SYSTEMATICS OF *LACTARIUS* IN THE ROCKY MOUNTAIN ALPINE ZONE

by

Edward Gilman Barge

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Plant Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2015

©COPYRIGHT

by

Edward Gilman Barge

2015

All Rights Reserved

ACKNOWLEDGMENTS

I would like to thank my major professor Dr. Cathy Cripps for taking me in and teaching me so much about mycology and life in general. Her dedication to her students is a rare and precious thing; she has had an extremely positive influence on all of us. I would like to thank Dr. Todd Osmundson for taking time out of his busy schedule to teach me and help me with molecular work. Thank you to members of my thesis committee, Dr. Matt Lavin and Carol Johnson. Thank you to friends and family that have assisted with various aspects of this project. My father, Marcy Barge, helped out immensely with field work, and spent countless hours searching for small, inedible mushrooms in alpine tundra landscapes. Don Bachman gave directions and often transportation to alpine sites; his knowledge and steady demeanor are an inspiration. While I conducted field work in Southwestern Colorado, my aunt and uncle Barbara and John Nolan provided lodging, warm meals and support. Thank you to mycologists and herbaria that supplied additional collections for this project. Dr. Ellen Larsson in Sweden, Dr. Pierre-Arthur Moreau in France, Jukka Vauras in Finland, and Dr. Ursula Peintner in Austria provided crucial European type locality collections. Dr. Egon Horak provided additional Rocky Mountain alpine collections. I gratefully acknowledge funding support from Montana Institute on Ecosystems, and Dr. John Sherwood of the Plant Science and Plant Pathology Department. Lastly, thank you to my wonderful girlfriend, Charissa Bujak, for being a loving partner throughout my graduate career.

TABLE OF CONTENTS

TABLE OF CONTENTS – CONTINUED

TABLE OF CONTENTS – CONTINUED

LIST OF TABLES

LIST OF TABLES – CONTINUED

Table Page

LIST OF FIGURES

LIST OF FIGURES – CONTINUED

Figure Page

LIST OF FIGURES – CONTINUED

ABSTRACT

Lactarius is an important component of the ectomycorrhizal community in cold-dominated arctic and disjunct alpine habitats where it associates primarily with the woody shrubs *Betula*, *Dryas*, and *Salix*. Little is known of the alpine fungi in the central and southern (Montana, Wyoming, Colorado) Rocky Mountain alpine zone (elevation 3,000—3,900 m) of North America. The goal of this study was to examine the genus *Lactarius* and here at least six species from subgenera *Russlularia* and *Piperites* are confirmed above treeline through molecular phylogenetic analyses of ITS and *rpb2* DNA in conjunction with detailed morphological examination. All (except two putative new species) appear to have broad intercontinental distributions in North America and Eurasia according to molecular comparison with type material, and collections from Europe, Greenland, Scandinavia, Svalbard, and Alaska. Rocky Mountain alpine collections of *L. lanceolatus* and the type from Alaska form a well-supported clade paraphyletic with respect to well-supported clades consisting of *L. aurantiacus* and several North American subalpine taxa. Rocky Mountain alpine collections of *L. nanus*, *L. glyciosmus, L. repraesentaneus*, and *L. salicis-reticulatae* all form well-supported clades with material from European type localities and other arctic-alpine material; although some clades contain nested (*L. hysginoides* within *L. nanus*) and possibly cryptic species (*L.* aff. *salicis-reticulatae* from Colorado). The well-known arctic-alpine *L. pseudouvidus/L. brunneoviolaceus* group of violet-staining species appears to be a complex possibly containing additional species. North American material originally described as part of this group, is well-separated phylogenetically and represents a putative new species (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*) so far confined to the Southern Rockies with shrub *Salix*. The monophyly of the violet-staining section *Uvidi* is supported. *Lactarius lanceolatus*, *L. nanus*, and *L. salicis-reticulatae* appear largely restricted to arctic-alpine habitats across their broad range, where they associate with dwarf and shrub *Salix*. *Lactarius glyciosmus* and *L. repraesentaneus* are not restricted to arctic-alpine areas and occur with *Betula* and krummholz *Picea* (possibly also *Salix*), respectively in the Rocky Mountains; the latter is also known with *Betula* in broader parts of its range. Species distributions are hypothesized to be tied to host ranges, glaciation, and long distance dispersal.

CHAPTER 1

LITERATURE REVIEW AND INTRODUCTION TO THE GENUS *LACTARIUS*

What is the Arctic-Alpine Zone?

The alpine zone covers roughly 3% of the Earth's land and consists of the vegetated areas above the natural altitudinal limit of tree growth in mountainous areas throughout the world (Körner 1999). The arctic zone covers roughly 5% of the Earth's land and consists of vegetated areas north of 65° to 70° N, between the climatic limit of tree growth and the limit of permanent ice (Bliss 1988, Chapin & Körner 1995). Arctic and alpine regions are similar in many ways, and together they are often referred to as the arctic-alpine biome (Bliss 1962, Billings 1973, Löve & Löve 1974, Bliss 1988, Chapin & Körner 1995, Murray 1995). However, alpine areas generally have more complex topography and higher habitat fragmentation (Körner 1999). Towards the equator, in the Northern Hemisphere, differences between the alpine and arctic become more pronounced. Along this north to south gradient, alpine areas generally display an increase in diurnal temperature fluctuations, length of growing season, maximum radiation and species richness (Billings 1973, Chapin & Körner 1995, Körner 1999). Furthermore, the altitude of tree line varies with latitude. Tree line occurs at sea level in polar areas, and increases towards the equator (Wielgolaski 1997a, Körner 1999). Tropical, subtropical, and southern hemisphere alpine areas are, overall, vastly different from arctic and northern hemisphere extratropical alpine areas in terms of flora and other factors (Billings 1974b, Körner 1999). Throughout this review, the term alpine will refer only to northern hemisphere, extratropical alpine areas unless stated otherwise. Sometimes the term arctic-alpine will be used, when considering arctic and alpine areas as a whole, and sometimes the two terms will be used separately, when discussing individual features of each.

Climate

In general, the climate in arctic-alpine areas is characterized by cold temperatures and more specifically, low mean air temperature during the growing season (Billings 1973, Körner 1999). Billings & Mooney (1968) display an annual mean temperature range of minus 3.3° C to minus 12.4° C and a July mean temperature range of 3.9° C to 8.3° C. Freezing temperatures commonly occur during the growing season, and they are especially common at night in alpine areas (Körner 1999). While the climate of the two zones is similar in many ways, alpine areas generally experience greater daily fluctuations in temperature and have longer growing seasons, whereas arctic areas experience more dramatic seasonal changes in temperature, due to nearly constant darkness in winter and steady daylight in summer (Billings 1973, Chapin & Körner 1995, Körner 1999).

Precipitation is variable in arctic-alpine areas. Areas in the low arctic (south of 72° N) are typically wetter than those in the high arctic (north of 72° N) (Barry & Hare 1974, Bliss 1988). As precipitation tends to increase with increasing altitude in mid and high latitude areas, higher elevation areas are typically wetter than the surrounding lowlands (Körner 1999). Throughout much of the year, precipitation occurs as snow in arctic-alpine areas. In the arctic, while snow is common, seasonal snowfall totals are often low (Barry & Ives 1974), whereas many alpine regions experience very high seasonal snowfall totals (Körner 1999). Furthermore, in both arctic and alpine areas, winter snow depth and time of snow melt are important determinants of the local distribution of species (Johnson & Billings 1962). In the summer, precipitation in arctic areas is largely due to upper level low pressure systems, whereas alpine areas tend to experience spottier, convective-based precipitation.

Arctic-alpine areas are often characterized as being extremely windy, but this is not always the case (Warren 1959, Körner 1999). However, regions subject to the westerly wind-belts, such as the Central and Southern Rockies, often do experience strong winds (Barry & Hare 1974, Körner 1999). For example, Billings & Mooney (1968) report an annual mean wind speed on Niwot Ridge, Colorado (altitude 3,749 m; latitude 40° N) of 29.6 km/hr. Because of the lack of trees and predominance of low-lying vegetation in arctic-alpine areas, wind speeds are typically greater closer to the ground than in many temperate regions (Warren 1959).

On average, warming is occurring globally (Jones et al. 1999) and, while variable, rates of warming in arctic-alpine areas are generally higher than the global average (Serreze et al. 2000, Böhm et al. 2001). Environmental changes in arctic-alpine areas associated with this warming include: reduced extent and thickness of sea ice (Parkinson et al. 1999), retreat of arctic and subarctic glaciers (Arendt et al. 2002), increased annual discharge from northward-flowing rivers (Peterson et al. 2002), and an Arctic-wide increase in permafrost temperatures (Romanovsky et al. 2002). Biological changes include: increased plant photosynthesis (Mynemi et al. 1997), northward movement of arctic treeline (Nicholls et al. 1996), altitudinal increase in alpine treeline (Pauli et al. 1996), and range expansion and increase in size of shrubs (Sturm et al. 2001). An increase in the height and abundance of shrubs and graminoids, a decrease in overall plant diversity, and a decline in lichens was correlated with experimental warming of plots across a wide range of arctic-alpine habitats including Niwot Ridge, CO, Svalbard and the Tibetan plateau (Walker et al. 2006).

Soils

Arctic-alpine soils form much the same way as in other biomes through an interaction between climate, parent material, topography, soil organisms and vegetation, over time (Jenny 1980, Bliss 1997, Campbell 1997). Soil parent material has a large effect on various soil properties including pH, nutrient content and texture, and is highly variable across the arctic and throughout the many mountainous regions of the world (Walker 2000). Due to the harsh climate of arctic-alpine areas, soils are generally poorly developed, and the process of soil formation occurs more slowly than in other biomes (Retzer 1974, Bliss 1988, Körner 1999). Cryoturbation, caused by freeze-thaw cycles, disrupts soil formation through movement and heaving of the soil and disturbance of plants (Körner 1999). Permafrost, which is present throughout the arctic and spotty in alpine areas, slows soil formation by reducing the vertical movement of water (Bliss 1988). Furthermore, soil conditions can vary considerably across the landscape due to the patchy nature of snow (Coulson et al. 1995). Surface soils can remain frozen for up to 74% of the year in the high arctic (Timling & Taylor 2012) and microbial activity is decreased, due to low temperatures which results in soil organic matter being accumulated at a slower rate than in many other regions (Körner 1999). However, as organic matter is also turned over more slowly, a large pool of organic carbon has accumulated and is present in mature arctic soils and permafrost (Ping et al. 2008).

Soil classification schemes vary widely throughout the world (Bliss 1997). Bliss (1988) described and classified the soils of Arctic North America as primarily Spodosols, Inceptisols and Histosols. Arctic podzols (Spodosols) occur in well-drained areas where dwarf shrub heath species and dwarf birch predominate (Bliss 1988). Spodosols are characterized by a subsurface accumulation of humus, complexed with Al and Fe, along with a leached E horizon (USDA classification). On uplands and dry ridges, poorly developed arctic brown soils (Inceptisols) are prevalent (Bliss 1988). Inceptisols are primarily characterized as having little horizon development and little organic matter accumulation (USDA classification). The most common soils in the Low Arctic are the tundra soils (Inceptisols) which occur in imperfectly drained areas of cottongrass-dwarf heath and sedge communities (Bliss 1988). Histosols, which are also found in the arctic, are composed largely of organic matter and occur in poorly drained or boggy areas dominated by sedge-moss or grass-moss communities (Bliss 1988). The surface layers of

Arctic podzols and arctic brown soils tend to be acidic (pH 6-4), low in available nutrients, and they are typically well-drained above the permafrost layer. Arctic tundra soils and Histosols are also typically acidic (pH 6.5-4.5 for Arctic tundra soils, pH 6.5-5.0 for Histosols) (Bliss 1988). In the high arctic, Arctic brown Inceptisols are common. These soils are often basic, with very low nitrogen and phosphorus content (Bliss 1988). Overall, pH is highly variable in arctic areas (Goryachkin et al. 2004), and nutrient contents (N, P, K) are generally low, while C is often high in the active layer and permafrost (Tarnocai 2009).

Literature describing pedogenesis of alpine soils is somewhat scarce. Many of the processes are believed to be similar to those in arctic areas, however dissimilarity in the environment, vegetation, and parent material, likely creates local differences (Campbell 1997). Alpine soils are highly variable, both across small distances and across mountain ranges; however they are typically shallow, with poorly developed horizons (Campbell 1997). The A horizon often contains large amounts of non-degraded organic matter (Campbell 1997). Also, large amounts of silt and clay are often present in the A horizon, possibly as a result of deposition by wind (Thorn & Darmody 1985). Alpine soils are often acidic, however those formed from sedimentary material such as limestone are basic (Eddleman & Ward 1984). The most common soils in alpine areas in North America, as in the low arctic, are the often acidic Inceptisols of well-vegetated areas (Retzer 1974, Campbell 1997). Less common are Mollisols, Spodosols, Entisols, and Histosols (Campbell 1997).

Microbial Activity

Overall, soil microbial activity is reduced in arctic-alpine areas, due to low temperatures. However, soil microbes in these areas still play key ecological roles and are phylogenetically diverse, spanning Bacteria, Eucarya, and Archaea (Nemergut et al. 2005, Margesin & Miteva 2011); recent research has shown many novel bacterial and fungal lineages (Schadt et al. 2003, Margesin & Miteva 2011). Microbial community composition and function vary across arctic-alpine areas due to variability in precipitation, temperature, vegetation, duration of plant growing season and many other factors (Nemergut et al. 2005). Arctic-alpine microbes are able to survive extreme temperatures, freeze-thaw cycles, variable precipitation and soil moisture, and low nutrient availability (Margesin & Miteva 2011). Seasonally, large shifts occur in soil microbial community composition, soil processes, and soil nutrient availability (Schadt et al. 2003, Nemergut et al. 2005). In the summer, soil microbial communities feed mainly on plant root exudates (Lipson et al. 2002), whereas in the fall, community composition shifts to become dominated by microbes which degrade matter from senescing plants (Nemergut et al. 2005). During the winter, arctic-alpine soil microbial activity continues, and can be quite high in areas insulated by snow. For example, Lipson et al. (1999) found microbial biomass to be highest in the winter at an alpine site and Grogen et al. (2001) measured high rates of microbial respiration under snow in the arctic. In the spring, snow melt is associated with a crash in the microbial population and subsequent release of nitrogen (Lipson et al. 1999, Nemergut et al. 2005).

Microbial activity and nutrient cycling in arctic-alpine areas is being affected by climate change, with warming causing increased microbial activity and thus increased available N (Aerts 2006) as well as faster carbon turnover (Shaver et al. 2006).

Flora

Approximately 4% of the global vascular plant flora occurs in the arctic-alpine zone (Chapin & Körner 1995, Willig et al. 2003), but there is low productivity in terms of biomass (Webber 1974). Roughly 1,500 arctic lowland plant species (Walker 1995) and 10,000 alpine plant species (total includes subtropical, tropical and southern hemisphere alpine plant species) (Körner 1995) have adapted to the harsh climate, poor soils and repeated glaciations of these regions. The most common vegetation types are low-lying perennial cushion plants, grasses, rosette plants and prostrate shrubs, whose morphologies act as heat traps for surviving the cold growing season temperatures and as litter traps for improved soil development (Billings 1974a, Körner 1999).

In the Northern hemisphere, tree line is most often formed by various conifer species, however in Fennoscandia, Russia, Iceland and Greenland, *Betula pubescens* Ehrh. can be dominant (Wielgolaski 1997a, b). Bliss (1997) divides the arctic into the “low” and “high-arctic tundra”. In the “low-arctic”, tundras predominate, followed by a transition from tundra to semideserts to polar deserts in the “high-arctic.” The “low-alpine” zone just above tree line in alpine areas frequently supports a thick cover of shrubs. The higher “mid-alpine” typically has less shrub cover and more graminoids and in the “high-alpine” there is little to no vegetation (Wielgolaski 1997a). Similarities exist between the vegetations of “low-alpine” and “low-arctic tundra”, between “mid-alpine” and “high-arctic tundra”, and between “high-alpine” and “high-arctic polar desert” (Bliss 1997, Wielgolaski 1997a). Near treeline in arctic-alpine areas, a “messy” forest-tundra ecotone often occurs, where islands of trees are interspersed with typical tundra habitat (Billings 1974a, Bliss 1988); there can be a significant overlap in plant species between the low-alpine zone and the subalpine zone (Wielgolaski 1997b). Yurtsev (1994) suggests that the lowest subzone of the arctic is an extension of the boreal forest without trees.

Overall, there are many similarities between arctic and alpine vegetation types (Billings 1973, Wielgolaski 1997a). However, in general, alpine areas have a greater diversity of regional endemic plants and display a more complex small-scale mosaic of plant communities due to the intricate terrain (Billings 1973, Barry & Ives 1974). Common classifications for alpine plant communities include open fellfield, cushion plant, various meadow and heath communities, shrub, snow-bed and wetland or bog communities (Dahl 1984, Billings 1988, Cooper et al. 1997). For the arctic, a number of different community classification schemes have been proposed, generally for specific countries or geographic regions such as Russia (e.g. Yurtsev 1994), North America (e.g. Bliss 1988), and Fennoscandia (e.g. Elvebakk 1999). A circumpolar arctic vegetation map, produced by Walker et al. (2005), delineates five major arctic community types, each with multiple subdivisions: barrens, graminoid tundras, prostrate-shrub tundras, erect-shrub tundras, and wetlands. Overall, the circumpolar arctic region has a more consistent core of plant species than the alpine, as well as more extensive wet tundras (Billings 1973, Walker et al. 2005).

Arctic-alpine plant species have a complex evolutionary and biogeographical history. Current north-temperate alpine plant species are hypothesized to have evolved through a number of pathways including adaptive radiations of older species through relocation and isolation by geologic processes, migration of arctic species during periods of glaciation, adaptations in lower elevation montane species, and survival of species in refugia during periods of glaciation (Löve & Löve 1974, Körner 1995, Murray 1995). With regard to the current arctic flora, fossil evidence suggests that no representatives of current arctic-alpine plant genera were present in the northern plains of North America during the Tertiary (Löve & Löve 1974). It appears that some elements evolved from a Tertiary nemoral flora by the late Miocene, and that prior to the onset of the Pliocene glaciations, an early arctic flora of about 1,500 species had achieved a circumpolar distribution (Löve & Löve 1974). During the Pliocene and Pleistocene glaciations (Pleistocene initiating around 2.5 million years ago) (Larsen & Barry 1974), northern hemisphere plant distributions were greatly affected by the advance and retreat of ice sheets (Abbott 2008). At the last glacial maximum (26,000-19,000 years ago) (Clark et al. 2009), ice covered much of northern North America and Eurasia, and glaciers extended southward into mountainous areas such as the Rocky Mountains, Alps and Himalayas (Heuberger 1974, Larsen & Barry 1974). During this time, arctic-alpine plant ranges were greatly altered due to climate change and physical disruption from glaciation. It has been hypothesized that arctic-alpine plants were pushed into isolated northern refugia (Hultén 1937) and nunataks (Dahl 1987), and/or spread into broad bands in suitable areas south of the ice (e.g. Birks 2008). Macrofossils of *Dryas integrifolia* Vahl, *Salix herbacea* L. and other tundra plants dated from the last glacial maximum from Beringia, suggest this area was a northern refugium (Goetcheus & Birks 2001). Beringia was a large unglaciated land bridge which spanned across the Bering Strait from Northeast Russia (125° E) to Northwest America (130° W). Other macrofossil and pollen records from the last glacial maximum reveal the presence of arctic- alpine plants in the Rocky Mountains south of the ice-sheets, in the high arctic and to the southeast and west of the ice-sheets in North America (Tremblay & Schoen 1999, Thompson & Anderson 2000), and to the west, south and east of the ice-sheets in Eurasia (Tarasov et al. 2000). As the climate warmed, it is suggested that competition gradually replaced these plants in some areas and they dispersed into suitable habitats, northward and/or upward into mountainous regions, resulting in the disjunct circumpolar/high mountain distribution pattern currently seen for many arctic-alpine plant species (Hultén 1937, 1968, 1971, Tremblay & Schoen 1999, Brochmann & Brysting 2008, Schmitt et al. 2010, Ronikier et al. 2012). Further range shifts due to ongoing climate change are occurring (Mynemi et al. 1997, Sturm et al. 2001) and are anticipated to continue into the future (Alsos et al. 2009, 2012).

Many arctic-alpine plants *appear* to lack long-distance dispersal mechanisms, therefore disjunct arctic-alpine distributions have traditionally been thought to represent fragments of past wider ranges (Weber 1965, 2003). However, evidence from recent molecular studies demonstrates that long-distance dispersal has played a prominent role in shaping present distribution patterns (Abbott & Brochmann 2003, Alsos et al. 2007). For example, it was shown that one of the chloroplast DNA haplotypes of the circumpolar wind-dispersed *Betula nana* is present in southern Norway, Svalbard, Greenland, northern Canada and Alaska, which is suggestive of at least some long-distance dispersal (Abbott & Brochmann 2003). Alsos et al. (2007) even suggest that long-distance dispersal mechanisms have been selected for in arctic flora due to repeated glaciations and the need to track "potential niches"; others have suggested the likelihood of increased wind-dispersal in open arctic and alpine landscapes (Bonde 1969). While long-distance dispersal appears to have influenced some current distribution patterns, it appears that the genetic constitution of alpine plant populations in remote (predominantly more Southern) mountain systems is often strongly divergent from arctic and more northern alpine populations (Schmitt et al. 2010), yet there is still a large overlap in species between arctic and alpine areas.

Rocky Mountain Alpine Zone

The alpine zone of the Rocky Mountains extends, semi-contiguously, above tree line on mountain tops, from the Brooks Range in northern Alaska to the Sangre de Cristo Range in northern New Mexico, with the elevation of tree line increasing toward the south (Billings 1988). Brouillet and Whetstone (1993) define four main biogeographical provinces in the Rocky Mountains: 1) the Brooks Range province which extends from western Alaska to eastern North West Territories 2) the Northern Rocky Mountain province which extends in a north-south direction from the Yukon to central Idaho and Montana 3) the Central Rocky Mountain province which extends from southern Montana to central Wyoming and 4) the Southern Rocky Mountain province which extends from central Wyoming and northern Utah through Colorado to central New Mexico. The bulk of mountain building occurred during the Late Cretaceous and early Tertiary (=Paleogene) as an upthrust through sedimentary rocks (Retzer 1956, Billings 1978). Erosion has since exposed primary rocks, the majority of which are granites. In addition, basalt, andesite and rhyolite occur, as well as pockets of sedimentary rocks, including shale, limestone, sandstone and quartzite (Retzer 1956).

Some of the only published alpine climatic data from the two lower provinces (a focus of this study) are from INSTAAR's long term ecological research station on Niwot Ridge for the Front Range of the southern Rockies in Colorado and from Johnson and Billings (1962) for the Beartooth Plateau in the central Rockies. For the Beartooth Plateau, Johnson and Billings (1962) report a July mean temperature of 48.5° F (9.2° C), a mid-June through August weekly average precipitation of 0.31 inches (0.79 cm) and a mid-June through August mean wind velocity of 11.3 miles per hour (18.2 km per hour) at 10,300 feet above sea level (3,139 meters above sea level) all in 1959; the highest temperatures occur between mid-July and mid-August and low temperatures can be experienced year-round. Annual mean temperature, precipitation and wind velocity are not published for alpine areas of the Beartooth Plateau. Although Niwot Ridge in Colorado is considerably further south, the climate is considered similar to that of the Beartooth Plateau (Cooper et al. 1997). Niwot ridge experiences low temperatures year-round as well as regular high winds. Precipitation is typically brought in by an upper westerly flow, although in winter and spring, significant precipitation can be delivered by easterly upslope flows. Maximum precipitation occurs in the spring, however, significant precipitation can also occur with the southwestern summer monsoon (Greenland 1989). The Niwot Ridge Long-Term Ecological Research Site (http://niwot.colorado.edu/site_info/climate /climate.html) reports an annual mean temperature of 25.34° F (-3.7° C), a July mean temperature of 46.8° F (8.2° C) and a mean annual precipitation of 36.61 inches (93 cm) at 12,280 feet above sea level (3,743 meters above sea level).

Periods of glaciation during the Pleistocene, beginning around 2.5 million years ago and ending around 12,000 years ago, greatly affected alpine regions in the Rocky Mountains (Larsen & Barry 1974, Billings 1978, Hadley 1987, Bowman & Damm 2002). At times, during this glacial period, much of the Northern Rocky Mountains was covered in or bounded by the Cordilleran Ice Sheet and much of the central Rockies was buried beneath a 1000 m thick layer of ice, while spottier, local mountain glaciers of various sizes affected the southern Rockies (Bowman & Damm 2002, Pierce 1979, 2003). Periods of climate fluctuation and glaciation from the Pleistocene to the present caused fluctuations in the size of alpine areas, and altered their connection to the arctic (Billings

1978, Hadley 1987, Bowman & Damm 2002). During glacial periods, the alpine zone was more broadly distributed and connected (Hadley 1987) and ice free areas likely served as important refugia for arctic-alpine plants (DeChaine & Martin 2005). Presently, alpine areas in much of the Rocky Mountains exist as a scattered chain of high elevation “islands” (Kuchler 1964) with plant species richness being strongly correlated with island size, proximity to other alpine areas and latitude (Hadley 1987).

Roughly 600 plant species occur in alpine areas of the central Rockies (Scott 1995) and about 418 species occur on the Beartooth Plateau (Anderson 1994). About 37% of the alpine plants of Colorado and about 47% of the alpine plants on the Beartooth Plateau also occur in the arctic (Bliss 1956, Johnson & Billings 1962). Toward the south, species diversity, and the number of endemic, locally unique species increases, perhaps as a result of greater adaptive radiation due to less interference by glaciation (Billings 1978, Takhtajan 1986, Hadley 1987). Overall, however, the floras of the northern, central and southern Rocky Mountain alpine are fairly similar (Hadley 1987).

Many alpine plant community types have been designated for the Rockies, and they typically parallel plant community types that have been designated for the arctic (Campbell 1997). Johnson & Billings (1962) recognize four distinct, but often intergrading alpine vegetation types on the Beartooth Plateau: Geum turf vegetation primarily on ridges and slopes; Deschampsia meadow vegetation primarily on well-drained, sheltered sites; Carex scopulorum bog primarily on wet sites (often below melting snowbanks); and Salix thicket vegetation primarily in alpine valley bottoms or along waterways. A more broad classification for the central and southern Rocky Mountain alpine plant community types delineates: open fellfield, cushion plant, meadows of various types, shrub, and wetland (Billings 1988, Cooper et al. 1997). Plant community types in the Rocky Mountain alpine zone vary, often sharply, as a result of changes in the physical environment, such as topography, aspect, and micro-relief (Johnson & Billings 1962). Local species distribution is also greatly affected by winter snow accumulation and persistence (Johnson & Billings 1962, Körner 1999), as well as by soil parent material (Billings 1978) and other factors. The major tree species found at tree line in the central and southern Rocky Mountains are *Abies lasiocarpa* (Hook.) Nutt., and *Picea engelmannii* Parry ex Engelm., with *Pinus albicaulis* Engelm. also occurring in the central Rockies and *Pinus aristata* Engelm. in the southern Rockies (Campbell 1997).

Ectomycorrhizal Plants of the Rocky Mountain Alpine Zone

The majority of arctic-alpine vascular plants engage in mutualistic relationships with fungi and form some type of mycorrhizae on their roots; a subset of these, primarily woody shrubs, are known to form ectomycorrhizae (Cripps & Eddington 2005). Ectomycorrhizal fungi (EMF) encase plant root tips and transfer soil nutrients and water to their plant hosts in exchange for photosynthetically derived sugars (Smith & Read 2008). Ectomycorrhizal (ECM) plants of arctic-alpine areas include: shrubby and dwarf/prostrate *Salix* species, including *Salix polaris* Wahlenb.*, S. herbacea* L.*, S. reticulata* L.*, S. rotundifolia* Trautv., *S. arctica* Pall.*, S. glauca* L.*, S. planifolia* Pursh, and *S. pulchra* Cham.; *Betula* species including *Betula nana* L. and the closely related *B. glandulosa* Michx.; *Dryas octopetala* L. and *D. integrifolia* Vahl; various *Kobresia* and *Arctostaphylos* species; *Bistorta vivipara* (L.) Delarbre (= *Polygonum viviparum* L.), and others (Haselwandter & Read 1980, Antibus et al. 1981, Miller 1982, Bledsoe et al. 1990, Kohn & Stasovski 1990, Gardes & Dahlberg 1996, Treu et al. 1996, Vare et al. 1992, 1997, Krpata et al. 2003, Ryberg et al. 2009, 2011, Gao & Yang 2010, Timling et al. 2012). In the central and southern Rocky Mountain alpine zone, ectomycorrhizae have been observed on the roots of *Betula glandulosa*, *Dryas octopetala*, *Bistorta vivipara*, *Salix arctica*, *S. glauca*, *S. planifolia*, and *S. reticulata* on the Beartooth Plateau (Lesica & Antibus 1986, Cripps & Eddington 2005, Cripps & Horak 2006b) and *Kobresia myosuroides* (Vill.) Fiori on Niwot Ridge in Colorado (Schadt 2002). Many ECM plants that occur in the Rocky Mountain alpine zone have wide-ranging, circumpolar or circumboreal distributions with disjunct components in mountainous or alpine areas to the south of the arctic (Hultén 1968). Species of interest for the Rocky Mountains will be discussed below.

Salix arctica Pall. This species is a highly variable arctic-alpine prostrate dwarf willow with a broad circumpolar distribution in arctic areas. It also occurs in alpine areas to the south of the arctic (Hultén 1968). In alpine areas of the Rocky Mountains, south to New Mexico, it inhabits open turf, moist areas near streams, and places of high snow accumulation (Scott 1995, Lesica 2012). Rocky Mountain plants are var. *petraea* Anderss. (Scott 1995).

Salix reticulata L. (= *Salix nivalis* Hook). This species is also a prostrate dwarf willow, and has a nearly circumpolar distribution in arctic areas, being absent only from Greenland (Hultén 1968), although it may have occurred there in the past (Bennike & Böcher 1999). It also occurs south of the arctic in dry fellfields to moist turf in alpine areas of some Eurasian mountain ranges, and in alpine areas of the Rockies south to New Mexico (Scott 1995, Lesica 2012). Plants in the central and southern Rockies are ssp. *nivalis* (Hook) Love et al. (Scott 1995).

Salix planifolia Pursh. This species is a small to medium shrubby willow, and occurs only in North America, from Yukon Territory south to California and New Mexico, through boreal Canada and into eastern Canada and New England (Scott 1995, Lesica 2012). It is not restricted to arctic-alpine areas. In western North America it is common in moist montane and alpine habitats. On the Beartooth Plateau it is often the dominant species in low alpine Salix thicket vegetations (Johnson & Billings 1962).

Salix glauca L. This species is a low to medium shrubby willow that has a circumpolar to circumboreal distribution and it also occurs to the south in isolated mountainous areas of Eurasia and North America (Hultén 1968). In the Rocky Mountains it occurs in subalpine and alpine areas, where it inhabits meadows, moist limestone talus slopes and streambanks south to New Mexico (Scott 1995, Lesica 2012).

Other *Salix* Species. Willows that occur in alpine areas of the central and southern Rocky Mountains that have not been investigated for the presence of ectomycorrhizae include the dwarf willow *Salix cascadensis* Cockll. and a variety of shrubby willows.

Betula glandulosa Michx. This shrubby species occurs in moist areas, swamps, bogs and fens from Alaska through boreal Canada and the Great Lakes region to Greenland, and south in the western mountains to California and Colorado (Hultén 1968, Lesica 2012). In the Rocky Mountains it occurs in wet montane areas up to low alpine areas (Lesica 2012). Its close relative *Betula nana* L. has a circumpolar to circumboreal distribution. The two species hybridize where their ranges overlap (Hultén 1968).

Dryas octopetala L. This species has a more or less circumpolar, arctic distribution and also occurs in alpine areas of Eurasia and North America (Hultén 1968, Scott 1995, Lesica 2012). In the Rocky Mountains it occurs on exposed slopes, fellfields and moist turf in alpine areas south to Colorado (Scott 1995, Lesica 2012). *Dryas octopetala* is most common on calcareous soils but can also occur on acidic soils in the Rockies (Lesica 2012). A related species, *Dryas integrifolia* M. Vahl cohabitates with and forms hybrids with *D. octopetala* in northern North America (Hultén 1968).

Bistorta vivipara (L.) Delarbre. This small, perennial, non-woody, herbaceous species has a circumpolar distribution (Scott 1995). In western North America, its distribution extends south in the Rocky Mountains to New Mexico. It inhabits moist stream banks, meadows, and willow thickets (Scott 1995).

Kobresia myosuroides (Vill.) Fiori. This perennial grass has a circumpolar distribution. In western North America, its distribution extends south from Alaska to mountainous regions in California, Idaho, Colorado, and New Mexico (Scott 1995). In the Rocky Mountains it inhabits fellfields and rocky ledges (Scott 1995).

Ectomycorrhizal Fungi

The first ectomycorrhizal (ECM) symbiosis may have had an ancient origin with Pinaceae or one of its ancestors (Hibbett & Matheny 2009). Since then, the ECM symbiosis has evolved and been maintained independently many times within different fungal lineages and has formed with a variety of gymnosperms and angiosperms (Hibbett & Matheny 2009). Ectomycorrhizal fungi (EMF) engage in associations with around 6,000 plant species (Brundrett 2009). Some prominent plant families involved include Pinaceae, Fagaceae, Betulaceae, Salicaceae, and Ulmaceae, which are major components of forest ecosystems in North America and Eurasia (Meyer 1973, Smith and Read 2008, Tedersoo et al. 2012). In these habitats, EMF play an important role in seedling establishment and tree survival and growth (Tedersoo et al. 2012). Also, as mentioned previously, some EMF associate with plants outside of the above listed families, such as *Bistorta vivipara* (Polygonaceae), *Dryas* spp. (Rosaceae), and *Kobresia* spp. (Cyperaceae) (Cripps & Eddington 2005). Ectomycorrhizal fungal richness is highest across northern temperate and boreal forest regions in Europe, America and Asia, where EMF can contribute up to 39% of the soil microbial biomass and 10-35% of soil respiration (Smith & Read 2008, Högberg et al. 2010, Tedersoo et al. 2012). Richness is lower in the tropics, contrasting with typical latitudinal patterns of biodiversity (Tedersoo et al. 2012). There are an estimated 20 – 25,000 species of EMF with the phylum Basidiomycota being the most diverse, followed by Ascomycota (Rinaldi et al. 2008, Brundrett 2009). Besides providing their host plants with nutrients, EMF also protect rootlets from pathogen invasion and prevent heavy metal uptake (Halling 2001). Furthermore, the sporocarps (specialized, multi-celled, spore-bearing structures) formed from sexual reproduction in Basidiomycota and Ascomycota (Hawksworth 2012) of many EMF serve as food for animals (Halling 2001). Specificity of particular EMF species for particular plant hosts varies. *Suillus* species for example, only associate with Pinaceae, whereas host specificity is often more broad for other EMF (Halling 2001, Ishida et al. 2007). EMF community composition is affected by a multitude of factors, including various site characteristics, such as litter quality, climate, soil type and soil nutrient status as well as interspecific interaction and plant identity, functional type, and successional stage (Molina et al. 1992, Ishida et al. 2007, Tedersoo et al. 2009, 2012). In a recent study by Tedersoo et al. (2012), host plant family had the largest effect on EMF community composition, and climate factors such as precipitation and temperature were most closely tied to EMF species richness.

Although EMF are generally thought of strictly as important components of forests, as previously mentioned, they occur with shrubs and even some herbaceous hosts above and beyond tree line.

Species Concepts Used for Fungi

A common species concept used by biologists is the evolutionary species concept (ESC). Under this concept, species are recognized as separately evolving metapopulation lineages (e.g. de Queiroz 2007). Adhering to this concept, fungal species have traditionally been recognized based on several different operational species recognition techniques: morphological species recognition (MSR), biological species recognition (BSR) and phylogenetic species recognition (PSR) (Taylor et al. 2000).

Historically, morphological species recognition has seen the most use for fungi and it serves as the basis for taxonomical classification. However, despite the usefulness of MSR, morphologically recognized species have been shown to often represent multiple biological or phylogenetic species (Taylor et al. 2000, 2006, Dettman et al. 2003). Furthermore, highly morphologically variable species can exist under several names, when in fact only one biological or phylogenetic species is present.

Biological species recognition, also commonly used, delineates fungal species based on their ability or inability to mate (reproductive isolation) (Taylor et al. 2000). However, BSR cannot be applied to all fungi. Many fungi do not sexually reproduce, some fungi are homothallic (self-fertile), many fungi will not mate *in vitro* and many fungi (including many ECM taxa) cannot be grown in culture (Taylor et al. 2000). Furthermore, BSR can lump fungi that are genetically isolated in nature into one species based on their ability to mate in culture, which measures potential gene flow rather than actual gene flow and thus does not adhere to the evolutionary species concept (Taylor et al. 2000).

Phylogenetic species recognition recognizes species based on evidence of genetic isolation as visualized on a phylogenetic tree (Taylor et al. 2000, 2006). PSR can be applied to fungi that will not mate in culture and fungi that will not grow in culture, and it measures actual genetic isolation rather than potential as in BSR (Taylor et al. 2000, 2006).

One form of PSR, termed geneological concordance phylogenetic species recognition (GCPSR), which uses multiple genetic loci to recognize species has been suggested as being the most accurate way of recognizing fungal species and has recently become widely used in studies of fungal phylogenetics and species recognition (Taylor et al. 2000, 2006, Dettman et al. 2003, Geml et al. 2008, Stubbe et al. 2010, 2012, Stubbe & Verbeken 2012, Grubisha et al. 2012, Van de Putte 2010, 2012, De Crop et al. 2013). One drawback of single-gene PSR is that individuals within a single species may be recognized as distinct phylogenetic species if one set of individuals possesses one allele of the sequenced region and another set possesses the other allele (Taylor et al. 2000). It is argued that GCPSR overcomes this by including more than one gene and as stated by Taylor et al. (2000), “conflict among the gene trees is likely to be due to recombination among individuals within a species, and the transition from concordance to conflict determines the limits of species."

Each of the species recognition concepts outlined above has its strengths and weaknesses and each has the potential to recognize a different number of species within a given set of individuals. In an attempt to recognize true, evolutionarily distinct species, each form of species recognition is relevant as each is based on real, observable characteristics of organisms (de Queiroz 2007). Hawksworth (2012) emphasizes the importance of taking into account, for example, both morphological and molecular data when recognizing fungal species.

The Molecular Era

While morphological species recognition has formed the foundation of fungal taxonomy and sporocarp surveys have served as the basis for fungal community ecology, many authors have realized their shortcomings: 1) Morphologically defined fungal species can consist of multiple phylogenetic species as delineated by DNA, and thus species distributions may be misrepresented and species richness may be underestimated using only morphological species recognition techniques (Taylor et al. 2000, 2006) and 2) The main body of the fungus resides below-ground and sporocarps are produced somewhat sporadically and are ephemeral, thus the actual fungal community composition and diversity cannot be accurately measured using morphological species recognition and sporocarp surveys (Gardes & Dahlberg 1996, Timling & Taylor 2012).

Molecular tools are providing another way to delineate fungal species, investigate their distribution, and explore below-ground community composition. Currently, the most commonly used gene region for studies of below-ground EMF communities, species delimitation, and EMF biogeography is the internal transcribed spacer (ITS) region (Horton & Bruns 2001, Nilsson et al. 2008, Schoch et al. 2012). The ITS region makes up part of the nuclear ribosomal repeat unit. It is roughly 550-basepairs in length, occurs in tandem repeats, and is made up of three different parts (Nilsson et al. 2008). The ITS1 typically evolves rapidly, the middle region, the 5.8S ribosomal RNA (rRNA) gene, is highly conserved, and the ITS2 typically evolves moderately rapidly to rapidly (Hershkovitz & Lewis 1996). The ITS region is flanked on either side by conserved regions of the small subunit (SSU) rRNA gene and large subunit (LSU) rRNA gene (Hillis & Dixon 1991). Furthermore, the ITS region is relatively easily amplified using generic primers from a broad range of materials such as environmental samples and herbarium specimens (Nilsson et al. 2008).

While the ITS region has been the most widely used gene region for studies of fungal biodiversity and lower-level fungal phylogenetics and species recognition, many other gene regions are used as well. The single-copy, protein encoding gene *rpb2,* which encodes the second largest subunit of RNA polymerase II has recently become popular for investigating fungal phylogenetics across broad taxonomic levels (Liu et al.

1999, Frøslev et al. 2005, Matheny 2005, Matheny et al. 2007, Stubbe et al. 2010, Van de Putte et al. 2012). In a study of phylogenetic relationships in the ECM genus *Cortinarius* by Frøslev et al. (2005) the region of *rpb2* studied (region between conserved domains 6 and 7), although almost entirely protein coding showed more variation yet was easier to align and provided more phylogenetic resolution than the ITS region. Phylogenies of other ECM taxa, *Inocybe* (Matheny 2005), *Lactarius* (Stubbe & Verbeken 2012), *Lactifluus* (Stubbe et al. 2010, 2012, Van de Putte et al. 2010, 2012, De Crop et al. 2013) have also benefited from the inclusion of *rpb2.*

Online DNA sequence repositories such as GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and Unite (https://unite.ut.ee/) provide researchers with access to ITS and *rpb2* DNA sequences as well as numerous other DNA sequences from other loci generated in previous studies.

Arctic-Alpine Ectomycorrhizal Fungi

In harsh environments, such as cold, nutrient poor arctic-alpine areas, EMF are believed to be of particular importance. In these systems they can provide their host plants with up to 86% of their nitrogen (Hobbie & Hobbie 2006). Furthermore, in arctic-alpine areas, the majority of plant biomass is represented by ECM genera, such as *Salix* and *Betula* (Chapin & Körner 1995).

Traditional Studies

Traditionally, studies of arctic-alpine EMF species diversity, host specificity, community structure and distribution have focused on sporocarp surveys, followed by the morphological recognition of species based on visible features of the sporocarps. Arctic-alpine EMF surveys have centered on areas of Greenland (Lange 1957, Kobayasi et al. 1971, Watling 1977, Lamoure et al. 1982, Borgen et al. 2006), Iceland (Christiansen 1941, Hallgrímsson & Eyjólfsdóttir 2004), Scandinavia (Kallio & Kankainen 1964, Kühner 1975, Gulden et al. 1985, Gulden 2005), Svalbard (Ohenoja 1971, Watling 1983, Gulden & Jenssen 1988, Gulden & Torkelsen 1996), the central European Alps (Favre 1955, Kühner 1975, Kühner & Lamoure 1986, Bon 1989, 1990, 1991, Senn-irlet 1993, Graf 1994, Moreau 2002, Jamoni 2008), and Scotland (Watling 1987). Less well-surveyed regions include Alaska (Laursen & Ammirati 1982, Miller 1982, 1987), Canada (Kernaghan & Harper 2001, Ohenoja & Ohenoja 2010), Siberia (Knudsen & Mukhin 1998, Karatygin et al. 1999), Kamchatka (Kalamees & Vaasma 1993), the Altai Mountains (Kalamees 2008, Gorbunova 2010), the Pyrenees Mountains (Corriol 2008), the Carpathians (Ronikier 2008), and the central and southern Rocky Mountains (Cripps & Horak 2008). These surveys have indicated the presence of all of the major sporocarp-producing EMF genera, and have revealed high EMF species diversity despite the limited diversity of ECM host plant species (Gardes & Dahlberg 1996). Sporocarps of the ectomycorrhizal genera *Cortinarius*, *Hebeloma*, *Inocybe*, *Lactarius* and *Russula* have been shown to be particularly abundant, while sporocarps of the genera *Amanita* and

Laccaria, as well as hypogeous taxa and Boletes are less common (Gardes & Dahlberg 1996). These studies have suggested that the majority of EMF taxa encountered in arctic-alpine areas have circumpolar distributions, centered in temperate or boreal regions, which extend into arctic-alpine areas (Lange 1957, Kallio & Kankainen 1964, Moser 1982, Gardes & Dahlberg 1996, Borgen et al. 2006). Some strictly arctic-alpine taxa have been described as well, however it is possible that some of these may represent commonly encountered temperate/boreal taxa that produce less pigmented, stunted or misshapen sporocarps in arctic-alpine areas (Gardes & Dahlberg 1996).

Molecular Ecology, Systematics and Biogeography of Arctic-Alpine Ectomycorrhizal Fungi

Through analysis of molecular data (mostly ITS region) from ECM root tips and soil clones from arctic-alpine regions in Svalbard (Fujiyoshi et al. 2011, Geml et al. 2012), Norway (Bjorbækmo et al. 2010), Sweden (Ryberg et al. 2011), Austria (Mühlmann & Peintner 2008), North America (Geml et al. 2009, Deslippe et al. 2011, Geml et al. 2012, Timling et al. 2012), and the Chinese Himalayas (Gao & Yang 2010), the genera *Thelephora*/*Tomentella*, *Inocybe* and *Cortinarius*, followed by *Hebeloma*, *Russula*, *Lactarius*, *Entoloma*, *Sebacina*, *Clavulina* and *Leccinum* have been shown to be the most common and species rich EMF genera in these areas (Timling & Taylor 2012). These results are similar to those obtained from sporocarp surveys, however they reveal the dominant genera *Thelephora*/*Tomentella* as well as *Sebacina*, which lack or produce cryptic sporocarps (Timling & Taylor 2012), and they imply a higher diversity of EMF species than suggested by sporocarp studies (Bjorbækmo et al. 2010, Timling et al. 2012).

Recent molecular studies also suggest that the distributions of mid-latitude EMF are more geographically structured than previously believed and that morphological species complexes of circumtemperate EMF from the Northern Hemisphere often include separate North American and Eurasian lineages (Geml et al. 2006, 2008, Taylor et al. 2006). For example, *Lactarius* sect. *Deliciosi* (Nuytinck et al. 2007), *Lactifluus* subg. *Gerardii* (Stubbe et al. 2010), and *Tricholoma populinum* (Grubisha et al. 2012) do not show intercontinental conspecificity between North America and Eurasia.

On the other hand, in arctic-alpine areas, large-scale environmental ITS region sequencing studies, as well as small-scale molecular systematics studies analyzing the ITS region are suggesting broad, ecologically diverse, intercontinental distributions for many EMF inhabiting these areas. In a study of the global biogeography of the /sebacina lineage, Tedersoo et al. (2014) found contrasting patterns of distribution and rate of evolution between the tropics and the Holarctic. Holarctic /sebacina taxa were generally broadly distributed in the northern hemisphere and showed slower rates of ITS evolution than their tropical counterparts. In agreement, Timling et al. (2012), reported that 73% of the EMF operational taxonomic units (OTUs, a proxy for species) recovered from *Dryas integrifolia* and *Salix arctica* root tips in arctic North America matched sequences in GenBank from other regions in the arctic or from outside the arctic. These findings were mirrored in Timling et al. (2014), where the majority of the most abundant OTUs appeared to occur across and outside the arctic, with some of the OTUs even occurring on every continent.

These studies present this as evidence for recent long-distance dispersal events and little dispersal limitation, which is in contrast to patterns observed in studies attempting to directly measure spore dispersal distances (e.g. Peay et al. 2010). In addition, Timling et al. (2014) measured a diversity of EMF fungi beyond the range of any woody species in the high arctic, which was likely based on a spore bank and not actual, functioning EMF species, suggesting the potential for arctic-alpine EMF to lie dormant as spores until suitable hosts are present. Long distance spore dispersal was also proposed in Geml et al. (2012) to explain high EMF ITS sequence similarity between Svalbard and arctic North America. However, Timling et al. (2014) suggest that some species likely have more restricted distributions than revealed in these studies, as a strict 97% ITS region similarity cutoff fails to differentiate some species. The value of a 97% cutoff for species delimitation is discussed later.

While ITS region data has suggested intercontinental, circumpolar distributions for arctic-alpine EMF, intercontinental divergence within morphologically defined taxa has been revealed for arctic-alpine EMF using other loci. Den Bakker et al. (2007), using two unlinked loci, looked at inter- and intraspecific phylogenetic relationships of arctic-alpine, boreal and temperate taxa in the EMF genus *Leccinum* sect. *Scabra*, which associates strictly with *Betula*. They called into question the validity of the *L. rotundifoliae* species concept, suggesting that it may represent an arctic-alpine ecotype of *L. scabrum*. They sampled representatives of *Leccinum* sect. *Scabra* from temperate, boreal, subalpine, and arctic regions in Europe, eastern North America, and Greenland "to explore phylogenetic relationships and phylogeographic patterns" using 5.8S-ITS2 rDNA and the glyceraldehyde-3-phosphate dehydrogenase gene (*gpd*). Results of *gpd* analyses displayed separate clades from Greenland and Eurasia for *L. rotundifoliae* and suggested that *L. rotundifoliae* is not conspecific with *L. scabrum*. On the other hand, 5.8S-ITS2 analyses suggested conspecificity between *L. rotundifoliae* and *L. scabrum* and did not reveal phylogeographic patterns for either taxa, highlighting the importance of examining more than one locus in phylogenetic studies (Den Bakker et al. 2007).

Broad patterns of host specificity for arctic-alpine EMF have been investigated as well. Kernaghan & Harper (2001) investigated ectomycorrhizal community structure across an alpine/subalpine ecotone in the Canadian Rockies by comparing PCR amplified ITS region DNA from mycorrhizal root tips to that of sporocarps through RFLP analysis. The subalpine forest was dominated by the conifer species *Abies lasiocarpa* and *Picea engelmannii*. The ecotone consisted of krummholz *Abies* and *Picea*, as well as angiosperms *Salix barrattiana* Hook., *S. glauca*, *S. arctica*, *Betula glandulosa*, and various ericaceous shrubs. Vegetation in the alpine zone was similar to the ecotone, but also included *Dryas octopetala*, *D. integrifolia* and fewer or no conifers. They observed a decrease in host-specificity of EMF with increasing elevation; EMF that occurred in the alpine zone, including certain species of *Cenococcum*, *Inocybe*, *Amphinema*, and

Tomentella were found to be non-host specific and shown to associate with both angiosperms and gymnosperms, although this was a limited study.

In agreement, using 97% similarity cutoff, Timling et al. (2014) found that the fungal community in the lowest arctic subzone included in their study, which was dominated by *Betula nana* was very similar to that of the adjacent boreal forest, however, they did note a large shift in the fungal community with movement to the next highest arctic subzone. Many other studies have shown low host specificity for arctic-alpine EMF and it has been suggested that low host specificity may be one factor that facilitates large geographic distributions (Kernaghan & Harper 2001, Ryberg et al. 2009, 2011, Fujimura & Egger 2012, Timling et al. 2012). However, while species with low host-specificity may be dominant in arctic-alpine areas, some species may be highly host-specific (e.g. Knudsen & Lamoure 1993).

While recent studies have generated large quantities of environmental molecular data (e.g. Bjorbækmo et al. 2010, Geml et al. 2012, Timling et al. 2012) and have provided much insight into large-scale patterns in arctic-alpine EMF diversity, distribution, host specificity and community structure, few studies have looked closely at species level recognition and phylogeny using molecular and morphological techniques, and many taxonomic problems exist. Typically, unidentified ITS sequences generated in environmental studies are grouped into Operational Taxonomic Units (OTU's) based on sequence similarity and then identified to some level based on top matches in the International Nucleotide Sequence Database (INSD) using the Basic Local

Alignment Search Tool (BLAST) (Nilsson et al. 2008). However, the ITS region has been sequenced for less than 1% of the estimated 1.5 million species of fungi (Hawksworth 2001, Nilsson et al. 2005), only 61% of ITS sequences in INSD are associated with a species name (Nilsson et al. 2009) and roughly 20% of these have the incorrect species name (Nilsson et al. 2006). Furthermore, intraspecies ITS variation is not equal for all fungi (Nilsson et al. 2008) and intragenome ITS variation between repeats is high within some individuals (Lindner & Banik 2011). Therefore, a set species level sequence similarity cutoff value (such as 97%) cannot accurately estimate species diversity and distribution for all groups of fungi (Nilsson et al. 2008). As such, "there is a need for case by case recognition of species by taxonomists, followed by the deposition of representative sequences in the database" (Nilsson et al. 2008, Begerow et al. 2010).

The necessity to inventory EMF diversity and distribution in arctic-alpine areas is further highlighted by the changing climate in these areas. ECM shrubs are increasing in abundance and expanding their range in the arctic (Sturm et al. 2001, 2005). Furthermore, a number of studies simulating climate change have shown that arctic-alpine EMF community composition changes with warming and increased nutrient availability. Deslippe et al. (2011) reported an increase in EMF diversity associated with the shrub *Betula nana* in arctic-alpine Alaska following warming. However, not all groups of EMF responded to treatments in the same way. Some groups of EMF (e.g. *Cortinarius* spp.) increased in abundance, whereas others (e.g. members of the Russulaceae) decreased in abundance. Fertilization to simulate N deposition slightly reduced EMF diversity and increased the abundance of saprotrophs (Deslippe et al. 2011). Sturm et al. (2005) suggests that increased shrub abundance will lead to greater snow depths, further insulating soils in the winter and leading to more microbial activity and greater plant-available nitrogen, which in turn further favors shrub growth. Deslippe et al. (2011) further suggest that increased mycorrhizal diversity and larger mycorrhizal networks may further increase shrub expansion in the arctic. Timling et al. (2014) suggest that with continued climate warming and northward advance of vascular plants, fungi symbiotic with plants are likely to become more abundant in arctic-alpine ecosystems, which will cause an overall shift in ecosystem functioning in these areas, highlighting the importance of EMF in this system.

Macrofungi of the Rocky Mountain Alpine Zone

Until recently, the diversity of macrofungi in the Rocky Mountain alpine zone has remained relatively unexplored. A few sporocarp surveys have included a limited number of alpine fungi (e.g. Overholtz 1919, Kauffman 1921, Seaver & Shope 1930, Solheim 1949, Moser & McKnight 1987, Moser 1993, Moser et al. 1994, 1995, Miller & Evenson 2001), and results of a more extensive sporocarp survey are reported in Cripps & Horak (2008). This survey covered the Beartooth Plateau in Montana/Wyoming in the central Rocky Mountains (lat 45° N), and the Front Range, San Juan Mountains, and Sawatch Range in the southern Rockies (lat 36°—38° N) in Colorado. Over 165 species in 46 genera and 11 families were recognized from ca 1500 collections. Over 75% of the species are known from other arctic-alpine regions and the remainder may be western North American endemics. Of these, it is estimated that 2-5% are unrecognized species, 75% are new records for the areas under study, and over half are new records for Colorado, Montana and Wyoming. Approximately 56% of the species are ectomycorrhizal taxa that associate primarily with *Salix* species and/or *Betula glandulosa*. Results suggest that the ectomycorrhizal families Cortinariaceae and Inocybaceae are extremely diverse in the Rocky Mountain alpine zone, with over 74 species of primarily *Inocybe* and *Cortinarius*. Results also suggest that the southern Rockies may have a greater diversity of macrofungi even though they may be at the southern extent of the distribution of many core arctic-alpine fungi in North America (Cripps & Horak 2008).

Studies spawned from this survey have further investigated fungal systematics, species recognition, biogeography and ecology of arctic-alpine fungi in the Rocky Mountains (Osmundson et al. 2005, Peintner 2008, Beker et al. 2010, Cripps and Horak 2006a, Cripps et al. 2010, Nauta 2010, Ronikier & Ronikier 2010, Cripps & Barge 2013, Larsson et al. 2014). Collections of fungi in the ectomycorrhizal genus *Inocybe* (*Mallocybe*) from the Rocky Mountain alpine zone were recognized as morphologically distinct species and were then compared phylogenetically to hypothetically conspecific taxa from alpine areas in northern Europe (Cripps et al. 2010). Results showed molecular congruence between most hypothetically conspecific taxa, suggesting broad intercontinental ranges in arctic-alpine areas, and also revealed occurrences in subalpine habitats. Larsson et al. (2014) found molecular congruence between North America and Europe for additional species of *Inocybe*, although many of the species studied were still only reported from Europe where more research has been done. Beker et al. (2010) examined the morphology and ITS region DNA of collections of *Hebeloma hiemale* Bres. from a broad geographic range, including collections from the Beartooth Plateau, in the central Rocky Mountains. Collections recognized as *H. hiemale* formed a monophyletic group despite high intraspecific ITS sequence variation and were recognized as one species, that appears to have a circumpolar distribution in arctic, alpine and temperate areas. Morphologically recognized collections of the saprobic *Rhizomarasmius epidryas* (Kühner ex A. Ronikier) A. Ronikier & M. Ronikier (= *Marasmius epidryas* Kühner ex A. Ronikier) suggest this species has a circumpolar range in the arctic, extending into alpine areas of the Himalayas, Alps and Rockies, a range possibly completely overlapping that of its *Dryas* host (Ronikier & Ronikier 2010, 2011). Other studies looking at distributions and incorporating Rocky Mountain alpine material reveal similar patterns and suggest a broad circumpolar/disjunct southern alpine distribution for *Arrhenia auriscalpium* (Fr.) Fr. (Cripps and Horak 2006a) and for *Amanita groenlandica* Bas ex Knudsen & T. Borgen and *A. nivalis* Grev. (Cripps & Horak 2010). These studies parallel findings from large-scale environmental sequencing studies investigating arctic areas (Geml et al. 2012, Timling et al. 2012, 2014). There are a few reports of *Lactarius* species in the Rocky Mountain alpine zone (Moser & McKnight 1987, Cripps & Horak 2008, Cripps & Barge 2013), but no detailed work has followed.

The Genus *Lactarius*

The Basidiomycota genus *Lactarius* Pers. is mostly comprised of ectomycorrhizal, mushroom-forming fungi that have a global distribution (Verbeken 2001) and are also very diverse and abundant in high latitude areas; certain species are reported in arctic-alpine areas (e.g. Kühner 1975, Knudsen & Borgen 1982, Laursen & Ammirati 1982, Gulden et al. 1985, Gardes & Dahlberg 1996, Heilmann-Clausen et al. 1998, Borgen et al. 2006, Knudsen & Vesterholt 2008, Geml et al. 2009, Ohenoja & Ohenoja 2010). The genus is currently placed in the order *Russulales* and family Russulaceae (Larsson & Larsson 2003, Larsson et al. 2004, Shimono et al. 2004, Binder et al. 2005, Miller et al. 2001, 2006, Hibbett 2007) along with the genera *Lactifluus* (Pers.) Roussel, *Multifurca* Buyck & Hoffstetter and *Russula* Pers. (Buyck et al. 2008, 2010). The genus *Lactarius* was originally described from European material by Persoon (1797) as *Lactaria* and *L. piperatus* was designated as the type. *Lactarius* has traditionally been recognized as a genus of mushroom-forming (agaricoid) fungi whose sporocarps exude a characteristic latex when damaged, contain clusters of round (isodiametric) cells called sphaerocysts, and produce light-colored spores with amyloid ornamentation (e.g. Neuhoff 1956, Hesler & Smith 1979, Bon 1980, Heilmann-Clausen et al. 1998, Basso 1999).

Lactarius is separated from the closely related genus *Russula* by the production of latex, along with a lack of sphaerocysts in the lamellae, and presence of pseudocystidia (e.g. Heilmann-Clausen et al. 1998). Furthermore, *Lactarius* species often have decurrent to subdecurrent lamellae, whereas *Russula* do not, although this is not true in all cases.

Recent molecular studies have demonstrated that *Lactarius* as historically recognized is paraphyletic and occurs in three clades within the Russulaceae (e.g. Buyck et al. 2008). The first clade is made up of species historically recognized in *Lactarius* subgenera *Russularia* (Fr.) Kauffman, *Piperites* (Fr.) Kauffman, *Plinthogalus* (Burl.) Hesler & A.H. Sm., *Colorati* (Bataille) Bon, *Tristes* Hesler & A.H. Sm. and *Rhysocybella* Bon. Altogether this clade contains about 80% of the species traditionally recognized as *Lactarius* and is mostly temperate to boreal in distribution (Buyck et al. 2008, 2010, Verbeken & Nuytinck 2013). The second clade is made up of species historically recognized in *Lactarius* subgenera *Lactifluus* (Burl.) Hesler & A.H. Sm., *Lactarius*, *Lactariopsis* (Henn.) R. Heim, *Gerardii* (A.H. Sm. & Hesler) Stubbe, *Russulopsis* Verbeken and section *Edules* Verbeken. Altogether this clade contains about 25% of the species traditionally recognized as *Lactarius* and is mostly temperate to tropical in distribution (Buyck et al. 2008, 2010, Verbeken & Nuytinck 2013). There is currently no published literature formally delineating the morphological differences between *Lactarius* and *Lactifluus,* a genus which also produces latex and shares many other morphological similarities with *Lactarius.* However, Verbeken & Nuytinck et al. (2013) have laid out several provisional synapomorphies for the two clades. *Lactifluus* can have thick-walled hyphae in the pileipellis and stipitipellis, lamprocystidia, as well as sphaerocysts in the hymenophoral trama, while this is very rarely seen in *Lactarius*. Furthermore, pleurotoid (without a stipe) species are only known from *Lactifluus*. The third clade is made up of former *Lactarius furcatus* Coker and *Russula* species formerly assigned to subsection *Ochricompactae* Bills & O.K. Mill. (Buyck et al. 2008, 2010, Verbeken & Nuytinck 2013).

A proposal to maintain the name *Lactarius* Pers. for the first monophyletic group with *Lactarius torminosus* (Schaeff.: Fr.) Pers. as the type, and adopt the name *Lactifluus* (Pers.) Roussel for the second monophyletic group has been submitted and accepted (Buyck et al. 2010, Verbeken & Nuytinck 2013). The genus *Multifurca* Buyck & Hoffstetter was created to house the third group (Buyck et al. 2008). Furthermore, molecular studies have demonstrated that gasteroid and secotioid species which exude latex and have traditionally been classified in the genera *Arcangeliella* Cavara, *Zelleromyces* Singer & A.H. Sm. and *Gastrolactarius* J.M. Vidal are nested within *Lactarius* (Calonge & Martin 2000, Miller et al. 2001, Desjardin 2003, Nuytinck et al. 2003, 2007, Eberhardt & Verbeken 2004, Buyck et al. 2010, Verbeken et al. 2014) and these genera have since been moved to *Lactarius* (Buyck et al. 2010). Out of the three clades presented above, gasteroid and secotioid species are only known from *Lactarius* (Verbeken & Nuytinck 2013). According to Verbeken (2001) there are more than 400 known species in *Lactarius* sensu lato, although Buyck et al. (2010) estimates that about 20% to 25% of these belong in *Lactifluus.*

Ecology and Host Associations

The genus *Lactarius sensu lato* is commonly cited as being a very important component of many forest ecosystems (Hesler & Smith 1979, Heilmann-Clausen et al. 1998, Buée et al. 2009, Geml et al. 2009) and molecular studies sequencing ectomycorrhizal root tips have confirmed the association of *Lactarius* with many prominent temperate and boreal tree and shrub genera such as *Abies* (Eberhardt et al. 2000), *Larix* (Leski & Rudawska 2012), *Pinus* (Taylor & Bruns 1999), *Picea* (Kraigher et al. 1995), *Tsuga* (Wright et al. 2009), *Pseudotsuga* (Burke et al. 2008), *Alnus* (Kennedy & Hill 2010), *Betula* (Deslippe et al. 2011), *Cistus* (Nuytinck et al. 2004), *Populus* (Bent et al. 2011), *Salix* (Ryberg et al. 2011), *Fagus* (Jany et al. 2003), *Quercus* (Courty et al. 2006), *Lithocarpus* (Bergemann & Garbelotto 2006), and *Castanea* (Peintner et al. 2007). Furthermore, *Lactarius* sporocarps serve as a source of food for forest mammals and some species are popular among mushroom hunters; they offer possibilities for drug production as well (Hesler & Smith 1979, Mlinaric et al. 2004, Kramer & Abraham 2012).

Heilmann-Clausen et al. (1998) suggest that, while the level of host specificity varies in the genus *Lactarius*, many species appear to associate with only one plant genus or species. Furthermore, they suggest that while some *Lactarius* species have wide distributions with their host plant(s), others have limited distributions within their host plant's range. For example, *L. hysginoides* Korhonen & T. Ulvinen and *L. subcircellatus* Kühner are only reported from boreal and subarctic/subalpine areas despite the wide distribution of their putative host genus *Betula* (Heilmann-Clausen et al. 1998), suggesting that their distributions are further limited by edaphic factors (Hansen 1988). In North America, Hesler & Smith (1979) report varying levels of host specificity for species in the genus. They report one group as commonly found with conifers, one group with *Betula*, one group from oak and beech forests, and another group with *Alnus*. Much of the diversity in tropical and subtropical Fabaceae and Dipterocarpaceae forest ecosystems previously attributed to *Lactarius* (Smith et al. 2011, Tedersoo et al. 2011, Stubbe et al. 2012), has since been recognized to be attributed to *Lactifluus* (Buyck et al. 2010). Heilmann-Clausen et al. (1998) suggest that host plant distribution, climatic conditions and soil conditions are especially important in determining individual species distributions in *Lactarius sensu lato*.

European Studies

There are too many European studies of *Lactarius* to cite them all, however a few prominent studies are cited here. The genus *Lactarius* was originally described in Europe by Persoon (1797). Other defining European treatments include those of Fries (1821), Gray (1821), Quélet (1888), Bataille (1908), Konrad (1935a, b), Neuhoff (1956) and Bon (1980, 1983). The most recently published, comprehensive identification guides devoted to *Lactarius* are those of Heilmann-Clausen et al. (1998), covering 96 taxa from northern Europe and Basso (1999) which also focuses on European taxa.

North American Studies

Early work in North America focused on Midwestern, Eastern (Peck 1885, Burlingham 1907a, b, 1908, 1910, Coker 1918, Kauffman 1918, Murrill 1938, 1948), and West coast (Burlingham 1913) *Lactarius* species. Hesler & Smith (1979) published a monograph on *Lactarius* species covering much of North America, including Alaska, Canada, the Pacific coast, the Rocky Mountains, the Midwest, and eastern and southeastern coasts. This monograph provided significant information on *Lactarius* in North America. It delineated many similar and conspecific taxa shared with Europe, described many North American endemics and showed little species overlap between Eastern and Western North America; it also revealed many taxonomic problems. Two hundred species and 60 varieties were recognized, with 112 of them being newly described by the authors. However, despite massive sampling efforts, many parts of North America were not covered and they did not sample arctic or alpine areas.

Hesler & Smith also published studies dealing with specific infrageneric sections of *Lactarius* (e.g. Hesler & Smith 1962 on North American species in subgenus *Plinthogalus*). Since then, Homola & Czapowskyj (1981) have provided information on the *Lactarius* species of Maine, Bills (1986a, b) dealt with *Lactarius* species in the high-elevation forests of the southern Appalachians, Methven (1985, 1992, 1993, 1997) has provided information on *Lactarius* in California, as well as infrageneric classification, Leacock (1993) covered *Lactarius* sect. *Dapetes* in Minnesota, and Bessette et al. (2009) published a field guide to North American species of *Lactarius*. Laursen and Ammirati

(1982) published a paper dealing specifically with *Lactarius* in arctic Alaska and Ohenoja and Ohenoja (1993, 2010) listed a few *Lactarius* species from arctic Canada. Geml et al. (2009) examined the phylogenetic diversity of *Lactarius* in boreal and arctic Alaska. Other than a checklist (Cripps & Horak 2008) and brief taxonomic write-up (Cripps & Barge 2013) no detailed studies have focused on *Lactarius* from alpine areas in the Rocky Mountains.

Macroscopic and Microscopic Characters

Species recognition and taxonomy for *Lactarius* have traditionally relied on macroscopic and microscopic characters of the sporocarp. Color of the sporocarp is considered very important for species differentiation, however color can be affected by age, weather and other environmental factors (Heilmann-Clausen et al. 1998). Surface texture, which is best observed in fresh specimens, is highly variable within the genus and important for species differentiation and infrageneric classification. Surface textures can be described macroscopically as sticky, slimy, dry, smooth, velvety, scaly and hairy, etc., and can be observed microscopically through examination of the structure of the pileipellis, which is the outermost layer of the pileus (cap) and through examination of the stipitipellis, the outermost layer of the stipe (stem) (Heilmann-Clausen et al. 1998). The surface texture of the stipe is often smooth, although in some species the stipe can have distinct features such as scrobicules (pits) (Heilmann-Clausen et al. 1998). Lamellae (gills) are decurrent to subdecurrent in most species, although the type of gill attachment, spacing, forking, anastomosing and color can help delineate some species (Heilmann-Clausen et al. 1998).

All species of *Lactarius* produce latex, which is exuded upon damage to the sporocarp. Some species appear to produce more latex than others and under dry conditions latex exudation can be scarce to nonexistent. A color change of the latex has been a very important tool for identification of some species and for infrageneric classification. The latex is usually white upon exudation, but the color can change in some species once exposed to air. In some taxa, the latex changes in isolation as well as in contact with the sporocarp, causing distinct discoloration of the flesh. However, in others, the latex changes colors only upon contact with the flesh, with other substances such as paper, or it does not change color at all (Hesler & Smith 1979, Heilmann-Clausen et al. 1998). Furthermore, changes in the color of flesh or latex with chemicals such as guaiac, guaiacol, iron sulphate, potassium hydroxide (KOH), ammonia and phenol, among others can be useful in species delimitation (Hesler & Smith 1979, Heilmann-Clausen et al. 1998).

Some species have a characteristic odor which often remains after collecting and drying that is useful in species identification (Heilmann-Clausen et al. 1998). Others have a characteristic taste that may be acrid, bitter or mild. It is typically recommended that taste be ascertained on fresh specimens (Heilmann-Clausen et al. 1998).

Microscopic features are useful for species identification and infrageneric classification as well. Spore size and shape have commonly been used for species differentiation. The spore ornamentation is best viewed in Melzer's as it is amyloid and darkens upon contact with this reagent. The height, shape, connectivity and overall pattern of spore ornamentation are often treated as important characters for species recognition. However, due to variation within species, spore ornamentation has been difficult to categorize (Heilmann-Clausen et al. 1998). Spore deposit color varies from white to cream to dark pinkish buff. However, even a single species can exhibit variation in spore deposit color (Hesler & Smith 1979). Characteristics of the plage (smooth area near the spore apiculus) can be useful for identification of some species. Basidia and pseudocystidia (extremities of lactiferous hyphae) are present in all species and are generally not taxonomically useful. For macrocystidia (sterile cells on the lamellae), presence, abundance, position, size and shape have been considered important characters. However, measurements should be treated with caution as macrocystidia size varies with sporocarp maturity (Heilmann-Clausen et al. 1998). Pileipellis structure shows much variation in the genus and is considered important in species recognition and infrageneric classification. Pileipellis types have been drawn and delineated according to the shape, thickness, incrustations, and arrangement of the hyphae (Hesler & Smith 1979, Heilmann-Clausen et al. 1998, Basso 1999).

Infrageneric Classification

A variety of infrageneric classification schemes have been developed for *Lactarius sensu lato*. Subgenera, sections and subsections were developed as an attempt at a natural classification of relationships within the genus and to aid in identification, but groups are not consistent across researchers (Quélet 1888, Neuhoff 1956, Singer 1975, Hesler & Smith 1979, Bon 1983, Heilmann-Clausen et al. 1998, Basso 1999) and some groups appear to be paraphyletic or polyphyletic in light of molecular phylogenetic studies (Eberhardt & Verbeken 2004, Nuytinck et al. 2004, Le et al. 2007). A recent infrageneric classification which is commonly cited is that of Heilmann-Clausen et al. (1998). Heilmann-Clausen et al. (1998) divide *Lactarius sensu stricto* into three subgenera and numerous sections and subsections. Overall, they suggest that the structure of the pileipellis is the most natural character for elucidating infrageneric relationships, although other characters are also used.

Species within their subgenus *Piperites* (Fr.) Kauffman are characterized by

> "caps sticky, slimy, rarely dry, often hairy and/or with concentric zones; stem dry or sticky, often pitted; pileipellis an ixocutis to an ixotrichoderm, more rarely a cutis to a trichoderm."

They include the subgenera '*Lactarius*' (*Dapetes*), *Colorati* (Bataille) Bon, *Piperites* and *Tristes* Hesler & A. H. Smith as recognized by Hesler & Smith (1979) and Bon (1983) within their concept of subgenus *Piperites* due to morphological similarities. Species within their concept of subgenus *Russularia* (Fr.) Kauffman are characterized by

> "caps dry or somewhat sticky, dominantly with orange, yellowish brown, orange brown or reddish brown colours; stem dry; milk white, cream or watery, in some species turning sulphur-yellow; pileipellis an oeodotrichoderm, a hyphoepithelium, a trichoepithelium or an epithelium, more rarely a trichoderm, in some species gelatinized."

They include subgenus *Rhysocybella* Bon within *Russularia*. Species within their concept of subgenus *Plinthogalus* (Burl.) Hesler & A. H. Smith are characterized by

> "caps dry or rarely sticky, often velvety; pileipellis a trichoepithelium to a palisadoepithelium, never with thick-walled elements, but often with a brown intracellular pigmentation; spores coarsely ornamented, typically winged and often with a distally amyloid plage."

Despite the vast amount of work that has gone into clarifying *Lactarius* taxonomy, there is a "lack of adequately precise species concepts, even for common species," morphological characters are affected by weather and growing conditions, and there are numerous complexes of closely related, somewhat poorly defined species (Hesler & Smith 1979, Heilmann-Clausen et al. 1998, Basso 1999). Furthermore, many taxa do not have designated types, especially the early taxa such as those described by Persoon and Fries (Hesler & Smith 1979).

Molecular Phylogenetic Studies

Molecular phylogenetic studies are making progress toward clarification of *Lactarius* taxonomy, along with contributing information on species diversity, distribution, host specificity and evolutionary relationships. Modern studies investigating taxonomy and species delimitation within *Lactarius* using molecular techniques include Eberhardt (2002), Eberhardt & Verbeken (2004), Nuytinck et al. (2003, 2004, 2006a, b, 2007), Nuytinck & Verbeken (2005), Le et al. (2007), Buyck et al. (2008), Geml et al. (2009), Stubbe & Verbeken (2012), Verbeken et al. (2014), Wisitrassameewong et al. (2014).

As stated previously, Buyck et al. (2010) delineates a monophyletic genus *Lactarius* made up of the historical subgenera *Piperites*, *Plinthogalus*, *Russularia*, *Colorati*, *Tristes* and *Rhysocybella*. However, in general, the monophyly of these subgenera remains poorly resolved. The most recent and comprehensive phylogenetic study of *Lactarius* recovered three major clades within *Lactarius* corresponding with subgenera *Piperites*, *Plinthogalus* and *Russularia* using ITS, LSU and *rpb2* DNA sequences, although support for the monophyly of *Piperites* was low and relationships between species were generally poorly supported, especially in subgenus *Piperites* (Verbeken et al. 2014). Reciprocal monophyly of subgenera *Piperites* and *Russularia* was also suggested in Le et al. (2007), and the monophyly of subgenus *Plinthogalus*, excluding ser. *Gerardii* has been recovered in numerous studies (Stubbe et al. 2010, 2012). Subgenus *Colorati* has received no support in molecular studies as its taxa are nested within subgenera *Piperites* and *Russularia* (Le et al. 2007, Verbeken & Nuytinck 2013). The subgenera *Tristes* and *Rhysocybella* are also often lumped in with subgenera *Piperites* and *Russularia* in modern treatments of *Lactarius* (e.g. Heilmann-Clausen et al. 1998), however a formal proposal to dissolve these subgenera has not been published.

The monophyly of sections within the subgenera discussed above has been in general, poorly investigated using molecular techniques. Le et al. (2007) suggested the monophyly of the violet staining section *Uvidi* (Konr.) Bon, within subgenus *Piperites*, although support was low; and Nuytinck et al. (2006a, b, 2007) and Nuytinck & Verbeken (2005) delineate a monophyletic section *Deliciosi*. Le et al. (2007) suggest polyphyly of sections *Zonarii* Quél. and *Piperites* Fr. within subgenus *Piperites*. The polyphyly of section *Piperites* was also suggested by Nuytinck et al. (2004).

Molecular phylogenetic delineation of morphologically defined species and investigation of their distribution using molecular methods have been carried out for only a fraction of *Lactarius* species, however recent studies have uncovered some interesting trends. Members of *Lactarius* section *Deliciosi* are easily recognized, however many taxa in this group have only subtle morphological differences and many nomenclatural problems exist. Furthermore, European names have often been used for taxa from locations such as Asia and Central America where the section is poorly studied, or for taxa whose sporocarps bear some resemblance to European specimens. Nuytinck et al. (2006a, b, 2007) and Nuytinck & Verbeken (2005) examined all known species in *Lactarius* section *Deliciosi*, sampled from boreal and temperate America, Europe, and Asia using the ITS region and part of the *gpd* gene. Their results show that section *Deliciosi* is monophyletic and nested within subgenus *Piperites*; their results also provided support for most of the morphologically-defined species analyzed. However, they also revealed taxonomic problems with the varieties of *L. deliciosus* (a species described from Europe) described from North America and suggest the need for detailed observations on macro- and micro-morphology to clearly delimit the North American taxa. The ITS and *gpd* datasets produced phylogenetic trees that supported mostly the same taxa, however the trees differed somewhat in their overall topology, and basal relationships, which were poorly resolved in both. They found that none of the North American samples with European names that were analyzed were phylogenetically conspecific with their European counterparts; overall, there was no intercontinental conspecificity between North American and Eurasian taxa. Their results also suggested low conspecificity between European and Asian members of the section (Nuytinck et al. 2007). Similarly, using ITS, LSU and *rpb2* sequences, Stubbe & Verbeken (2012) demonstrated no intercontinental conspecificity between Europe and Southern Asia and between Europe and North America in *Lactarius* subgenus *Plinthogalus*. Overall, intercontinental conspecificity has only been suggested for *L. controversus* (Nuytinck & Verbeken 2013), several boreal and arctic-alpine species in Geml et al. (2009), and several arctic-alpine species in Geml et al. (2012), although the latter two studies did not focus on taxonomy and the species were not well-defined.

A number of recent, detailed phylogenetic studies using molecular techniques have also focused on the recently proposed genus *Lactifluus* (Buyck et al. 2008, Stubbe et al. 2010, 2012, Van de Putte et al. 2010, 2012, De Crop et al. 2013), which appears to have a higher rate of molecular evolution than *Lactarius* (Verbeken & Nuytinck 2013). Stubbe et al. (2010), performed a phylogenetic analysis using ITS, LSU and *rpb*2 gene sequences from species in the *Lactifluus gerardii* complex, sampled from tropical to temperate America and Asia. They found no intercontinental conspecificity between North America and Asia and also indicated high cryptic species diversity within the complex. They suggest that the high frequency of sister relationships between North America and Asia points toward multiple migration and speciation events across the continents (Stubbe et al. 2010). Lack of intercontinental conspecificity was also recovered in the *Lactifluus piperatus* complex (De Crop et al. 2013) and high cryptic species diversity has been recovered in the *Lactifluus volemus* complex (Van de Putte et al. 2012).

These studies found separate American and Eurasian lineages for the tropical and temperate *Lactarius* and *Lactifluus* species examined and revealed contrasting rates of molecular evolution between the two genera. However, few studies (Geml et al. 2009, 2012) have used molecular techniques to investigate the distribution of arctic-alpine *Lactarius*, or test hypotheses regarding their intercontinental conspecificity and no studies have used molecular techniques to delineate species of *Lactarius* inhabiting alpine areas in the Rocky Mountains.

Lactarius in Arctic-Alpine Habitats

In reviewing a sample of pertinent literature (56 references), 70 *Lactarius* taxa have been reported from arctic-alpine areas, mostly through morphological species recognition (Table 1). Many taxa in this table have been synonymized and some taxonomic issues have been subsequently addressed, rendering some of the nomenclature outdated. However, besides showing distribution and ecological patterns, the purpose of this table is to provide a link to original reports of various *Lactarius* taxa, regardless of whether the nomenclature is still valid. The estimate of 70 taxa may be an overestimate of species diversity due to synonymies and also to nomenclatural misapplications, misidentifications, and the possibility of some reports actually coming from subalpine or subarctic areas, however it could also be argued that 70 taxa could be an underestimate, because of the potential for cryptic species diversity.

Table 1. *Lactarius* taxa reported from arctic-alpine habitats. References are listed below the table.

Taxa	**Arctic-alpine locations***										**Host ****
	AK	RM	CAN	GNLD	ICLD	BRIT ISL.	EUR ALPS	SCNDV	SVBD	N RUS	ALTAI	
L. alpigines										29		A
L. alpinus				9			6, 25, 26			31, 35		A
L. aquizonatus							6					D
L. aspideoides	42			32		55						D, S
L. aspideus	42							30, 36				Bd, S
L. aurantiacus				9, 32, 40, 53				6, 38				B, S
L. brunneo-hepaticus				9			2, 6, 25					A
L. brunneo-violaceus				9, 24	19, 24, 36		10, 24, 57	20, 24, 36	24		28	D, S
L. circellatus	15											
L. citriolens					19		38, 39					
L. controversus						55						S
L. dryadophilus	15		34, 47, 48	8, 9, 24, 34, 53	19, 34, 36		1, 6, 10, 24, 25, 26, 38, 39, 52,	20, 24, 34, 36, 38	16, 23	31	18, 28	D, S
L. dryadophilus v. *saliceticola*							25, 26					Sd
L. duplicatus				9								
L. flavidus				41								
L. flexuosus					19			30			17	
L. fuliginosus								30		31		B, S
L. fuscus				9, 32								Bd
L. glyciosmus		11, 12	47, 48	8, 9, 13, 32, 40, 41	19		6	6, 30, 38	16, 46	31		B, S
L. griseus				14								
L. groenlandicus				32, 40, 41, 51				6, 25				B, D, S
L. helvus						55		30		31		Bd
L. hysginus								30				
L. hysginoides				14	19							
L. kühneri							2					A
L. lacunarum						55	38			31		A, S
L. lanceolatus	24, 42, 43	12	24, 47, 48	9, 24		56		6, 24, 36	16, 21	24, 31, 35, 45		B, Sd
L. lapponicus				13				6		35		A, B
L. lepidotus							2, 6, 25, 26			29		A, S
L. lignyotus											17	

Table 1 Continued.

Taxa	**Arctic-alpine locations***											**Host ****
	AK	RM	CAN	GNLD	ICLD	BRIT ISL.	EUR ALPS	SCNDV	SVBD	N RUS	ALTAI	
L. luteus							6					Sd
L. mammosus					19			30				
L. mitissimus				41						45		
L. nanus	15	11, 12, 44	47, 48	9, 24, 32, 40	19, 36		2, 6, 7, 14, 24, 25, 38, 39, 49, 50	22, 24, 36, 38	20	45	31	S
L. necator				9, 13	19			30				B
L. obscuratus				9, 32			6, 25	30		29		A
L. obscuratus v. *radiatus*							25					
L. obscuratus v. *subalpinus*							26					
L. pallidus				37								
L. picinus					19							
L. pilatii				9								
L. pseudouvidus			47, 48	9, 24, 40	19, 24, 36		2, 6, 24, 25, 26, 38, 39	20, 22, 24, 36, 38	23, 24	31, 35, 45		Bd, Sd
L. pubescens	15, 42	11		9, 13, 32, 33, 40	19, 36			30, 36		31	16	B, S
L. pusillus				32								A
L. pyrogalus	42											B
L. radiatus f. *alnobetulae*								6				
L. repraesentaneus	15, 42	11, 12		8, 9, 13, 32, 54	19			6, 30, 38		31	17	B, Ss
L. robertianus							3, 5, 6, 25, 26, 50		23	35		S
L. rufus			47, 48	8, 9, 13, 32, 41		55		30, 36		31		B, S
L. salicis-herbaceae			47, 48	9, 24, 32, 40	19, 36		6, 10, 24, 25, 26, 38, 39	19, 23, 24, 38, 36		31, 35, 45		S
L. salicis-herbaceae v. *immutabilis*							4, 6, 38, 39					Sd
L. salicis-reticulatae		11, 12		9, 24, 40			1, 6, 24, 25, 26, 38, 39	20, 24, 36, 38		31, 45		B, S
L. scoticus					19					31		

Table 1 Continued.

Taxa	**Arctic-alpine locations***											**Host ****
	AK	RM	CAN	GNLD	ICLD	BRIT ISL.	EUR ALPS	SCNDV	SVBD	N RUS	ALTAI	
L. scrobiculatus								30				B
L. spinosulus					19							
L. subcircellatus	15, 42			9, 32	19, 36			6, 22, 24, 36		31		Bd
L. subdulcis				37								
L. subtorminosus				33								Bd
L. tabidus				9, 41	19			38			17	B, Sd
L. theiogalus				13, 32				30, 38	54			B, S
L. torminosulus			48	8, 9	19, 36			6, 36		31, 35	17	B
L. torminosus	42		47, 48	9, 32, 33	19, 36			30, 36, 38	16	31	17	B, S
L. torminosus v. *gracillimus*				41								B
L. trivialis				8, 9, 13				29, 38		31		B
L. utilis				8, 9, 13								B
L. uvidus				9, 32, 40, 41	19		2, 39	30, 38	46	31		S
L. uvidus f. *alpigines*							6, 25, 27, 45					Sd
L. vellereus					54							
L. vietus				8, 9, 13	19, 36			6, 30, 36, 38		31	17	B
L. violascens							14					Sd

*AK = Alaska, RM = Rocky Mountains, CAN = Canada, GNLD = Greenland, ICLD = Iceland, BRIT IS = British Isles, EUR ALP includes Alps, Pyrenees and Carpathians, SNDNV = Scandinavia, SVBD = Svalbard, N RUSS = northern Russia, ALTAI = Altai Mountains.

**Hosts: A = *Alnus*, B = *Betula*, Bd = dwarf *Betula*, D = *Dryas*, Pg = *Polygonum*, S = *Salix*, Sd = dwarf *Salix*, Ss = shrubby *Salix*

References: 1. Ballará 1997; 2. Bon 1985a; 3. Bon 1985b; 4. Bon 1989; 5. Bon 1991; 6. Bon 1998; 7. Bon & Ballará 1996; 8. Borgen 2006; 9. Borgen et al. 2006; 10. Corriol 2008; 11. Cripps & Horak 2008; 12. Cripps & Barge 2013; 13. Elbourne & Knudsen 1990; 14. Favre 1955; 15. Geml et al. 2009; 16. Geml et al. 2012; 17. Gorbunova 2010; 18. Gorbunova & Taylakov 2011; 19. Eyjolfsdottir pers. comm.; 20. Gulden 2005; 21. Gulden & Jenssen 1988; 22. Gulden et al. 1985; 23. Gulden & Torkelsen 1996; 24. Heilmann-Clausen et al. 1998; 25. Jamoni 1995; 26. Jamoni 2008; 27. Jamoni & Bon 1991; 28. Kalamees 2008; 29. Kalamees & Vaasma 1993; 30. Kallio & Kankainen 1964; 31. Karatygin et al. 1999; 32. Knudsen & Borgen 1982; 33. Knudsen & Borgen 1994. 34. Knudsen & Lamoure 1993; 35. Knudsen & Mukhin 1998; 36. Knudsen & Vesterholt 2008; 37. Kobayasi et al. 1971; 38. Kühner 1975; 39. Kühner & Lamoure 1986; 40. Lamoure et al. 1982; 41. Lange 1957; 42. Laursen & Ammirati 1982; 43. Miller & Laursen 1973; 44. Moser & McKnight 1987; 45. Nezdoiminogo 1997; 46. Ohenoja 1971; 47. Ohenoja & Ohenoja 1993; 48. Ohenoja & Ohenoja 2010; 49. Ronikier 2008; 50. Senn-Irlet 1993; 51. Terkelsen 1956; 52. Vila et al. 1997; 53. Watling 1977; 54. Watling 1983; 55. Watling 1987; 56. Watling et al. 2001.

Due to many changes in nomenclature through time, authorities were not included in the table, but they can be found in the original reports in the literature.

Some prominent studies cited in Table 1, which provided significant taxonomic information on *Lactarius* in arctic-alpine habitats include Cripps & Barge (2013) for the Rocky Mountains, Miller & Laursen (1973), Laursen & Ammirati (1982) and Geml et al. (2009) for Alaska, Ohenoja & Ohenoja (1993, 2010) for Canada, Lange (1957), Kobayasi et al. (1971), Knudsen & Borgen (1982), Lamoure et al. (1982), Elborne & Knudsen (1990), Knudsen & Lamoure (1993), Knudsen & Borgen (1994) and Borgen et al. (2006) for Greenland, Heilmann-Clausen et al. (1998) and Eyjolfsdottir (pers. comm.) for Iceland, Watling (1987, 2001) for the British Isles, Favre (1955), Kühner (1975), Bon (1985), Jamoni & Bon (1991), Senn-Irlet (1993) and Jamoni (1995, 2008) for the European Alps, Bon & Ballará (1996) for the Pyrenees, Ronikier (2008) for the Carpathians, Kallio & Kankainen (1964), Gulden et al. (1985), Heilmann-Clausen et al. (1998), Gulden (2005) and Knudsen & Vesterholt (2008) for Scandinavia, Gulden & Jenssen (1988) and Gulden & Torkelsen (1996) for Svalbard, Karatygin et al. (1998) and Knudsen & Muhkin (1998) for Northern Russia, and Kalamees (2008), Gorbunova (2010) and Gorbunova & Taylakov (2011) for the Altai Mountains.

There are taxonomic problems for several species complexes including the one comprised of *L. aurantiacus* (Pers.: Fr.) Gray (syn.: *L. mitissimus* (Fr.) Fr.), *L. lanceolatus* O.K. Mill. & Laursen, *L. luculentus* Burl., *L. luculentus* v. *laetus* Hesler & A. H. Sm., *L. subflammeus* Hesler & A. H. Sm., *L. substriatus* A. H. Sm. and *L. subviscidus* Hesler & A.

H. Sm. (Hesler & Smith 1979, Heilmann-Clausen et al. 1998). Another problematic complex includes *L. pubescens* Fr., *L. torminosus* (Schaeff.: Fr.) Pers. and *L. torminosulus* Knudsen & Borgen (Knudsen & Borgen 1994). In addition, there is considerable confusion surrounding certain names such as *L. theiogalus* (Idzerda & Noordeloos 1997), and some taxa (e.g. Friesian) which were described long ago, such as *L. glyciosmus* (Fr.) Fr. and *L. helvus* (Fr.) Fr. lack type specimens, making it impossible to study the original material (Heilmann-Clausen et al. 1998).

Furthermore, it has been suggested that some species such as *L. lanceolatus* and *L. torminosulus* reported as unique to the arctic-alpine biome may in fact be commonly encountered boreal or temperate species, which produce less pigmented, stunted or misshapen sporocarps in arctic-alpine areas due to harsher conditions (Gardes & Dahlberg 1996, Heilmann-Clausen et al. 1998). With the exception of one report of *Lactarius vellereus* from Iceland, which is now classified in *Lactifluus*, all *Lactarius* species reported from arctic-alpine areas belong in subgenera *Piperites, Russularia* or *Plinthogalus* (with the majority belonging to *Piperites* and *Russularia*) (Table 1) and are thus likely members of the genus *Lactarius* as currently recognized. However this has only been molecularly confirmed for a few of the taxa reported from arctic-alpine areas.

Host reports for *Lactarius* species collected in arctic-alpine areas are typically based on the proximity of *Lactarius* sporocarps to ECM plants such as *Alnus spp.*, *Betula glandulosa*, *B. nana*, *Dryas spp.*, and various dwarf and shrubby *Salix spp.* (Table 1). However, accurate identification of host plant based on sporocarp proximity is often difficult, due to heterogeneous patches of ECM plant species in these habitats. Molecular analyses of ECM root tips collected in arctic-alpine areas, however, have provided a way to unambiguously determine host-plant identity; *Lactarius* species have been confirmed on the roots of arctic-alpine plants *Betula nana* (Deslippe et al. 2011), *Dryas integrifolia* (Timling et al. 2012), *D. octopetala* (Bjorbækmo et al. 2010), *Salix herbacea* (Ryberg et al. 2011), *S. polaris* (Fujiyoshi et al. 2011), *S. reticulata* (Ryberg et al. 2009), *Kobresia spp.* (Gao & Yang 2010), and a *Polygonum* sp. (Unpublished, GenBank number JQ347149).

Traditionally, a core group of *Lactarius* are often cited as being restricted to arctic-alpine areas with various dwarf and shrubby *Salix* and/or *Dryas* species (Knudsen & Borgen 1982, Heilmann-Clausen et al. 1998, Basso 1999, Knudsen & Vesterholt 2008). However, most of the *Lactarius* species reported in arctic-alpine areas (Table 1) are also known from temperate or boreal areas with plants in the Betulaceae or Pinaceae as well as other families (Knudsen & Borgen 1982, Heilmann-Clausen et al. 1998, Basso 1999, Knudsen & Vesterholt 2008). These results parallel findings from recent molecular studies examining soil cores or ectomycorrhizal root tips such as Timling et al. (2014), where the most abundant fungi in their data set were not restricted to arctic-alpine areas.

Broad, intercontinental, circumpolar distributions are suggested for at least some taxa, most of which are well known in arctic-alpine habitats; for example, *L. dryadophilus* Kühner, *L. glyciosmus*, *L. lanceolatus*, *L. nanus* J. Favre, *L. pseudouvidus*

Kühner, *L. pubescens*, *L. repraesentaneus* Britzelm., *L. salicis-herbaceae* Kühner, *L. subcircellatus* Kühner and *L. torminosus* (Table 1). Based on ITS region data and 97% sequence similarity thresholds for species recognition, Geml et al. (2009, 2012) suggest intercontinental conspecificity between Svalbard and arctic Alaska and possibly between boreal or temperate North America and Svalbard for at least some *Lactarius* species, a pattern different than that observed by Nuytinck et al. (2007) and Stubbe et al. (2010) for *Lactarius* sampled from low to mid-latitudes and non-arctic-alpine habitats. Circumpolar distribution of host plant species in the Salicaceae and Betulaceae as well as long distance dispersal have been posited as reasons explaining the seemingly broad distribution of many arctic-alpine *Lactarius* species (Laursen & Ammirati 1982, Geml et al. 2012).

In North America, *Lactarius* species have been described and reported from arctic Canada and Alaska by Geml et al. (2009), Laursen & Ammirati (1982), Miller (1987), Miller & Laursen (1973), and Ohenoja & Ohenoja (1993, 2010). Laursen & Ammirati (1982) describe and report 10 *Lactarius* taxa from arctic Alaska: *L.* aff. *aspideoides* Burl., *L.* aff. *aspideus* (Fr.) Fr., *L. pubescens*, *L.* aff. *pubescens*, *L. repraesentaneus* Britz. sensu Neuhoff, *L. torminosus*, *L.* aff. *pyrogalus* (Fr.) Fr., *L. subcircellatus*, *L. lanceolatus* and *L.* aff. *lanceolatus*. Geml et al. (2009) analyzed ITS sequences from sporocarps and soil cores from boreal and arctic Alaska to investigate *Lactarius* diversity and ecology. They detected at least 43 putative phylogroups, and 38 distinct OTUs based on 97% ITS sequence similarity and these estimates were considered conservative, as species accumulation curves were non-asymptotic. Some OTUs matched identified sequences on GenBank, while many did not. Overall, *Lactarius* species showed strong habitat partitioning; a certain set occurred in arctic tundra, another set in black spruce forests, and another in mixed birch-aspen-white spruce forests. *Lactarius* diversity was higher in boreal sites than in arctic tundra (Geml et al. 2009). However, taxonomic issues were left unresolved, and broad distribution patterns of arctic-alpine *Lactarius* outside of polar regions were not investigated. To see the taxa reported from arctic-alpine areas of Alaska in this study see Table 1. From arctic Canada, Ohenoja & Ohenoja (1993, 2010) describe and report *L. dryadophilus*, *L. glyciosmus*, *L. lanceolatus*, *L. nanus*, *L. pseudouvidus*, *L. rufus* (Scop.) Fr., *L. salicis-herbaceae*, *L. torminosulus*, and *L. torminosus* (Table 1).

Rocky Mountain Alpine *Lactarius*

Cripps & Horak (2008) and Cripps & Barge (2013) previously reported six morphologically defined *Lactarius* species from the Rocky Mountain alpine zone: *Lactarius glyciosmus*, *L. lanceolatus*, *L. nanus*, *L. pubescens*, *L. repraesentaneus* and *L. salicis-reticulatae*; preliminary work has recognized an additional morphologically defined species identified tentatively as *L.* aff. *pseudouvidus* (*Lactarius pallidomarginatus* Barge & C.L. Cripps *ad int.*). Most of these taxa were originally described from Europe (except for *L. lanceolatus* which was described from Alaska) and records suggest most have a broad, intercontinental distribution (Table 1). The primary goals of this thesis are to 1) determine how many species of *Lactarius* occur in the Rocky

Mountain alpine zone, 2) determine if their identity based on morphological species recognition is concordant with phylogenetic species recognition, 3) investigate their infrageneric placement in light of phylogenetic analyses, 4) provide insight into their worldwide distribution and relative confinement to arctic-alpine environments and 5) provide insight into their specificity as to particular host plants. This will be achieved using traditional morphological species recognition techniques, molecular phylogenetic analyses of ITS and *rpb2* DNA using PAUP*(Swofford 2001), raxmlGUI (Stamatikis 2006, Silvestro & Michalak 2012) and MrBayes (Ronquist & Huelsenbeck 2003), the online molecular databases GenBank and Unite, and geographic and host plant identity data. Furthermore, detailed descriptions, drawings and images and a key to *Lactarius* species occurring in the Rocky Mountain alpine zone are provided, along with a discussion of their taxonomy, ecology and distribution in light of molecular phylogenetic analyses.

CHAPTER 2

SYSTEMATICS OF *LACTARIUS* IN THE ROCKY MOUNTAIN ALPINE ZONE

Introduction

Much progress has been made toward delineating and understanding broad patterns in arctic-alpine ectomycorrhizal fungal diversity, distribution and host specificity (Cripps & Horak 2008, Cripps et al. 2010, Geml et al. 2012, Timling et al. 2012, 2014, Dahlberg & Bültmann 2013, Larsson et al. 2014). However, species level taxonomy using a combination of traditional morphological and molecular phylogenetic techniques remains relatively poorly investigated, especially for isolated alpine regions such as the Rocky Mountain alpine zone (Osmundson et al. 2005, Cripps & Horak 2008, 2010, Beker et al. 2010, Cripps et al. 2010, Kasuya 2010, Larsson et al. 2014). These kinds of studies are important for inferring the evolution of arctic-alpine fungi, examining and understanding their biodiversity, biogeography and ecology, as well as for building an accurate molecular database for recognizing fungal species from both environmental samples and sporocarps.

The arctic-alpine zone covers roughly 8% of the Earth's land and consists of the vegetated areas beyond the altitudinal or climatic limit of tree growth (Bliss 1988, Chapin & Körner 1995, Körner 1999). The Rocky Mountain alpine zone extends southward from the true arctic and exists as a chain of scattered, high elevation islands of tundra (Billings 1988). From a mycological perspective, arctic-alpine areas are of particular interest as they are climatically very harsh and devoid of trees, yet they contain a significant diversity of ectomycorrhizal fungi that associate with dwarf *Betula*, shrubby and dwarf *Salix*, *Bistorta*, *Kobresia* and *Dryas* (e.g. Cripps & Eddington 2005). Furthermore, climate warming is facilitating the expansion of shrubs in arctic-alpine areas, which highlights the potential importance of ectomycorrhizal fungi in this process (Deslippe et al. 2011).

Many plant and ectomycorrhizal fungal species that inhabit the arctic-alpine zone appear to have broad distributions, occurring throughout the arctic as well as in northern hemisphere alpine, subalpine, boreal or temperate areas outside the arctic (Bliss 1956, Lange 1957, Johnson & Billings 1962, Kallio & Kankainen 1964, Moser 1982, Gardes & Dahlberg 1998, Borgen et al. 2006, Geml et al. 2012, Timling et al. 2014). Although only a few reports have actually confirmed broad intercontinental distrubutions of some ectomycorrhizal fungal species in arctic-alpine habitats with detailed taxonomic examination and molecular phylogenetic analyses (Peintner 2008, Cripps et al. 2010, Larsson et al. 2014). Numerous regional endemic plants and fungi are reported as well, especially in more southern alpine areas (Billings 1978, Takhtajan 1986, Hadley 1987, Cripps & Horak 2008).

One genus of ectomycorrhizal fungi which is abundant and diverse in arctic-alpine areas is *Lactarius* (Table 1). Reports of *Lactarius* species from arctic-alpine areas include the Rocky Mountain alpine zone of North America (Cripps & Horak 2008, Cripps & Barge 2013), Alaska (Laursen & Ammirati 1982, Geml et al. 2009), Canada (Ohenoja

1993, 2010), Iceland (Heilmann-Clausen et al. 1998, Eyjolfsdottir pers. comm.), Greenland (Lange 1957, Kobayasi et al. 1971, Knudsen & Borgen 1982, Lamoure et al. 1982, Elbourne & Knudsen 1990, Knudsen & Lamoure 1993, Knudsen & Borgen 1994, Heilmann-Clausen et al. 1998, Borgen et al. 2006), the British Isles (Watling 1987, 2001), the European Alps (Favre 1955, Kühner 1975, Bon 1985a, b, Jamoni & Bon 1991, Senn-Irlet 1993, Jamoni 1995, 2008), the Pyrenees (Bon & Ballará 1996), the Carpathians (Ronikier 2008), Scandinavia (Kallio & Kankainen 1964, Gulden et al. 1985, Heilmann-Clausen et al. 1998, Gulden 2005, Knudsen & Vesterholt 2008), Svalbard (Gulden & Jenssen 1988, Gulden & Torkelsen 1996), Northern Russia (Karatygin et al. 1999, Knudsen & Muhkin 1998), and the Altai Mountains (Kalamees 2008, Gorbunova 2010, Gorbunova & Taylakov 2011), yet none of these (except for Geml et al. 2009, 2012) have contributed molecular phylogenetic data.

Putative host plants for *Lactarius* in arctic-alpine areas include various *Alnus* spp., *Betula glandulosa*, *B. nana*, *Dryas* spp., and various dwarf and shrubby *Salix* spp. (Table 1). Ectomycorrhizal root tip sequencing of plants in arctic-alpine areas has confirmed *Lactarius* species on the roots of *Betula nana* (Deslippe et al. 2011), *Dryas integrifolia* (Timling et al. 2012), *D. octopetala* (Bjorbækmo et al. 2010), *Salix herbacea* (Ryberg et al. 2011), *S. polaris* (Fujiyoshi et al. 2011), *S. reticulata* (Ryberg et al. 2009), *Kobresia* spp. (Gao & Yang 2010), and a *Polygonum* sp. (Unpublished, GenBank number JQ347149).

From sporocarp surveys, many *Lactarius* species from arctic-alpine areas appear to have broad, intercontinental distributions (Table 1). These data also suggest that some species may be restricted to arctic-alpine areas with dwarf and shrubby *Salix* and/or *Dryas* species (Knudsen & Borgen 1982, Heilmann-Clausen et al. 1998, Basso 1999, Knudsen & Vesterholt 2008), however, the majority of species are also known from outside the arctic-alpine zone with plants in the Betulaceae or Pinaceae as well as other plant families (Knudsen & Borgen 1982, Heilmann-Clausen et al. 1998, Basso 1999, Knudsen & Vesterholt 2008). Using 97% ITS region similarity cutoffs for recognizing species, environmental sequencing studies have also shown broad, intercontinental ranges, with little confinement to arctic-alpine habitats for arctic-alpine inhabiting ectomycorrhizal fungi, including *Lactarius* (Geml et al. 2012, Timling et al. 2012, 2014). However, 97% ITS region similarity cutoffs for species recognition may fail to differentiate some species (Nilsson et al. 2008), thus inflating their ranges. Interestingly, recent molecular phylogenetic studies of *Lactarius* sampled from lower-latitude areas have shown narrow species ranges and a lack of intercontinental conspecificity (Nuytinck et al. 2007, Stubbe & Verbeken 2012).

In the Rocky Mountain alpine zone of North America, Cripps & Horak (2008) and Cripps & Barge (2013) previously reported six morphologically defined *Lactarius* species: *Lactarius glyciosmus*, *L. lanceolatus*, *L. nanus*, *L. pubescens*, *L. repraesentaneus*, and *L. salicis-reticulatae*; preliminary work has recognized an additional morphologically defined species identified tentatively as *L.* aff. *pseudouvidus* (*L. pallidomarginatus* Barge

& C.L. Cripps *ad int.*). Most of these taxa were originally described from Europe (except for *L. lanceolatus* which was described from Alaska) and records suggest most have broad, intercontinental distributions (Table 1). However, morphologically defined species can harbor more than one genetically distinct species (Geml et al. 2006, 2008, Taylor et al. 2006), but these species' distributions have not been rigorously tested in a molecular phylogenetic context. Furthermore, there are taxonomic problems for certain arctic-alpine *Lactarius* species groups such as the complex surrounding *L. lanceolatus*, which aslso includes *L. aurantiacus*, (syn.: *L. mitissimus*), *L. subflammeus*, *L. substriatus*, and *L. subviscidus* (Hesler & Smith 1979, Heilmann-Clausen et al. 1998), and the delineation between *L. pseudouvidus* and *L. brunneoviolaceus* (syn.: *L. robertianus*) is unclear (Heilmann-Clausen et al. 1998, Basso 1999).

This study investigates morphological and molecular systematics of arctic-alpine members of the genus *Lactarius*, with a focus on collections from above tree-line in the central and southern Rocky Mountain alpine zone. The primary goals of this thesis are to 1) determine the number of *Lactarius* species that occur in the Rocky Mountain alpine zone, 2) determine if their identity based on morphological species recognition is concordant with molecular data, 3) investigate their infrageneric placement in light of molecular phylogenetic analyses, 4) provide insight into their worldwide distribution and relative confinement to arctic-alpine environments and 5) provide insight into their host associations and specificity to particular host plants.

Materials and Methods

Study Sites

The Rocky Mountain alpine zone occurs as scattered, high elevation islands on mountaintops in the U.S.A. and Canada from the Brooks Range in Northern Alaska to the Sangre de Cristo Range in northern New Mexico (Billings 1988). This study was performed in alpine areas of the Central and Southern Rocky Mountains as classified by Brouillet and Whetstone (1993). Central Rocky Mountain sites are located above treeline on the Beartooth Plateau in Southern Montana and Northern Wyoming and include: Birch Site, Highline Trail, Frozen Lakes, Gardner Lake and Solifluction Terraces (Table 2, Figure 1). Southern Rocky Mountain sites are all located above treeline in Colorado and include: Blue Lake, Brainard Lake and Loveland Pass in the Front Range; Cottonwood Pass and Independence Pass in the Sawatch Range; Black Bear Pass, Cinnamon Pass, Imogene Pass, Maggie Gulch and Stony Pass in the San Juan Range (Table 2, Figure 1).

Published climate data is somewhat scarce for alpine sites in the Rockies. For the Beartooth Plateau, Johnson and Billings (1962) report a July mean temperature of 48.5° F (9.2° C), a mid-June through August weakly average precipitation of 0.31 inches (0.79 cm) and a mid-June through August mean wind velocity of 11.3 miles per hour (18.2 km per hour) at 10,300 feet above sea level (3,139 meters above sea level) all in 1959; the highest temperatures occur between mid-July and mid-August and low temperatures can be experienced year-round. Annual mean temperature, precipitation and wind velocity are not published for alpine areas of the Beartooth Plateau. Although Niwot Ridge in Colorado is considerably further south, the climate is considered similar to that of the Beartooth Plateau (Cooper et al. 1997) as well as other Colorado sites. Niwot ridge experiences low temperatures year-round as well as regular high winds. Precipitation is typically brought in by an upper westerly flow, although in winter and spring, significant precipitation can be delivered by easterly upslope flows. Maximum precipitation occurs in the spring, however, significant precipitation can also occur with the southwestern summer monsoon (Greenland 1989). The Niwot Ridge Long-Term Ecological Research Site (http://niwot.colorado.edu/site_info/climate /climate.html) reports an annual mean temperature of 25.34° F (-3.7° C), a July mean temperature of 46.8° F (8.2° C) and a mean annual precipitation of 36.61 inches (93 cm) at 12,280 feet above sea level (3,743 meters above sea level).

Overall, granites are the most abundant rock type, however the geology is complex and basalt, andesite, rhyolite, shale, limestone, sandstone and quartzite are locally abundant as well (Retzer 1956).

Known ectomycorrhizal plants in the Central and Southern Rocky Mountain alpine zone include *Betula glandulosa* Michx., *Dryas octopetala* L., *Bistorta vivipara* (L.) Delarbre, *Salix arctica* Pall., *S. glauca* L., *S. planifolia* Pursh, *S. reticulata* L. and *Kobresia myosuroides* (Vill.) Fiori (Lesica & Antibus 1985, Schadt 2002, Cripps & Eddington 2005, Cripps & Horak 2006b). A few other *Salix* species present in the Central and Southern Rocky Mountain alpine zone are also likely ectomycorrhizal, but this has not yet been confirmed. In general, the same ectomycorrhizal host plants occur at the various study sites, however a greater abundance of *Salix glauca* was encountered at sites in Colorado and only one isolated *Betula glandulosa* shrub was encountered on the Beartooth Plateau, while *B. glandulosa* was observed at several alpine to subalpine sites in Colorado.

Table 2. Locations and elevations of the 15 Rocky Mountain alpine sites investigated in this study.

Site	**State**	**Range**	**Latitude, Longitude**	**Elevation**
Birch Site	MT	Beartooth	45° 01.444 N, 109° 24.486 W	2990-3020 m
Highline Trail	MT	Beartooth	45° 00.356 N, 109° 24.387 W	3060-3100 m
Frozen Lakes	WY	Beartooth	44° 57.932 N, 109° 28.998 W	3190-3200 m
Gardner Lake	WY	Beartooth	44° 57.949 N, 109° 27.199 W	3030 m
Solifluction Terraces	WY	Beartooth	44° 58.372 N, 109° 26.810 W	3258 m
Blue Lake	CO	Front	40° 05.400 N, 105° 37.200 W	3560 m
Brainard Lake	CO	Front	40° 04.200 N, 105° 34.200 W	3300 m
Loveland Pass	CO	Front	39° 39.600 N, 105° 52.800 W	3650 m
Cottonwood Pass	CO	Sawatch	38° 49.200 N, 106° 24.000 W	3700 m
Independence Pass	CO	Sawatch	39° 06.600 N, 106° 33.600 W	3650 m
Black Bear Pass	CO	San Juan	37° 24.000 N, 107° 42.000 W	3900 m
Cinnamon Pass	CO	San Juan	37° 55.800 N, 107° 31.800 W	3840 m
Imogene Pass	CO	San Juan	37° 55.800 N, 107° 43.200 W	3900 m
Maggie Gulch	CO	San Juan	37° 51.000 N, 107° 34.200 W	3600 m
Stony Pass	CO	San Juan	37° 46.800 N, 107° 31.800 W	3700 m

Taxon Sampling and Processing

Sporocarps were collected at the alpine sites described above from late July to late August, from 1997 to 2014. All collections were described in detail when fresh, and select collections were photographed. Ectomycorrhizal host plants in close proximity to sporocarps were noted for each collection. Sporocarps were dried on an electric, warm-air dryer and deposited in the Montana State University herbarium (MONT), or the

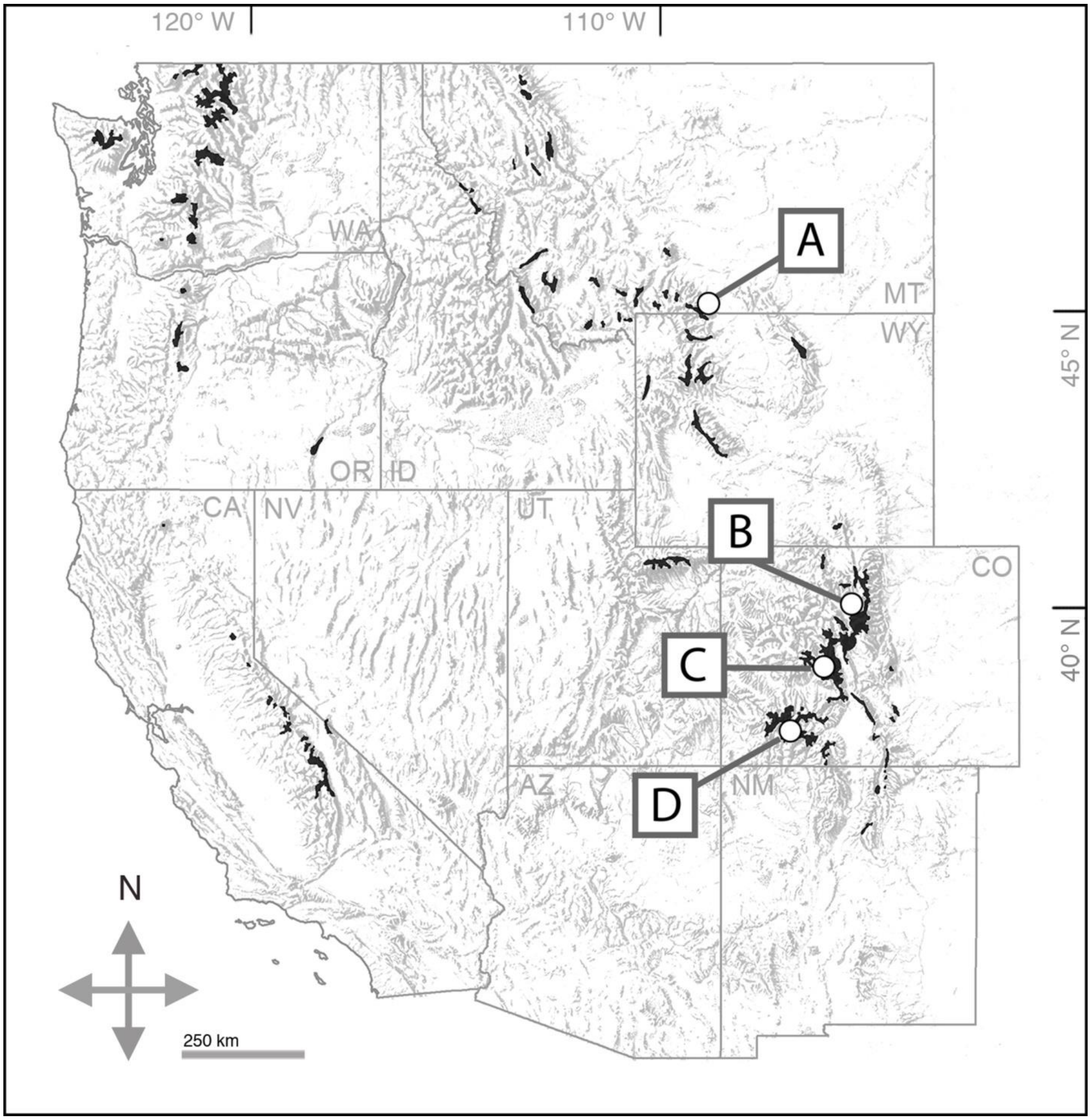


Figure 1. Map illustrating the distribution of alpine areas (shaded black) in the western USA according to Kuchler (1964) and the locations of study sites. A: Beartooth Plateau (Birch Site, Highline Trail, Frozen Lakes, Gardner Lake and Solifluction Terraces), B: Front Range (Blue Lake, Brainard Lake, Loveland Pass), C: Sawatch Range (Cottonwood Pass, Independence Pass) and D: San Juan Range (Black Bear Pass, Cinnamon Pass, Imogene Pass, Maggie Gulch, Stony Pass). The alpine vegetation layer of Kuchler (1964) was downloaded at http://databasin.org/datasets/1c7a301c8e6843f2b4fe63fdb3a9fe39, the map was generated in ArcMap 9.2 (ESRI 2009) and edited in Adobe Photoshop CS6 (Adobe Systems Inc.) by E. Barge.

Institute of Integrative Biology, ETH, Zurich, Switzerland (ZT). Collections were identified to species based on morphology using various keys (Jamoni 1995, 2008, Bon 1998, Heilmann-Clausen et al. 1998, Basso 1999). The literature was consulted and herbarium specimens of type collections, putative conspecific and taxonomically similar species were obtained from the herbarium of Universität Innsbruck, Innsbruck, Austria (IB), the Herbarium Universitatis Tartuensis, Tartu, Estonia (TU), the Botanical Museum, Oslo, Norway (O), Herbarium GB, University of Gothenburg, Göteborg, Sweden (GB), the herbarium of Åbo Akademi University, Turku, Finland (TURA), the Mycological Herbarium of the Natural History Museum of Denmark, Copenhagen, Denmark (C), the herbarium of Université de Lille, France (LIP), the University of Michigan fungal herbarium, Ann Arbor, Michigan (MICH), and the Denver Botanic Gardens' Sam Mitchel Herbarium of Fungi, Denver, Colorado (DBG). The type collections of *Lactarius pseudouvidus* Kühner, *L. salicis-reticulatae* Kühner and *L. nanus* J. Favre were obtained from the herbarium of the Conservatoire et Jardin botaniques de la Ville de Genève, Geneva, Switzerland (G). The type collection of *L. lanceolatus* O.K. Mill. & Laursen was obtained from Massey Herbarium, Virginia Tech, Blacksburg, Virginia (VPI) and is now at the herbarium of the New York Botanical Garden (NY). Herbarium acronyms follow Thiers http://sweetgum.nybg.org/ih/ (continuously updated). The collection locations of specimens that were examined and (or) included in phylogenetic analyses in this study are shown in relation to Rocky Mountain alpine study sites in Figure 2.

Morphological Descriptions

Descriptions of macromorphological features were made from fresh and a few dry sporocarps. Micromorphological descriptions were made from dried material for several representative collections of each putative species and for the corresponding type specimens (when available). Specimens whose morphological features were examined are shown in Table 3 denoted by superscript 1 after each taxon. Dried material was reconstituted in ethanol and thin sections were prepared. Spores were examined in Melzer's reagent (0.5 g iodine, 1.5 g potassium iodide, 20 mL dH_2O, 2mL chloral hydrate) to test for an amyloid reaction; basidia, macrocystidia and pileipellis were examined in 2.5% potassium hydroxide (KOH). Length and width was measured with an ocular micrometer under a 100X oil-immersion lens (1000X magnification) for a random sample of 25 basidiospores per collection, excluding ornamentation, the hilar appendix and giant spores from 2-spored basidia; mean spore length and width were calculated for each collection. The length to width ratio (Q) was calculated for each basidiospore, and mean length to width ratio (Q_m) was calculated for each collection. The maximum height of the spore ornamentation was measured and recorded and the ornamentation was measured and recorded and the ornamentation was described following Heilmann-Clausen et al. (1998). Length and width (at widest point) were measured under a 40X lens (400X magnification) for a random sample of ten basidia (including sterigmata and excluding 2-spored basidia), ten pleuromacrocystidia and ten cheilomacrocystidia per collection. *Lactarius* macrocystidia are defined as hymenial

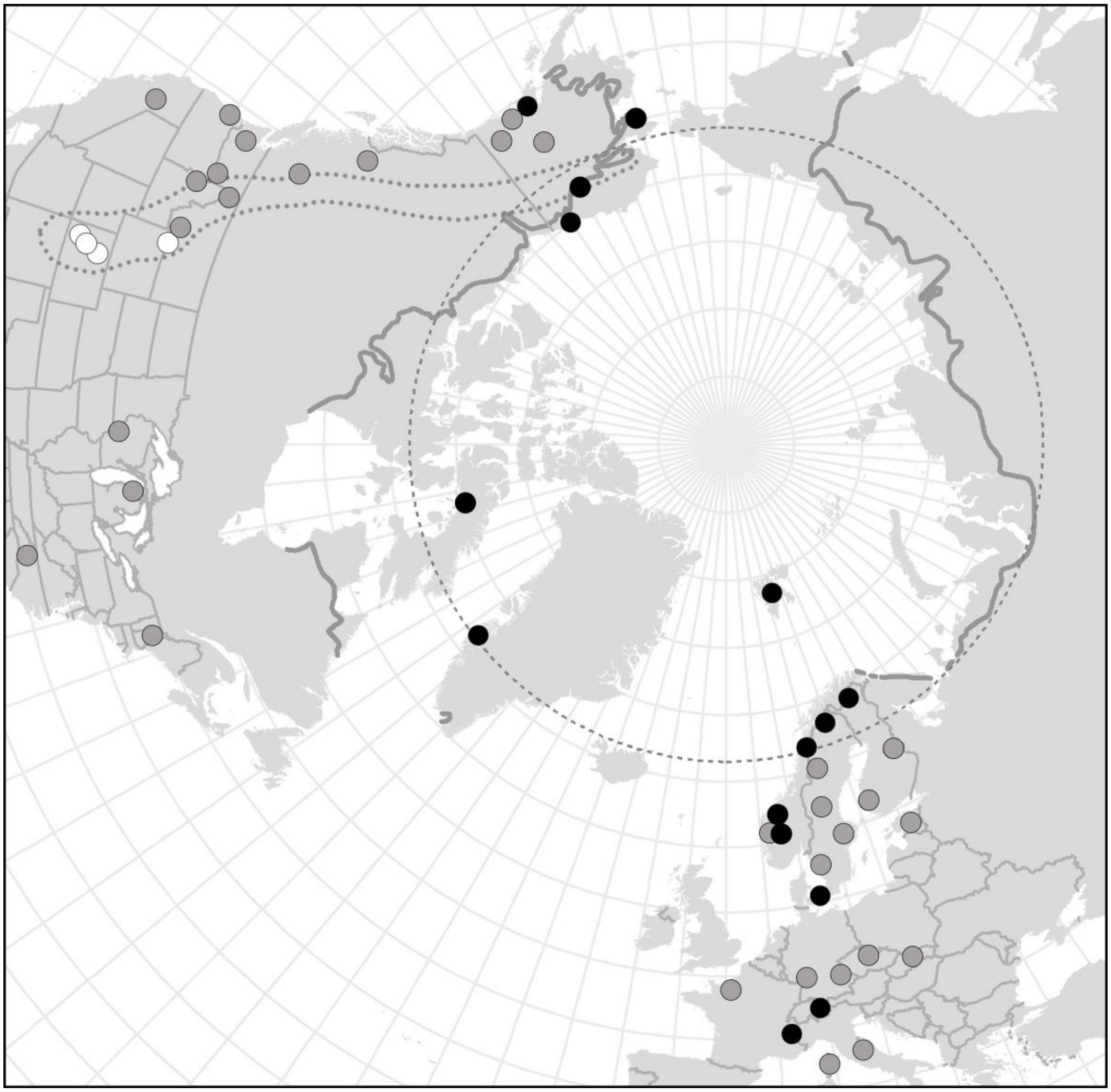


Figure 2. Map illustrating the collection locations of specimens which were examined and (or) included in molecular analyses (if collection location known). Rocky Mountain alpine study sites are indicated by white dots; black dots indicate other arctic, subarctic or alpine sites; and gray dots indicate subalpine sites. Several collections from outside of the map area are not shown. The dotted line delineates the Rocky Mountains; the arctic-circle is indicated by the dashed line; and the northern tree line which corresponds with the southern extent of arctic tundra is indicated by the dark gray line (Brown et al. 2001). The northern tree line data layer was downloaded at ftp://sidads.colorado.edu/pub/DATASETS/ fgdc/ggd318_map_ circumarctic, the map was generated in ArcMap 9.2 (ESRI 2009) and edited in Adobe Photoshop CS6 (Adobe Systems Inc.) by E. Barge.

cells that are thin-walled, generally fusiform to conical in shape, with an obtuse to acute to moniliform apex, possessing guttulate, needle-like or granular contents and basal septa (Heilmann-Clausen et al. 1998). Pseudocystidia are differentiated from macrocystidia in that they are directly connected with the lactiferous system and thus lack basal septa (Heilmann-Clausen et al. 1998). Paracystidia have basal septa but are differentiated from macrocystidia in that they lack guttulate, needle-like or granular contents and have a more rounded apex (Heilmann-Clausen et al. 1998). Drawings of basidiospores, pleuromacrocystidia and cheilomacrocystidia were prepared from one representative collection of each morphological species as well as for their corresponding type specimen (when available) using a Leica drawing tube attached to a Leica DMLS research microscope.

DNA Extraction

Rocky Mountain alpine *Lactarius* specimens collected in this study, their corresponding type specimens (when available), putative conspecific taxa from a broad geographic area, and morphologically similar taxa were subjected to molecular methods. Dried tissue was ground to a fine powder by placing a small (roughly 1 g), clean piece of dried sporocarp tissue into a 2.0 mL screw cap microcentrifuge tube, adding several 5 mm glass beads, and beating for 10 to 20 sec. with a Mini-beadbeater (Biospec Products) set to the lowest speed setting or using a Retsch Mixer Mill MM300 (Qiagen) set at 30 beats/sec. for 30 sec., repeating once or twice if necessary to ensure powdering of tissue. Ground tissue was then centrifuged down. DNA was extracted

Table 3. Specimens whose morphological features were examined and/or were used in phylogenetic analyses. Type specimens are bolded. **Yes** is bolded in the ITS or *rpb2* collum for sequences generated in this study. Superscripts after taxa: 1 = morphological features examined; 2 = in broad phylogenetic analysis; A = in phylogenetic analysis of clade A; B = in phylogenetic analysis of clade B; C = in phylogenetic analysis of clade C. Herbarium acronynms follow Thiers http://sweetgum.nybg.org/ih/ (continuously updated)

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. akahatsu^{2}	JN2004-141 (GENT)	NA	Thailand	NA	KF133269	KF133333
L. aspideoides^{1,2,C}	RL Shaffer 6957 (MICH)	10 Aug 1973	Emmet Co., MI, U.S.A.	Beach-maple forest	**Yes**	NA
L. atroviridis^{2}	AV05-306 (GENT)	NA	U.S.A.	NA	KF133270	KF133334
L. aurantiacus^{1}	JHC97-251 (C)	16 Oct 1997	Broby Vesterskov, Denmark	*Picea*	NA	NA
L. aurantiacus^{A}	Hue179 (TUB)	19 Oct 1998	Baden-Württemberg, Germany	*Picea*	UDB000312	NA
L. aurantiacus^{A}	A. Pergolini 2090	24 Sept 1997	Italy	NA	JF908299	NA
L. aurantiacus^{A}	TU106115 (TU)	10 Oct 2007	Võru, Estonia	NA	UDB011687	NA
L. aurantiacus^{1}	JHC00-057 (C)	11 Sept 1999	Leikanger, Norway	*Betula* and *Corylus*	NA	NA
L. aurantiacus^{1,2,A}	JV94-422 (C)	8 Sept 1994	Uppland, Sweden	*Picea*, *Populus*, *Betula*	**Yes**	**Yes (partial)**
L. auriolla^{2}	RW1601 (GENT)	NA	Sweden	NA	KF133257	KF133321
L. brunneohepaticus^{2,A}	PAM08090315 (LIP)	NA	Corse-du-Sud, France	*Alnus alnobetula* subsp. *suaveolens*	HQ714726	HQ714858
L. brunneoviolaceus^{1,,C}	JV28448F (TURA)	30 Aug 2011	Koillismaa, Finland	*Picea abies*, *Betula*, *Salix*	NA	**Yes**
L. aff. *brunneoviolaceus*^{1,C}	CLC2133 (MONT)	10 Aug 2005	Finse, Norway	Alpine *Salix*	**Yes**	**Yes**
L. camphoratus^{2}	UE04.09.2004-5 (UPS)	NA	Sweden	NA	DQ422009	DQ421933
L. chrysorrheus^{2}	UE04.10.2002-8 (UPS)	NA	Italy	NA	KF133261	KF133325
L. citriolens^{2}	UE20.09.2004-03 (UPS)	NA	Sweden	NA	DQ422003	DQ421931
L. cyathuliformis^{2,A}	PAM08100409 (LIP)	NA	Orne, France	*Alnus glutinosa*	HQ714738	HQ714869
L. deliciosus^{2}	JN2001-046 (GENT)	NA	Slovakia	NA	KF133272	KF133337
L. dryadophilus^{1,C}	CLC2729 (MONT)	5 Aug 2011	AK, U.S.A.	NA	**Yes**	**Yes**
L. dryadophilus^{C}	E. Cautero 1195	27 Aug 1997	Italy	NA	JF908292	NA

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. dryadophilus[1,2,C]	EL57-10 (GB)	14 Aug 2010	Latnjavagge, Sweden	Alpine *Salix reticulata*, *Dryas octopetala*	**Yes**	**Yes**
***L. dryadophilus* (Holotype)**[1]	K 67-17 (G)	30 July 1967	Hordaland, Norway	NA	NA	NA
L. dryadophilus[1,C]	CLC2744 (MONT)	7 Aug 2011	Palmer Creek Road, AK, U.S.A.	Alpine *Salix reticulata*	**Yes**	**Yes**
L. flavopalustris[C]	TU118356 (TU)	6 Sept 2011	Võru, Estonia	*Salix*	UDB015606	NA
L. flavopalustris[2,C]	TU118426 (TU)	4 Sept 2012	Võru, Estonia	*Salix*	UDB017888	NA
L. flavopalustris[C]	TU101514 (TU)	3 Sept 2009	Nord-Trondelag, Norway	*Picea*, *Betula*, *Corylus*, *Ulmus*	UDB019705	NA
L. flavopalustris[1,2,C]	JV23334 (TURA)	31 Aug 2005	Koillismaa, Finland	*Picea, Pinus*, *Betula*, *Alnus*	**Yes**	**Yes**
L. flavopalustris[C]	TU101619 (TU)	30 Aug 2007	Koillismaa, Finland	*Betula*, *Salix*, *Alnus*, *Picea*	UDB011119	NA
L. flexuosus[2]	UE06.09.2002-1 (UPS)	NA	Sweden	NA	DQ421992	DQ421925
L. formosus[2,C]	LTH382 (CMU)	16 Oct 2005	Thailand	*Castanopsis armata*, *Pinus kesiya*	EF141549	NA
L. fuscus[B]	E. Campo 14652	28 Aug 2002	Italy	NA	JF908316	NA
L. glyciosmus[B]	OUC99130 (DAVFP)	NA	British Columbia, Canada	Interior Cedar Hemlock zone	DQ097872	NA
L. glyciosmus[1,B]	TU118535 (TU)	17 Sept 2012	Saare, Estonia	Grassland	UDB017965	NA
L. glyciosmus[1,B]	TU106737 (TU)	12 Sept 2010	Tartu, Estonia	Mixed forest	UDB015747	NA
L. glyciosmus[1,B]	TU106724 (TU)	13 Sept 2010	Tartu, Estonia	Mixed forest	UDB015740	
L. glyciosmus[B]	E. Cautero 1315	2 Oct 1997	Italy	NA	JF908294	NA
L. glyciosmus[B]	O73706	NA	Svalbard	Arctic tundra	GU234027	NA
L. glyciosmus[1,2,B]	M. Moser 19810148 (IB)	9 Aug 1981	Femsjö, Sweden (Type locality)	*Betula*	**Yes**	NA
L. glyciosmus[1,B]	M. Moser 19780234 (IB)	16 Aug 1978	Femsjö, Sweden (Type locality)	*Betula*	**Yes**	NA
L. glyciosmus[1,B]	M. Moser 19780191 (IB)	13 Aug 1978	Femsjö, Sweden (Type locality)	*Betula*, *Salix*	**Yes**	NA
L. glyciosmus[B]	UP559	NA	Uppsala, Sweden	Mixed forest	EF493307	NA
L. glyciosmus[1]	CLC1134 = ZT6096 (MONT)	29 July 1997	Birch Site, Beartooth Plateau, MT, U.S.A.	Alpine *Betula glandulosa*	NA	NA

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. glyciosmus[1]	CLC1217 (MONT)	8 Aug 1998	Birch Site, Beartooth Plateau, MT, U.S.A.	Alpine *Betula glandulosa*	NA	NA
L. glyciosmus[1]	CLC1380 (MONT)	19 Aug 1999	Birch Site, Beartooth Plateau, MT, U.S.A.	Alpine *Betula glandulosa*	NA	NA
L. glyciosmus[1,2,B]	TWO269 (MONT)	10 Aug 1999	Birch Site, Beartooth Plateau, MT, U.S.A.	Alpine *Betula glandulosa*	**Yes**	**Yes**
L. glyciosmus[1,B]	ZT12723 (MONT)	13 Aug 2007	Birch Site, Beartooth Plateau, MT, U.S.A.	Alpine *Betula glandulosa*	**Yes**	NA
L. glyciosmus[1,B]	EB133 (MONT)	13 Aug 2012	Ironton Park, San Juan Range, CO, U.S.A.	*Betula glandulosa*	**Yes**	**Yes**
L. glyciosmus[1,2,B]	CLC1624 (MONT)	2 Aug 2001	Blue Lake, Front Range, CO, U.S.A.	Alpine *Betula glandulosa*	**Yes**	**Yes**
L. glyciosmus[1,B]	20923 (DBG)	13 Aug 2000	Brainard Lake, Front Range, CO, U.S.A.	Alpine *Salix*, *Betula*	**Yes**	**Yes**
L. glyciosmus[B]	JMP0043 (UWL)	NA	West Salem, WI, U.S.A.	Temperate forest	EU819484	NA
L. glyciosmus[1,2,B]	CLC2874 (MONT)	27 Aug 2012	Fjell Ailigas, Finland	Alpine *Betula nana*	**Yes**	**Yes**
L. helvus[2]	UE08.09.2004-1 (UPS)	NA	Sweden	NA	KF133263	KF133327
L. hysginoides[B]	TU101865	22 A ug 2011	Koillismaa, Finland	Mixed forest	UDB016019	NA
L. hysginoides[1,2,B]	JV28432 (TURA)	28 Aug 2011	Koillismaa, Finland	*Picea abies*, *Betula*	**Yes**	**Yes**
L. lanceolatus[1,A]	JV15112F (TURA)	14 Aug 1999	Nordland, Norway	Alpine *Salix*, *Betula nana*	**Yes**	**Yes**
L. lanceolatus[1,2,A]	CLC1885 (MONT)	18 Aug 2003	Longyearbyen, Svalbard	Arctic *Salix polaris*	**Yes**	**Yes**
***L. lanceolatus* (Holotype)** [1,2,A]	F4239 (VPI)	30 July 1971	Beaufort Lagoon, AK, U.S.A.	Mesic tundra	**Yes**	**Yes**
L. lanceolatus[1,A]	CLC2743 (MONT)	7 Aug 2011	Palmer Creek Road, AK, U.S.A.	Alpine *Salix spp.*	**Yes**	**Yes**
L. lanceolatus[1,A]	CLC1389 (MONT)	20 Aug 1999	Highline Trail, Beartooth Plateau, MT, U.S.A.	Alpine *Salix reticulata*	**Yes**	**Yes**
L. lanceolatus[1]	CLC1578 (MONT)	20 July 2001	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	Alpine *Salix reticulata*	NA	NA

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. lanceolatus[1,A]	CLC2319 (MONT)	13 Aug 2007	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	Alpine *Salix reticulata*	**Yes**	**Yes**
L. lanceolatus[1,A]	CLC2358 (MONT)	5 Aug 2008	Highline Trail, Beartooth Plateau, MT, U.S.A.	Alpine *Salix reticulata*	**Yes**	**Yes**
L. lanceolatus[1,2,A]	EB105-13 (MONT)	17 Aug 2013	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	Alpine *Salix planifolia*, *S. arctica*, *S. reticulata*	**Yes**	**Yes**
L. lanceolatus[A]	O73608	NA	Svalbard	NA	GU234010	NA
L. lanceolatus[A]	IA-F20	3 Sept 1998	Svalbard	Arctic *Salix polaris*, *Dryas octopetala*	UDB002454	NA
L. cf. *lanceolatus*[1]	ZT6214 (MONT)	1 Aug 1997	Highline Trail, Beartooth Plateau, MT, U.S.A.	Alpine dwarf and shrubby *Salix*	NA	NA
L. cf. *lanceolatus*	ZT6412 (MONT)	7 Aug 1998	Highline Trail, Beartooth Plateau, MT, U.S.A.	*Salix* spp.	NA	NA
L. lepidotus[2]	PAM08090304 (LIP)	NA	Corse-du-Sud, France	*Alnus alnobetula* subsp. *suaveolens*	HQ714722	HQ714854
L. lignyotus[2]	UE06.09.2003-5 (UPS)	NA	Sweden	NA	DQ421993	DQ421926
L. lilacinus[2]	EDB08101401 (LIP)	NA	France	*Alnus glutinosa*	HQ714748	HQ714879
L. luculentus[A]	F15192 (UBC)	NA	British Columbia, Canada	NA	DQ384582	NA
L. luculentus[1,A]	AH Smith 79943 (MICH)	14 Nov 1970	Polk Co., OR, U.S.A. (Near type locality)	NA	**Yes**	NA
L. luculentus[1,A]	AH Smith 90905 (MICH)	11 Oct 1980	Tucannon River, WA, U.S.A.	Conifers	**Yes**	NA
L. luculentus v. *laetus*[A]	SMI221 (UBC)	NA	British Columbia, Canada	Sub-boreal spruce zone	FJ845419	NA
L. luculentus v. *laetus*[1,2,A]	DBG-F-024643 (DBG)	23 Aug 2008	Larimer Co., CO, U.S.A. (Near type locality)	*Abies*, *Picea*	**Yes**	**Yes**
L. luculentus v. *laetus*[1,A]	DBG-F-022653 (DBG)	27 Aug 2011	El Paso., Co., CO	*Picea, Populus*	**Yes**	**Yes**
***L. luculentus* v. *laetus* (Paratype)**[1]	AH Smith 84923 (MICH)	13 Aug 1974	Perigo, Front Range, Gilpin Co., CO, U.S.A.	Spruce-pine woods	NA	NA

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. luridus[2,C]	OB11-011 (GENT)	NA	Belgium	NA	KF241547	NA
L. luridus[C]	TU106140 (TU)	11 Sept 2007	Saare, Estonia	NA	UDB011691	NA
L. mammosus[B]	TU106314 (TU)	28 Sept 2008	Hiiu, Estonia	Pine forest	UDB011464	NA
L. mammosus[B]	TU106617 (TU)	10 Sept 2009	Võru, Estonia	NA	UDB015711	NA
L. mammosus[2,A,B]	UE09.09.2004-5 (UPS)	NA	Sweden	NA	KF133265	KF133329
L. mammosus[B]	GG415-86	NA	Svalbard	Arctic tundra	GU234062	NA
L. mitissimus[A]	ue179	NA	Germany	*Picea abies*	AF157412	NA
***L. montanus* (paratype)**[1,C]	AH Smith 81954 (MICH)	14 Sept 1972	Bonner County, ID, U.S.A.	Conifers and *Betula*	**Yes**	NA
L. montanus[1,2,C]	EB120-13 (MONT)	3 Sept 2013	Spanish Peaks, Gallatin Range, MT, U.S.A.	*Picea engelmanni, Abies lasiocarpa*	**Yes**	**Yes**
L. montanus[1,C]	CLC3001 (MONT)	3 Sept 2013	Tobacco Root Mountains, MT, U.S.A.	Conifers, *Salix planifolia*	**Yes**	**Yes**
***L. nanus* (Holotype)**[1]	ZA192c (G)	20 Aug 1943	Grisons, Switzerland	Alpine *S. herbacea*	NA	NA
L. nanus[1,B]	CLC1403 (MONT)	21 Aug 1999	Frozen Lakes, Beartooth Plateau, WY, U.S.A.	*Salix arctica*	**Yes**	**Yes**
L. nanus[1,B]	EB125 (MONT)	11 Aug 2012	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	Alpine *Salix planifolia*, *S. arctica*, *S. reticulata*	NA	**Yes**
L. nanus[1]	CLC1221 (MONT)	7 Aug 1998	Highline Trail, Beartooth Plateau, MT, U.S.A.	Alpine shrubby and dwarf *Salix*	NA	NA
L. nanus[1]	ZT6422 (MONT)	8 Aug 1998	Highline Trail, Beartooth Plateau, MT, U.S.A.	Alpine *Salix reticulata* and *Salix* spp.	NA	NA
L. nanus[1,2,B]	EB106-13 (MONT)	18 Aug 2013	Highline Trail, Beartooth Plateau, MT, U.S.A.	Alpine *Salix planifolia*, *S. arctica*, S. *reticulata*	**Yes**	**Yes**
L. nanus[1]	ZT9529 (MONT)	11 Aug 2001	Black Bear Pass, San Juan Range, CO, U.S.A.	Alpine *Salix reticulata*	NA	NA

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. nanus[1,B]	CLC1716 (MONT)	11 Aug 2001	Black Bear Pass, San Juan Range, CO, U.S.A.	Alpine *Salix arctica*	**Yes**	**Yes**
L. nanus[1,B]	CLC1801 (MONT)	27 July 2002	Cinnamon Pass, San Juan Range, CO, U.S.A.	Alpine *Salix arctica*	**Yes**	**Yes**
L. nanus[1,B]	EB138 (MONT)	15 Aug 2012	Cinnamon Pass, San Juan Range, CO, U.S.A.	Alpine *Salix arctica*	**Yes**	**Yes**
L. nanus[1,2,B]	CLC2134 (MONT)	5 Aug 2010	Finse, Norway	Alpine *Salix spp.*	**Yes**	**Yes**
L. nanus[1,B]	CLC1829 (MONT)	29 July 2002	Imogene Pass, San Juan Range, CO, U.S.A.	Alpine *Salix arctica*	**Yes**	**Yes**
L. nanus[1,2,B]	CLC1471 (MONT)	6 Aug 2000	Independence Pass, Sawatch Range, CO, U.S.A.	NA	**Yes**	**Yes**
L. nanus[1,B]	Bon 89093 (LIP)	24 Aug 1989	Savoie, France (Type locality)	Alpine prairie	**Yes**	NA
L. nanus[1,B]	JV15148 (TURA)	15 Aug 1999	Torne Lappmark, Sweden	Alpine heath	**Yes**	**Yes**
L. nanus[1,B]	CLC1896 (MONT)	20 Aug 2002	Near Longyearbyen, Svalbard	Arctic *Salix polaris*, *Dryas octopetala*	**Yes**	**Yes**
L. nanus[B]	O73576	NA	Svalbard	Arctic tundra	GU234019	NA
L. cf. *nanus*[B]	GAL 8739 (GAL)	NA	Nome, AK, U.S.A.	Arctic tundra	FJ607374	NA
L. necator[2]	AV04-231 (GENT)	NA	France	NA	KF133276	KF133341
L. pallescens[C]	OUC97047 (DAVFP)	NA	British Columbia, Canada	Interior Cedar Hemlock forest	DQ093852	NA
***L. pallescens* (Holotype)**[1,2,C]	AH Smith 81936 (MICH)	12 Sept 1972	Boundary County, ID, U.S.A.	Conifers	**Yes**	NA
L. pallidomarginatus Barge & C.L. Cripps *ad int*[1,C]	CLC1470 (MONT)	6 Aug 2000	Independence Pass, Sawatch Range, CO, U.S.A.	Alpine *Salix spp.*	**Yes**	**Yes**
L. pallidomarginatus Barge & C.L. Cripps *ad int*[1,2,C]	EB0041 (MONT)	13 Aug 2011	Stony Pass, San Juan Range, CO, U.S.A.	Alpine *Salix planifolia*	**Yes**	**Yes**
L. pallidomarginatus Barge & C.L. Cripps *ad int* [1]	ZT9093 (MONT)	9 Aug 2000	Independence Pass, Sawatch Range, CO, U.S.A	Alpine *Salix planifolia*, *S. glauca*	NA	NA
L. pallidomarginatus Barge & C.L. Cripps *ad int* [1]	ZT5229 (MONT)	22 Aug 1994	Union Peak, Wind River Range, WY, U.S.A.	Alpine *Salix glauca*	NA	NA
L. pseudouvidus[1,C]	U. Peintner 20070035 (IB)	3 July 2007	Tyrol, Austria (Near type locality)	Alpine *Polygonum, Salix*	NA	**Yes**
L. pseudouvidus[1,,C]	JV10468 (TURA)	16 Aug 1995	Inarin Lappi, Finland	Alpine *Salix*, *Betula*	**Yes**	**Yes**

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
***L. pseudouvidus* (Holotype)**[1]	Va 7 1971 (G)	13 Aug 1971	Isère, France	Alpine	NA	NA
L. pseudouvidus[1,C]	E. Søyland 73867 (O)	11 Aug 2011	Ulvik, Norway	NA	**Yes**	**Yes**
L. pseudouvidus[1,C]	P. Larsen 361395 (O)	26 Aug 2006	Stordal, Norway	Arctic-alpine *Salix reticulata*, *Dryas*, *Betula*	**Yes**	**Yes**
L. pseudouvidus[1,2,C]	EL101-11 (GB)	4 Aug 2011	Latnjajaure, Sweden	Alpine *Salix herbacea*	**Yes**	**Yes**
L. pseudouvidus[1,C]	EL63-10 (GB)	17 Aug 2010	Latnjavagge, Sweden	Alpine *Salix herbacea*	**Yes**	**Yes**
L. pseudouvidus[1,C]	TWO809 (MONT)	18 Aug 2002	Svalbard	Arctic-alpine	**Yes**	**Yes**
L. aff. *pseudouvidus*[1,2,C]	CLC1910 (MONT)	20 Aug 2002	Svalbard	Arctic *Salix polaris*	**Yes**	**Yes**
L. pubescens[2]	UE15.09.2002-2 (UPS)	NA	Sweden	NA	DQ421996	DQ421929
L. quieticolor[2]	UE10.09.2004-1 (UPS)	NA	Sweden	NA	DQ422002	DQ421930
L. quietus[2]	UE16.09.2004 (UPS)	NA	Sweden	NA	KF133264	KF133328
L. repraesentaneus[C]	NA	NA	Baffin Island, Canada	Arctic *Dryas integrifolia*	JX630967	NA
L. repraesentaneus[C]	TU106146 (TU)	13 Sept 2007	Saare, Estonia	NA	UDB011701	NA
L. repraesentaneus[C]	TU106676 (TU)	30 Aug 2009	Saare, Estonia	NA	UDB015723	NA
L. repraesentaneus[C]	Nl1180 (TUB)	4 Oct 1987	Germany	Mixed forest	UDB000367	NA
L. repraesentaneus[C]	D. & M. Antonini 12511	31 July 1998	Italy	NA	JF908312	NA
L. repraesentaneus[1,C]	CLC2318 (MONT)	13 Aug 2007	Birch Site, Beartooth Plateau, MT, U.S.A.	Alpine shrubby *Salix*	**Yes**	**Yes**
L. repraesentaneus[1,2,C]	EB107-13 (MONT)	18 Aug 2013	Birch Site, Beartooth Plateau, MT, U.S.A.	*Salix glauca*, *S. arctica*, krummholz *Picea engelmannii*	**Yes**	**Yes**
L. repraesentaneus[1,C]	EB0048 (MONT)	16 Aug 2011	Cottonwood Pass, Sawatch Range, CO, U.S.A.	*Salix glauca*, krummholz *Picea engelmannii*	**Yes**	**Yes**
L. repraesentaneus[1,C]	JV21671 (TURA)	16 Aug 2004	Enontakiö, Finland	Alpine *Betula nana*	**Yes**	**Yes**
L. repraesentaneus[1,2,C]	CLC1747 (MONT)	14 Aug 2001	Independence Pass, Sawatch Range, CO, U.S.A.	Alpine *Salix glauca*	**Yes**	**Yes**

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. repraesentaneus[1,C]	EL92-07 (GB)	12 Aug 2007	Latnjavagge, Sweden	Alpine *Dryas octopetala*	**Yes**	**Yes**
L. repraesentaneus[C]	JN 2001-023 (GENT)	NA	Sweden	Mixed forest	AY331011	NA
L. repraesentaneus[1,2,C]	JV13837F (TURA)	9 Aug 1998	Varsinais-Suomi, Finland	*Picea*, *Pinus*, *Betula*, *Ulmus*	**Yes**	**Yes**
L. robertianus[1]	PAM930831 (LIP)	31 Aug 1993	Bourg-Saint-Maurice, France (Near type locality)	Alpine *Salix herbacea*	NA	NA
L. robertianus[1,C]	U. Peintner 20040156 (IB)	20 Aug 2004	Trentino, Italy (Near type locality)	Alpine dwarf *Salix*, *Dryas*, *Polygonum*	**Yes**	**Yes**
L. romagnesii[2]	UE29.09.2006-6 (UPS)	NA	France	NA	DQ421989	DQ421923
***L. salicis-herbaceae* (Holotype)**[1]	K71-89 (G)	22 Aug 1971	Savoie, France	Alpine *Salix herbacea*	NA	NA
L. salicis-herbaceae[1,2,C]	CLC1536 (MONT)	19 Aug 2000	Sismiut, Greenland	Arctic *Salix herbacea*	**Yes**	**Yes (partial)**
***L. salicis-reticulatae* (Holotype)**[1]	K72-104 (G)	12 Aug 1972	Mt. Laktatjäkko, Sweden	Alpine *Salix spp.*, *Dryas*	NA	NA
L. salicis-reticulatae[1,C]	CLC1211 (MONT)	8 Aug 1998	Highline Trail, Beartooth Plateau, MT, U.S.A.	Alpine dwarf *Salix spp.*	**Yes**	NA
L. salicis-reticulatae[1,2,C]	CLC2776 (MONT)	17 Aug 2011	Birch Site, Beartooth Plateau, MT, U.S.A.	Alpine *Salix reticulata*	**Yes**	**Yes**
L. salicis-reticulatae[1,C]	EB0057-14 (MONT)	16 Aug 2014	Gardner Lake, Beartooth Plateau WY, U.S.A.	Alpine shrubby *Salix*	**Yes**	**Yes**
L. salicis-reticulatae[1,C]	CLC2885 (MONT)	28 Aug 2012	Near Utsjoki, Finland	NA	**Yes**	**Yes**
L. salicis-reticulatae[1,C]	CLC2745 (MONT)	7 Aug 2011	Palmer Creek Road, Alaska, U.S.A.	Alpine *Salix reticulata*	**Yes**	**Yes**
L. salicis-reticulatae[1,2,C]	JV15133 (TURA)	15 Aug 1999	Mt. Laktatjäkko, Sweden (Type locality)	Alpine *Salix*	**Yes**	**Yes**
L. aff. *salicis-reticulatae*[1,C]	CLC1710 (MONT)	10 Aug 2001	Cinnamon Pass, San Juan Range, CO, U.S.A.	Alpine *Salix reticulata*	**Yes**	**Yes**
L. aff. *salicis-reticulatae*[1,C]	CLC1741 (MONT)	13 Aug 2001	Independence Pass, Sawatch Range, CO, U.S.A.	Alpine *Salix spp.*	**Yes**	**Yes**
L. aff. *salicis-reticulatae*[1,2,C]	EB0036 (MONT)	11 Aug 2011	Maggie Gulch, San Juan Range, CO, U.S.A.	Alpine *Salix planifolia*	**Yes**	**Yes**

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
L. aff. *salicis-reticulatae*[1,C]	CLC1689 (MONT)	9 Aug 2001	Stony Pass, San Juan Range, CO, U.S.A.	Alpine *Salix spp.*	**Yes**	**Yes**
L. aff. *salicis-reticulatae*[1,C]	EB0039 (MONT)	13 Aug 2011	Stony Pass, San Juan Range, CO, U.S.A.	Alpine *Salix planifolia*	**Yes**	**Yes**
L. speciosus[2,C]	A. Verbeken04/206 (GENT)	17 July 2005	North Carolina, U.S.A.	NA	EF141547	NA
L. sphagneti[2,A]	PL2805 (pers. herb. P. Leonard)	NA	United Kingdom	NA	KF133268	KF133332
L. spinosulus[2,B]	AT2003068 (UPS)	NA	Sweden	NA	KF133262	KF133326
L. subdulcis[2,A,B,C]	JV2006-024 (GENT)	NA	Belgium	NA	KF133279	KF133344
***L. subflammeus* (Holotype)**[1,2,A]	AH Smith 83602 (MICH)	9 Nov 1972	Tillamook County, OR, U.S.A.	Sand dunes	**Yes**	NA
L. subsericatus[2,A]	UE11.10.2004-8 (UPS)	NA	Sweden	NA	DQ422011	DQ421934
L. substriatus[A]	src438 (UC), (OSC)	NA	Yuba County, CA, U.S.A.	*Quercus douglasii*	DQ974746	NA
L. substriatus[1,A]	AH Smith 83693 (MICH)	12 Nov 1972	Tillamook Co., OR, U.S.A.	NA	**Yes**	NA
L. substriatus[1,A]	AH Smith 83694 (MICH)	12 Nov 1972	Tillamook Co., OR, U.S.A.	NA	**Yes**	NA
***L. subviscidus* (Paratype)**[1,A]	AH Smith 83066 (MICH)	30 Oct 1972	Lewis Co., WA, U.S.A.	On rotten conifer logs	**Yes**	NA
***L. subviscidus* (Paratype)**[1,A]	AH Smith 83331 (MICH)	1 Nov 1972	Lewis Co., WA, U.S.A.	On rotten conifer logs	**Yes**	NA
L. thyinos[2]	A. Voitk23-08-2004 (GENT)	NA	Canada	NA	KF133271	KF133336
L. torminosus[2]	RW3183 (GENT)	NA	Czech Republic	NA	KF133281	KF133346
L. trivialis[B]	UP575 (UPS)	NA	Jamtländ, Sweden	Mixed forest	EF493308	NA
L. trivialis[2]	UE27.08.2002-17a (UPS)	NA	Sweden	NA	DQ421991	DQ421924
L. uvidus[2,C]	mh0963 (TUB)	NA	Germany	Mixed forest	AY606957	NA
L. uvidus[C]	KVP10-027 (GENT)	NA	Russia	NA	KF241546	NA
L. uvidus[B]	O73621	NA	Svalbard	Arctic tundra	GU234147	NA
L. vietus[2]	UE11.19.2004-1 (UPS)	NA	Sweden	NA	KF133267	KF133331
L. violascens[2,C]	TU118591 (TU)	14 Sept 2012	Saare, Estonia	*Quercus*	UDB017998	NA
Lactarius sp.[A]	GAL13852 (GAL)	NA	AK, U.S.A.	NA	EU711569	NA
Lactarius sp.[A]	GAL18572 (GAL)	NA	AK, U.S.A.	Maritime forest	EU711607	NA
Lactarius sp.[B]	NA12 P31 I22	NA	AK, U.S.A.	Arctic tundra	FJ607382	
Lactarius sp.[C]	GAL15392 (GAL)	NA	Fairbanks, AK, U.S.A.	Upland boreal forest	EU711582	NA
Lactarius sp.[C]	GAL14139 (GAL)	NA	Nome, AK, U.S.A.	Arctic tundra	FJ607367	NA

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
Lactarius sp.^{C}	GAL14850 (GAL)	NA	Toolik Lake, AK, U.S.A.	Arctic tundra	FJ607373	NA
Lactarius sp.^{C}	GAL18580 (GAL)	NA	Valdez, AK, U.S.A.	Maritime forest	EU711609	NA
Lactarius sp.^{C}	NA	NA	Svalbard	Arctic tundra	HQ215798	NA
Environmental sample^{A}	NA	NA	AK, U.S.A.	Boreal forest	EU292558	NA
Environmental sample^{A}	NA	NA	China	Alpine *Kobresia* sp.	JQ347147	NA
Environmental sample^{A}	NA	NA	Norway	Alpine *Dryas octopetala*	HQ445527	NA
Environmental sample^{A}	NA	NA	Norway	Alpine *Dryas octopetala*	HQ445510	NA
Environmental sample^{A}	NA	NA	Svalbard	Arctic tundra	JF304414	NA
Environmental sample^{B}	NA	NA	Bartlett, NH, U.S.A.	Temperate forest	HQ021853	NA
Environmental sample^{B}	NA	NA	Bonanza Creek, AK, U.S.A.	Boreal forest	EU711624	NA
Environmental sample^{B}	NA	NA	Bonanza Creek, AK, U.S.A.	Boreal forest	EU711685	NA
Environmental sample^{B}	NA	NA	Bonanza Creek, AK, U.S.A.	Boreal forest	EU711622	NA
Environmental sample^{B}	NA	NA	Bonanza Creek, AK, U.S.A.	Boreal forest	EU711635	NA
Environmental sample^{B}	NA	NA	Finland	*Pinus sylvestris*	AJ633116	NA
Environmental sample^{B}	NA	NA	Sweden	*Picea abies*	FM992937	NA
Environmental sample^{B}	NA	NA	Sweden	NA	AY097042	NA
Environmental sample^{B}	NA	2 July 2008	Hokkaido, Japan	*Betula platyphylla*	AB669497	NA
Environmental sample^{B}	NA	2 June 2011	ID, U.S.A.	*Alnus rhombifolia* or *Betula occidentalis*	JX198526	NA
Environmental sample^{B}	NA	NA	Svalbard	Arctic tundra	JF304415	NA
Environmental sample^{B}	NA	July 2007	Toolik Lake, AK, U.S.A.	Arctic *Betula nana*	GU998223	NA
Environmental sample^{B}	NA	July 2007	Toolik Lake, AK, U.S.A.	Arctic *Betula nana*	GU998695	NA
Environmental sample^{B}	NA	NA	AK, U.S.A.	NA	JF304344	NA
Environmental sample^{B}	NA	NA	China	Alpine *Kobresia* sp.	JQ347141	NA
Environmental sample^{C}	NA	NA	Italy	NA	JX625292	NA
Environmental sample^{C}	NA	Aug 2008	Ny-Alesund, Svalbard	Arctic *Salix polaris*	AB588960	NA

Table 3 Continued.

Taxon	**Voucher**	**Date**	**Location**	**Host or Habitat**	**ITS**	***rpb2***
Environmental sample^{C}	NA	NA	Svalbard	Arctic tundra	JF304416	NA
Lactifluus piperatus^{2}	UE09.08.2004-6 (UPS)	NA	Sweden	NA	DQ422035	DQ421937
Lactifluus volemus^{2}	90804-5 (GENT)	NA	Sweden	NA	JN388959	JN375612
Multifurca furcata^{2}	RH7804 (NY)	NA	Costa Rica	NA	DQ421994	DQ421927
M. ochricompacta^{2}	BB02.107 (PC)	NA	U.S.A.	NA	DQ421984	DQ421940
Russula camarophylla^{2}	PAM01081108 (PC)	NA	France	NA	DQ421982	DQ421938
R. nigricans^{2}	UE20.09.2004-07 (UPS)	NA	Sweden	NA	DQ422010	DQ421952

from dried sporocarp tissue using the method of Wang et al. (1993) or using the DNeasy Plant Mini Kit (Qiagen). Using the method of Wang et al. (1993), discussed in Osmundson et al. (2013), 495 µL of 100 mM TRIS pH 8.0 was added to 1.5 mL microcentrifuge tubes and set aside. Next, 200 µL of 0.5 M NaOH was added to the 2.0 mL screw cap tubes containing the ground tissue. These tubes were then vortexed for 30 sec. to 1 min. to ensure mixing of ground tissue and NaOH solution and not allowed to sit for more than 10 min. Within 10 min. 5 µL of this solution (containing the extracted DNA) was added to the TRIS solution and stored at -30° C, or quickly used in downstream applications. When using the DNeasy Plant Mini Kit, the manufacturer's instructions were followed except that final elutions of 30 µL were made for type specimens and for older or poorly preserved specimens instead of the protocol 100 µL. This was done to increase the final concentration of eluted DNA.

PCR Amplification of the ITS Region

PCR amplification (Mullis et al. 1986, Saiki et al. 1988) of the ITS region was performed using primers ITS1-F and ITS4 (White et al. 1990, Gardes & Bruns 1993). Primer sequences are: ITS1-F (CTTGGTCATTTAGAGGAAGTAA); ITS4 (TCCTCCGCTTATTG ATATGC) (Integrated DNA Technologies). Two different PCR reaction mixes were used. The first method was carried out at the Matteo Garbelotto lab at UC Berkeley following a protocol used by Dr. Todd Osmundson (pers. comm.). The second method was carried out in the mycology lab at Montana State University. The reaction mix for the first method consisted of 9.8µL sterile ddH_2O, 5 µL 5X buffer, 2.5 µL dNTP's, 2.5 µL BSA, 2 µL $MgCl_2$, 1 µL ITS1-F (10 µM), 1 µL ITS4 (10 µM), 0.2 µL Taq and 1 µL template DNA per well. The reaction mix for the second method consisted of 9.5 µL sterile ddH_2O, 1 µL ITS1-F (10 µM), 1 µL ITS4 (10 µM), 12.5 µL REDtaq ReadyMix (Sigma-Aldrich), and 1 µL template DNA per well. The thermocycler program used for both PCR reaction mixes was 94° C for 2 min., followed by 30 cycles of 94° C for 30 sec., 55° C for 1 min. and 72° C for 1 min.; followed by a final elongation step of 72° C for 5 min (Osmundson pers. comm.).

When the previous methods failed (often for old herbarium specimens), PCR amplification of the ITS region was performed using two sets of primers: ITS1-F – ITS2 (GCTGCGTTCTTCATCGATGC) and ITS3 (GCATCGATGAAGAACGCAGC) – ITS4 (Integrated DNA Technologies) (White et al. 1990) with one 25 µL reaction per primer pair being performed. The reaction mixture was the same as for the second method described above except 3-4 µL of template DNA and 6.5-7.5 µL ddH$_2$O was used. The thermocycler program used for older material began at 94° C for 85 sec., followed by 35 cycles of varying conditions. The first 13 cycles consisted of 95° C for 35 sec., 55° C for 55 sec., and 72° C for 45 sec. Cycles 14-26 and 27-35 were the same except that the extension (72° C) steps were lengthened to 120 and 180 sec, respectively (Gardes & Bruns 1993, Larsson & Jacobsson 2004).

PCR Amplification of the Region Between Conserved Domains 6 and 7 of the *rpb2* Gene

PCR amplification of the region between conserved domains 6 and 7 of the second largest subunit of the RNA polymerase II (*rpb2*) gene was performed using degenerate primers bRPB2-6f (TGGGGYATGGTNTGYCCYGC) and fRPB2-7cR (CCCATRGCTTGYTTRCCCAT) (Integrated DNA Technologies) (Liu et al. 1999, Matheny 2005). The PCR reaction mixture consisted of 5.5 µL sterile ddH$_2$O, 3 µL bRPB2-6f (10 µM), 3µL fRPB2-7cR (10 µM), 12.5 µL REDtaq ReadyMix, and 1 µL template DNA per well. The thermocycler program consisted of 94° C for 90 sec. followed by 40 cycles of 94° C for 30 sec., 55° C for 90 sec. and 68° C for 3 min., concluding with a 5 min. final extension at 68° C (Osmundson pers. comm.).

When the previous method failed (often for old herbarium specimens), PCR amplification of the region between conserved domains 6 and 7 of the second largest subunit of the RNA polymerase II (*rpb2*) gene was performed using two sets of primers: bRPB2-6f – pri2r-RPB2 (ACVGTYTCYTCTTCYTCRGCRTC) (pri2r-RPB2 is a slightly modified version of i2r-RPB2 from Stubbe et al. 2010) and fRPB2-7cR - ilf-RPB2 (GCARAARAAGCACGTDMGGTGGC) (Integrated DNA Technologies) (Stubbe et al. 2010) with one 25 µL reaction per primer pair being performed. The reaction mixtures and thermocycler programs used were the same as in the previous paragraph. Modifications to i2r-RPB2 (ACVGTTTCCTCTTCTTCRGCRTC) were made by examining an alignment of *Lactarius* subgenus *Piperites* and *Russularia rpb2* sequences extracted from GenBank and adjusting the primer sequence by replacing positions 6 (=T), 9 (=C) and 15 (=T) with Y.

Visualization, Purification and Quantification of PCR Products

PCR products were visualized on 1.5% agarose gels with 0.003% ethidium bromide. Prior to sequencing, PCR products were purified by adding 7 µL of PCR product, 0.5 µL ExoSAP-IT (USB, USA) and 1.5 µL sterile ddH_2O to a 0.2 mL tube. Tubes were placed in a thermocycler at 37° C for 15 min., followed by 80° C for 15 min. Alternatively, PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's instructions. The DNA concentration in purified PCR products was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

DNA Sequencing

DNA sequencing was performed either at the University of California Berkeley or at the Berkeley Sequencing Facility (http://mcb.berkeley.edu/barker/dnaseq /home). At the University of California Berkeley, the cycle sequencing reaction was performed using the ABI Prism BigDye v.3.1. cycle sequencing kit (Applied Biosystems, Foster City, CA). The reaction mix consisted of 5.7 µL sterile ddH_2O, 1.8 µL 5X BigDye sequencing buffer, 1.0 µL primer (1 µM), 0.5 µL BigDye v.3.1 and 1.0 µL cleaned PCR product. Separate reactions were assembled for the forward (ITS1-F or bRPB2-6f) and reverse (ITS4 or fRPB2-7cR) primers. The thermocycler was run using the following program: 1) 25 cycles of 96° C for 10 sec.; 50° C for 5 sec.; 60° C for 4 min. and 2) Hold at 4° C until plate is removed. Sequencing products were cleaned with magnetic beads using the Agencourt CleanSeq system (Agencourt Bioscience Corporation), following the manufacturer's instructions. Sequencing products were analyzed using an ABI 3130xl capillary sequencer (Applied Biosystems, Foster City, CA). Alternatively, samples were sent to the Berkeley Sequencing Facility where sequencing was performed. Prior to shipment to the facility, template DNA and primer solutions were prepared following the facility's instructions, except that twice the recommended amount of primer was added when using degenerate primers. Separate reactions were assembled for the forward and reverse primers.

Sequence chromatograms were examined and reverse complements of the ITS2, ITS4, pri2r-RPB2 and fRPB2-7cR primed sequences were generated using either GeneiousPro (Drummond et al. 2009) or SeqTrace v. 0.8.1 (Stucky 2012). Forward and reverse-complemented reverse sequences were aligned and edited to produce contig consensus sequences using either GeneiousPro (Drummond et al. 2009) or SeqTrace v.

0.8.1 (Stucky 2012). Poor nucleotide calls at the 5' and 3' ends of sequences were trimmed, and ambiguous nucleotide calls throughout the sequences were replaced with N's. Positions with unambiguous (no more than 2) overlapping peaks were coded as W (A or T), S (C or G), M (A or C), K (G or T), R (A or G), or Y (C or T). In total, 79 ITS sequences and 66 *rpb2* squences were generated (Table 3, Appendix D).

Rational for Multiple Phylogenetic Analyses

Phylogenetic analyses were performed on four separate datasets in order to address different questions and to overcome ITS sequence alignment problems.

Broad Phylogenetic Analyses. As mentioned previously, recent molecular phylogenetic analyses have shown that *Lactarius* Pers. and *Russula* Pers. as historically treated are paraphyletic and that Russulaceae is composed of four main clades (genera): *Russula, Multifurca*, *Lactarius* and *Lactifluus* (Buyck et al. 2008, 2010, Stubbe et al. 2010, Van de Putte et al. 2012). The morphological identity of Rocky Mountain alpine taxa collected in this study places them in *Lactarius* subgenera *Piperites* and *Russularia* as recognized by Heilmann-Clausen et al. (1998) and thus in the genus *Lactarius* as recognized by Buyck et al. (2008, 2010). In order to confirm their placement in the genus *Lactarius* as currently recognized and to investigate infrageneric phylogenetic relationships, molecular phylogenetic analyses were carried out on a dataset which included one to two representatives of each (six) Rocky Mountain alpine *Lactarius* morphological species, as well as taxa from *Multifurca*, *Lactifluus*, *Russula* and *Lactarius* subgenera *Piperites*, *Russularia* and *Plinthogalus* as recognized in Verbeken et al. (2014). ITS and *rpb2* sequences were either generated from sporocarps using the methods outlined above or obtained from GenBank and Unite (Kõljalg et al. 2005, 2013, Abarenkov 2010) with a focus on sequences from vouchered collections published in manuscripts investigating *Lactarius* systematics. Large subunit (LSU) rDNA sequences downloaded from GenBank and Unite were also used in preliminary analyses, however this data did not noticeably affect tree topology nor did it improve phylogenetic resolution, and because data were missing for most of the taxa of interest it was not included in the final analysis. The initial intent was to include all of the sequences generated in this study in one phylogenetic analysis, however, due to problems aligning the ITS region across the wide diversity of taxa included, and the loss of phylogenetic information resulting from the removal of ambiguously aligned regions in the ITS alignment, only one to two sequenced collections per taxa were included, and species level relationships were not investigated in depth with this tree. Outgroups were not enforced for Bayesian inference (BI) or maximum likelihood (ML) analyses. The results of BI and ML analyses were used to select *Lactifluus piperatus*, *Lactifluus volemus*, *Multifurca furcata*, *M. ochricompacta*, *Russula camarophylla* and *R. nigricans* as outgroup taxa for maximum parsimony (MP) analyses. Specimens used in this analysis are shown in Table 3 as denoted by superscript 2 after each taxon.

Phylogenetic Analyses of Clades A-C. To generate alignments without ambiguously aligned regions, delineate species, investigate biogeography and host specificity and examine affiliation with publicly available sequences on GenBank and Unite (including environmental sequences), phylogenetic analyses of ITS and *rpb2* data were carried out on three separate groups, based on clades (A, B, C) identified in the above investigation (broad phylogenetic analyses). All of the sequences generated from Rocky Mountain alpine collections along with sequences from putatively conspecific taxa from a broad geographic range and some sequences from taxa with morphological affinities to Rocky Mountain alpine taxa were included. Furthermore, the ITS sequence from a representative specimen of each Rocky Mountain alpine morphological species was subjected to megaBLAST searches on GenBank and Unite and greater than or equal to 97% matches were tabulated (see results section) and their ITS sequences downloaded (when available) for use in the phylogenetic analyses using members of clades A, B, C as backbones. Seemingly redundant (greater than two), identical environmental sequences from the same geographic region are not shown in the table nor were they included in the phylogenetic analysis. After preliminary analyses, some terminal nodes with redundant taxon names and identical sequences were pruned and analyses rerun. Also, some accessions that jumped around in successive analyses were omitted.

Phylogenetic analyses of clade A focused on Rocky Mountain alpine collections identified as *Lactarius lanceolatus* as well as related taxa. Phylogenetic analyses of clade B focused on Rocky Mountain alpine collections identified as *Lactarius nanus* and *L. glyciosmus* as well as related taxa. Phylogenetic analyses of clade C focused on Rocky

Mountain alpine collections identified as *Lactarius* aff. *pseudouvidus* (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*), *L. repraesentaneus*, and *L. salicis-reticulatae* as well as related taxa. Outgroups for the three analyses were selected based on results from the broad phylogenetic analyses. For the phylogenetic analyses of clade A, *Lactarius mammosus* was used as an outgroup. For the phylogenetic analyses of clades B and C, *L. subdulcis* was used as an outgroup. Specimens used for the phylogenetic analyses of clades A, B and C are shown in Table 3 as denoted by superscripts A, B, C after taxa names.

Sequence Alignment and Phylogenetic Analyses

ITS and *rpb2* sequences were compiled into .fasta files and aligned using MUSCLE (Edgar 2004) under default settings and edited manually using SeAl: Sequence Alignment Editor (Rambaut 1995). The ITS alignment was trimmed to the “CATTA” motif on the 5’ end and to near the start of the LSU gene (represented by “TTGA”, “-TGA” or “TTGG” in this dataset) on the 3’ end. For the broad phylogeny, ambiguously aligned regions of the ITS region were highlighted and removed using the online version of GBlocks v0.91b (Castresana 2000) under the least-stringent settings. This was done in order to minimize bias and to allow repeatability of the removal of ambiguously aligned regions. For the phylogenetic analyses of clades A and B the alignments contained no ambiguously aligned regions after minor manual editing. In clade C a large 268-269 base pair insertion present in the ITS1 region of some species (AB588960, AH Smith 81954, CLC2133, CLC1470, CLC1910, CLC3001, EB0041, EB120-13, EL63-10, EL101-11, E Søyland

73867, JV10468, JX625292, OB11-011, OUC97047, P Larsen 361395, TU106140, TWO809, and U Peintner 20040156), as well as a small ambiguously aligned region were manually removed prior to running phylogenetic analyses.

Each aligned dataset was then formatted as a nexus file and uploaded to PAUP* 4.0b10 (Swofford 2001). Topological incongruence between the ITS and *rpb2* datasets was examined using the partition homogeneity test (PHT) (Farris et al. 1995) as implemented in PAUP*. The null hypothesis of congruence was rejected if $P < 0.05$. Maximum parsimony (MP) analyses were carried out separately for ITS and *rpb2* loci and also on the combined data sets with 100 repetitions using heuristic searches performed separately on the ITS and *rpb2* datasets and on the combined datases with random addition of sequences, TBR branch swapping in effect, USENONMIN option set to “yes”, two trees held at each step during stepwise addition, STEEPEST option set to “yes”, MULTREES set to “yes”, and MAXTREES set to 5000. Gaps were treated as missing data and characters were treated as unordered and equally weighted. Clade support was assessed with 1000 bootstrap replicates using heuristic searches with random addition of sequences, TBR branch swapping in effect, confidence level set to 50, GRPFREQ option set to “no” and MULTREES option set to “no” (Lavin pers. comm.).

Maximum likelihood (ML) analyses were carried out using raxmlGUI 1.3.1. (Stamatikis 2006, Silvestro & Michalak 2012). An ML search combined with rapid bootstrapping was run until bootstrap convergence or 1,000 bootstrap replicates using the autoMRE option. The GTRGAMMA model was estimated separately for ITS1, 5.8S, ITS2, *rpb2* exon 1st, 2nd and 3rd codon positions and *rpb2* intron for the broad phylogenetic analyses. For the phylogenetic analyses of clades A, B and C the GTRCAT model was estimated separately for ITS1, 5.8S, ITS2, *rpb2* exon 1st, 2nd and 3rd codon positions and *rpb2* intron.

For Bayesian inference (BI) analyses the best-fitting substitution model for the evolution of ITS1, 5.8S, ITS2, *rpb2* exon 1st, 2nd and 3rd codon positions and *rpb2* intron was estimated from 24 models of evolution by the Akaike information criterion (AIC) with MrModeltest 2.3 (Nylander 2004) in PAUP* 4.0b10. BI was executed in MrBayes v3.2 (Ronquist & Huelsenbeck 2003). See table 4 for models selected for each partition within each analysis. Parameters were unlinked between partitions and the overall rate was allowed to vary across partitions. On preliminary analyses, consensus trees had unrealistically long branch-lengths, however summary statistics appeared to indicate that the analyses had run smoothly. The long branch-length problem was fixed by setting a branch length prior of 0.01 for all analyses (see Marshall 2010 for more information about this problem). BI was executed separately for ITS (ITS1+5.8S+ITS2) and *rpb2* and also on the combined datasets. All BI analyses consisted of two independent runs with four chains (3 heated, 1 cold) each run until the average standard deviation of split frequencies between runs was less than 0.02 which totaled between 10M and 30M generations, sampling every 500th tree. The plot of generation number versus the log probability of observing the data output by MrBayes was used to determine how many samples to discard as burn-in. For all runs, the default burn-in value of 25% was deemed sufficient. The program TreeGraph 2 (Stöver & Müller 2010) was used to draw, edit and finalize the resulting trees.

Table 4. Best-fitting substitution models used in Bayesian analyses.

Partition	**Broad**	**Clade A**	**Clade B**	**Clade C**
ITS1	GTR+G	SYM+G	K80+I	HKY+I+G
5.8S	SYM	K80	K80	JC
ITS2	GTR+I+G	KHY+G	HKY+G	GTR+I
rpb2 1st codon	GTR+I+G	F81+I	HKY+I	HKY+I
rpb2 2nd codon	GTR+I+G	F81+I	HKY	HKY
rpb2 3rd codon	GTR+G	GTR+I	HKY+I	GTR+G
rpb2 intron	K80+I	HKY	K80	K80

Results

Broad Phylogenetic Analysis

The broad phylogenetic analysis includes 70 specimens, and contains 70 ITS (100%) and 59 *rpb2* (84%) sequences. A total of 29 of the ITS (41%) and 26 of the *rpb2* (44%) sequences were generated in this study. The initial ITS alignment (after minor manual adjustment and trimming) contained many ambiguously aligned regions and included 1045 positions of which 445 were removed for a final alignment length of 600 positions (57% of the original number of positions) of which 303 were constant, 96 were variable but parsimony-uninformative and 201 were parsimony-informative (33%). The *rpb2* alignment did not contain any ambiguously aligned regions (after minor manual adjustment and trimming) and included 842 positions of which 487 were constant, 56 were variable but parsimony-uninformative and 299 were parsimony-informative (36%).

The ITS maximum parsimony (MP) analysis resulted in 5000 most parsimonious trees of 934 steps, consistency index (CI) = 0.4786, retention index (RI) = 0.6727 and rescaled consistency index (RC) = 0.3220. The *rpb2* MP analysis resulted in 4999 most parsimonious trees of 1243 steps, consistency index (CI) = 0.4521, retention index (RI) = 0.6204, and rescaled consistency index (RC) = 0.2805. The partition homogeneity test (PHT) indicated that the ITS and *rpb2* loci were producing significantly different phylogenetic signals (*P* = 0.03), and based on visual inspection of the resulting trees there were minor, poorly supported conflicts with regard to the placement of some of the terminal branches. However, these conflicts received less than 75% bootstrap support and pertained only to the placement of a few terminal branches, thus the combined dataset was analyzed. The combined (ITS + *rpb2*) MP analysis resulted in 5000 most parsimonious trees of 2205 steps, consistency index (CI) = 0.4576, retention index (RI) = 0.6356, and rescaled consistency index (RC) = 0.2908. The maximum likelihood (ML) tree combining ITS and *rpb2* DNA is shown in Figure 3.

Due to missing data and because single gene trees were poorly resolved, this section will focus on results of the broad phylogenetic analysis combining ITS and *rpb2* DNA. Single gene ML trees are included in the appendix (see Appendix A for ITS tree and Appendix B for *rpb2* tree). Furthermore, Bayesian, MP and ML analyses varied in the overall topology, yet agreed on the well-supported clades. For simplicity, and because the ML topology most closely agreed with the most recent comprehensive phylogeny of *Lactarius* (Verbeken et al. 2014), only the ML topology will be discussed in detail and displayed (Figure 3) (see Appendix C for combined Bayesian tree). Bootstrap values generated in PAUP* are abbreviated as BP, bootstrap values generated in RAxML are abbreviated as BS and Bayesian posterior probability is abbreviated as PP.

Rocky Mountain alpine specimens included in this analysis fall out in a strongly supported clade (BP 100/BS 100/PP 1.0) corresponding with the genus *Lactarius* Pers. as currently recognized (indicated as *Lactarius* in Figure 3), and distinct from *Lactifluus* (BP 100, unresolved and basal in ML and Bayesian analyses), *Multifurca* (BP 97/BS 100/PP 1.0) and *Russula* (BP 100/BS 100/PP 1.0). Within *Lactarius*, subgenus *Plinthogalus*, as represented by *L. romagnesii* Bon and *L. lignyotus* Fr. is well-supported (indicated as Subg. *Plinthogalus* in Figure 3) (BP 100/BS 100/PP 1.0); no Rocky Mountain alpine specimens occur in this clade. Subgenus *Russularia* as recognized by modern phylogenetic studies (Verbeken et al. 2014) is well-supported (BP 85/BS 100/PP 1.0) (indicated as Subg. *Russularia* in Figure 3). *Lactarius lanceolatus* (EB105-13) from the Rocky Mountain alpine zone falls out within subgenus *Russularia*. Subgenus *Piperites* is monophyletic in the ML tree but received low support (BS<50) (indicated as Subg. *Piperites* in Figure 3) and is polyphyletic in MP and Bayesian analyses, although with low support (see Appendix C for Bayesian combined tree). Rocky Mountain alpine collections identified as *Lactarius glyciosmus* (TWO269, CLC1624), *L. nanus* (EB106-13, CLC1471), *L. repraesentaneus* (EB107-13, CLC1747), *L.* aff. *pseudouvidus* (*L. pallidomarginatus*, EB0041), and *L. salicis-reticulatae* (CLC2776, EB0036) fall out in subgenus *Piperites* (Figure 3).

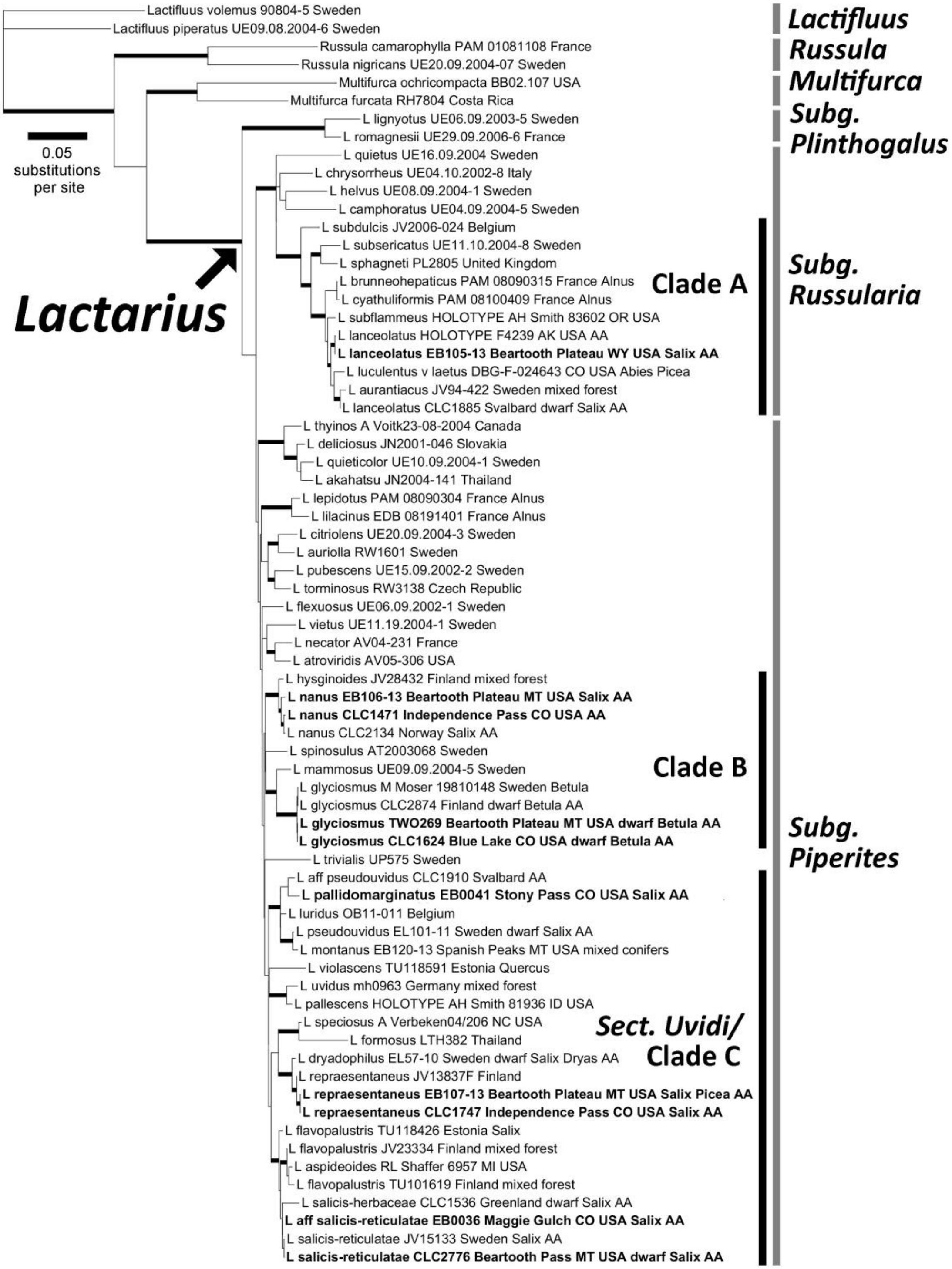


Figure 3. ML tree combining ITS and *rpb2* DNA. Thickened branches lead up to clades receiving ≥75% bootstrap support from either PAUP* or raxml and Bayesian posterior probabilities ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

Selection of Backbone Clades (A, B, C). Within subgenus *Russularia*, a smaller, well-supported clade (BP 100/BS 100/PP 1.00) (labeled as clade A in Figure 3) was identified to serve as a backbone for carrying out species level phylogenetic analyses focusing on Rocky Mountain alpine collections identified as *L. lanceolatus*. Taxa from this clade as well as greater than or equal to 97% megaBLAST matches to *L. lanceolatus* (EB105-13) (Table 5), additional collections of *L. lanceolatus* and several other related taxa were used in these analyses. Results for these analyses are shown in Figures 4-7 and outlined in a subsequent section.

Within subgenus *Piperites*, a poorly supported clade (BP<50/BS<50/PP 0.69) (labeled as clade B in Figure 3) was identified to serve as a backbone for carrying out species level phylogenetic analyses focusing on Rocky Mountain alpine collections identified as *L. glyciosmus* and *L. nanus*. Taxa from clade B, along with greater than or equal to 97% megaBLAST matches to *L. glyciosmus* (TWO269) and *L. nanus* (EB106-13) (Table 5), and additional collections of these taxa were used in these analyses. Results for these analyses are shown in Figures 8, 9 and outlined in a subsequent section.

Also within subgenus *Piperites*, another clade (not recovered in MP tree/BS<50/PP 0.99) (indicated as sect. *Uvidi*/clade C in Figure 3) was identified to serve as a backbone for carrying out species level phylogenetic analyses focusing on Rocky Mountain collections identified as *L.* aff. *pseudouvidus* (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*), *L. repraesentaneus*, and *L. salicis-reticulatae*. Interestingly, this clade exclusively included members of the violet-staining section *Uvidi* of subgenus *Piperites*, suggesting the monophyly of this section. This clade was not resolved in the single gene analyses. Taxa from clade C, along with greater than or equal to 97% megaBLAST matches to *L. repraesentaneus* (EB107-13), *L.* aff. *pseudouvidus* (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*, EB0041), and *L. salicis-reticulatae* (CLC2776) (Table 5), additional collections of these taxa and some additional related taxa were used in these analyses. Results for these analyses are shown in Figures 10-13 and outlined in a subsequent section.

MegaBLAST Results for Select Rocky Mountain Alpine Specimens

Greater than or equal to 97% megaBLAST matches to ITS sequences from Rocky Mountain alpine *Lactarius* specimens *L. glyciosmus* (TWO269), *L. lanceolatus* (EB105-13), *L. nanus* (EB106-13), *L.* aff. *pseudouvidus* (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*, EB0041), *L. repraesentaneus* (EB107-13) and *L. salicis-reticulatae* (CLC2776) span a diversity of taxa beyond any particular query sequence identity. Furthermore all of the query taxa have greater than or equal to 97% matches with accessions from arctic-alpine as well as subalpine areas in North America and Eurasia. Results from these searches are shown in Table 5. Select sequences matching *L. lanceolatus* were included in the phylogenetic analysis of clade A, select sequences matching *L. nanus* and *L. glyciosmus* were included in the phylogenetic analysis of clade B and select sequences matching *L.* aff. *pseudouvidus* (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*), *L. repraesentaneus* and *L. salicis-reticulatae* were included in the phylogenetic analysis of clade C. The sequences that were downloaded and included in these phylogenetic analyses are further discussed in sections dealing with the individual clades A-C.

Table 5. Results for 97% or higher megaBLAST matches to the ITS region of one representative specimen of each Rocky Mountain alpine *Lactarius* morphological species identified in this study (excluding *L.* aff. *salicis-reticulatae*). Searched reference databases include GenBank and Unite. Bolded accession numbers represent sequences used in species-level phylogenetic analyses. ES = Environmental sequence; *B.* = *Betula*, *B. platyph.* = *Betula platyphylla*, *D. integrif.* = *Dryas integrifolia*, *Pin. sylv.* = *Pinus sylvestris*, *Q.* = *Quercus*, *S.* = *Salix*; AA = arctic-alpine, Bor For = boreal forest, Con For = conifer forest, Mar For = maritime forest, Mix For = mixed forest, Rip For = riparian forest ,Temp For = temperate forest; Brtsh Col = British Columbia, N Hamp = New Hampshire, Wiscnsn = Wisconsin.

***Lactarius lanceolatus* EB105-13**							
Accession	**Identification**	**Score**	**Prcnt**	**Source**	**Host**	**Habitat**	**Location**
JF304414	ES	1295	99.58	Soil	NA	AA	Svalbard
FJ845419	*L. luculentus* v. *laetus*	1273	97.96	Sporocarp	NA	Bor For	Brtsh Col
JQ347147	ES	1264	97.70	Root tip	*Kobresia* sp.	AA	China
JQ347138	ES	1245	97.54	Root tip	*Kobresia* sp.	AA	China
UDB000887	*L. aurantiacus*	1227	97.51	Sporocarp	NA	NA	NA
UDB000312	*L. aurantiacus*	1221	97.50	Sporocarp	*Picea* sp.	NA	Germany
JF908299	*L. aurantiacus*	1221	97.50	Sporocarp	NA	NA	Italy
AF157412	*L. mitissimus*	1221	97.50	Sporocarp	*Picea abies*	NA	Germany
UDB011687	*L. aurantiacus*	1164	97.12	Sporocarp	NA	NA	Estonia
EF493295	*L. mitissimus*	1144	97.08	Sporocarp	NA	Mix For	Italy
GU234010	*L. lanceolatus*	1136	99.52	Sporocarp	NA	AA	Svalbard
EU711607	*Lactarius* sp.	1134	99.52	Sporocarp	NA	Mar For	Alaska
DQ974746	*L. substriatus*	1114	97.00	Sporocarp	*Q. douglasii*	Temp For	California
EU711569	*Lactarius* sp.	1109	98.87	Sporocarp	NA	NA	Alaska
UDB000819	*L. lapponicus*	1101	97.25	Sporocarp	NA	NA	NA
UDB002454	*L. lanceolatus*	1094	97.38	Sporocarp	NA	AA	Svalbard
EU292558	ES	1094	97.10	Soil	NA	Bor For	Alaska
***Lactarius nanus* EB106-13**							
Accession	**Identification**	**Score**	**Prcnt**	**Source**	**Host**	**Habitat**	**Location**
GU234147	*L. uvidus*	1203	98.66	Sporocarp	NA	AA	Svalbard
UDB016019	*L. hysginoides*	1203	98.67	Sporocarp	NA	Bor For	Finland
JF304415	ES	1184	99.09	Soil	NA	AA	Svalbard
EU711622	ES	1109	98.41	Soil	NA	Bor For	Alaska
EU711635	ES	1098	97.94	Soil	NA	Bor For	Alaska
GU234062	*L. mammosus*	1072	98.35	Sporocarp	NA	AA	Svalbard
GU234019	*L. nanus*	1064	98.34	Sporocarp	NA	AA	Svalbard
***Lactarius glyciosmus* TWO269**							
Accession	**Identification**	**Score**	**Prcnt**	**Source**	**Host**	**Habitat**	**Location**
GU998223	ES	1186	99.84	Root tip	*B. nana*	AA	Alaska
HQ021853	ES	1181	99.69	Soil	NA	Temp For	N Hamp
GU998695	ES	1181	99.69	Root tip	*B. nana*	AA	Alaska
JX198526	ES	1175	99.53	Root tip	NA	Rip For	Idaho

Table 5 Continued.

DQ097872	*L. glyciosmus*	1175	99.53	Sporocarp	NA	Temp For	Brtsh Col
EF493307	*L. glyciosmus*	1173	99.38	Sporocarp	NA	Mix For	Sweden
EU819484	*L. glyciosmus*	1168	99.38	Sporocarp	NA	Temp For	Wiscnsn
UDB015747	*L. glyciosmus*	1153	98.91	Sporocarp	NA	Mix For	Estonia
UDB015705	*L. glyciosmus*	1153	98.91	Sporocarp	NA	NA	Estonia
AB669497	ES	1147	98.76	Root tip	*B. platyph.*	Temp For	Japan
JF908294	*L. glyciosmus*	1147	98.76	Sporocarp	NA	NA	Italy
UDB017965	*L. glyciosmus*	1146	98.76	Sporocarp	NA	NA	Estonia
UDB015740	*L. glyciosmus*	1146	98.76	Sporocarp	NA	Mix For	Estonia
FJ607374	*L.* cf. *nanus*	1142	99.68	Sporocarp	NA	AA	Alaska
FJ607382	ES	1134	99.52	Soil	NA	AA	Alaska
UDB011530	*L. glyciosmus*	1134	98.45	Sporocarp	NA	NA	Estonia
FM992937	ES	1127	98.15	Root tip	*Picea abies*	Con For	Sweden
AF476975	ES	1127	98.15	Root tip	NA	Con For	Sweden
AJ633116	ES	1127	98.15	Root tip	*Pin. sylv.*	Con For	Finland
JF908316	*L. fuscus*	1123	98.00	Sporocarp	NA	NA	Italy
UDB015711	*L. mammosus*	1122	97.99	Sporocarp	NA	NA	Estonia
EU711624	ES	1120	99.19	Soil	NA	Bor For	Alaska
UDB011464	*L. mammosus*	1118	97.85	Sporocarp	NA	Con For	Estonia
EU711685	ES	1107	98.87	Soil	NA	Bor For	Alaska
UDB000834	*L. mammosus*	1101	98.11	Sporocarp	NA	NA	NA
JF304344	ES	1081	99.66	Soil	NA	AA	Alaska
EF493308	*L. trivialis*	1068	98.21	Sporocarp	NA	Mix For	Sweden
AY097042	ES	1066	98.05	Soil	NA	Con For	Sweden
EU711566	ES	1064	97.58	Soil	NA	Bor For	Alaska
GU234027	*L. glyciosmus*	1044	99.31	Sporocarp	NA	AA	Svalbard
***Lactarius* aff. *pseudouvidus* EB0041 (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*)**							
Accession	**Identification**	**Score**	**Prcnt**	**Source**	**Host**	**Habitat**	**Location**
UDB018409	*L. brunneoviolaceus*	1624	98.08	NA	NA	NA	NA
UDB000846	*L. luridus*	1624	98.08	NA	NA	NA	NA
KF241547	*L. luridus*	1618	97.97	Sporocarp	NA	NA	Belgium
UDB000833	*L. luridus*	1616	98.37	NA	NA	NA	NA
AB588960	ES	1615	99.66	Root tip	*S. polaris*	AA	Svalbard
UDB018415	*L. pseudouvidus*	1615	97.54	NA	NA	NA	NA
UDB018414	*L. pseudouvidus*	1609	97.33	NA	NA	NA	NA
UDB000925	*L. aspideus*	1598	99.43	NA	NA	NA	NA
UDB011691	*L. luridus*	1596	98.15	Sporocarp	NA	NA	Estonia
UDB018416	*L. pseudouvidus*	1587	97.33	NA	NA	NA	NA
UDB018408	*L. brunneoviolaceus*	1574	97.91	NA	NA	NA	NA
JX625292	ES	1563	98.11	Root tip	NA	NA	Italy
DQ093852	*L. pallescens*	1426	97.71	Sporocarp	NA	Con For	Brtsh Col
GU234088	*L. nanus*	1205	98.96	Sporocarp	NA	AA	Svalbard
GU234059	*L. violascens*	1037	98.80	Sporocarp	NA	AA	Svalbard
GU234051	*L. pseudouvidus*	1035	97.07	Sporocarp	NA	AA	Svalbard
***Lactarius repraesentaneus* EB107-13**							
Accession	**Identification**	**Score**	**Prcnt**	**Source**	**Host**	**Habitat**	**Location**
UDB011701	*L. repraesentaneus*	1334	100.0	Sporocarp	NA	NA	Estonia
UDB000871	*L. repraesentaneus*	1330	100.0	Sporocarp	NA	NA	NA
UDB000367	*L. repraesentaneus*	1330	100.0	Sporocarp	NA	Mix For	Germany
JF304416	ES	1295	99.58	Soil	NA	AA	Svalbard
UDB015723	*L. repraesentaneus*	1277	99.43	Sporocarp	NA	NA	Estonia
JF908292	*L. dryadophilus*	1267	99.43	Sporocarp	NA	NA	Italy

Table 5 Continued.

AY331011	*L. repraesentaneus*	1240	99.71	Sporocarp	NA	Mix For	Sweden
JF908312	*L. repraesentaneus*	1236	100.0	Sporocarp	NA	NA	Italy
FJ607373	*Lactarius* sp.	1236	100.0	Sporocarp	NA	AA	Alaska
JX630967	*L. repraesentaneus*	1227	99.41	Root tip	*D. integrif.*	AA	Baffin Is.
FJ607367	*Lactarius* sp.	1214	99.40	Sporocarp	NA	AA	Alaska
UDB000888	*L. subcircellatus*	1186	97.82	Sporocarp	NA	NA	NA
UDB000977	*L. dryadophilus*	1160	99.22	Sporocarp	NA	NA	NA
Lactarius salicis-reticulatae **CLC2776**							
Accession	**Identification**	**Score**	**Prcnt**	**Source**	**Host**	**Habitat**	**Location**
UDB015606	*L. flavopalustris*	1184	98.52	Sporocarp	*Salix*	NA	Estonia
UDB017888	*L. flavopalustris*	1173	98.22	Sporocarp	*Salix*	For	Estonia
UDB011119	*L. flavopalustris*	1153	98.05	Sporocarp	NA	Bor For	Finland
EU711609	*Lactarius* sp.	1131	99.05	Sporocarp	NA	Mar For	Alaska
UDB000856	*L. salicis-herbaceae*	1122	97.85	Sporocarp	NA	NA	NA
UDB000881	*L. salicis-reticulatae*	1105	99.50	Sporocarp	NA	NA	NA
EU711582	*Lactarius* sp.	1105	98.41	Sporocarp	NA	Bor For	Alaska
HQ215798	ES	1068	99.49	Soil	NA	AA	Svalbard

Phylogenetic Analyses of Clades A-C

Bayesian, MP and ML analyses have similar topologies and in general support the same clades. However, in some analyses there are subtle, poorly supported differences between MP, ML and Bayesian trees. In instances where there are differences, the combined (ITS + *rpb2*) Bayesian tree for that particular dataset was used to make taxonomic inference. For simplicity, only Bayesian topologies are displayed where all three trees agreed, however, in instances where the ML and the MP tree differed significantly from the Bayesian tree, differences are presented. Support values from Bayesian, MP and ML analyses are presented in their respective sections and displayed on phylogenetic trees.

Phylogenetic Analysis of Clade A

The phylogenetic analysis of clade A focused on Rocky Mountain alpine collections identified as *Lactarius lanceolatus* as well as closely related species and included 40 specimens, and contained 40 ITS (100%) and 17 *rpb2* (43%) sequences. No major edits were made to the initial ITS or *rpb2* alignments besides minor manual adjustment and trimming. The final ITS alignment length was 691 positions of which 597 were constant, 54 were variable but parsimony-uninformative and 40 were parsimony-informative (5%). The final *rpb2* alignment length was 825 positions of which 706 were constant, 60 were variable but parsimony-uninformative and 59 were parsimony-informative (7%). The ITS MP analysis resulted in 5000 most-parsimonious trees of 121 steps, CI = 0.8017, RI = 0.8681, and RC = 0.6959. The *rpb2* MP analysis resulted in 5000 most-parsimonious trees of 153 steps, CI = 0.8693, RI = 0.8305, and RC = 0.7219. The PHT indicated that the ITS and *rpb2* loci were not producing significantly different phylogenetic signals ($P = 0.44$), although minor, poorly supported conflict was seen upon visual inspection of the resulting trees. The combined (ITS + *rpb2*) MP analysis resulted in 5000 most-parsimonious trees of 277 steps, CI = 0.8303, RI = 0.8412, rescaled consistency index RC = 0.6985.

<u>Clade A, Summary.</u> Based on BI (Figures 4, 6, 7), ML (Figures 5, 7), and MP (not shown) phylogenetic analyses we recognize 14 species-level clades and (or) terminal branches (labeled in Figure 4). Species recognized include *L. mammosus* (outgroup), *L. subdulcis*, *L. subsericatus*, *L. sphagneti*, *L. subviscidus*, *L. brunneohepaticus*, *L. cyathuliformis*, *L. substriatus*/*L. subflammeus*, *Lactarius* sp. 1, *Lactarius* sp. 2, *L. lanceolatus*, *L. luculentus* v. *laetus*, *L. luculentus*, and *L. aurantiacus*. Specimens originally identified as *Lactarius lanceolatus* do not form a monophyletic group in single-

gene or combined MP, ML, or BI analyses, there is minor conflict between MP, ML and BI analyses and there is minor conflict between ITS and *rpb2* trees, although there was no statistical support for these conflicts.

Lactarius lanceolatus: Combined Analyses. In the combined Bayesian analysis (Figure 4), collections originally identified as *L. lanceolatus* are polyphyletic. Rocky Mountain alpine collections identified as *L. lanceolatus* (EB105-13, CLC1389, CLC2358, CLC2319), the holotype from Alaska (F4239), and several samples from Svalbard (JF304414, O73608) and Alaska (GAL13852, GAL18572) occur in an unresolved group paraphyletic with respect to *Lactarius* sp. 2 (src438 misidentified as *L. substriatus*), *L. luculentus* v. *laetus* (DBG-F-024643, DBG-F-022653) (PP 1.0), *L. luculentus* (AH Smith 79943, AH Smith 90905, SMI221 misidentified as *L. luculentus* v. *laetus*) (PP 0.97), and a well-supported clade (PP 1.0) containing several other collections originally identified as *L. lanceolatus* (IA-F20, JV15112F, CLC1885, CLC2743) from Alaska, Scandinavia and Svalbard, specimens originally identified as *L. aurantiacus* (JV94-422, TU106115, A Pergolini 2090, Hue179), *L. mitissimus* (ue179, syn.: *L. aurantiacus*), and an environmental sample isolated from the roots of *Kobresia* in alpine China (JQ347147). The combined ML (Figure 5) and MP (not shown) trees differ subtly from the combined Bayesian tree in the placement of Rocky Mountain alpine collections identified as *L. lanceolatus*. In the ML analysis, these specimens are split into two poorly supported clades which contain the same sequences that occur in the paraphyletic *L. lanceolatus* group recovered in the Bayesian analysis. One clade contains Rocky Mountain alpine specimen CLC2358, the *L. lanceolatus* holotype (F4239), an environmental sample from Svalbard (JF304414), and a specimen from Alaska (GAL18572). The other clade contains three other Rocky Mountain alpine speciemens identified as *L. lanceolatus* (CLC1389, CLC2319, EB105-13), as well as a specimen from Svalbard (O73608) and one from Alaska (GAL13852). The remaining topology is identical to that recovered in the Bayesian analysis.

Lactarius lanceolatus: ITS Analyses. The topology of the Bayesian ITS analysis (Figure 6) is identical to the Bayesian combined analysis except for the placement of *L. subdulcis*. Results of the MP and ML ITS analyses (not shown) are also nearly identical.

Lactarius lanceolatus: *rpb2* Analyses. In the Bayesian *rpb2* analysis (Figure 7), all collections identified as *L. lanceolatus* including the holotype as well as all collections identified as *L. aurantiacus* and *L. mitissimus* form a strongly supported clade (PP 1.0), paraphyletic with respect to *L. brunneohepaticus*/*L. cyathuliformis* (PP 1.0) and *L. luculentus* v. *laetus* (PP 1.0). More resolution was obtained in the ML (Figure 7) and MP (not shown) *rpb2* analyses because clades occurring in less than 50% of the sample trees were not collapsed as in the Bayesian analysis. However, support for these differences was low. In the *rpb2* ML and MP analyses, Rocky Mountain alpine collections identified

Figure 4. Bayesian 50% majority rule tree of clade A combining ITS and *rpb2* DNA. Support values (BP/BS/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

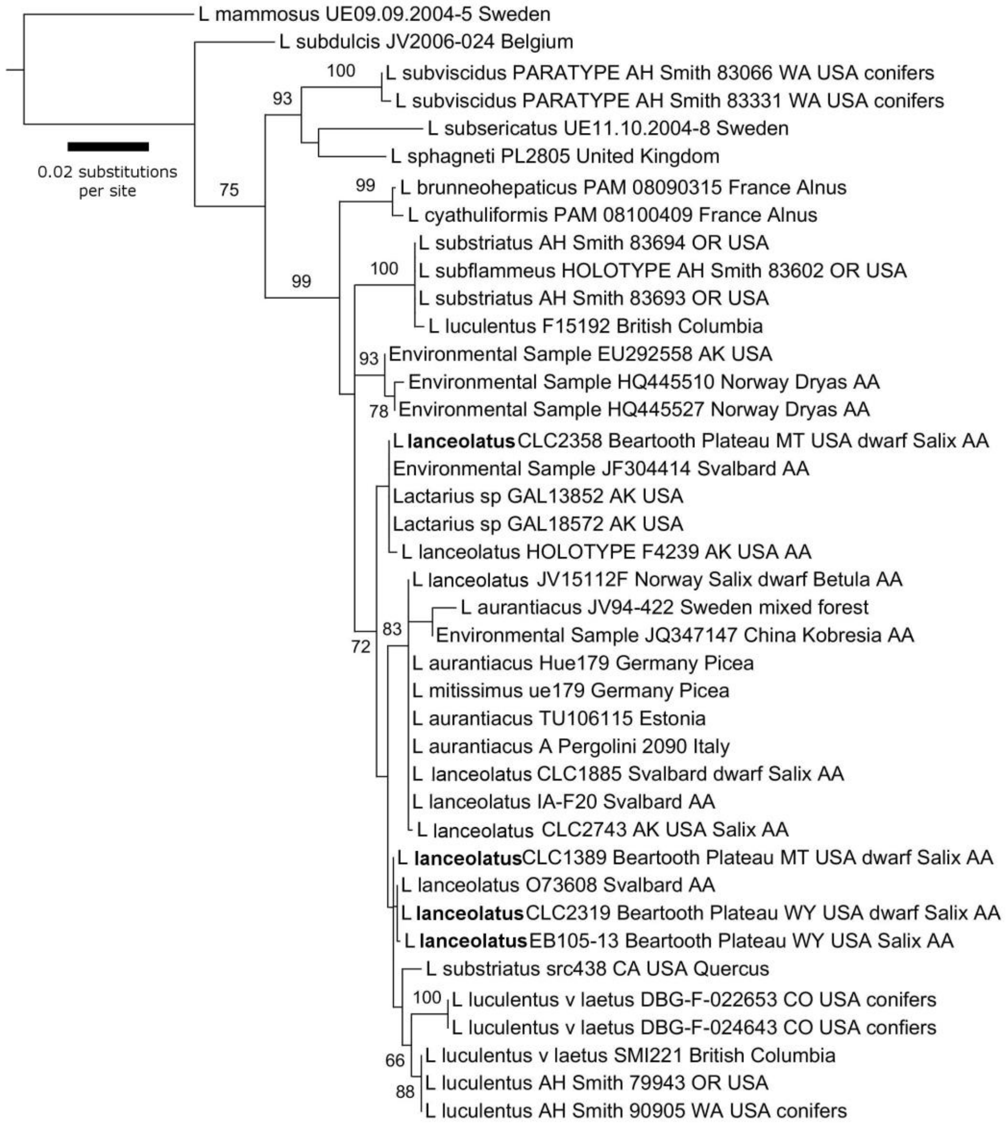


Figure 5. ML tree of clade A combining ITS and *rpb2* DNA. Support values (BS) ≥60% are indicated above or below branches leading up to clades. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

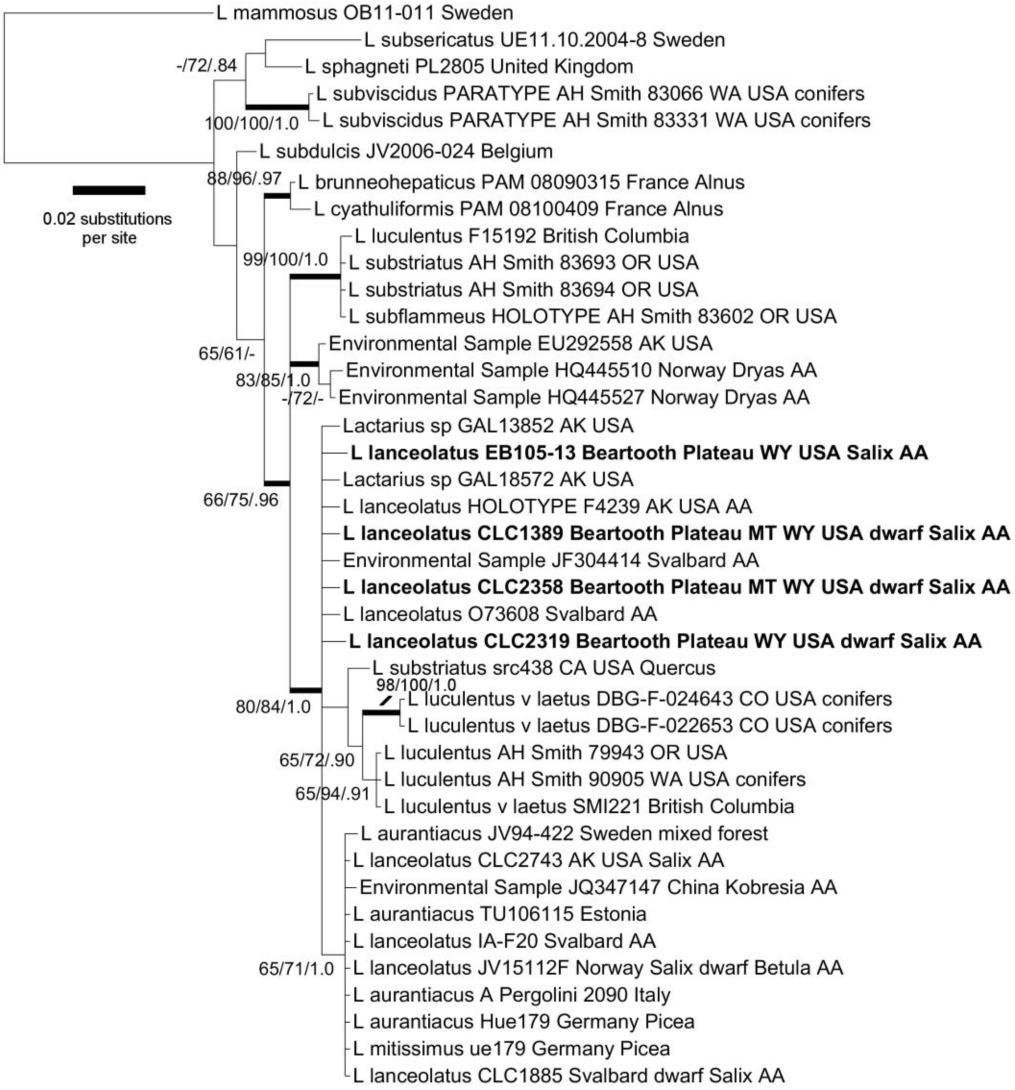


Figure 6. Bayesian single-gene ITS tree of clade A. Support values (BP/BS/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

Clade A: Bayesian rpb2 Tree

Clade A: ML rpb2 Tree

Figure 7. Clade A: Bayesian and ML single-gene *rpb2* trees. For the Bayesian tree, support values (BP/BS/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. For the ML tree, support values (BS) ≥60% are indicated above or below branches leading up to clades. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

as *L. lanceolatus* are polyphyletic and scattered in several poorly supported clades. In the ML analysis, Rocky Mountain alpine *L. lanceolatus* collection CLC2358 forms a poorly supported clade (BS<50) with a specimen originally identified as *L. lanceolatus* (JV15112F) from Norway, and the other Rocky Mountain alpine colletions form a poorly supported clade (BS<50) with *L. aurantiacus* as well as specimens originally identified as *L. lanceolatus* from Alaska (CLC2743) and from Svalbard (CLC1885); the *L. lanceolatus* holotype occupies its own branch separate from the two previously mentioned clades.

Unfortunately, *rpb2* data was missing for numerous samples for which there was ITS data.

Clade A, Rationale for Nomenclatural Assignments. Due to the unresolved nature of the analyses and incongruence between ITS and *rpb2* trees with respect to *L. lanceolatus* it is difficult to make a firm decision on species limits for the group comprising the taxa *L. lanceolatus*, *Lactarius* sp. 2, *L. luculentus*, *L. luculentus* v. *laetus*, and *L. aurantiacus*. Several different species hypotheses are suggested by the different topologies. Because *Lactarius* demonstrates relatively slow molecular evolution (Verbeken & Nuytinck 2013), which makes recently diverged species difficult to separate, and because several clades in this group are correlated with morphological and ecological differences (i.e. *L. luculentus* v. *laetus* and *L. luculentus*) the hypothesis suggesting at least five species appears to be most strongly supported. Under this hypothesis *Lactarius lanceolatus* is represented by the paraphyletic group in the combined Bayesian analysis (Figure 4) which contains the *L. lanceolatus* holotype (F4239), Rocky Mountain alpine collections identified as *L. lanceolatus* (EB105-13, CLC1389, CLC2358, CLC2319), and samples GAL13852, GAL18572, JF304414, and O73608; *Lactarius* sp. 2 is represented by the collection misidentified as *L. substriatus* (src438); *L. luculentus* v. *laetus* is represented by the clade containing speciemns DBG-F-024643 and DBG-F-022653 collected near the type locality; *L. luculentus* is represented by the clade containing specimens AH Smith 7943 and AH Smith 90905 collected near the type locality; *L. aurantiacus* is represented by the clade recovered in ITS and combined analyses that includes specimens identified as *L. aurantiacus*, *L. mitissimus*, several collections originally identified as *L. lanceolatus* (CLC2743, IA-F20, JV15112F, CLC1885) and an environmental sequence from China (JQ347147). This hypothesis is preferred until more data is obtained and more analyses are carried out as it requires very few nomenclatural rearrangements. Rational for nomenclatural assignments are as follows:

1) The name *L. lanceolatus* is applied to the group recovered in the Bayesian combined and ITS analyses (Figures 4, 6) as the *L. lanceolatus* holotype occurs here. Since this group is somewhat poorly defined at the *rpb2* locus in ML and MP analyses, it should be investigated using more loci.

2) The name *Lactarius* sp. 2 is temporarily applied to the singleton misidentified as *L. substriatus* (src438). The identity of this sequence is unknown and the collection was

not personally examined. *Lactarius substriatus* collected by the authors of that species occurs in a separate clade (Figures 4, 5, 6)

3) The name *L. luculentus* v. *laetus* is applied to the clade with collections DBG-F-024643 and DBG-F-022653 as these collections are from the type locality and match the original description of *L. luculentus* v. *laetus* (Hesler & Smith 1974). This hypothesis suggests that *L. luculentus* v. *laetus* likely should be raised to the species level as *L. laetus*, a change that should not be made until more collections are analyzed.

4) The name *L. luculentus* is applied to the clade containing collections AH Smith 7943 and AH Smith 90905 as these collections are from near the type locality and match the original description discussed in Hesler & Smith (1974). The collection identified as *L. luculentus* v. *laetus* (SMI221) in this clade is likely misidentified and should be *L. luculentus*.

5) The name L. *aurantiacus* is applied to the clade containing collection JV94-422. This collection fits the concept of this taxon well, it is from Sweden, the country where *L. aurantiacus* was originally described and *L. aurantiacus* is the oldest, accepted name for members of this clade. *Lactarius aurantiacus* has already been synonymized with *L. mistissimus* by other authors (e.g. Heilmann-Clausen et al. 1998). Despite having smaller basidiocarps, the collections identified as *L. lanceolatus* in this clade were morphologically identical to *L. aurantiacus* (JV94-422).

Clade A, Other Taxa. Several other clades containing taxa of interest due to their morphological or ecological similarity to *L. lanceolatus* emerge in the combined

Bayesian (Figure 4), ML (Figure 5) and MP (not shown) trees. *Lactarius subviscidus* forms a distinct, well-supported clade (BP 100/BS 100/PP 1.0). The *L. subflammeus* Hesler & A.H. Smith holotype as well as *L. substriatus* A.H. Sm. specimens collected by the author of the species form a well-supported clade (BP 99/BS 100/PP 1.0), and are likely synonymous. The name *Lactarius substriatus* likely should be assigned to this species as it was described in 1960, prior to *L. subflammeus*, which was described in 1979, however more specimens should be examined before this change is made. One specimen identified as *L. luculentus* (F15192) in this clade is likely misidentified as *L. luculentus* collections (AH Smith 90905, AH Smith 79943) from near the type locality occur in a different strongly supported clade. Two environmental samples isolated from the root-tips of *Dryas octopetala* from arctic-alpine Norway (Bjorbækmo et al. 2010), and an environmental sequence from Alaska which have high ITS sequence similarity to *L. lanceolatus* (EB105-13) form a well-supported clade without taxonomic affiliation (BP 82/BS 93/PP 1.0) (indicated as *Lactarius sp.* 1 in Figure 4).

Phylogenetic Analysis of Clade B

The phylogenetic analysis of clade B focused on Rocky Mountain alpine collections identified as *L. nanus* and *L. glyciosmus* and included 58 specimens, and contained 57 ITS (98%) and 20 *rpb2* (34%) sequences. No major edits were made to the initial ITS or *rpb2* alignments besides minor manual adjustment and trimming. The final ITS alignment length was 684 positions of which 607 were constant, 39 were variable but parsimony-uninformative and 38 were parsimony-informative (5%). The final *rpb2* alignment length was 820 characters of which 715 were constant, 65 were variable but parsimony-uninformative and 40 were parsimony-informative (5%). The ITS MP analysis resulted in 5000 most-parsimonious trees of 95 steps, CI = 0.8947, RI = 0.9791, and RC = 0.8761. The *rpb2* MP analysis resulted in 5000 most-parsimonious trees of 134 steps, CI = 0.8806, RI = 0.8841, RC = 0.7785. The PHT indicated that the ITS and *rpb2* loci were not producing significantly different phylogenetic signals ($P = 0.14$). The combined (ITS + *rpb2*) MP analysis resulted in 5000 most-parsimonious trees of 233 steps, CI = 0.8712, RI = 0.9514, RC = 0.8289.

<u>Clade B, Summary</u>. Based on BI (Figures 8, 9), MP (not shown) and ML (not shown) phylogenetic analyses, seven species are recognized (labeled in Figure 8). Species recognized include *L. subdulcis* (outgroup), *L. hysginoides*, *L. nanus*, *L. spinosulus*, *L.* aff. *glyciosmus*, *L. mammosus*, and *L. glyciomsus*. The identity of Rocky Mountain alpine collections originally identified as *L. glyciosmus* is confirmed. The identity of Rocky Mountain alpine collections originally identified as *L. nanus* is supported, however it is still somewhat unclear due to paraphyly with *L. hysginoides*.

<u>*Lactarius nanus*: Combined Analyses.</u> In the combined phylogenetic analyses of clade B (Figure 8), Rocky Mountain alpine collections identified as *L. nanus* occur in a well-supported clade (BP 98/BS 94/PP 1.0) with *L. nanus* from the type locality (Bon 89093, French Alps), arctic-alpine collections of *L. nanus* and environmental sequences from Svalbard and Scandinavia as well as two apparently misidentified accessions (*L.*

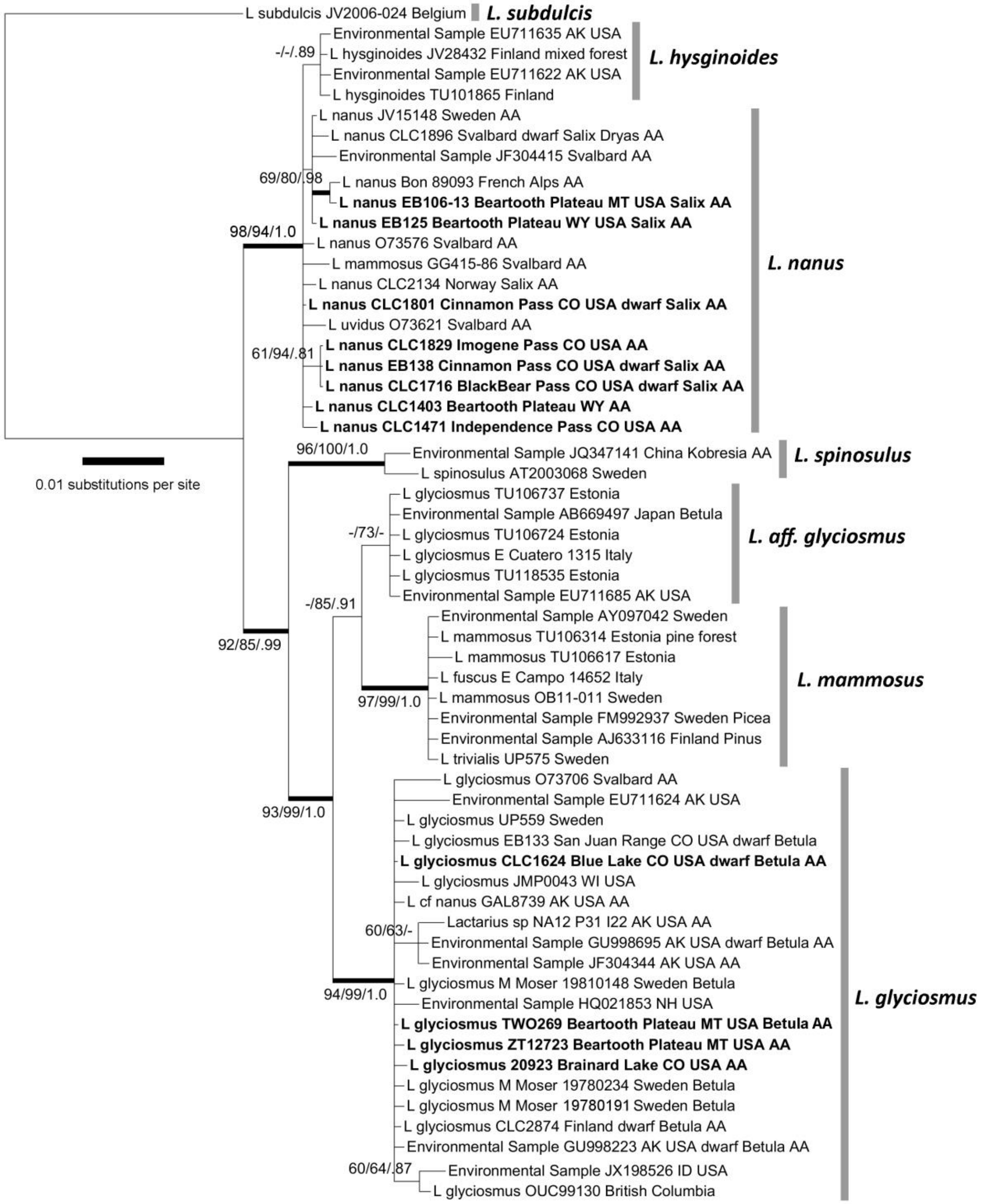
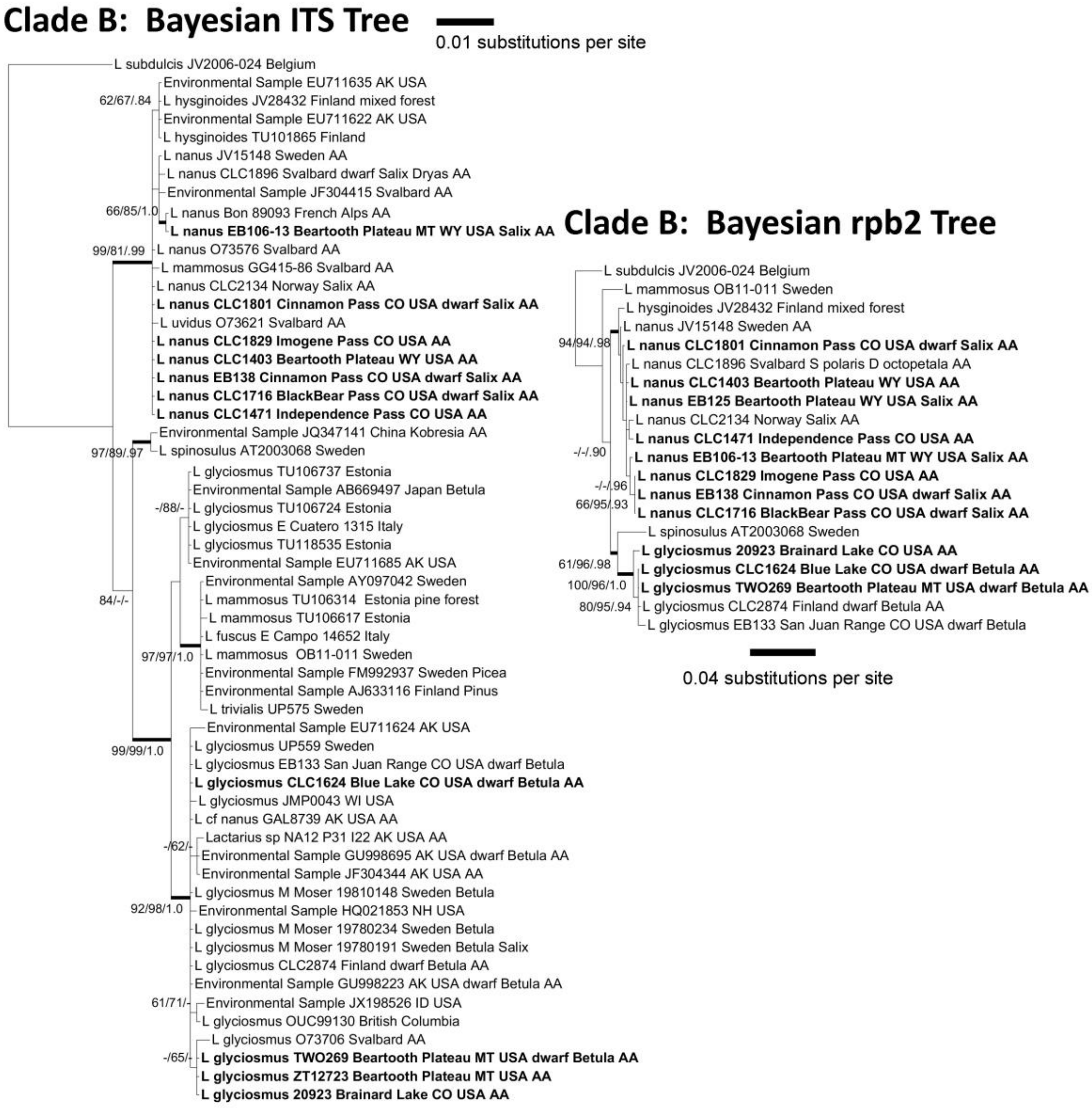


Figure 8. Bayesian 50% majority rule tree of clade B combining ITS and *rpb2* DNA. Support values (BP/BP/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.



Clade B: Bayesian ITS Tree

Clade B: Bayesian rpb2 Tree

Figure 9. Single-gene Bayesian 50% majority rule trees of clade B. Support values (BP/BS/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

mammosus GG415-86, *L. uvidus* O73621). Another clade (BP<50/BS<50/PP 0.89) containing *L. hysginoides* from subalpine Finland as well as two environmental sequences from black spruce (*Picea mariana*) forests in Alaska also occurs within the *L. nanus* clade.

Lactarius nanus: ITS Analyses. In single-gene analyses of ITS DNA (Figure 9) as in the combined analyses, the *L. hysginoides* clade is nested within the *L. nanus* clade. Despite this, *L. nanus* and *L. hysginoides* appear to be distinct species as there are sufficient morphological differences between them, and they appear to occupy different ecological niches. Relative to other species examined in this study, there appears to be high genetic diversity within the *L. nanus* clade and the species concept may be too broad, however all collections are morphologically indistinguishable and further study is needed with more material and perhaps using more loci to confirm this either way.

Lactarius nanus: *rpb2* Analyses. Single-gene analyses of *rpb2* DNA (Figure 9) paint basically the same picture as the combined analyses, however, here *L. hysginoides* is distinct, although with low support.

Lactarius glyciosmus: Combined Analyses. In combined phylogenetic analyses of clade B (Figure 8), *L. glyciosmus* as traditionally recognized is polyphyletic. Rocky Mountain alpine specimens identified as *L. glyciosmus* occur in a well-supported clade (BP 94/BS 99/PP 1.0) with collections of *L. glyciosmus* (M. Moser 19780234, 19780191, 19810148) from Femsjö, Sweden, the locality from which Fries originally described *L.*

glyciosmus in the 1800's. Other samples included in this clade are from Uppsala, Sweden (subalpine), Finland (arctic-alpine), Svalbard (arctic-alpine), British Columbia (subalpine), Idaho (subalpine), Wisconsin (subalpine), New Hampshire (subalpine) and Alaska (arctic-alpine and subalpine). Another clade contains specimens originally identified as *L. glyciosmus* (BS 70/PP 0.53, unresolved in MP analyses) from subalpine areas in Estonia and Italy as well as an environmental sequence from Alaska and a sequence isolated from the roots of *Betula platyphylla* in Japan. This clade is sister to a well-supported clade (BP 97/BS 99/PP 1.0) likely corresponding to *L. mammosus.*

Lactarius glyciosmus: ITS Analyses. The ITS analyses (Figure 9) shows the same topology regarding the placement of *L. glyciosmus* as the combined analyses.

Lactarius glyciosmus: *rpb2* Analyses. In *rpb2* phylogenetic analyses (Figure 9), the same *L. glyciosmus* clade is shown as in the ITS analyses (BP 100/BS 96/PP 1.0). Unfortunately, the potential polyphyly of this taxon could not be investigated with *rpb2* data as these data are missing for members of the *L.* aff. *glyciosmus* clade that is shown in combined and ITS analyses (Figures 8, 9).

Clade B, Other Taxa. While no specimens in the *L. mammosus* clade (Figures 8, 9) were personally examined, we hypothesize that this clade represents *L. mammosus* as it contains a specimen cited in Verbeken et al. (2014) which originated from Sweden, where *L. mammosus* was originally described. It also contains several environmental sequences isolated from the roots of members of the Pinaceae, which is the suggested host for *L. mammosus* and images of specimens TU106314 and TU106617 on the Unite database match the morphological concept of *L. mammosus*. *Lactarius trivialis* (UP575), appearing in the *L. mammosus* clade is likely misidentified and *L. fuscus* (E Campo 14652) has been synonymized with *L. mammosus*. Interestingly, *L. spinosulus*, which is morphologically quite different from *L. glyciosmus* and *L. nanus*, appears to be phylogenetically close (Figures 8, 9). It forms a strongly supported clade (labeled as *L. spinosulus* in Figure 8) in combined (BP 96/100/PP 1.0) and ITS (BP 97/BS 89/PP 0.97) analyses with an ITS sequence (JQ347141) isolated from the roots of *Kobresia* from alpine China.

Phylogenetic Analysis of Clade C

Phylogenetic analyses of clade C focused on Rocky Mountain alpine collections originally identified as *L.* aff. *pseudouvidus* (*L. pallidomarginatus* Barge & C.L. Cripps *ad int.*), *L. repraesentaneus* and *L. salicis-reticulatae* and included 69 specimens, and contained 67 ITS (97%) and 38 *rpb2* (55%) sequences. No major edits were made to the initial ITS or *rpb2* alignments besides minor manual adjustment, trimming and the removal of a 268-269 base-pair insertion present in the ITS1 region of *Lactarius* sp. 4 (CLC1910), *Lactarius* sp. 5 (AB588960), *Lactarius pallidomarginatus* Barge & C.L. Cripps *ad int.* (CLC1470, EB0041), *L. montanus* (AH Smith 81954, OUC97047, CLC3001, EB120-13), *L.* aff. *pseudouvidus* group (EL101-11, EL63-10, E Søyland 73867, JV10468, TWO809, U Peintner 20040156), *L.* aff. *brunneoviolaceus* (CLC2133, P Larsen 361395), and *L. luridus* (JX625292, OB11-011, TU106140). Sequence chromatograms of the ITS region of two collections (U Peintner 20040156, JV28448F) in the *L.* aff. *pseudouvidus* group had large, unreadable stretches with double peaks suggesting divergent ITS copies within these individuals. These sequences were unsalvageable and were not used in phylogenetic analyses. Many individuals in this group also had *rpb2* sequences heterozygous for many alleles. The final ITS alignment length was 696 positions of which 576 were constant, 47 were variable but parsimony-uninformative and 73 were parsimony-informative (10%). The final *rpb2* alignment length was 835 positions of which 707 were constant, 46 were variable but parsimony-uninformative and 82 were parsimony-informative (10%). The ITS MP analysis resulted in 5000 most-parsimonious trees of 199 steps, CI = 0.7085, RI = 0.9200, RC = 0.6519. The *rpb2* MP analysis resulted in 5000 most-parsimonious trees of 195 steps, CI = 0.7590, RI = 0.9015, RC = 0.6842. The PHT indicated that the ITS and *rpb2* loci were not producing significantly different phylogenetic signals ($P = 0.41$). The combined (ITS + *rpb2*) MP analysis resulted in 5000 most-parsimonious trees of 401 steps, CI = 0.7207, RI = 0.9068, RC = 0.6535.

Clade C, Summary. Based on BI (Figures 10, 12, 13), ML (Figure 11) and MP (not shown) phylogenetic analyses 17 well-defined clades or singletons representing species are recognized (labeled in Figure 10). Well-defined species include *L. subdulcis* (outgroup), *L. formosus*, *L. speciosus*, *L. dryadophilus*, *L. repraesentaneus*, *L. violascens*, *L. pallescens*, *L. uvidus*, *L. salicis-herbaceae*, *Lactarius* sp. 3, *L.* aff. *flavopalustris*, *L. aspideoides/flavopalustris*, *L. salicis-reticulatae*, *Lactarius* sp. 4, *Lactarius pallidomarginatus* Barge & C.L. Cripps *ad int.*, *L. montanus*, and *L. luridus*. Several poorly defined groups or species were also recovered and include *L.* aff. *salicis-reticulatae*, *Lactarius* sp. 5, *L.* aff. *pseudouvidus* group, *L.* aff. *brunneoviolaceus* and two environmental sequences with affinities to *L. dryadophilus* (also labeled in Figure 10). Rocky Mountain alpine collections originally identified as *L.* aff. *pseudouvidus* appear to be a distinct species, and taxonomic problems were revealed for the *L. pseudouvidus*/*brunneoviolaceus* group. The identity of Rocky Mountain alpine collections originally identified as *L. repraesentaneus* are confirmed. The identity of collections of *Lactarius salicis-reticulatae* collected at Beartooth Plateau, MT/WY sites are also confirmed, however phylogenetic analyses suggest that collections originally identified as *L. salicis-reticulatae* from Colorado may represent a distinct taxon, hereto informally referred to as *L.* aff. *salicis-reticulatae*; More information is needed to formally confirm this.

Lactarius pallidomarginatus and Allies. In combined (Figures 10, 11) and ITS (Figure 12) analyses, Rocky Mountain alpine collections identified as *L*. aff. *pseudouvidus* fell out in a strongly supported clade (combined BP 97/BS 93/PP 1.0, ITS BP 97/BS 98/PP 1.0) with other similar taxa, identified as *L. brunneoviolaceus*, *L. luridus*, *L. montanus*, *L. pseudouvidus* and *L. robertianus*. All specimens in this clade for which ITS data was available have a similar, large insertion present in the ITS1 region. Within this clade, Rocky Mountain alpine collections identified as *L.* aff. *pseudouvidus* form a well-supported clade (combined BP 82/BS 100/PP 0.98, ITS BP 61/BS 96/PP 0.94) sister to an environmental sequence from Svalbard (AB588960) isolated from the roots of *Salix* *polaris* (Fujiyoshi et al. 2011). All other collections identified as *L. pseudouvidus* (all obtained from European countries and including a specimen from near the type locality) are phylogenetically distinct from Rocky Mountain alpine material as are other similar taxa such as *L. brunneoviolaceus* and *L. robertianus*. It is thus hypothesized that Rocky Mountain material represents an undescribed species, informally referred to here as *Lactarius pallidomarginatus* Barge & C.L. Cripps *ad int.* until it is formally named. Another specimen with affinities to *L. pseudouvidus*, CLC1910, collected in Svalbard, putatively associated with *S. polaris* is phylogenetically close to Rocky Mountain alpine material, yet distinct; this species is referred to as *Lactarius* sp. 4. The environmental sample (AB588960) isolated from *Salix polaris* in Svalbard is very closely related to *Lactarius pallidomarginatus* Barge & C.L. Cripps *ad int.*, and further work is needed to determine whether or not it actually represents a distinct species. It is hereto informally referred to as *Lactarius* sp. 5. While *rpb2* data was missing for numerous specimens in this group, results of the *rpb2* analyses (Figure 13) are similar results of ITS and combined analyses.

Other collections proposed to be *L. pseudouvidus* (all obtained from European countries), while distinct from Rocky Mountain alpine material, do not form a monophyletic group and there are discrepancies between the ITS and *rpb2* trees; overall there is a lot more variation present in the *rpb2* locus as compared to the ITS region in

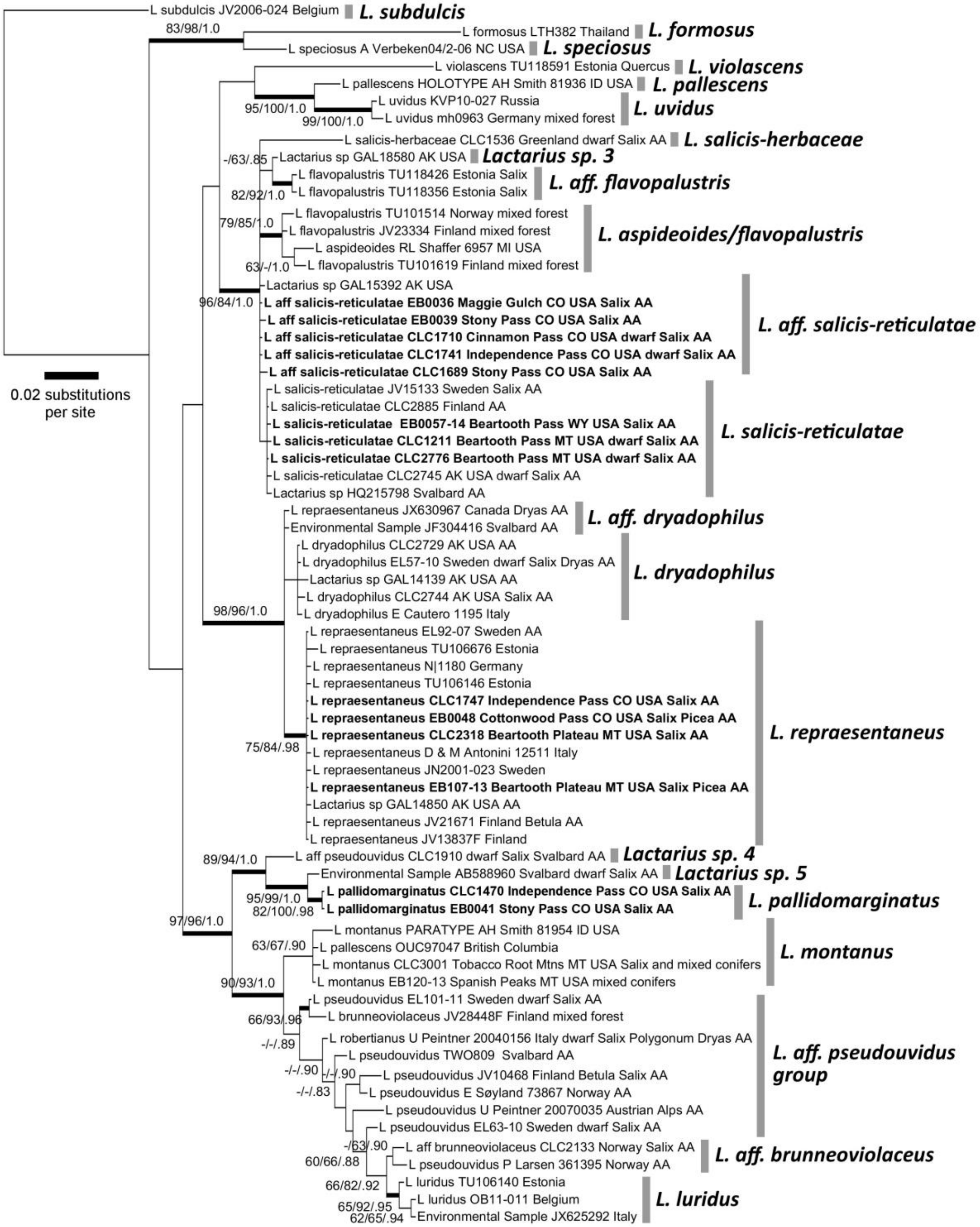


Figure 10. Bayesian 50% majority rule tree of section *Uvidi*/clade C combining ITS and *rpb2* DNA. Support values (BP/BP/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

Figure 11. ML tree of section *Uvidi*/clade C combining ITS and *rpb2* DNA. Support values (BS) ≥60% are indicated above or below branches leading up to clades. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

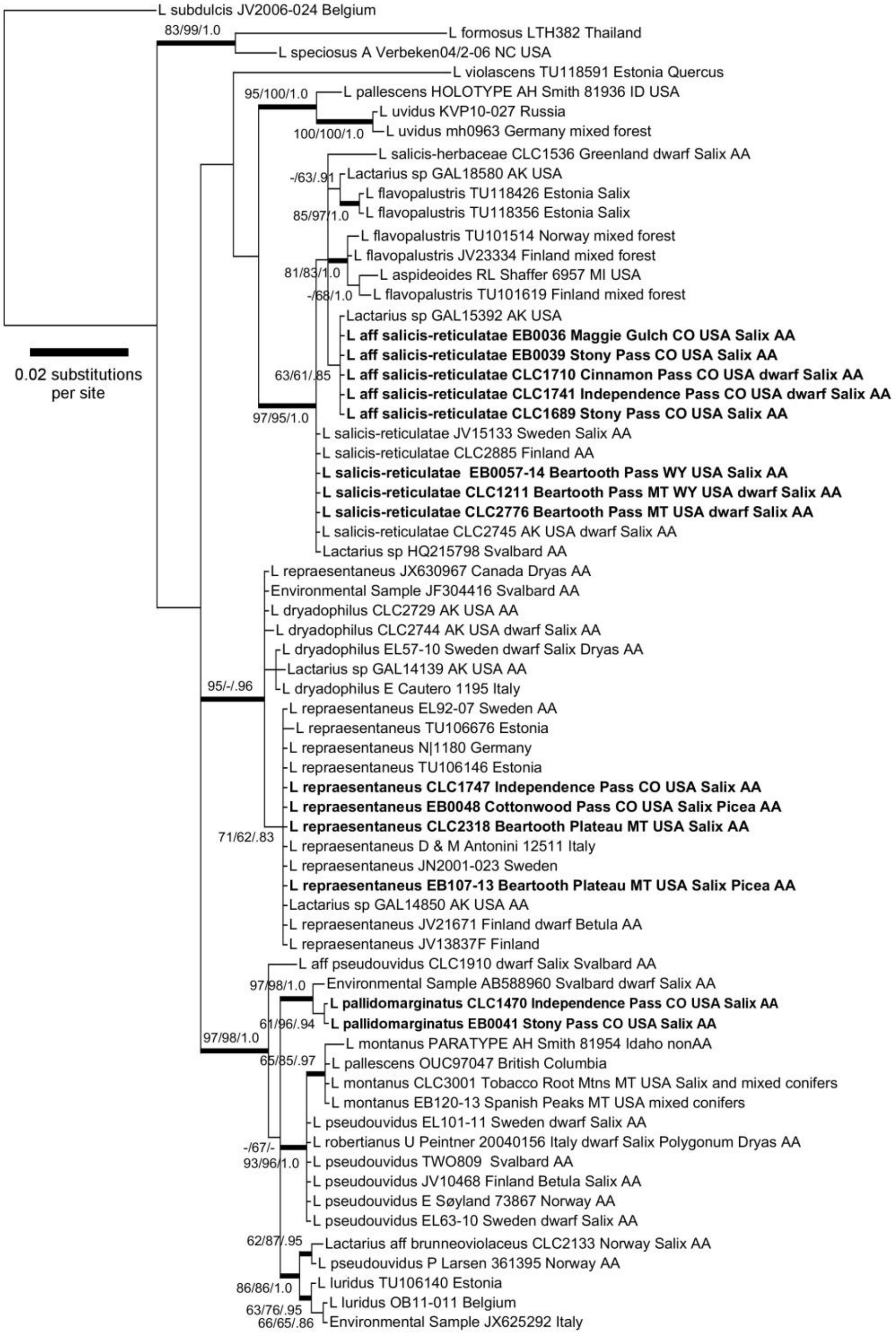


Figure 12. Bayesian 50% majority rule ITS tree of section *Uvidi*/clade C. Support values (BP/BS/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

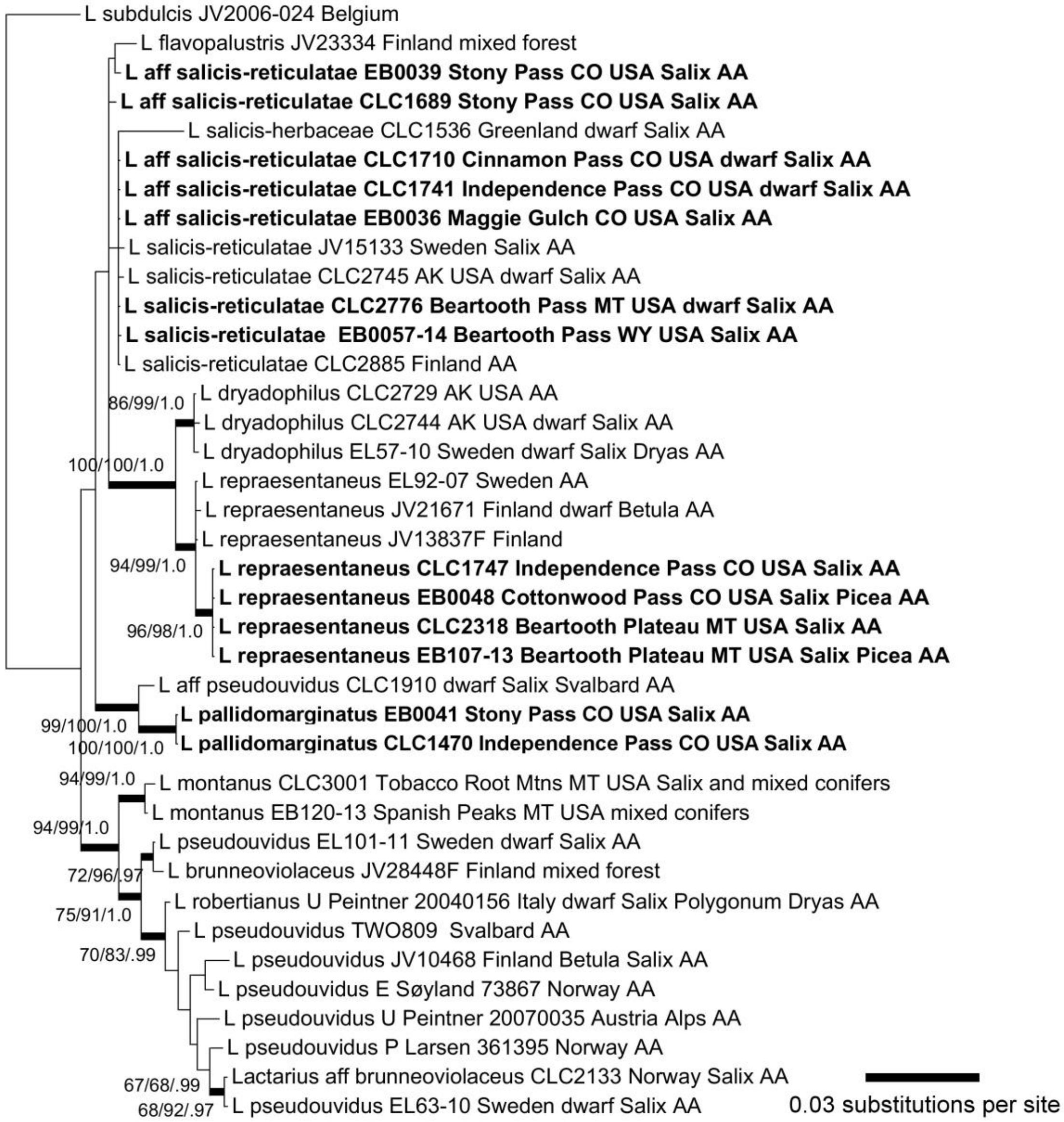


Figure 13. Bayesian 50% majority rule *rpb2* tree of section *Uvidi*/clade C. Support values (BP/BS/PP) ≥60% (BP/BS) and ≥0.80 (PP) are indicated above or below branches leading up to clades. Thickened branches lead up to clades receiving ≥75% bootstrap support (BP or BS) and Bayesian posterior probabilities (PP) ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.
(Verbeken et al. 2014).

these collections. In combined and *rpb2* analyses (Figures 10, 11, 13), these collections along with two collections identified as *L. brunneoviolaceus* and 1 collection identified as *L. robertianus* (considered synonymous of each other by some authors) form a polyphyletic gradient (indicated as *L.* aff. *pseudouvidus* group and *L.* aff. *brunneoviolaceus* in Figure 10) between an *L. montanus* clade (combined BP 63/BS 67/PP 0.90) and an *L. luridus* clade (combined BP 65/BS 92/PP 0.95, *rpb2* data missing for *L. luridus*). Clades emerging within this gradient generally do not receive strong support. In the ITS analyses, most collections in the gradient mentioned above occur in a well-supported clade (BP 93/BS 96/PP 1.0) paraphyletic with respect to *L. montanus*. This group includes a specimen matching *L. pseudouvidus* collected near the type locality (U Peintner 20070035, Austrian Alps) and is referred to here as the *L.* aff. *pseudouvidus* group. It appears likely that *L. brunneoviolaceus* (JV28448F) and *L. robertianus* (U Peintner 20040156) which fall out in this group are misidentified. Two collections (*L.* aff. *brunneoviolaceus* CLC2133 and *L. pseudouvidus* P Larsen 361395) occur in a strongly supported clade (BP 62/BS 87/PP 0.95) sister to *L. luridus* in the ITS analysis. This clade is also recovered in the combined analyses (BP<50/BS 63/PP 0.90). These specimens bare strong resemblance to *L. brunneoviolaceus* as described in Heilmann-Clausen et al. (1998), thus this clade is tentatively referred to as *L.* aff *brunneoviolaceus* and it is likely that the collection P Larsen 361395 is misidentified. In the *rpb2* analysis *L. pseudouvidus* (EL63-10) also occurrs within this clade, however morphologically it fits better with other members of the *L.* aff. *pseudouvidus* group.

Other species in this group which are morophologically somewhat similar to *L. pseudouvidus s.l.* and *L. pallidomarginatus* include *L. luridus* and *L. montanus*. While no collections in the *L. luridus* clade were personally examined, we tentatively assign the name to this clade as the image of TU106140 on the Unite database matches well with the morphological concept of this species and specimen OB11-011 was used in recent study by leading experts on *Lactarius* taxonomy (Verbeken et al. 2014). The clade designated as *L. montanus* clearly represents this species as paratype specimen AH Smith 81954 is included; the GenBank accession *L. pallescens* (DQ093852) occurring in this clade is clearly misidentified.

Lactarius uvidus and *L. pallescens* also bear morphological resemblance to *L. pseudouvidus s.l.* and *L. pallidomarginatus*. *Lactarius uvidus* forms a strongly supported clade (combined BP 99/BS 100/PP 1.0, ITS BP 100/BS 100/PP 1.0) (Figures 10, 11, 12) and although neither of the specimens in this clade were personally examined, both have been used in previous studies investigating *Lactarius* systematics and the molecular data were generated by leading experts on *Lactarius* taxonomy (Eberhardt & Verbeken 2004, Verbeken et al. 2014). The identity of the *L. pallescens* collection (AH Smith 81936) included in the phylogenetic analysis is accurate as it is the holotype specimen. Interestingly, while both *L. pallescens* and *L. uvidus* bear strong morphological affinities to *L. brunneoviolaceus s.l.*, *L. pallidomarginatus*, *L. pseudouvidus s.l.*, *L. luridus* and *L. montanus*, they are phylogenetically quite distant (with closer affinities to the *L. salicis-reticulatae*/*L. flavopalustris* group) and they lack the ITS1 insertion present in the other taxa.

GenBank ITS sequences, *L. nanus* (GU234088), *L. violascens* (GU234059) and *L. pseudouvidus* (GU234051), all from Svalbard, are 97.07—98.96% similar to *L. pallidomarginatus* (EB0041) (Table 5), however, they were not included because they are represented only by partial ITS sequences and in preliminary analyses they jumped around. However, in all preliminary analyses they were clearly distinct from *L. pallidomarginatus*. Unite ITS sequences of *L. brunneoviolaceus* (UDB018408, UDB018409), and *L. pseudouvidus* (UDB018414, UDB018415, UDB018416) were 97.33—98.08% similar to *L. pallidomarginatus* (EB0041) (Table 5), however they were locked and could not be included in phylogenetic analyses. Interestingly, a locked Unite ITS sequence, *L. aspideus* (UDB000925, collection location unknown) was 99.43% similar to *L. pallidomarginatus* (EB0041) (Table 5). Also, in preliminary analyses, ITS and *rpb2* data personally extracted from *L. aspideus* (JV24534) fell out very close to *L. pallidomarginatus* (not shown in final phylogenetic analyses). However, *Lactarius aspideus* is a large, yellow species, that is morphologically similar to other violet-staining, yellow taxa such as *L. flavopalustris* and *L. salicis-reticulatae*. This peculiarity should be examined in the future, however it is outside the scope of this study.

<u>Lactarius repraesentaneus</u> <u>and Allies.</u> In combined (ITS + *rpb2*) and ITS analyses (Figures 10, 11, 12), Rocky Mountain alpine collections identified as *L. repraesentaneus* occur in a moderately to strongly supported clade (combined BP 75/BS 84/PP 0.98, ITS

BP 71/BS 62/PP 0.83) with other collections identified as *L. repraesentaneus* from arctic-alpine and subalpine areas in Scandinavia, Estonia, Italy and Germany (the lectotype country for *L. repraesentaneus*), and an unidentified *Lactarius* sp. from arctic-alpine Alaska. This clade is confirmed to represent *L. repraesentaneus.* It is sister to a somewhat unresolved group containing collections identified as *L. dryadophilus* from arctic-alpine Alaska, Sweden and Italy, an accession apparently misidentified as *L. repraesentaneus* from arctic-alpine Canada with *Dryas integrifolia*, an environmental sequence from arctic-alpine Svalbard, and an unidentified *Lactarius* sp. from arctic-alpine Alaska. In the combined analyses (Figures 10, 11), *L. dryadophilus* from arctic-alpine Alaska, Sweden and Italy and the *Lactarius* sp. from Alaska form a poorly supported clade (BP<50/BS 51/PP 0.61). In the ITS analyses (Figure 12), only *L. dryadophilus* from Sweden and Italy and the *Lactarius* sp. from Alaska form a poorly supported clade (BP 54/PP 0.62, not supported in ML analysis) and the rest are unresolved. The clade containing these speciemens in the combined analyses (Figures 10, 11) is hypothesized to represent *L. dryadophilus*. The environmental sequence named *L. repraesentaneus* (JX630967) from Canada, isolated from the roots of *Dryas integrifolia* and the environmental sequence (JF304416) from Svalbard may be conspecific with *L. dryadophilus*, however they do not fall out with other collections identified as *L. dryadophilus* in combined or ITS analyses (*rpb2* data lacking), thus their identity cannot be confirmed. These accessions are labeled as *L.* aff. *dryadophilus* in Figure 10.

In the *rpb2* phylogenetic analyses (Figure 13), results are similar, however support for the separation of *L. repraesentaneus* and *L. dryadophilus* is stronger. Here, members of the *L. repraesentaneus* clade discussed above for which *rpb2* data was available form a strongly supported clade (BP 94/BS 99/PP 1.0) sister to a strongly supported clade (BP 86/BS 99/PP 1.0) with *L. dryadophilus* from arctic-alpine Alaska and Sweden.

Two other taxa worth mentioning due to their morphological similarity to *L. repraesentaneus* are *L. formosus* and *L. speciosus*. The relationships between *L. formosus*/*L. speciosus*, and *L. dryadophilus*/*L. repraesentaneus*, all of which have bearded pileus margins differed between analyses. In the combined Bayesian analysis (Figure 10), *L. formosus* and *L. speciosus* are basal to the rest of section *Uvidi*. In the combined ML analysis (Figure 11), they are basal to the *L.* aff. *pseudouvidus*/*L. montanus* group. In the broad analyses (Figure 3, Appendix C), they are sister to *L. repraesentaneus*/*L. dryadophilus*.

<u>*Lactarius salicis-reticulatae* and Allies.</u> In all analyses, Rocky Mountain alpine collections identified as *L. salicis-reticulatae* do not form a monophyletic group. In the combined analyses (Figures 10, 11), collections identified as *L. salicis-reticulatae* from the Beartooth Plateau form a poorly supported clade (BP<50/BS<50/PP 0.55) with a collection from the type locality of Mt. Laktatjäkko, Sweden, a collection from Finland, a collection from Alaska and a collection from Svalbard, all from arctic-alpine locations. This clade is hypothesized to represent *L. salicis-reticulatae*.

Collections initially identified as *L. salicis-reticulatae* from alpine areas in Colorado formed an unresolved group outside the above-mentioned clade in the combined Bayesian analysis (Figure 10) and are referred to as *L.* aff. *salicis-reticulatae*.. In the combined ML tree (Figure 11), results are similar, however here, Colorado collections from Stony Pass (EB0039, CLC1689) and the unidentified *Lactarius* from Alaska (GAL15392) form a separate poorly supported clade near the *L.* aff. *flavopalustris* and *L. aspideoides/flavopalustris* clades, while other Colorado collections form a sister clade to the core *L. salicis-reticulatae* clade.

In the ITS analyses (Figure 12), the Colorado collections and sequence from Alaska form a moderately supported clade (BP 63/BS 61/PP 0.86), while the core *L. salicis-reticulatae* clade mentioned above is paraphyletic and basal to a larger clade containing the *L.* aff. *flavopalustris* and *L. aspideoides/flavopalustris* clades and Colorado collections. In *rpb2* analyses (Figure 13), the members of the core *L. salicis-reticulate* clade form a poorly supported clade with some of the Colorado specimens, however, the two collections from Stony Pass fall outside this clade, with their placement varying between analyses.

Other taxa similar to *L. salicis-reticulatae* include *L. aspideoides*, *L. flavopalustris* and *L. salicis-herbaceae*. Although represented by only one specimen, *L. salicis-herbaceae* is clearly distinct from *L. salicis-reticulatae* in all analyses (Figures 10-13). In combined and ITS analyses (Figures 10-12), specimens identified as *L. flavopalustris* from Finland, the country from which the species was described, and Norway form a moderately to strongly supported clade (combined BP 79/BS 84/PP 1.0, ITS BP 81/BS 83/PP 1.0) along with *L. aspideoides.* In *rpb2* analyses (Figure 13), data were missing for two of the three collections of *L. flavopalustris* included in the other analyses as well as for *L. aspideoides*. Here, *L. flavopalustris* forms a poorly supported clade with one or both of the collections of *L.* aff. *salicis-reticulatae* from Stony Pass, Colorado, depending on the analysis.

Two other collections identified as *L. flavopalustris* from Estonia putatively associated with *Salix*, downloaded from the Unite database form a separate clade. In combined and ITS analyses (Figures 10-12) (*rpb2* data missing) these sequences form a well-supported clade (combined BP 82/BS 92/PP 1.0, ITS BP 85/BS 97/PP 1.0) sister to an unidentified ITS sequence from Alaska (GAL18580) (labeled as *Lactarius* sp. 3 in Figure 10).

Interestingly, *Lactarius aspideus*, which looks similar to and has traditionally been considered to be closely related to *L. salicis-reticulatae* appears fairly distantly related in preliminary analyses. In preliminary combined and single-gene analyses, *Lactarius aspideus* (JV24534) fell out close to Rocky Mountain alpine *L. pallidomarginatus*. At first it was thought that contamination was the cause of this unexpected result, however, this specimen was sequenced several times with the same result. This is interesting and bears further scrutiny, however it is outside the scope of the current study and for simplicity JV24534 was not included in the final phylogenetic analyses.

Taxonomy of Rocky Mountain Alpine *Lactarius*

All descriptions are based on Rocky Mountain alpine material.

Lactarius lanceolatus O.K. Mill. & Laursen Figures 3-7, 14-16, 34, 36

Canadian Journal of Botany 51: 43 (1973)

Diagnosis: *Lactarius lanceolatus* is recognized by its small to medium size and bright, light to deep orange pileus, pale cream to pale orange lamellae discoloring brownish in age or where damaged, pale orange stipe, watery, unchanging latex and mild taste; growing in arctic-alpine (possibly also subalpine) areas with *Salix* species.

Macromorphology: Pileus 10—45 mm in diameter, convex at first, becoming plane to infundibuliform with depressed center, with or without a small papilla, smooth, sometimes faintly scaly toward center, viscid, deep orange-brown to deep orange when immature becoming light to deep orange, often blotchy, often darker toward center; margin incurved to straight when immature, becoming straight to upturned and often slightly wavy when mature. Lamellae adnate to subdecurrent, slightly crowded, pale cream to pale yellow to pale orange, discoloring brownish orange in age or where damaged. Stipe 10—20 × 2.5—7.5 mm, equal to clavate, central, smooth, dry, at first covered by faint whitish pubescence, pale orange, discoloring dingy orange to dingy light brown where damaged, hollow. Latex scarce, watery, unchanging, mild. Context of pileus and stipe pale orange. Odor mild. Taste mild. Spore deposit not observed.

Micromorphology: Basidiospores 8—10 × 6—8 µm, average = 8.6 × 7 µm, Q = 1.14—1.38, Q average = 1.24, hyaline in 2.5% KOH, broadly ellipsoid; ornamentation of isolated warts connected by fine lines to subreticulate, up to 0.5 µm high. Basidia 38.1—66.0 × 8.9—10.2 µm, 4-spored. Pleuromacrocystidia 73.7—127 × 6.4—10.2 µm, narrowly fusiform to lanceolate, with acute to moniliform apex, thin-walled, scattered to abundant. Cheilomacrocystidia 35.6—89 × 5.1—10.2 µm, conical to fusiform, with acute to moniliform apex, thin-walled, sparse to abundant. Pileipellis an ixotrichoderm with well-developed gelatinous layer.

Habit, Habitat and Distribution: Scattered, growing singly or in small clusters. In alpine areas on the Beartooth Plateau, it is recorded with *Salix reticulata* and possibly other *Salix* species (*S. arctica*, *S. planifolia*). Collections from alpine areas on the Beartooth Plateau are molecularly very close to the holotype collection from Alaska and phylogenetic analyses further suggest its presence in Svalbard (Figures 4-6). It has previously been reported from Alaska (Miller & Laursen 1973, Laursen & Ammirati 1982, Heilmann-Clausen et al. 1998), Canada (Ohenoja & Ohenoja 1993, 2010), Greenland (Heilmann-Clausen et al. 1998, Borgen et al. 2006), Scandinavia (Bon 1998, Heilmann-Clausen et al. 1998, Knudsen & Vesterholt 1998), Svalbard (Gulden & Jenssen 1988, Geml et al. 2012), the British Isles (Watling 2001), and Siberia (Nezdoiminogo 1997, Heilmann-Clausen et al. 1998, Karatygin et al. 1999, Knudsen & Mukhin 1998) (Table 1), however, at least some of these reports are likely to be *L. aurantiacus*. *Lactarius lanceolatus* has not been reported from alpine areas in southern Rocky Mountains.

Host reports include dwarf *Salix spp.* as well as *Betula* (Table 1), however *Betula* is an unlikely host based on patterns seen in other arctic-alpine *Lactarius spp.*, where species associated with *Salix* do not associate with *Betula* and vice-versa.

Specimens Examined: U.S.A. ALASKA. North Slope County, Beaufort Lagoon, 30 July 1971 *F4239* (Holotype of *L. lanceolatus*, VPI). MONTANA. Carbon County, Beartooth Plateau, Highline Trail, 1 Aug 1997 *ZT6214* (MONT), 7 Aug 1998 *ZT6412* (MONT), 20 Aug 1999 *CLC 1389* (MONT), 5 Aug 2008 *CLC 2358* (MONT), date not available *CLC 1139* (MONT). WYOMING, Park County, Beartooth Plateau, Solifluction Terraces, 20 July 2001 *CLC 1578* (MONT), 13 Aug 2007 *CLC 2319* (MONT), 17 Aug 2013 *EB 105-13* (MONT). Rocky Mountain alpine collections of *L. lanceolatus* examined, with collection data and ectomycorrhizal host plants are shown in Table 6.

Similar Taxa Examined: DENMARK. Broby Vesterskov, 16 Oct 1997 *JHC97-251* (*L. aurantiacus*, C). NORWAY. Leikanger, 11 Sept 1999 *JHC00-057* (*L. aurantiacus*, C). Nordland, 14 Aug 1999 *JV15112F* (*L. aurantiacus*, misidentified as *L. lanceolatus*, TURA). Svalbard, Longyearbyen 18 Aug 2003 *CLC 1885* (*L. aurantiacus*, misidentified as *L. lanceolatus*, MONT). SWEDEN, Uppland, 8 Sept 1994 *JV94-422* (*L. aurantiacus*, C). U.S.A. ALASKA. Kenai Peninsula County, Palmer Creek Road, 7 Aug 2011 *CLC 2743* (*L. aurantiacus*, misidentified as *L. lanceolatus*, MONT). COLORADO. El Paso County, 27 Aug 2011 *DBG-F-022653* (*L. luculentus* v. *laetus*, DBG). Gilpin County, Perigo, 13 Aug 1974 *AH Smith 84923* (Paratype of *L. luculentus* v. *laetus*, MICH). Larimer County, 23 Aug 2008

DBG-F-024643 (*L. luculentus* v. *laetus*, DBG). OREGON. Polk County, 14 Nov 1970 *AH Smith 79943* (*L. luculentus*, MICH). Tillamook County, 12 Nov 1972 *AH Smith 83693* (MICH), 12 Nov 1972 *AH Smith 83694* (MICH). WASHINGTON. Lewis County, 30 Oct 1972 *AH Smith 83066* (Paratype of *L. subviscidus*, MICH), 1 Nov 1972 *AH Smith 83331* (Paratype of *L. subviscidus*, MICH). Tucannon River, 11 Oct 1980 *AH Smith 90905* (*L. luculentus*, MICH).

Comments: *Lactarius lanceolatus* belongs to a taxonomically problematic group of species with orange, orange-brown, or orange-red pilei. This complex includes *L. aurantiacus* (Pers.: Fr.) Gray (syn.: *L. mistissimus* (Fr.) Fr., *L. aurantiofulvus* J. Blum), *L. luculentus* Burl., *L. luculentus* v. *laetus* Hesler & A.H. Sm., *L. subflammeus* Hesler & A.H. Sm., *L. substriatus* A.H. Sm., and *L. subviscidus* Hesler & A.H. Sm. (See Table 7 for comparison of morphological and ecological features between these taxa).

Traditionally *L. lanceolatus* and *L. aurantiacus* have been separated based mainly on ecology, with *L. aurantiacus* being restricted to a variety of subalpine habitats, and *L. lanceolatus* being restricted to arctic-alpine habitats with *Salix spp*., however it is clear from the phylogenetic analyses in this thesis that *L. aurantiacus* also inhabits arctic-alpine habitats, likely associating with *Salix* as well as *Kobresia*. It is also clear that the ranges of the two species overlap at least in arctic-alpine areas in Alaska and Svalbard. Interestingly, *rpb2* data places the two species in the same clade, whereas ITS data separates them.

As for morphological differences, in subalpine areas *L. aurantiacus* can attain a larger size (pileus up to 90 mm diam, Basso 1999) than is reported for *L. lanceolatus* (pileus up to to 45 mm diam) which appears restricted to arctic-alpine areas. Within arctic-alpine areas, the two species appear to be morphologically very similar. It has been suggested that *L. lanceolatus* has longer macrocystidia and larger spores than *L. aurantiacus* (e.g. Heilmann-Clausen et al. 1998), however this does not appear to be the case. *Lactarius aurantiacus* collections (including arctic-alpine collections from Scandinavia and Svalbard misidentified as *L. lanceolatus*) measured in this study had pleuromacrocystidia ranging from 68.6—119.4 × 7.6—12.7 µm, with the longest pleuromacrocystidia measurement coming from a collection of *L. aurantiacus* (JV94-422) from subalpine Sweden. These measurements along with cheilocystidia measurements strongly overlap with those of *L. lanceolatus* including the holotype. Furthermore, spore sizes for *L. aurantiacus* ranged from 7—11.5 × 6—8 µm (average 8.7 × 6.9 µm) which overlaps and actually exceeds the upper limit measured for *L. lanceolatus.*

It has been suggested that *Lactarius lanceolatus* is often darker and more reddish, with more cracking of the pileus than *L. aurantiacus* (e.g. Heilmann-Clausen et al. 1998). In comparison of exsiccatae and images of fresh specimens this did not appear to be the case (see Figure 34 for images of fresh specimens of *L. lanceolatus* and *L. aurantiacus*). The pileus color of exsiccatae of both species was quite variable and overlapped in tone; the pileus color of young to medium aged basidiomes of *L.*

aurantiacus (CLC1885, misidentified as *L. lanceolatus*) from arctic-alpine Svalbard was actually darker red than any *L. lanceolatus* basidiomes examined. Overall, both species exhibit strong overlap in morphological features and no definitive macro- or micromorphological characters were found to separate them.

However, it was not possible to examine many of the collections in fresh condition and some potential differences that should be further investigated are suggested here. Based on personal observations of fresh collections from the Beartooth Plateau, as well as observations by others (Hesler & Smith 1979), *L. lanceolatus* can have a very well-developed gelatinous layer over the pileus cuticle, a feature lacking in descriptions of *L. aurantiacus* (e.g. Heilmann-Clausen et al. 1998). Also, *L. lanceolatus* appears to lack a regularly crenulate, striate pileus margin (not observed on fresh collections from the Beartooth Plateau or mentioned in the type description of Miller & Laursen 1973), a feature often but not always reported for *L. aurantiacus*. While pileus color can overlap, comparison of images of *L. lanceolatus* from the Beartooth Plateau with images of *L. aurantiacus* suggest that *L. lanceolatus* may generally have a brighter orange pileus than *L. aurantiacus*. Laursen & Ammirati (1982) report a *Lactarius* aff. *lanceolatus* from arctic-alpine Alaska growing near *Betula nana* and *Salix alaxensis* that could be *L. aurantiacus*. They state that it has a “dull burnt-orange” pileus as opposed to the “bright orange” pileus of *L. lanceolatus*. They also note a lighter colored stipe and “the lamellae, particularly when dried, are lighter and silver-blond in appearance.” Unfortunately, *L. aurantiacus* (CLC2743, misidentified as *L. lanceolatus*) from an alpine area with *Salix* in Alaska and appearing in the phylogenetic analysis was not observed in fresh condition, however, in exsiccatae, the lamellae were lighter than those of the *L. lanceolatus* holotype and *L. lanceolatus* from the Beartooth Plateau. The lamellae of other dried collections confirmed as *L. aurantiacus* from arctic-alpine areas, however, varied from lighter than or concolorous to the lamellae of dried *L. lanceolatus*.

Further comparison of fresh basidiomes of *L. lanceolatus* and *L. aurantiacus* along with sequence data is necessary for finding consistent morphological differences, if any exist between the two species. Further studies should be carried out to determine if they are independent species. Ecological differences, such as host requirements could also be further investigated. For example, *L. aurantiacus* is not restricted to arctic-alpine areas and appears to have a broader host range than *L. lanceolatus*, putatively associating with *Salix* and possibly other angiosperms as well as members of the Pinaceae. In arctic-alpine areas, examination of the hosts utilized by each species, perhaps through ectomycorrhizal root-tip sequencing could reveal any fine-scale ecological differences between them.

Lactarius luculentus is morphologically distinct from *L. lanceolatus* in having a browner pileus (ochraceous-tawny to ochraceous-buff to rich reddish cinnamon), a slowly acrid taste, and shorter cheilocystidia (23—45 × 4—6 µm) (Hesler & Smith 1979). However, pictures of this taxon in Bessette & Bessette (2009) depict a bright orange species that looks very similar to *L. lanceolatus*. It is clear that further comparison of morphological features with other taxa in this group is needed in order to make firm conclusions regarding morphological differences between species. Ecologically, *L. luculentus* is distinct from *L. lanceolatus* in that it inhabitats subalpine areas, and putatively associates with Douglas fir (Hesler & Smith 1979). In exsiccatae, basidiomes are paler, and more yellow-tan than those of *L. lanceolatus*. In phylogenetic analyses, *L. luculentus* was closely related to, but distinct from *L. lanceolatus* (Figures 4-6).

Lactarius luculentus v. *laetus* Hesler & A.H. Sm. is also very similar to *L. lanceolatus*, and comparison with a paratype collection (AH Smith 84923) from Colorado, other collections from Colorado (DBG-F-024643, DBG-F-022653), and the description in Hesler & Smith (1979), revealed that morphological differences between the species were subtle. In exsiccatae, collections of *L. luculentus* v. *laetus* had more crowded lamellae than *L. lanceolatus* and shorter (15—30 × 4—8 µm) cheilocystidia (also noted in Hesler & Smith 1979). Furthermore, its current species concept confines it to coniferous or alder habitats in western North America (Hesler & Smith 1979). Sequencing of the paratype collection from Colorado was unsuccessful, however other collections from Colorado that were sequenced formed a well-supported clade, closely related to but distinct from *L. lanceolatus* (Figures 4-7). It also appears that this variety should be raised to the species level, possibly as *L. laetus*, although further work is necessary to support this. Habitat seems the most useful method for separating *L. lanceolatus* from *L. luculentus* and *L. luculentus* v. *laetus*. Historically, *Lactarius luculentus* v. *laetus* differs from *L. luculentus* in having a bitter, rather than acrid taste, as well by its brighter colors (Hesler & Smith 1979), although further work is needed to clarify these taxa.

Lactarius substriatus differs morphologically from *L. lanceolatus* in having a redder pileus, that is glutinous when wet, with a translucent-striate margin, latex that slowly becomes straw-yellow, a slowly acrid taste, lamellae color that does not change in age or when damaged, shorter cheilocystidia and by its occurrence under conifers in western North America (Hesler & Smith 1979). *Lactarius subflammeus* can be morphologically separated from *L. lanceolatus* by the presence of short translucent striations along the pileus margin at maturity, scarlet fruiting bodies when young, slightly smaller spores, a slowly acrid taste, and occurrence in conifer forests in the Pacific Northwest (pers. obs. of holotype AH Smith 83602, Hesler & Smith 1979). *Lactarius subflammeus* appears to be conspecific with *L substriatus* based on specimens used in the phylogenetic analyses. The only major morphological difference between the taxa is the yellowing latex in *L. substriatus*, which appears to be a taxonomically uninformative character in this case, as it is apparently present in some collections and not others. As *L. substriatus* was described prior to *L. subflammeus*, *L. substriatus* should be the current name used for this species, although the holotype for *L. substriatus* still needs to be sequenced to confirm this. In phylogenetic analyses, *L. substriatus/subflammeus* was closely related to but distinct from *L. lanceolatus* (Figures 4-6). A GenBank sequence from a specimen identified as *L. substriatus* from California with *Quercus douglasii* (src438) was phylogenetically close to *L. lanceolatus* (Figures 4-

6), however, it was apparently misidentified based on its distance from the true *L. substriatus* clade. The identity of this species in unknown.

Lactarius subviscidus can be separated from *L. lanceolatus* by its mahogany-red to brick-red to onion-skin pink pileus that is rugulose when dry, a slightly acrid taste, its lamellae that do not discolor where damaged, a white latex that stains white paper yellow (not always), shorter cheilocystidia and its occurrence on coniferous debris and humus in conifer forests in western North America (Hesler & Smith 1979); in exsiccatae, basidiomes are much more brown than those of *L. lanceolatus*. For its phylogenetic position see Figures 4-6.

Lactarius duplicatus and *L. lapponicus* are somewhat similar to *L. lanceolatus*. They are synonymized under *L. duplicatus* by Heilmann-Clausen et al. (1998) and whether they are synonymous or not, both taxa are distinct from *L. lanceolatus* in having strongly yellowing latex. As yellowing latex appears to be variable between individuals in *L. substriatus*/*L. subflammeus*, it would be interesting to investigate these taxa in a molecular phylogenetic context, however, molecular data was lacking.

Two other species with morphological affinities to *L. lanceolatus* include *L. alpinus* Peck and *L. alpinus* v. *mitis* Hesler & A.H. Sm. *Lactarius alpinus* has a tomentose-squamulose, ochraceous or tawny-yellow pileus, yellowish lamellae, an acrid taste and smaller, inconspicuous pleurocystidia (Hesler & Smith 1979). *Lactarius alpinus* v. *mitis* has a vinaceous-brown (onion-skin pink) or fulvous-isabelline (light pinkish-cinnamon), strongly hygrophanous pileus, but has microscopic features similar to *L. lanceolatus*

(Hesler & Smith 1979). Furthermore, *Lactarius alpinus* and *L. alpinus* v. *mitis* appear to be strict associates of *Alnus* (Hesler & Smith 1979, Rochet et al. 2011). Molecular data are lacking for these taxa.

Interestingly, phylogenetic analyses recovered another species closely related to, but distinct from *L. lanceolatus*, isolated from *Dryas octopetala* in Norway, represented only by environmental sequences on GenBank (HQ445510, HQ4455527, Bjorbækmo et al. 2010) that may be an undescribed species (Figures 4-6). Some reports of *L. theiogalus* (a name commonly misapplied to yellow-orange to orange-brown *Lactarii*) from arctic-alpine areas (e.g. Kobayasi et al. 1968), likely represent *L. lanceolatus* (Gulden & Jenssen 1988) and possibly also *L. aurantiacus*.

Overall, more work is needed on defining species and finding morphological differences between species in the clade containing *L. substriatus*/*L. subflammeus*, *Lactarius* sp. 1, *L. lanceolatus*, *Lactarius* sp. 2, *L. luculentus*, *L. luculentus* v. *laetus* and *L. aurantiacus*. This research has made a start at showing phylogenetic differences between species, however, no fresh collections of species other than those of *L. lanceolatus* were personally examined and morphological comparisons were largely made with data obtained from the literature and dried material.

Table 6. Rocky Mountain alpine *Lactarius lanceolatus* collections examined, showing collection locations, putative host associations, and loci sequenced. Collections are designated with C.L. Cripps (CLC), E. Barge (EB), or E. Horak (ZT) collection numbers and are stored at MONT herbarium.

Voucher	**Date**	**Location**	**Host(s)**	**ITS**	***rpb2***
CLC1389	20 Aug 1999	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	*Salix reticulata*	Yes	Yes
CLC1578	20 July 2001	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	*Salix reticulata*	NA	NA
CLC2319	13 Aug 2007	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	*Salix reticulata*	Yes	Yes
CLC2358	5 Aug 2008	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	*Salix reticulata*	Yes	Yes
EB105-13	17 Aug 2013	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	*Salix planifolia*, *S. arctica*, *S. reticulata*	Yes	Yes
ZT6214	1 Aug 1997	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	Dwarf and shrubby *Salix*	NA	NA
ZT6412	7 Aug 1998	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	*Salix* spp.	NA	NA

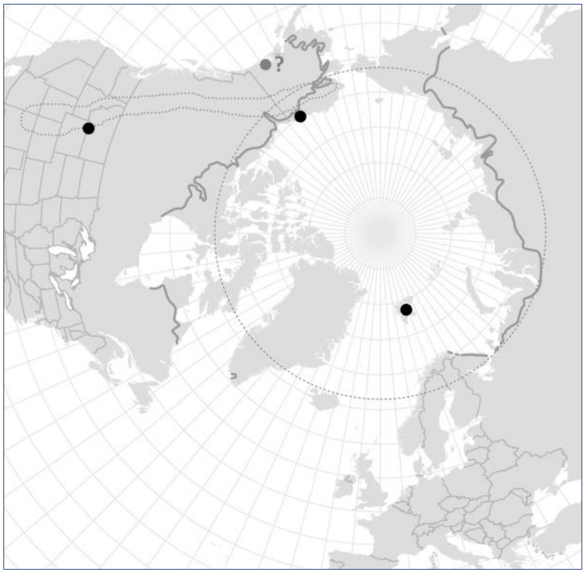


Figure 14. Confirmed distribution of *L. lanceolatus* based on available molecular data. Black dots indicate arctic-alpine sites, gray dots indicate non-arctic-alpine sites. The Rocky Mountains are outlined by a dotted gray line. The northern extent of trees is indicated by a thick gray line. Arctic circle = 67.5° N.

Figure 15. *L. lanceolatus* (EB105-13) growing with *Salix spp.*, Solifluction Terraces, Beartooth Plateau, Wyoming. Scale bar = 2 cm.

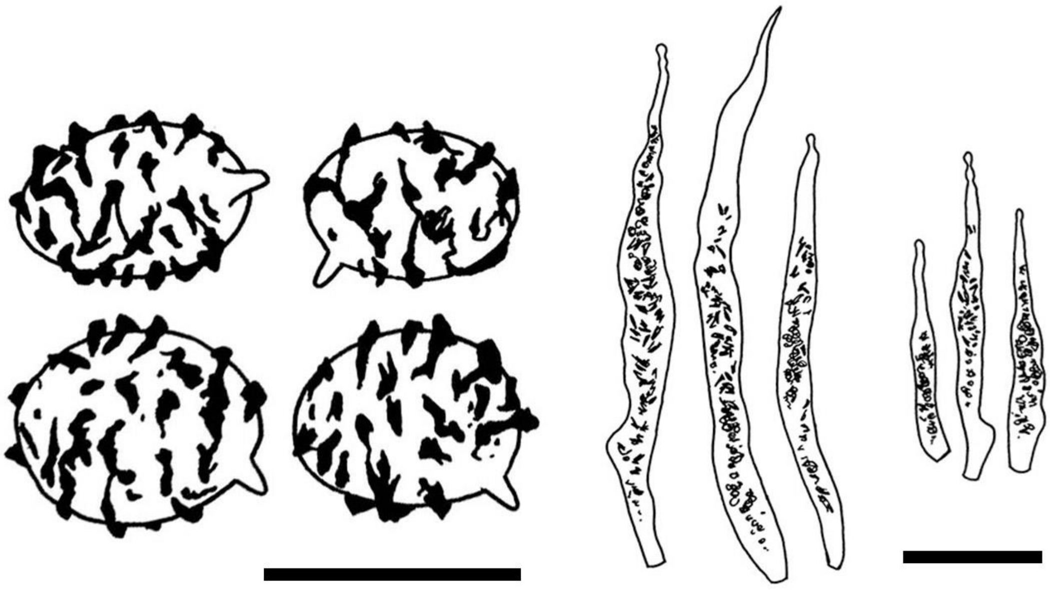


Figure 16. Spores, pleurocystidia (left) and cheilocystidia (right) of *L. lanceolatus* (CLC2319). Bars = 10 µm for spores and 20 µm for cystidia.

Table 7. Comparison of morphological features between *L. lanceolatus* and select closely related taxa. EUR = Eurasia, NA = North America, SVLBD = Svalbard, W. = Western, AK = Alaska, PNW = Pacific Northwest.

Taxon	**Spores**	**Pleurocystidia/ Cheilocystidia**	**latex**	**Pileus Characters**	**Distribution/ Ecology**
L. lanceolatus	8—10 × 6—8 µm	73.7—127 × 6.4—10.2 µm/ 35.6—89 × 5.1—10.2 µm	White, unchanging	Orange, margin smooth	SVLBD, NA; arctic-alpine, possibly subalpine; *Salix*
L. aurantiacus =*L. mitissimus*	7—11.5 × 6—8 µm	68.6—119.4 × 7.6—12.7 µm/ 40.6—91.4 × 6.4—10.2 µm	White, unchanging	Dull to bright orange, margin +/- crenulate	EUR, AK; subalpine/arctic-alpine. *Salix*, *Kobresia*, others?
*L. duplicatus**	6.4—10.0 × 5.6—7.4 µm	50—105 × 9—13 µm/ 25—70 × 5.5—8 µm	White, becoming sulphur yellow	Orange-brown to brick	EUR, NA; low arctic/boreal; *Betula*
*L. luculentus***	7.5—9 × 6—7.5 µm	(45)67—90 × (5)7.5—11 µm/ 23—45 × 4—6 µm	White, unchanging	Ochraceous tawny to rich reddish cinnamon	W. NA; subalpine; Douglas Fir
L. luculentus v. *laetus****	8—10 × 7—8.5 µm	56—90 × 9—14 µm/ 15—30 × 4—8 µm	White, unchanging	Orange	W. NA; subalpine; conifers
*L. substriatus/ subflammeus***	6.5—9 × 6—8 µm	(38)55—93 × (3)5—9(12) µm/ 30—45 × 3—6 µm	White, unchanging or becoming straw yellow	Scarlet to orange; margin translucent-striate at maturity	PNW of W. NA; subalpine; conifers
*L. subviscidus***	8—10 × 7—8 µm	60—75 × 7—10 µm/ 37 × 5 µm	White, unchanging	Dark mahogany-red to onion-skin pink	PNW of W. NA; conifers
Lactarius sp. 1	NA	NA	NA	NA	SVLBD, AK?; arctic-alpine, subalpine?; *Dryas octopetala*
Lactarius sp. 2	NA	NA	NA	NA	California; subalpine; *Quercus*

*Data from Heilmann-Clausen et al. (1998)

**Data from Hesler & Smith (1979)

***Data from this study and Hesler & Smith (1979)

<u>*Lactarius nanus* J. Favre</u> Figures 3, 8, 9, 17-20, 34, 36

Ergebnisse der wissenschaftlichen Untersuchungen des schweizerischen National Parks 5: 205 (1955)

<u>Diagnosis:</u> *Lactarius nanus* is recognized by its typically small size, its gray-brown to liver-brown pileus typically with a glaucous coating at least when young, watery, unchanging latex, mild odor, mild to slightly acrid taste and occurrence with *Salix* species in arctic-alpine areas.

<u>Macromorphology:</u> Pileus 10—50 mm in diameter, shallowly convex to plano-convex becoming plane to infundibuliform with or without a depressed center and small papilla, smooth, slightly viscid to dry, with whitish glaucous coating when immature, easily rubbing away or fading in age, often in patches, deep brown to gray-brown to liver brown with lighter and darker areas present, often lighter toward margin, becoming lighter overall upon dessication; margin straight when immature becoming upturned and wavy to convoluted when mature. Lamellae adnate to subdecurrent, subdistant to slightly crowded, cream when immature becoming dingy cream to light tan in age, sometimes with a faint pinkish tinge, discoloring to dingy tan where damaged. Stipe 5—30 × 3—15 mm, equal to clavate, central, smooth, dry, at first covered with whitish glaucous coating as in pileus, pale apricot to dingy cream-tan, frost line sometimes visible on upper stipe, hollow. Latex scarce, watery, unchanging, mild.

Context of pileus and stipe dingy cream to brownish. Odor mild. Taste mild to slightly acrid. Spore deposit not observed.

Micromorphology: Basidiospores 7—10.5(—12) × 5—8 µm, average = 8.4 × 6.6 µm, Q = 1.07—1.60, Q average = 1.27, hyaline in 2.5% KOH, subglobose to ellipsoid; ornamentation of scattered ridges and warts forming an incomplete reticulum with some completely reticulate areas, to 0.7 µm high. Basidia 40.6—76.2 × 8.9—15.2 µm, mostly 4-spored, some 2-spored. Pleuromacrocystidia 53.3—96.5(—114.3) × 6.4—11.4 µm, lanceolate, with acute to moniliform apex, scattered to abundant. Cheilomacrocystidia 26.0—91.4 × 7.6—10.2 µm, subulate to linear with undulating margin, with acute to rounded apex, scattered to abundant. Pileipellis an (ixo-) cutis with some ascending hyphae embedded in a very faint gelatinous layer.

Habit, Habitat and Distribution: *Lactarius nanus* is one of the more commonly encountered *Lactarius* species in alpine areas of the central and southern Rocky Mountains, where it is found growing singly or in small clusters with *Salix arctica*, *S. reticulata* and *S. planifolia*. Rocky Mountain alpine collections identified as *L. nanus* morphologically match the holotype specimen (ZA192c, sequencing unsuccessful), collected with *Salix herbacea* in the Swiss Alps and form a clade with a specimen from the type locality (French Alps). Phylogenetic analyses further confirm its presence in arctic-alpine areas of Svalbard and Scandinavia, putatively associated with *S. polaris* and possibly other *Salix* species (Figures 3, 8, 9). It has previously been reported from arctic-alpine areas in Canada (Ohenoja & Ohenoja 1993, 2010), Greenland (Knudsen & Borgen 1982, Lamoure et al. 1982, Heilmann-Clausen et al. 1998, Borgen et al. 2006), Iceland (Eyjolfsdottir pers. comm., Knudsen & Vesterholt 2008), the European Alps (Favre 1955, Kühner 1975, Kühner & Lamoure 1986, Senn-Irlet 1993, Bon 1985a, 1998, Bon & Ballará 1996, Heilmann-Clausen et al. 1998, Jamoni 1995), the Carpathians (Ronikier 2008), Scandinavia (Kühner 1975, Gulden et al. 1985, Heilmann-Clausen et al. 1998, Knudsen & Vesterholt 2008), Svalbard (Gulden 2005), Siberia (Nezdoiminogo 1997), and alpine areas in the Altai Mountains (Karatygin et al. 1999) with *Salix* (Table 1); but these reports are not yet molecularly confirmed. Phylogenetic analyses suggest that the only report from Alaska (Geml et al. 2009) is actually *L. glyciosmus*.

Specimens Examined: FRANCE. Savoie, 24 Aug 1989 *Bon 89093* (LIP). NORWAY. Finse, 5 Aug 2010 *CLC 2134* (MONT). Svalbard, Longyearbyen, 20 Aug 2002 *CLC 1896* (MONT). SWEDEN. Torne Lappmark, 15 Aug 1999 *JV15148* (TURA). SWITZERLAND. Grisons, 20 Aug 1943 *ZA192c* (Holotype of *L.nanus*, G).U.S.A. COLORADO. Pitkin County, Sawatch Range, Independence Pass, 6 Aug 2000 *CLC 1471* (MONT). San Juan County, San Juan Range, Black Bear Pass, 11 Aug 2001 *CLC 1716* (MONT), 11 Aug 2001 *ZT9529* (MONT). Cinnamon Pass, 27 July 2002 *CLC 1801* (MONT), 15 Aug 2012 *EB138* (MONT). San Miguel County, San Juan Range, Imogene Pass, 29 July 2002 *CLC 1829* (MONT). MONTANA. Carbon County, Beartooth Plateau, Highline Trail, 7 Aug 1998 *CLC 1221* (MONT), 8 Aug 1998 *ZT6422* (MONT), 18 Aug 2013 *EB106-13* (MONT). WYOMING. Park County, Beartooth Plateau, Frozen Lakes, 21 Aug 1999 *CLC 1403* (MONT). Solifluction

Terraces, 11 Aug 2012 *EB125* (MONT). Rocky Mountain alpine collections of *L. nanus* examined, with collection data and ectomycorrhizal host plants are shown in Table 8.

Similar Taxa Examined: FINLAND. Koillismaa, 28 Aug 2011 *JV28432* (*L. hysginoides*, TURA).

Comments: Species similar to *L. nanus* include *L. hysginoides* Korhonen & T. Ulvinen, *L. glyciosmus* (Fr.) Fr. *sensu lato* (*s.l.*), *L. mammosus* Fr., *L. pallidomarginatus* Barge & C.L. Cripps *ad int.*, *L. brunneoviolaceus* M.P. Christ. (Syn.: *L. robertianus* Bon), *L. pseudouvidus* Kühner *sensu lato* (*s.l.*) and *L. uvidus* fo. *alpigines* Jamoni & Bon (See Table 10 for comparison of morphological and ecological features between *L. nanus*, *L. hysginoides*, *L. glyciosmus* and *L. mammosus*).

Lactarius hysginoides can be morphologically distinguished from *L. nanus* by a more cinnamon-toned pileus and smaller spores (Heilmann-Clausen et al. 1998; personal measurement of *L. hysginoides* (JV28432) spores = 6—8 × 5—7 µm, ave 6.8 × 5.7 µm). *Lactarius hysginoides* is generally reported from boreal areas with *Betula*, *Picea* and *Salix* (Heilmann-Clausen et al. 1998), however its range likely overlaps with that of *L. nanus* in alpine-subalpine transition areas. *Lactarius nanus* and *L. hysginoides* are very closely related; *L. hysginoides* is nested within *L. nanus* in combined and ITS analyses (Figures 8, 9), and distinct (with low support) in *rpb2* analyses (Figure 9). *Lactarius hysginoides* has not been reported from the Rocky Mountains.

Lactarius glyciosmus is a similar taxon which occurs in the Rocky Mountain alpine zone and other arctic-alpine areas, however it has an odor of coconut, smaller spores, smaller and differently shaped macrocystidia, and it associates with *Betula* (pers. obs. and Heilmann-Clausen et al. 1998). According to phylogenetic analyses in this thesis, the only previously published report of *L. nanus* from Alaska (*Lactarius* cf. *nanus* GAL8739) in Geml et al. (2009) is actually *L. glyciosmus* (Figures 8, 9).

However, collections identified as *Lactarius mammosus* (GG415-86) and *L. uvidus* (O73621), reported from Svalbard in Geml et al. (2012) and appearing in the phylogenetic analysis (Figures 8, 9) are actually *L. nanus*. *Lactarius mammosus* is a more robust species with a coconut odor (Heilmann-Clausen et al. 1998) that associates with members of the Pinaceae (see comments under *L. glyciosmus* in the following section) and is phylogenetically distinct (Figures 8, 9). *Lactarius uvidus* is a more robust, violet-staining species with larger (typically), differently ornamented spores (Heilmann-Clausen et al. 1998). Other violet-staining species which are similar to *L. nanus* include *L. pallidomarginatus*, *L. brunneoviolaceus* (syn.: *L. robertianus*), *L. pseudouvidus s.l.* and *L. uvidus* fo. *alpigines.* These species typically stain violet, wherease *L. nanus* does not, and they have different microscopic features (pers. obs. and Kühner 1975, Bon 1985b, Jamoni & Bon 1991, Heilmann-Clausen et al. 1998).

Table 8. Rocky Mountain alpine *Lactarius nanus* collections examined, showing collection locations, putative host associations, and loci sequenced. Collections are designated with C.L. Cripps (CLC), E. Barge (EB), or E. Horak (ZT) collection numbers and are stored at MONT herbarium.

Voucher	**Date**	**Location**	**Host(s)**	**ITS**	***rpb2***
CLC1221	7 Aug 1998	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	Shrubby and dwarf *Salix*	NA	NA
CLC1403	21 Aug 1999	Frozen Lakes, Beartooth Plateau, WY, U.S.A.	*Salix arctica*	Yes	Yes
CLC1471	6 Aug 2000	Independence Pass, Sawatch Range, CO, U.S.A.	NA	Yes	Yes
CLC1716	11 Aug 2001	Black Bear Pass, San Juan Range, CO, U.S.A.	*Salix arctica*	Yes	Yes
CLC1801	27 July 2002	Cinnamon Pass, San Juan Range, CO, U.S.A.	*Salix arctica*	Yes	Yes
CLC1829	29 July 2002	Imogene Pass, San Juan Range, CO, U.S.A.	*Salix arctica*	Yes	Yes
EB106-13	18 Aug 2013	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	*Salix planifolia*, *S. arctica*, *S. reticulata*	Yes	Yes
EB125	11 Aug 2012	Solifluction Terraces, Beartooth Plateau, WY, U.S.A.	*Salix planifolia*, *S. arctica*, *S. reticulata*	NA	Yes
EB138	15 Aug 2012	Cinnamon Pass, San Juan Range, CO, U.S.A.	*Salix arctica*	Yes	Yes
ZT6422	8 Aug 1998	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	*Salix reticulata* and *Salix* spp.	NA	NA
ZT9529	11 Aug 2001	Black Bear Pass, San Juan Range, CO, U.S.A.	*Salix reticulata*	NA	NA

Figure 17. *L. nanus* (EB106-13) growing with *Salix* spp., Highline Trail, Beartooth Plateau, Montana/Wyoming. Note frost line mark on stipe. Scale bar = 2 cm.

Figure 18. *L. nanus* (EB138) growing with *Salix arctica*, Cinnamon Pass, San Juan Range, Colorado. Scale bar = 2 cm.

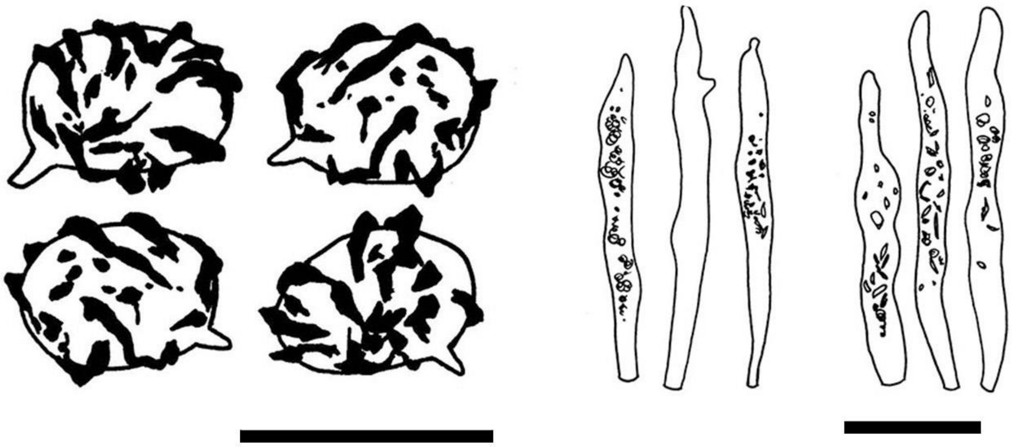


Figure 19. Spores, pleurocystidia (left) and cheilocystidia (right) of *L. nanus* (CLC1471). Bars = 10 µm for spores and 20 µm for cystidia.

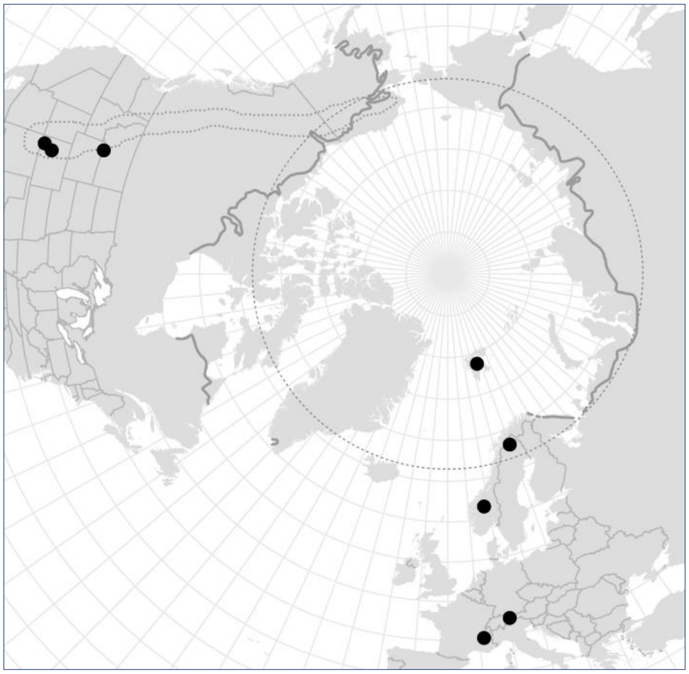


Figure 20. Confirmed distribution of *L. nanus* based on available molecular data. Black dots indicate arctic-alpine sites. The Rocky Mountains are outlined by a dotted gray line. The northern extent of trees is indicated by a thick gray line. Arctic circle = 67.5° N.

<u>*Lactarius glyciosmus* (Fr.) Fr.</u> Figures 3, 8, 9, 21-23, 34, 36

Epicrisis Systematis Mycologici (Upsaliae): 348 (1838) [1836-1838]

<u>Diagnosis:</u> *Lactarius glyciosmus* is recognized by its small to medium size, pale gray-brown to mauve pileus, typically with a glaucous coating at least when young, watery, unchanging latex, odor of coconuts and association with *Betula.*

<u>Macromorphology:</u> Pileus 15—50 mm in diameter, broadly convex, later becoming plane with or without a depressed center and small papilla, smooth, dry, pale gray-brown to mauve (violet-brown) with whitish glaucous coating when immature, fading in age, azonate to lightly zoned especially near margin; margin incurved when young and remaining so or becoming straight when mature. Lamellae adnate to subdecurrent, slightly crowded to crowded, cream to pale pinkish to pale yellow-orange. Stipe 10—40 × 3—12 mm, equal to slightly clavate, often curved, central to eccentric, smooth, dry, buff or pale salmon with faint glaucous coating at first as in pileus, stuffed or hollow, often white-mycelioid toward base. Latex scarce, watery, unchanging. Context of pileus and stipe pale cream. Odor of coconut. Taste mild. Spore deposit not observed.

<u>Micromorphology:</u> Basidiospores 7—9 × 5—7 µm, average = 7.7 × 6.0 µm, Q = 1.17—1.42, Q average = 1.28, hyaline in 2.5% KOH, broadly ellipsoid to ellipsoid; ornamentation of scattered ridges and warts forming an incomplete to complete reticulum in places, to 0.5 µm high. Basidia 38.1—63.5 × 7.6—10.2 µm, mostly 4-

spored, some 2-spored. Pleuromacrocystidia 45.7—63.5 × 6.4—7.6 µm, subclavate to lanceolate, with rounded to occasionally mucronate apex, scarce to scattered. Cheilomacrocystidia 33—66 × 5.1—8.9 µm, cylindric to subclavate, with rounded apex, scattered to abundant. Pileipellis a cutis with tufts of ascending hyphae.

Habit, Habitat and Distribution: Scattered to gregarious, growing singly or in small clusters. In subalpine and low alpine areas of the central and southern Rocky Mountains, *L. glyciosmus* appears to associate with *Betula glandulosa*. The taxon also occurs in subalpine and arctic-alpine areas elsewhere in North America as well as Eurasia with *Betula* (Hesler & Smith 1979, Heilmann-Clausen et al. 1998). It was described from the Femsjö region of Sweden (Fries 1838) but lacks a type specimen. It has also been introduced with *Betula* into areas in the southern hemisphere such as New Zealand (McNabb 1971). Previous reports from arctic-alpine areas include the central and southern Rocky Mountains (Cripps & Horak 2008, Cripps & Barge 2013), Canada (Ohenoja & Ohenoja 1993, 2010), Greenland (Lange 1957, Knudsen & Borgen 1982, Lamoure et al. 1982, Elbourne & Knudsen 1990, Borgen 2006, Borgen et al. 2006), Iceland (Eyjolfsdottir pers. comm.), the European Alps (Bon 1998), Scandinavia (Kallio & Kankainen 1964, Kühner 1975, Bon 1998), Svalbard (Ohenoja 1971, Geml et al. 2012), and Siberia (Karatygin et al. 1999) with *Betula* and possibly also *Alnus* and *Salix* species (Table 1) although the latter are unlikely hosts.

Based on phylogenetic analyses in this study, *L. glyciosmus s.l.* is polyphyletic and actually represents at least two species. Rocky Mountain alpine collections occur in a well-supported clade with specimens from the type locality and thus are considered to represent the actual *L. glyciosmus*, which appears to occur at least in subalpine and arctic-alpine areas elsewhere in North America, Svalbard, and Scandinavia. The other clade (*L.* aff. *glyciosmus*) occurs in subalpine areas in Alaska, Italy, Estonia and Japan, although its range may be much broader (Figures 8, 9).

How the distribution and ecology of the two species that fall under the name *L. glyciosmus* differs is unclear. However, it does appear possible that 1) *L. glyciosmus* occurs in the Rocky Mountain alpine zone and *L.* aff. *glyciosmus* is absent, 2) that the ranges of the two species may not overlap in the USA outside of Alaska and 3) that *L.* aff. *glyciosmus* may be restricted to subalpine areas, whereas *L. glyciosmus* occupies both subalpine and arctic-alpine areas, potentially due in part to host species differences. *Betula platyphylla* is a confirmed host for *L.* aff. *glyciosmus* in Japan (Hashimoto et al. 2012); Estonian collections analyzed in this study were likely associated with *B. pendula* or *B. pubescens* as they are the only *Betula* species present at the collection locations (Irja Saar pers. comm.); and *Betula neoalaskana* is a possible host in Alaska (Geml et al. 2009). *Lactarius glyciosmus sensu stricto* appears to associate with *Betula glandulosa* in the Rocky Mountain alpine zone, is confirmed with *B. nana* in Alaska (GU998223, GU998695), is suspected with *B. nana* in Finland and is confirmed with either *Alnus rhombifolia* or *B. occidentalis* in Idaho (JX198526, Bogar & Kennedy 2013), with *B. occidentalis* being the more likely host. Unfortunately, definitive host data is lacking for environmental sequences and vouchers for members of the *L. glyciosmus* clade from

British Columbia, Wisconsin, New Hampshire, Svalbard and Sweden, thus potential differences in host requirements of the two species cannot be further explored at this time. In order to further investigate the distribution and ecology of each species, sequencing should focus on collections from a broader geographic area than included in this study; areas where *L. glyciosmus* has been reported but not sequenced, such as northern Canada, Greenland and Russia should be targeted. Furthermore, detailed host data should be recorded for each collection, including the sequencing of ectomycorrhizal root tips.

Specimens Examined: FINLAND. Fjell Ailigas, 27 Aug 2012 *CLC 2874* (MONT). SWEDEN. Femsjö, 13 Aug 1978 *M. Moser 19780191* (Type locality, IB), 16 Aug 1978 *M. Moser 19780234* (Type locality, IB), 9 Aug 1981 *M. Moser 19810148* (Type locality, IB). U.S.A. COLORADO. Boulder County, Front Range, Blue Lake, 2 Aug 2001 *CLC 1624* (MONT). Brainard Lake, 13 Aug 2000 *20923* (DBG). San Juan Range, Ironton Park, 13 Aug 2012 *EB133* (MONT). MONTANA. Carbon County, Beartooth Plateau, Birch Site, 29 July 1997 *CLC 1134 = ZT6096* (MONT), 8 Aug 1998 *CLC 1217* (MONT), 10 Aug 1999 *TWO 269* (MONT), 19 Aug 1999 *CLC 1380* (MONT), 13 Aug 2007 *ZT12723* (MONT). Rocky Mountain alpine collections of *L. glyciosmus* examined, with collection data and ectomycorrhizal host plants are shown in Table 9.

Similar Taxa Examined: ESTONIA. Saare Co., Lümanda Comm., 17 Sept 2012 *TU118535* (*L.* aff. *glyciosmus*, TU). Tartu Co., Võnnu Comm., 12 Sept 2010 *TU106737* (*L.* aff. *glyciosmus*, TU), 13 Sept 2010 *TU106724* (*L.* aff. *glyciosmus*, TU).

Comments: As mentioned previously, *L. glyciosmus s.l.* is polyphyletic and likely contains two species (Figures 8, 9). One of the clades contains specimens from the type locality, thus the name *L. glyciosmus* can be reliably applied to this clade. This clade also contains Rocky Mountain alpine collections.

The original description of *L. glyciosmus* is quite broad and lacking in detail (Fries 1838). Modern descriptions depict a variable mushroom with a velutinous to squamulose pileus with colors ranging from “pale to dark pinkish buff, grayish pink, clay-pink, dark grayish buff, smoke-grey, grayish brown or brownish olive” (Heilmann-Clausen et al. 1998) or “cream to pinkish-gray, pale grayish-brown tinged with lilac, brown when moist” (Basso 1999) or “buttons vinaceous-gray retaining this color more or less and drying between vinaceous-buff and avellaneous (pinkish gray) (Hesler & Smith 1979). All descriptions mention a distinct odor of coconut. The descriptions of *L. glyciosmus* cited above are based on material from Europe (Heilmann-Clausen et al. 1998, Basso 1999) and Alaska (Hesler & Smith 1979) where both species appear to occur, thus it is quite possible that these descriptions are based on both species.

Based on comparison of microscopic and macroscopic morphological features no definitive differences were detected between *L. glyciosmus* and *L.* aff. *glyciosmus*. The pilei of fresh specimens confirmed as *L. glyciosmus* were dark (pale gray-brown to mauve) at all stages of maturity. Images and exsiccatae of *L.* aff. *glyciosmus* (TU106737, TU106724, TU118535) from Estonia are typically lighter in color suggesting the possibility that pileus color could be a distinguishing feature between the two species. However, the pilei of young specimens of *L.* aff. *glyciosmus* (TU118535) shown on Unite are quite dark and exsiccatae of *L. glyciosmus* from the type locality (M. Moser 19810148, 19780234, 19780191) were fairly light. Images in Dähncke (1993) show the pilei of individual basidiomes within one collection of *L. glyciosmus* (molecular data lacking) varying from dark when young to light at maturity. Microscopic differences between *L. glyciosmus* and *L.* aff. *glyciosmus* were not detected.

Until more work is conducted, a new name is not suggested for the *L.* aff. *glyciosmus* clade. Macro- and micromorphological features of each species should be examined in detail in order to ascertain morphological differences between the two species, if any exist. Data suggest the possibility that the two putative species occupy different ecological niches and may not overlap in reference to the species of *Betula* with which they associate. Detailed observation of habitat characteristics and sequencing of ectomycorrhizal root-tips could reveal fine-scale ecological differences between them and help better distinguish them. Furthermore, it is possible that the two clades are not actually distinct species, but rather two groups containing divergent ITS alleles. Future work should also focus on sequencing more loci to test this.

Other species similar to *L. glyciosmus* include *L. mammosus* (syn.: *L. fuscus* Rolland), *L. vietus* (Fr.) Fr., *L. trivialis* (Fr.) Fr. and *L. nanus* (See Table 10 for comparison of morphological and ecological features between *L. nanus*, *L. hysginoides*, *L. glyciosmus* and *L. mammosus*). *Lactarius mammosus* can be separated from *L. glyciosmus* as it has a browner pileus, stouter stature and more elongate, more reticulate spores (Heilmann-Clausen et al. 1998). Sequences isolated from ectomycorrhizal root tips of *Pinus sylvestris* (AJ633116, Heinonsalo et al. 2007) and *Picea abies* (FM992937, Kjøller & Clemmensen 2009) and appearing in the phylogeny confirm *L. mammosus*' association with members of the Pinaceae (Figures 8, 9), however it has also been reported with *Betula* (Heilmann-Clausen et al. 1998). *Lactarius mammosus* is phylogenetically distinct from *L. glyciosmus* (Figures 8, 9), it has not been reported from the Rocky Mountains and it may not even occur in North America (Hesler & Smith 1979).

Lactarius vietus differs from *L. glyciosmus* in having a distinct pale zone near the stipe apex, latex that dries greenish gray, longer pleurocystidia and a mild to slightly fruity odor (Heilmann-Clausen et al. 1998). It is phylogenetically distinct from *L. glyciosmus* (Figure 3) and has not been reported from alpine areas in the Rocky Mountains, although it has been reported from elsewhere (mostly northern areas) in North America with *Betula* (Hesler & Smith 1979).

Lactarius trivialis, another similar species, is also phylogenetically distinct from *L. glyciosmus* (Figure 3) and can be morphologically separated as it is typically much larger (pileus to 150 mm diam) and has distinct vinaceous to vinaceous-brown dots on the pileus when young, pale cream latex turning orange-yellow in KOH and it lacks an odor of coconut (Heilmann-Clausen et al. 1998). *Lactarius trivialis* has not been reported from alpine areas in the Rocky Mountains, although it is reported from conifer forests in western North America (Alaska, Idaho, Washington) (Hesler & Smith 1979).

Interestingly, *L. spinosulus* Quél. is phylogenetically somewhat close to *L. glyciosmus* (Figures 3, 8, 9), however, morphologically they are very different. *Lactarius spinosulus* has a distinctly zoned, coarsely squamulose, pinkish to salmon to brick colored pileus with a finely hairy margin and fruity odor (Heilmann-Clausen et al. 1998). See comments under *L. nanus* for distinguishing features between *L. nanus* and *L. glyciosmus*.

Table 9. Rocky Mountain alpine *Lactarius glyciosmus* collections examined, showing collection locations, putative host associations, and loci sequenced. Collections are designated with C.L. Cripps (CLC), T.W. Osmundson (TWO), or E. Horak (ZT) collection numbers and are stored at MONT herbarium. Voucher 20923 was colleted by V. Evenson and is stored at DBG herbarium.

Voucher	**Date**	**Location**	**Host(s)**	**ITS**	***rpb2***
20923	13 Aug 2000	Brainard Lake, Front Range, CO, U.S.A.	*Salix*, *Betula*	Yes	Yes
CLC1134 = ZT6096	29 July 1997	Birch Site, Beartooth Plateau, MT, U.S.A.	*Betula glandulosa*	NA	NA
CLC1217	8 Aug 1998	Birch Site, Beartooth Plateau, MT, U.S.A.	*Betula glandulosa*	NA	NA
CLC1380	19 Aug 1999	Birch Site, Beartooth Plateau, MT, U.S.A.	*Betula glandulosa*	NA	NA
CLC1624	2 Aug 2001	Blue Lake, Front Range, CO, U.S.A.	*Betula glandulosa*	Yes	Yes
TWO269	10 Aug 1999	Birch Site, Beartooth Plateau, MT, U.S.A.	*Betula glandulosa*	Yes	Yes
ZT12723	13 Aug 2007	Birch Site, Beartooth Plateau, MT, U.S.A.	*Betula glandulosa*	Yes	NA

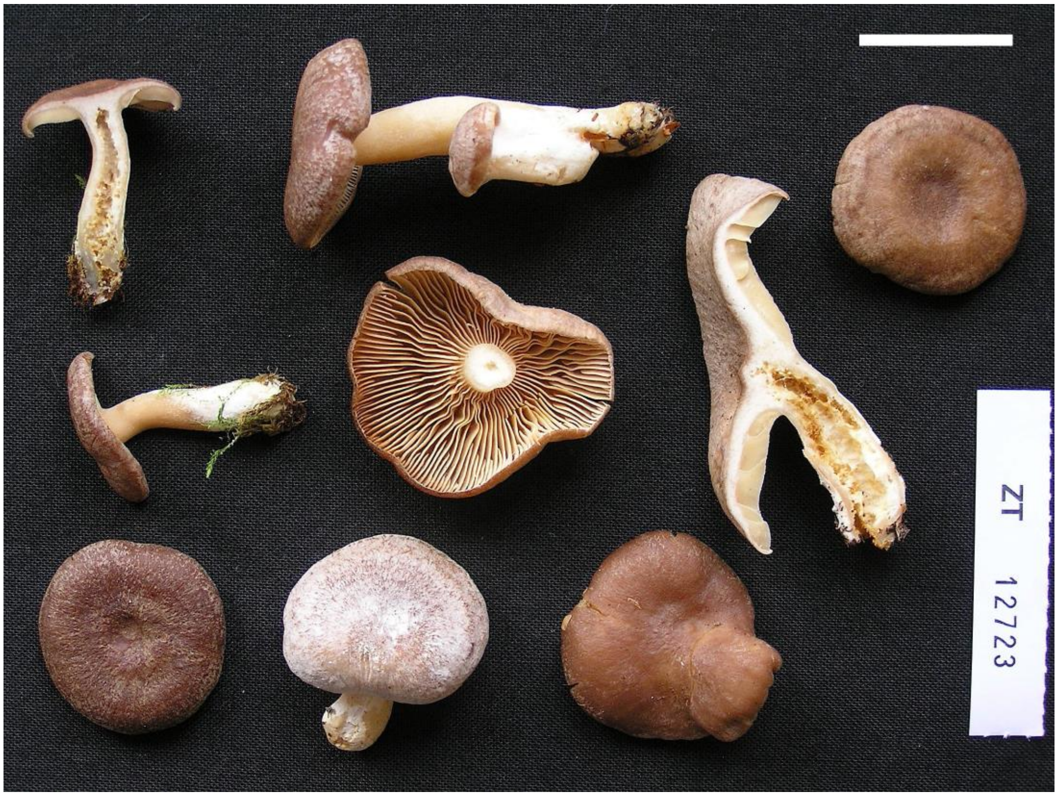


Figure 21. *Lactarius glyciosmus* (ZT12723), collected near *Betula glandulosa*, Birch Site, Beartooth Plateau, Montana. Scale bar = 2 cm.

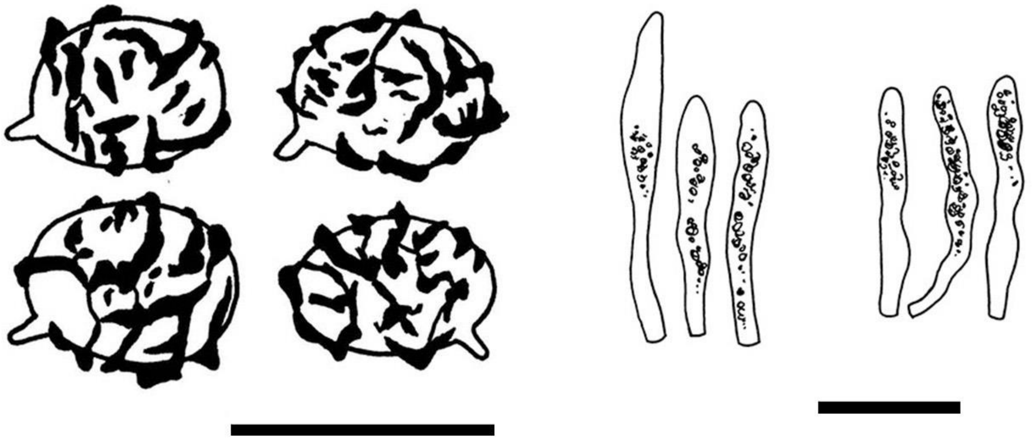


Figure 22. Spores, pleurocystidia (left) and cheilocystidia (right) of *L. glyciosmus* (TWO269). Bars = 10 µm for spores and 20 µm for cystidia.

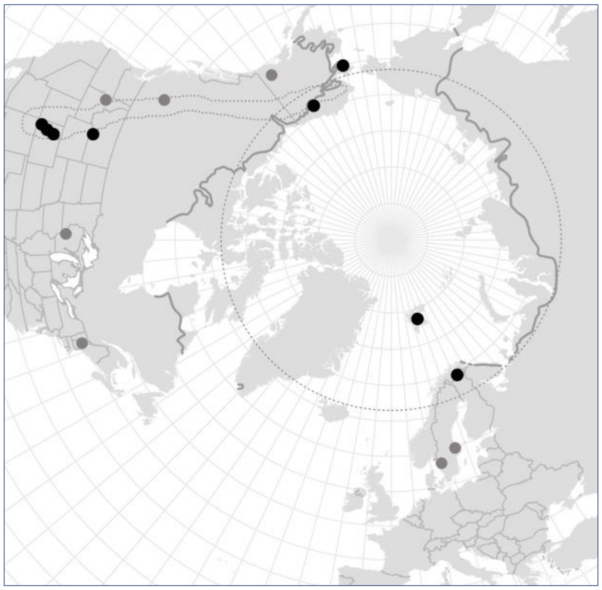


Figure 23. Confirmed distribution of *L. glyciosmus* based on available molecular data. Black dots indicate arctic-alpine sites, gray dots indicate non-arctic-alpine sites. The Rocky Mountains are outlined by a dotted gray line. The northern extent of trees is indicated by a thick gray line. Arctic circle = 67.5° N.

Table 10. Comparison of morphological features between *L. glyciosmus*, *L. nanus*, and select closely related taxa. EUR = Eurasia, NA = North America, AK = Alaska.

Taxon	**Spores**	**Pleurocystidia/ Cheilocystidia**	**Odor**	**Fruitbody characters**	**Distribution Ecology**
L. glyciosmus	7—9 × 5—7 µm	45.7—63.5 × 6.4—7.6 µm/ 33—66 × 5.1—8.9 µm	Coconut	Pileus pale gray-brown to mauve; basidiome fairly small and fragile	EUR, NA, introduced elsewhere; subalpine, arctic-alpine; *Betula*
L. aff. *glyciosmus*	7—8 × 5—6.5 µm	NA	NA	Same as above?	EUR, AK, Japan; subalpine; *Betula*
*L. mammosus**	6—8.8 × 4.8—6.6 µm	45—75 × 6.5—9.5 µm/ 20—50 × 5.5—9.5 µm	Coconut	Pileus gray-brown; basidiome fairly stout	EUR; subalpine, arctic-alpine?; conifers, *Betula*?
L. nanus	7—10.5(—12) × 5—8 µm	53.3—96.5(—114.3) × 6.4—11.4 µm/ 26.0—91.4 × 7.6—10.2 µm	Mild	Pileus gray-brown to liver-brown; basidiomes fairly small	EUR, NA; arctic-alpine; *Salix*
*L. hysginoides**	6—8 × 5—7 µm	56—77.5 × 7—8 µm/ 48—64 × 6—8 µm	Mild	Pileus cinnamon; basidiomes fairly small	EUR, AK; subalpine; *Picea*, *Betula*, *Salix*

*Some data from Heilmann-Clausen et al. (1998)

Lactarius pallidomarginatus Barge & C.L. Cripps *ad int.* Figures 3, 10-13, 24-26, 35, 36

Diagnosis: *Lactarius pallidomarginatus* is recognized by its small to medium size, blotchy light tan to medium brown pileus, which is typically lighter toward the margin, white to pale yellow-cream lamellae, pale cream stipe, violet staining tissue and association with *Salix* in low alpine areas.

Macromorphology: Pileus 20—50 mm in diameter, convex to broadly convex, becoming broadly convex to plane with or without a depressed center, smooth, dry to slightly viscid, azonate, blotchy light tan to medium brown, developing violet stains, lighter (to cream) toward margin; margin incurved to downturned when young, remaining downturned or becoming nearly straight when mature. Lamellae adnate to subdecurrent, subdistant to slightly crowded, white to pale yellow-cream, staining violet where damaged. Stipe 10—40 × 5—10 mm, equal to slightly clavate, central, smooth, dry, white to cream, staining violet where damaged, hollow. Latex scarce, watery, staining tissue violet. Context of pileus and stipe white to cream, staining violet where damaged. Odor mild. Taste mild. Spore deposit not observed.

Micromorphology: Basidiospores 8—10 × 6.5—8 µm, average = 8.7 × 7.1 µm, Q = 1.14—1.36, Q average = 1.23, hyaline in 2.5% KOH, broadly ellipsoid to ellipsoid; ornamentation of thin ridges and a few isolated warts forming an incomplete to dense reticulum, to 0.5 µm high. Basidia 50.8—71.1 × 10.2—12.7 µm, 4-spored. Pleuromacrocystidia 81.3—111.8 × 8.9—10.2 µm, cylindric to lanceolate, with acute to moniliform apex, scarce to scattered. Cheilomacrocystidia 48.3—101.6 × 7.6—12.7 µm, cylindric to fusiform to lanceolate, with acute to moniliform apex, scattered. Pileipellis an (ixo-) cutis with some ascending hyphae embedded in a very faint gelatinous layer.

Habit, Habitat and Distribution: Scattered, growing singly. *Lactarius pallidomarginatus* is rare, and is here reported from the low alpine zone on Independence Pass in the Sawatch Range and Stony Pass in the San Juan Range in Colorado with *Salix planifolia* and possibly other *Salix* species. *Lactarius* sp. 5, isolated from the roots of *S. polaris* in Svalbard may be conspecific, however more research is needed to confirm this. An additional collection (ZT5229) from Union Peak in the Wind River Range of Wyoming growing with *Salix glauca* was examined and likely represents *Lactarius pallidomarginatus*, however it could not be molecularly confirmed. As historically defined, the similar *Lactarius pseudouvidus s.l.* has been reported from arctic-alpine areas in Canada (Ohenoja & Ohenoja 1993, 2010), Greenland (Lamoure et al. 1982, Heilmann-Clausen et al. 1998, Borgen et al. 2006), Iceland (Eyjolfsdottir pers. comm., Heilmann-Clausen et al. 1998, Knudsen & Vesterholt 2008), European Alps (Kühner 1975, Bon 1985a, 1998, Kühner & Lamoure 1986, Heilmann-Clausen et al. 1998, Jamoni 1995, Jamoni 2008), Scandinavia (Kühner 1975, Gulden et al. 1985, Heilmann-Clausen et al. 1998, Gulden 2005, Knudsen & Vesterholt 2008), Svalbard (Gulden & Torkelsen 1996, Heilmann-Clausen et al. 1998), Russia (Karatygin et al. 1999, Knudsen & Mukhin 1998, Nezdoiminogo 1997) with dwarf *Salix* and possibly also dwarf *Betula* (Table 1). Phylogenetic analyses in this study reveal the presence of several species closely fitting the morphological criteria of *L. pseudouvidus*. Collections identified as *L. pseudouvidus* from Svalbard, Scandinavia and the Austrian Alps included in phylogenetic analyses in this study are phylogenetically distinct from *L. pallidomarginatus*, and do not form a monophyletic group. The *L. pseudouvidus* collections were also from arctic-alpine areas with *Salix*. How the distribution and ecology of these species differs is still unclear.

<u>Specimens Examined:</u> U.S.A. COLORADO. Pitkin County, Sawatch Range, Independence Pass, 6 Aug 2000 *CLC 1470* (MONT), 9 Aug 2000 *ZT9093* (MONT). San Juan County, San Juan Range, Stony Pass, 13 Aug 2011 *EB0041* (MONT). WYOMING. Sublette County, Wind River Range, Union Peak, 22 Aug 1994 *ZT5229* (MONT). Rocky Mountain alpine collections of *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* examined, with collection data and ectomycorrhizal host plants are shown in Table 11.

<u>Similar Taxa Examined:</u> AUSTRIA. Tyrol, 3 July 2007 *U. Peintner 20070035* (*L. pseudouvidus*, IB). FINLAND. Inarin Lappi, 16 Aug 1995 *JV10468* (*L. pseudouvidus*, TURA). Koillismaa, *JV28448F* (*L. brunneoviolaceus*, TURA). FRANCE. Bourg-Saint-Maurice, 31 Aug 1990 *PAM900830* (*L. pseudouvidus*, LIP), 31 Aug 1993 *PAM930831* (*L. robertianus*, LIP), Isère, 13 Aug 1971 *Va 7 1971* (Holotype of *L. pseudouvidus*, G). ITALY. Trentino, 20 Aug 2004 *U. Peintner* 20040156 (*L. robertianus*, IB). NORWAY. Finse, 10 Aug 2005 *CLC 2133* (*Lactarius* aff. *brunneoviolaceus*, MONT). Stordal, 26 Aug 2006 *P. Larsen 361395* (*L. pseudouvidus*, O) Svalbard, 18 Aug 2002 *TWO809* (*L. pseudouvidus*, MONT), 20 Aug 2002

CLC 1910 (*L.* aff. *pseudouvidus* 1, MONT). Ulvik, E. Søland 73867 (*L. pseudouvidus*, O). SWEDEN. Latnjajaure, 4 Aug 2011 *EL101-11* (*L. pseudouvidus*, GB). Latnjavagge, 17 Aug 2010 *EL63-10* (*L. pseudouvidus*, GB).

Comments: The name *Lactarius pallidomarginatus* is used in an ad interim manner for purposes of this thesis; effective publication of this taxon in accordance with the International Code of Nomenclature for Algae, Fungi and Plants (McNeill et al. 2012) will be established in a subsequent published paper. Based on phylogenetic analyses (Figures 10-13), *Lactarius pallidomarginatus* occurs in a complex of at least four or five arctic-alpine species with affinities to *L. pseudouvidus* and *L. brunneoviolaceus*: *Lactarius* sp. 4, *Lactarius* sp. 5, *Lactarius pallidomarginatus*, the *L.* aff. *pseudouvidus* group (may be multiple species), and *L.* aff. *brunneoviolaceus*. However, there are only three names available for members of this group; *L. pseudouvidus*, *L. brunneoviolaceus* and *L. robertianus*, and many authors consider the latter two synonymous (e.g. Heilmann-Clausen et al. 1998) (See Table 12 for comparison of morphological and ecological features between *L. pallidomarginatus*, *L. pseudouvidus*, *L.* aff. *brunneoviolaceus*, *Lactarius* sp. 4 and *Lactarius* sp. 5).

Lactarius pseudouvidus was first described by Kühner (1975) from the French Alps with *Salix herbacea*; Kühner (1975) also reported it in association with *S. reticulata* and *S. retusa*. There are no images or drawings of the type specimen in fresh condition and the description is vague. The holotype collection (Va 7 1971) consists of several sub-collections from near the same area. These sub-collections consist of very small, dried basidiomes which are dull-brown; differences between these sub-collections were not detected. Sequencing of these holotype collections was attempted but was unsuccessful and in phylogenetic analyses, collections identified as or with affinities to *L. pseudouvidus* were polyphyletic. Overall, the morphological features of Rocky Mountain alpine material are very similar to those of the *L. pseudouvidus* holotype. However, spores of *L. pallidomarginatus* are smaller (8—10 × 6.5—8 µm, as opposed to 7.5—12 × 6.5—8(8.5) µm) and have narrower ridges forming a less dense reticulum (Figure 36). Other specimens (including a specimen from near the type locality, U Peintner 20070035, Austrian Alps) identified as *L. pseudouvidus* that fell out in the *L.* aff. *pseudouvidus* group (Figures 10-13) had larger spores. Solid macroscopic differences between *L. pseudouvidus* and *L. pallidomarginatus* were not detected in this study, however, fresh material identified as *L. pseudouvidus* from Europe was not examined. The specimens identified as *L. pseudouvidus* obtained for this study were phylogenetically distinct from Rocky Mountain alpine material. The group with the closest affinities to *L. pseudouvidus* formed a distinct group paraphyletic with respect to *L. montanus* in ITS analyses, however in analyses of the *rpb2* locus, there was a large amount of molecular variation within this group. It is uncertain whether the *L.* aff. *pseudouvidus* group depicted in phylogenetic analyses in this study represents one or several species.

Lactarius brunneoviolaceus M.P. Christ. was described from Iceland (Christiansen 1941) and has traditionally been separated from *L. pseudouvidus* by a typically darker, more vinaceous pileus, without a light margin, more pale-cream lamellae as opposed to yellow-buff or yellow-orange, a smell and taste of cedar, more elongate spores often with an amyloid plage and ridges that are highly variable in width and form sharper angles (Heilmann-Clausen et al. 1998). *Lactarius brunneoviolaceus* is synonymized by some authors with *L. robertianus* Bon (e.g. Heilmann-Clausen et al. 1998), which was described from the French Alps with *Salix herbacea* (Bon 1985b). The clade with specimens bearing the strongest affinities to *L. brunneoviolaceus* as described in Heilmann-Clausen et al. (1998) is labeled as *L.* aff. *brunneoviolaceus* in Figure 10. This clade was distinct from the *L.* aff. *pseudouvidus* group in ITS analyses, however it was not clearly distinct in *rpb2* analyses. *Lactarius brunneoviolaceus* has much larger, more elongate spores (to 13 µm long), as well as a different spore ornamentation pattern than *L. pseudouvidus s.l.* and *Lactarius pallidomarginatus.* Interestingly, the two specimens with the closest affinities to *L. brunneoviolaceus* examined in this study displayed green staining on their dried pilei context when 2.5% KOH was applied. Green-staining in KOH has not been noted for *L. brunneoviolaceus*, *L. pseudouvidus*, *L. robertianus* or for any other *Lactarius* species inhabiting arctic-alpine areas. However, it is recorded for *L. montanus*, which appears to be closely related according to the phylogenetic analyses. Fresh collections and herbarium specimens identified as *L. brunneoviolaceus* and *L. pseudouvidus* should be tested for the green staining reaction to KOH.

Lactarius sp. 4, which may be an undescribed species can be distinguished from *Lactarius pallidomarginatus* as it has a more mottled, darker brown pileus, although only one collection was examined and it was only seen in fresh condition through one image (Figure 35). Microscopic features of the two species appear identical, and the pileus of both species is lighter near the margin, a key feature also commonly mentioned for *L. pseudouvidus* (Kühner 1975, Basso 1999).

Lactarius sp. 5, which also may be an undescribed species, is very closely related to *L. pallidomarginatus.* It is represented only by an environmental sequence isolated from the roots of *Salix polaris* in Svalbard. Further analyses are needed to determine whether or not it is conspecific with *L. pallidomarginatus.*

Other species superficially similar to *L. pallidomarginatus* Barge include other gray-brown, violet staining taxa such as *L. cordovaensis* Hesler & A.H. Sm., *L. luridus* (Pers.) Gray, *L. montanus* (Hesler & A.H. Sm.) Montoya & Bandala, *L. pallescens* Hesler & A.H. Sm., *L. pallescens* v. *palmerensis* Hesler & A.H. Sm., *L. uvidus* (Fr.) Fr., *L. uvidus* fo. *alpigines* Jamoni & Bon, and *L. violascens* (J. Otto) Fr. Most of these species, however, have a different ecology, different microscopic features and are generally much larger (Hesler & Smith 1979, Heilmann-Clausen et al. 1998, Basso 1999).

Lactarius montanus, a violet staining species that occurs in the Rocky Mountains differs from *L. pallidomarginatus* in its larger and more stout stature, darker, grayish vinaceous to vinaceous-brown pileus, different spores and green staining flesh in 2.5% KOH (pers. obs. of paratype, and Hesler & Smith 1979, Montoya & Bandala 2003) (Figure

35). It also has a different ecology (subalpine with conifers), although it could potentially overlap with *L. pallidomarginatus* in transition areas between alpine and subalpine. For example, *L. montanus* (CLC3001) was found at high elevation near krummholz conifers but in a patch of *Salix*. It is closely related to *L. pallidomarginatus*, but distinct (Figures 3, 10-13).

Lactarius luridus is also phylogenetically close to *L. pallidomarginatus* (Figures 3, 10-12). It has larger sporocarps, different microscopic features, a subalpine ecology and may be restricted to Eurasia (Heilmann-Clausen et al. 1998); it has not been reported from the Rocky Mountains or elsewhere in North America.

Lactarius pallescens, another phylogenetically distinct (Figures 3, 10-12), violet staining species that occurs in the Rocky Mountains differs from *L. pallidomarginatus* by its larger and more stout stature and different spores (pers. obs. of holotype, and Hesler & Smith 1979). It also has a different ecology (subalpine with conifers), although it could also potentially overlap with *L. pallidomarginatus* in transition areas between alpine and subalpine as it occurs in conifer forests in western North America (Hesler & Smith 1979).

Lactarius pallescens v. *palmerensis* and *L. cordovaensis* both have larger sporocarps and different microscopic features and have only been reported from subalpine areas in Alaska (Hesler & Smith 1979).

Lactarius violascens is phylogenetically distinct (Figures 3, 10-12) and has larger sporocarps, different microscopic features and a subalpine ecology (Heilmann-Clausen et al. 1998). It has not been reported from the Rocky Mountains or elsewhere in North America.

Lactarius uvidus is phylogenetically distinct (Figures 3, 10-12), has larger sporocarps with a “pale pinkish buff to grayish pink to pale gray to light vinaceous gray pileus” and different microscopic features (Heilmann-Clausen et al. 1998). *Lactarius uvidus* fo. *alpigines* is basically a small, alpine version of *L. uvidus* (Jamoni & Bon 1991). *Lactarius uvidus* fo. *alpigines* has not been reported from the Rocky Mountains. See *L. nanus* section for distinguishing features between it and *L.* aff. *pseudouvidus* 2.

Table 11. Rocky Mountain alpine *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* collections examined, showing collection locations, putative host associations, and loci sequenced. Collections are designated with C.L. Cripps (CLC), E. Barge (EB), or E. Horak (ZT) collection numbers and are stored at MONT herbarium.

Voucher	**Date**	**Location**	**Host(s)**	**ITS**	***rpb2***
CLC1470	6 Aug 2000	Independence Pass, Sawatch Range, CO, U.S.A.	*Salix* spp.	Yes	Yes
EB0041	13 Aug 2011	Stony Pass, San Juan Range, CO, U.S.A.	*Salix planifolia*	Yes	Yes
ZT5229	22 Aug 1994	Union Peak, Wind River Range, WY, U.S.A.	*Salix glauca*	NA	NA
ZT9093	9 Aug 2000	Independence Pass, Sawatch Range, CO, U.S.A.	*Salix planifolia*, *S. glauca*	NA	NA

Figure 24. *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* (EB0041), growing with *Salix planifolia*, Stony Pass, San Juan Range, Colorado. Scale bar = 2 cm.

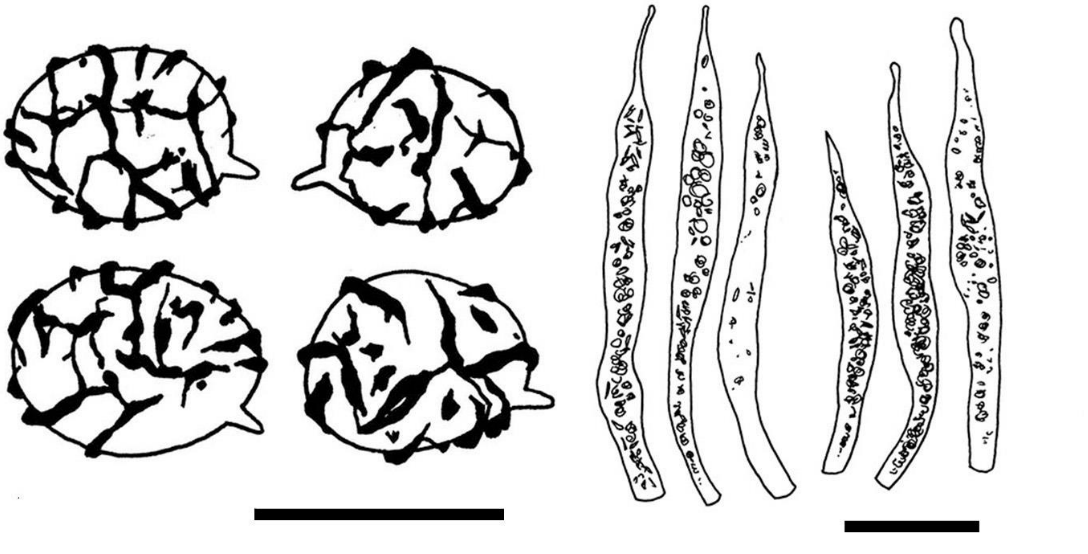


Figure 25. Spores, pleurocystidia (left) and cheilocystidia (right) of *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* (EB0041). Bars = 10 µm for spores and 20 µm for cystidia.

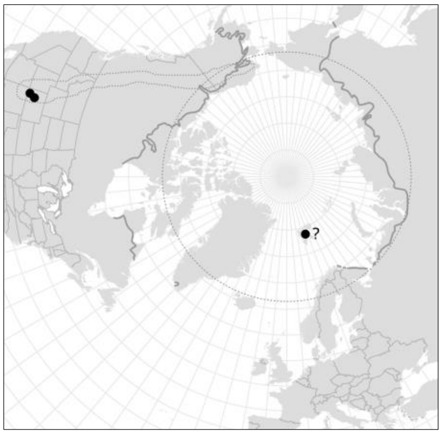


Figure 26. Confirmed distribution of *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* based on available molecular data. Black dots indicate arctic-alpine sites. The Rocky Mountains are outlined by a dotted gray line. The northern extent of trees is indicated by a thick gray line. Arctic circle = 67.5° N.

Table 12. Comparison of morphological features between *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* and select closely related taxa. NA = North America, SVLBD = Svalbard, EUR = Eurasia.

Taxon	**Spores**	**Pleurocystidia/ Cheilocystidia**	**Odor**	**Fruitbody characters**	**Distribution Ecology**
Lactarius pallidomarginatus* Barge & C.L. Cripps *ad int.	8—10 × 6.5—8 µm	81.3—111.8 × 8.9—10.2 µm/ 48.3—101.6 × 7.6—12.7 µm	Mild	Pileus blotchy tan to brown, lighter toward margin	NA, SVLBD?; low alpine, arctic?; *Salix*
Lactarius sp. 4	8.5—11.5 × 7—9 µm	94—132 × 7.6—10.2 µm/ 61—109.2 × 7.6 µm	Mild	Pileus gray-brown to vinaceous, lighter toward margin	SVLBD; arctic-alpine; *Salix polaris*?
Lactarius sp. 5	NA	NA	NA	NA	SVLBD; arctic-alpine; *Salix polaris*
L. pseudouvidus (Holotype)*	7.5—12 × 6.5—8(8.5) µm	58.4—88.9 × 7.6—10.2 µm/ 53.3—83.8 × 7.6 µm	Mild	Pileus dull brownish-gray, brownish-yellowish, lighter toward margin	EUR; arctic-alpine; dwarf *Salix*
L. aff. *pseudouvidus* group	8.5—11 × 6.5—8.5 µm	61.3—124.5 × 7.6—10.2 µm/ 50.8—94 × 6.4—12.7 µm	Mild	Not seen in fresh condition	EUR; arctic-alpine, subarctic; *Salix*
L. aff. *brunneoviolaceus* group	10—13 × 7—9 µm	83.8—114.3 × 10.2—12.7 µm/ 76.2—104.1 × 7.6—12.7 µm	Cedar	Pileus vinaceous; dried material green in 2.5% KOH	EUR; arctic-alpine; *Salix*

*Some data from Kühner (1975)

<u>*Lactarius repraesentaneus* Britzelm.</u> Figures 3, 10-13, 27-29, 35, 36

Bericht des Naturhistorischen Vereins in Augsburg 28: 136 (1885)

<u>Diagnosis:</u> *Lactarius repraesentaneus* is recognized by its medium to large size, orange-brown to yellow-brown, hairy pileus with a heavily bearded margin, violet staining latex and tissue, spicy-floral odor and association with *Betula*, *Picea* and possibly shrubby *Salix* species. In alpine areas in the Rocky Mountains it occurs in low-alpine areas near *S. glauca* and krummholz *Picea*.

<u>Macromorphology:</u> Pileus 60—100 mm in diameter, convex to plano-convex usually with a depressed center and sometimes with a papilla, smooth at center, becoming increasingly hairy to bearded toward margin, dry to viscid, azonate, surface orange-brown at center, becoming pale yellow-brown to cream toward margin; hairs pale yellow-brown to red-brown; margin strongly invcurved, remaining so or becoming nearly straight when mature. Lamellae adnate to decurrent, crowded, cream to pale-yellow, staining violet where damaged. Stipe 30—70 × 15—35 mm, stout, equal to clavate, central, dry to viscid, cream to pale-yellow surface often with numerous dingy yellow, golden yellow, yellow-brown, or light orange-brown pits, hollow. Latex scarce to abundant, white, becoming violet; taste spicy-floral. Context of pileus and stipe white, staining violet where damaged. Odor spicy-floral. Taste spicy-floral to slightly acrid. Spore deposit not observed.

Micromorphology: Basidiospores 8—10.5 × 6—8.5 µm, average = 9.3 × 7.3 µm, Q = 1.13—1.43, Q average = 1.27, hyaline in 2.5% KOH, broadly ellipsoid to ellipsoid; ornamentation of narrow to broad ridges and isolated warts forming an incomplete to dense reticulum, to 1 µm high. Basidia 61.0—76.2 × 10.2—11.4 µm, 4-spored. Pleuromacrocystidia 78.7—144.8 × 8.9—12.7 µm, subfusiform to lanceolate, thin-walled, with acute to moniliform apex, scattered to abundant. Cheilomacrocystidia 55.9—139.7 × 7.6—12.7 µm, subfusiform to lanceolate, thin-walled, with acute to moniliform apex, scattered to abundant. Pileipellis an ixotrichoderm with well-developed gelatinous layer.

Habit, Habitat and Distribution: Scattered to gregarious, growing singly. In the Rocky Mountain alpine zone *L. repraesentaneus* occurs in low alpine areas near *Salix glauca* and krummholz *Picea*. It is also reported from the spruce-fir zone in the central and southern Rocky Mountains, the northern United States and southern Canada where spruce is present (Hesler & Smith 1979) and Eurasia with *Picea* and *Betula* (Heilmann-Clausen et al. 1998). It was described from Germany, however it lacks a type specimen. A lectotype from Germany is designated in Heilmann-Clausen et al. (1998). Previous reports from arctic-alpine areas include the Rocky Mountains (Cripps & Horak 2008, Cripps & Barge 2013), Alaska (Laursen & Ammirati 1982, Geml et al. 2009), Greenland (Knudsen & Borgen 1982, Watling 1983, Elbourne & Knudsen 1990, Borgen 2006, Borgen et al. 2006), Iceland (Eyjolfsdottir pers. comm.), Scandinavia (Kallio & Kankainen

1964, Kühner 1975, Bon 1998), Siberia (Karatygin et al. 1999) and the Altai Mountains (Gorbunova 2010) with *Betula* and possibly shrubby *Salix* species (Table 1).

Phylogenetic analyses confirm previous reports of *L. repraesentaneus* from alpine areas in the Rocky Mountains (Cripps & Horak 2008, Cripps & Barge 2013) and also confirm its presence in arctic-alpine areas in Alaska and Scandinavia, as well as subalpine areas in Scandinavia and central and southern Europe with *Betula nana* and *Picea* as putative hosts (Figures 3, 10-13). In alpine areas in the Rocky Mountains, it may be associated with *Salix glauca*, however, it is more likely associated with the roots of krummholz *Picea* extending into alpine areas. It has not been reported with pure *Betula glandulosa* in alpine areas in the Rocky Mountains, however *B. glandulosa* is a possible host.

<u>Specimens Examined:</u> FINLAND. Enontakiö, 16 Aug 2004 *JV21671* (TURA). Varsinais-Suomi, 9 Aug 1998 *JV13837F* (TURA). SWEDEN. Latnjavagge, 12 Aug 2007 *EL92-07* (GB). U.S.A. COLORADO. Chaffee County, Sawatch Range, Cottonwood Pass, 4 Aug 2001 *CLC 1643* (MONT), 16 Aug 2011 *EB0048* (MONT). Pitkin County, Sawatch Range, Independence Pass, 14 Aug 2001 *CLC 1747* (MONT), *ZT9837* (MONT), 15 Aug 2001 *ZT9537* (MONT). MONTANA. Carbon County, Beartooth Plateau, Birch Site, 12 Aug 2002 *CLC 1971* (MONT), 13 Aug 2007 *CLC 2318* (MONT), 18 Aug 2013 *EB107-13* (MONT). WYOMING. Park County, Beartooth Plateau, Frozen Lakes, 21 Aug 1999 *CLC 1394*

(MONT). Rocky Mountain alpine collections of *L. repraesentaneus* examined, with collection data and ectomycorrhizal host plants are shown in Table 13.

Similar Taxa Examined: NORWAY. Hordland Scandanavie, 30 July 1967 *K 67-17* (Holotype of *L. dryadophilus*, G). SWEDEN. Latnjavagge, 14 Aug 2010 *EL57-10* (*L. dryadophilus*, GB). U.S.A. ALASKA, 5 Aug 2011 *CLC 2729* (*L. dryadophilus*, MONT). Kenai Peninsula County, Palmer Creek Road, 7 Aug 2011 *CLC 2744* (*L. dryadophilus*, MONT).

Comments: The combination of large size, pale yellow-brown to orange-brown pileus with a bearded margin and violet staining latex clearly separate *L. repraesentaneus* from most other *Lactarius* species that have been reported from alpine areas in the central and southern Rocky Mountains. Morphologically, *L. repraesentaneus* is somewhat similar to *L. dryadophilus* Kühner, however *L. dryadophilus* has a more cream to yellow, smoother pileus with hairs restricted to near the margin and it possesses a different spore ornamentation with thinner ridges (pers. obs. of holotype K 67-17, and Kühner 1975, Heilmann-Clausen et al. 1998). *Lactarius dryadophilus* is reported as being associated with *Dryas*, *Salix* and possibly also *Betula* (Kühner 1975, Heilmann-Clausen et al. 1998); its association with *Dryas* and *Salix* is supported in the phylogenetic analyses (Figures 3, 10-13) (See Table 14 for comparison of morphological and ecological features between *L. repraesentaneus* and *L. dryadophilus*).

Lactarius dryadophilus is morphologically distinct from *L. repraesentaneus*, however, interestingly, the ITS region of several accessions on GenBank (FJ607367, JF908292) which fall out as *L. dryadophilus* in the phylogeny were around 99.4% similar to that of *L. repraesentaneus* (EB107-13) from the Rocky Mountain alpine zone (Table 5). These results highlight the utility of phylogenetic analyses for differentiating species in comparison to using strict percent-similarity cutoffs, such as 97% as used in ecological studies. Results also reinforce claims from previous studies that there can be low genetic variability, yet high morphological variability between *Lactarius* species in contrast to other genera (Verbeken & Nuytinck 2013). *Lactarius dryadophilus* appears to be fairly widespread in arctic areas, however it has not been reported from alpine areas in the central and southern Rocky Mountains.

Lactarius formosus H.T. Le & Verbeken and *L. speciosus* Burl. are morphologically somewhat similar to *L. repraesentaneus* in that they are violet staining and have a bearded pileus margin, however they are clearly phylogenetically distinct (Figures 3, 10-12) and sporocarps are smaller and more slender with a zonate, grayish-yellow to yellow-buff pileus, different microscopic features, and distribution in Thailand for the former (Le et al. 2007) and a duller, zonate pileus and seemingly more southern North American distribution for the latter (Hesler & Smith 1979). Neither species has been reported from the Rocky Mountains.

Hesler & Smith (1979) note the presence of several “varieties” of *L. repraesentaneus* in North America with different spore sizes. In collections examined in this study, spore size was highly variable within collections and somewhat variable between collections, yet all collections were phylogenetically conspecific. However, in the future, phylogenetic analyses should investigate a greater ecological and geographical breadth of North American representatives of *L. repraesentaneus* to investigate the possibility of cryptic species.

Table 13. Rocky Mountain alpine *Lactarius repraesentaneus* collections examined, showing collection locations, putative host associations, and loci sequenced. Collections are designated with C.L. Cripps (CLC), E. Barge (EB), or E. Horak (ZT) collection numbers and are stored at MONT herbarium.

Voucher	**Date**	**Location**	**Host(s)**	**ITS**	***rpb2***
CLC1394	21 Aug 1999	Frozen Lakes, Beartooth Plateau, WY, U.S.A.	NA	NA	NA
CLC1643	4 Aug 2001	Cottonwood Pass, Sawatch Range, CO, U.S.A.	NA	NA	NA
CLC1747	14 Aug 2001	Independence Pass, Sawatch Range, CO, U.S.A.	*Salix glauca*	Yes	Yes
CLC1971	12 Aug 2002	Birch Site, Beartooth Plateau, MT, U.S.A.	*Salix*	NA	NA
CLC2318	13 Aug 2007	Birch Site, Beartooth Plateau, MT, U.S.A.	Shrubby *Salix*	Yes	Yes
EB0048	16 Aug 2011	Cottonwood Pass, Sawatch Range, CO, U.S.A.	*Salix glauca*, krummholz *Picea engelmannii*	Yes	Yes
EB107-13	18 Aug 2013	Birch Site, Beartooth Plateau, MT, U.S.A.	*Salix glauca*, *S. arctica*, krummholz *Picea engelmannii*	Yes	Yes
ZT9837	14 Aug 2001	Independence Pass, Sawatch Range, CO, U.S.A.	*Salix glauca*	NA	NA
ZT9537	15 Aug 2001	Independence Pass, Sawatch Range, CO, U.S.A.	*Salix glauca*	NA	NA
ZT9093	9 Aug 2000	Independence Pass, Sawatch Range, CO, U.S.A.	*Salix planifolia*, *S. glauca*	NA	NA

Figure 27. *Lactarius repraesentaneus* (EB107-13) growing with *Salix glauca* and krummholz *Picea engelmannii*, Birch Site, Beartooth Plateau, Montana. Scale bar = 2 cm.

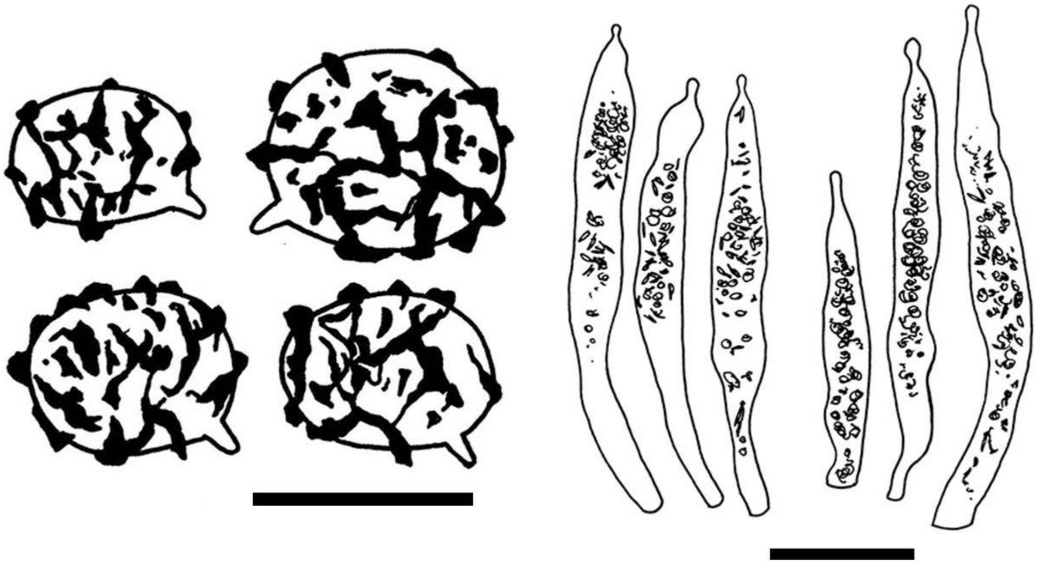


Figure 28. Spores, pleurocystidia (left) and cheilocystidia (right) of *L. repraesentaneus* (CLC1747). Bars = 10 µm for spores and 20 µm for cystidia.

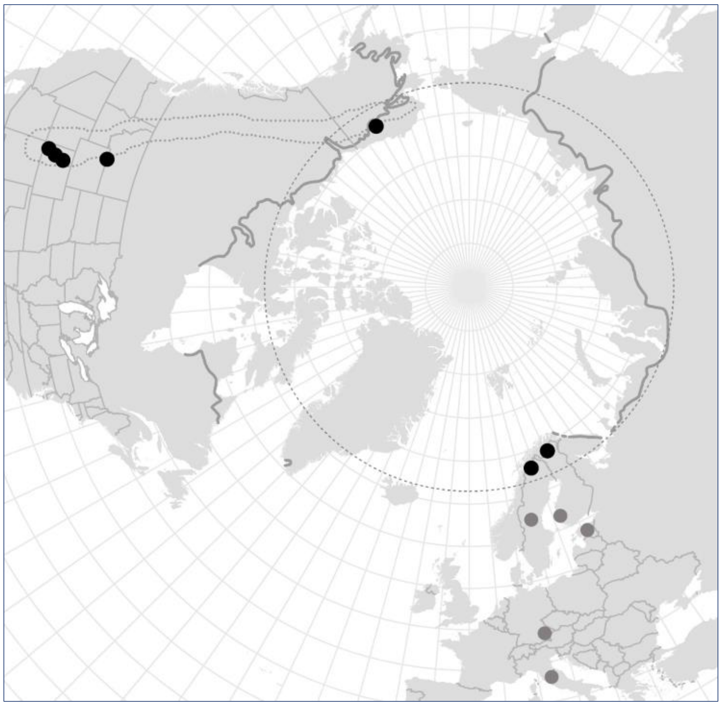


Figure 29. Confirmed distribution of *L. repraesentaneus* based on available molecular data. Black dots indicate arctic-alpine sites, gray dots indicate non-arctic-alpine sites. The Rocky Mountains are outlined by a dotted gray line. The northern extent of trees is indicated by a thick gray line. Arctic circle = 67.5° N.

Table 14. Comparison of morphological features between *L. repraesentaneus* and select closely related taxa. EUR = Eurasia, NA = North America.

Taxon	**Spores**	**Pleurocystidia/ Cheilocystidia**	**Fruitbody characters**	**Distribution Ecology**
L. repraesentaneus	8—10.5 × 6—8.5 µm, ornamentation of dense, thick ridges	78.7—144.8 × 8.9—12.7 µm/ 55.9—139.7 × 7.6—12.7 µm	Pileus orange-brown to yellow-brown, hairy, margin heavily bearded	EUR, NA; arctic-alpine, subalpine; *Betula*, *Picea*, *Salix*?
*L. dryadophilus**	9.3—11.8 × 7.2—9.2 µm, ornamentation of very fine, narrow ridges	80—120 × 10—15 µm/ 40—60 × 7—9 µm	Pileus pale cream to warm buff; hairs restricted to margin	EUR, NA; arctic-alpine; *Dryas*, *Salix*

*Data from Heilmann-Clausen et al. (1998)

<u>*Lactarius salicis-reticulatae* Kühner</u> Figures 3, 10-13, 30, 31, 33, 35, 36

Bulletin trimestriel de la Société mycologique de France 91: 20 (1975)

<u>Diagnosis:</u> *Lactarius salicis-reticulatae* is recognized by its small to medium, cream, pale yellow or pale ocher sporocarps with pale yellowish, well-spaced lamellae often with a pinkish-buff tint, violet staining latex and tissue, mild to slightly sweet odor, mild taste and association with dwarf (*S. reticulata*) and possibly also shrubby *Salix* species.

<u>Macromorphology:</u> Pileus 20—40 mm in diameter, convex to broadly convex, usually with a depressed center, smooth, dry to viscid, azonate, cream, pale yellow, or pale ocher, sometimes darker toward center, staining violet where damaged; margin invcurved, remaining so or becoming downturned when mature. Lamellae adnate to subdecurrent, fairly well-spaced, cream, pale yellow, or pale orange-ocher often with a pinkish-buff tint, staining violet where damaged. Stipe 15—20 × 10—15 mm, equal to clavate, central, dry to viscid, hollow, with a cream to pale-yellow surface, staining violet where damaged. Latex scarce, watery-white, becoming violet. Context of pileus and stipe white, staining violet where damaged. Odor mild to slightly sweet. Taste mild. Spore deposit not observed.

<u>Micromorphology:</u> Basidiospores (7—)8.5—11.5 × (7—)8—10 µm, average = 10.0 × 8.8 µm, Q = (1.00—)1.13—1.43, Q average = 1.25, hyaline in 2.5% KOH, sublgobose to ellipsoid; ornamentation of narrow ridges and a few isolated warts

forming an incomplete reticulum, to 0.3 µm high. Basidia 73.7—88.9 × 10.2—12.7 µm, 4-spored. Pleuromacrocystidia 76.2—101.6 × 7.6—11.4 µm, subfusiform to fusiform, thin-walled, with rounded to acute to moniliform apex, scarce. Cheilomacrocystidia 68.6—91.4 × 7.6—10.2 µm, subfusiform to fusiform, thin-walled, with acute to rounded to moniliform apex, scattered to abundant. Pileipellis an ixocutis.

Habit, Habitat and Distribution: Scattered, growing singly or in small clusters. In alpine areas in the central Rocky Mountains *L. salicis-reticulatae* occurs with dwarf (*Salix reticulata*) and possibly also shrubby *Salix* species. *Lactarius salicis-reticulatae* has been previously reported from alpine areas in the central and southern Rocky Mountains (Cripps & Horak 2008, Cripps & Barge 2013). Reports from the Beartooth Plateau are molecularly confirmed as *L. salicis-reticulatae* in this study, however reports from Colorado with *S. reticulata* and *S. planifolia* may represent a distinct species, referred to here as *L.* aff. *salicis-reticulatae* (Figures 10-13). *Lactarius salicis-reticulatae* has also been reported from arctic-alpine areas in Greenland (Lamoure et al. 1982, Heilmann-Clausen et al. 1998, Borgen et al. 2006), the European Alps (Kühner 1975, Kühner & Lamoure 1986, Jamoni 1995, 2008, Ballará 1997, Bon 1998, Heilmann-Clausen et al. 1998), Scandinavia (Kühner 1975, Heilmann-Clausen et al. 1998, Gulden 2005, Knudsen & Vesterholt 2008) and Siberia (Nezdoiminogo 1997, Karatygin et al. 1999) with *Salix* and possibly also *Betula* (Table 1). Phylogenetic analyses carried out in this study suggest it may be restricted to arctic-alpine areas with *Salix*, and confirm its presence in alpine areas on the Beartooth Plateau in the central Rocky Mountains as well as arctic-alpine areas in Alaska, Svalbard, Finland and Sweden (Figures 3, 10-13). Some previous reports of *L. aspideoides* from arctic-alpine areas in Alaska (Laursen & Ammirati 1982), Greenland (Knudsen & Borgen 1982) and the British Isles (Watling 1987) may represent *L. salicis-reticulatae*.

Specimens Examined: FINLAND. Near Utsjoki, 28 Aug 2012 *CLC 2885* (MONT). SWEDEN. Mt. Laktatjäkko, 12 Aug 1972 *K72-104* (Holotype of *L. salicis-reticulatae*, G). Mt. Laktatjäkko, 15 Aug 1999 *JV15133* (TURA). U.S.A. ALASKA. Kenai Peninsula County, Palmer Creek Road, 7 Aug 2011 *CLC 2745* (MONT). MONTANA. Carbon County, Beartooth Plateau, Birch Site, 17 Aug 2011 *CLC 2776* (MONT). Highline Trail, 8 Aug 1998 *CLC 1211* (MONT). WYOMING. Gardner Lake, 16 Aug 2014 *EB0057-14* (MONT). Rocky Mountain alpine collections of *L. salicis-reticulatae* examined and molecularly confirmed, with collection data and ectomycorrhizal host plants are shown in Table 15.

Similar Taxa Examined: FINLAND. Koillismaa, 31 Aug 2005 *JV23334* (*L. flavopalustris*, TURA). FRANCE. Savoie, 22 Aug 1971 *K71-89* (Holotype of *L. salicis-herbaceae*, G). GREENLAND. Sismiut, 19 Aug 2000 *CLC 1536* (*L. salicis-herbaceae*, MONT). U.S.A. MICHIGAN. Emmet County, 10 Aug 1973 *RL Shaffer 6957* (*L. aspideoides*, MICH).

L. aff. *salicis-reticulatae* Specimens Examined: U.S.A. COLORADO. Pitkin County, Sawatch Range, Independence Pass, 13 Aug 2001 *CLC 1741* (MONT). San Juan County, San Juan Range, Cinnamon Pass, 10 Aug 2001 *CLC 1710* (MONT). Maggie Gulch, 11 Aug

2011 *EB0036* (MONT). Stony Pass, 9 Aug 2001 *CLC 1689* (MONT), 13 Aug 2011 *EB0039* (MONT). Rocky Mountain alpine collections of *L.* aff. *salicis-reticulatae* examined and molecularly confirmed, with collection data and ectomycorrhizal host plants are shown in Table 16.

Unconfirmed Specimens: U.S.A. COLORADO. Pitkin County, Sawatch Range, Independence Pass, 13 Aug 2001 *ZT9536* (MONT). San Juan County, San Juan Range, Black Bear Pass, 11 Aug 2001 *ZT9538* (MONT), Stony Pass, 9 Aug 2001 *ZT9792* (MONT). Summit County, Front Range, Loveland Pass, 14 Aug 1997 *CLC1155* (MONT), 8 Aug 1999 *ZT8094* (MONT). MONTANA. Carbon County, Birch Site, 30 July 1997 *ZT6099* (MONT).

Comments: Some taxonomic confusion has been caused by the fact that Kühner (1975) originally named *L. salicis-reticulatae* as *L. aspideoides* Kühner, but then found out that name had already been applied to a fungus named by Burlingham (1907a) and Kühner thus renamed his species *L. salicis-reticulatae*. *Lactarius aspideoides* Burl. can be differentiated from *L. salicis-reticulatae* in that it generally has larger, somewhat zonate sporocarps with more crowded lamellae that lack any salmon colored hues, slightly smaller spores with broader ridges, a bitter to slightly acrid taste and an apparently subalpine ecology (pers. obs. of RL Shaffer 6957, and Burlingham 1907a, Hesler & Smith 1979). *Lactarius aspideoides* Burl. is clearly distinct from *L. salicis-reticulatae* in phylogenetic analyses (Figures 3, 10-12).

Other species similar to *L. salicis-reticulate* include *L. aspideus* (Fr.) Fr., *L. dryadophilus*, *L. flavidus* Boud., *L. flavoaspideus* Kytöv., *L. flavopalustris* Kytöv., *L. pseudoaspideus* Hesler & A.H. Sm. and *L. salicis-herbaceae* Kühner (See Table 17 for comparison of morphological and ecological features between *L. salicis-reticulatae* and *L.* aff. *salicis-reticulatae*, *L. aspideus*, *L. aspideoides*, *L. flavopalustris*, *L. flavoaspideus*, and *L. salicis-herbaceae*). *Lactarius aspideus* can be differentiated from *L. salicis-reticulate* in that it has smaller spores with denser reticulation, more crowded, creamier colored lamellae and habitat in subalpine areas (Heilmann-Clausen et al. 1998). However, apparently it associates with *Salix* as does *L. salicis-reticulatae* and has been reported from arctic-alpine areas (Kallio & Kankainen 1964, Laursen & Ammirati 1982, Knudsen & Vesterholt 2008). While some of these reports may in fact represent *L. salicis-reticulatae*, it is also possible that the range of *L. aspideus* and *L. salicis-reticulatae* overlaps in low arctic-alpine areas.

Lactarius dryadophilus can be distinguished from *L. salicis-reticulatae* in that it typically has a creamier colored pileus, is often larger and has a tomentose to bearded margin (Heilmann-Clausen et al. 1998). However, it can easily be confused with *L. salicis-reticulatae* if not examined closely or if the margin is not obviously tomentose. While *L. dryadophilus* occurs in similar habitats as *L. salicis-reticulatae*, and appears to be fairly widespread in arctic-alpine habitats, it has not been reported from alpine areas in the central and southern Rocky Mountains. It is clearly phylogenetically distinct (Figures 3, 10-13).

Lactarius flavidus can be differentiated from *L. salicis-reticulatae* in that it has larger sporocarps with zones of darker spots on the pileus sometimes forming concentric zones, more crowded, creamier colored lamellae, darker violet staining flesh, spores with higher ornamentation and a distally amyloid plage, a slightly bitter to acrid taste and subalpine ecology with *Carpinus*, *Quercus* and *Fagus* in Europe (Heilmann-Clausen et al. 1998). Molecular data is lacking for *L. flavidus*.

Lactarius flavoaspideus can be differentiated from *L. salicis-reticulatae* in that it has slightly smaller spores on average with a denser reticulum and subalpine ecology with *Salix* (Kytövuori 2009). Kytövuori (2009) mentions that another differentiating feature is that *L. salicis-reticulatae* has cream colored gills, while *L. flavoaspideus* has white to pale pinkish buff gills. However, in the type description of *L. salicis-reticulatae* (Kühner 1975), yellow ocher (subsaumones) is mentioned suggesting the lamellae of the two species may actually be similarly colored. Molecular data is lacking for *L. flavoaspideus*, however the phylogenetic relationship between it and *L. salicis-reticulatae* should be investigated in the future. *Lactarius flavoaspideus* has only been reported from Northern Europe with *Salix*, however the possibility of its presence in North America should be investigated as well.

Lactarius flavopalustris can be differentiated from *L. salicis-reticulatae* in that it has larger, often brighter yellow basidiocarps with faint concentric zones of “ochre watery spots”, more crowded lamellae, a sometimes “weakly scrobiculate” stipe, smaller spores with thicker ridges forming a denser reticulum and subalpine ecology in

Picea abies, *Betula*, *Salix* forests, although it has been reported from above treeline (Kytövuori 2009). Thus far it has only been reported from Northern Europe. Combined and ITS analyses show three collections of *L. flavopalustris* (with two coming from near the type locality) nested within a moderately to well-suported clade with *L. aspideoides* from North America (Figures 3, 10-13). The possibility of conspecificity between the two taxa should be further investigated, but is outside the scope of this study. Another clade containing collections identified as *L. flavopalustris* (from Estonia) was also recovered. These collections may be misidentified. Both clades containing collections identified as *Lactarius flavopalustris* are clearly phylogenetically distinct from the core *L. salicis-reticulatae* clade (Figures 3, 10-13).

Lactarius pseudoaspideus is a somewhat obscure North American species described by Hesler & Smith (1979). It can be differentiated from *L. salicis-reticulatae* in that it has larger basidiocarps, latex turning grayish, a very acrid taste and it has only been reported from North Carolina, suggesting it has a different ecology and distribution (Hesler & Smith 1979). Molecular data is lacking for this species.

Lactarius salicis-herbaceae, an arctic-alpine species that associates with *Salix* can be differentiated from *L. salicis-reticulatae* in that it typically has smaller, darker yellow to yellow-brown sporocarps, pale cream to grayish buff lamellae and more densely reticulate spores (Heilmann-Clausen et al. 1998). In combined, ITS and *rpb2* analyses it is clearly distinct from *L. salicis-reticulatae* (Figures 3, 10-13). It is apparently fairly widespread in arctic-alpine areas but has not been reported from alpine areas in the central and southern Rocky Mountains.

Collections initially identified as *L. salicis-reticulatae* from alpine areas in Colorado with dwarf and shrubby *Salix* may be a distinct species based on phylogenetic analyses (Figures 10-13). These collections, referred to as *L.* aff. *salicis-reticulatae*, are morphologically very similar to other collections of *L. salicis-reticulatae*. However, some have larger sporocarps (to 90 mm) than is described for *L. salicis-reticulatae* and possibly more crowded lamellae (Figures 32, 35). Other than that, morphological features strongly overlap. Differences were not detected regarding the color of the basidiocarps, or for the spore size and ornamentation pattern. *Lactarius flavoaspideus* is very similar to *L.* aff. *salicis-reticulatae*, however it has slightly smaller, more densely reticulate spores. *Lactarius* sp. (EU711582) from upland boreal forest in Alaska clusters with *L.* aff. *salicis-reticulatae* in phylogenetic analyses (Figures 10-12). Before describing *L.* aff. *salicis-reticulatae* as a new species, more collections should be examined and more loci should be included in phylogenetic analyses.

Table 15. Rocky Mountain alpine *Lactarius salicis-reticulatae* collections examined and molecularly confirmed; showing collection locations, putative host associations, and loci sequenced. Collections are designated with C.L. Cripps (CLC), or E. Barge (EB) collection numbers and are stored at MONT herbarium.

Voucher	**Date**	**Location**	**Host(s)**	**ITS**	***rpb2***
CLC1211	8 Aug 1998	Highline Trail, Beartooth Plateau, MT/WY, U.S.A.	Dwarf *Salix* spp.	Yes	NA
CLC2776	17 Aug 2011	Birch Site, Beartooth Plateau, MT, U.S.A.	*Salix reticulata*	Yes	Yes
EB0057-14	16 Aug 2014	Gardner Lake, Beartooth Plateau, WY, U.S.A.	Shrubby *Salix*	Yes	Yes

Figure 30. *L. salicis-reticulatae* (EB0057-14) growing with *Salix* cf. *glauca*, Gardner Lake, Beartooth Plateau, Wyoming. Scale bar = 2 cm.

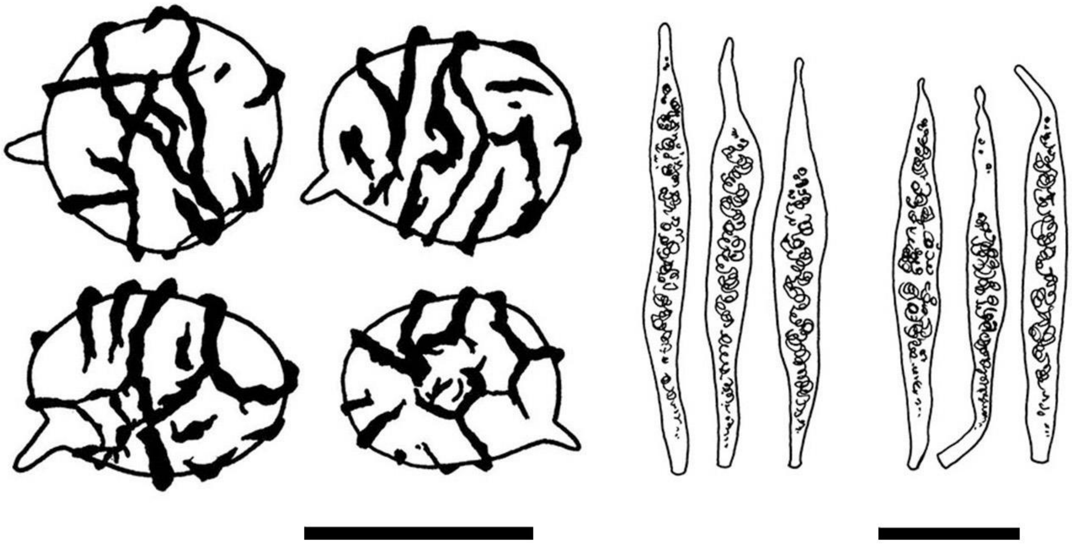


Figure 31. Spores, pleurocystidia (left) and cheilocystidia (right) of *L. salicis-reticulatae* (CLC2776). Bars = 10 µm for spores and 20 µm for cystidia.

Table 16. Rocky Mountain alpine *Lactarius* aff. *salicis-reticulatae* collections examined and molecularly confirmed; showing collection locations, putative host associations, and loci sequenced. Collections are designated with C.L. Cripps (CLC) or E. Barge (EB) collection numbers and are stored at MONT herbarium.

Voucher	**Date**	**Location**	**Host(s)**	**ITS**	***rpb2***
CLC1689	9 Aug 2001	Stony Pass, San Juan Range, CO, U.S.A.	*Salix* spp.	Yes	Yes
CLC1710	10 Aug 2001	Cinnamon Pass, San Juan Range, CO, U.S.A.	*Salix reticulata*	Yes	Yes
CLC1741	13 Aug 2001	Independence Pass, Sawatch Range, CO, U.S.A.	*Salix reticulata*	Yes	Yes
EB0036	11 Aug 2011	Maggie Gulch, San Juan Range, CO, U.S.A.	*Salix planifolia*	Yes	Yes
EB0039	13 Aug 2011	Stony Pass, San Juan Range, CO, U.S.A.	*Salix planifolia*	Yes	Yes

Figure 32. *L.* aff. *salicis-reticulatae* (EB0036) growing with *Salix planifolia*, Maggie Gulch, San Juan Range, Colorado. Scale bar = 2 cm.

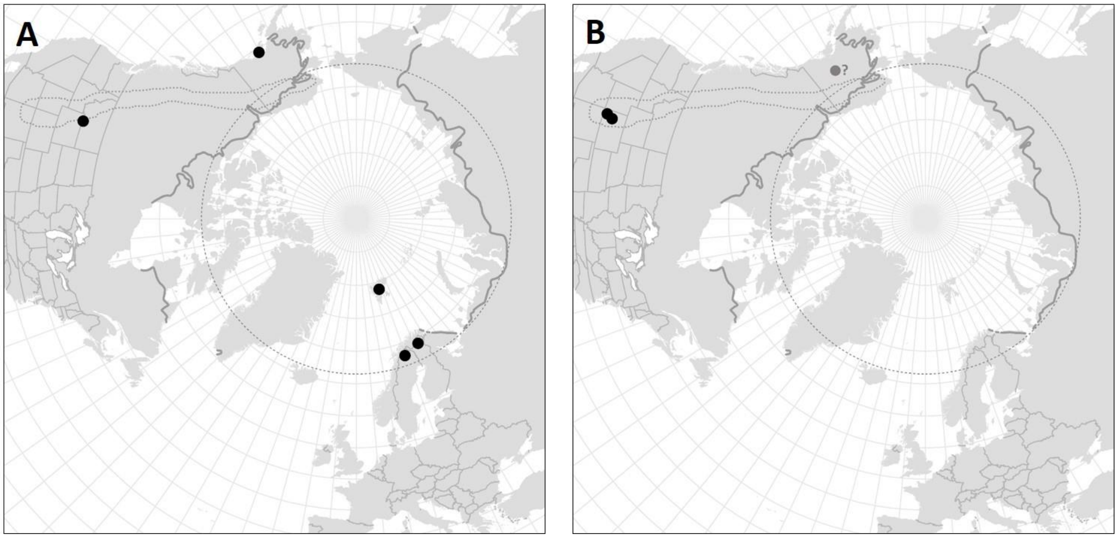


Figure 33. Confirmed distribution of A. *L. salicis-reticulatae* and B. *L.* aff. *salicis-reticulatae* based on available molecular data. Black dots indicate arctic-alpine sites, gray dots indicate non-arctic-alpine sites. The Rocky Mountains are outlined by a dotted gray line. The northern extent of trees is indicated by a thick gray line. Arctic circle = 67.5° N.

Table 17. Comparison of morphological features between *L. salicis-reticulatae* and select closely related taxa. EUR = Eurasia, NA = North America, CO = Colorado, AK = Alaska, GRNLND = Greenland.

Taxon	**Spores**	**Pleurocystidia/ Cheilocystidia**	**Fruitbody characters**	**Distribution Ecology**
L. salicis-reticulatae	(7—)8.5—11.5 × (7—)8—10 µm	76.2—101.6 × 7.6—11.4 µm/ 68.6—91.4 × 7.6—10.2 µm	Pileus cream, pale yellow, or pale ocher, to 40 mm in diameter	EUR, NA; arctic-alpine; *Salix*
L.* aff. *salicis-reticulatae	8.5—11.5 × 7—10 µm	80—127 × 7.6—12.7 µm/ 55.9—99.1 × 7.6—10.2 µm	Pileus cream, pale yellow, or pale ocher, to 90 mm in diameter	NA (CO, AK?); low alpine, subalpine?; *Salix*
*L. aspideus**	7.9—9.5 × 6.3—7.9 µm	54—90 × 8—11(—12) µm/33—73 × 7—10 µm	Pileus whitish alutaceous, pale brownish yellowish	EUR; subalpine; *Salix*
*L. flavoaspideus**	8.4—10.2 × 6.3—7.9 µm	45—90 × 7.5—11(—12.5) µm/30—73(—82) × 7—10 µm	Pileus pale citrine	EUR; subalpine; *Salix*

Table 17 Continued.

Taxon	**Spores**	**Pleurocystidia/ Cheilocystidia**	**Fruitbody characters**	**Distribution Ecology**
*L. flavopalustris**	7.7—9.3(—10.2) × 6.1—7.3 µm	66—93 × 9—14.5 µm/ 35—80 × 6—11 µm	Pileus pale citrine yellow to bright yellow; slimy	EUR; subalpine, rarely arctic-alpine; *Betula*, *Salix*?, *Picea*?
*L. aspideoides*** (may be conspecific with *L. flavopalustris*)	7—9(—10) × 7—8 µm	52—90 × 9—14 µm/cheilocystidia similar	Pileus pale yellow; slimy-viscid	NA, EUR?, subalpine; conifer forests
*L. salicis-herbaceae****	8.3—11.4 × 6.6—8.4 µm	55—80 × 8—11 µm/30—50 × 8—10 µm	Pileus cream to buff-yellow	EUR, NA (GRNLND); arctic-alpine; *Salix*

*Data from Kytövuori et al. (2009)
**Data from Hesler & Smith (1979)
***Data from Heilmann-Clausen et al. (1998)

Key to Rocky Mountain Alpine *Lactarius* Species with Notes on Select Related Taxa

1. Latex turning violet to dull lilac or flesh staining violet to dull lilac 2

1. Latex not turning violet to dull lilac; flesh not staining violet to dull lilac..................... 4

2. Fruiting body robust; pileus 60—100 mm in diameter, yellow-brown to orange-brown, hairy, especially near margin; lamellae cream to pale-yellow; stipe 30—70 × 15—35 mm, stout, cream to pale-yellow, often with numerous yellow-brown pits; odor spicy-floral; taste spicy-floral to mildly acrid; spores 8—10.5 × 6—8.5 µm, broadly ellipsoid to ellipsoid; ornamentation of relatively thick, dense ridges; putatively associated with *Betula* and *Picea* (possibly also *Salix*); in Rocky Mountain alpine zone and subalpine and arctic-alpine areas in

North America and Eurasia .... ***L. repraesentaneus* (Figures 27-29, 35, 36)**

> See also ***L. dryadophilus***: Pileus pale cream to pale yellow, hairy only at margin; tissue staining violet; spores with relatively thin, sparse ridges; putatively associated with *Salix* and *Dryas* in arctic-alpine areas in North America and Eurasia; not reported from the Rocky Mountain alpine zone outside of Alaska.

2. Fruiting body smaller; pileus smooth; margin smooth 3

3. Pileus 20—40 mm in diameter, pale yellow to pale ocher; lamellae cream, pale yellow, or pale orange-ocher, often with a pinkish-buff tint; stipe 15—20 × 10—15 mm, cream to pale yellow; odor mild to slightly sweet; taste mild; spores (7—)8.5—11.5 × (7—)8—10 µm; ornamentation of narrow ridges and isolated warts forming an incomplete reticulum; in Rocky Mountain alpine zone and other arctic-alpine areas in North America and Eurasia with *Salix* .... ***L. salicis-reticulatae* (Figures 30, 31, 33, 35, 36)**.

> See also ***L.* aff. *salicis-reticulatae* (Figures 32, 33, 35):** Pileus to 90 mm wide; tissue staining violet; occurring in alpine areas in Colorado with *Salix*, possibly also occurring in Alaska.
>
> ***L. aspideoides/flavopalustris***: Fruiting bodies robust (to 130 mm wide); pale yellow to bright yellow; slimy; tissue staining violet; subalpine (rarely arctic-alpine) in Europe (*L. flavopalustris*) and North America (*L. aspideoides*) with *Betula* and possibly *Picea* and *Salix*.
>
> ***L. aspideus*** and ***L. flavoaspideus***: Fruiting bodies smaller than *L. flavopalustris*; pale yellow to pale citrine; viscid; tissue staining violet; subalpine in Europe (possibly North America?) with *Salix*.
>
> ***L. salicis-herbaceae***: Fruiting bodies small; yellow-brown; lamellae crowded, pale cream to grayish buff; tissue staining violet; spores densely reticulate; arctic-alpine in North America (at least Greenland) and Eurasia with dwarf *Salix*.

3. Pileus 20—50 mm in diameter, blotchy light tan to medium brown, lighter toward margin; lamellae white to pale yellow-cream; stipe 10—40 × 5—10 mm, white to cream;

odor mild, taste mild; spores 8—10 × 6.5—8 µm; ornamentation of thin ridges and a few isolated warts forming an incomplete to dense reticulum; in low alpine areas in Colorado with *Salix planifolia* and possibly Wyoming with *Salix glauca* .... ***Lactarius pallidomarginatus*** **Barge & C.L. Cripps** ***ad int.***

See also ***L. pseudouvidus*****:** Very similar to *Lactarius pallidomarginatus*; tissue staining violet; spores slightly larger (7.5—12 × 6.5—8(8.5) µm) with thicker ridges; arctic-alpine (and subarctic) in Eurasia and possibly North America (Greenland?) with dwarf *Salix; Lactarius pseudouvidus s.l.* may actually represent a complex of species.

L. brunneoviolaceus **(Figure 35):** Pileus vinaceous; tissue staining violet; dried tissue (possibly also fresh tissue) green in 2.5% KOH; odor of cedar; spores large (to 13 µm long); arctic-alpine in Eurasia and North America (at least Greenland) with *Salix*.

L. montanus **(Figure 35):** Fruiting bodies robust; pileus to 130 mm in diameter, gray-brown to vinaceous; tissue staining violet; tissue green in 2.5% KOH; subalpine (can be found in krummholz zone) in mountains of western North America with *Picea.*

L. luridus**:** Fruiting bodies robust; pileus to 75 mm wide, gray-brown to vinaceous; subalpine in Europe with deciduous trees.

L. pallescens**:** Fruiting bodies robust; pileus 100 mm wide, white to pale gray; subalpine in mountainous and coastal areas of western North America.

L. uvidus**:** Fruiting bodies robust; pileus to 130 mm wide, pale gray to gray-vinaceous; subalpine in Europe and possibly North America with *Betula*, *Picea* and possibly *Salix* .

Lactarius **sp. 4:** Very similar to *L. pallidomarginatus* Barge & C.L. Cripps *ad int.*; pileus darker (gray-brown to vinaceous); spores slightly larger (8.5—11.5 × 7—9 µm); only reported from arctic-alpine Svalbard with *Salix polaris*

***Lactarius* sp. 5:** Morphological features currently unknown; probably similar to and possibly even conspecific with *L. pallidomarginatus* Barge & C.L. Cripps *ad int.*; only reported from arctic-alpine Svalbard with *Salix polaris*

4. Pileus 10—45 mm in diameter, bright orange to orange-brown; margin smooth; lamellae pale cream to pale yellow with faint orange tint; stipe 10—20 × 2.5—7.5 mm, pale orange, discoloring dingy orange to dingy light brown where damaged; odor mild; taste mild; in Rocky Mountain alpine zone, arctic-alpine Alaska (possibly also subalpine) to Svalbard with *Salix*... ***L. lanceolatus* (Figures 14-16, 34, 36)**

See also ***L. aurantiacus* (Figure 34):** Pileus dull orange to bright orange; margin sometimes crenulate; subalpine and arctic-alpine in Alaska to Svalbard and Eurasia; found in a variety of subalpine forest types and occurs with *Salix* and *Kobresia* in arctic-alpine areas.

***L. substriatus*/*L. subflammeus*:** Pileus scarlet when young, becoming orange, slimy-viscid; margin translucent-striate; latex sometimes becoming straw yellow; taste slowly acrid; subalpine in conifer forests in the Pacific Northwest of North America.

***L. subviscidus*:** Pileus red-brown to pinkish in age, subviscid, rugulose when dry; subalpine in and around well-decayed conifer debris in the Pacific Northwest of North America.

***L. luculentus*:** Pileus ochraceous, cinnamon-red, or pale orange, slimy-viscid, smooth when dry; cheilocystidia shorter (23—45 × 4—6 µm) than in *L. lanceolatus*; taste slowly acrid; subalpine in conifer forests in western North America.

***L. luculentus* v. *laetus*:** Pileus pale orange, or bright orange (apparently brighter than *var. luculentus*), slimy-viscid, smooth when dry; taste bitter; cheilocystidia shorter (15—30 × 4—8 µm) than in *L. lanceolatus*; subalpine in conifer forests in western North America.

L. duplicatus/L. lapponicus: Pileus brick to orange-brown to yellowish-brown, viscid; latex becoming sulphur-yellow; taste bitter; low-arctic to subalpine in *Betula* stands or mixed forests of North America and Eurasia.

Lactarius **sp. 1**: Morphological features currently unknown, but probably similar to *L. lanceolatus* based on close phylogenetic relationship; arctic-alpine in Svalbard (possibly also subalpine Alaska) with *Dryas octopetala*.

Lactarius **sp. 2:** Morphological features currently unknown, but probably similar to *L. lanceolatus* based on close phylogenetic relationship; subalpine in California under *Quercus*.

4. Pileus gray-brown, brown or mauve.. 5

5. With odor of coconuts; pileus 15—50 mm in diameter, pale pinkish buff to pale gray-brown, with glaucous coating when young; lamellae cream to pale yellow-orange; stipe 10—40 × 3—12 mm, buff or pale salmon; taste mild; spores 7—9 × 5—7 µm; pleurocystidia 45.7—63.5 × 6.4—7.6 µm; in Rocky Mountain alpine zone and subalpine and arctic-alpine areas in

North America and Eurasia with *Betula* .... ***L. glyciosmus*** **(Figures 21-23, 34, 36)**

See also ***L. mammosus***: Basidiomes typically more robust than *L. glyciosmus*; pileus to 120 mm in diameter, gray-brown; subalpine (possibly also alpine) in Europe and possibly North America with members of Pinaceae (possibly also *Betula*).

5. Without odor of coconuts; pileus 10—50 mm in diameter, gray-brown to liver-brown, with whitish glaucous coating when young; lamellae cream when immature becoming dingy cream to light tan in age, sometimes with a faint pinkish tinge, discoloring to dingy tan where damaged. Stipe 5—30 × 3—15 mm, pale apricot to dingy cream-tan, frost line sometimes visible on upper stipe; taste mild to slightly acrid; spores 7—10.5(—12) ×

5—8 µm; pleurocystidia 53.3—96.5(—114.3) × 6.4—11.4 µm; in Rocky Mountain alpine zone and arctic-alpine areas in Eurasia and likely other arctic-alpine areas in North America with *Salix*... ***L. nanus*** **(Figures 17-20, 34, 36)**

> See also ***L. hysginoides***: Pileus yellow-brown to cinnamon, spores 6—8 × 5—7 µm; subalpine in Alaska and Northern Europe, putatively with *Picea*, *Betula* and *Salix*.

Figure 34. A. *L. lanceolatus* (EB105-13), B. *L. aurantiacus* (JV15112F) in an arctic-alpine habitat in Scandinavia (originally identified as *L. lanceolatus*), C. *L. nanus* (EB106-13), D. *L. nanus* (EB138), E. *L. glyciosmus* (ZT12723), F. *L. glyciosmus* in subalpine habitat (EB133). Photographs by E. Barge (A, C, D, F), J. Vauras (B), E. Horak (E). Scale bars = 2 cm.

Figure 35. A. *L. salicis-reticulatae* (EB0057-14), B. *L.* aff. *salicis-reticulatae* (EB0036), C. *L. repraesentaneus* (EB107-13), D. Also EB107-13 showing close-up of bearded pileus margin, E. *L. montanus* (EB0072-14), F. *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* (EB0041), G. *L.* aff. *brunneoviolaceus* (CLC2133), H. *Lactarius* sp. 4 (CLC1910), Photographs by E. Barge (A-F), C. Cripps (G, H). Scale bars = 2 cm.

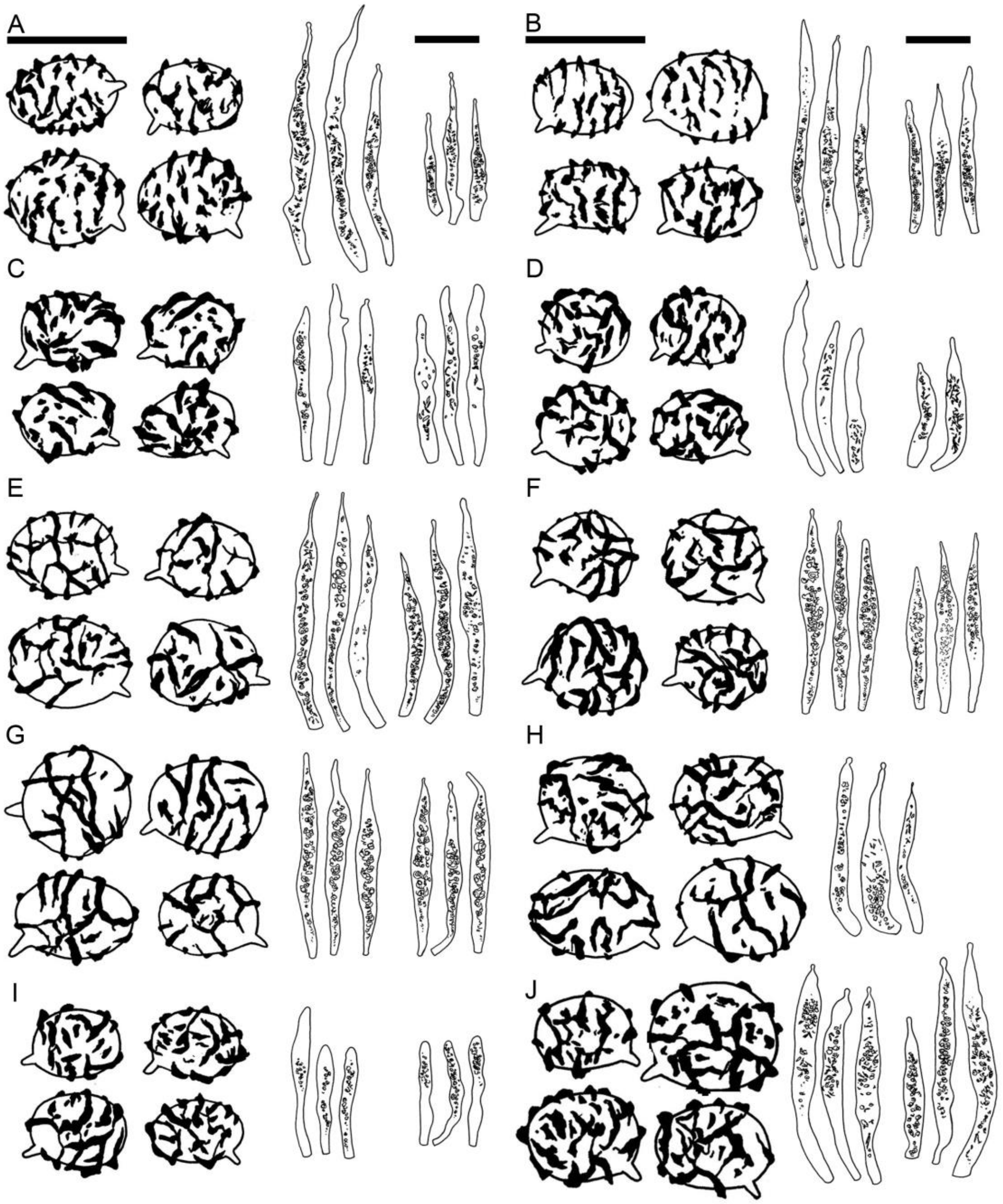


Figure 36. Comparison of micromorphological features of Rocky Mountain alpine specimens and Holotypes (when available). Spores, pleurocystidia (left) and cheilocystidia (right) of A. *Lactarius lanceolatus* (CLC2319), B. *L. lanceolatus* (F 4239, HOLOTYPE), C. *L. nanus* CLC1471, D. *L. nanus* (ZA 192c, HOLOTYPE), E. *L. pallidomarginatus* Barge & C.L. Cripps *ad int.* (EB0041), F. *L. pseudouvidus* (Va 71971, HOLOTYPE), G. *L. salicis-reticulatae* (CLC2776), H. *L. salicis-reticulatae* (K72-104, HOLOTYPE, no cheilocystidia drawn), I. *L. glyciosmus* (TWO269), J. *L. repraesentaneus* (CLC1747). Bars = 10 µm for spores and 20 µm for cystidia. Drawings by E. Barge.

Discussion

Species of *Lactarius* in the Rocky Mountain Alpine Zone

The number of *Lactarius* species reported from the Rocky Mountain alpine zone represents only a fraction of the total number of *Lactarius* species reported from arctic-alpine areas, however, nearly half of the core arctic-alpine species are now confirmed in continental North America. Core species often cited as being restricted to arctic-alpine areas with dwarf and shrubby *Salix* and (or) *Dryas* species include *L. brunneoviolaceus*, *L. dryadophilus*, *L. lanceolatus*, *L. nanus*, *L. pseudouvidus*, *L. salicis-herbaceae* and *L. salicis-reticulatae* (Knudsen & Borgen 1982, Heilmann-Clausen et al. 1998, Basso 1999, Knudsen & Vesterholt 2008). Phylogenetic analyses and morphological examination confirm three of these species for the Rocky Mountain alpine zone (*L. lanceolatus*, *L. nanus*, *L. salicis-reticulatae*), and support the hypothesis that they are largely restricted to arctic-alpine areas with *Salix*. One definitive new species, *L. pallidomarginatus* was revealed, along with a putative new cryptic species, *L.* aff. *salicis-reticulatae*.

A majority of *Lactarius* species that have been reported from arctic-alpine areas are also commonly reported from subalpine or boreal areas with Betulaceae and (or) Pinaceae (Heilmann-Clausen et al. 1998, Knudsen & Vesterholt 2008). From this set of *Lactarii*, we report two species from the Rocky Mountain alpine zone: *L. glyciosmus*, which associates with *Betula glandulosa* in the Rocky Mountain alpine zone and which may be restricted to *Betula* spp. across the rest of its distribution, and *L.*

repraesentaneus which is commonly reported with *Betula* and *Picea* (Hesler & Smith 1979, Heilmann-Clausen et al. 1998) and appears to associate with *Salix glauca* and (or) perhaps krummholz *Picea* in the Rocky Mountain alpine zone.

Taxonomic uncertanties were revealed in the phylogenetic analyses of clades A, B, and C. In the phylogenetic analyses of clade A, certain collections obtained for this study originally identified as *L. lanceolatus* from arctic-alpine areas in Svalbard, Alaska and Scandinavia fall out with *L. aurantiacus*, and this study is the first to confirm that *L. aurantiacus* occurs in arctic-alpine areas, although so far it is not reported from the Rocky Mountain alpine zone. However, solid morphological differences between *L. lanceolatus* and *L. aurantiacus* were not detected. In the phylogenetic analyses of clade B, a possible cryptic species fitting the morphological concept of *L. glyciosmus* was recovered; it does not appear to occur in the Rocky Mountain alpine zone. In the phylogenetic analyses of clade C, the whole *L. pseudouvidus*/*L. brunneoviolaceus* group is poorly defined and more work is needed here to clarify the number and taxonomic affiliation of species; it appears that there may be one or two undescribed species within this complex.

In contrast to other genera, there is relatively little molecular variation between closely related species studied in this thesis, which has previously been reported for the genus *Lactarius* (Verbeken & Nuytinck 2013); in some cases, even morphologically distinct species do not separate clearly using molecular techniques. This is the case for *L. repraesentaneus* and *L. dryadophilus*, and while *L. dryadophilus* has not been reported in the Rocky Mountain alpine zone, the two species are sometimes confused in other areas, but they are clearly morphologically distinct. ITS sequences between the two species are as much as 99.4 percent similar. The two species separate more clearly when *rpb2* DNA is compared.

Conflicts between ITS and *rpb2* gene trees (although not statistically supported) as seen for *L. lanceolatus*, *L. hysginoides*, the *L.* aff. *brunneoviolaceus*/*pseudouvidus* group and *L. salicis-reticulatae*/aff. *salicis-reticulatae* may have been due to incomplete lineage sorting of alleles. Incomplete lineage sorting is a widely cited occurrence when inferring evolutionary relationships between recently diverged species and can lead to problems interpreting species boundaries (Tajima 1983, Takahata and Nei 1985, Hudson 1992, Carsten & Knowles 2007). The probability that genes sampled from a species are monophyletic is positively correlated with the age of the species and negatively correlated with the species' effective population size (Kingman 1982). Recently diverged species will have acquired few differences in their gene sequences, which can result in paraphyly and polyphyly of single species (Nichols 2001). To attempt to overcome this problem, future studies should include greater taxon sampling with more type specimens, more loci, less missing data and perhaps explore the use of techniques incorporated into programs such as BEAST 1.7 (Drummond et al. 2012) to infer species trees from gene trees.

Comparison of ITS and *rpb2* Loci

The two gene regions studied in this thesis, the ITS region and a portion of *rpb2*, differed overall in their phylogenetic utility; each had strengths and weaknesses. Regarding ease of PCR amplification, the ITS region outperformed *rpb2*, likely due to its multi-copy nature. Generally, both ITS and *rpb2* DNA readily amplified for specimens collected within the last 30 years. In specimens older than 30 years both loci amplified somewhat sporadically. For specimens up to 40 years old, the ITS region typically amplified when broken up into two smaller parts, whereas *rpb2* DNA typically did not. Thus, the ITS region was more useful for older type specimens. Unfortunately, some old collections, such as Kühner's types, collected circa 1970 did not yield useful DNA for amplification of either the ITS or *rpb2* regions, and poor storage conditions might possibly have played a role.

The ITS and *rpb2* regions differed in their utility for species-level phylogenetics. For closely related species, such as *L. repraesentaneus*/*L. dryadophilus*, and *L. nanus*/*L. hysginoides*, *rpb2* DNA sequence phylogenies provide clearer delimitation and better reflect historical taxonomic expectations. For *L. lanceolatus*/*L. luculentus*/*L. luculentus* v. *laetus*/*L. aurantiacus*, the resulting ITS gene tree better reflects what was expected than the *rpb2* gene tree, although *rpb2* data was missing for *L. luculentus*. For *L. salicis-reticulatae*/*L.* aff. *salicis-reticulatae*, neither locus provided strongly supported delimitation. Interestingly, many of the taxa examined (*L. lanceolatus*, *L. aurantiacus*, *L.* aff. *brunneoviolaceus*, members of the *L.* aff. *pseudouvidus* group) had *rpb2* sequences heterozygous for many alleles, which may have had a negative impact on obtaining a strong phylogenetic signal for species recognition; this has been encountered at the *rpb2* locus in other fungal genera, such as *Rhizopogon* (Dowie et al. 2012).

A positive aspect of using the ITS region is that it is the most commonly sequenced locus from environmental samples, and it is the most widely used barcode region for fungi; thus many fungal ITS sequences are available in online databases (such as GenBank and Unite) which can be downloaded and incorporated into phylogenetic analyses. This was extremely useful for this study as it provided additional information on species distributions and ecology. Overall, both loci were similar in length and provided a similar number of parsimony-informative sites for each of the species-level phylogenetic analyses.

The ITS region and *rpb2* locus also differed in their utility for broad phylogenetic analyses, which incorporated several genera. The *rpb2* locus was much easier to align across genera, contained very few indels, and 36% of the sites were informative in the alignment used in this study. The ITS region on the other hand was not easy to align across multiple genera in broad phylogenetic analyses. The initial alignment included 1045 positions, however, 445 ambiguously aligned positions were removed, whereas no ambiguously aligned regions were present in the *rpb2* alignment. The final ITS alignment also provided fewer informative sites than the *rpb2* alignment. However, the combined analysis of both loci provided a more resolved tree than either locus alone, although backbone support was generally low even in the combined analyses.

Infrageneric Classification

The intent of this thesis was not to provide an exhaustive revision of infrageneric classification in *Lactarius*, however infrageneric taxonomy is discussed here in light of phylogenetic analyses to provide some insight as well as direction for future research. This discussion focuses mainly on the phylogenetic classification of Verbeken et al. (2014), as well as on the morphological classification schemes of Hesler & Smith (1979), Heilmann-Clausen et al. (1998) and Basso (1999).

Verbeken et al. (2014) delineate three major clades within *Lactarius* corresponding with the subgenera *Piperites*, *Plinthogalus* and *Russularia* and these groups are largely concordant with the two most recent morphological classification schemes proposed by Heilmann-Clausen et al. (1998) and Basso (1999). However, in Verbeken et al. (2014), *Piperites* received low statistical support, taxon sampling was low and there were subtle differences when results were compared to the aforementioned morphological classification schemes. The three clades corresponding with subgenera *Piperites*, *Russularia* and *Plinthogalus* recovered in Verbeken et al. (2014) were also recovered in combined (ITS + *rpb2*) maximum likelihood (ML) phylogenetic analyses in this thesis. Support for the clade corresponding with subgenus *Piperites* was low in this thesis as well as in Verbeken et al. (2014), and single-gene trees as well as combined maximum parsimony and Bayesian analyses showed polyphyly of subgenus *Piperites*, although with no statistical support. Furthermore, as in Verbeken et al. (2014), support for relationships between species within subgenus *Piperites* was low in general.

A monophyletic clade within subgenus *Piperites* was recovered in the broad, combined Bayesian and ML phylogenetic analyses, corresponding with section *Uvidi* (labeled as Sect. *Uvidi* in Figure 3) as recognized by Heilmann-Clasusen et al. (1998). Taxa included in this section have latex which stains the sporocarp flesh violet. The monophyly of this section was previously suggested in Le et al. (2007), however, taxon sampling was low. Rocky Mountain alpine violet-staining species *Lactarius pallidomarginatus*, *L. repraesentaneus*, *L. salicis-reticulatae* and *L.* aff. *salicis-reticulatae* clearly fall out in this clade.

The placement of *Lactarius lanceolatus* in subgenus *Russularia* (Fr.) Kauffmann by Hesler & Smith (1979), Heilmann-Clausen et al. (1998) and Basso (1999) is supported by phylogenetic analyses and agrees with other modern molecular treatments of *Russularia* (Verbeken et al. 2014, Wisitrassameewong 2014). Its sectional placement varies by author; Hesler & Smith (1979) and Heilmann-Clausen et al. (1998) place it in section *Russularia* Fr., while Basso (1999) places it in section *Mitissimi* Neuhoff ex Bon, which contains a subset of the species treated in section *Russularia* by the other two authors. Each author has a different concept of these sections and each includes different species. Sectional and in some cases subgeneric placements within a phylogenetic context are still poorly investigated in *Lactarius* and are in need of a unifying treatment. The monophyly of sections *Russularia* or *Mitissimi* was not investigated in depth in this study, however, phylogenetic analyses suggest that these sections are not monophyletic. *Lactarius lanceolatus* formed a clade with *L. aurantiacus*, *L. luculentus*, *L. luculentus* v. *laetus*, and *L. substriatus/subflammeus*, *L. brunneohepaticus*, *L. cyathuliformis*, *L. subviscidus*, *L. sphagneti*, *L. subsericatus* and *L. subdulcis*. Most of these taxa are placed in section *Russularia* by either Hesler & Smith (1979) or Heilmann-Clausen et al. (1998), however, four of these (*L. brunneohepaticus*, *L. cyathuliformis*, *L. subviscidus*, and *L. substriatus*) are placed in other sections by these authors. *Lactarius quietus*, which has historically been placed in section *Russularia*, appears distantly related to all of the above-mentioned taxa based on phylogenetic analyses. Basso (1999) does not cover species such as *L. luculentus*, *L. luculentus* v. *laetus*, *L. substriatus/subflammeus*, and *L. subviscidus* likely because they have not been reported from Europe. Basso's (1999) section *Mitissimi* includes *L. aurantiacus* (syn.: *L. mitissimus*), *L. lanceolatus* and *L. subsericatus* (syn.: *L. fulvissimus*). Based on phylogenetic analyses this sectional classification is polyphyletic with respect to *L. subsericatus*.

The placement of *Lactarius nanus* in subgenus *Piperites* (Fries) Kauffman by Heilmann-Clausen et al. (1998) and Basso et al. (1999) (not treated in Hesler & Smith 1979) was supported by phylogenetic analyses in this study. Both Heilmann-Clausen et al. (1998) and Basso (1999) place *L. nanus* in section *Glutinosi* Quél. The monophyly of this section is unresolved, however, phylogenetic analyses in this study suggest it may not be monophyletic. *Lactarius nanus* appears most closely related to *L. hysginoides*, *L.*

glyciosmus, *L. mammosus*, and *L. spinosulus*, however support for these relationships is low. *Lactarius hysginoides* has historically been placed in the same section as *L. nanus*, however *L. glyciosmus*, and *L. mammosus* have historically been placed in section *Colorati* (Bat.) Hesler & A.H. Sm., and *L. spinosulus* has historically been placed in section *Piperites* Fr.

Lactarius glyciosmus is placed in subgenus *Piperites* (Fr.) Kauffman by Heilmann-Clausen et al. (1998), subgenus *Russularia* (Fr.) Kauffman by Basso (1999) and subgenus *Tristes* Hesler & A.H. Sm. by Helser & Smith (1979). All three works place *Lactarius glyciosmus* in section *Colorati* (Bat.) Hesler & A.H. Sm. Heilmann-Clausen et al. (1998) lump subgenus *Tristes* in with subgenus *Piperites*, which is supported by Verbeken et al. (2014). Phylogenetic analyses in this study place *L. glyciosmus* in subgenus *Piperites* and agree with the results of Verbeken et al. (2014) in regard to lumping subgenus *Tristes* with subgenus *Piperites*. Based on phylogenetic analyses in this study, section *Colorati* appears to be non-monophyletic. *Lactarius glyciosmus* appears most closely related to *L. hysginoides*, *L. mammosus*, *L. nanus* and *L. spinosulus* (with low support), however *L. hysginoides* and *L. nanus* have historically been placed in section *Glutinosi*, and *L. spinosulus* has historically been placed in section *Piperites*.

Distribution and Ecology of Rocky Mountain Alpine *Lactarius* Species

All Rocky Mountain alpine *Lactarius* species delineated in this study appear to have broad intercontinental distributions except for *L. pallidomarginatus*, which is only documented from low alpine areas in Colorado and Wyoming, and *L.* aff. *salicis-reticulatae*, which is only documented from alpine areas in Colorado and possibly also Alaska. This is in contrast to patterns recently observed through molecular phylogenetic analyses of *Lactarius*, where different species from other habitats were found to be restricted to either North America or Eurasia (Verbeken et al. 2007, Stubbe & Verbeken 2012). However, results here are largely in agreement with molecular studies investigating the distribution of other boreal and arctic-alpine fungal species (Peintner 2008, Beker et al. 2010, Cripps et al. 2010, Geml et al. 2012, Timling et al. 2012, 2014, Tedersoo et al. 2014, Larsson et al. 2014), as well as with historical distribution concepts for arctic-alpine ectomycorrhizal fungi (e.g. Knudsen & Borgen 1982, Knudsen & Lamoure 1993, Gardes & Dahlberg 1996, Heilmann-Clausen et al. 1998) that show broad intercontinental distributions.

In the Rocky Mountain alpine zone, *Lactarius lanceolatus* was found on the Beartooth Plateau with *Salix reticulata* as the only suitable host in close proximity, however it is also reported with mixed-species *Salix* patches. A particular host has not yet been confirmed for *L. lanceolatus* through ECM root tip sequencing. Interestingly, *L. lanceolatus’* close relative *L. aurantiacus* appears to associate with *S. polaris* on Svalbard (although *S. reticulata* is a possible host as it also occurs on Svalbard) and *Kobresia* in alpine China, as well as with a variety of trees in subalpine Eurasia.

Lactarius nanus appears to associate with *Salix arctica* in alpine areas in the Rocky Mountains, although other *Salix* species such as *S. reticulata* and *S. planifolia* cannot be ruled out as they are often in proximity. In Svalbard, *S. polaris* appears to be a possible host, although again *S. reticulata* cannot be ruled out as it too occurs on Svalbard. A particular host has not yet been confirmed for *L. nanus* through ECM root tip sequencing.

Lactarius pallidomarginatus appears to associate with *Salix planifolia* and perhaps *S. glauca* in low alpine areas of Colorado and Wyoming based on proximity to these hosts. A closely related if not conspecific species (*Lactarius* sp. 5) has been isolated from the roots of *S. polaris* in Svalbard.

Lactarius salicis-reticulatae appears to associate with *S. reticulata* and *S. glauca* in alpine areas in the central Rockies, however, other *Salix* species such as *S. arctica* and *S. planifolia* are often nearby; in Alaska, it is reported with *S. reticulata*. Kühner (1975) reports *L. salicis-reticulatae* with *S. reticulata* in the Alps and shrubby willows (possibly *S. glauca*) in Scandinavia. This species has not been isolated from ECM roots.

The distribution and ecological amplitude of *L.* aff. *salicis-reticulatae* is still somewhat ambiguous. It occurs in low alpine areas in Colorado, possibly with *Salix planifolia* and *S. reticulata* and based on phylogenetic analyses in this study, possibly also Alaska. Further phylogenetic analyses incorporating more loci are needed to determine whether or not it is conspecific with *L. salicis-reticulatae sensu stricto*, which would expand its range.

In the Rocky Mountain alpine zone, the host association of *L. repraesentaneus* is somewhat ambiguous; it is possibly associated with the roots of krummholz *Picea*, although *Salix glauca* cannot be ruled out as it is always present and it appears to be an ectomycorrhizal host for other species of *Lactarius*. *Lactarius repraesentaneus* has not been reported with *B. glandulosa* in the Rocky Mountain alpine zone. Ectomycorrhizal root tip sequencing has not definitively tied *L. repraesentaneus* to any host.

Lactarius glyciosmus appears to associate with *B. glandulosa* in the Rocky Mountain alpine zone and phylogenetic placement of ectomycorrhizal root tip sequences (GU998223, GU998695) confirm *Betula nana* as a host in Alaska. Across the rest of its range in subalpine North America and Eurasia, possible hosts include *B. pubescens* (Elborne & Knudsen 1990, Basso 1999), *B. papyrifera* (Durall et al. 2006), and *B. occidentalis* (Bogar & Kennedy 2013). *Betula glandulosa* is somewhat rare in the central Rockies and more common in the southern Rockies, however, extensive surveys of *Betula* were not conducted in this study. Cripps & Horak (2008) report *L. pubescens* from *Betula glandulosa* in Colorado (these collections were not included in this study) and it is possible that other *Lactarius* species associate with *Betula* in the Rocky Mountain alpine zone, but more extensive surveys are necessary.

Other detailed morphological and molecular systematics studies have also found broad, intercontinental distributions for arctic-alpine inhabiting, ectomycorrhizal species of *Inocybe* (Cripps et al. 2010, Larsson et al. 2014), *Cortinarius* (Peintner 2008) and *Hebeloma* (Beker et al. 2010). In these studies, as with the present study, some species appear somewhat host-specific and restricted to arctic-alpine areas, whereas others were much less tied to a particular host and occurred in subalpine habitats as well.

Overall, distributional results from this study largely agree with distributional patterns found for other arctic-alpine ectomycorrhizal fungi. However, based on a 97% ITS region similarity cutoff for species recognition from environmental samples collected in the North American arctic, Timling et al. (2012, 2014) found that the most abundant ectomycorrhizal fungi (species of *Cortinarius*, *Inocybe*, *Thelephora*, etc.) had very little host specificity and were not restricted to arctic-alpine areas.

Our findings suggests that the universal 97% ITS region similarity cutoff for species recognition is too broad for *Lactarius* and that at least some *Lactarius* species are quite host-specific and are largely restricted to arctic-alpine areas. In this thesis, *L. repraesentaneus* and *L. dryadophilus* were found to be phylogenetically and morphologically distinct species yet their ITS regions are up to 99.4% similar. If all 97% or more similar BLAST results to *L. repraesentaneus* (Table 5) are treated as one species, it would lump *L. dryadophilus* and *L. repraesentaneus* together as one species which would appear to associate with *Dryas, Salix*, *Betula* and perhaps *Picea* in North America and Eurasia. Yet, it is clear from phylogenetic analyses that *L. dryadophilus* is a distinct species, likely confined to arctic-alpine areas with *Dryas* and *Salix* and that *L. repraesentaneus* inhabits arctic-alpine and subalpine areas with *Betula, Picea* and possibly *Salix*. Furthermore, ITS region BLAST results show that for every Rocky Mountain alpine species recognized, at least one other species was 97% or more similar (Table 5). Thus, using a 97% ITS region similarity cutoff for recognizing species fails to differentiate closely related *Lactarius* species and can inflate inferred ecological and geographical distributions. This problem may be exacerbated in northerly latitudes, as it has been shown that the ITS region in the /sebacina lineage evolves more slowly in high latitude in contrast to low latitude areas (Tedersoo et al. 2014), which could be true for other lineages as well.

Many misidentified sequences were also encountered on GenBank. The problem here is that studies are increasingly relying upon molecular tools and online databases to identify fungi. Thus, publicly available, misidentified sequences are used in subsequent studies and misidentifications are propagated through time, leading to incorrect assumptions about species' distributions and ecology. In total, at least four out of the nine fully identified (genus, species) sequences from arctic-alpine areas that were downloaded from GenBank and used in phylogenetic analyses in this study were misidentified.

The current distribution of arctic-alpine inhabiting *Lactarius* species examined in this study was likely shaped by a number of biotic and abiotic factors including host dispersal and distribution, climate change, glaciation and geography. As *Lactarius* is obligately ectomycorrhizal, it seems likely that the distribution of each species was largely shaped by the distribution of suitable host plants, many of which also appear to have very broad distributions. While information on evolutionary and biogeographic histories are lacking for many of the plant species utilized by the Rocky Mountain alpine *Lactarius* species identified in this study, recent studies suggest complex and contrasting evolutionary and biogeographic histories for *Betula nana*, *Salix herbacea*, and *S. arctica*.

It is currently unclear how closely the biogeographic history of particular ectomycorrhizal fungi mirrors that of their host plants.

Betula nana, a circumpolar species, closely related to *B. glandulosa,* which is absent from Rocky Mountain alpine sites examined in this study, shows little genetic variation (at least at a cpDNA locus) over Norway, Svalbard, Greenland, northern Canada, and Alaska, which is evidence for long distance dispersal for this species (Abbott & Brochmann 2003). In contrast, *Salix herbacea*, which also has a circumpolar distribution, but is absent from the Rocky Mountain alpine zone shows strong population differentiation between West Greenland/Canada and East Greenland/Europe, which may represent a divide predating the last glacial maximum (Alsos et al. 2009). Regional population divergence in *Salix arctica* (also with a circumpolar distribution and disjunct distribution in the Rocky Mountain alpine zone) in the Canadian high arctic suggests that post-glacial recolonization has come from multiple point sources (Mimura et al. 2013). This has also been shown molecularly for both *Betula nana* and *Salix herbacea*, which apparantly recolonized Svlabard after the last glacial maximum from multiple point sources on mainland Eurasia (Alsos et al. 2007), again indicating regular long-distance dispersal of these species.

It has been demonstrated that ectomycorrhizal fungi likely play an important role during primary succession in the arctic (Fujiyoshi et al. 2011) and the ability of some arctic-alpine plant species such as *Salix arctica* and *S. polaris* to rapidly recolonize rocky, previously glaciated terrain (Fujiyoshi et al. 2011, Mimura et al. 2013, Boulanger-

Lapionte et al. 2014), along with long-distance dispersal might further facilitate broad distributions of fungus and plant host. While arctic-alpine ectomycorrhizal fungi appear to be long-distance dispersers (Geml et al. 2012), it is still unclear if the population structure of arctic-alpine ectomycorrhizal fungi follows host patterns; this is further complicated by the fact that many arctic-alpine ectomycorrhizal fungi appear to associate with more than one host species and many appear unrestricted to arctic-alpine areas. Furthermore, detailed information on the biogeographical history of *Salix* and *Betula* species present during glaciations in the Rocky Mountains was not found. However, pollen data from Blacktail Pond, Yellowstone National Park suggest that montane, mid-elevations in the northern Greater Yellowstone Ecosystem were occupied by an alpine tundra vegetation (*Betula*, *Salix*, many herbaceous taxa) similar to that of the present Rocky Mountain alpine tundra vegetation during the late glacial period (14,000—11,500 calibrated years before present) (Krause & Whitlock 2013). Alpine tundra also appears to have domintated areas in the southern Rocky Mountains from around 17,500—12,600 calibrated years before present (Minckley 2014). Both of these studies suggest the presence of broad, low-elevation glacial refugia for arctic-alpine life-forms in the Rocky Mountains along the glacial front. However, how this tundra fluctuated in size and connectedness with other western tundra as well as the Canadian and Eurasian arctic-alpine zone throughout the Pleistocene is unclear.

While reconstructing the biogeographical history of *Lactarius* species in the Rocky Mountain alpine zone is currently not possible, two broad patterns emerge; one set of Rocky Mountain alpine *Lactarius* species (*L. nanus*, *L. lanceolatus*, *L. pallidomarginatus* Barge & C.L. Cripps *ad int.*, *L. salicis-reticulatae*, and *L.* aff. *salicis-reticulatae*) appear to be largely restricted to arctic-alpine areas with *Salix* and another set appears to occur in both subalpine and arctic-alpine areas with *Picea*, *Betula* and possibly *Salix* (*L. repraesentaneus*) or *Betula* only (*L. glyciosmus*). Furthermore, all the Rocky Mountain alpine *Lactarius* species delineated in this study definitively occur in both North America and Eurasia (except perhaps *L. pallidomarginatus* and *L.* aff. *salicis-reticulatae*). The disjunct southern alpine/arctic distribution pattern seen for the arctic-alpine *Salix* associates lends support to the hypothesis that these species may have occupied tundra areas with *Salix* south of the ice sheets during cooler weather during the last glacial maximum and then dispersed upward in elevation and northward as the glaciers and *Salix* hosts retreated (Löve & Löve 1974). Different host associations and different physiological tolerances may have allowed *L. glyciosmus* and *L. repraesentaneus* to persist in subalpine, forested areas as well as to track the tundra as it moved upward in elevation and north following the retreat of the ice sheets. In order for all of these species to have achieved and maintained such broad distributions, gene flow must have been possible over long distances during the Pleistocene, perhaps mediated by long-distance dispersal (Geml et al. 2012), range shifts due to climate change and glacial expansion and retreat (Löve & Löve 1974, Birks et al. 2008), glacial and interglacial refugia (Billings 1974a, Löve & Löve 1974) and the Bering Land Bridge (Shapiro et al. 2004).

With ongoing climate change, arctic-alpine ectomycorrhizal fungal community structure and species' distributions will continue to change. Currently, ectomycorrhizal shrubs are increasing in abundance and expanding their range in the arctic (Sturm et al. 2001, 2005) as well as in the Rocky Mountain alpine zone (Formica et al. 2014) and studies have shown that experimental warming alters the composition of ectomycorrhizal fungal communities (Sturm et al. 2005, Deslippe et al. 2011). Deslippe et al. (2011) reported an increase in ectomycorrhizal fungal diversity associated with the shrub *Betula nana* in arctic-alpine Alaska following experimental warming. However, not all ectomycorrhizal taxa responded in the same way. Some groups of ectomycorrhizal fungi, such as *Cortinarius* became more diverse with warming, whereas others, such as members of the Russulaceae (of which *Lactarius* is a member) decreased in abundance (Deslippe et al. 2011). Deslippe et al. (2011) suggests that overall increased mycorrhizal diversity and larger mycorrhizal networks may further increase shrub expansion in arctic-alpine areas (Deslippe et al. 2011). However, the role of particular *Lactarius* species in this changing scenario is unclear.

Suggestions for Further Research

1) In order to further our understanding of *Lactarius* evolution and better define species within the genus it is clear that analyses including more genetic loci are necessary. Many closely related species were not strongly supported as distinct in phylogenetic analyses in this thesis and it has been suggested that the rate of molecular evolution is relatively slow in *Lactarius* (and perhaps slower at higher

latitudes). A search for additional phylogenetically informative loci for *Lactarius* should be initiated.

2) More species level phylogenetic studies, in conjunction with detailed morphological studies should be conducted for *Lactarius* in order to provide a high quality molecular database for ecological studies.
3) As many older collections (>30-40 yrs.) were not successfully sequenced, recently collected material should be targeted for sequencing with a focus on material from type localities. Alternatively, every effort should be made to improve methods for sequencing older material, particularly types.
4) This thesis made progress towards delineating *Lactarius* species present in the Rocky Mountain alpine zone in reference to arctic-alpine species from other regions, and clarified an aspect of their worldwide distribution and ecology, however their complete biogeographical history is still unknown. In order to better understand the history of these species and predict their future in a changing climate, population level studies should be initiated.

REFERENCES CITED

Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vrålstad T, Liimatainen K, Peintner R, Kõljalg U. 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytologist 186(2): 281-285.

Abbott RJ. 2008. History, evolution and future of arctic and alpine flora: overview. Plant Ecology and Diversity 1: 129-133.

Abbott RJ, Brochmann C. 2003. History and evolution of the arctic flora: in the footsteps of Eric Hultén. Molecular Ecology 12: 299-313.

Aerts R. 2006. The freezer defrosting: global warming and litter decomposition rates in cold biomes. Journal of Ecology 94(4): 713-724.

Alsos IG, Alm T, Normand S, Brochmann C. 2009. Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modeling. Global Ecology and Biogeography 18: 223-239.

Alsos IG, Ehrich D, Thuiller W, Eidesen PB, Tribsch A, Schönswetter P, Lagaye C, Taberlet P, Brochmann C. 2012. Genetic consequences of climate change for northern plants. Proceedings of the Royal Society B: Biological Sciences 279(1735): 2042-2051.

Alsos IG, Eidesen PB, Ehrich D, Skrede I, Westergaard K, Jacobsen GH, Landvik JY, Taberlet P, Brochmann C. 2007. Frequent long-distance plant colonization in the changing Arctic. Science 316: 1606-1609.

Anderson B. 1994. *Beartooth Country: Montana's Absaroka and Beartooth Mountains*. Unicorn Publishing Inc., Billings, Montana. 120 p.

Antibus RK, Croxdale JG, Miller OK Jr., Linkins AE. 1981. Ectomycorrhizal fungi of *Salix rotundifolia* III. Resynthetized mycorrhizal complexes and their surface phosphatase activities. Canada Journal of Botany 59: 2458-2465.

Arendt AA, Echelmeyer KA, Harrison WD, Lingle CS, Valentine VB. 2002. Rapid wastage of Alaska glaciers and their contribution to rising sea level. Science 297(5580): 382-386.

Ballará J. 1997. Nou Estudi D' species Fungiques Interessants Dels Estatges Alpi I Subalpi Dels Pirineus Catalans. Revista Catalana de Micologia 20: 1-24.

Barry RG, Hare FK. 1974. Arctic climate. Pp. 17-54. In: Barry R, Ives JD, (eds.). *Arctic and Alpine Environments*. Methuen, London.

Barry RG, Ives JD. 1974. Introduction. Pp. 1-11. In: Barry RG, Ives JD, (eds.). *Arctic and Alpine Environments.* Methuen, London.

Basso MT. 1999. *Lactarius* Pers. Fungi Europaei 7. Mykoflora, Alassio, Italy. 845 p.

Bataille F. 1908. Flore monographique des Astérosporés. Lactaires et Russules. Mémoires de la Société d'émulation du Doubs, 8 sér. 2: 163-260.

Begerow D, Nilsson H, Unterseher M, Maier W. 2010. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. Applied Microbiology and Biotechnology 87: 99-108.

Beker HJ, Eberhardt U, Vesterholt J. 2010. *Hebeloma hiemale* Bres. in Arctic/Alpine Habitats. North American Fungi 5(5): 51-65.

Bennike O, Böcher J. 1999. Forest-tundra neighbouring the North Pole: plant and insect remains from the Plio-Pleistocene Kap København Formation, North Greenland. Arctic 43(4): 331-338.

Bent E, Kiekel P, Brenton R, Taylor DL. 2011. Root-associated ectomycorrhizal fungi shared by various boreal forest seedlings naturally regenerating after a fire in interior Alaska and correlation of different fungi with host growth responses. Applied and environmental microbiology 77(10): 3351-3359.

Bergemann SE, Garbelotto M. 2006. High diversity of fungi recovered from the roots of mature tanoak (*Lithocarpus densiflorus*) in northern California. Botany 84(9): 1380-1394.

Bessette AE, Harris DB, Bessette AR. 2009. *Milk Mushrooms of North America*: *A Field Identification Guide to the Genus Lactarius*. Syracuse University Press, Syracuse, New York. 297 p.

Billings WD. 1973. Arctic and alpine vegetation: similarities, differences, and susceptibility to disturbance. BioScience 23: 697–704.

Billings WD. 1974a. Adaptations and origins of alpine plants. Arctic and Alpine Research 6: 129-142.

Billings WD. 1974b. Plant adaptations to cold summer climates. Pp. 1-11. In: Barry RG, Ives JD, (eds.). *Arctic and Alpine Environments.* Methuen, London.

Billings WD. 1978. Alpine phytogeography across the Great Basin. Great Basin Naturalist Memoirs 2: 105-117.

Billings WD. 1988. Alpine vegetation. Pp. 392-420. In: Barbour MG, Billings WD, (eds.). *North American terrestrial vegetation*. Cambridge University Press, New York.

Billings WD, Mooney HA. 1968. The ecology of arctic and alpine plants. Biological Reviews 43(4): 481-529.

Bills GF. 1986. Notes on *Lactarius* in the high-elevation forests of the southern Appalachians. Mycologia 78(1): 70-79.

Bills GF. 1986. Distribution of *Lactarius* in the high-elevation forests of the southern Appalachians. Mycologia 78(1): 80-85.

Binder M, Hibbet DS, Larsson K-H, Larsson E, Langer E, Langer G. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). Systematics and Biodiversity 3: 113-157.

Birks HH. 2008. The Late-Quaternary history of arctic and alpine plants. Plant Ecology & Diversity 1(2): 135-146.

Bjorbækmo MFM, Carlson T, Brysting A, Vrålstad, Høiland K, Ugland KI, Geml J, Schumacher T, Kauserud H. 2010. High diversity of root associated fungi in both alpine and arctic *Dryas octopetala*. BMC Plant Biology 10: 244.

Bledsoe C, Klein P, Bliss LC. 1990. A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. Canadian Journal of Botany 68: 1848-1856.

Bliss LC. 1956. A comparison of plant development in microenvironments of arctic and alpine tundras. Ecological Monographs 26(4): 303-337.

Bliss LC. 1962. Adaptations of arctic and alpine plants to environmental conditions. Arctic 15: 117-144.

Bliss LC. 1988. Arctic tundra and polar desert biome. Pp. 1-32. In: MG Barbour, WD Billings, (eds.). *North American Terrestrial Vegetation*. Cambridge University Press, New York.

Bliss LC. 1997. Arctic ecosystems of North America. Pp. 551-683. In: Wielgolaski FE, (ed.). *Ecosystems of the World 3*. *Polar and Alpine Tundra*. Elsevier, Amsterdam, Netherlands.

Bogar LM, Kennedy PG. 2013. New wrinkles in an old paradigm: neighborhood effects can modify the structure and specificity of *Alnus*-associated ectomycorrhizal fungal communities. FEMS Mycrobiology Ecology 83(3): 767-777.

Böhm R, Auer I, Brunetti M, Maugeri M, Nanni T, Schöner W. 2001. Regional temperature variability in the European Alps: 1760-1998 from homogenized instrumental time series. International Journal of Climatology 21(14): 1779-1801.

Bon M. 1980. Clé monographique du genre *Lactarius* (Pers. ex Fr.) SF Gray. Documents Mycologiques 10: 1-85.

Bon M. 1983. Notes sur la systematique du genre *Lactarius*. Documents Mycologiques 13: 15-26.

Bon M. 1985a. Stage mycologie alpine: Lanslebourg (Savoie) du 1 au 3 septembre 1984. Bulletin de la Féderation Mycologique du Daupiné-Savoie 96: 19-25.

Bon M. 1985b. Quelques nouveaux taxons de la flore mycologique alpine. Bulletin de la Féderation Mycologique du Daupiné-Savoie 97: 23-30.

Bon M. 1989. Quelques Agaricomycetes intéressants de la zone alpine récolteés dans le Tessin. Mycologica Helvetica 3(3): 315-330.

Bon M. 1990. Mycologie alpine. Bulletin de la Féderation Mycologique du Dauphiné-Savoie 116: 25-30.

Bon M. 1991. Inventaires des espéces récoltees au stage de mycology alpine. Bulletin de la Féderation Mycologique du Dauphiné-Savoie 122: 25-28.

Bon M. 1998. Clé monographique des lactaires alpines. Bulletin de la Féderation Mycologique du Dauphiné-Savoie 150: 15-22.

Bon M, Ballará J. 1996. Aportació a L'estudi de la microflora alpine dels Pirineus (2^a part). Revista Catalana de Mycologia 19: 139-153.

Bonde EK. 1969. Plant disseminules in wind-blown debris from a glacier in Colorado. Arctic and Alpine Research 1: 135-140.

Borgen T. 2006. Distribution of selected basidiomycetes in oceanic dwarf-scrub heaths in Paamiut area, low arctic South Greenland. Pp. 25-36. In: Boertmann D, Knudsen H, (eds.). *Arctic and Alpine Mycology 6*. Meddelelser om Grønland Bioscience. Museum Tusulanum Press, Greenland.

Borgen T, Elbourne SA, Knudsen H. 2006. A checklist of the Greenland basidiomycetes. Pp. 37-59. In: Boertmann D, Knudsen H, (eds.). *Arctic and Alpine Mycology 6*. Meddelelser om Grønland Bioscience. Museum Tusulanum Press, Greenland.

Boulanger-Lapointe N, Lévesque E, Boudreau S, Henry GHR, Schmidt NM. Population structure and dynamics of Arctic willow (*Salix arctica*) in the High Arctic. Journal of Biogeography 41: 1967-1978.

Bowman WD, Damm M. 2002. Causes and consequences of alpine vascular plant diversity in the Rocky Mountains. Pp. 35-47. In: Körner C, Spehn E, (eds.). *Global mountain biodiversity assessment*. Parthenon/CRC Press, New York, New York, USA.

Britzelmayr M. 1885. Hymenomyceten aus Südbayern. Teil 6, 9. Bericht des Naturhistorischen Vereins in Augsburg 28: 119-160.

Brochmann C, Brysting AK. 2008. The Arctic - an evolutionary freezer? Plant Ecology & Diversity 1: 181-195.

Brouillet L, Whetstone RD. 1993. Climate and physiography. Pp. 15-46. In: Flora of North America Editorial Committee, (eds.). *Flora of North America North of Mexico*. Oxford University Press, Oxford.

Brown J, Ferrians OJ Jr, Heginbottom JA, Melnikov ES. 1998, revised February 2001. Circum-arctic map of permafrost and ground ice conditions. Boulder, CO: National Snow and Ice Data Center. Digital media.

Brundrett MC. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320(1-2): 37-77.

Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F. 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytologist 184(2): 449-456.

Burke DJ, Dunham SM, Kretzer AM. 2008. Molecular analysis of bacterial communities associated with the roots of Douglas fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi. FEMS Microbiology Ecology 65(2): 299-309.

Burlingham GS. 1907a. Some *Lactarii* from Windham County, Vermont. Memoirs of the Torrey Botanical Club 34: 85-95.

Burlingham GS. 1907b. Suggestions for the Study of the *Lactariae*. Torreya 7: 118-123.

Burlingham GS. 1908. A study of the *Lactariae* of the United States. Memoirs of the Torrey Botanical Club 14: 1-109.

Burlingham GS. 1910. *Lactaria*. Pers. North American Flora 9: 172-200.

Burlingham GS. 1913. The *Lactariae* of the Pacific Coast. Mycologia 5: 305-307.

Buyck B, Hofstetter V, Eberhardt U, Verbeken A, Kauff F. 2008. Walking the thin line between *Russula* and *Lactarius*: the dilemma of *Russula* subsect. *Ochricompactae*. Fungal Diversity 28: 15-40.

Buyck B, Hofstetter V, Verbeken A, Walleyn R. 2010. Proposal to conserve *Lactarius* nom. cons. (Basidiomycota) with a conserved type. Taxon 59: 295-296.

Calonge FD, Martin MP. 2000. Morphological and molecular data on the taxonomy of *Gymnomyces*, *Martellia* and *Zelleromyces* (Russulaceae). Mycotaxon 76: 9-15.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540-552.

Campbell JS. 1997. North American alpine ecosystems. Pp. 211-261. In: Wielgolaski FE, (ed.). *Ecosystems of the World 3*. *Polar and Alpine Tundra*. Elsevier, Amsterdam, Netherlands.

Carsten BC, Knowles LL. 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. Systematic Biology 56: 400-411.

Chapin FS, III, Körner C. 1995. Patterns, causes, changes, and consequences of biodiversity in arctic and alpine ecosystems. Pp. 313-320. In: Chapin FS, Korner CH, (eds.). *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences*. Ecological Studies 113. Springer-Verlag, New York.

Christiansen MP. 1941. Studies in the Larger Fungi of Iceland. The Botany of Iceland 3(2): 187-227.

Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, Mitrovica JX, Hostetler SW, McCabe AM. 2009. The last glacial maximum. Science 325: 710-714.

Coker WC. 1918. The *Lactarias* of North Carolina. Journal of the Elisha Mitchell Scientific Society 34: 1-61.

Cooper SV, Lesica P, Page-Dumroese D. 1997. *Plant community classification for alpine vegetation on the Beaverhead National Forest, Montana*. USDA Forest Service General Technical Report INT-GTR-362, Ogden, UT, USA.

Corriol G. 2008. Checklist of Pyrenean alpine-stage macrofungi. Sommerfeltia 31: 29-99.

Coulson SJ, Hodkonson ID, Strathdee AT, Block W, Webb NR, Bale JS, Worland MR. 1995. Thermal environments of Arctic soil organisms during winter. Arctic, Antarctic, and Alpine Research 27: 364-370.

Courty PE, Pouysegur R, Buée M, Garbaye J. 2006. Laccase and phosphatase activities of the dominant ectomycorrhizal types in a lowland oak forest. Soil biology and biochemistry 38(6): 1219-1222.

Cripps CL, Barge E. 2013. Notes on the genus *Lactarius* from the Rocky Mountain alpine zone in regard to Finnish arctic-alpine species. *Karstenia* 53: 29-37.

Cripps CL, Eddington LH. 2005. Distribution of mycorrhizal types among alpine vascular plant families on the Beartooth Plateau, Rocky Mountains, USA, in reference to large-scale patterns in arctic-alpine habitats. Arctic, Antarctic, and Alpine Research 37: 177-188.

Cripps CL, Horak E. 2006a. Ecology and world-wide distribution of *Arrhenia auriscalpium*: a true arctic-alpine fungus. Pp. 17-24. In: Boertmann D, Knudsen H, (eds.). *Arctic and Alpine Mycology 6*. Meddelelser om Grønland Bioscience. Museum Tusulanum Press, Greenland.

Cripps CL, Horak E. 2006b. Agarics associated with *Dryas octopetala* (Rose family) in the Rocky Mountain alpine zone: in reference to arctic-alpine habitats world-wide. Mycological Society of America Meeting, Baton Rouge, Aug. 3-7, 2007.

Cripps CL, Horak E. 2008. Checklist and ecology of the Agaricales, Russulales and Boletales in the alpine zone of the Rocky Mountains (Colorado, Montana, Wyoming) at 3000-4000 A.S.L. Sommerfeltia 31: 101-123.

Cripps CL, Horak E. 2010. *Amanita* in the Rocky Mountain alpine zone, USA: New records for *A. nivalis* and *A. groenlandica*. North American Fungi 5(5): 9-21.

Cripps CL, Larsson E, Horak E. 2010. Subgenus *Mallocybe* (*Inocybe*) in the Rocky Mountain alpine zone with molecular reference to European arctic-alpine material. North American Fungi 5(5): 97-126.

Dahl E. 1984. En oversikt over plantesamfunn på Finse. Rapporter Høgfjellsøkologisk forkningsstasjon, Finse. Norge 1: 1-30.

Dahl E. 1987. The nunatak theory reconsidered. Ecological Bulletin 38: 77-94.

Dahlberg A, Bültmann H. 2013. Chapter 10. Fungi. Pp 292-309. In: Meltofte H, (ed.). *Arctic Biodiversity Assessment – Status and Trends in Arctic Biodiversity*. Conservation of Arctic Flora and Fauna, Akureyri.

Dähncke RM. 1993. *1200 Pilze: in Farbfotos*. AT Verlag.

DeChaine EG, Martin AP. 2005. Marked genetic divergence among sky island populations of *Sedum lanceolatum* (Crassulaceae) in the Rocky Mountains. American Journal of Botany 92(3): 477-486.

De Crop E, Nuytinck J, Van de Putte K, Lecomte M, Eberhardt U, Verbeken A. 2013. *Lactifluus piperatus* (Russulales, Basidiomycota) and allied species in Western Europe and a preliminary overview of the group worldwide. Mycological Progress 13: 493-511.

De Groot WJ, Thomas PA, Wein RW. 1997. *Betula nana* L. and *Betula glandulosa* Michx. Journal of Ecology 85: 241-264.

De Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology 56(6): 879-886.

Den Bakker HC, Zuccarello GC, Kuyper TW, Noordeloos ME. 2007. Phylogeographic patterns in *Leccinum* sect. *Scabra* and the status of the arctic-alpine species *L. rotundifoliae*. Mycological Research 111: 663-672.

Deslippe JR, Hartmann M, Mohn WW, Simard SW. 2011. Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. Global Change Biology 17: 1625-1636.

Desjardin DE. 2003. A unique ballistosporic hypogeous sequestrate *Lactarius* from California. Mycologia 95: 148-155.

Dettman JR, Jacobson DJ, Taylor JW. 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. Evolution 57(12): 2703-2720.

Dowie NJ, Hemenway JJ, Miller SL. 2012. Identity, genetic lineages and putative hybrids of an obligate mycobiont associated with the mycoheterotrophic plant *Pterospera andromedea* in the south-central Rocky Mountains. Fungal Ecology 5(2): 137-146.

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A. 2009. Geneious v4.7. Available at http://www.geneious.com/.

Drummond AJ, Suchard MC, Xie D, Rambaut A. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969-1973.

Durall DM, Gamiet S, Simard SW, Kudrna L, Sakakibara SM. 2006. Effects of clearcut logging and tree species composition on the diversity and community composition of epigeous fruit bodies formed by ectomycorrhizal fungi. Canadian Journal of Botany 84: 966-980.

Eberhardt U. 2002. Molecular kinship analyses of the agaricoid Russulaceae: correspondence with mycorrhizal anatomy and sporocarp features in the genus *Russula*. Mycological Progress 1(2): 201-223.

Eberhardt U, Verbeken A. 2004. Sequestrate *Lactarius* species from tropical Africa: *L. angiocarpus* sp. nov. and *L. dolichocaulis* comb. nov. Mycological Research 108: 1042-1052.

Eberhardt U, Oberwinkler F, Verbeken A, Rinaldi AC, Pacioni G, Comandini O. 2000. *Lactarius* ectomycorrhizae on *Abies alba*: morphological description, molecular characterization, and taxonomic remarks. Mycologia 92(5): 860-873.

Eddleman LE, Ward RT. 1984. Phytoedaphic relationships in alpine tundra of north-central Colorado, USA. Arctic and Alpine Research 16: 343-359.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research 32(5): 1792-1797.

Elborne SA, Knudsen H. 1990. Larger fungi associated with *Betula pubescens* in Greenland. Meddelelser om Grønland, Bioscience 33: 77-80.

Elvebakk A. 1999. Bioclimatic delimitation and subdivision of the Arctic. Det Norsk Videnskaps-Akademi. I Matematisk Naturvitenskapelige Klasse, Skrifter, Ny seri 38: 81-112.

ESRI (Environmental Systems Resource Institute). 2009. ArcMap 9.2. ESRI, Redlands, California.

Farris JS, Källersjö M, Kluge AG, Bult C. 1995. Constructing a significance test for incongruence. Systematic Biology 44(4): 570-572.

Favre J. 1955. Les Champignons supérieurs de la zone alpine du Parc National Suisse. Resultats des recherches scientifiques enterprises au Parc National Suisse. Ergebnisse der wissenschaftlichen Untersuchungen des schweizerischen National Parks 5: 1-212.

Fries EM. 1821. *Systema Mycologicum*. Vol. 1. Greifswald.

Fries EM. 1836-1838. *Epicrisis Systematis Mycologici*. Typographia Academica, Upsaliae.

Formica A, Farrer EC, Ashton IW, Suding KN. 2014. Shrub expansion over the past 62 years in Rocky Mountain alpine tundra: Possible causes and consequences. Arctic, Antarctic, and Alpine Research 46(3): 616-631.

Frøslev TG, Matheny PB, Hibbett DS. 2005. Lower level relationships in the mushroom genus *Cortinarius* (Basidiomycota, Agaricales): A comparison of RPB1, RPB2, and ITS phylogenies. Molecular Phylogenetics and Evolution 37(2): 602-618.

Fujimura KE, Egger KN. 2012. Host plant and environment influence community assembly of High Arctic root-associated fungal communities. Fungal Ecology 5(4): 409-418.

Fujiyoshi M, Yoshitake S, Watanabe K, Murota K, Tsuchiya Y, Uchida M, Nakatsubo T. 2011. Successional changes in ectomycorrhizal fungi associated with the polar willow *Salix polaris* in a deglaciated area in the High Arctic, Svalbard. Polar Biology 34: 667-673.

Gao Q, Yang ZL. 2010. Ectomycorrhizal fungi associated with two species of *Kobresia* in an alpine meadow in the eastern Himalaya. Mycorrhiza 20: 281-287.

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

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

Geml J, Laursen GA, O'Neill K, Nusbaum HC, Taylor DL. 2006. Beringian origins and cryptic speciation events in the fly agaric (*Amanita muscaria*). Molecular Ecology 15: 225-239.

Geml J, Laursen GA, Timling I, McFarland JM, Booth G, Lennon N, Nusbaum C, and Taylor DL. 2009. Molecular phylogenetic biodiversity assessment of arctic and boreal ectomycorrhizal *Lactarius* Pers. (Russulales; Basidiomycota) in Alaska, based on soil and sporocarp DNA. Molecular Ecology 18(10): 2213–2227.

Geml J, Timling I, Robinson C, Lennon N, Nusbaum HC, Brochmann C, Noordeloos ME, Taylor DL. 2012. An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. Journal of Biogeography 39: 74-88.

Geml J, Tulloss RE, Laursen GA, Sazanova NA, Taylor DL. 2008. Evidence for strong inter- and intracontinental phylogeographic structure in *Amanita muscaria*, a wind-dispersed ectomycorrhizal basidiomycete. Molecular Phylogenetics and Evolution 48(2): 694-701.

Goetcheus VG, Birks HH. 2001. Full-glacial upland tundra vegetation preserved under tephra in the Beringia National Park, Seward Peninsula, Alaska. Quaternary Science Reviews 20(1): 135-147.

Gorbunova IA. 2010. Macromycetes of alpine area of Altai. Turczaninowia 13(3): 125-134.

Gorbunova IA, Taylakov AA. 2011. New species of Russulaceae in the mycobiota of south Siberia. Turczaninowia 14(3): 87-90.

Goryachkin SV, Blume HP, Beyer L, Campbell I, Claridge G, Bockheim JG, Karavaeva NA, Targulin V, Tarnocai C. 2004. Similarities and differences in Arctic and Antarctic soil zones. Pp 49-70. In: Kimble JM, (ed.). *Cryosols*. Springer-Verlag.

Graf F. 1994. Ecology and sociology of macromycetes in snow-beds with *Salix herbacea* L. in the alpine valley of Radönt (Grisons, Switzerland). Dissertationes Botanicae 235: 1-242.

Gray SF. 1821. *A Natural Arrangement of British Plants*. Vol. 1.

Greenland D. 1989. The climate of Niwot Ridge, Front Range, Colorado USA. Arctic and Alpine Research 21: 380-391.

Griekspoor A, Groothuis T. 2006. 4Peaks. Available at http://mekentosj.com.

Grogen P, Illeris L, Michelsen A, Jonasson S. 2001. Respiration of recently-fixed plant carbon dominates mid-winter ecosystem CO_2 production in sub-arctic heath tundra. Climatic Change 50(1-2): 129-142.

Grubisha LC, Levsen N, Olson MS, Taylor DL. 2012. Intercontinental divergence in the *Populus*-associated ectomycorrhizal fungus, *Tricholoma populinum*. New Phytologist 194: 548-560.

Gulden G. 2005. A preliminary guide to the macromycetes in the Finse area, Hardangervidda, Norway. Prepared for ISAM VII, July 2005. 81 p.

Gulden G, Jenssen KM, Stordal J. 1985. *Arctic and alpine fungi. 1.* Soppkonsulenten, Oslo. 62 p.

Gulden G, Jenssen KM. 1988. *Arctic and alpine fungi. 2.* Soppkonsulenten, Oslo. 58 p.

Gulden G, Torkelsen AE. 1996. Fungi I. Basidiomycota: Agaricales, Aphyllophorales, Exobasdiales, Dacrymycetales and Tremellales. Pp. 173-206. In: Elvebakk A, Prestrud P, (eds.). *A catalogue of Svalbard plants, fungi, algae and cyanobacteria*. Norsk Polarinstitt Skrifter 198.

Hadley KS. 1987. Vascular alpine plant distributions within the central and southern Rocky Mountains, USA. Arctic and Alpine Research 19(3): 242-251.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series 41: 95-98. Available at http://www.mbio.ncsu.edu/bioedit/bioedit.html.

Hallgrímsson H, Eyjólfsdóttir GG. 2004. Checklist of Icelandic Fungi I. Microfungi. Fjölrit Náttúrufræðistofnunar 45: 1-189.

Halling RE. 2001. Ectomycorrhizae: co-evolution, significance, and biogeography. Annals of the Missouri Botanical Garden 88(1): 5-13.

Hansen P. 1988. Prediction of macrofungal occurrence in Swedish beech forests from soil and litter variable models. Vegetatio 78: 31-44.

Haselwandter K, Read DJ. 1980. Fungal associations of roots of dominant and sub-dominant plants in high-alpine vegetation systems with special reference to mycorrhizae. Oecologia 45: 57-62.

Hashimoto Y, Fukukawa S, Kunishi A, Suga H, Richard F, Sauve M, Selosse MA. 2012. Mycoheterotrophic germination of *Pyrola asarifolia* dust seeds reveals convergences with germination in orchids. New Phytologist 195(3): 620-630.

Hawksworth DL. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological research 105(12): 1422-1432.

Hawksworth DL. 2012. Integrating morphological and molecular data in fungal systematics. Pp. 1-14. In: Misra JK, Tewari JP, Deshmukh SK, (eds.). *Systematics and Evolution of Fungi*. Science Publishers, Enfield, NH, USA.

Heilmann-Clausen J, Verbeken A, Vesterholt J. 1998. *The genus Lactarius*. Danish Mycological Society, Denmark.

Heinonsalo J, Koskiahde I, Sen R. 2007. Scots pine bait seedling performance and root colonizing ectomycorrhizal fungal community dynamics before and during the 4 years after forest clear-cut logging. Canadian Journal of Forest Research 37: 415-429.

Hershkovitz MA, Lewis LA. 1996. Deep-level diagnostic value of the rDNA-ITS region. Molecular Biology and Evolution 13(9): 1276-1295.

Hesler LR, Smith AH. 1962. Studies on *Lactarius*—III the North American species of section *Plinthogali*. Brittonia 14: 369-440.

Hesler LR, Smith AH. 1979. *North American species of Lactarius*. University of Michigan Press. Ann Arbor Michigan, USA. 841 p.

Heuberger H. 1974. Alpine Quaternary glaciations. Pp. 1-11. In: Barry RG, Ives JD, (eds.). *Arctic and Alpine Environments.* Methuen, London.

Hibbett D. 2007. After the gold rush, or before the flood? Evolutionary morphology of mushroom-forming fungi (*Agaricomycetes*) in the early 21st century. Mycological Research 111: 1001-1018.

Hibbett D, Matheny PB. 2009. The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. BMC biology 7(1): 13.

Hillis DM, Dixon MT. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. The Quarterly Review of Biology 66(44): 411-446.

Hobbie JE, Hobbie EA. 2006. ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. Ecology 87(4): 816-822.

Högberg, MN, Briones MJI, Keel SG, Metcalfe DB, Campbell C, Midwood AJ, Thornton B, Hurry V. 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187(2): 485-493.

Homola RL, Czapowskyj MM. 1981. Ectomycorrhizae of Maine 2. A listing of *Lactarius* with the associated hosts (with additional information on edibility). Life Sciences and Agriculture Experiment Station Bulletin 779. University of Maine, Orono.

Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10(8): 1855-1871.

Hudson RR. 1992. Gene trees, species trees and segregation of ancestral alleles. Genetics 131: 509-512.

Hultén E. 1937. *Outline of the history of arctic and boreal biota during the Quaternary period*. Aktiebolaget Thule, Stockholm. 168 p.

Hultén E. 1968. *Flora of Alaska and Neighboring Territories*. Stanford: Stanford University Press, CA. 1008 p.

Hultén E. 1971. *Circumpolar Plants II. Dicotyledons*. Almqvist & Wiksell, Stockholm. 463 p.

Idzerda S, Noordeloos ME. 1997. Studies in *Lactarius* sect. *Tabidi*. Österreichische Mykologische Gesellschaft, Austria 6: 71-89.

Ishida TA, Nara K, Hogetsu T. 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. New Phytologist 174(2): 430-440.

Jamoni PG. 1995. Russulaceae della zona alpine. Proposta di chiavi di determinazione per le specie crescent nella zona alpine delle Alpi. Rivista Di Micologia 2: 75-80.

Jamoni PG. 2008. *Fungi alpini, delle zone alpine superiori e inferiori*. Association Micologica Bresadola, Fondazione centro Studi Micologici, Trento. 543 p.

Jamoni PG, Bon M. 1991. Note di micologia alpina repperti rari e nuovi della zona alpina del Massiccio del Monte Rosa (1^a parte). Associzione Micologica Bresadola 3: 255-274.

Jany JL, Martin F, Garbaye J. 2003. Respiration activity of ectomycorrhizas from *Cenococcum geophilum* and *Lactarius* sp. in relation to soil water potential in five beech forests. Plant and Soil 255(2): 487-494.

Jenny H. 1980. *The Soil Resource: Origin and Behavior*, Ecological Studies No. 37. Springer, New York.

Johnson PL, Billings WD. 1962. The alpine vegetation of the Beartooth Plateau in relation to cryopedogenic processes and patterns. Ecological Monographs 32: 105-135.

Jones PD, New M, Parker DE, Martin S, Rigor IG. 1999. Surface air temperature and its changes over the past 150 years. Reviews of Geophysics 37(2): 173-199.

Kalamees K. 2008. Some agarics in the subalpine and alpine belts of the Altaj (Russia) and Tyan'-Shan' (Kyrgyzstan) Mountains. Sommerfeltia 31: 133-138.

Kalamees K, Vaasma M. 1993. Mycobiota of alpine and subalpine sites of Kamchatka. Pp. 121-131. In: Petrini O, Laursen GA, (eds.). *Arctic and Alpine Mycology 3-4*. J Cramer, Stuttgart, Berlin.

Kallio P, Kankainen E. 1964. Notes on the Macromycetes of Finnish Lapland and adjacent Finnmark. Reports from the Kevo Subarctic Research Station 1: 178-235.

Karatygin EL, Nezdoiminogo EL, Novozhilov YK, Zhurbenko MP. 1999. Russian arctic fungi. Checklist (In Russian). Academy of Chemistry and Pharmacology, St. Petersburg.

Kasuya T. 2010. Lycoperdaceae (Agaricales) on the Beartooth Plateau, Rocky Mountains, U.S.A. North American Fungi 5(5): 159-171.

Kauffman CH. 1918. *The Agaricaceae of Michigan*. Vol. 1. Michigan Geological and Biological Survey, Lansing, Michigan. 918 p.

Kauffman CH. 1921. The mycological flora of the higher Rockies of Colorado. Papers of the Michigan Academy of Science, Arts and Letters 1: 101-150.

Kennedy PG, Hill LT. 2010. A molecular and phylogenetic analysis of the structure and specificity of *Alnus rubra* ectomycorrhizal assemblages. Fungal Ecology 3(3): 195-204.

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

Kingman JFC. 1982. The coalescent. Stochastic Processes and their Applications 13: 235-248.

Kjøller R, Clemmensen KE. Belowground ectomycorrhizal fungal communities respond to liming in three southern Swedish coniferous forest stands. Forest Ecology and Management 257(11): 2217-2225.

Knudsen H, Borgen T. 1982. Russulaceae in Greenland. Pp. 216-244. In: Laursen GA, Ammirati JF, (eds.). *Arctic and Alpine Mycology 1*. University of Washington Press, Seattle.

Knudsen H, Borgen T. 1994. The *Lactarius torminosus*-group in Greenland. Mycologia Helvetica 6: 49-56.

Knudsen H, Lamoure D. 1993. Notes on *Lactarius dryadophilus* Kühner and *L. groenlandicus* Terkelsen. Bibliotheca Mycologica 150: 147-154.

Knudsen H, Mukhin VA. 1998. The arctic-alpine agaric element in the Polar Urals and Yamal, Western Siberia. Pp. 152-162. In: Mukhin VA, Knudsen H, (eds.). *Arctic and alpine Mycology 5*. Yekaterinburg Publications, Russia.

Knudsen H, Vesterholt J, (eds.). 2008. *Funga Nordica: Agaricoid*, *Boletoid and Cyphelloid Genera*. Nordsvamp, Copenhagen. 965 p.

Kobayasi Y, Hiratsuka N, Otani Y, Tubaki K, Udagawa S, Sugiyama J, Konno K. 1971. Mycological Studies of the Angmagssalik Region of Greenland. Bulletin of the National Science Museum Tokyo 14: 1-96.

Kohn LM, Stasovski E. 1990. The mycorrhizal status of plants at Alexandria Fjord, Ellsmere Island, Canada, a high Arctic site. Mycologia 82: 23-35.

Kõjalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM. 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166: 1063-1068.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiß M, Larsson K-H. 2013. Towards a unified paradigm for sequence-based identification of Fungi. Molecular Ecology 22: 5271-5277.

Konrad MP. 1935a. Les lactaires. Notes critiques et essai de classification. Bulletin trimestriel de la Société mycologique de France 51: 160-191.

Konrad MP. 1935b. Classification du genre *Lactarius*. Schweizerische Zeitschrift für Pilzkunde 9: 134-141.

Körner C. 1995. Alpine plant diversity: A global survey and functional interpretations. Pp. 45-62. In: Chapin FS, Körner C, (eds.). *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences.* Ecological Studies 113. Springer-Verlag, New York.

Körner C. 1999. *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems.* Springer-Verlag, Berlin. 338 p.

Kraigher H, Agerer R, Javornik B. 1995. Ectomycorrhizae of *Lactarius lignyotus* on Norway spruce, characterized by anatomical and molecular tools. Mycorrhiza 5(3): 175-180.

Kramer R, Abraham WR. 2012. Volatile sesquiterpenes from fungi: what are they good for? Phytochemistry Reviews 11(1): 15-37.

Krause TR, Whitlock C. 2013. Climate and vegetation change during the late-glacial/early-Holocene transition inferred from multiple proxy records from Blacktail Pond, Yellowstone National Park, USA. Quaternary Research 79(3): 391-402.

Krpata D, Mühlmann O, Kuhnert R, Ladurner H, Göbl F, Peintner U. 2003. High diversity of ectomycorrhizal fungi associated with *Arctostaphylos uva-ursi* in subalpine and alpine zones: Potential inoculum for afforestation, Forest Ecology and Management 250(3): 167-175.

Kuchler AW. 1964. Potential natural vegetation of the Conterminous United States. American Geographical Society, Special Publication No. 36.

Kühner R. 1975. Agaricales de la zone alpine: *Lactarius*. Bulletin trimestriel de la Société mycologique de France 91: 5-69.

Kühner R, Lamoure D. 1986. Catalogue des Agaricales (Basidiomycètes) de la zone alpine du Parc Nationale de la Vanoise et des régions limitrophes. Traveaux scientifiques du Parc national de la Vanoise 15: 103-187.

Kytövuori I. 2009. Two new *Lactarius* species, *L. flavopalustris* and *L. flavoaspideus*, in Fennoscandia. Karstenia 49: 19-31.

Lamoure D, Lange M, Petersen PM. 1982. Agaricales found in the Godhavn Area, West Greenland. Nordic Journal of Botany 2: 85-90.

Lange M. 1957. Den Botaniske Ekspedition til Vestgrønland 1946, Macromycetes, Part III, I. Greenland Agaricales (pars) Macromycetes caeteri, II. Ecological and Plant Geographical Studies. Meddelelser om Grønland 138(2): 1-125.

Larsen JA, Barry RG. 1974. Palaeoclimatology. Pp. 253-276. In: Barry RG, Ives JD, (eds.). *Arctic and Alpine Environments.* Methuen, London.

Larsson E, Jacobsson S. 2004. Controversy over *Hygrophorus cossus* settled using ITS sequence data from 200 year-old type material. Mycological Research 108(7): 781-786.

Larsson E, Larsson K-H. 2003. Phylogenetic relationships of russuloid basidiomycetes with emphasis on aphyllophoralean taxa. Mycologia 95: 1037-1065.

Larsson K-H, Larsson E, Koljalg U. 2004. High phylogenetic diversity among corticioid homobasidiomycetes. Mycological Research 108: 983-1002.

Larsson E, Vauras J, Cripps CL. 2014. *Inocybe leiocephala*, a species with an intercontinental distribution range – disentangling the *I. leiocephala – subbrunnea – catalunica* morphological species complex. *Karstenia* 54: 15-39.

Laursen GA, Ammirati JF. 1982. Lactarii in Alaskan Arctic tundra. Pp. 245-281. In: Laursen GA, Ammirati JF, Redhead SA, (eds.). *Arctic and Alpine Mycology 1*. University of Washington Press, Seattle.

Le HT, Nuytinck J, Verbeken A, Lumyong S, Desjardin DE. 2007. *Lactarius* in Northern Thailand 1: *Lactarius* subgenus *Piperites*. Fungal Diversity 24: 173-224.

Leacock PR. 1993. *The* Lactarius *Species of Minnesota: Biosystematics and Biogeography of Section Dapetes*. University of Minnesota. 176 p.

Lesica P. 2012. *Manual of Montana Vascular Plants*. Botanical Research Institute of Texas, Texas. 771 p.

Lesica P, Antibus RK. 1986. Mycorrhizae of alpine fell-field communities on soils derived from crystalline and calcareous parent materials. Canadian Journal of Botany 64(8): 1691-1697.

Leski T, Rudawska M. 2012. Ectomycorrhizal fungal community of naturally regenerated European larch (*Larix decidua*) seedlings. Symbiosis 56(2): 45-53.

Linder DL, Banik MT. 2011. Intragenomic variation in the ITS rDNA region obscures phylogenetic relationships and inflates estimates of operational taxonomic units in the genus *Laetiporus*. Mycologia 103(4): 731-740.

Lipson DA, Schadt CW, Schmidt SK. 2002. Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. Microbial Ecology 43(3): 307-314.

Lipson DA, Schmidt SK, Monson RK. 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80(5): 1623-1631.

Liu YL, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799-1808.

Löve A, Löve D. 1974. Origin and evolution of the arctic and alpine floras. Pp. 571-603. In: Barry R, Ives JD, (eds.). *Arctic and Alpine Environments*. Methuen, London.

Margesin R, Miteva V. 2011. Diversity and ecology of psychrophilic microorganisms. Research in Microbiology 162 (3): 346-361.

Marshall DC. 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: lost in the land of long trees. Systematic Biology 59: 108-117.

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). Molecular Phylogenetics and Evolution 35: 1-20.

Matheny PB, Wang Z, Binder M, Curtis FM, Woon Lim Y, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Frøslev T, Ge Z-H, Kerrigan RW, Slot JC, Yang Z-L, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS. 2007. Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Molecular Phylogenetics and Evolution 43(2): 430-451.

McNabb RFR. 1971. The Russulaceae of New Zealand 1. *Lactarius* DC ex S.F. Gray. New Zealand Journal of Botany 9(1): 46-66.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen S, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland NJ. 2012. *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code)*. Regnum Vegetabile 154. Koeltz Scientific Books. 240 p.

Methven AS. 1985. New and interesting species of *Lactarius* from California. The New York Botanical Garden 77(3): 472-482. 78 p.

Methven AS. 1992. An introduction to the infrageneric classification of *Lactarius*. McIlvainea 10: 29-40.

Methven AS. 1993. Contributions to a study of the North American species of *Lactarius*. McIlvainea 11: 26-34.

Methven AS. 1997. *The Agaricales (Gilled Fungi) of California. 10 Russulaceae. II Lactarius.* Mad River Press, Eureka, California.

Meyer FH. 1973. Distribution of ectomycorrhizae in native and man-made forests. Pp. 79-105. In: Marks GC, Kozlowski TT, (eds.). *Ectomycorrhizae: their ecology and physiology*. Academic Press, London.

Miller OK Jr. 1982. Mycorrhizae, mycorrhizal fungi, and fungal biomass in subalpine tundra at Eagle Summit, Alaska. Holarctic Ecology 5: 125-134.

Miller OK Jr. 1987. Higher fungi in tundra and subalpine tundra from the Yukon Territory and Alaska. Pp. 287-297. In: Laursen GA, Ammirati JF, (eds.). *Arctic and Alpine Mycology 2*. Plenum Press, New York.

Miller OK Jr, Evenson VS. 2001. Observations on the alpine tundra species of *Hebeloma* in Colorado. Harvard Papers in Botany 6(1): 155-162.

Miller OK Jr, Laursen GA. 1973. Arctic and alpine agarics from Alaska and Canada. Canadian Journal of Botany 51: 43-49.

Miller SL, Larsson E, Larsson K-H, Verbeken A, Nuytinck J. 2006. Persepectives in the new Russulales. Mycologia 98(6): 960-970.

Miller SL, McClean TM, Walker JF, Buyck B. 2001. A molecular phylogeny of the Russulales including agaricoid, gasteroid and pleurotoid taxa. Mycologia 93(2): 344-354.

Mimura M, Mori AS, Uchida M, Kanda H. 2013. Colonization of vegetation-rich moraines and inference of multiple sources of colonization in the High Arctic for *Salix arctica*. Conservation Genetics 14: 223-229.

Minckley TA. 2014. Postglacial vegetation history of southestern Wyoming, U.S.A. Rocky Mountain Geology 49: 61-74.

Mlinaric A, Kac J, Fatur T, Filipic M. 2004. Anti-genotoxic activity of the mushroom *Lactarius vellereus* extract in bacteria and in mammalian cells *in vitro*. Die Pharmazie-An International Journal of Pharmaceutical Sciences 59(3): 217-221.

Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: Community-ecological consequences and practical implications. Pp. 357-423. In: Routledge AMF, (ed.). *Mycorrhizal functioning, and integrative plant-fungal process*. Chapman & Hall, New York, USA.

Montoya L, Bandala VM. 2003. Studies on *Lactarius*: A new combination and two new species from Mexico. Mycotaxon 85: 393-407.

Moreau PA. 2002. Á la découverte des champignons de zone alpine. Bulletin Mycologique et Botanique, Daupiné-Savoie 166: 5-38.

Moser MM. 1982. Mycoflora of the transitional zone from subalpine forests to alpine tundra. Pp. 371-384. In: Laursen GA, Ammirati JF, (eds.). *Arctic and Alpine Mycology 1*. University of Washington Press, Seattle.

Moser MM. 1993. Studies on North American Cortinarii. 3. The *Cortinarius* flora of dwarf and shrubby *Salix* associations in the alpine zone of the Windriver Mountains, Wyoming, USA. Sydowia 45: 275-306.

Moser MM, McKnight K. 1987. Fungi (Agaricales, Russulales) from the alpine zone of Yellowstone National Park and Beartooth Mountains with emphasis on *Cortinarius*. Pp. 299-318. In: Laursen GA, Ammirati JF, Redhead SA, (eds.). *Arctic and Alpine Mycology 2*. Plenum Press, New York.

Moser MM, McKnight KH, Ammirati JF. 1995. Studies on North American Cortinarii I. New and interesting taxa from the greater Yellowstone area. Mycotaxon 60: 301-346.

Moser MM, McKnight KH, Sigl M. 1994. The genus *Cortinarius* (Agaricales) in the Greater Yellowstone Area: mycorrhizal host associations and taxonomic considerations. Pp. 239-246. In: Despain DG, (ed.). *Plants and their environments*: Proceedings of the first biennial scientific conference. Greater Yellowstone Ecosystem.

Mühlmann O, Peintner U. 2008. Mycobionts of *Salix herbacea* on a glacier forefront in the Austrian Alps. Mycorrhiza 18(4): 171-180.

Mullis KB, Faloona FA, Scharf SJ, Saiki RK, Horn GT, Erlich H. 1986. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology, Vol. LL: 263-273.

Murray DF. 1995. Causes of arctic plant diversity: origin and evolution. Pp. 21-32. In: Chapin III FS, Körner C, (eds.). *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences.* Springer-Verlag, Berlin.

Murrill WA. 1938. New Florida Agarics. Mycologia 30 (4): 360-362.

Murrill WA. 1948. Species of Florida *Lactarius* Fr. Lloydia 11: 86-98.

Mynemi RB, Keeling CD, Tucker CJ, Asrar G, Nemani RR. 1997. Increased plant growth in the northern high latitudes from 1981 to 1991. Nature 386: 698-702.

Nauta M. 2010. Notes on Mollisioid Ascomycetes from the Beartooth Plateau, Rocky Mountains USA. North American Fungi 5(5): 181-186.

Nemergut DR, Costello EK, Meyer AF, Pescador MY, Weintraub N, Schmidt SK. 2005. Structure and function of alpine and arctic soil microbial communities. Research in microbiology 156(7): 775-784.

Neuhoff W. 1956. *Die Milchlinge* (*Pilzgattung Lactarius*). Bad Heilbrunn Obb., Julius Klinkhardt. 248 p.

Nezdoiminogo EL. 1997. Homobasidiomycetes of natural reserves of the Russian Arctic. Mikologiia I Fitopatologiia 31(5): 40-43.

Nicholls N, Gruza GV, Jouzel J, Karl TR, Ogallo LA, Parker DE. 1996. Observed climate variability and change. Pp. 137-192. In: Houghton JT, Meira Filho LG, Callander BA, Harris N, Kattenberg A, Maskell K, (eds.). *Climate Change 1995, The Science of Climate Change, Contribution of Working Group I to the Second Assessment of the Intergovernmental Panel on Climate Change* (Ch. 3). Cambridge University Press, Cambridge, U.K.

Nichols R. 2001. Gene trees and species trees are not the same. Trends in Ecology and Evolution 16: 358-364.

Nilsson RH, Kristiansson E, Ryberg M, Larsson K-H. 2005. Approaching the taxonomic affiliation of unidentified sequences in public databases-an example from the mycorrhizal fungi. BMC Bioinformatics 6: 178.

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H. 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS ONE 1: e59.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H. 2008. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evolutionary Bioinformatics Online 4: 193.

Nilsson RH, Ryberg M, Abarenkov K, Sjökvist E, Kristiansson E. 2009. The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. FEMS Microbiology Letters 296: 97-101.

Nuytinck J, Miller SL, Verbeken A. 2006a. A taxonomical treatment of the North and Central American species in *Lactarius* sect. *Deliciosi*. Mycotaxon 96: 261-307.

Nuytinck J, Verbeken A, Delarue S, Walleyn R. 2003. Systematics of European sequestrate lactarioid Russulaceae with spiny spore ornamentation. Belgian Journal of Botany 136: 145-153.

Nuytinck J, Verbeken A, Rinaldi AC, Leonari M, Pacioni G, Comandini O. 2004. Characterization of *Lactarius tesquorum* ectomycorrhizae on *Cistus* sp. and molecular phylogeny of related European *Lactarius* taxa. Mycologia 96(2): 272-282.

Nuytinck J, Verbeken A. 2005. Morphology and taxonomy of the European species in *Lactarius* sect. *Deliciosi* (*Russu-Russulales*). Mycotaxon 92: 125-168.

Nuytinck J, Verbeken A, Miller SL. 2007. Worldwide phylogeny of *Lactarius* section *Deliciosi* inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 99(6): 820-832.

Nuytinck J, Wang XH, Verbeken A. 2006b. Descriptions and taxonomy of the Asian representatives of *Lactarius* sect. *Deliciosi*. Fungal Diversity 22: 171-203.

Nylander JAA. 2004. MrModeltest v2.3. Distributed by the author 2.3rd edition.

Ohenoja E. 1971. The larger fungi of Svalbard and their ecology. Reports from the Kevo Subarctic Research Station 8: 122-147.

Ohenoja E, Ohenoja M. 1993. *Lactarii* of the Franklin and Keewatin Districts of the Northwest Territories, Arctic Canada. Pp. 179-192. In: Petrini O, Laursen GA, (eds.). *Arctic and alpine mycology 3-4*. J Cramer, Stuttgart, Berlin.

Ohenoja E, Ohenoja M. 2010. Larger Fungi of the Canadian Arctic. North American Fungi 5(5): 85-96.

Osmundson TW, Cripps CL, Mueller GM. 2005. Morphological and molecular systematics of Rocky Mountain alpine *Laccaria*. Mycologia 97(5): 949-972.

Osmundson TW, Eyre CA, Hayden KM, Dhillon J, Garbelotto MM. 2013. Back to basics: an evaluation of NaOH and alternative rapid DNA extraction protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and oomycete samples.

Østrem G. 1974. Present alpine ice cover. Pp. 225-252. In: Barry RG, Ives JD, (eds.). *Arctic and Alpine Environments.* Methuen, London.

Overholtz L. 1919. Some Colorado fungi. Mycologia 11: 245.

Parkinson CL, Cavalieri DJ, Gloersen P, Zwally HJ, Comiso JC. 1999. Arctic sea ice extents, areas, and trends, 1978-1996. Journal of Geophysical Research: Oceans (1978-2012) 104(C9): 20837-20856.

Pauli H, Gottfried M, Grabherr G. 1996. Effects of climate change on mountain ecosystems – upward shift of alpine plants. World Resource Review 8: 382-390.

Peay KG, Garbelotto M, Bruns TD. 2010. Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree island. Ecology 91: 3631-3640.

Peck C. 1885. New York species of *Lactarius*. Reports of the Botanist, Annual reports of the New York State Museum 38: 111-133.

Peintner U. 2008. *Cortinarius alpinus* as an example for morphological and phylogenetic species concepts in ectomycorrhizal fungi. Sommerfeltia 31: 161-177.

Peintner U, Iotti M, Klotz P, Bonuso E, Zambonelli A. 2007. Soil fungal communities in a *Castanea sativa* (chestnut) forest producing large quantities of *Boletus edulis* sensu lato (porcini): where is the mycelium of porcini? Environmental microbiology 9(4): 880-889.

Peterson BJ, Holmes RM, McClelland JW, Vorosmarty CJ, Lammers RB, Shiklomanov AI, Shiklomanov IA, Rahmstorf S. 2002. Increasing river discharge to the Arctic Ocean. Science 298(5601): 2171-2173.

Persoon HC. 1797. Tentamen dispositionis methodica fungorum in classes, ordines, genera et familias cum suppl. adjecta. Lipsiae.

Pierce KL. 1979. History and dynamics of glaciation in the northern Yellowstone National Park area: U.S. Geological Survey Professional Paper 729-F. 90 p.

Pierce KL. 2003. Pleistocene glaciations of the Rocky Mountains. Developments in Quaternary Sciences 1: 63-76.

Ping CL, Michaelson GJ, Jorgenson MT, Kimble JM, Epstein H, Romanovsky VE, Walker DA. 2008. High stocks of soil organic carbon in the North American Arctic region. Nature Geoscience 1(9): 615-619.

Quélet L. 1888. *Flore mycologique de la France et des pays limitrophes*. Paris.

Rambaut A. 1995. Se-Al, Sequence Alignment Editor. Oxford University, Oxford, UK.

Retzer JL. 1956. Alpine soils of the Rocky Mountains. Journal of Soil Science 7: 22-32.

Retzer JL. 1974. Alpine soils. Pp. 771-802. In: Ives JD, Barry RG, (eds.). *Arctic and Alpine Environments*. Methuen, London.

Rinaldi AC, Comandini O, Kuyper TW. 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity 33: 1-45.

Romanovsky V, Burgess M, Smith S, Yoshikawa K, Brown J. 2002. Permafrost temperature records: indicators of climate change. Eos, Transactions American Geophysical Union 83(50): 589-594.

Ronikier A. 2008. Contribution to the biogeography of arctic-alpine fungi: first records in the Southern Carpathians (Romania). Sommerfeltia 31: 191-211.

Ronikier A, Ronikier M. 2010. Biogeographical patterns of arctic-alpine fungi: distribution analysis of *Marasmius epidryas*, a typical circumpolar species of cold environments. North American Fungi 5(5): 23-50.

Ronikier M, Ronikier A. 2011. *Rhizomarasmius epidryas* (Physalacriaceae): phylogenetic placement of an arctic-alpine fungus with obligate saprobic affinity to *Dryas spp.* Mycologia 103(5): 1124-1132.

Ronikier M, Schneeweiss GM, Schönswetter P. 2012. The extreme disjunction between Beringia and Europe in *Ranunculus glacialis* s.l. (Ranunculaceae) does not coincide with the deepest genetic split-a story of the importance of temperate mountain ranges in arctic-alpine phylogeography. Molecular ecology 21(22): 5561-5578.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Ryberg M, Andreasen M, Bjork RG. 2011. Weak habitat specificity in ectomycorrhizal communities associated with *Salix herbacea* and *Salix polaris* in alpine tundra. Mycorrhiza 21: 289-296.

Ryberg M, Larsson E, Molau U. 2009. Ectomycorrhizal diversity on *Dryas octopetala* and *Salix reticulata* in an alpine cliff ecosystem. Arctic, Antarctic, and Alpine Research 41: 506-514.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487-491.

Schadt CW. 2002. Studies on the fungal associations of the alpine sedge *Kobresia myosuroides*. Ph.D. Dissertation, University of Colorado, Boulder.

Schadt CW, Martin AP, Lipson DA, Schmidt SK. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301: 1359-1361.

Schmitt T, Muster C, Schönswetter P. 2010. Are disjunct alpine and arctic-alpine animal and plant species in the Western Palearctic really “relics of a cold past”? Pp. 239-

252. In: Habel JC, Assmann C, (eds.). *Relict species: phylogeography and conservation biology*. Springer, Heidelberg, Berlin.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. 2012. Nuclear ribosomal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. Proceedings of the National Academy of Sciences of the United States of America 109(16): 6241-6246.

Scott RW. 1995. *The alpine flora of the Rocky Mountains; volume 1. The Middle Rockies*. University of Utah Press, Salt Lake City, Utah, USA. 901 p.

Seaver R, Shope PF. 1930. A mycological foray through the mountains of Colorado, Wyoming and South Dakota. Mycologia 22: 1-8.

Senn-Irlet B. 1993. The mycoflora of Alpine mire communities rich in *Salix*. Pp. 235-429. In: Petrini O, Laursen GA, (eds.). *Arctic and alpine Mycology 3-4*. J Cramer, Stuttgart, Berlin.

Serreze MC, Walsh JE, Chapin FS. 2000. Observational evidence of recent change in the northern high-latitude environment. Climate Change 46: 159-207.

Shapiro B, Drummond, AJ, Rambaut A, Wilson MC, Matheus PE, Sher AV, Pybus OG, Gilbert MTP, Barnes I, Binladen J, Willerslev E, Hansen AJ, Baryshnikov GF, Burns FA, Davydov S, Driver JC, Froese DG, Harington CR, Keddie G, Kosintsev P, Kunz ML, Martin LD, Stephenson RO, Storer J, Tedford R, Zimov S, Cooper A. 2004. Rise and fall of the Beringian steppe bison. Science 306(5701): 1561-1565.

Shaver GR, Giblin AE, Nadelhoffer KJ, Thieler KK, Downs MR, Laundre JA, Rastetter EB. 2006. Carbon turnover in Alaskan tundra soils: effects of organic matter quality, temperature, moisture and fertilizer. Journal of Ecology 94(4): 740-753.

Shimono Y, Kato M, Takamatsu S. 2004. Molecular phylogeny of Russulaceae (Basidiomycetes; Russulales) inferred from the nucleotide sequences of nuclear large subunit rDNA. Mycoscience 45: 303-316.

Silvestro D, Michalak I. 2012. raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12: 335-337.

Singer R. 1975 *The Agaricales in modern taxonomy*. 3rd ed. Cramer, Vaduz. 912 p.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. Academic Press, San Diego, California, USA.

Smith ME, Henkel TW, Aime MC, Fremier AK, Vilgalys R. 2011. Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. New Phytologist 192(3): 699-712.

Solheim WG. 1949. Studies on Rocky Mountain fungi. 1. Mycologia 41: 623-631.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688-2690.

Stöver BC, Müller KF. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11: 7.

Stubbe D, Nuytinck J, Verbeken A. 2010. Critical assessment of the *Lactarius gerardii* species complex (*Russulales*). Fungal Biology 114: 271-283.

Stubbe D, Le HT, Wang X-H, Nuytinck J, Van de Putte K, Verbeken A. 2012. The Australasian species of *Lactarius* subgenus *Gerardii* (*Russulales*). Fungal Diversity 52: 141-167.

Stubbe D, Verbeken A. 2012. *Lactarius* subg. *Plinthogalus*: the European taxa and American varieties of *L. lignyotus* re-evaluated. Mycologia 104(6): 1490-1501.

Stucky B. 2012. SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. Journal of biomolecular techniques 23(3): 90-93.

Sturm M, Racine C, Tape K. 2001. Climate change: increasing shrub abundance in the Arctic. Nature 411(6837): 546-547.

Sturm M, Schimel J, Michaelson G, Welker JM, Oberbauer SF, Liston GE, Fahnestock J, Romanovsky VE. 2005. Winter biological processes could help convert Arctic tundra to shrubland. BioScience 55(1): 17-26.

Swofford DL. 2001. PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b command reference. Sunderland, Massachusetts: Sinauer Associates, Inc. 142 p.

Tajima F. 1983. Evolutionary relationships of DNA sequences in finite populations. Genetics 105: 437-460.

Takahata N, Nei M. 1985. Gene geneology and variance of inter-populational nucleotide differences. Genetics 110: 325-344.

Takhtajan A. 1986. *Floristic Regions of the World*. University of California Press, Berkeley. 522 p.

Tarasov PE, Volkova VS, Webb T, Guiot J, Andreev AA, Bezusko LG, Bezusko TV, Bykova GV, Dorofeyuk NI, Kvavadze EV, Osipova IM, Panova NK, Sevastyanov DV. 2000. Last glacial maximum biomes reconstructed from pollen and plant macrofossil data from northern Eurasia. Journal of Biogeography 27: 609-620.

Tarnocai C. 2009. Arctic permafrost soils. Pp. 3-16. In: Margesin R, (ed.). *Permafrost Soils*. Springer-Verlag, Heidelberg, Berlin.

Taylor DL, Bruns TD. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology 8: 1837-1850.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31: 21-32.

Taylor JW, Turner E, Townsend JP, Dettman JR, Jacobson D. 2006. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. Philosophical Transactions of the Royal Society B: Biological Sciences 361(1475): 1947-1963.

Tedersoo L, Bahram M, Jairus T, Bechem E, Chinoya S, Mpumba R, Leal M, Randrianjohany E, Razafimandimbison S, Sadam A, Naadel T, Kõljalg U. 2011. Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of Continental Africa and Madagascar. Molecular Ecology 20: 3071-3080.

Tedersoo L, Bahram M, Ryberg M, Otsing E, Kõljalg U, Abarenkov K. 2014. Global biogeography of the ectomycorrhizal /sebacina lineage (Fungi, Sebacinales) as revealed from comparative phylogenetic analyses. Molecular Ecology 23: 4168-4183.

Tedersoo L, Bahram M, Toots M, Diédhiou, Henkel TW, Kjøller R, Morris MH, Nara K, Nouhra E, Peay KG, Põlme S, Ryberg M, Smith ME, Kõljalg U. 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Molecular Ecology 21(17): 4160-4170.

Tedersoo L, Suvi T, Jairus T, Ostonen I, Põlme S. 2009. Revisiting ectomycorrhizal fungi of the genus *Alnus*: differential host specificity, diversity and determinants of the fungal community. New Phytologist 182(3): 727-735.

Terkelsen F. 1956. *Lactarius groenlandicus* sp. nov. Friesia 5: 417-419.

Thompson RS, Anderson KH. 2000. Biomes of western North America 18,000, 6000 and 0 14C yr BP reconstructed from pollen and packrat midden data. Journal of Biogeography 27(3): 555-584.

Thorn CE, Darmody RG. 1985. Grain-size distribution of the insoluble component of contemporary eolian deposits in the alpine zone, Front Range, Colorado, USA. Arctic and Alpine Research 17(4): 433-442.

Timling I, Taylor DL. 2012. Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi. Fungal Ecology 5(4): 419-429.

Timling I, Dahlberg A, Walker DA, Gardes M, Charcosset JY, Welker JM, Taylor DL. 2012. Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. Ecosphere 3: 1-25.

Timling I, Walker DA, Nusbaum C, Lennon NJ, Taylor DL. 2014. Rich and cold: diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. Molecular Ecology 23: 3258-3272.

Tremblay NO, Schoen DJ. 1999. Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. Molecular Ecology 8: 1187-1198.

Treu R, Laursen GA, Stephenson SL, Landolt JC, Densmore R. 1996. Mycorrhizae from Denali National Park and Preserve, Alaska. Mycorrhiza 6: 21-29.

Van de Putte K, Nuytinck J, Das K, Verbeken A. 2012. Exposing hidden diversity by concordant genealogies and morphology—a study of the *Lactifluus volemus* (Russulales) species complex in Sikkim Himalaya (India). Fungal Diversity 55: 171-194.

Van de Putte K, Nuytinck J, Stubbe D, Le HT, Verbeken A. 2010. *Lactarius volemus* sensu lato (Russulales) from northern Thailand: morphological and phylogenetic species concepts explored. Fungal Diversity 45: 99-130.

Vare H, Vestberg M, Eurola S. 1992. Mycorrhiza and root-associated fungi in Spitsbergen. Mycorrhiza 1: 93-104.

Vare H, Vestberg M, Ohtonen R. 1997. Shifts in mycorrhiza and microbial activity along an oroarctic altitudinal gradient in northern Fennoscandia. Arctic and Alpine Research 29: 93-104.

Verbeken A. 2001. Worldwide systematics of *Lactarius*: a state of the art. Micologia e vegetazione Mediterranea 16: 71-88.

Verbeken A, Nuytinck J. 2013. Not every milkcap is a *Lactarius*. Scripta Botanica Belgica 51: 162-168.

Verbeken A, Stubbe D, Van de Putte K, Eberhardt U, Nuytinck J. 2014. Tales of the unexpected: angiocarpous representatives of the *Russulaceae* in tropical South East Asia. Persoonia 32: 13-24.

Vila J, Llistosella J, Llimona X. 1997. Contribucio al Coneixement Dels Fongs De L'estage Alpi Dels Pirineus De Catalunya. Revista Catalana de Mycologia 20: 221-232.

Walker DA. 2000. Hierarchical subdivision of Arctic tundra based on vegetation response to climate, parent material and topography. Global Change Biology 6(S1): 19-34.

Walker DA, Raynolds MK, Daniëls FJA, Einarsson E, Elvebakk A, Gould WA, Katenin AE, Kholod SS, Mardon CJ, Melnikov ES, Moskalenko NG, Talbot SS, Yurtsev BA, CAVM Team. 2005. The circumpolar Arctic vegetation map. Journal of Vegetation Science 16: 267-282.

Walker MD. 1995. Patterns and causes of arctic plant community diversity. Pp. 3-20. In: Chapin FS, Körner, (eds.). *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences.* Ecological Studies 113. Springer-Verlag, New York.

Walker MD, Wahren CH, Hollister RD, Henry GH, Ahlquist LE, Altalo JM, Bret-Harte MS, Calef MP, Callaghan TV, Carroll AB, Epstein HE, Jónsdóttir IS, Klein JA, Magnússon B, Molau U, Oberbauer SF, Rewa SP, Robinson CH, Shaver GR, Suding KN, Thompson CC, Tolvanen A, Totland O, Turner PL, Tweedie CE, Webber PJ, Wookey PA. 2006. Plant community responses to experimental warming across the tundra biome. Proceedings of the National Academy of Sciences of the United States of America 103(5): 1342-1346.

Wang H, Qi M, Cutler AJ. 1993. A simple method for preparing plant samples for PCR. Nucleic Acids Research 21: 4153-4154.

Warren WJ. 1959. Notes on wind and its effects in arctic-alpine vegetation. Journal of Ecology. 47: 45-427.

Watling R. 1977. Larger fungi from Greenland. Astarte 10: 61-71.

Watling R. 1983. Larger cold-climate fungi. Sydowia 36: 308-325.

Watling R. 1987. Larger arctic-alpine fungi in Scotland. Pp. 17-46. In: Laursen G, Ammirati JF, Redhead SA, (eds.). *Arctic and Alpine Mycology 2*. Plenum Press, New York.

Watling R, King R, Riddiford N. 2001. New and interesting records of fungi from Shetland. Transactions and Proceedings of the Botanical Society of Edinburgh and Botanical Society of Edinburgh Transactions 53: 57-64.

Webber PJ. 1974. Tundra primary productivity. Pp. 445-473. In: Ives JD, Barry RG, (eds.). *Arctic and Alpine Environments*. Methuen, London.

Weber WA. 1965. Plant geography in the southern Rocky Mountains. Pp. 453-468. In: Wright HE, Frey DG, (eds.) *The Quaternary of the United States*. Princeton University Press, Princeton.

Weber WA. 2003. The Middle Asian element in the southern Rocky Mountain Flora of the western United States: a critical review. Journal of Biogeography 30: 649-685.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, (eds.). *PCR Protocols: a Guide to Methods and Applications*. Academic Press, Inc.

Wielgolaski FE. 1997a. Inroduction. Pp. 1-5. In: Wielgolaski FE (ed.). *Ecosystems of the World 3*. *Polar and Alpine Tundra*. Elsevier, Amsterdam, Netherlands.

Wielgolaski FE. 1997b. Fennoscandian Tundra. Pp. 27-83. In: Wielgolaski FE (ed.). *Ecosystems of the World 3*. *Polar and Alpine Tundra*. Elsevier, Amsterdam, Netherlands.

Willig MR, Kaufman DM, Stevens RD. 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. Annual Review of Ecology, Evolution, and Systematics 34: 273-309.

Wisitrassameewong K, Nuytinck J, Hyde KD, Verbeken A. 2014. *Lactarius* subgenus *Russularia* (Russulaceae) in Southeast Asia: 1. Species with very distant gills. Phytotaxa 158(1): 23-42.

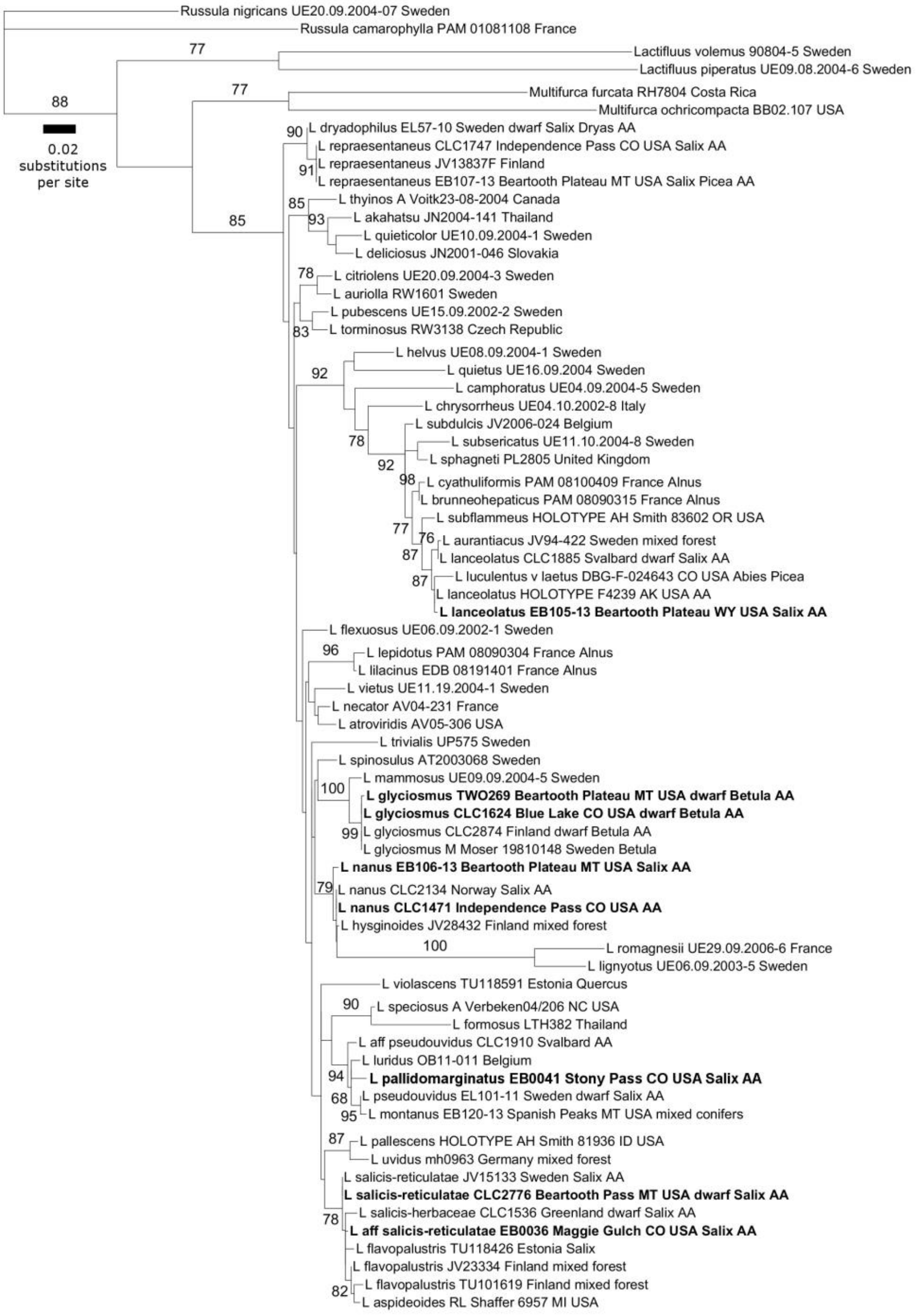
Wright SH, Berch SM, Berbee ML. 2009. The effect of fertilization on the below-ground diversity and community composition of ectomycorrhizal fungi associated with western hemlock (*Tsuga heterophylla*). Mycorrhiza 19(4): 267-276.

Yurtsev BA. 1994. Floristic division of the Arctic. Journal of Vegetation Science 5(6): 765-776.

APPENDICES

APPENDIX A

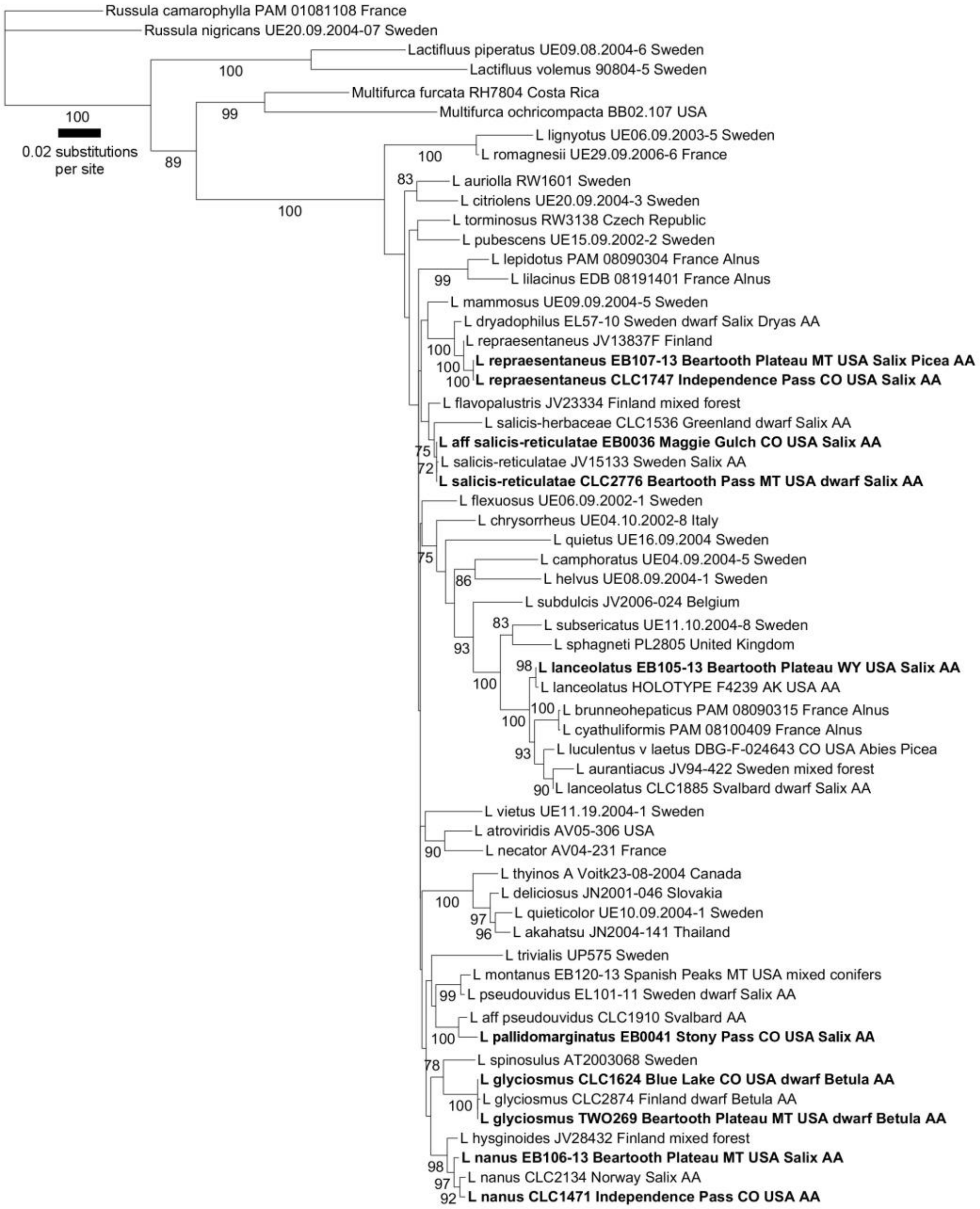
BROAD PHYLOGENETIC ANALYSIS: MAXIMUM-LIKELIHOOD TREE OF ITS DNA



Appendix A. Broad phylogenetic analysis: ML tree of ITS DNA. Bootstrap values generated in RAxML ≥ 70% are indicated above or below branches. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

APPENDIX B

BROAD PHYLOGENETIC ANALYSIS: MAXIMUM-LIKELIHOOD TREE OF RPB2 DNA



Appendix B. Broad phylogenetic analysis: ML tree of *rpb2* DNA. Bootstrap values generated in RAxML ≥ 70% are indicated above or below branches. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

APPENDIX C

BROAD PHYLOGENETIC ANALYSIS: BAYESIAN 50% MAJORITY RULE TREE COMBINING ITS AND RPB2 DNA

Appendix C. Broad phylogenetic analysis: Bayesian 50% majority rule tree combining ITS and *rpb2* DNA. Thickened branches lead up to clades receiving ≥75% bootstrap support from either PAUP* or raxml and Bayesian posterior probabilities ≥0.95. Bolded tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

APPENDIX D

SEQUENCES TO BE ACCESSIONED INTO GENBANK

Appendix D. Sequences to be accessioned into GenBank.

Taxon	Voucher	ITS	*rpb2*
L. aspideoides	RL Shaffer 6957 (MICH)	Yes	NA
L. aurantiacus	JV94-422 (C)	Yes	Yes
L. brunneoviolaceus	JV28448F (TURA)	NA	Yes
L. aff. *brunneoviolaceus*	CLC2133 (MONT)	Yes	Yes
L. dryadophilus	CLC2729 (MONT)	Yes	Yes
L. dryadophilus	EL57-10 (GB)	Yes	Yes
L. dryadophilus	CLC2744 (MONT)	Yes	Yes
L. flavopalustris	JV23334 (TURA)	Yes	Yes
L. glyciosmus	TWO269 (MONT)	Yes	Yes
L. glyciosmus	ZT12723 (MONT)	Yes	NA
L. glyciosmus	CLC1624 (MONT)	Yes	Yes
L. glyciosmus	20923 (DBG)	Yes	Yes
L. glyciosmus	EB133 (MONT)	Yes	Yes
L. glyciosmus	M Moser 19810148 (IB)	Yes	NA
L. glyciosmus	M Moser 19780234 (IB)	Yes	NA
L. glyciosmus	M Moser 19780191 (IB)	Yes	NA
L. glyciosmus	CLC2874 (MONT)	Yes	Yes
L. hysginoides	JV28432 (TURA)	Yes	Yes
L. lanceolatus (Holotype)	F4239 (VPI)	Yes	Yes
L. lanceolatus	CLC1389 (MONT)	Yes	Yes
L. lanceolatus	CLC2319 (MONT)	Yes	Yes
L. lanceolatus	CLC2358 (MONT)	Yes	Yes
L. lanceolatus	EB105-13 (MONT)	Yes	Yes
L. lanceolatus	JV15112F (TURA)	Yes	Yes
L. lanceolatus	CLC1885 (MONT)	Yes	Yes
L. lanceolatus	CLC2743 (MONT)	Yes	Yes
L. luculentus	AH Smith 79943 (MICH)	Yes	NA
L. luculentus	AH Smith 90905 (MICH)	Yes	NA
L. luculentus v. *laetus*	DBG-F-024643 (DBG)	Yes	Yes
L. luculentus v. *laetus*	DBG-F-022653 (DBG)	Yes	Yes
L. montanus (Paratype)	AH Smith 81954 (MICH)	Yes	NA
L. montanus	EB120-13 (MONT)	Yes	Yes
L. montanus	CLC3001 (MONT)	Yes	Yes
L. nanus	CLC1403 (MONT)	Yes	Yes
L. nanus	EB125 (MONT)	NA	Yes
L. nanus	EB106-13 (MONT)	Yes	Yes
L. nanus	CLC1716 (MONT)	Yes	Yes
L. nanus	CLC1801 (MONT)	Yes	Yes
L. nanus	EB138 (MONT)	Yes	Yes
L. nanus	CLC1829 (MONT)	Yes	Yes
L. nanus	CLC1471 (MONT)	Yes	Yes
L. nanus	CLC2134 (MONT)	Yes	Yes
L. nanus	Bon 89093 (LIP)	Yes	NA
L. nanus	JV15148 (TURA)	Yes	Yes
L. nanus	CLC1896 (MONT)	Yes	Yes
L. pallescens (Holotype)	AH Smith 81936 (MICH)	Yes	NA

Appendix D Continued.

Taxon	Voucher	ITS	*rpb2*
L. pallidomarginatus ad int.	CLC1470 (MONT)	Yes	Yes
L. pallidomarginatus ad int.	EB0041 (MONT)	Yes	Yes
L. pseudouvidus	U. Peintner 20070035 (IB)	NA	Yes
L. pseudouvidus	JV10468 (TURA)	Yes	Yes
L. pseudouvidus	E. Søyland 73867 (O)	Yes	Yes
L. pseudouvidus	P. Larsen 361395 (O)	Yes	Yes
L. pseudouvidus	EL101-11 (GB)	Yes	Yes
L. pseudouvidus	EL63-10 (GB)	Yes	Yes
L. pseudouvidus	TWO809 (MONT)	Yes	Yes
L. aff. *pseudouvidus*	CLC1910 (MONT)	Yes	Yes
L. repraesentaneus	CLC2318 (MONT)	Yes	Yes
L. repraesentaneus	EB107-13 (MONT)	Yes	Yes
L. repraesentaneus	EB0048 (MONT)	Yes	Yes
L. repraesentaneus	CLC1747 (MONT)	Yes	Yes
L. repraesentaneus	JV21671 (TURA)	Yes	Yes
L. repraesentaneus	EL92-07 (GB)	Yes	Yes
L. repraesentaneus	JV13837F (TURA)	Yes	Yes
L. robertianus	U. Peintner 20040156 (IB)	Yes	Yes
L. salicis-herbaceae	CLC1536 (MONT)	Yes	Yes
L. salicis-reticulatae	CLC1211 (MONT)	Yes	NA
L. salicis-reticulatae	CLC2776 (MONT)	Yes	Yes
L. salicis-reticulatae	EB0057-14 (MONT)	Yes	Yes
L. salicis-reticulatae	CLC2885 (MONT)	Yes	Yes
L. salicis-reticulatae	CLC2745 (MONT)	Yes	Yes
L. salicis-reticulatae	JV15133 (TURA)	Yes	Yes
L. aff. *salicis-reticulatae*	CLC1710 (MONT)	Yes	Yes
L. aff. *salicis-reticulatae*	CLC1741 (MONT)	Yes	Yes
L. aff. *salicis-reticulatae*	EB0036 (MONT)	Yes	Yes
L. aff. *salicis-reticulatae*	CLC1689 (MONT)	Yes	Yes
L. aff. *salicis-reticulatae*	EB0039 (MONT)	Yes	Yes
L. subflammeus (Holotype)	AH Smith 83602 (MICH)	Yes	NA
L. substriatus	AH Smith 83693 (MICH)	Yes	NA
L. substriatus	AH Smith 83694 (MICH)	Yes	NA
L. subviscidus (Paratype)	AH Smith 83066 (MICH)	Yes	NA
L. subviscidus (Paratype)	AH Smith 83331 (MICH)	Yes	NA