Systematics of the ectomycorrhizal genus *Lactarius* in the Rocky Mountain alpine zone

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Abstract: *Lactarius* (Russulales) is an important component of ectomycorrhizal fungal communities in cold-dominated contiguous arctic and disjunct alpine habitats where it associates primarily with *Betula*, *Dryas* and *Salix*. However, little is known of this genus in the central and southern Rocky Mountain alpine zone (3000–3900 m) of North America. Molecular phylogenetic analyses of nuc rDNA ITS1-5.8S-ITS2 (ITS barcode) and the second largest subunit of the RNA polymerase II gene (*RPB2*) partial sequences in conjunction with detailed morphological examination confirm at least six species occurring above treeline. Most have intercontinental distributions in North America and Eurasia according to molecular comparison with type material and collections from Europe, Fennoscandia, Svalbard and Alaska. Rocky Mountain collections of *L. lanceolatus* (subgenus *Russularia*), along with the type from Alaska are paraphyletic with respect to *L. aurantiacus* and North American taxa *L. luculentus* and *L. luculentus* v. *laetus*. Rocky Mountain collections of *L. nanus*, *L. glycosmus*, *L. repraesentaneus* and *L. salicis-reticulatae* (subgenus *Piperites*) all form clades with European material from type localities and other arctic-alpine habitats. The arctic-alpine *L. pseudowidius*/*L. brunneoviolaceus* group appears to be a complex containing additional taxa. North American material originally described as part of this group is well-separated phylogenetically and is described here as *L. pallidomarginatus* sp. nov. *Lactarius lanceolatus*, *L. nanus* and *L. salicis-reticulatae* appear largely restricted to arctic-alpine habitats with *Salix*. *Lactarius glycosmus* and *L. repraesentaneus* occur in arctic-alpine, subalpine and boreal habitats with *Betula* and also *Picea* and possibly *Salix* for the latter. Species distributions are hypothesized to be shaped by host ranges, glaciation and long distance dispersal.

Key words: Arctic-alpine, *Betula*, ITS, *RPB2*, Russulaceae, *Salix*

INTRODUCTION

The arctic-alpine region encompasses cold-dominated vegetated areas above or beyond the altitudinal or latitudinal limit of tree growth (Chapin and Körner 1995). Here ectomycorrhizal fungi associate with shrubs including *Betula*, *Dryas* and *Salix* and sedges and herbs such as *Kobresia* and *Bistorta* (Cripps and Eddington 2005, Dahlberg and Bültmann 2013). The ectomycorrhizal basidiomycete genus *Lactarius* Pers. (Russulales, Russulaceae) is well-documented in arctic-alpine habitats, although a comprehensive study had not been made of species in this genus occurring in the Rocky Mountain alpine zone. As originally defined *Lactarius* includes species with sporocarps that exude a latex when damaged, in addition to the amyloid ornamented basidiospores characteristic of the family. The genus is now recognized as nonmonophyletic and has been split into three genera within Russulaceae: *Lactarius*, *Lactifluus* (Pers.) Roussel and *Multifurca* Buyck & Hoffstetter (Buyck et al. 2008, 2010). *Lactarius sensu stricto* (s.s.) contains about 80% of the traditional species, most of which are temperate to boreal in distribution (Buyck et al. 2008, 2010; Verbeken and Nuytinck 2013). Most species from arctic-alpine habitats fall in *Lactarius* s.s. subgenera *Piperites* (Fr.) Kauffman and *Russularia* (Fr.) Kauffman based on morphological characteristics, although the monophyly of these subgenera has not been thoroughly investigated with molecular data.

More than 65 species and varieties of *Lactarius* have been reported from arctic-alpine areas in the northern hemisphere, mostly through sporocarp surveys (SUPPLEMENTARY TABLE I). However, due to synonymy, nomenclatural misapplications, misidentifications and the possibility that some reports actually came from subalpine or subarctic areas, it is likely that the actual number of species inhabiting arctic-alpine areas is much lower.

Lactarius species have been reported from arctic-alpine areas in Alaska (Laursen and Ammirati 1982, Geml et al. 2009), Canada (Ohenoja and Ohenoja 1993, 2010), Iceland (Heilmann-Clausen et al. 1998, Eyjolfsson pers. comm), Greenland (Terkelsen 1956, Lange 1957, Kobayasi et al. 1971, Knudsen and Borgen 1982, Lamoure et al. 1982, Elbourne and Knudsen 1990, Knudsen and Lamoure 1993, Knudsen and Borgen 1994, Watling 1997, Heilmann-Clausen et al. 1998, Borgen et al. 2006), the British Isles (Watling 1987, Watling et al. 2001), the Alps (Favre

1955; Kühner 1975; Bon 1985a, b, 1989, 1991; Jamoni and Bon 1991; Senn-Irlet 1993; Jamoni 1995, 2008), the Pyrenees (Bon and Ballarà 1996, Vila et al. 1997, Corriol 2008), the Carpathians (Ronikier 2008), Fennoscandia (Kallio and Kankainen 1964, Gulden et al. 1985, Heilmann-Clausen et al. 1998, Gulden 2005, Knudsen and Vesterholt 2008), Svalbard (Gulden and Jenssen 1988, Gulden and Torkelsen 1996), northern Russia (Kalamees and Vaasma 1993, Knudsen and Muhkin 1998, Karatygin et al. 1999) and the Altai Mountains (Kalamees 2008, Gorbunova 2010, Gorbunova and Taylakov 2011).

Many of the *Lactarius* species reported in these treatments appear to have broad, intercontinental distributions in North America and Eurasia, but this hypothesis remains largely untested. In addition, some species appear largely restricted to arctic-alpine areas while reports suggest others have wider distributions that extend into subalpine temperate and boreal habitats (Knudsen and Borgen 1982, Heilmann-Clausen et al. 1998, Knudsen and Vesterholt 2008). A few studies have confirmed broad intercontinental distributions for species in other ectomycorrhizal genera in arctic-alpine habitats using morphological and molecular data (Peintner 2008, Beker et al. 2010, Cripps et al. 2010, Larsson et al. 2014). Environmental sequencing studies (some of which include *Lactarius*) also suggest intercontinental conspecific taxa, although they allow less stringent species delineation (Geml et al. 2012; Timling et al. 2012, 2014). In contrast, recent molecular phylogenetic studies of *Lactarius* s.s. sampled from subalpine temperate habitats reveal narrow species ranges and a lack of intercontinental conspecificity (Nuytinck et al. 2007, Stubbe and Verbeken 2012).

Until recently, relatively little was known of the macrofungal mycota of the Rocky Mountain alpine zone of North America. An extensive sporocarp survey conducted by Cripps and Horak (2008) in the central and southern Rocky Mountain alpine zone of USA (Montana, Wyoming, Colorado) revealed the presence of more than 165 fungal species in 46 genera and 11 families. More than 75% appear to be species known from other arctic-alpine regions, and the remainder may be western endemics. Detailed studies stemming from this survey include those on the molecular and morphological systematics of arctic-alpine *Cortinarius* (Peintner 2008), *Hebeloma* (Beker et al. 2010), *Inocybe* (Cripps et al. 2010, Larsson et al. 2014), *Laccaria* (Osmundson et al. 2005) and morphological systematics of arctic-alpine *Amanita* (Cripps and Horak 2010) and Lycoperdaceae (Kasuya 2010).

Lactarius is well-studied in North America (e.g. Hessler and Smith 1979, Methven 1997, Bessette et al. 2009), however, outside Alaska and Canada arctic-alpine taxa are largely unaddressed. Preliminary

research tentatively identified six morphologically defined *Lactarius* species from the Rocky Mountain alpine zone (Cripps and Horak 2008, Cripps and Barge 2013). All species originally were described from Europe, except for *L. lanceolatus* O.K. Mill. & Laursen which was described from Alaska. Here collections of *Lactarius* from the Rocky Mountain alpine zone are evaluated through detailed morphological study and phylogenetic analyses of sequences from two genetic loci: nuclear rDNA ITS1–5.8S–ITS2 (ITS barcode) and the gene region between conserved domains six and seven of the second largest subunit of the RNA polymerase II (*RPB2*). Data were compared to type specimens, specimens from type localities, collections from arctic-alpine and subalpine habitats in North America, Europe, Fennoscandia, Svalbard, Alaska, Greenland and related taxa represented by sequences in databases (GenBank, UNITE). Insight into worldwide distribution, degree of confinement to arctic-alpine habitats and host specificity also is provided for these and related taxa.

MATERIALS AND METHODS

Study sites.—The Rocky Mountain alpine zone occurs as scattered, high elevation islands of open landscape above treeline on mountaintops in USA and Canada, extending southward from arctic 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 (FIG. 1) and include: Birch Site (45°01.444N, 109°24.486W, 2990–3020 m), Highline Trail (45°00.356N, 109°24.387W, 3060–3100 m), Frozen Lakes (44°57.932N, 109°28.998W, 3190–3200 m), Gardner Lake (44°57.949N, 109°27.199W, 3030 m) and Solifluction Terraces (44°58.372N, 109°26.810W, 3258 m). Southern Rocky Mountain sites are all above treeline in Colorado and include: Blue Lake (40°05.400N, 105°37.200W, 3560 m), Brainard Lake (40°04.200N, 105°34.200W, 3300 m) and Loveland Pass (39°39.600N, 105°52.800W, 3650 m) in the Front Range; Cottonwood Pass (38°49.200N, 106°24.000W, 3700 m) and Independence Pass (39°06.600N, 106°33.600W, 3650 m) in the Sawatch Range; and Black Bear Pass (37°24.000N, 107°42.000W, 3900 m), Cinnamon Pass (37°55.800N, 107°31.800W, 3840 m), Imogene Pass (37°55.800N, 107°43.200W, 3900 m), Maggie Gulch (37°51.000N, 107°34.200W, 3600 m), and Stony Pass (37°46.800N, 107°31.800W, 3700 m) in the San Juan Range (FIG. 1). These sites are characterized by a relatively windy, dry continental climate with large diurnal fluctuations in temperature and large differences between winter and summer average temperatures, although freezing temperatures are often experienced even during the growing season. The vegetation consists of low-lying perennial cushion plants, grasses, rosette plants and prostrate and erect shrubs. Plant community types in the Rocky Mountain alpine zone vary, often sharply, as a result of

FIG. 1. Map of the collection locations of specimens that were examined and/or included in molecular analyses (if collection locations are known). Rocky Mountain alpine study sites are indicated by white dots; black dots indicate other arctic-alpine sites; and gray dots indicate subalpine sites. Several collections from outside the map area (China, Japan, Thailand) are not included. The black dotted line delineates the Rocky Mountains; the arctic circle (67.5°N) is indicated by the dashed gray line; and the northern tree line which corresponds with the southern extent of arctic tundra is indicated by the solid dark gray line (Brown et al. 2001).

changes in the physical environment, such as topography, aspect and microrelief (Johnson and Billings 1962, Cooper et al. 1997). Ectomycorrhizal host species in the Rocky Mountain alpine zone include shrubby willows *Salix glauca* L. and *S. planifolia* Pursh, dwarf willows *S. arctica* Pall. and *S. reticulata* L., the bog birch *Betula glandulosa* Michx., the recumbent mat plant *Dryas octopetala* L. and the perennial sedge *Kobresia myosuroides* (Vill.) Fiori (Schadt 2002, Cripps and Eddington 2005). In general the same ectomycorrhizal host plants occur across the 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 sites in Colorado.

Taxon sampling and processing.—Sporocarps were collected from late Jul–late Aug, 1997–2014. All collections were described in detail when fresh, and select collections were photographed. Ectomycorrhizal host plants near sporocarps were noted for each collection. Sporocarps were dried on

an electric, warm-air dryer and deposited in the Montana State University herbarium (MONT). Collections initially were identified to species primarily using Jamoni (1995, 2008), Bon (1998), Heilmann-Clausen et al. (1998) and Basso (1999). Herbarium specimens of type collections, putative conspecific and taxonomically similar species were obtained from the herbarium of the Conservatoire et Jardin botaniques de la Ville de Genève, Geneva, Switzerland (G), the herbarium of Universität Innsbruck, Innsbruck, Austria (IB), the Herbarium Universitatis Tartuens, 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

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 (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 (FIG. 1).

Morphological descriptions.—Descriptions of macromorphological and micromorphological features were made from fresh and dry material, respectively. For micromorphological examination, dried material was reconstituted in ethanol and thin sections were prepared. Basidiospores were examined in Melzer's reagent; basidia, macrocystidia and pileipellis tissue were examined in 2.5% KOH. Length and width of micromorphological features were measured with an ocular micrometer under an oil-immersion lens at 1000 $\times$ magnification for a random sample of 25 basidiospores per collection, excluding ornamentation, the hilar appendix and giant basidiospores from two-spored basidia; mean basidiospore length and width were calculated for each collection. The length to width ratio ($\bar{Q}$) was calculated for each basidiospore, and mean length to width ratio ($\bar{Q}$) was calculated for each collection. The maximum height of the basidiospore ornamentation was measured and recorded and the ornamentation described. Length and width (at widest point) were measured at 400 $\times$ magnification for a random sample of 10 basidia (including sterigmata and excluding two-spored basidia), 10 pleuromacrocystidia and 10 cheilomacrocystidia per collection. Drawings of basidiospores, pleuromacrocystidia and cheilomacrocystidia were prepared from one representative collection of each Rocky Mountain alpine *Lactarius* species as well as for their corresponding type specimen (when available) with a Leica drawing tube attached to a Leica DMLS research microscope. Terminology is according to Heilmann-Clausen et al. (1998).

DNA extraction, PCR amplification and sequencing.—DNA was extracted from dried, ground sporocarp tissue following Osmundson et al. (2013) or using the DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions. PCR amplification of the ITS region was performed with primers ITS1-F and ITS4 or ITS1-F and ITS2 or ITS3 and ITS4 (White et al. 1990, Gardes and Bruns 1993). The *RPB2* gene segment was amplified with primers bRPB2-6f and fRPB2-7cR or bRPB2-6f and i2r-RPB2 or fRPB2-7cR and ilf-RPB2 (Liu et al. 1999, Matheny 2005, Stubbe et al. 2010). Primer i2r-RPB2 of Stubbe et al. (2010) was made slightly less specific by replacing primer positions 6 (= T), 9 (= C) and 15 (= T) with Y, yielding the primer consensus sequence 5'-ACV GTY TCY TCT TCY TCR GCR TC-3'. The thermo-cycler program used for ITS amplification was 94 C for 2 min, followed by 30 cycles of 94 C for 30 s, 55 C for 1 min and 72 C for 1 min; followed by a final elongation step of 72 C for 5 min. The thermo-cycler program used for *RPB2* amplification was 94 C for 90 s followed by 40 cycles of 94 C for 30 s, 55 C for 90 s and 68 C for 3 min, concluding with a 5 min final extension at 68 C. PCR products were cleaned with ExoSAP-IT (USB, USA) or

the QIAquick PCR Purification Kit (QIAGEN) following the manufacturer's instructions. PCR products were sequenced on both strands with the same primers used for PCR. Raw sequences were edited and contigs constructed with either Geneious Pro (Drummond et al. 2009) or SeqTrace 0.8.1 (Stucky 2012).

Sequence alignment and phylogenetic analyses.—In addition to the sequences generated in this study, sequences exhibiting 97% or higher BLAST matches to the ITS region of a representative specimen of each Rocky Mountain alpine taxon were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/>) and UNITE (<https://unite.ut.ee/>) as were a number of ITS and *RPB2* sequences mostly from Buyck et al. (2008), Verbeken et al. (2014) and the UNITE database. Locality, voucher information and accession numbers of sequences generated in this study (TABLE I) and of all sequences used in phylogenetic analyses (SUPPLEMENTARY TABLE II) are provided. Due to difficulty unambiguously aligning the ITS region across the genus *Lactarius*, four separate datasets were assembled. One broad ITS+*RPB2* dataset was constructed in an attempt to ascertain the phylogenetic position of each Rocky Mountain alpine taxon within the genus *Lactarius* (referred to as the "broad dataset"). This resulted in the identification of three clades that served to guide the construction of three subsequent species-level ITS+*RPB2* datasets (referred to as "datasets A, B, C"). Alignments were performed with MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>) with default parameters. For the broad dataset, ambiguously aligned regions of the ITS alignment were highlighted and removed with the online version of GBLOCKS 0.91b (Castresana 2000) under the least stringent settings. For datasets A, B, and C the alignments were edited with SeAl (Rambaut 1995). For dataset C, a 268–269 base-pair insertion present in the ITS1 region of a number of specimens (see SUPPLEMENTARY TABLE II) was removed along with a small ambiguously aligned region present in taxa lacking the insertion.

For each dataset, topological incongruence between the ITS and *RPB2* loci was examined with the partition homogeneity test (Farris et al. 1995) as implemented in PAUP* (Swofford 2001). 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 for the combined datasets with PAUP* using heuristic searches including 100 random addition sequence replicates and tree bisection reconnection branch swapping. Clade support was assessed with 1000 bootstrap replicates with heuristic searches, tree bisection reconnection branch swapping and one random addition sequence per replicate.

Maximum likelihood (ML) analyses were carried out with raxmlGUI 1.3.1 (Stamatidis 2006, Silvestro and Michalak 2012). An ML search combined with rapid bootstrapping was run until bootstrap convergence or 1000 bootstrap replicates was reached, using the autoMRE option. The GTRGAMMA model was estimated separately for ITS1, 5.8S, ITS2, *RPB2* exon first, second and third codon positions, and *RPB2* introns for the broad dataset. For datasets A, B and C the GTRCAT model was estimated separately for ITS1, 5.8S, ITS2, *RPB2* exon first, second and third codon positions

TABLE I. Voucher, locality information and GenBank accession numbers for DNA sequences generated in this study. Herbarium acronyms follow Thiers <http://sweetgum.nybg.org/ih/> (continuously updated). Rocky Mountain alpine specimens are shown in boldface

Taxon	Voucher	Location	ITS	<i>RPB2</i>
<i>L. aspideoides</i>	RL Shaffer 6957 (MICH)	Emmet County, MI, USA	KR090893	NA
<i>L. aspideus</i>	JV24534 (TURA)	Varsinais-Suomi, Finland	KR090894	KR090972
<i>L. aurantiacus</i>	JV94-422 (C)	Uppland, Sweden (type country)	KR090896	KR090974
<i>L. cf. aurantiacus</i> ¹	JV15112F (TURA)	Nordland, Norway	KR090895	KR090973
<i>L. cf. aurantiacus</i> ¹	CLC1885 (MONT)	Longyearbyen, Svalbard	KR090897	KR090975
<i>L. cf. aurantiacus</i> ¹	CLC2743 (MONT)	Palmer Creek Road, AK, USA	KR090898	KR090976
<i>L. aff. brunneoviolaceus</i>	CLC2133 (MONT)	Finse, Norway	KR090899	KR090977
<i>L. aff. brunneoviolaceus</i> ²	P. Larsen 361395 (O)	Stordal, Norway	KR090900	KR090978
<i>L. dryadophilus</i>	CLC2729 (MONT)	AK, USA	KR090901	KR090979
<i>L. dryadophilus</i>	EL57-10 (GB)	Latnjavagge, Sweden	KR090902	KR090980
<i>L. dryadophilus</i>	CLC2744 (MONT)	Palmer Creek Road, AK, USA	KR090903	KR090981
<i>L. flavopalustris</i>	JV23334 (TURA)	Koillismaa, Finland (type country)	KR090904	KR090982
<i>L. glyciosmus</i>	TWO269 (MONT)	Beartooth Plateau, MT, USA	KR090905	KR090983
<i>L. glyciosmus</i>	ZT12723 (MONT)	Beartooth Plateau, MT, USA	KR090906	NA
<i>L. glyciosmus</i>	CLC1624 (MONT)	Front Range, CO, USA	KR090907	KR090984
<i>L. glyciosmus</i>	20923 (DBG)	Brainard Lake, Front Range, CO, USA	KR090908	KR090985
<i>L. glyciosmus</i>	EB133 (MONT)	San Juan Range (subalpine), CO, USA	KR090909	KR090986
<i>L. glyciosmus</i>	M. Moser 19810148 (IB)	Femsjö, Sweden (type locality)	KR090910	NA
<i>L. glyciosmus</i>	M. Moser 19780234 (IB)	Femsjö, Sweden (type locality)	KR090911	NA
<i>L. glyciosmus</i>	M. Moser 19780191 (IB)	Femsjö, Sweden (type locality)	KR090912	NA
<i>L. glyciosmus</i>	CLC2874 (MONT)	Fjell Ailigas, Finland	KR090913	KR090987
<i>L. hysginoides</i>	JV28432 (TURA)	Koillismaa, Finland (type country)	KR090914	KR090988
<i>L. lanceolatus</i> (HOLOTYPE)	F4239 (VPI)	Beaufort Lagoon, AK, USA	KR090915	KR090989
<i>L. lanceolatus</i>	CLC1389 (MONT)	Beartooth Plateau, MT, USA	KR090916	KR090990
<i>L. lanceolatus</i>	CLC2319 (MONT)	Beartooth Plateau, WY, USA	KR090917	KR090991
<i>L. lanceolatus</i>	CLC2358 (MONT)	Beartooth Plateau, MT, USA	KR090918	KR090992
<i>L. lanceolatus</i>	EB105-13 (MONT)	Beartooth Plateau, WY, USA	KR090919	KR090993
<i>L. luculentus</i>	AH Smith 79943 (MICH)	OR, USA (near type locality)	KR090920	NA
<i>L. luculentus</i>	AH Smith 90905 (MICH)	Tucannon River, WA, USA	KR090921	NA
<i>L. luculentus</i> v. <i>laetus</i>	DBG-F-024643 (DBG)	CO, USA (near type locality)	KR090922	KR090994
<i>L. luculentus</i> v. <i>laetus</i>	DBG-F-022653 (DBG)	El Paso. County, CO (near type locality)	KR090923	KR090995
<i>L. montanus</i> (PARATYPE)	AH Smith 81954 (MICH)	Bonner County, ID, USA	KR090924	NA
<i>L. montanus</i>	EB120-13 (MONT)	Madison Range, MT, USA	KR090925	KR090996
<i>L. montanus</i>	CLC3001 (MONT)	Tobacco Root Mountains, MT, USA	KR090926	KR090997
<i>L. nanus</i>	CLC1403 (MONT)	Beartooth Plateau, WY, USA	KR090927	KR090998
<i>L. nanus</i>	EB125 (MONT)	Beartooth Plateau, WY, USA	NA	KR090999
<i>L. nanus</i>	EB106-13 (MONT)	Beartooth Plateau, MT, USA	KR090928	KR091000
<i>L. nanus</i>	CLC1716 (MONT)	San Juan Range, CO, USA	KR090929	KR091001
<i>L. nanus</i>	CLC1801 (MONT)	San Juan Range, CO, USA	KR090930	KR091002
<i>L. nanus</i>	EB138 (MONT)	San Juan Range, CO, USA	KR090931	KR091003
<i>L. nanus</i>	CLC1829 (MONT)	San Juan Range, CO, USA	KR090932	KR091004
<i>L. nanus</i>	CLC1471 (MONT)	Sawatch Range, CO, USA	KR090933	KR091005
<i>L. nanus</i>	CLC2134 (MONT)	Finse, Norway	KR090934	KR091006
<i>L. nanus</i>	Bon 89093 (LIP)	Savoie, France (near type locality)	KR090935	NA
<i>L. nanus</i>	JV15148 (TURA)	Torne Lappmark, Sweden	KR090936	KR091007

TABLE I. Continued

Taxon	Voucher	Location	ITS	<i>RPB2</i>
<i>L. nanus</i>	CLC1896 (MONT)	Longyearbyen, Svalbard	KR090937	KR091008
<i>L. pallescens</i> (HOLOTYPE)	AH Smith 81936 (MICH)	Boundary County, ID, USA	KR090938	NA
<i>L. pallidomarginatus</i> sp. nov.	CLC1470 (MONT)	Sawatch Range, CO, USA	KR090939	KR091009
<i>L. pallidomarginatus</i> sp. nov.	EB0041 (MONT)	San Juan Range, CO, USA	KR090940	KR091010
<i>L. aff. pseudouvidus</i>	U. Peintner 20070035 (IB)	Tyrol, Austria (near type locality)	NA	KR091011
<i>L. aff. pseudouvidus</i> ³	JV28448F (TURA)	Koillismaa, Finland	NA	KR091012
<i>L. aff. pseudouvidus</i>	JV10468 (TURA)	Inarin Lappi, Finland	KR090941	KR091013
<i>L. aff. pseudouvidus</i> ⁴	U. Peintner 20040156 (IB)	Trentino, Italy (near type locality)	KR090942	KR091014
<i>L. aff. pseudouvidus</i>	E. Søyland 73867 (O)	Ulvik, Norway	KR090943	KR091015
<i>L. aff. pseudouvidus</i>	EL101-11 (GB)	Latnjajaure, Sweden	KR090944	KR091016
<i>L. aff. pseudouvidus</i>	EL63-10 (GB)	Latnjavagge, Sweden	KR090945	KR091017
<i>L. aff. pseudouvidus</i>	TWO809 (MONT)	Svalbard	KR090946	KR091018
<i>L. repraesentaneus</i>	CLC2318 (MONT)	Beartooth Plateau, MT, USA	KR090948	KR091020
<i>L. repraesentaneus</i>	EB107-13 (MONT)	Beartooth Plateau, MT, USA	KR090949	KR091021
<i>L. repraesentaneus</i>	EB0048 (MONT)	Sawatch Range, CO, USA	KR090950	KR091022
<i>L. repraesentaneus</i>	CLC1747 (MONT)	Sawatch Range, CO, USA	KR090951	KR091023
<i>L. repraesentaneus</i>	JV21671 (TURA)	Enontakiö, Finland	KR090952	KR091024
<i>L. repraesentaneus</i>	EL92-07 (GB)	Latnjavagge, Sweden	KR090953	KR091025
<i>L. repraesentaneus</i>	JV13837F (TURA)	Varsinais-Suomi, Finland	KR090954	KR091026
<i>L. salicis-herbaceae</i>	CLC1536 (MONT)	Sismiut, Greenland	KR090955	KR091027
<i>L. salicis-reticulatae</i>	CLC1211 (MONT)	Beartooth Plateau, MT, USA	KR090956	NA
<i>L. salicis-reticulatae</i>	CLC2776 (MONT)	Beartooth Plateau, MT, USA	KR090957	KR091028
<i>L. salicis-reticulatae</i>	EB0057-14 (MONT)	Beartooth Plateau WY, USA	KR090958	KR091029
<i>L. salicis-reticulatae</i>	CLC2885 (MONT)	Near Utsjoki, Finland	KR090959	KR091030
<i>L. salicis-reticulatae</i>	CLC2745 (MONT)	Palmer Creek Road, Alaska, USA	KR090960	KR091031
<i>L. salicis-reticulatae</i>	JV15133 (TURA)	Sweden (near type locality)	KR090961	KR091032
<i>L. aff. salicis-reticulatae</i>	CLC1710 (MONT)	San Juan Range, CO, USA	KR090962	KR091033
<i>L. aff. salicis-reticulatae</i>	CLC1741 (MONT)	Sawatch Range, CO, USA	KR090963	KR091034
<i>L. aff. salicis-reticulatae</i>	EB0036 (MONT)	San Juan Range, CO, USA	KR090964	KR091035
<i>L. aff. salicis-reticulatae</i>	CLC1689 (MONT)	San Juan Range, CO, USA	KR090965	KR091036
<i>L. aff. salicis-reticulatae</i>	EB0039 (MONT)	San Juan Range, CO, USA	KR090966	KR091037
<i>L. subflammeus</i> (HOLOTYPE)	AH Smith 83602 (MICH)	Tillamook County, OR, USA	KR090967	NA
<i>L. substriatus</i>	AH Smith 83693 (MICH)	Tillamook County, OR, USA	KR090968	NA
<i>L. substriatus</i>	AH Smith 83694 (MICH)	Tillamook County, OR, USA	KR090969	NA
<i>L. subviscidus</i> (PARATYPE)	AH Smith 83066 (MICH)	Lewis County, WA, USA	KR090970	NA
<i>L. subviscidus</i> (PARATYPE)	AH Smith 83331 (MICH)	Lewis County, WA, USA	KR090971	NA
<i>Lactarius</i> sp.	CLC1910 (MONT)	Svalbard	KR090947	KR091019

¹ Originally identified as *L. lanceolatus*.² Originally identified as *L. pseudouvidus*.³ Originally identified as *L. brunneoviolaceus*.⁴ Originally identified as *L. robertianus*.

and *RPB2* introns. Single-gene and combined ML analyses were performed on each dataset.

For Bayesian inference (BI) analyses the best-fitting substitution model for the evolution of ITS1, 5.8S, ITS2, *RPB2* exon first, second and third codon positions and *RPB2* intron was estimated from 24 models by the Akaike information

criterion (AIC) with MrModeltest 2.3 (Nylander 2004). Models selected for each partition within each dataset are provided (SUPPLEMENTARY TABLE III). BI analyses were executed in MrBayes 3.2 (Ronquist and Huelsenbeck 2003). Parameters were unlinked between partitions and the substitution rate was allowed to vary across partitions. Preliminary

Bayesian trees were an order of magnitude longer but had nearly identical topologies to maximum-likelihood trees. This issue was addressed by setting a branch length prior of 0.01 for all analyses (see Marshall 2010 for a discussion of this problem). BI was executed separately for ITS and *RPB2* and also on the combined datasets. All BI analyses consisted of two independent runs with four chains (three heated, one cold) each run for 10 million 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. Topological convergence of independent program runs was assessed with AWTY (Nylander et al. 2008). The program TreeGraph 2 (Stöver and Müller 2010) was used to draw, edit and finalize the resulting trees.

RESULTS

In total 79 ITS sequences and 66 *RPB2* sequences from 82 collections were generated in this study (TABLE I). Sequences were obtained from six type collections, including that of *Lactarius lanceolatus* O.K. Mill. & Laurén. Sequences were not successfully obtained from types of *L. nanus* J. Favre, *L. pseudowidius* Kühner and *L. salicis-reticulatae* Kühner; *L. glyciosmus* (Fr.) Fr. and *L. repraesentaneus* Britzelm. lack physical type material; however, sequences were obtained from type locality material for all five taxa. An overview of the datasets used for phylogenetic analyses is provided (SUPPLEMENTARY TABLE IV). Alignments and multigene (ITS+*RPB2*) trees were deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S17922>).

Convergence diagnostics for the Bayesian analyses of the combined (ITS+*RPB2*) datasets indicated that each analysis achieved topological convergence; although sliding window analyses indicate a moderate degree of within-run instability, the cumulative posterior probability plots and between-run comparison plot indicate convergence (SUPPLEMENTARY FIGS. 1–4).

For the broad dataset (*Lactarius* and related genera) the partition homogeneity test indicated that the ITS and *RPB2* loci were resolving significantly different phylogenetic relationships ($P = 0.03$). However, these conflicts generally received low support (bootstrap support < 75%, posterior probability < 0.95) and pertained only to the placement of a few terminal branches. Therefore the broad, combined (ITS+*RPB2*) dataset was analyzed and the Bayesian 50% majority-rule consensus tree is displayed (FIG. 2). Two clades corresponding with *Lactarius* subgenera *Plinthogalus* (Burl.) Hesler & A.H. Sm. (well-supported) and *Russularia* (well-supported) as recognized in Verbeken et al. (2014) and a monophyletic clade corresponding to section *Uvidi* (Konr.) Bon (poorly supported) were obtained, as were clades that were used to guide the construction of datasets A, B and C. *Lactarius* subgenus

Piperites was not resolved as monophyletic in the Bayesian (FIG. 2) or MP (not shown) analyses, however, it was resolved as monophyletic, with low support in the ML analysis (not shown).

For datasets A ($P = 0.44$), B ($P = 0.14$) and C ($P = 0.41$) the partition homogeneity test indicated that the ITS and *RPB2* loci did not produce conflicting signals; thus combined datasets were analyzed. Combined (ITS+*RPB2*) Bayesian 50% majority-rule consensus trees for datasets A (FIG. 3), B (FIG. 4) and C (FIG. 5) are shown.

For dataset A (subgenus *Russularia* p.p.) collections originally identified as *L. lanceolatus* from the Rocky Mountain alpine zone, as well as the holotype collection from Alaska, form a polytomy within a well-supported clade that also includes *Lactarius* sp. 2, *L. luculentus* Burl., *L. luculentus* v. *laetus* Hesler & A.H. Sm. and *L. aurantiacus* (Pers.) Gray (FIG. 3). Several arctic-alpine collections originally identified as *L. lanceolatus* from Norway (JV15112F), Svalbard (CLC1885, IA-F20) and Alaska (CLC2743) group with *L. aurantiacus* (syn.: *L. mitissimus* [Fr.] Fr.) from the type country in a well-supported clade. Two collections identified as *L. luculentus* v. *laetus* (DBG-F-024643, DBG-F-022653) from the type locality form a well-supported clade sister to a well-supported clade containing two collections of *L. luculentus* (AH Smith 79943 from type locality, AH Smith 90905) and a collection identified as *L. luculentus* v. *laetus* (SMI221) from British Columbia, suggesting there may be two species in this group. Other species closely related to *L. lanceolatus* include *L. substriatus* A.H. Sm./*L. subflammeus* Hesler & A.H. Sm. (taxa that may be conspecific), and *Lactarius* sp. 1, represented by unidentified environmental GenBank sequences from subalpine Alaska and arctic-alpine Norway with *Dryas octopetala*. GenBank sequence DQ974746 (voucher src438, *Lactarius* sp. 2) is likely misidentified as *L. substriatus* because specimens collected by the author of *L. substriatus* form a separate clade (FIG. 3). Results of the ITS (SUPPLEMENTARY FIG. 5) analyses are similar to the combined analysis, however, in the *RPB2* analysis (SUPPLEMENTARY FIG. 6) *L. aurantiacus* does not form a clade separate from the Rocky Mountain alpine and holotype *L. lanceolatus* collections.

For dataset B (subgenus *Piperites* p.p.), Rocky Mountain alpine collections initially identified as *L. nanus* occur in a well-supported clade with an alpine specimen collected by Marcel Bon from the French Alps (type locality) along with a number of collections from arctic-alpine areas elsewhere in Europe (FIG. 4); sequencing of the holotype was unsuccessful. In multigene (FIG. 4), ITS (SUPPLEMENTARY FIG. 7) and *RPB2* (SUPPLEMENTARY FIG. 8) analyses *L. nanus* is paraphyletic with respect to *L. hysginoides* and the two

FIG. 2. Combined (ITS+RPB2) Bayesian 50% majority-rule consensus tree based on analysis of the broad dataset (*Lactarius* and related genera). Thickened branches lead to clades receiving $\geq 75\%$ bootstrap support from either PAUP* or RAxML and Bayesian posterior probabilities ≥ 0.95 . Boldface tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

consistently differ at only one nucleotide position in the ITS region and at four positions and one indel in the RPB2 locus. Despite high sequence similarity, the two taxa are maintained as distinct in this study because basidiospore size and habitat appear to be consistently different.

Also in dataset B Rocky Mountain alpine collections originally identified as *L. glyciosmus* occur in a well-supported clade with several specimens collected by Meinhard Moser from Femsjö, Sweden

(type locality), as well as with collections from arctic-alpine and subalpine areas elsewhere in North America and Europe (FIG. 4). Several GenBank and UNITE sequences from specimens identified as *L. glyciosmus* (labeled as *L. aff. glyciosmus* in FIG. 4) from subalpine Alaska, Europe and Japan occur in a separate, poorly supported clade, and appear to represent a distinct, cryptic species. No definitive morphological or geographical differences were detected between *L. glyciosmus* and *L. aff. glyciosmus*;

FIG. 3. Combined (ITS+RPB2) Bayesian 50% majority-rule consensus tree of dataset A (*Lactarius* subgenus *Russularia* p.p.). Support values (PAUP* bootstrap/RAXML bootstrap/Bayesian posterior probability) $\geq 60\%$ (bootstrap) and ≥ 0.80 (posterior probability) are indicated above or below branches leading to clades. Thickened branches lead to clades receiving $\geq 75\%$ bootstrap support and Bayesian posterior probabilities ≥ 0.95 . Boldface tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine. *Originally identified as *L. lanceolatus*.

however, the *L. aff. glyciosmus* clade does not contain any arctic-alpine specimens (FIG. 4).

In dataset C (subgenus *Piperites*, section *Uvidi*), collections originally identified as *L. aff. pseudowidius* from the Rocky Mountain alpine zone are well-separated from the majority of collections (mostly from Europe) labeled the *L. pseudowidius* group (FIG. 5); the latter appear in the BI consensus tree as paraphyletic to *L. brunneoviolaceus* M.P. Christ. and *L. luridus* (Pers.) Gray. Instead Rocky Mountain alpine material forms a well-supported clade close to the morphologically very different species *L. aspidius* (Fr.) Fr. and an arctic-alpine environmental ITS sequence isolated from the roots of *Salix polaris* Wahlenb. in Svalbard (AB588960, Fujiyoshi et al. 2012) (FIG. 5). Thus based

on morphological and molecular differences the Rocky Mountain alpine material is recognized here as the new species *L. pallidomarginatus* Barge & Cripps sp. nov. (see TAXONOMY). An additional collection from Svalbard with *Salix polaris* (labeled *Lactarius* sp. 3 in FIG. 5) is sister to the clade containing *L. aspidius*/*L. pallidomarginatus*.

The ITS region of the European *L. pseudowidius* group has little variation and is paraphyletic with respect to *L. montanus* (Hesler & A.H. Sm.) Montoya & Bandala (SUPPLEMENTARY FIG. 9). However, the RPB2 locus is highly variable both between and within most collections examined (SUPPLEMENTARY FIG. 10). It is not possible to definitively provide names to taxa within this group in that sequencing of the *L.*

FIG. 4. Combined (ITS+RPB2) Bayesian 50% majority-rule consensus tree of dataset B (*Lactarius* subgenus *Piperites* p.p.). Support values (PAUP* bootstrap/RAXML bootstrap/Bayesian posterior probability) $\geq 60\%$ (bootstrap) and ≥ 0.80 (posterior probability) are indicated above or below branches leading up to clades. Thickened branches lead to clades receiving $\geq 75\%$ bootstrap support and Bayesian posterior probabilities ≥ 0.95 . Boldface tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine.

pseudowidius holotype was unsuccessful and cryptic taxa may be present. Collections matching the morphological concept of the similar arctic-alpine taxon *L. brunneoviolaceus* (syn.: *L. robertianus* Bon) occur in a

moderately supported clade (labeled *L. aff. brunneoviolaceus*) in the combined (FIG. 5) and ITS analyses (SUPPLEMENTARY FIG. 9), but these are paraphyletic with the *L.*

FIG. 5. Combined (ITS+RPB2) Bayesian 50% majority-rule consensus tree of dataset C (*Lactarius* subgenus *Piperites*, section *Uvidi*). Support values (PAUP* bootstrap/RAxML bootstrap/Bayesian posterior probability) $\geq 60\%$ (bootstrap) and ≥ 0.80 (posterior probability) are indicated above or below branches leading up to clades. Thickened branches lead to clades receiving

pseudowidius group in *RPB2* analyses (SUPPLEMENTARY FIG. 10). Collections of *Lactarius pallidomarginatus*, *Lactarius* sp. 3, *L. aspideus*, *L. montanus*, *L. luridus*, *L. aff. pseudowidius* and *L. aff. brunneoviolaceus* for which ITS data are available all have a similar 268–269 base-pair insertion in the ITS1 region. There is variation within the base-pair content of the insertion between collections; however, inclusion of this insertion did not increase phylogenetic resolution in preliminary analyses and it therefore was omitted. The insertion itself is highly similar to an insertion in *Russula olivacea* (e.g. GenBank accessions AF418634, AY061699) as to length, sequence (approximately 90% similar) and placement within the ITS1 region.

Also in dataset C Rocky Mountain alpine collections initially identified as *L. repraesentaneus* form a well-supported clade with a collection of *L. repraesentaneus* from Germany (the lectotype country), as well as with numerous collections from subalpine and arctic-alpine areas elsewhere in Eurasia and Alaska (FIG. 5). This clade is sister to a poorly supported *L. dryadophilus* Kühner clade. The two GenBank sequences JX630967 from arctic Canada with *Dryas* and JF394416 from arctic Svalbard which occur just outside the *L. dryadophilus* clade are likely conspecific with *L. dryadophilus*, although this cannot be confirmed based on present results. *Lactarius repraesentaneus* and *L. dryadophilus* consistently differ at two nucleotide positions in the ITS locus and at four positions in the *RPB2* locus and have consistent morphological and host differences.

In dataset C collections originally identified as *L. salicis-reticulatae* from alpine areas in the Rocky Mountains are paraphyletic with regard to *L. salicis-herbaceae* Kühner, *L. aspideoides* Burl./*L. flavopalustris* Kytöv. and *L. aff. flavopalustris* (FIG. 5). Collections of *L. salicis-reticulatae* from alpine areas on the Beartooth Plateau (central Rocky Mountains) form a poorly supported clade with an alpine collection of *L. salicis-reticulatae* from Mt. Låktajäkka, Sweden, the type locality (sequencing of the holotype of *L. salicis-reticulatae* was not successful), as well as with other arctic-alpine collections of *L. salicis-reticulatae* from Finland and Alaska and an unidentified collection from Svalbard (FIG. 5); this clade is hypothesized to represent *L. salicis-reticulatae*. Collections from southern Rocky Mountain alpine areas in Colorado are larger than reported for *L. salicis-reticulatae* and form an unresolved group that consistently differs at one nucleotide position in the ITS region but no positions in the *RPB2*

locus; they are tentatively referred to as *L. aff. salicis-reticulatae* pending data from additional loci.

Overall phylogenetic analyses support the recognition of six to seven *Lactarius* species from the Rocky Mountain alpine zone: *Lactarius lanceolatus* (FIG. 3), *L. nanus*, *L. glyciosmus* (FIG. 4), *L. pallidomarginatus* sp. nov., *L. repraesentaneus*, *L. salicis-reticulatae* and possibly *L. aff. salicis-reticulatae* (FIG. 5). Violet-staining taxa *L. pallidomarginatus*, *L. repraesentaneus*, *L. salicis-reticulatae* and *L. aff. salicis-reticulatae* are resolved as a poorly supported clade exclusively containing other members of the violet-staining section *Uvidi*, supporting the monophyly of this section (FIG. 2).

TAXONOMY

All descriptions are based on Rocky Mountain alpine material.

Lactarius lanceolatus O.K. Mill. & Laursen FIGS. 6, 7

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

Pileus 10–45 mm diam, convex at first, becoming plane to infundibuliform with a 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, discolored 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, discolored dingy orange to dingy light brown where damaged, hollow. Latex scarce, watery, unchanging. Context of pileus and stipe pale orange. Odor mild. Flavor mild. Basidiospore deposit not observed. Basidiospores 8–10 × 6–8 µm, average = 8.6 × 7 µm, *Q* = 1.14–1.38, *Q̄* = 1.24, hyaline in 2.5% KOH, broadly ellipsoid; ornamentation amyloid, of isolated warts connected by fine lines, up to 0.5 µm high. Basidia 38.1–66.0 × 8.9–10.2 µm, four-spored. Pleuromacrocyttidia 73.7–127 × 6.4–10.2 µm, narrowly

←

≥75% bootstrap support and Bayesian posterior probabilities ≥ 0.95. Boldface tip labels represent Rocky Mountain alpine specimens. AA = arctic-alpine. *See TABLE I for original identifications.

fusiform to lanceolate, with acute to moniliform apex, thin-walled, scattered to abundant. Cheilomacrocytidia $35.6\text{--}89 \times 5.1\text{--}10.2 \mu\text{m}$, conical to fusiform, with acute to moniliform apex, thin-walled, sparse to abundant. Pileipellis an ixotrichoderm with well-developed gelatinous layer.

Distribution and habitat: *Lactarius lanceolatus* originally was described from arctic-alpine Alaska (Miller and Laursen 1973) and phylogenetic analyses (including the holotype) further confirm its presence in the central Rocky Mountain alpine zone and Svalbard. In alpine areas on the Beartooth Plateau it occurs with *Salix reticulata* and possibly other *Salix* species (*S. arctica*, *S. planifolia*); it is not reported from the southern Rocky Mountains. Overall *L. lanceolatus* appears largely confined to arctic-alpine habitats, however, an ITS sequence from a maritime forest in Alaska (GAL18572) clusters with the arctic-alpine collections in phylogenetic analyses (FIG. 3).

Previous arctic-alpine reports include Canada (Ohenoja and Ohenoja 1993, 2010), Alaska (Miller and Laursen 1973, Laursen and Ammirati 1982, Heilmann-Clausen et al. 1998), Beartooth Plateau in the Rocky Mountain alpine zone (Cripps and Barge 2013), Greenland (Heilmann-Clausen et al. 1998, Borgen et al. 2006), Fennoscandia (Bon 1998, Heilmann-Clausen et al. 1998, Knudsen and Vesterholt 2008), Svalbard (Gulden and Jenssen 1988, Geml et al. 2012), the British Isles (Watling et al. 2001) and Siberia (Nezdoiminogo 1997, Heilmann-Clausen et al. 1998, Karatygin et al. 1999, Knudsen and Mukhin 1998); however, phylogenetic analyses (FIG. 3) suggest that some of these reports may represent *L. aurantiacus* (Pers.: Fr.) Gray.

Specimens examined: USA. ALASKA: North Slope County, Beaufort Lagoon, 30 Jul 1971, *F4239* (Holotype, 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); 27 Jul 1997, *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).

Other specimens 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. cf. aurantiacus*, originally identified as *L. lanceolatus*, TURA); Svalbard, Longyearbyen, 18 Aug 2003, *CLC 1885* (*L. cf. aurantiacus*, originally identified as *L. lanceolatus*, MONT). SWEDEN. Uppland, 8 Sept 1994, *JV94-422* (*L. aurantiacus*, C). USA. ALASKA: Kenai Peninsula County, Palmer Creek Road, 7 Aug 2011, *CLC 2743* (*L. cf. aurantiacus*, originally identified 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). 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 including *L. aurantiacus*, *L. luculentus*, *L. luculentus* v. *laetus*, *L. subflammeus* and *L. substriatus*.

Traditionally *L. lanceolatus* and *L. aurantiacus* have been separated mainly by ecology, with *L. aurantiacus* being restricted to a variety of subalpine habitats (although it has been reported from arctic-alpine habitats) and *L. lanceolatus* to arctic-alpine habitats with *Salix* spp.; however, it is clear from phylogenetic analyses (FIG. 3) that *L. aurantiacus* also may occur in arctic-alpine habitats, likely associating with *Salix* as well as *Kobresia*, and that the ranges of the two taxa overlap at least in arctic-alpine areas of Alaska and Svalbard. It has been suggested that *L. lanceolatus* has longer macrocytidia and larger basidiospores than *L. aurantiacus* (e.g. Heilmann-Clausen et al. 1998); however, collections designated as *L. aurantiacus* measured in this study have pleuromacrocytidia that overlap and actually exceed the upper limit measured for *L. lanceolatus* including the holotype (see Barge 2015). Basidiospore sizes for *L. aurantiacus* also overlap and actually exceed the upper limit measured for *L. lanceolatus* in the collections examined (Barge 2015). Overall solid morphological differences between the two taxa were not detected and further study may reveal that they are conspecific; however, provisionally *L. lanceolatus* may tend to have a thicker gelatinous layer over the pileipellis, as well as a smooth pileus margin in contrast to the crenulate pileus margin reported for *L. aurantiacus*. *Lactarius aurantiacus* is not reported from the Rocky Mountains.

For North American taxa *Lactarius luculentus* has a browner pileus, a slowly acrid flavor, shorter cheilomacrocytidia ($23\text{--}45 \times 4\text{--}6 \mu\text{m}$), and exsiccata are paler and more yellow-tan (Hesler and Smith 1979). *Lactarius luculentus* v. *laetus* is similar in appearance to *L. lanceolatus* but also has shorter cheilomacrocytidia ($15\text{--}30 \times 4\text{--}8 \mu\text{m}$) and possibly more crowded lamellae (Hesler and Smith 1979). *Lactarius substriatus* has a redder pileus that is glutinous when wet, with a translucent-striate margin, latex that slowly becomes straw yellow, shorter cheilomacrocytidia and an acrid flavor (Hesler and Smith 1979). *Lactarius subflammeus* also has a redder pileus (at least when young), short translucent striations along its pileus margin, shorter cheilomacrocytidia and a slowly

FIG. 6. A. *L. lanceolatus* (EB105-13), B. *L. nanus* (EB106-13), C. *L. nanus* (EB138), D. *L. gbyciosmus* (ZT12723), E. *L. pallidomarginatus* Barge & Cripps sp. nov. (EB0041, HOLOTYPE), F. *L. repraesentaneus* (EB107-13), G. *L. salicis-reticulatae* (EB0057-14), H. *L. aff. salicis-reticulatae* (EB0036), Photographs by E. Barge (A-C, E-H), E. Horak (D). Bars = 2 cm.

acid taste (pers obs of holotype AH Smith 83602, Hesler and Smith 1979). Molecular phylogenetic analyses (FIG. 3) suggest that *L. subflammeus* is conspecific with *L. substriatus*; however, sequencing of the holotype of *L. substriatus* is needed to confirm this observation. *Lactarius luculentus*, *L. luculentus* v. *laetus*, *L. subflammeus* and *L. substriatus* have been reported only from subalpine areas with conifers in western North America (Hesler and Smith 1979).

Lactarius duplicatus A.H. Sm. (syn.: *L. lapponicus* Harmaja), a North American and European *Betula* associate, is also morphologically close to *L. lanceolatus*. However, it can be distinguished by the production of strongly yellowing latex. We did not procure any molecular data from material representing this taxon, and no molecular data is publicly available on GenBank or UNITE. However, *L. duplicatus* should be included in future phylogenetic analyses investigating the *L. lanceolatus*/*L. aurantiacus* group.

Lactarius nanus J. Favre

FIGS. 6, 7

Short description: *Lactarius nanus* is recognized by its typically small size, gray-brown to liver brown pileus typically with a glaucous coating at least when young, cream to pinkish tan lamellae, pale cream-tan to pale apricot stipe, watery, unchanging latex, mild odor, mild to slightly acid flavor and occurrence with *Salix* species in arctic-alpine areas.

Pileus 10–50 mm diam, 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, hollow. Latex scarce, watery, unchanging. Context of pileus and stipe dingy cream to brownish. Odor mild. Flavor mild to slightly acid. Basidiospore deposit not observed. Basidiospores 7–10.5(–12) × 5–8 µm, av. = 8.4 × 6.6 µm, $Q = 1.07$ –1.60, $\bar{Q} = 1.27$, hyaline in 2.5% KOH, subglobose to ellipsoid; ornamentation of amyloid 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 four-spored, some two-spored.

Pleuromacrocyttidia 53.3–96.5(–114.3) × 6.4–11.4 µm, lanceolate, with acute to moniliform apex, scattered to abundant. Cheilomacrocyttidia 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.

Distribution and habitat: *Lactarius nanus* originally was described by Jules Favre from the Swiss Alps with *Salix herbacea* L. (Favre 1955). Phylogenetic analyses including a specimen from near the type locality confirm this species in the Rocky Mountain alpine zone, where it is one of the most commonly encountered *Lactarius* species; it associates with *Salix arctica*, *S. reticulata* and *S. planifolia*. Overall *L. nanus* appears to be largely restricted to arctic-alpine areas with *Salix*.

Previous arctic-alpine reports include Canada (Ohe-noja and Ohe-noja 1993, 2010), the Rocky Mountain alpine zone (Moser and McKnight 1987, Cripps and Horak 2008, Cripps and Barge 2013), Greenland (Knudsen and Borgen 1982, Lamoure et al. 1982, Heilmann-Clausen et al. 1998, Borgen et al. 2006), Iceland (Eyjolfssdottir pers comm, Knudsen and Vesterholt 2008), the European Alps (Favre 1955; Kühner 1975; Kühner and Lamoure 1986; Senn-Irlet 1993; Bon 1985a, 1998; Heilmann-Clausen et al. 1998; Jamoni 1995), the Pyrenees (Bon and Ballará 1996), the Carpathians (Ronikier 2008), Fennoscandia (Kühner 1975, Gulden et al. 1985, Heilmann-Clausen et al. 1998, Gulden 2005, Knudsen and Vesterholt 2008), Svalbard (Gulden et al. 1985), Siberia (Nezdoiminogo 1997), and alpine areas in the Altai Mountains (Karatygin et al. 1999).

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, G). USA. 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); San Juan County, San Juan Range, 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); Park County, Beartooth Plateau, Solifluction Terraces, 11 Aug 2012, EB125 (MONT).

Other specimens examined: FINLAND. Koillismaa, 28 Aug 2011, JV28432 (*L. hyssiginoides*, TURA).

Comments: *Lactarius nanus* is morphologically and phylogenetically close to *L. hyssiginoides*; however, the latter has a more cinnamon-toned pileus, smaller basidiospores ($6\text{--}8 \times 5\text{--}7 \mu\text{m}$, av. $6.8 \times 5.7 \mu\text{m}$) and is generally reported from boreal areas with *Betula*, *Picea* and *Salix* in Europe (Heilmann-Clausen et al. 1998), although phylogenetic placement of environmental sequences extend its range into boreal Alaska (FIG. 4). Its range likely overlaps that of *L. nanus* in alpine-subalpine transition areas. It is not reported from the Rocky Mountains. *Lactarius mammosus* is a more robust species with a coconut odor (Heilmann-Clausen et al. 1998) that associates with members of the Pinaceae; it has not been reported from the Rocky Mountains. Differences between *L. glyciosmus* and *L. nanus* are discussed in the taxonomy section for *L. glyciosmus*.

Lactarius glyciosmus (Fr.) Fr. FIGS. 6, 7

Short description: *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, cream, pale pinkish or pale yellow-orange lamellae, buff or pale salmon stipe, watery, unchanging latex, odor of coconuts, mild flavor, and association with *Betula*.

Pileus 15–50 mm diam, 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 $\times$ 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. Flavor mild. Basidiospore deposit not observed. Basidiospores $7\text{--}9 \times 5\text{--}7 \mu\text{m}$, av. $= 7.7 \times 6.0 \mu\text{m}$, $Q = 1.17\text{--}1.42$, $\bar{Q} = 1.28$, hyaline in 2.5% KOH, broadly ellipsoid to ellipsoid; ornamentation of amyloid scattered ridges and warts forming an incomplete to nearly complete reticulum, to $0.5 \mu\text{m}$ high. Basidia $38.1\text{--}63.5 \times 7.6\text{--}10.2 \mu\text{m}$, mostly four-spored, some two-spored. Pleuromacrocystidia $45.7\text{--}63.5 \times 6.4\text{--}7.6 \mu\text{m}$, subclavate to lanceolate, with rounded to occasionally mucronate apex, scarce to scattered. Cheilomacrocystidia $33\text{--}66 \times 5.1\text{--}8.9 \mu\text{m}$, cylindrical to subclavate, with rounded apex, scattered to abundant. Pileipellis a cutis with tufts of ascending hyphae.

Distribution and habitat: *Lactarius glyciosmus* was first described by Fries from a hemiboreal to boreal habitat

in the Femsjö region of Sweden and the species lacks a type specimen. Phylogenetic analyses including specimens from the type locality confirm this species in the central and southern Rocky Mountain alpine zone and suggest it has a broad ecological and geographical distribution confirmed from subalpine and arctic-alpine areas in Alaska, western Canada, Idaho, Wisconsin, New Hampshire, Svalbard and Fennoscandia. In subalpine and alpine areas of the Rocky Mountains *L. glyciosmus* associates with *Betula glandulosa*. Phylogenetic placement (FIG. 4) of ectomycorrhizal root tip sequences confirm *Betula nana* L. as a host in Alaska (GU998223, GU998695) and likely *B. occidentalis* Hook. in Idaho (JX198526, Bogar & Kennedy 2013). Elsewhere it is reported with *B. pubescens* Ehrh. (e.g. Elborne & Knudsen 1990) and *B. papyrifera* Marshall (e.g. Durall et al. 2006).

Previous reports from arctic-alpine areas include Canada (Ohenoja and Ohenoja 1993, 2010), the Rocky Mountain alpine zone (Cripps and Horak 2008, Cripps and Barge 2013), Greenland (Lange 1957, Knudsen and Borgen 1982, Lamoure et al. 1982, Elbourne and Knudsen 1990, Borgen 2006, Borgen et al. 2006), Iceland (Eyjolfsson pers comm), the Alps (Bon 1998), Fennoscandia (Kallio and Kankainen 1964, Kühner 1975, Bon 1998), Svalbard (Ohenoja 1971, Geml et al. 2012) and Siberia (Karatygin et al. 1999). At least some reports might be *L. aff. glyciosmus* as defined here.

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). USA. COLORADO: Boulder County, Front Range, Blue Lake, 2 Aug 2001 CLC 1624 (MONT); Boulder County, Front Range, Brainard Lake, 13 Aug 2000 20923 (DBG); Ouray County, 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).

Other specimens examined: ESTONIA. Saare County, Lümända Commune, 17 Sep 2012, TU118535 (*L. aff. glyciosmus*, TU); Tartu County, Võnnu Commune, 12 Sept 2010, TU106737 (*L. aff. glyciosmus*, TU); 13 Sept 2010, TU106724 (*L. aff. glyciosmus*, TU).

Comments: *Lactarius glyciosmus* is morphologically and phylogenetically similar to *L. mammosus*; however, the latter has a browner pileus, stouter stature and more elongate, more reticulate basidiospores (Heilmann-Clausen et al. 1998). Phylogenetic placement of sequences isolated from ectomycorrhizal root tips of *Pinus sylvestris* L. (AJ633116, Heinonsalo et al. 2007) and *Picea abies* (L.) Karst. (FM992937, Kjølner and Clemmensen 2009) (FIG. 4) confirm that *L. mammosus*

FIG. 7. Comparison of micromorphological features of Rocky Mountain alpine specimens and Holotypes (where available). Basidiospores, pleuromacrocytidia (left) and cheilomacrocytidia (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 & Cripps sp. nov. (EB0041, HOLOTYPE), F. *L. pseudourvidus* (Va 71971, HOLOTYPE), G. *L. salicis-reticulatae* (CLC2776), H. *L. salicis-reticulatae*.

associates with members of the Pinaceae; however, it also has been reported with *Betula* mainly in subalpine areas (Heilmann-Clausen et al. 1998). *Lactarius vietus* (Fr.) Fr. is also similar but has a distinct pale zone near the stipe apex, latex that dries greenish gray, longer pleuromacrocystidia and a mild to slightly fruity odor (Heilmann-Clausen et al. 1998). It 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 and Smith 1979). The similar-looking *L. nanus* also occurs in alpine areas of the Rocky Mountains and has larger basidiospores, larger and differently shaped macrocystidia, lacks an odor of coconut, and associates with *Salix*. *Lactarius spinosulus* is phylogenetically close to *L. glyciosmus* (as well as *L. nanus*) (FIG. 4); however, morphologically they are very different. *Lactarius spinosulus* has a distinctly zoned, coarsely squamulose, pinkish, salmon- or brick-colored pileus with a finely hairy margin and fruity odor (Heilmann-Clausen et al. 1998); it has not been reported from North America. No definitive morphological differences were detected between *L. glyciosmus* and *L. aff. glyciosmus*, and the latter is not reported from the Rocky Mountain alpine zone.

Lactarius pallidomarginatus Barge & C.L. Cripps, sp. nov. FIGS. 6, 7
Mycobank MB812246

Typification: USA. COLORADO: San Juan County, San Juan Range, Stony Pass, scattered, solitary on soil under *Salix planifolia*, 13 Aug 2011, *EB0041* (**holotype**, MONT). GenBank: KR090940 (ITS) and KR091010 (*RPB2*).

Etymology: Referring to the pale pileus margin.

Diagnosis: *Lactarius pallidomarginatus* is recognized by its small to medium size, blotchy light tan to light brown to gray-brown pileus, which is typically lighter toward the margin, white to pale yellow-cream lamellae, pale cream stipe, violet staining tissue, mild odor and flavor, and association with *Salix*. Morphologically it is close to *L. pseudoviduus* Kühner but differs by having smaller basidiospores with an ornamentation of thinner ridges as well as macrocystidia that become more lanceolate (as opposed to subfusiform) and attenuated. Phylogenetically it is close to *L. aspideus* (Fr.) Fr. but differs consistently in two nucleotide positions in the rDNA ITS locus and at eight nucleotide positions in the *RPB2* locus; morphologically *L. aspideus* has larger, yellow basidiomata.

Pileus 20–50 mm diam, 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 light brown to gray-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. Flavor mild. Basidiospore deposit not observed. Basidiospores 8–10 × 6.5–8 µm, av. = 8.7 × 7.1 µm, Q = 1.14–1.36, $\bar{Q}$ = 1.23, hyaline in 2.5% KOH, broadly ellipsoid to ellipsoid; ornamentation amyloid, 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, four-spored. Pleuromacrocystidia 81.3–111.8 × 8.9–10.2 µm, cylindrical to lanceolate, with acute to moniliform apex, scarce to scattered. Cheilomacrocystidia 48.3–101.6 × 7.6–12.7 µm, cylindrical to lanceolate, with acute to moniliform apex, scattered. Pileipellis an (ixo-)cutis with some ascending hyphae embedded in a faint gelatinous layer.

Distribution and habitat: Rare in low alpine areas with *Salix planifolia*, reported from Independence Pass in the Sawatch Range and Stony Pass in the San Juan Range in Colorado. An additional collection (ZT5229) from Union Peak in the Wind River Range of Wyoming with *Salix glauca* is likely conspecific, however, this hypothesis has not been confirmed with molecular data. Due to confusion with *L. pseudoviduus* it is possible that *L. pallidomarginatus* is more widespread than confirmed here.

Specimens examined: USA. 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* (Holotype, MONT). WYOMING: Sublette County, Wind River Range, Union Peak, 22 Aug 1994, *ZT5229* (MONT).

Other specimens examined: AUSTRIA. Tyrol, 3 July 2007, *U. Peintner 20070035* (*L. aff. pseudoviduus* from near type locality, IB). FINLAND. Inarin Lappi, 16 Aug 1995, *JV10468* (*L. aff. pseudoviduus*, TURA); Koillismaa, *JV28448F*, 30 Aug 2011 (*L. aff. pseudoviduus*, originally identified as *L. brunneoviolaceus*, TURA). FRANCE. Isère, 13 Aug 1971, *Va 7 1971* (Holotype of *L. pseudoviduus*, G). ITALY. Trentino, 20 Aug 2004, *U.*

←

(K72-104, HOLOTYPE, no cheilocystidia drawn), *I. L. glyciosmus* (TWO269), *J. L. repraesentaneus* (CLC1747). Bars = 10 µm for basidiospores and 20 µm for cystidia. Drawings by E. Barge.

Peintner 20040156 (*L. aff. pseudovidus* from near type locality, originally identified as *L. robertianus*, IB). NORWAY. Finse, 10 Aug 2005, CLC 2133 (*Lactarius aff. brunneoviolaceus*, MONT); Stordal, 26 Aug 2006, P. Larsen 361395 (*L. aff. brunneoviolaceus*, originally identified as *L. pseudovidus*, O); Svalbard, 18 Aug 2002, TWO809 (*L. aff. pseudovidus*, MONT); 20 Aug 2002, CLC 1910 (*Lactarius* sp. 5, MONT); Ulvik, 11 Aug 2011, E. Søyland 73867 (*L. aff. pseudovidus*, O). SWEDEN: Lamjajaure, 4 Aug 2011, EL101-11 (*L. aff. pseudovidus*, GB); Latnjavagge, 17 Aug 2010, EL63-10 (*L. aff. pseudovidus*, GB).

Comments: Morphological features of *L. pallidomarginatus* fit the holotype description of *L. pseudovidus* and both are described as lighter near the pileus margin (Kühner 1975, Basso 1999); however, basidiospores of *L. pallidomarginatus* are slightly smaller ($8\text{--}10 \times 6.5\text{--}8 \mu\text{m}$ as opposed to $7.5\text{--}12 \times 6.5\text{--}8(8.5) \mu\text{m}$) and have narrower ridges that form a less dense reticulum; and macrocystidia take on a more lanceolate form with greater attenuation of the apex (FIG. 7). In phylogenetic analyses exsiccati identified as *L. pseudovidus* from Europe (including specimens from near the type locality) appear as distinct from Rocky Mountain alpine material and may represent a species complex. *Lactarius* sp. 3 (CLC1910), collected in Svalbard, has darker, more vinaceous pilei than *L. pallidomarginatus* and may represent another undescribed species, although only one collection was morphologically examined. Members of the *L. aff. brunneoviolaceus* clade (FIG. 5) that were examined, differ from *L. pallidomarginatus* in having darker, more vinaceous pilei, larger, more elongate basidiospores (to $13 \mu\text{m}$), and green staining of exsiccati in 2.5% KOH, a feature not mentioned previously for *L. brunneoviolaceus*, but which could be of taxonomic interest. Based on taxa included in the phylogenetic analyses (FIG. 5) *Lactarius aspideus* appears closely related to *L. pallidomarginatus*; however, the former has more yellow coloration and typically larger sporocarps (pileus 10–70 mm diam) and smaller basidiospores ($6.7\text{--}9.5 \times 5.6\text{--}7.8 \mu\text{m}$); traditionally *L. aspideus* has been placed with other yellow, violet-staining taxa such as *L. flavidus* Boud., *L. salicis-reticulatae* and *L. salicis-herbaceae* (Heilmann-Clausen et al. 1998), but it is separated here.

Lactarius montanus, another violet-staining species originally described from the Rocky Mountains, differs from *L. pallidomarginatus* by its larger stature, darker grayish vinaceous to vinaceous-brown pileus and green staining flesh in 2.5% KOH; it is typically found in subalpine habitats with conifers but also occurs in the krummholz zone. The violet-staining *Lactarius pallescens* Hesler & A.H. Sm. occurs in the Rocky Mountains, but its sporocarps are larger and lighter in color, and it is reported from subalpine conifer forests (Hesler and Smith 1979). Other

gray-brown to brownish violet-staining species such as *L. luridus*, *L. uvidus* (Fr.) Fr., *L. violascens* (J. Otto) Fr., *L. pallescens* v. *palmerensis* Hesler & A.H. Sm. and *L. cordovaensis* Hesler & A.H. Sm. are also larger and have a subalpine ecology; none have been reported from the central or southern Rocky Mountains (Hesler and Smith 1979, Heilmann-Clausen et al. 1998). *Lactarius cistophilus* Bon & Trimbach typically has larger basidiomata, larger basidiospores ($10\text{--}14 \times 7.2\text{--}9 \mu\text{m}$), and occurs under *Cistus* in the Mediterranean region (Basso 1999). The small, pale gray-brown, European alpine taxon *L. uvidus* f. *alpigenes* Jamoni & Bon has microscopic features that match those of *L. uvidus* (Jamoni and Bon 1991) but not *L. pallidomarginatus*. The small, non-violet staining *L. nanus* has basidiospores with thicker, more jagged ridges, and differently shaped macrocystidia (often with more rounded apices) (FIG. 7).

Lactarius repraesentaneus Britzelm.

FIGS. 6, 7

Short description: *Lactarius repraesentaneus* is recognized by its medium to large size, orange-brown to yellow-brown, hairy pileus with a heavily bearded margin, cream to pale-yellow lamellae, stout, cream to pale-yellow, often pitted stipe, violet staining latex and tissue, spicy-floral odor, slightly acrid flavor, and association with *Betula*, *Picea* and possibly shrubby *Salix* species such as *S. glauca*.

Pileus 60–100 mm diam, 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 incurved, remaining so or becoming nearly straight when mature. Lamellae adnate to decurrent, crowded, cream to pale-yellow, staining violet where damaged. Stipe 30–70 $\times$ 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. Context of pileus and stipe white, staining violet where damaged. Odor spicy-floral. Flavor slightly acrid. Basidiospore deposit not observed. Basidiospores $8\text{--}10.5 \times 6\text{--}8.5 \mu\text{m}$, av. = $9.3 \times 7.3 \mu\text{m}$, $Q = 1.13\text{--}1.43$, $\bar{Q} = 1.27$, hyaline in 2.5% KOH, broadly ellipsoid to ellipsoid; ornamentation amyloid, of narrow to broad ridges and isolated warts forming an incomplete to dense reticulum, to $1 \mu\text{m}$ high. Basidia $61.0\text{--}76.2 \times 10.2\text{--}11.4 \mu\text{m}$, four-spored. Pleuromacrocystidia $78.7\text{--}144.8 \times 8.9\text{--}12.7 \mu\text{m}$, subfusiform to lanceolate, thin-walled, with acute to moniliform apex, scattered to abundant.

Cheilomacrocyttidia $55.9\text{--}139.7 \times 7.6\text{--}12.7 \mu\text{m}$, subfusiform to lanceolate, thin-walled, with acute to moniliform apex, scattered to abundant. Pileipellis an ixotrichoderm with well-developed gelatinous layer.

Distribution and habitat: *Lactarius repraesentaneus* first was described from Germany (Britzelmayer 1885). Phylogenetic analyses including material from Germany confirm *L. repraesentaneus* from alpine areas of the Rocky Mountains and overall reinforce the traditional concept that this species has a broad ecological and geographic range in arctic-alpine and subalpine habitats in North America, northern, central and southern Europe. In alpine areas of the Rocky Mountains it associates with *Salix glauca*; however, krummholz *Picea* also is typically (maybe always) present; it is reported with *Picea* and *Betula* elsewhere.

Previous reports from arctic-alpine areas, mainly with *Betula*, include Alaska (Laursen and Ammirati 1982, Geml et al. 2009), the Rocky Mountain alpine zone (Cripps and Horak 2008, Cripps and Barge 2013), Greenland (Knudsen and Borgen 1982, Watling 1983, Elbourne and Knudsen 1990, Borgen 2006, Borgen et al. 2006), Iceland (Eyjolfssdottir pers comm), Fennoscandia (Kallio and Kankainen 1964, Kühner 1975, Bon 1998), Siberia (Karatygin et al. 1999) and the Altai Mountains (Gorbunova 2010).

Specimens examined: FINLAND. Enontakiö, 16 Aug 2004, JV21671 (TURA); Varsinais-Suomi, 9 Aug 1998, JV13837F (TURA). SWEDEN. Latnjavagge, 12 Aug 2007, EL92-07 (GB). USA. 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).

Other specimens examined: SWEDEN. Latnjavagge, 14 Aug 2010, EL57-10 (*L. dryadophilus*, GB). USA. 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 a large pale yellow-brown to orange-brown pileus with a bearded margin and violet staining latex clearly separates *L. repraesentaneus* from other *Lactarius* species of the central and southern Rocky Mountain alpine zone. Phylogenetically close, the arctic-alpine taxon *L. dryadophilus* is morphologically similar but has a cream to yellow, smoother pileus with hairs restricted to near the margin, basidiospore ornamentation consisting of sparse, thinner ridges, and ecological associations with *Dryas* and *Salix*. It has not been reported from the Rocky Mountains.

Lactarius salicis-reticulatae Kühner

FIGS. 6, 7

Short description: *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, cream to pale yellow stipe, violet staining latex and tissue, mild to slightly sweet odor, mild flavor and association with dwarf (*S. reticulata*) and possibly also shrubby *Salix* species.

Pileus 20–40 mm diam, 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 $\times$ 10–15 mm, equal to clavate, central, dry to viscid, cream to pale-yellow, staining violet where damaged, hollow. Latex scarce, watery-white, becoming violet. Context of pileus and stipe white, staining violet where damaged. Odor mild to slightly sweet. Flavor mild. Basidiospore deposit not observed. Basidiospores $(7\text{--})8.5\text{--}11.5 \times (7\text{--})8\text{--}10 \mu\text{m}$, av. = $10.0 \times 8.8 \mu\text{m}$, $Q = (1.00\text{--})1.13\text{--}1.43$, $\bar{Q} = 1.25$, hyaline in 2.5% KOH, subglobose to ellipsoid; ornamentation amyloid, of narrow ridges and a few isolated warts forming an incomplete reticulum, to $0.3 \mu\text{m}$ high. Basidia $73.7\text{--}88.9 \times 10.2\text{--}12.7 \mu\text{m}$, four-spored. Pleuromacrocyttidia $76.2\text{--}101.6 \times 7.6\text{--}11.4 \mu\text{m}$, subfusiform to fusiform, thin-walled, with rounded to acute to moniliform apex, scarce. Cheilomacrocyttidia $68.6\text{--}91.4 \times 7.6\text{--}10.2 \mu\text{m}$, subfusiform to fusiform, thin-walled, with acute to rounded to moniliform apex, scattered to abundant. Pileipellis an ixocutis.

Distribution and habitat: *Lactarius salicis-reticulatae* first was described from alpine tundra with *Salix* on Mt. Låktatjåkka, Sweden (Kühner 1975). Phylogenetic analyses including material from the type locality confirm this species from alpine areas on the Beartooth Plateau with *Salix reticulata* and shrubby *Salix* spp. and in arctic-alpine areas of Alaska, Svalbard and Finland. Overall *L. salicis-reticulatae* appears largely confined to arctic-alpine habitats. Colorado collections with *S. reticulata* and *S. planifolia* may represent a distinct taxon, although support is low.

Previous arctic-alpine reports include Greenland (Lamoure et al. 1982, Heilmann-Clausen et al. 1998, Borgen et al. 2006), the Rocky Mountain alpine zone (Cripps and Horak 2008, Cripps and Barge 2013), the Alps (Kühner 1975, Kühner and Lamoure 1986, Jamoni 1995, 2008, Bon 1998, Heilmann-Clausen et al. 1998), the Pyrenees (Ballará 1997), Fennoscandia (Kühner 1975, Heilmann-Clausen et al. 1998, Gulden 2005, Knudsen and

Vesterholt 2008) and Siberia (Nezdoiminogo 1997, Karatygin et al. 1999); previous reports of *L. aspidioides* Burl. from arctic-alpine areas in Alaska (Laurson and Ammirati 1982), Greenland (Knudsen and Borgen 1982) and the British Isles (Watling 1987) may refer to this species.

Specimens examined: FINLAND. Near Utsjoki, 28 Aug 2012, CLC 2885 (MONT). SWEDEN. Mt. Låktatjåkka, 12 Aug 1972, K72-104 (Holotype, G); 15 Aug 1999, JV15133 (Type locality, TURA). USA. 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); Carbon County, Beartooth Plateau, Highline Trail, 8 Aug 1998, CLC 1211 (MONT). WYOMING: Park County, Beartooth Plateau, Gardner Lake, 16 Aug 2014, EB0057-14 (MONT).

Other specimens examined: FINLAND. Koillismaa, 31 Aug 2005, JV23334 (*L. flavopalustris*, TURA). GREENLAND. Sisimiut, 19 Aug 2000, CLC 1536 (*L. salicis-herbaceae*, MONT). USA. COLORADO: Pitkin County, Sawatch Range, Independence Pass, 13 Aug 2001, CLC 1741 (*L. aff. salicis-reticulatae*, MONT); San Juan County, San Juan Range, Cinnamon Pass, 10 Aug 2001, CLC 1710 (*L. aff. salicis-reticulatae*, MONT); San Juan County, San Juan Range, Maggie Gulch, 11 Aug 2011, EB0036 (*L. aff. salicis-reticulatae*, MONT); San Juan County, San Juan Range, Stony Pass, 9 Aug 2001, CLC 1689 (*L. aff. salicis-reticulatae*, MONT); 13 Aug 2011, EB0039 (*L. aff. salicis-reticulatae*, MONT). MICHIGAN: Emmet County, 10 Aug 1973, RL Shaffer 6957 (*L. aspidioides*, MICH).

Comments: Some taxonomic confusion has been caused by the fact that Kühner (1975) originally named this species *L. aspidioides* Kühner but subsequently renamed it because the name had been previously applied; *L. aspidioides* Burl. (1907) is generally larger, with a somewhat zonate pileus, more crowded lamellae that lack salmon-colored hues, slightly smaller basidiospores with broader ridges, a bitter to slightly acrid flavor, and a subalpine ecology apparently in eastern and western North America (pers obs of RL Shaffer 6957, Hesler and Smith 1979). *Lactarius flavopalustris* is also similar; however, it has a larger, more brightly yellow pileus with faint zonation, more crowded lamellae, a weakly scrobiculate stipe (sometimes), smaller basidiospores with thicker ridges, and a subalpine ecology (Kytövuori 2009). Phylogenetic analyses (FIG. 5) reveal three collections of *L. flavopalustris* (two from near the type locality in Finland) nested in a well-supported clade with *L. aspidioides* from a subalpine forest in northern Michigan, suggesting the possible conspecificity of these taxa. Neither is reported from the Rocky Mountains. Two other collections identified as *L. flavopalustris* (from Estonia) were resolved as distinct from the *L. aspidioides/flavopalustris* clade (FIG. 5); their identity is unclear.

Lactarius aspidioides is differentiated from *L. salicis-reticulatae* by its smaller basidiospores with denser reticulation, more crowded, creamier-colored lamellae, and habitat mainly in subalpine areas (Heilmann-Clausen et al. 1998), although an environmental sequence isolated from an *S. polaris* root tip from arctic Svalbard clusters with *L. aspidioides* in phylogenetic analyses (FIG. 5). *Lactarius dryadophilus* is often larger with a cream-colored pileus that has a tomentose to bearded margin (Heilmann-Clausen et al. 1998). *Lactarius flavidus* is larger with a sparsely zonate pileus, more crowded, cream-colored lamellae, darker violet-staining context, basidiospores with more prominent ornamentation and a distally amyloid plage and subalpine ecology (Heilmann-Clausen et al. 1998). *Lactarius flavoaspidioides* Kytöv. has slightly smaller basidiospores on average with a denser reticulum and subalpine ecology with *Salix* (Kytövuori 2009); it has been reported only from northern Europe. *Lactarius salicis-herbaceae*, a similar arctic-alpine *Salix* associate, has smaller (typically), darker yellow to yellow-brown sporocarps, pale cream to grayish buff lamellae and more densely reticulate basidiospores (pers obs Heilmann-Clausen et al. 1998). It is fairly widespread in arctic-alpine areas but is not reported from alpine areas in the central and southern Rocky Mountains. *Lactarius* aff. *salicis-reticulatae* from Colorado may be a distinct taxon, and sporocarps can obtain a larger size (pileus to 90 mm diam) but otherwise morphological features strongly overlap.

KEY TO ROCKY MOUNTAIN ALPINE *LACTARIUS* SPECIES

1. Latex white, turning violet to dull lilac or flesh staining violet to dull lilac2
1. Latex watery to white, unchanging and flesh not staining violet to dull lilac4
2. Fruiting body robust; pileus 60–100 mm diam, yellow-brown to orange-brown, hairy, especially near margin; lamellae cream to pale-yellow; stipe stout, cream to pale-yellow, often with numerous yellow-brown pits; odor spicy-floral; flavor mildly acrid; basidiospores 8–10.5 × 6–8.5 µm; 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. repaerentaneus*
2. Fruiting body smaller, 20–50 mm diam; pileus and margin smooth3
3. Pileus pale yellow to pale ocher; lamellae cream, pale yellow, or pale orange-ocher; stipe cream to pale yellow; odor mild to slightly sweet; flavor mild; basidiospores 8.5–11.5 × 8–10 µm; in Rocky Mountain alpine zone and other arctic-alpine areas in North America and Eurasia with *Salix* *L. salicis-reticulatae*
3. Pileus blotchy light tan to light brown to gray-brown; margin lighter; lamellae white to pale yellow-cream;

stipe white to cream; odor mild, flavor mild; basidiospores $8\text{--}10 \times 6.5\text{--}8 \mu\text{m}$; in low alpine areas in Colorado with *Salix planifolia* and possibly Wyoming with *Salix glauca* *L. pallidomarginatus*

4. Pileus bright orange to orange-brown; 10–45 mm diam; margin smooth; lamellae pale cream to pale orange; stipe pale orange; odor mild; flavor mild; in Rocky Mountain alpine zone, arctic-alpine Alaska (possibly also subalpine) to Svalbard with *Salix* *L. lanceolatus*
4. Pileus pinkish buff, gray-brown, brown or mauve 5

5. With odor of coconuts; pileus 15–50 mm diam, pale pinkish buff to pale gray-brown, with glaucous coating; lamellae cream to pale yellow-orange; stipe buff or pale salmon; flavor mild; basidiospores $7\text{--}9 \times 5\text{--}7 \mu\text{m}$; in Rocky Mountain alpine zone and subalpine/arctic-alpine areas in North America and Eurasia with *Betula* *L. glyciosmus*
5. Without odor of coconuts; pileus 10–50 mm diam, gray-brown to liver-brown, with whitish glaucous coating; lamellae cream to light tan, sometimes with pinkish tinge, discoloring to dingy tan where damaged. Stipe pale apricot to dingy cream-tan; flavor mild to slightly acrid; basidiospores $7\text{--}10.5 \times 5\text{--}8 \mu\text{m}$; in Rocky Mountain alpine zone and arctic-alpine areas in Eurasia and likely other arctic-alpine areas in North America with *Salix* *L. nanus*

DISCUSSION

The number of *Lactarius* species reported from the Rocky Mountain alpine zone represents only a fraction of the total number of *Lactarius* species reported elsewhere from arctic-alpine areas; however, nearly half of the core arctic-alpine species are now confirmed for 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. pseudoviduus*, *L. salicis-herbaceae* and *L. salicis-reticulatae* (Knudsen and Borgen 1982, Heilmann-Clausen et al. 1998, Basso 1999, Knudsen and Vesterholt 2008). Phylogenetic analyses and morphological examination confirm three out of seven of these species for the Rocky Mountain alpine zone (*L. lanceolatus*, *L. nanus*, *L. salicis-reticulatae*) and support the hypothesis that these species are largely restricted to arctic-alpine areas with *Salix*. One definitive new species, *L. pallidomarginatus* (with *Salix*), was revealed along with a possible cryptic species, *L. aff. salicis-reticulatae* (also with *Salix*). Many *Lactarius* species previously reported from arctic-alpine areas are also commonly reported from subalpine areas with Betulaceae and/or Pinaceae

as well as with other plant families (Heilmann-Clausen et al. 1998). From this set of species we report: *L. glyciosmus*, which associates with *Betula glandulosa* in the Rocky Mountain alpine zone and *Betula* spp. across the rest of its distribution, and *L. repraesentaneus*, which is commonly reported with *Betula* and *Picea* (Hesler and 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.

Several taxa of interest were not molecularly distinct. In dataset A *L. lanceolatus* did not form a monophyletic group but was paraphyletic with respect to *Lactarius* sp. 2, the *L. luculentus* group and *L. aurantiacus*. In dataset B, *L. nanus* and *L. hyssiginoides* were not clearly distinct. In dataset C *L. salicis-reticulatae* did not form a clear monophyletic group and *L. pseudoviduus*, *L. brunneoviolaceus* and *L. luridus* were not clearly distinguished from each other, especially at the *RPB2* locus; poor molecular resolution also has been encountered for the latter three taxa by Das et al. (2015).

Overall there was little genetic variation distinguishing closely related species in this study. These findings are similar to reports from other researchers who suggest that there is, in general, relatively little molecular variation yet high morphological variation in genus *Lactarius* (Verbeken and Nuytinck 2013). While many of the species could be morphologically distinguished, all of the Rocky Mountain alpine taxa delineated in this study are 97% or more similar at the ITS region to one or more species. For example, *L. repraesentaneus* and *L. dryadophilus* are as much as 99.4% similar; *L. salicis-reticulatae* is approximately 98% similar to *L. salicis-herbaceae* and *L. flavopalustris*; *L. pallidomarginatus* is approximately 97–98% similar to *L. aff. brunneoviolaceus*, *L. luridus*, *L. montanus* and members of the *L. aff. pseudoviduus* group and approximately 99% similar to *L. aspideus*; *L. glyciosmus* is approximately 98% similar to *L. mammosus*; *L. nanus* is approximately 98% similar to *L. hyssiginoides*; and *L. lanceolatus* is approximately 97% similar to *L. substriatus/subflammeus*, *L. aurantiacus*, *L. luculentus* v. *laetus* and *Lactarius* sp. 1 and 2 and approximately 98% similar to *L. luculentus*. This high degree of similarity could be problematic for environmental sequencing studies that use a strict 97% ITS region similarity cutoff for recognizing species (e.g. Geml et al. 2012; Timling et al. 2012, 2014). In addition, Tedersoo et al. (2014) suggested that the ITS region in the /sebacina lineage evolves more slowly in high latitude areas, which could be true for other genera. The *RPB2* locus proved better for differentiating some species, and overall phylogenetic analyses revealed several taxonomic issues that we were not able to resolve. A search for additional phylogenetically informative loci for *Lactarius* should be initiated, along with more species-level phylogenetic

studies to provide a high quality molecular database for ecological studies. Furthermore, improvement of methods for sequencing older material, such as type specimens, is critical.

Most of the Rocky Mountain alpine *Lactarius* species delineated in this study (except *L. pallidomarginatus* and *L. aff. salicis-reticulatae*, for which further study could reveal broader ranges) appear to have broad, intercontinental distributions in North America and Eurasia. The current distributions of these species likely were shaped by a number of biotic and abiotic factors that include host dispersal and distribution, climate, glaciation and geography. Because the genus *Lactarius* is obligately ectomycorrhizal it is likely that the distribution of each fungal species was shaped largely by the distribution of suitable host plants, many of which also have broad, intercontinental distributions. Long-distance dispersal is hypothesized to have played an important role in structuring present distribution patterns of arctic-alpine plants, although many appear clonal (Abbott and Brochmann 2003, Alsos et al. 2007) and ectomycorrhizal fungi (Geml et al. 2012, Timling et al. 2014). It also has been suggested that long-distance dispersal mechanisms have been selected for in arctic flora due to repeated glaciations and the need to track potential niches (Alsos et al. 2007). Pollen data from Blacktail Pond, Yellowstone National Park, suggest that montane, mid-elevations in the northern greater Yellowstone ecosystem (Montana, Wyoming) were occupied by alpine tundra (*Betula*, *Salix*, many herbaceous taxa) similar to that of the present Rocky Mountain alpine vegetation during the last glacial period (14 000–11 500 calibrated years before present) (Krause and Whitlock 2013). Alpine vegetation also appears to have dominated areas in the southern Rocky Mountains (Wyoming, Colorado) 17 500–12 600 calibrated years before present (Minckley 2014). Both studies suggest the presence of broad, low-elevation glacial refugia for arctic-alpine life in the Rocky Mountains along the glacial front and, along with this study, lend support to the idea that arctic-alpine *Lactarius* species (and many other ectomycorrhizal fungi) may have occupied tundra areas south of the icesheets during cooler weather during the last glacial maximum and then dispersed upward in elevation and northward as the glaciers and ectomycorrhizal host plants retreated. Broader ecological tolerances of taxa such as *L. glyciosmus* and *L. repraesentaneus*, which are not restricted to arctic-alpine habitats, likely let these species persist in tundra and non-tundra areas during the last glacial maximum as well as to track arctic-alpine vegetation as it moved upward in elevation and northward following the retreat of the icesheets. For 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 (Birks 2008), glacial and interglacial refugia (Billings 1974) and the Bering land bridge (Shapiro et al. 2004, Geml et al. 2006).

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