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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APPROVAL

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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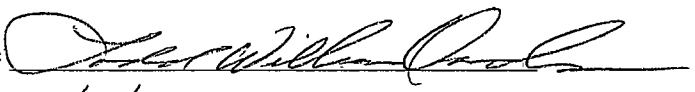
Signature 

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ABSTRACT

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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).

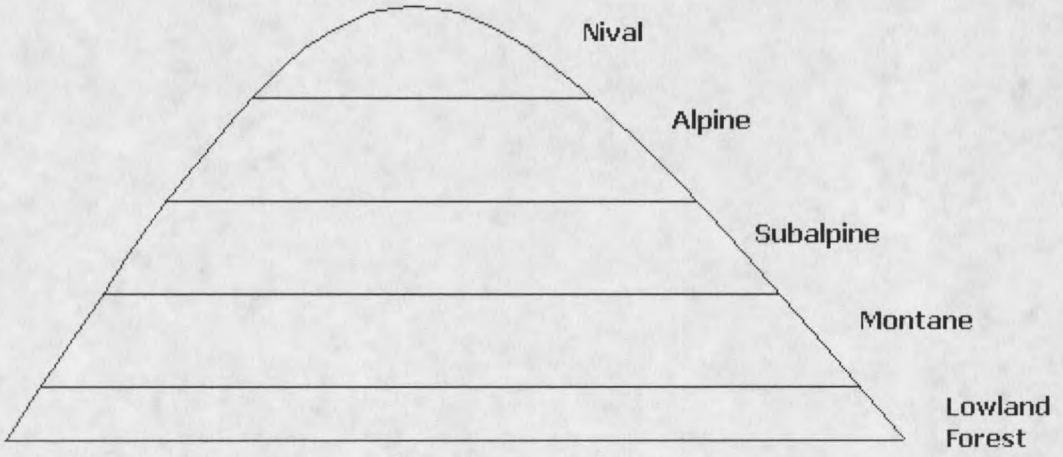

Figure 1. Schematic diagram showing elevational gradient of mountain vegetation zones.

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
Arctostaphylos alpina	Michelsen et al. 1996
Arctostaphylos rubra	Miller et al. 1982
Betula glandulosa	Eddington & Cripps (manuscript in preparation), Lange 1957
Betula nana	Michelsen et al. 1996, Miller et al. 1982, Treu et al 1996, Väre et al. 1997
Cassiope tetragona	Miller 1982, Miller & Laursen 1974, Kohn & Stasovski 1990, Stutz 1972
Crepis aurea	Read & Haselwandter 1981
Daphne striata	Read & Haselwandter 1981
Dryas integrifolia	Bledsoe et al. 1990, Kohn & Stasovski 1990, Miller & Laursen 1974, Read & Haselwandter 1981, Stutz 1972, Väre et al. 1992
Dryas octopetala	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
Festuca rubra	Read & Haselwandter 1981
Helianthemum oelandicum	Read & Haselwandter 1981
Homogyne alpina	Read & Haselwandter 1981
Kobresia bellardii (=*K. myosuroides*)	Haselwandter & Read 1980, Kohn & Stasovski 1990, Massicotte et al. 1998, Read & Haselwandter 1981
Pedicularis capitata	Kohn & Stasovski 1990
Pedicularis dasyantha	Väre et al. 1992
Pedicularis hirsuta	Stutz 1972
Polygonum viviparum	Haselwandter & Read 1980, Lesica & Antibus 1986, Massicotte et al. 1998, Michelsen et al. 1996, Read & Haselwandter 1981, Treu et al 1996
Potentilla hyparctica	Bledsoe et al. 1990, Haselwandter & Read 1980
Potentilla reptans	Read & Haselwandter 1981
Potentilla stricta	Read & Haselwandter 1981
Pyrola grandiflora	Kohn & Stasovski 1990
Salix 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
Saxifraga oppositifolia	Kohn & Stasovski 1990, Stutz 1972
Saxifraga paniculata	Read & Haselwandter 1981
Silene acaulis	Read & Haselwandter 1981
Vaccinium uligionosum	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_2 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 conditons, 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 additonal 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 inaccessability 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 latu*) to most *Laccaria* species lacking violet basidiocarp pigments, therefore underestimating true species diversity. Nomenclatural synonomy, 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.ohiensis* (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 synonomy 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^2 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 synonomy, 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.

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

Table 4, continued.

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

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

Species	Distribution
L. amethysteo-occidentalis G.M. Mueller	Western North America
L. amethystina Cooke	Eastern North America, Europe, Central and South America
L. bicolor (Maire) Orton	Western North America, Great Lakes region, Europe
L. bullulifera Singer	Mexico
L. canaliculata (Cooke & Massee) Pegler	under native Australasian trees
L. chibiensis Michal	USSR
L. fibrillosa McNabb	New Zealand
L. fraterna (Cooke & Massee) Pegler	Australia, widely distributed where *Eucalyptus* introduced
L. galerinoides Singer	South America
L. gomezii Singer & G.M Mueller	Costa Rica, Colombia
L. grabripes McNabb	New Zealand
L. impolita Vellinga & G.M. Mueller	under native European trees
L. laccata (Scop..Fr.) Cooke var *laccata*	Europe, North America; rare
L. laccata var *moelleri* Singer	Europe
L. laccata var. *pallidifolia* (Peck) Peck	North America, Europe
L. lilacina Stevenson	New Zealand
L. longipes G.M. Mueller	North America (Great Lakes region)
L. maritima (Teod.) Singer ex Huhtinen	Eastern North America (rare), Northern Europe
L. masonii var. *brevispinosa* McNabb	New Zealand
L. masonii var *masonii* Stevenson	New Zealand
L. montana Singer	cosmopolitan in arctic, alpine, boreal habitats
L. murina Imai	Japan
L. nana Massee	England
L. nigra Hongo	Japan
L. nobilis G.M. Mueller	Western North America, Great Lakes region
L. oblongospora G M Mueller	North America (Gulf Coast states)
L. ochropurpurea (Berk.) Peck	Eastern North America
L. ohiensis (Mont.) Singer	Eastern North America, Central and South America, Europe
L. ohiensis var. *paraphysata* McNabb	New Zealand
L. proxima (Boud) Pat	cosmopolitan
L. proximella Singer	Southern Argentina, Chile
L. pumila Fayod	cosmopolitan in arctic, alpine, boreal habitats
L. purpureobadia Reid	Western Europe
L. striatula (Peck) Peck	Eastern North America
L. tortilis (Bolt.) Cooke	North and South America, Europe
L. trichodermophora G M. Mueller	SE North America, Mexico, Central America
L. trullissata (Ellis) Peck	Eastern North America
L. vinaceoavellanea Hongo	Japan
L. vinaceobrunnea G.M. Mueller	USA (Gulf Coast states)
L. violaceoniger 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 synonomy 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 synonomy 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
L. altaica Singer	Western Alps	Kühner & Lamoure 1986, Trimbach 1978
	Altai Mountains, USSR	Singer 1967
	Svalbard	Skifte 1979
	Northwest Territories, Canada	Lahaie 1981
	Baffin Island, Canada	Lahaie 1981
L. avachaensis Kalamees	Rodygino, Kamchatka	Kalamees & Vaasma 1993
L. bicolor (Maire) Orton	Kronok Nature Res., Kamchatka	Kalamees & Vaasma 1993
	Radont Valley, Switzerland	Graf 1994
	Godhavn area, W. Greenland	Lamoure et al. 1982
	Scotland	Watling 1987
L. laccata (Scop.: Fr.) Cooke	Greenland	Lange 1957, Lamoure et al. 1982, Wathng 1983
	Uzon Caldera, Kamchatka [ss. Mueller and Vellinga]	Kalamees & Vaasma 1993
	Western Italian Alps	Lo Bue et al. 1994
	Western Alps	Kühner & Lamoure 1986
	French Alps	Eynard 1977
	Swiss Alps	Senn-Irlet 1992, 1993
	Iceland	Hallgrímsson 1981,1998, Watling 1983
	Norway	Lange & Skifte 1967
	Alaska	Linkins & Antibus 1982
	Svalbard	Gulden &Torkelsen 1996, Ohenoja 1971, Väre et al. 1992
L. laccata var. *subalpina* Singer	Scotland	Watling 1987
L. maritima (Theodor) Singer	Iceland	Hallgrímsson 1998
	Greenland	Lange 1955 (see *L. trullisata* var. *rugulospora*)
	North Sea coastal	Redhead 1989

Taxon	Location	References
L. montana Singer	Fiera di Primiero, Italy	Bon 1987
	Uzon Caldera, Kamchatka	Kalamees & Vaasma 1993
	Mt. Rae, Alberta, Canada	Kernaghan & Currah 1998
	Northwest Territories, Canada	Lahaie 1981
	Ellesmere Island, Canada	Lahaie 1981
	Baffin Island, Canada	Lahaie 1981
	Svalbard	Gulden &Torkelsen 1996, Väre et al 1992, Osmundson (unpublished)
	Iceland	Hallgrímsson 1998
	Radont Valley, Switzerland	Graf 1994
	Eastern Swiss Alps	Favre 1955
	Central Swiss Alps	Senn-Irlet 1987
L. ohiensis (Mont.) Singer	Greenland	Lamoure et al. 1982, Watling 1977
	Svalbard	Watling & Watling 1988
L. proxima (Boud.) Pat.	Radont Valley, Switzerland	Graf 1994
	Eastern Swiss Alps	Favre 1955
	Godhavn area, W. Greenland	Lamoure et al. 1982
	Western Italian Alps	Lo Bue et al. 1994
	Umiat, Alaska	Kobayasi et al. 1967
	Finnish Lapland	Kallio & Kankainen 1964
	Norway	Lange & Skifte 1967
L. proximella Singer	Scotland	Watling 1987
L. pumila Fayod	Svalbard	Gulden &Torkelsen 1996, Väre et al. 1992, Osmundson (unpublished)
	Western Italian Alps	Lo Bue et al. 1994
	Eastern Swiss Alps	Favre 1955
	Iceland	Hallgrímsson 1998
	Switzerland	Senn-Irlet 1988, 1992
	Alaska	Mueller 1992
L. striatula (Peck) Peck	Alaska	Laursen & Ammirati 1982, Laursen & Chmielewski 1982, Linkins & Antibus 1982
	Svalbard	Reid 1979
L. tetraspora Singer	Switzerland	Senn-Irlet 1988
	Baffin Island	Parmalee 1969
	Alaska	Kobayasi et al. 1967, Miller et al. 1982
	Svalbard	Ohenoja 1971
	Greenland	Lange 1957
L. tortilis (Bolton) Cooke	Greenland	Lamoure et al. 1982, Lange 1957
	Western Italian Alps	Lo Bue et al. 1994
	Alaska	Kobayasi et al. 1967, Laursen & Chmielewski 1982, Miller et al.1982
	Svalbard	Ohenoja 1971
	Iceland	Hallgrímsson 1981,1998
	Kamchatka	Kalamees & Vaasma 1993
L. trullisata (Ellis) Peck f *rugulospora* 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 synonomy. 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 consistant 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 μm, e.g., *L. maritima, L. proxima, L. oblongospora*), moderately echinulate (1-1.5 μm, e.g., *L. bicolor, L. laccata, L. montana, L. pumila*), or strongly echinulate (>1.5 μm, 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 μm) 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 pleisiomorphic 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 dedikaryotized 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^2 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_2O, 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^2) 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 ≤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) μm; subglobose to broadly ellipsoidal (Q^m = 1.08-1.18), with moderately coarse echinulae (≤ 1.5 (-2) μm in length, 0.3-1 (-1.25) μm 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 course echinulae (≤ 1.5 (-2) μm in length, 0.3-1.1 μm wide at base) ..... *Laccaria laccata* var. *pallidifolia* (Peck) Peck

3. Basidiocarps small (pileus 0.5-2 (-3.5) cm in diameter, stipe generally ≤ 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 x 8.7-9.1 μm) with moderately course echinulae (≤ 1.7 (-2.5) μm in length, 0.3-1.1 μm 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 x 6.8-8.3 μm), having finer echinulae than above (≤ 1 (-1.7 at spore apex) μm in length, 0.3-0.6 μm 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 (≤ 1.5 (-2.5) μm in length, 0.4-1.2 μm 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 ≤ 1 μm in length with occasional long echinulae (1-1.8 μm long) at apex; echinulae 0.2-0.75 μm 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 μm; absent to abundant. Basidia clavate, hyaline, 26-44 x 8-11 μm, tetrasterigmate; sterigmata ≤ 7 μm in length. Basidiospores 5.4-8 (-9.5) x (4.1-) 5.5-7.7 (-8.5) μm (mean = 6.2-8 x 5.5-7 μm), Q = 1-1.32 (Q^m = 1.06 – 1.15), subglobose to broadly ellipsoidal, hyaline, echinulate; echinulae ≤ 1.5 (-2.5) μm in length, (0.2-) 0.4-1.2 μm 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.

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

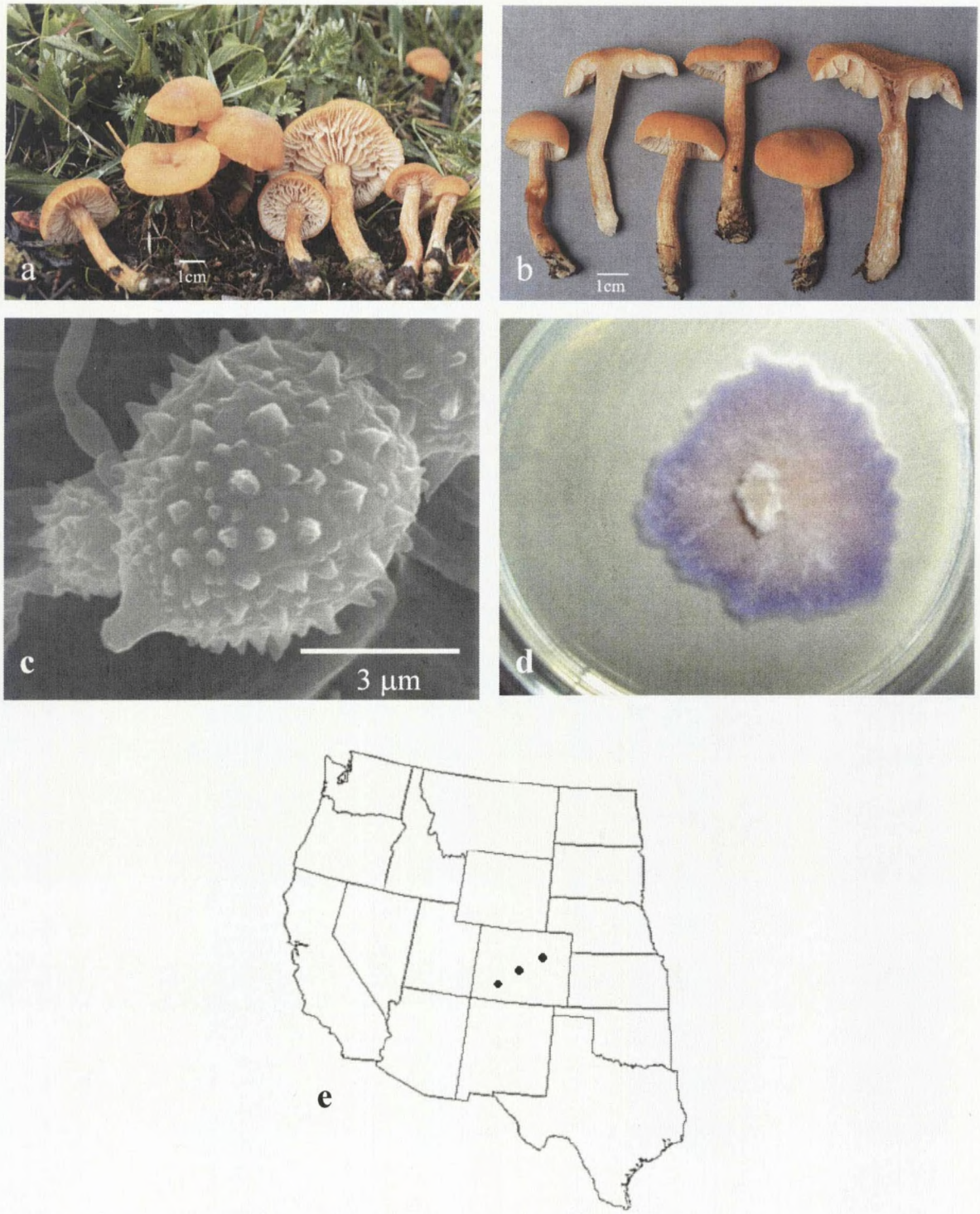

Figure 2. *Laccaria bicolor* (Maire) Orton **a, b**. Basidiocarps (CLC 1672). **c**. Scanning electron micrograph of basidiospores (CLC 1482). **d**. Culture morphology on PDA medium (CLC 1825). **e**. Rocky Mountain alpine distribution map.

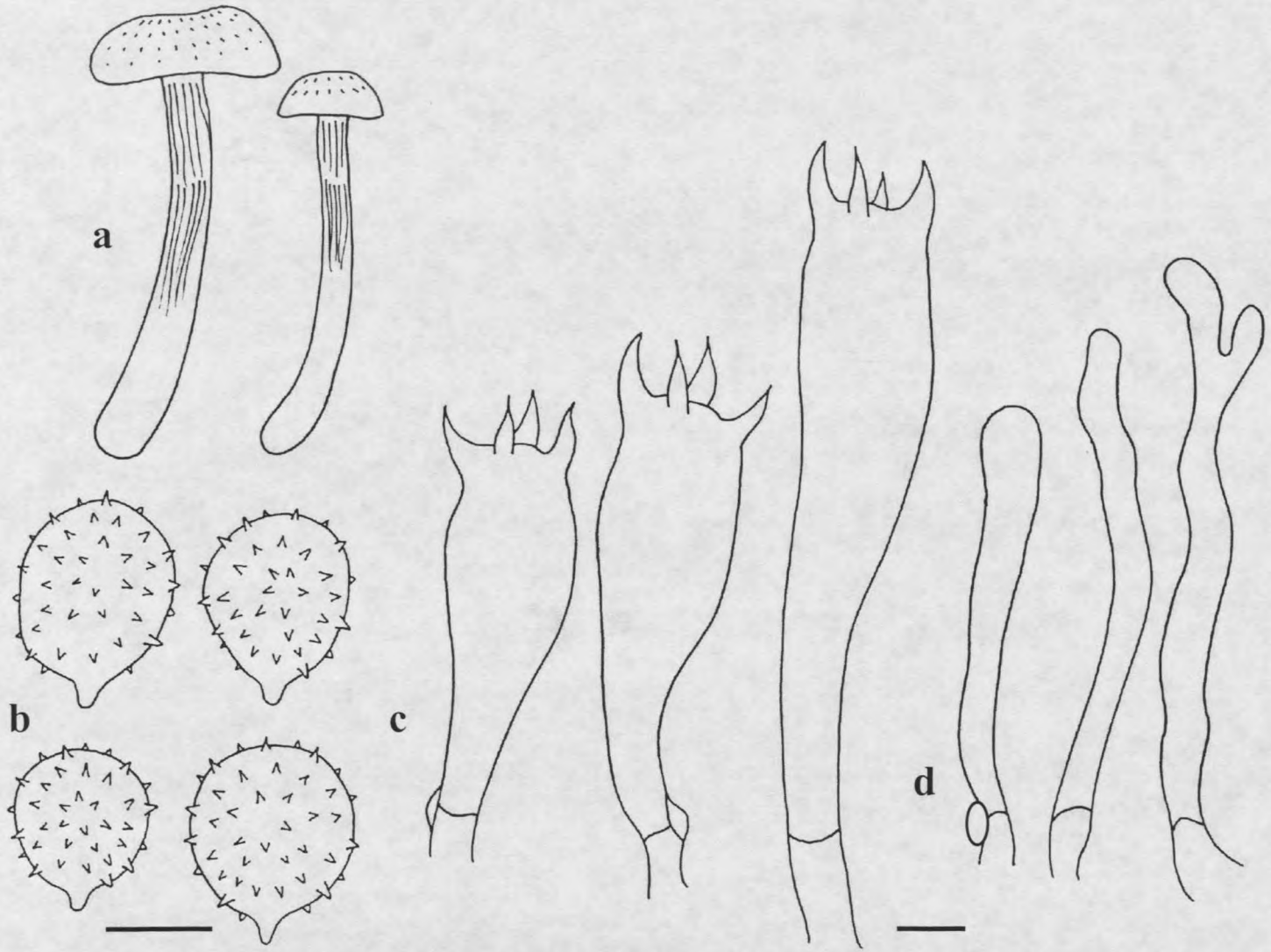

Figure 3. *Laccaria bicolor* (Maire) Orton, CLC 1445. a. Basidiomata, actual size. b. Basidiospores. c. Basidia. d. Cheilocystidia. Scale bars = 10 μm.

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 μm; rare to absent. Basidia clavate, hyaline, 26-50 x 9-12 μm, tetrasterigmate; sterigmata $\leq$ 8 (-11) μm in length. Basidiospores (5-) 6.2-10

(-10.9) x (4.1-) 5.6-9.8 (-10.8) μm (mean = 6.2-8.8 x 5.5-8.8 μm), Q = 1-1.22 (-1.32) (Q^m = 1-1.07 (– 1.18), subglobose to broadly ellipsoidal, hyaline, echinulate; echinulae ≤ 1.5 (-2) μm in length, 0.3-1.1 μm 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.

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

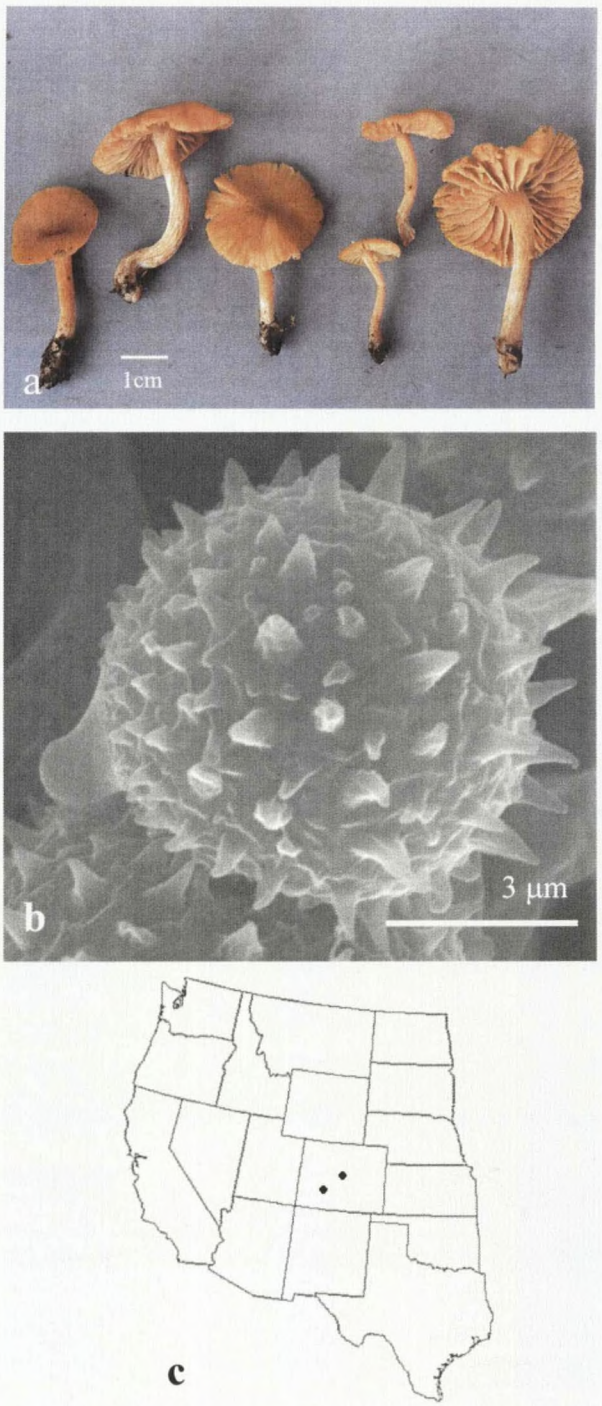

Figure 4. *Laccaria laccata* var. *pallidifolia* (Peck) Peck. **a**. basidiocarps (CLC1655). **b**. Scanning electron micrograph of basidiospores (CLC 1633). **c**. Rocky Mountain alpine distribution map.

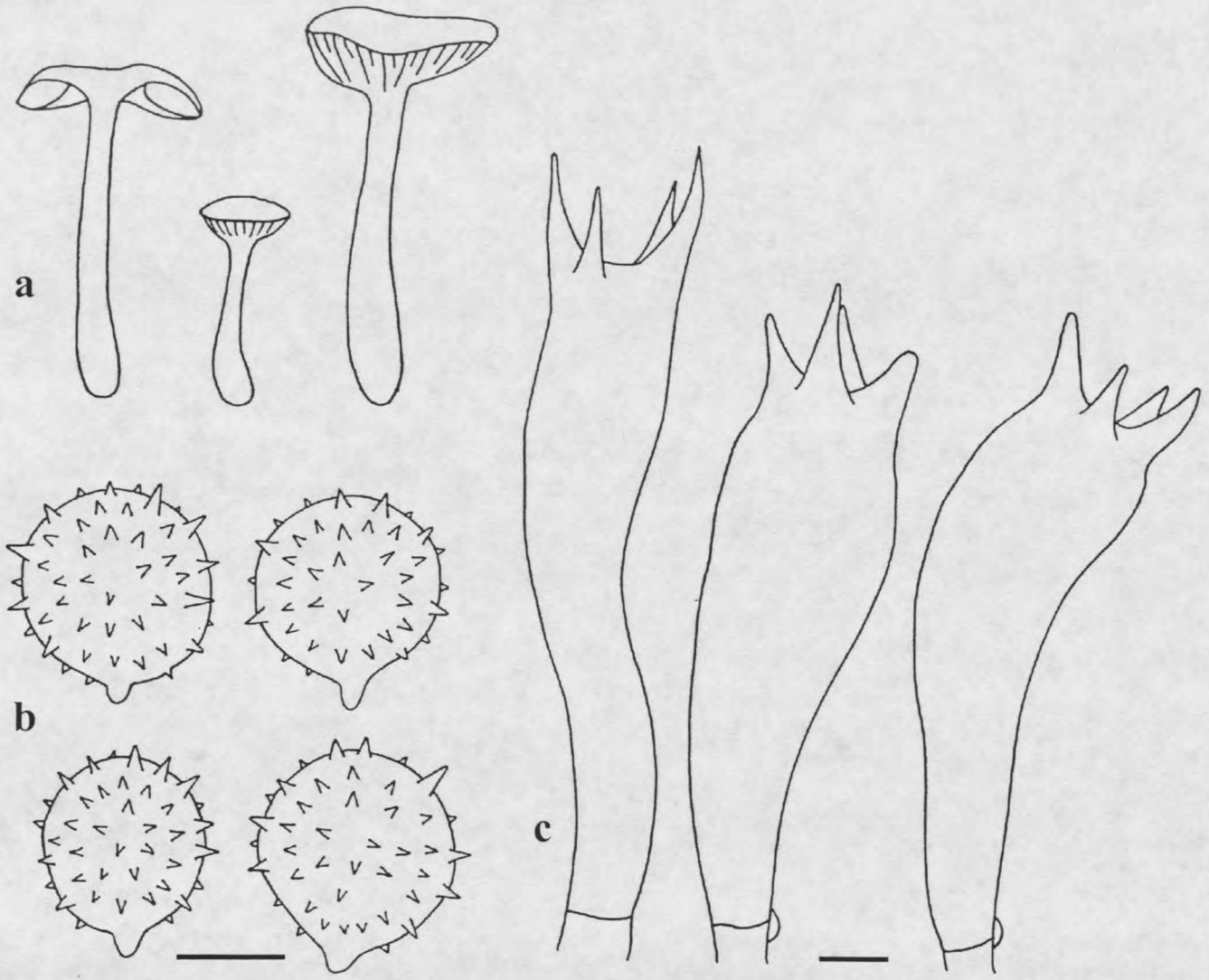

Figure 5. *Laccaria laccata* var. *pallidifolia* Peck (Peck), CLC 1370. a. Basidiomata, actual size. b. Basidiospores. c. Basidia. Scale bars = 10 μm.

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 ("Pompeiian 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, ± equal, solid becoming hollow in age, glabrous to minutely fibrillose, ± 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 μm; absent in some collections while scattered to abundant in others. Basidia clavate, hyaline, 32-51 (-65) x (9-) 11-14 (-17) μm, tetrasterigmate; sterigmata ≤ 10 (-12) μm in length. Basidiospores (8-) 9-11 (-12) x (7-) 7.9-9.5 (-10.5) μm (mean = 9.1-10.1x 8.7-9.1 μm), Q = 1-1.29 (Q^m = 1.04 – 1.16), subglobose to broadly ellipsoidal or occasionally globose, hyaline, echinulate; echinulae ≤ 1.7 (-2.5) μm in length, 0.3-1.1 (-1.3) μm 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.

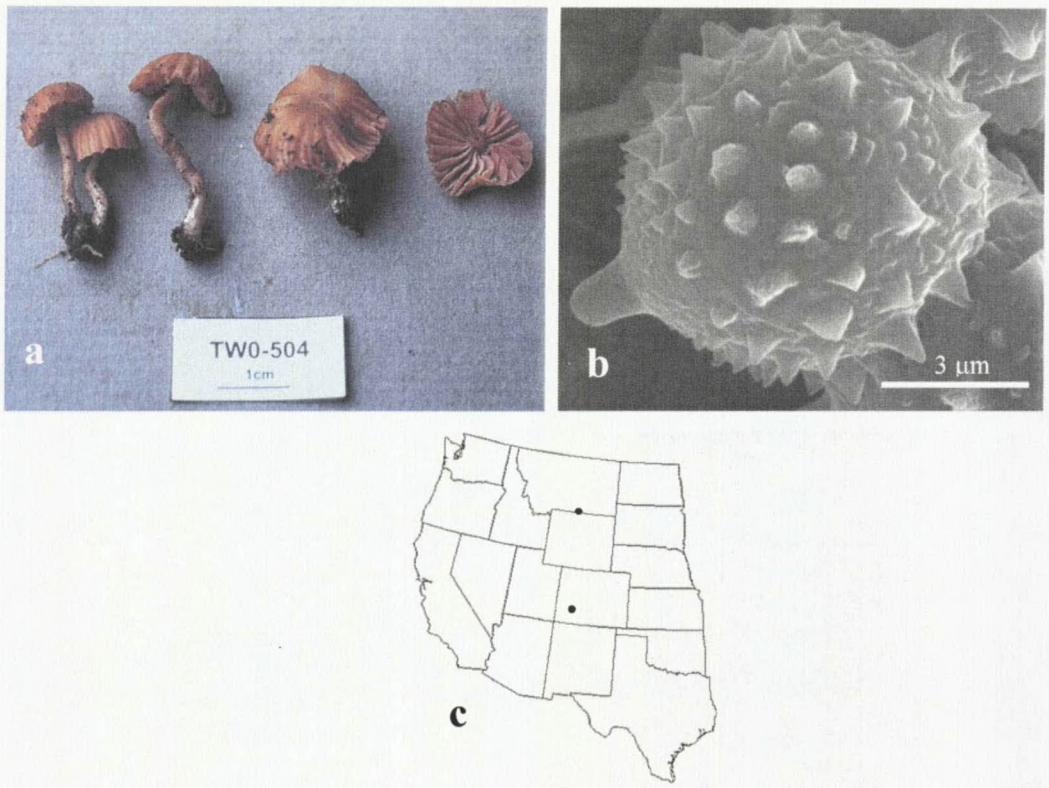

Figure 6. *Laccaria montana* Singer. **a**. Basidiocarps (TWO 504). **b**. Scanning electron micrograph of basidiospores (TWO 613). **c**. Rocky Mountain alpine distribution map.

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

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

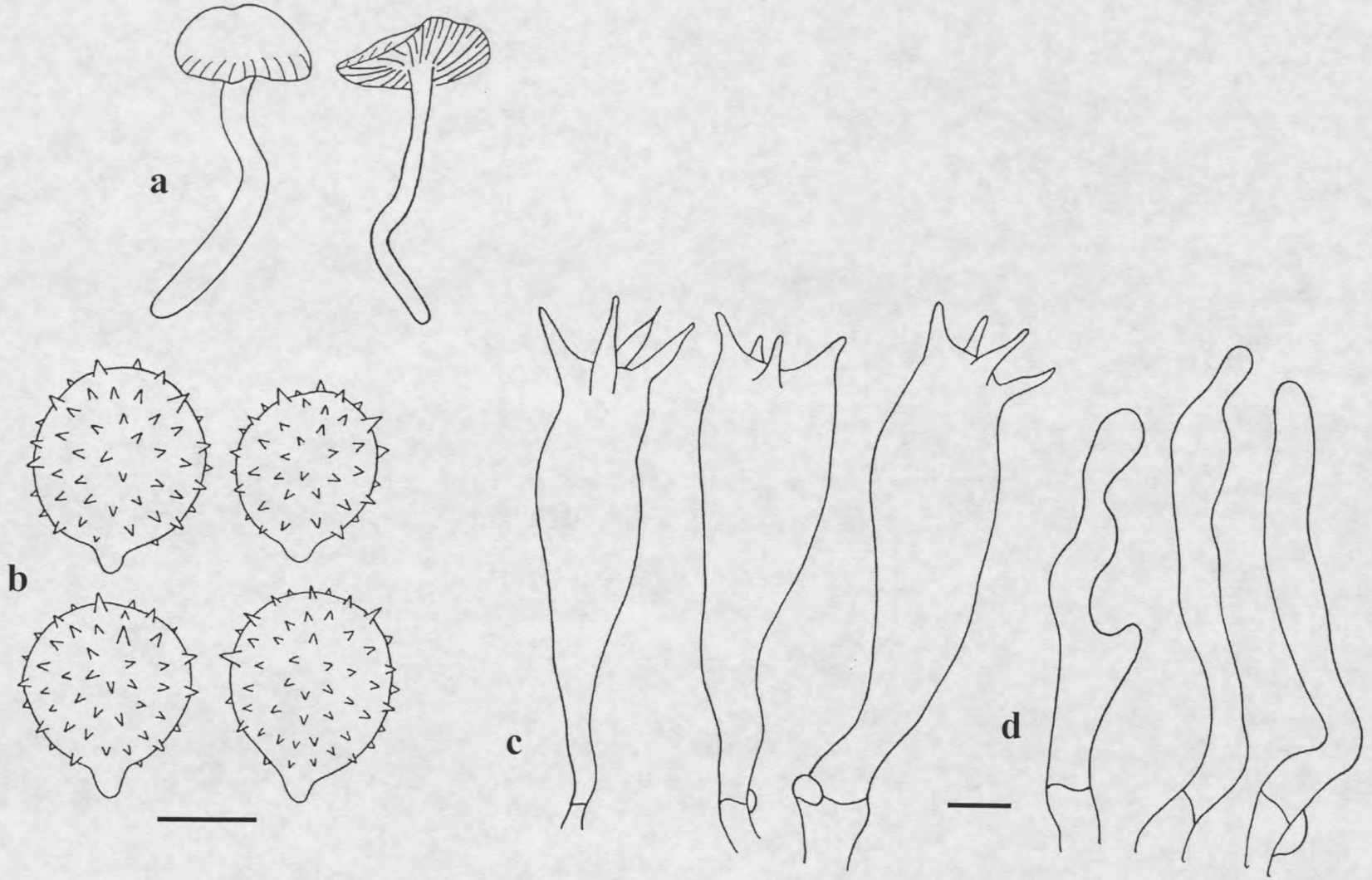

Figure 7. *Laccaria montana* Singer a. Basidiomata, actual size (TWO 441, left; TWO 561, right). b. Basidiospores (TWO 540). c. Basidia (TWO 540). d. Cheilocystidia (TWO 540). Scale bars = 10 μm.

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, ± 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 μm 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 μm; uncommon. Basidia clavate, hyaline, (28-) 32-44 (-50) x 8-15 μm, bisterigmate; sterigmata ≤ 13 μm in length.

Basidiospores (8-) 9-13.5 (-15) x (6.8-) 7.5-10.5 (-14.5) μm (mean = 9.5-11.4 x 8.1-9.5 (-10.5) μm), Q = 1-1.3 (Q^m = 1.08 – 1.18), subglobose to broadly ellipsoidal or occasionally globose, hyaline, echinulate; echinulae ≤ 1.5 (-2) μm in length, 0.3-1 (-1.25) μm 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.