCGT	C--CATTGAC	TTG-GACAA-
L. laccatal1603	TATCTATCTA	CACTGTGGAT	G-TGAAGCGG	-----T	G---TTCAGC	TTCTAACCGT	C--TATT-A-	TTG--ACTTA
L. bicolor1672	TAATTATCTA	CGCCGTGGGT	G-TGAAGCAG	CT----TTAT	GAAGTTCCTGC	TTCTAACCGT	C--CATTGAC	TTG-GACAA-
L. bicolor1304	TAATTATCTA	CGCCGTGGGT	G-TGAAGCAG	CT----TTAT	GAAGTTCCTGC	TTCTAACCGT	C--CATTGAC	TTG-GACAA-
L. bicolor1482	TAATTATCTA	CGCCGTGGGT	G-TGAAGCAG	CT----TTAT	GAAGTTCCTGC	TTCTAACCGT	C--CATTGAC	TTG-GACAA-
L. bicolor1445	TAATTATCTA	CGCCGTGGGT	G-TGAAGCAG	CT----TTAT	GAAGTTCCTGC	TTCTAACCGT	C--CATTGAC	TTG-GACAA-
L. montana591	TAATTATCTA	CGCTGTGGAT	G-TGAAGCAT	AT----TTAT	GAAGTTCAGC	TTCTAATCGT	C--CATTGAC	TTG-GACAA-
L. sp.1771	TAATTATCTA	CGCCGTGGAT	T-TGAAGCAG	CT----TTAT	GAAGTTCAGC	CTCTAACCGT	C--CATTGAC	TTG-GACAA-
L. laccatal370	TATCTATCTA	CACTGTGGAT	G-TGAAGCGG	-----T	G---TTCAGC	TTCTAACCGT	C--TATT-A-	TTG--ACTTA
L. pumila1252	TAATTATCTA	CGCCGTGGAT	G-TGAAGCAG	CT----TTAT	GAAGTTCAGC	TTCTAATCGT	C--CATTGAC	TTG-GACAA-
L. montana319	TAATTATCTA	CGCTGTGGAT	G-TGAAGCAT	AT----TTAT	GAAGTTCAGC	TTCTAATCGT	C--CATTGAC	TTG-GACAA-
L. pumila501	TAATTATCTA	CGCCGTGGAT	G-TGAAGCAG	CT----TTAT	GAAGTTCAGC	TTCTAATCGT	C--CATTGAC	TTG-GACAA-
L. laccatal724	TATCTATCTA	CACTGTGGAT	G-TGAAGCGG	-----T	G---TTCAGC	TTCTAACCGT	C--TATC-A-	GTG--ACTTA
L. laccatal655	TATCTATCTA	CACTGTGGAT	G-TGAAGCAG	-----T	G---TTCAGC	TTCTAACCGT	C--TATT-A-	TTG--ACTTA
L. bicolor1469	TAATTATCTA	CGCCGTGGGT	G-TGAAGCAG	CT----TTAT	GAAGTTCCTGC	TTCTAACCGT	C--CATTGAC	TTG-GACAA-
L. laccatal633	TATCTATCTA	CACTGTGGAT	G-TGAAGCGG	-----T	G---TTCAGC	TTCTAACCGT	C--TATT-A-	TTG--ACTTA
L. bicolor752	TAATTATCTA	CGCCGTGGAT	G-TGAAGCAG	CT----TTAT	GAAGTTCCTGC	TTCTAACCGT	C--CATTGAC	TTG-GACAA-
T. unifactum	TAATTATCTA	CGCC--ATT	GC-GAAGCAG	CTTTAATAAT	GGGGTTCAGC	TTCTAACCGT	CCCC-TTCAC	--GGGACAA-
T. portentosum	TAATTATCTA	CGCT-T-ATT	GCTGAA--AG	TG----ACTT	G--GTACAGC	TTCTAATCGT	CTTCATTTAT	TTGAGACAA-
Ingrp. Consensus	TA**TATCTA	C*C*GTGG*T	*-TGAAGC**	**-----***T	G***TTC*GC	*TCTAA*CGT	C--*AT**A*	*TG-*AC***

	..... .....	..... .....	..... .....
	645	655	665
L.sp.1625	-TTTT---GA	CAATTTGACC	TCAAATCAG
L.laccata1603	CTTTT---GA	CCATTTGACC	TCAAATCAG
L.bicolor1672	-TTTT---GA	CAATTTGACC	TCAAATCAG
L.bicolor1304	-TTTT---GA	CAATTTGACC	TCAAATCAG
L.bicolor1482	-TTTT---GA	CAATTTGACC	TCAAATCAG
L.bicolor1445	-TTTT---GA	CAATTTGCCC	TCAAATCAG
L.montana591	TTTTT---GA	CAATTTGACC	TCAAATCAG
L.sp.1771	-TTTT---GA	CAATTTGACC	TCAAATCAG
L.laccata1370	CTTTT---GA	CCATTTGACC	TCAAATCAG
L.pumila1252	TTTTT---GA	CAATTTGACC	TCAAATCAG
L.montana319	TTTTT---GA	CAATTTGACC	TCAAATCAG
L.pumila501	TTTTT---GA	CAATTTGACC	TCAAATCAG
L.laccata1724	CTTTT---GA	CCATTTGACC	TCAAATCAG
L.laccata1655	CTTTT---GA	CCATTTGACC	TCAAATCAG
L.bicolor1469	-TTTT---GA	CAATTTGACC	TCAAATCAG
L.laccata1633	CTTTT---GA	CCATTTGACC	TCAAATCAG
L.bicolor752	-TCTT---GA	TAATTTGACC	TCAAATCAG
T.unifactum	CTCTC--TGA	CATTTTGACC	TCAAATCAG
T.portentosum	TTCATAATGA	CAATTTGACC	TCAAATCAA
Ingrp.Consensus	*T*TT---GA	**ATTG*CC	TCAAATCAG

APPENDIX D

PLANT COMMUNITY CHARACTERIZATION FOR BEARTOOTH PLATEAU

ALPINE SAMPLING PLOTS

Appendix D. Plant community characterization for Beartooth Plateau alpine sampling plots. Only herbaceous forbs listed. Ectomycorrhizal host species appears at top of columns.

Plant Species	EM Host Species:																
	Site-Plot:	<i>Salix reticulata</i>			<i>Salix arctica</i>				<i>Salix planifolia</i>		<i>Salix glauca</i>			<i>Betula glandulosa</i>	<i>Dryas octopetala</i>		
		2-3	2-7	3-2	3-1	3-3	4-1	4-2	1-2	2-5	2-1	2-2	1-3	1-4**	1-1	2-4*	2-6*
<i>Antennaria lanata</i> (Hook.) Greene												X					
<i>Antennaria</i> sp.			X														
<i>Artemisia scopulorum</i> Gray		X	X	X	X		X	X				X		X			
<i>Aster alpigenus</i> (T&G.) Gray			X									X					
<i>Aster</i> sp.															X	X	
<i>Astragalus</i> sp.																	X
<i>Bupleurum americanum</i> Coult. & Rose											X				X		
<i>Caltha leptosepala</i> DC.								X						X			
<i>Castelleja</i> sp.			X						X		X	X					
<i>Cerastium</i> sp.					X												
<i>Geum rossii</i> (R.Br.) Ser. in DC.		X	X		X		X				X				X	X	
<i>Lewisia pygmaea</i> (Gray) Robins.											X	X					
<i>Lupine</i> sp.				X							X				X		X
<i>Mertensia</i> sp.				X													
<i>Minuartia obtusiloba</i> (Rydb.) House															X	X	
<i>Pedicularis groenlandica</i> Retz									X		X	X				X	
<i>Pedicularis oderi</i> Vahl ex Homem.					X	X											
<i>Penstemon procerus</i> Doug. Ex Grah.									X		X						
<i>Phleum alpinum</i> L.												X					
<i>Phlox</i> sp.																	X
<i>Phylodoce glanduliflora</i> (Hook.) Cov.														X			
<i>Polygonum bistortoides</i> Pursh		X	X	X		X	X	X			X	X			X		X
<i>Polygonum viviparum</i> L.														X		X	X
<i>Potentilla diversifolia</i> Lehm.									X			X					
<i>Potentilla</i> sp.					X					X				X	X		
<i>Rumex paucifolius</i> Nutt.					X												

Appendix D., continued

	EM Host Species:																
	Site-Plot:	<i>Salix reticulata</i>			<i>Salix arctica</i>				<i>Salix planifolia</i>		<i>Salix glauca</i>			<i>Betula glandulosa</i>	<i>Dryas octopetala</i>		
		2-3	2-7	3-2	3-1	3-3	4-1	4-2	1-2	2-5	2-1	2-2	1-3	1-4**	1-1	2-4*	2-6*
<i>Sedum lanceolatum</i> Torr.		X	X														X
<i>Sedum rhodanthum</i> Gray									X					X			
<i>Senecio cymbalarioides</i> Buek												X					
<i>Senecio</i> sp.		X												X	X		X
<i>Sibaldia procumbens</i> L.											X						
<i>Silene acaulis</i> (L.) Jacq.					X												X
<i>Trifolium parryi</i> Gray				X			X	X				X					X
<i>Trifolium</i> sp.		X	X														X X
<i>Veronica nutans</i> Bong.												X					
<i>Zigedanus elegans</i> Pursh.																	X

\* Mixed plot including *Salix reticulata*

\*\* Mixed plot including *Salix reticulata* and *Salix planifolia*

APPENDIX E

LIST OF BEARTOOTH PLATEAU PLOT SURVEY DATES

Appendix E: List of Beartooth Plateau Survey Dates. \* Denotes dates that plots were surveyed. Sites visited appear in parentheses after date. Excursions joined by left brackets.

1999

- { July 20 (Quad Creek, Highline Trailhead)
- { July 22 (Quad Creek)
- { August 9\* (Highline Trailhead)
- { August 10\* (Quad Creek)
- { August 11\* (Frozen Lake)

2000

- { July 17\* (Quad Creek, Gardner Headwall)
- { July 18\* (Highline Trailhead, Frozen Lake)
- { July 31\* (Quad Creek)
- { August 1\* (Highline Trailhead)
- { August 2\* (Frozen Lake, Gardner Headwall)
- { August 21\* (Quad Creek)
- { August 22\* (Highline Trailhead)
- { August 23\* (Frozen Lake, Gardner Headwall)

2001

- { July 13\* (Quad Creek, Gardner Headwall)
- { July 14\* (Highline Trailhead, Frozen Lake)
- { July 19\* (Quad Creek)
- { July 20\* (Highline Trailhead)
- { July 21\* (Frozen Lake, Gardner Headwall)
- { July 23\* (Quad Creek)
- { July 24\* (Highline Trailhead)
- { July 28\* (Quad Creek)
- { July 29\* (Gardner Headwall)
- { July 30\* (Quad Creek, Highline Trailhead)
- { July 31\* (Quad Creek, Frozen Lake, Gardner Headwall)
- { August 1\* (Solifluction Terraces)
- { August 3\* (Quad Creek, Frozen Lake, Highline Trailhead)
- { August 4\* (Frozen Lake, Gardner Headwall)
- { August 5\* (Quad Creek, Highline Trailhead)

2001 (continued)

- { August 16\* (Quad Creek, Highline Trailhead)
- { August 17\* (Frozen Lake, Gardner Headwall)
- { August 19\* (Highline Trailhead, Solifluction Terraces)
- { August 20\* (Quad Creek)
- { August 21\* (Quad Creek, Gardner Headwall, Frozen Lake)
- { September 1\* (Quad Creek, Frozen Lake)
- { September 2\* (Highline Trailhead)

2002

- July 3 (Quad Creek)
- { July 17\* (Quad Creek)
- { July 18\* (Gardner Headwall, Highline Trailhead)
- { July 19\* (Frozen Lake)
- { July 29\* (Quad Creek)
- { July 30\* (Highline Trailhead)
- { July 31\* (Solifluction Terraces, Frozen Lake, Gardner Headwall)
- { August 14\* (Quad Creek)
- { August 15\* (Highline Trailhead)
- { August 16\* (Gardner Headwall, Frozen Lake)
- { August 27\* (Quad Creek, Gardner Headwall)
- { August 28\* (Highline Trailhead, Solifluction Terraces)
- { August 29\* (Frozen Lake)

APPENDIX F

SPECIMENS COLLECTED ON BEARTOOTH PLATEAU  
SAMPLING PLOTS

Appendix F. Specimens collected on Beartooth Plateau sampling plots.

Specific Locations	Plot	Genus	Species	Collector	ID	Day	Month	Year	Plant associations
Quad Creek	2	<i>Entoloma</i>	sp. #2	TWO	503	3	08	2001	Sn
Quad Creek	2	<i>Entoloma</i>	sp. #2	TWO	543	16	08	2001	Sn
Quad Creek	2	<i>Laccaria</i>	<i>montana</i>	TWO	264	10	08	1999	Sn
Quad Creek	2	<i>Laccaria</i>	<i>montana</i>	TWO	441	28	07	2001	Sn
Quad Creek	2	<i>Russula</i>	<i>pascua</i>	TWO	546	16	08	2001	Sn
Quad Creek	2	<i>Russula</i>	<i>pascua</i>	TWO	583	01	09	2001	Sn
Quad Creek	2	<i>Russula</i>	<i>pascua</i>	TWO	721	27	08	2002	Sn
Quad Creek	2	<i>Russula</i>	<i>pascua</i>	TWO	689	14	08	2002	Sn
Quad Creek	3	<i>Bryoglossum</i>	<i>gracile</i>	TWO	696	14	08	2002	mo
Quad Creek	3	<i>Bryoglossum</i>	<i>Bryoglossum</i>	TWO	310	31	07	2000	mo
Quad Creek	3	<i>Cortinarius</i>	<i>absarokensis</i>	TWO	672	29	07	2002	Sg
Quad Creek	3	<i>Cortinarius</i>	<i>absarokensis</i>	TWO	263	10	08	1999	Sg
Quad Creek	3	<i>Cortinarius</i>	<i>absarokensis</i>	TWO	534	5	08	2001	Sg
Quad Creek	3	<i>Cortinarius</i>	cf. <i>anomalous</i>	TWO	692	14	08	2002	Sg
Quad Creek	3	<i>Cortinarius</i>	sp. #2 (Telamonia)	TWO	535	5	08	2001	Sg
Quad Creek	3	<i>Cortinarius</i>	sp. #4 (Telamonia)	TWO	349	21	08	2000	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	691	14	08	2002	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	266	10	08	1999	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	350	21	08	2000	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	693	14	08	2002	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	698	14	08	2002	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	549	16	08	2001	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	CLC	1379	19	8	1999	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	444	28	07	2001	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	382	19	07	2001	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	537	5	08	2001	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	722	27	08	2002	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	674	29	07	2002	Sg
Quad Creek	3	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	313	31	07	2000	Sg
Quad Creek	3	<i>Hebeloma</i>	cf. <i>mesophaeum</i>	TWO	697	14	08	2002	Sg
Quad Creek	3	<i>Hebeloma</i>	cf. <i>mesophaeum</i>	TWO	695	14	08	2002	Sg

Specific Locations	Plot	Genus	Species	Collector	ID	Day	Month	Year	Plant associations
Quad Creek	3	<i>Laccaria</i>	<i>pumila</i>	TWO	717	27	08	2002	Sg
Quad Creek	3	<i>Laccaria</i>	<i>pumila</i>	TWO	265	10	08	1999	Sg
Quad Creek	3	<i>Laccaria</i>	<i>pumila</i>	TWO	348	21	08	2000	Sg
Quad Creek	3	<i>Laccaria</i>	<i>pumila</i>	TWO	442	28	07	2001	Sg
Quad Creek	3	<i>Laccaria</i>	<i>pumila</i>	TWO	520	5	08	2001	Sg
Quad Creek	3	<i>Laccaria</i>	<i>pumila</i>	TWO	465	30	07	2001	Sg
Quad Creek	3	<i>Mycena</i>	sp.	TWO	440	28	07	2001	mo
Quad Creek	3	<i>Russula</i>	<i>norvegica</i>	TWO	690	14	08	2002	Sg
Quad Creek	3	<i>Russula</i>	<i>norvegica</i>	TWO	309	31	07	2000	Sg
Quad Creek	3	<i>Russula</i>	<i>norvegica</i>	TWO	544	16	08	2001	Sg
Quad Creek	4	<i>Clitocybe</i>	sp. #1	TWO	311	31	07	2000	gr
Quad Creek	4	<i>Clitocybe</i>	sp. #1	TWO	550	16	08	2001	gr
Quad Creek	4	<i>Clitocybe</i>	sp. #1	TWO	381	19	07	2001	gr
Quad Creek	4	<i>Cortinarius</i>	sp. #1 (Telamonia)	TWO	536	5	08	2001	Bg, Sn, Sr
Quad Creek	4	<i>Cortinarius</i>	sp. #2 (Telamonia)	TWO	482	31	07	2001	Bg, Sn, Sr
Quad Creek	4	<i>Dermocybe</i>	<i>cinnamomeoluteus</i>	TWO	619	29	07	2002	Bg, Sn, Sr
Quad Creek	4	<i>Entoloma</i>	sp. #3	TWO	551	16	08	2001	Bg, Sn, Sr
Quad Creek	4	<i>Entoloma</i>	sp. #3	TWO	271	10	08	1999	Bg, Sn, Sr
Quad Creek	4	<i>Entoloma</i>	sp. #3	TWO	312	31	07	2000	Bg, Sn, Sr
Quad Creek	4	<i>Inocybe</i>	<i>giacomii</i> group	TWO	270	10	08	1999	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	584	01	09	2001	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	269	10	08	1999	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	523	5	08	2001	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	621	29	07	2002	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	694	14	08	2002	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	548	16	08	2001	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	479	31	07	2001	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	TWO	450	28	07	2001	Bg, Sn, Sr
Quad Creek	4	<i>Lactarius</i>	<i>glyciosmus</i>	CLC	1380	19	8	1999	Bg, Sn, Sr
Quad Creek	4	<i>Leccinum</i>	cf. <i>rotundifoliae</i>	TWO	582	01	09	2001	Bg, Sn, Sr
Quad Creek	4	<i>Rickenella</i>	cf. <i>fibula</i>	TWO	315	31	07	2000	mo
Quad Creek	4	<i>Russula</i>	<i>norvegica</i>	CLC	1381	19	8	1999	Bg, Sn, Sr
Frozen Lake	1	<i>Laccaria</i>	<i>montana</i>	TWO	553	17	08	2001	Si

Specific Locations	Plot	Genus	Species	Collector	ID	Day	Month	Year	Plant associations
Frozen Lake	1	<i>Russula</i>	<i>pascua</i>	TWO	554	17	08	2001	Si
Frozen Lake	2	<i>Cortinarius</i>	sp. #3 (Telamonia)	TWO	662	31	07	2002	Sr
Frozen Lake	2	<i>Cortinarius</i>	<i>favrei</i>	TWO	661	31	07	2002	Sr
Frozen Lake	2	<i>Cortinarius</i>	<i>favrei</i>	TWO	617	19	07	2002	Sr
Frozen Lakes	2	<i>Inocybe</i>	aff. <i>salicis</i>	CLC	1402	21	8	1999	Sr
Frozen Lakes	2	<i>Lepista</i>	aff. <i>irina</i>	CLC	1399	21	8	1999	gr
Frozen Lakes	3	<i>Laccaria</i>	<i>pumila</i>	CLC	1404	21	8	1999	Si
Frozen Lake	3	<i>Laccaria</i>	<i>montana</i>	TWO	477	31	07	2001	Si
Frozen Lakes	3	<i>Lactarius</i>	cf. <i>nanus</i>	CLC	1403	21	8	1999	Si
Frozen Lakes	3	<i>Omphalina</i>	<i>rivulicola</i>	CLC	1397	21	8	1999	mo
Highline Trail	1	<i>Amanita</i>	<i>absarokensis</i>	CLC	1392	20	8	1999	Sg
Highline Trail	1	<i>Amanita</i>	<i>absarokensis</i>	TWO	538	16	08	2001	Sg
Highline Trail	1	<i>Amanita</i>	<i>absarokensis</i>	TWO	678	30	07	2002	Sg
Highline Trail	2	<i>Amanita</i>	<i>absarokensis</i>	TWO	539	16	08	2001	Sg
Highline Trail	2	<i>Cortinarius</i>	<i>absarokensis</i>	TWO	699	15	08	2002	Sg
Highline Trail	3	<i>Amanita</i>	<i>absarokensis</i>	TWO	733	28	08	2002	Sr
Highline Trail	3	<i>Amanita</i>	<i>absarokensis</i>	TWO	397	20	07	2001	Sr
Highline Trail	3	<i>Amanita</i>	<i>absarokensis</i>	TWO	677	30	07	2002	Sr
Highline Trail	3	<i>Cortinarius</i>	<i>favrei</i>	TWO	401	20	07	2001	Sr
Highline Trail	3	<i>Inocybe</i>	<i>giacomii</i> group	TWO	398	20	07	2001	Sr
Highline Trail	4	<i>Cortinarius</i>	<i>tenebricus</i>	TWO	372	14	07	2001	Do
Highline Trail	5	Ascomycete	(pale yellow, stalked cup)	TWO	735	28	08	2002	Sn
Highline Trail	5	<i>Entoloma</i>	sp. #1	TWO	643	30	07	2002	Sn
Highline Trail	5	<i>Hebeloma</i>	cf. <i>mesophaeum</i>	TWO	728	28	08	2002	Sn
Highline Trail	5	<i>Mycena</i>	<i>citrinomarginata</i>	TWO	645	30	07	2002	mo
Highline Trail	6	<i>Clitocybe</i>	sp. #2	TWO	654	30	07	2002	gr, Do, Sr
Highline Trail	7	<i>Amanita</i>	<i>absarokensis</i>	TWO	524	5	08	2001	Sr

Sg = *Salix glauca* ; Si = *Salix arctica* ; Sn = *Salix planifolia* ; Sr = *Salix reticulata* ; Do = *Dryas octopetala* ; Bg = *Betula glandulosa* ; gr = grass; mo = moss

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