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Molecular phylogenetic biodiversity assessment of arctic and boreal ectomycorrhizal *Lactarius* Pers. (Russulales; Basidiomycota) in Alaska, based on soil and sporocarp DNA

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Abstract

Despite the critical roles fungi play in the functioning of ecosystems, especially as symbionts of plants and recyclers of organic matter, their biodiversity is poorly known in high-latitude regions. In this paper, we discuss the molecular diversity of one of the most diverse and abundant groups of ectomycorrhizal fungi: the genus *Lactarius* Pers. We analysed internal transcribed spacer rDNA sequences from both curated sporocarp collections and soil polymerase chain reaction clone libraries sampled in the arctic tundra and boreal forests of Alaska. Our genetic diversity assessment, based on various phylogenetic methods and operational taxonomic unit (OTU) delimitations, suggests that the genus *Lactarius* is diverse in Alaska, with at least 43 putative phylogroups, and 24 and 38 distinct OTUs based on 95% and 97% internal transcribed spacer sequence similarity, respectively. Some OTUs were identified to known species, while others were novel, previously unsequenced groups. Non-asymptotic species accumulation curves, the disparity between observed and estimated richness, and the high number of singleton OTUs indicated that many *Lactarius* species remain to be found and identified in Alaska. Many *Lactarius* taxa show strong habitat preference to one of the three major vegetation types in the sampled regions (arctic tundra, black spruce forests, and mixed birch-aspen-white spruce forests), as supported by statistical tests of UniFrac distances and principal coordinates analyses (PCoA). Together, our data robustly demonstrate great diversity and nonrandom ecological partitioning in an important boreal ectomycorrhizal genus within a relatively small geographical region. The observed diversity of *Lactarius* was much higher in either type of boreal forest than in the arctic tundra, supporting the widely recognized pattern of decreasing species richness with increasing latitude.

Keywords: Alaska, fungi, internal transcribed spacer region, ribosomal large subunit gene, soil microbes

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Introduction

Approximately 80 000 species of Eumycota (the true Fungi) have been described, but an often-cited estimate of their true diversity is 1.5 million species (Hawksworth 1991). In recent years, DNA-based studies of soil fungal communities have provided valuable insights into the biodiversity and ecology of fungi and provided evidence for an incredible amount of fungal diversity in soils that is still mostly unknown (Schadt *et al*. 2003; O'Brien *et al*. 2005; Lynch & Thorn 2006). The same is true, although to a much lesser extent, for fungi with known sporocarp morphology, where previously unknown, morphologically cryptic species are often detected when molecular tools are applied (e.g. Taylor *et al*. 2006, and the references therein). Perhaps more important than their diversity *per se* are the critical roles that fungi play in the functioning of ecosystems, especially

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as mutualists of plants (i.e. mycorrhizae) and recyclers of organic matter.

Due to their relative importance to the global climate system and position at the forefront of climate change, we have targeted arctic and boreal regions of Alaska for extensive surveys of fungal molecular biodiversity. Although critical for the functioning of ecosystems, fungi are particularly poorly known in high-latitude regions (Callaghan *et al*. 2004). With the exception of *Amanita muscaria* (Geml *et al*. 2006; Geml *et al*. 2008a) and the genus *Agaricus* (Geml *et al*. 2008b), present information on species distributions, relationships to taxa in other regions, within species genetic diversity, and phylogeographical origins of Alaskan fungi is scarce.

One of the most diverse and abundant groups of ectomycorrhizal fungi in the northern high latitudes is the genus *Lactarius* Pers. Based on their sheer abundance and wide distribution, they are presumed to have great ecological importance as mycorrhizal partners of all major tree and shrub genera in Alaska (e.g. *Picea, Betula, Populus, Salix*, etc.) (personal observation). *Lactarius*, a very diverse genus, is placed in the family Russulaceae, with approximately 350 described species worldwide (Kirk *et al*. 2001). Although the genus is closely related to the genus *Russula* Pers. and has traditionally been separated based on the extension of the lactiferous (milky fluid producing) system in the hymenium, former phylogenetic studies show *Lactarius* to be monophyletic (Shimono *et al*. 2004; Miller *et al*. 2006). Despite being one of the genera best-studied by fungal systematists in temperate Europe and North America (e.g. Nuytinck & Verbeken 2005; Miller *et al*. 2006; Nuytinck *et al*. 2006), diversity studies on sporocarps and ectomycorrhizal root tips, focusing on previously unsampled geographical areas, often report unidentified and/or previously unknown *Lactarius* taxa (Laursen & Ammirati 1982; Eberhardt & Verbeken 2004; Riviere *et al*. 2007).

We have analysed internal transcribed spacer (ITS) rDNA sequences generated in a high throughput fashion from both curated sporocarp collections and soil polymerase chain reaction (PCR) clone libraries to assess the phylogenetic diversity of *Lactarius* in Alaska. The herbarium collections were gathered from across Alaska over a 35-year period. Soil sampling for clone-library construction was carried out at various plots throughout the Bonanza Creek Long Term Ecological Research programme (BNZ LTER, www.lter.uaf.edu/) in Interior Alaska, representing characteristic types and successional stages of the boreal region, and along the Alaskan portion of the North American Arctic Transect (NAAT, Walker *et al*. 2004; Raynolds *et al*. 2008), spanning three arctic tundra subzones. Our results provide the first diversity assessment of *Lactarius* in Alaska, and reveal several clades that have not been documented previously. Also, our data indicate that the species composition of *Lactarius* communities differ significantly between major plant community types, while localities sharing similar vegetation did not statistically differ from each other regardless of geographical distance.

Materials and methods

The study regions

Interior Alaska. The Intermontane Boreal Forest ecoregion (Nowacki *et al*. 2001) of Interior Alaska is bordered by the Alaska Range to the south, the arctic tree line in the Brooks Range to the north and to climatic tree line in western Alaska. This ecoregion represents the westernmost end of the boreal belt spanning the North American continent. Interior Alaska is an area of discontinuous permafrost, with approximately 75–80% of the area underlain by permafrost, except on south-facing slopes (Osterkamp & Romanovsky 1999). The region has a continental climate with low annual precipitation (286 mm on average), extreme temperatures ranging from −60 to 35 °C, and snow covering the ground for 6–9 months of the year (Slaughter & Benson 1986; Hinzman *et al*. 2005). Despite the fact that most of Interior Alaska was not glaciated, there is little morphological development in the soils, most of them being Inceptisols, Entisols, Histosols, or Gelisols (Ahrens *et al*. 2004; Hollingsworth *et al*. 2006).

The area's forest vegetation consists of a mosaic of different forest types, formed predominantly as a result of slope, aspect, elevation, parent material and succession following disturbance (mostly fire and flooding). Forest types include various black spruce (*Picea mariana* [Mill] Britton, Sterns et Poggenburg) communities on permafrost-dominated north-facing slopes and lowlands, and mixed birch-aspen-white spruce (*Betula neoalaskana* Sarg., *Populus tremuloides* Michx., *Picea glauca* [Moench] Voss) forests on well-drained south-facing slopes (Viereck *et al*. 1992; Hollingsworth *et al*. 2006).

We collected soil samples at various sites monitored by the BNZ LTER (Table 1). Among these, the upland mixed birch-aspen-white spruce stands (indicated by UP numbers) are located in the Bonanza Creek Experimental Forest (BCEF), approximately 20 km southwest of Fairbanks, Alaska. The sampled black spruce types are broadly distributed among multiple sites, including the BCEF, the Caribou-Poker Creek Research Watershed (40 km northeast of Fairbanks), as well as areas in the vicinity of Delta Junction and Fairbanks, Alaska. Plant community and soil characteristics of these sites are described in detail by Hollingsworth *et al*. (2006). Sporocarps were collected from different forested and tundra regions of Alaska. The map of locations for these and other collections was published previously (Fig. 1 in Geml *et al*. 2008b).

Northern Alaska. The circumpolar Arctic is divided into five bioclimatic subzones (A–E, cold to warm). Each of

Table 1 Habitat, clone library names, plot numbers, and locations [with global positioning system (GPS) coordinates] for soil samples used in this study. For each habitat, three plots were sampled (50 soil cores per plot) and the extracted DNAs were pooled for PCR clone library constructions. The process was replicated two times: in 2004 and 2005

Habitat	Clone library (sampling year)	Plot number	Stand age (years)	Location (latitude, longitude)
Early successional upland mixed forest	UP1 (2004) and UP4 (2005)	UP1 A	23–25	Bonanza Creek LTER site, Parks Hwy. (64.73473541; -148.2976791)
		UP1 B	23–25	Bonanza Creek LTER site, Parks Hwy. (64.73195762; -148.2974016)
		UP1 C	23–25	Bonanza Creek LTER site, Parks Hwy. (64.73195762; -148.2974016)
Mid-successional upland mixed forest	UP2 (2004) and UP5 (2005)	UP2A	93–98	Bonanza Creek LTER site, Parks Hwy. (64.69390269; -148.3537914)
		UP2 B	93–98	Bonanza Creek LTER site, Parks Hwy. (64.68945831; -148.3599027)
		UP2 C	93–98	Bonanza Creek LTER site, Parks Hwy. (64.68862521; -148.3790685)
Late successional upland mixed forest	UP3 (2004) and UP6 (2005)	UP3A	225–230	Bonanza Creek LTER site, Parks Hwy. (64.7666796; -148.2740661)
		UP3 B	225–230	Bonanza Creek LTER site, Parks Hwy. (64.75973477; -148.2446238)
		UP3 C	225–230	Bonanza Creek LTER site, Parks Hwy. (64.72445795; -148.324901)
Black spruce, acidic, dry	TKN7 (2004) and TKN11 (2005)	TKN-0012	200–210	Washington Creek, Elliott Hwy. (65.16721667; -147.8941333)
		TKN-0122	90–100	Delta Junction, Alaskan Hwy. (63.90620584; -145.3711972)
		TKN-0001	95–100	Bonanza Creek LTER site, Parks Hwy. (64.76572442; -148.295527)
Black spruce, acidic, wet	TKN8 (2004) and TKN12 (2005)	TKN-0015	170–180	Washington Creek, Elliott Hwy. (65.15451667; -147.8631667)
		TKN-0022	150–180	Babe Creek, Elliott Hwy. (64.99653333; -147.65305)
		TKN-0109	90–104	Caribou Poker Creek Research Watershed, Steese Hwy. (65.16166012; -147.4878514)
Black spruce, non-acidic, dry	TKN9 (2004) and TKN13 (2005)	TKN-0039	130–190	Ballaine Rd., Fairbanks (64.91003773; -147.8218001)
		TKN-0123	97–100	Delta Junction, Alaskan Hwy. (63.92459496; -145.3167362)
		TKN-0126	120–130	Delta Junction, Alaskan Hwy. (63.84655372; -145.7208714)
Black spruce, non-acidic, wet	TKN10 (2004) and TKN14 (2005)	TKN-0051	68–91	UAF Arboretum, Fairbanks (64.86588323; -147.8736745)
		TKN-0119	280–320	Delta Junction, Alaskan Hwy. (63.81379184; -144.9532331)
		TKN-0040	179–216	Ballaine Rd., Fairbanks (64.9148493; -147.8297945)
Arctic tundra, subzone E tussock-sedge, erect dwarf shrubs	NA12 (2007)	HV-zonal	n/a	Happy Valley, Dalton Hwy. (69.1468611; -148.8480555)
Arctic tundra, subzone D sedges, prostrate dwarf shrubs, lichens	NA9 and NA10 (2007)	FB-zonal	n/a	Franklin Bluffs, Dalton Hwy. (69.6745833; -148.7211111)
Arctic tundra, subzone C bare soil, lichens, prostrate dwarf shrubs	NA10 and NA11 (2007)	HI-zonal	n/a	Howe Island, Beaufort Sea (70.3151667; -147.9936111)

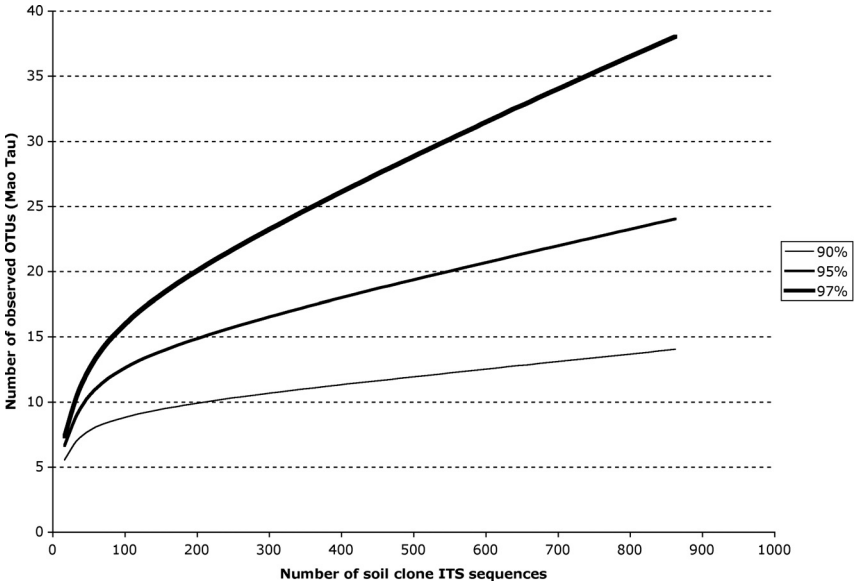


Fig. 1 Species accumulation curves for *Lactarius* OTUs from Interior Alaska based on 90%, 95%, and 97% ITS sequence similarity. The observed number of OTUs is given as Mao Tau estimates.

these subzones has its own climate and distinctive vegetation (Walker *et al*. 2005), and can be characterized using the Summer Warmth Index (SWI, sum of monthly mean air temperature > 0 °C). Subzone A (SWI < 6 °C) is sparsely vegetated, with no shrubs or sedges, with mosses and lichens as dominant groups. Subzone B (SWI 6–9 °C) is characterized by greater cover of plants, including prostrate dwarf-shrubs and sedges. Subzone C (SWI 9–12 °C) has mostly complete plant cover, and hemi-prostrate shrubs up to 15 cm tall commonly occur, especially in snowbed communities. Subzone D (SWI 12–20 °C) is almost completely vegetated, and dwarf shrubs up to 40 cm tall occur. Subzone E (SWI 20–35 °C) has very little bare ground. Shrubs are commonly 20–50 cm tall, and can reach up to 2 m in protected areas along streams (Raynolds *et al*. 2008). Of these five bioclimatic subzones, the southernmost three (C–E) can be found in Alaska. A more complete description of the typical vegetation found in each subzone can be found in Walker *et al*. (2005).

Soil sampling locations were chosen along the arctic bioclimatic gradient, including one location in each of the three arctic bioclimatic subzones represented in Alaska: Howe Island (Subzone C), Franklin Bluffs (Subzone D), and Happy Valley (Subzone E) (Table 1). At each location, soil samples were taken from the zonal vegetation of the area, defined as areas that were typical of the local vegetation, with fine-grained soils, and no extremes of moisture, slope, soil chemistry or disturbance (Raynolds *et al*. 2008).

Isolates and DNA extraction

We sampled three upland forest (UP) and four black spruce forest (B) subtypes: early successional (UP1), mid-successional (UP2), and late successional (UP3) upland forests and acidic, dry (BAD), acidic, wet (BAW), non-acidic, dry (BND), and non-acidic, wet (BNW) black spruce sites (Table 1). In each subtype, three replicate plots were sampled. In each plot, 50 soil cores, 1.8 cm in diameter and 10 to 20 cm deep, always containing both organic and mineral horizons, were taken along four parallel, 100 m transects in such a way that cores were at least 10 m from each other to minimize the probability of sampling the same genet repeatedly. Samples were separated into organic and mineral horizons. For every 50×100 m plot, the 50 cores sampled as described above were pooled for each horizon, which resulted in two separate DNA extractions per plot. Thus, 18 separate extractions were made for the upland sites and 24 for the black spruce sites. We replicated the entire process in the following year.

In each of three arctic tundra bioclimatic subzones, we sampled soils from five frost boils and adjacent interboil areas. Due to the high spatial heterogeneity of soil fungal communities, we collected 20 cores within each boil and interboil, which were then pooled and mixed, and frozen as quickly as possible following sampling. The arctic soil samples were not divided due to the lack of distinct organic and mineral horizons. DNAs were extracted separately for each frost boil and interboil, resulting in a total of 30 DNA extractions from the three arctic bioclimatic subzones. For this paper, sequences from frost boils and interboils were pooled, partly because of the low number of sequences identified as *Lactarius*, and partly because later papers will deal with total fungal community comparisons between frost boils and interboils.

Soils were deposited in 50 mL Falcon tubes, and stored at −80 °C until lyophilization. The soils were then ground

either on a ball mill at –20 °C, with 0.8 mm steel beads, or using a Vortex-2-Genie-Vortexer (Scientific Industries). Genomic DNA was extracted from 1–5 g of soil from each of the two composite samples using the Mo Bio Powersoil kit following the manufacturer's instructions. The soil DNA extracts were normalized to 2.5 ng/μL after Picogreen (Molecular Probes) quantification. Methods have been partially described in Taylor *et al*. (2007) and Taylor *et al*. (2008).

In addition, sporocarps of 383 specimens of *Lactarius* were collected and deposited in G. A. Laursen Mycological Herbarium (GAL) at the University of Alaska Fairbanks (UAF). To reduce redundancy, fifty-five of these, representing morphological groups and geographical areas of origin among the collections, were selected for molecular work (Table 2). DNA was extracted from small samples of dried specimens using the E–Z 96 Fungal DNA Kit (Omega Bio-tek, Inc.) or the DNeasy Plant Mini Kit (QIAGEN, Inc.).

PCR, DNA sequencing, and contig assembly

The entire ITS and partial LSU regions were PCR amplified in reaction mixtures containing 1.75 μL Ultrapure Water (Invitrogen), 1 μL 10× Herculase PCR buffer (Stratagene), 0.05 μL 100 mM dNTP mixture, 25 mM of each dNTP (Applied Biosystems), 0.2 μL Herculase DNA polymerase (Stratagene), 2 μL of 1 μM forward primer, ITS1F (Gardes & Bruns 1993) and reverse primer, TW13 (White *et al*. 1990), and 3 μL of template DNA at a concentration of 0.1 ng/μL. PCRs were performed using the following temperature programme: 95 °C/2 min, 25 or 34 cycles of 95 °C/0.5 min, 54 °C/1 min, 72 °C/2 min; and 72 °C/10 min. For each soil DNA extract, seven replicate PCRs were performed and pooled. To minimize the formation of chimeras, 25 PCR cycles were utilized for soil DNA extracts. We applied a molecular tagging strategy to mark PCR products from various sources with DNA tags, which can then be pooled before library sequencing (Taylor *et al*. 2008). Thus, the identities of many samples were preserved while processing a feasibly low number of clone libraries. Tagging was achieved through the addition of 10-base extensions at the 5′ end of the TW13 primer. We used a slightly elongated version of ITS1F in order to increase specificity and balance primer annealing temperature. The pooled PCR products were cloned into the pCR4-TOPO vector from Invitrogen. The resulting PCR libraries were shipped frozen to the Broad Institute of MIT and Harvard, where transformation, plating, colony picking, Templiphi reactions and sequencing were carried out on automated equipment, using M13 forward and reverse external vector primers and ITS4 and CTB6 (White *et al*. 1990) as internal sequencing primers (sporocarps only) to generate bidirectional coverage of the 1100–2000 bp inserts. We sequenced at least 1536 clones per library, which provides a more detailed characterization of fungal diversity and community composition than is publicly available for any biome. Sequence data obtained for both strands of each locus were edited and assembled for each isolate using CodonCode Aligner version 1.3.4 (CodonCode Inc.).

Delimitation of OTUs

The delimitation of OTUs based on an arbitrary cut-off value for pairwise sequence similarity has been widely used in diversity assessment studies (e.g. O'Brien *et al*. 2005; Lynch & Thorn 2006; Higgins *et al*. 2007). Generally, 90–97% ITS sequence similarity values have been used in previous papers as a proxy for species boundaries (e.g. O'Brien *et al*. 2005; Arnold & Lutzoni 2007; Higgins *et al*. 2007). Because the degree of inter- and intraspecific ITS divergence and mutation rate variability across fungal taxonomic groups are mostly unknown, we used 90%, 95% and 97% similarity values for OTU delimitations.

Soil clone sequences were identified to genera using FASTA (Pearson & Lipman 1988) similarity searches against an in-house reference database containing all fungal ITS sequences from GenBank (www.borealfungi.uaf.edu/). In total, 918 ITS sequences of Alaskan *Lactarius* species were obtained from soil and herbarium DNA extracts. The number of OTUs was estimated based on 863 soil clones. Herbarium sequences were excluded as they were not randomly sampled. Pairwise sequence similarity-based groupings of ITS sequences identified as *Lactarius* were estimated by Cap3 (Huang & Madan 1999). Note that Cap3 uses a single-linkage clustering algorithm, meaning that sequences less than, for example, 95% similar will be grouped together if an intermediate sequence greater than 95% similar to both is found. Species accumulation curves and bootstrap estimates of total richness were computed using EstimateS (version 7.5, R. K. Colwell, purl.oclc.org/estimates).

Phylogenetic analysis of Lactarius *sequences*

Of the total 918 *Lactarius* ITS sequences from soil clone libraries and herbarium specimens, a representative subset of 189 was chosen for more detailed phylogenetic analyses and was deposited in GenBank (EU711563–EU711723, FJ607365–FJ607394). In addition, because our goal was to conduct the most complete phylogenetic analyses possible, all (as of 2008 February) *Lactarius* ITS sequences were downloaded from GenBank, including unidentified environmental samples, from which we selected two to four representatives of every identified taxon, plus the unidentified sequences that were at least 600 bp long. Multiple sequence alignments were generated using ClustalW (Thompson *et al*. 1997). Phylogenetic analyses were conducted using maximum-likelihood (ML) and Bayesian methods in Garli

Table 2 Herbarium specimen number, macro-morphological identification, collecting location, and habitat of *Lactarius* sporocarps sequenced in this study

Specimen	Identification*	Location	Habitat†
GAL5424	*L.* cf. *citriolens*	Denali National Park, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL8739	*L.* cf. *nanus*	Nome, Western Alaska	Arctic tundra
GAL8876	*L.* sp.	Denali National Park, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL12566	*L.* cf. *deliciosus*	Kobuk National Park, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL12608	*L.* cf. *circellatus*	Kobuk National Park, Interior Alaska	Arctic tundra
GAL12636	*L.* cf. *deliciosus*	Kobuk National Park, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL13852	*L.* cf. *luculentus*	Dalton Hwy., Interior Alaska	n/a
GAL13967	*L. rufus*	Steese Hwy., Interior Alaska	n/a
GAL13975	*L. necator*	Steese Hwy., Interior Alaska	n/a
GAL14116	*L.* cf. *dryadophilus*	Nome, Western Alaska	Arctic tundra
GAL14139	*L.* cf. *dryadophilus*	Nome, Western Alaska	Arctic tundra
GAL14142	*L.* cf. *subcircellatus*	Nome, Western Alaska	Arctic tundra
GAL14169	*L.* cf. *subcircellatus*	Nome, Western Alaska	Arctic tundra
GAL14255	*L. trivialis*	Interior Alaska	n/a
GAL14289	*L.* sp.	Interior Alaska	n/a
GAL14498	*L.* cf. *torminosus*	Kobuk National Park, Interior Alaska	n/a
GAL14544	*L.* cf. *deterrimus*	Kobuk National Park, Interior Alaska	n/a
GAL14578	*L. trivialis*	Kobuk National Park, Interior Alaska	n/a
GAL14662	*L.* cf. *pubescens*	Toolik Lake LTER site, Northern Alaska	Arctic tundra
GAL14826	*L.* sp.	Toolik Lake LTER site, Northern Alaska	Arctic tundra
GAL14843	*L.* sp.	Toolik Lake LTER site, Northern Alaska	Arctic tundra
GAL14850	*L.* cf. *repraesentaneus*	Toolik Lake LTER site, Northern Alaska	Arctic tundra
GAL15053	*L.* cf. *repraesentaneus*	Nome, Western Alaska	Arctic tundra
GAL15195	*L.* cf. *lanceolatus*	Sitka, Southeastern Alaska	Maritime forest (Ps)
GAL15231	*L.* sp.	Sitka, Southeastern Alaska	Maritime forest (Ps)
GAL15287	*L.* sp.	Sitka, southeastern Alaska	Maritime forest (Ps)
GAL15392	*L.* sp.	Fairbanks, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL15684	*L. trivialis*	Bonanza Creek LTER site, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL15690	*L.* cf. *scrobiculatus*	Bonanza Creek LTER site, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL16724	*L.* cf. *deliciosus*	Fairbanks, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL16877	*L. pubescens*	Fairbanks, Interior Alaska	Lowland boreal forest (Pm, Ll)
GAL16885	*L.* cf. *deliciosus*	Fairbanks, Interior Alaska	Lowland boreal forest (Pm, Ll)
GAL17021	*L. repraesentaneus*	Denali National Park, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17024	*L.* cf. *scrobiculatus*	Denali National Park, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17049	*L.* cf. *scrobiculatus*	Denali National Park, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17071	*L.* cf. *deterrimus*	Anchorage, Southcentral Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17100	*L.* sp.	Anchorage, Southcentral Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17112	*L.* sp.	Anchorage, Southcentral Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17113	*L.* cf. *uvidus*	Anchorage, Southcentral Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17116	*L.* cf. *rufus*	Anchorage, Southcentral Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17121	*L.* cf. *scrobiculatus*	Anchorage, Southcentral Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17122	*L.* cf. *deterrimus*	Anchorage, Southcentral Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL17141	*L. torminosus*	Bonanza Creek LTER site, Interior Alaska	Lowland boreal forest (Pm, Ll)
GAL17144	*L.* cf. *deterrimus*	Bonanza Creek LTER site, Interior Alaska	Lowland boreal forest (Pm, Ll)
GAL17353	*L.* cf. *deliciosus*	Bonanza Creek LTER site, Interior Alaska	Lowland boreal forest (Pm, Ll)
GAL17359	*L.* sp.	Bonanza Creek LTER site, Interior Alaska	Lowland boreal forest (Pm, Ll)
GAL17749	*L. torminosus*	Fairbanks, Interior Alaska	Lowland boreal forest (Pm, Pg, Ll)
GAL17954	*L.* cf. *deliciosus*	Bonanza Creek LTER site, Interior Alaska	Floodplain boreal forest (Pb, Pg)
GAL17957	*L. repraesentaneus*	Bonanza Creek LTER site, Interior Alaska	Lowland boreal forest (Pm)
GAL17972	*L.* cf. *pubescens*	Bonanza Creek LTER site, Interior Alaska	n/a
GAL18054	*L.* cf. *resimus*	Fairbanks, Interior Alaska	Upland boreal forest (Pg, Pt, Bn)
GAL18572	*L.* sp.	Valdez, Southcentral Alaska	Maritime forest (Pb, Ps)
GAL18573	*L. repraesentaneus*	Valdez, Southcentral Alaska	Maritime forest (Pb, Ps)
GAL18580	*L.* sp.	Valdez, Southcentral Alaska	Maritime forest (Pb, Ps)
GAL18611	*L.* cf. *deterrimus*	Fairbanks, Interior Alaska	Lowland boreal forest (Pm, Ll)
GAL18818	*L. rufus*	Homer, Southcentral Alaska	Maritime forest (Ps)

*Species were field identified, thus the given names refer to morphological similarities, not necessarily to true identities.
†Abbreviations: Bn, *Betula neoalaskana*; Ll, *Larix laricina*; Pg, *Picea glauca*; Pm, *Picea mariana*; Ps, *Picea sitchensis*; Pb, *Populus balsamifera*; Pt, *Populus tremuloides*; n/a, not available.

0.94 (Zwickl 2006) and MrBayes (Huelsenbeck & Ronquist 2001), respectively. The best-fit evolutionary model was determined by comparing different evolutionary models with varying values of base frequencies, substitution types, α-parameter for the γ-distribution of variable sites, and proportion of invariable sites via hierarchical likelihood ratio tests using PAUP* (Swofford 2002) and ModelTest 3.06 (Posada & Crandall 1998). Gaps were scored as 'missing data'. To compare different tree topologies, two-tailed Shimodaira–Hasegawa tests (SHT) were used (Shimodaira & Hasegawa 1999). The High Performance Computing cluster maintained by the UAF Biotechnology Computing Research Group (http://biotech.inbre.alaska.edu/) was used for all analyses.

Comparing Lactarius *communities among vegetation types*

We used sequences generated in our study to test for statistical differences among assemblages of *Lactarius* species in different vegetation types in Alaska. The same alignment was used as above, except that sporocarps collected in maritime habitats in Southeast Alaska or with no specified information on vegetation type of collection site were excluded from the analyses. The alignment was subsequently corrected manually and was subject to ML analyses in Garli. The resulting tree was used to carry out unweighted UniFrac analyses (Lozupone & Knight 2005). UniFrac distance metric tests, *P*-tests (Martin 2002), and principal coordinates analyses (PCoA, Lozupone & Knight 2005) were calculated to assess differences among *Lactarius* communities of the sampled vegetation types. The UniFrac distance is calculated as the percentage of branch length leading to descendants from only one of the environments represented in the phylogenetic tree, and reflects differences in the phylogenetic lineages in one environment vs. the others. The *P*-test estimates similarity between communities as the number of parsimony changes (transitions from one environment to another on the tree) to explain the distribution of sequences between the different environments in the tree. PCoA is a multivariate statistical technique to find the most important axes along which the sequences vary. PCoA calculates the distance matrix for each pair of environments using the UniFrac metric. It then turns these distances into points in a space with n–1 dimensions, n being the number of samples.

Results

Delimitation of OTUs

We recovered 14, 24, and 38 distinct OTUs based on 90%, 95%, and 97% ITS sequence similarity, respectively (Table 3). Species accumulation curves remained non-asymptotic regardless of the degree of ITS sequence similarity used for OTU delimitation (Fig. 1). Bootstrap estimates of species richness significantly exceeded the observed species richness under the 97% OTU delimitation (Table 2). Under the 90% and 95% OTU definitions, the majority of ITS types occurred more than once, while the opposite was true for the 97% sequence similarity OTUs, where 55.26% of the OTUs were singletons.

Table 3 Species richness among 863 ITS sequences of *Lactarius* from soil DNA extracts, as a function of operational taxonomic units based on 90%, 95%, and 97% ITS sequence similarity.

Sequence similarity	S_{obs}	S_{obs} SD	Bootstrap (%)	Singletons N (percentage)
90%	14	2.40	15.83	5 (35.71)
95%	24	4.08	28.02	11 (45.83)
97%	38	5.3	45.93*	21 (55.26)

S_{obs} indicates observed species richness. S_{obs} SD indicates standard deviation based on 100 randomizations per sample. Bootstrap values indicate the estimate of total species richness. Bootstrap estimates marked by asterisk (*) are significantly greater than the observed species richness ($B > S_{obs} + 1$ SD).

Phylogenetic analysis

The ITS data set consisted of 376 sequences, including 189 newly generated sequences from Alaskan *Lactarius* species. There were 813 characters, including gaps, of which 387 were parsimony informative. The Hasegawa–Kishino–Yano model (Hasegawa *et al*. 1985), with calculated proportion of invariable sites ($I = 0.2099$) and estimated α-parameter ($\alpha = 0.5515$) of γ-distribution (HKY + I + G), was selected as the best-fit evolutionary model. ML analyses resulted in a single tree (–ln L = 16359.98216) (Fig. 2). The SHT revealed that the phylograms generated by ML and Bayesian methods were not significantly different from each other (values ranging from $P = 0.1574$ to $P = 0.5475$). Phylogenetic groups were identified as the smallest clades supported by Bayesian posterior probability ≥ 0.95 (Fig. 2). In some cases, phylogroups were paraphyletic with respect to other well-supported phylogroups.

We detected 43 phylogroups of Alaskan *Lactarius* taxa, which were widely distributed on the genus-wide tree and grouped with several major infrageneric groups (Fig. 2). In many cases, Alaskan sequences grouped in phylogroups together with previously published sequences with known identity, such as *Lactarius helvus* [Fr.] Fr. (phylogenetic group 1), *L. scrobiculatus* [Scop.] Fr. (2), *L. auriolla* Kytöv. (3), *L. alnicola* A.H. Sm. (7), *L. rufus* [Scop.] Fr. (8), *L. scoticus* Berk. et Broome (15), *L. torminosus* [Schaeff.] Gray and *L. torminosulus* Knudsen et T. Borgen (16), *L. aurantiosordidus* Nuytinck & S.L. Mill. and *L. chelidonium* Peck (20), *L. glyciosmus*

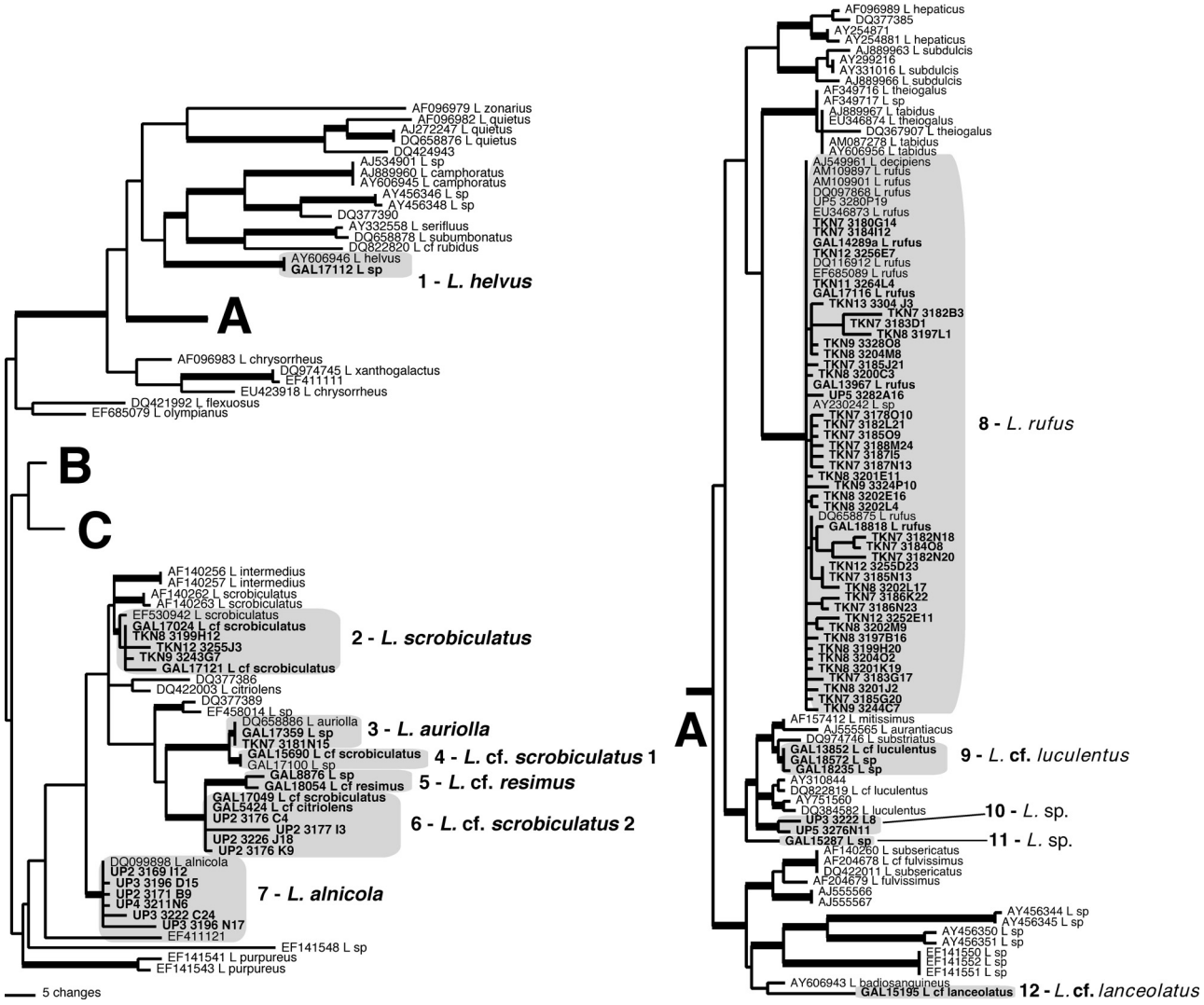


Fig. 2 The maximum-likelihood phylogram showing the phylogenetic breadth of arctic and boreal Alaskan *Lactarius* sequences (in bold) generated in this study among representatives of *Lactarius* taxa in GenBank. Sequences with GAL numbers were derived from herbarium specimens (for easier viewing, arctic and boreal specimens are labelled ArcGAL and GAL, respectively), while UP, TKN, and ArcNA sequences in bold are from soil clone libraries of upland boreal forest, lowland boreal forest, and arctic tundra, respectively. Branches with Bayesian posterior probability support ≥ 0.95 are thickened. Phylogroups including Alaskan sequences are indicated with grey boxes. GenBank sequences with no name attached are from unidentified environmental samples. Clades marked by 'A', 'B', and 'C' are shown in detail in expansions 2b, 2c, 2d, respectively.

[Fr.] Fr. (26), *L. trivialis* [Fr.] Fr. (29), *L. uvidus* [Fr.] Fr. (31), *L. repraesentaneus* Britzelm. (34), and *L. necator* [Bull.] Pers. (36). Groups 22 and 23 could not be identified to known species, but matched published, unidentified sequences in GenBank.

In other cases, some Alaskan sequences formed unique, unidentified clades, with or without known sister groups. Such singletons or groups were *L.* cf. *scrobiculatus* (4 and 6), *L.* cf. *resimus* (5), *L.* cf. *luculentus* Burl (9), *L.* cf. *lanceolatus* O.K. Mill. & Laursen (12), *L.* cf. *pubescens* [Fr.] Fr. (13 and 14), *L.* cf. *torminosus* (17), *L.* cf. *deliciosus* [L.] Gray (19, 22, 23, and 24), *L.* cf. *deterrimus* Gröger (21), *L.* cf. *circellatus* Fr. (30), *L.* cf. *subcircellatus* Kühner (35), and unidentified *L.* sp. (10, 11, 18, 25, 27, 28, 32, 33, 37, 38, 39, 40, 41, 42, and 43).

Partitioning of phylogenetic diversity among vegetation types

UniFrac analyses of soil clones revealed that the *Lactarius* communities significantly differed in phylogenetic constituency among the three major types: arctic tundra, black spruce forests and mixed birch-aspen-white spruce forests. For the entire data set, taxa significantly clustered according to vegetation types (UniFrac metric: $P < 0.01$; P-test: $P < 0.01$).

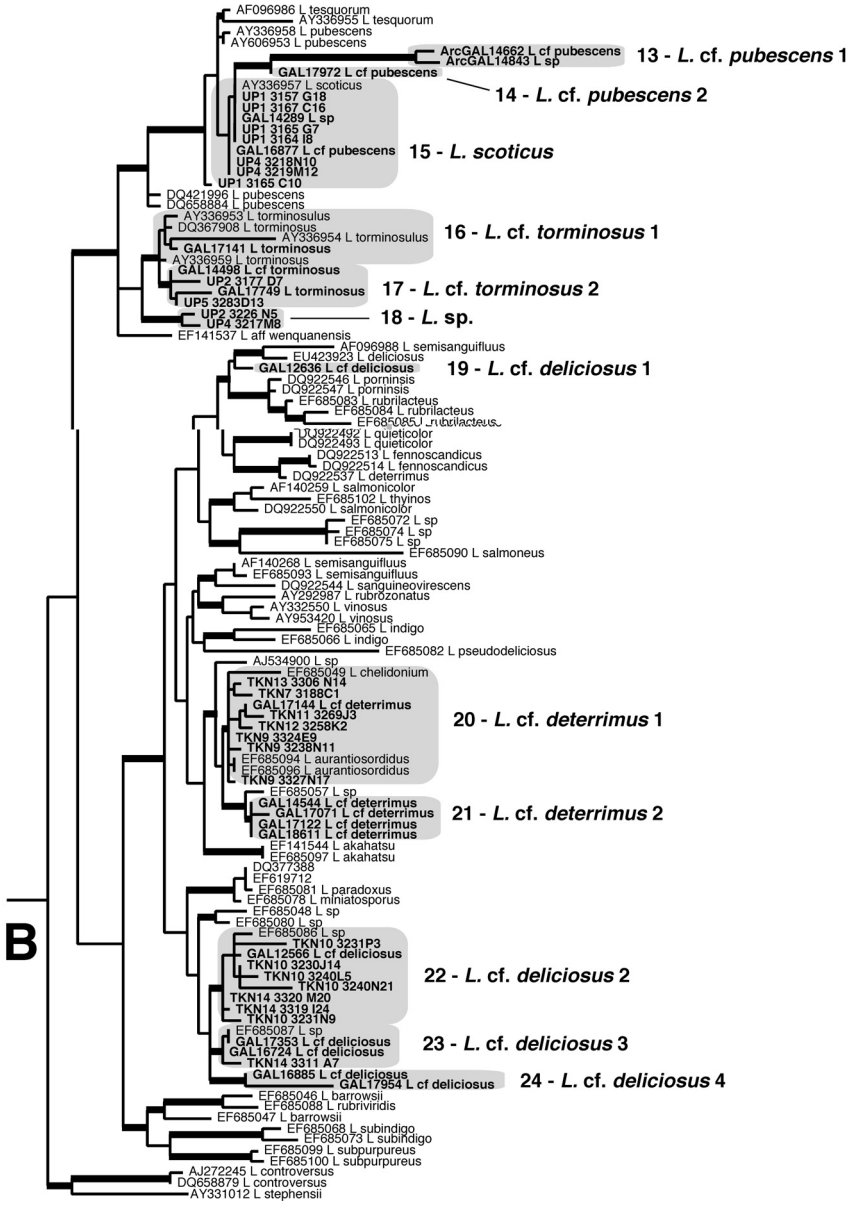


Fig. 2 *Continued*

Pairwise comparisons of these vegetation types also revealed significant differences in the phylogenetic structure of the species assemblages (all $P < 0.03$). On the other hand, communities of different forest subtypes with varying age, soil acidity, and moisture, etc., did not differ significantly from each other. This finding was confirmed by PCoA (Fig. 3), where communities tended to group together according to the major vegetation types.

Discussion

Given the estimated millions of species of fungi on Earth, studies based on large-scale DNA sequencing have immense potential to augment our current knowledge of fungal diversity. Our paper is the first to assess the phylogenetic diversity of *Lactarius*, one of the most diverse ectomycorrhizal genera, in Alaska. Boreal and arctic plant communities are frequently described as relatively species poor and having simpler patterns than those in more southern biomes (e.g. Whittaker 1975; Scott 1995; Hollingsworth *et al*. 2006). Our genus-wide diversity assessment suggests that the genus *Lactarius* is diverse in Alaska, particularly when comparing our data to other estimates of basidiomycete diversity in soil, which typically detected zero to five *Lactarius* species (O'Brien *et al*. 2005; Allison *et al*. 2007; Porter *et al*. 2008).

Based on the phylogenetic breadth of our sequences, most, if not all, major phylogenetic clades of *Lactarius*, as

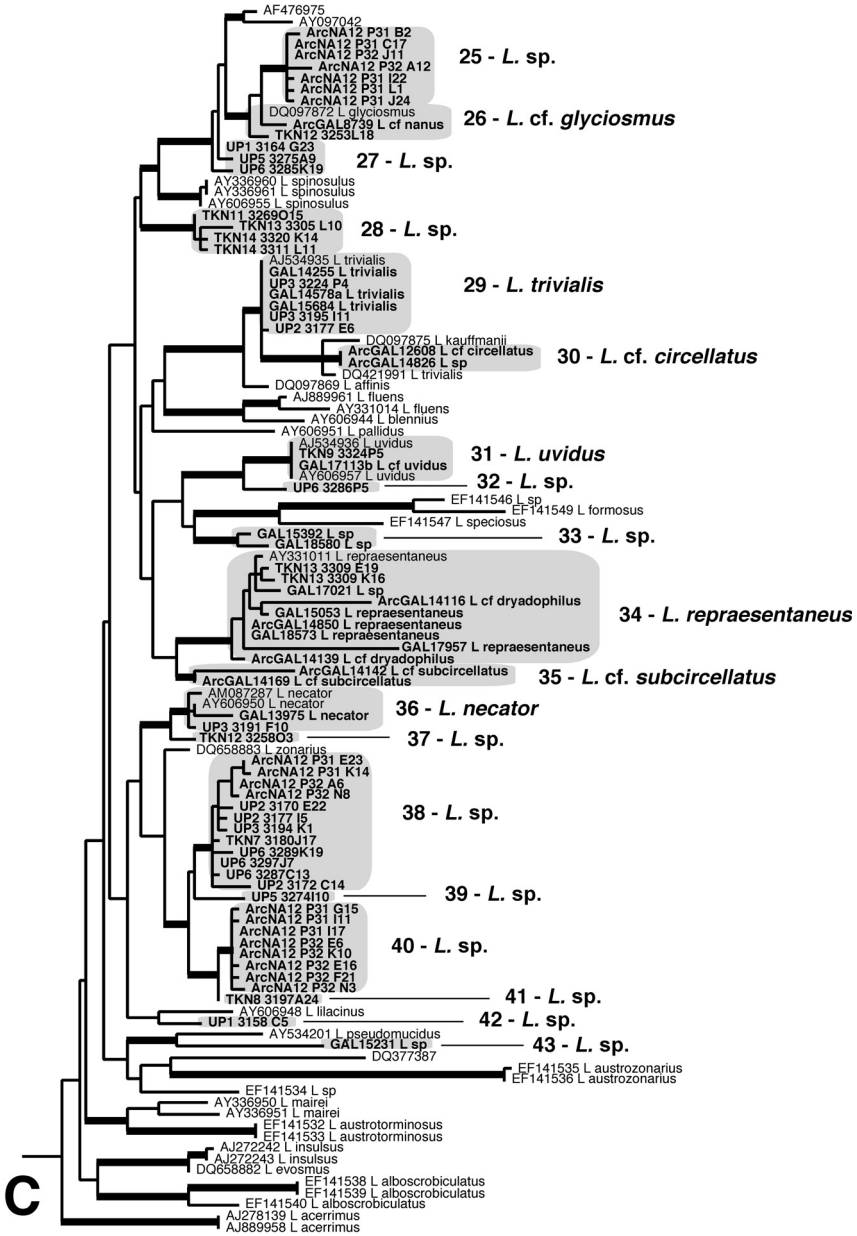
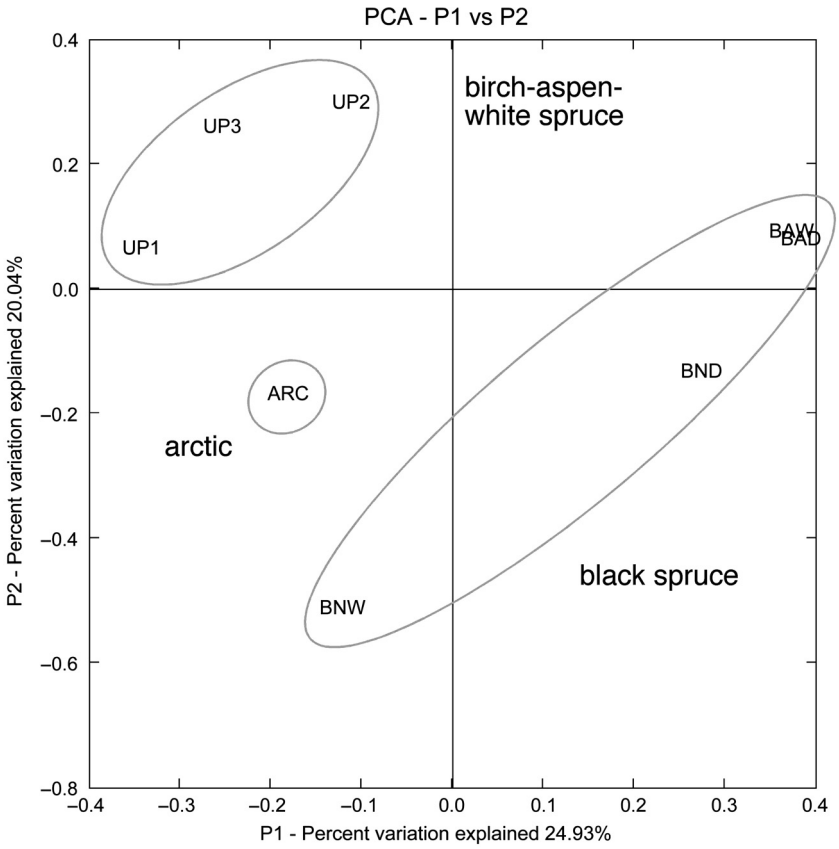


Fig. 2 *Continued*

published in Miller *et al*. (2006), Nuytinck & Verbeken (2005), and Nuytinck *et al*. (2006), are represented in Alaska. This is in sharp contrast to the trend seen in the nonmycorrhizal saprotrophic *Agaricus* L.Fr., the only other Alaskan genus that has undergone a similar assessment of molecular diversity (Geml *et al*. 2008b). In *Agaricus*, only two taxonomic section-level phylogenetic clades have multiple species in Interior Alaska, while a third one is represented by a single species, leaving more than half of the major subgeneric groups missing from Alaska.

Some of the Alaskan OTUs clearly matched known species and some were unique, currently of unknown identity. These latter may or may not represent newly discovered species, the formal description of which is beyond the scope of this paper. Nonasymptotic species accumulation curves (Fig. 1), the disparity between observed and estimated richness (Table 3), and the high number of singleton OTUs at 95% and 97% OTU recognition indicate that many *Lactarius* species have yet to be found in these forests despite the relatively low plant diversity. When comparing the number of phylogroups (Fig. 2) detected by different sampling methods, we observed that 30 (69.8%) and 27 (55.8%) of the 43 groups were found in sporocarp and soil clone sequences, respectively. Sixteen groups (37.1%) were represented only by sporocarps, 13 (30.2%) only by soil clones, and 14 (32.6%) by both. Four of the 16 'sporocarp-only' groups ('1', '11',



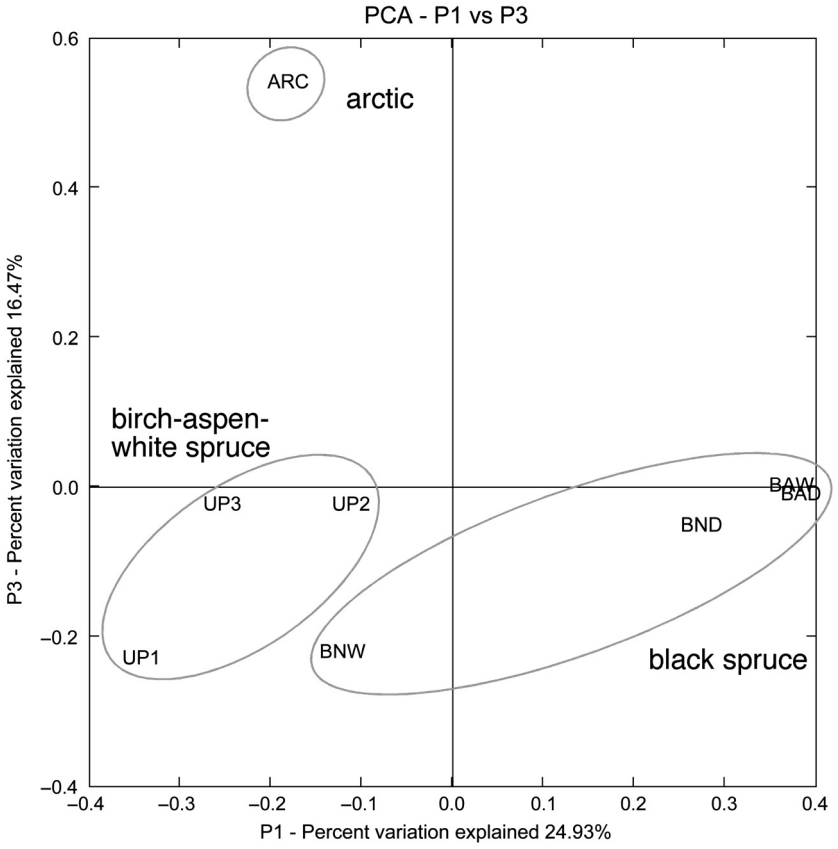


Fig. 3 Principal coordinates analysis (PCoA) ordination plot for the *Lactarius* communities from arctic tundra (*Betula nana, Dryas* spp., *Salix* spp.), black spruce (*Picea mariana*) and upland birch-aspen-white spruce (*Betula neoalaskana, Picea glauca, Populus tremuloides*) forest types. The percentage of variation explained by each principal component is indicated on the axes. Labels: BAD, black spruce, acidic, dry; BAW, black spruce, acidic, wet; BND, black spruce, non-acidic, dry; BNW, black spruce, non-acidic, wet; UP1, early successional upland birch-aspen; UP2, mid-successional upland birch-aspen-white spruce; UP3, late successional upland birch-aspen-white spruce.

'12', '43') were found only in southcentral and southeastern Alaska, and their absence in the clone libraries may be because they do not occur in Interior and northern Alaska and/or they might be relatively rare species. Nonetheless, the remaining 12 are present in the ecoregions sampled, but were not detected in the clone libraries. This may be explained by the findings of Horton & Bruns (2001), who emphasized the clustered distribution of many ectomycorrhizal fungi and reported that most species actually occur in less than 10% of all samples. However, it has to be noted, that our sporocarp collecting effort spanned 35 years, while the soil samples were taken in two consecutive, although climatologically very different years. Our results not only imply that combined sporocarp and soil sampling should be used for biodiversity assessments in fungi, but also suggest that the true diversity of arctic and boreal *Lactarius* in Alaska may be higher than what we observed. On the other hand, although we have not recovered all *Lactarius* species present in the study area, the phylogenetic breadth of our samples, and the shape of the accumulation curve and the bootstrap diversity estimates of the 90% ITS similarity OTUs suggest that we have likely detected most of the major infrageneric groups inhabiting Interior and northern Alaska.

Vegetation/habitat greatly influences the composition of *Lactarius* species assemblages. *Lactarius* groups often showed strong habitat preference to one of the three major vegetation types as supported by UniFrac analyses (Fig. 3). Differences between these three vegetation types (i.e. black spruce, upland birch-aspen-white spruce, and arctic tundra) were much greater than differences among vegetation subtypes (e.g. early- vs. late-successional upland forests). In our example, the vegetation types overlap with respect to some host tree and shrub genera. For example, *Picea* and *Populus* can be found in both major boreal forest types, while *Alnus, Betula,* and *Salix* occur not only in both lowland and upland boreal forests, but in the low arctic tundra as well. Therefore, the specificity of some phylogenetic groups of *Lactarius* to a certain vegetation type suggests that complex ecological properties other than host specificity *per se* may be among the major factors shaping these ectomycorrhizal communities.

The majority of *Lactarius* groups, either defined as phylogroups or as 97% ITS sequence-similarity groupings, showed clear preference to either lowland or upland forests (Table 4). For example, taxa predominantly found at upland forest sites included *Lactarius trivialis, L.* cf. *uvidus, L. scoticus, L.* cf. *torminosus,* and unidentified species in phylogroups 10 and 42. A similar list for black spruce sites contained *L. rufus, L. deterrimus, L. deliciosus, L. repraesentaneus*, and unidentified phylogroup 28. Furthermore, some OTUs seemed to prefer one soil horizon to the other. For example, *L. deliciosus* was found only within the organic horizon (29 clones), and not in the mineral layer (see Table 4). On other hand, *L. glyciosmus* was predominantly found in the mineral horizon (25 clones) at five boreal and arctic sites, with seven clones at one site in the organic horizon.

All four OTUs seemingly exceptional to this rule (contigs 12, 13, 19, and 20 in Table 4) turned out to contain multiple well-supported phylogroups that differed from each other in their habitat preference. For example, the 97% similarity group contig 13 included soil clones in phylogroups *Lactarius scrobiculatus* (2), *L. auriolla* (3), *L.* cf. *scrobiculatus* (6), and *L. alnicola* (7). Each of these phylogroups were detected in only one of the two major vegetation types: in black spruce (phylogroups 2 and 3) or in upland forest (6 and 7). This lumping of phylogroups arises from the fact that similarity-based searches are insensitive to phylogenetic signals in the data set and that they tend to group sequences less similar than the cut-off value when intermediate sequences sufficiently similar to the otherwise divergent groups are found. Therefore, even 97% ITS sequence similarity groupings seem to be rather conservative estimates of diversity in *Lactarius* and may mask important ecological differences among closely related species.

The observed diversity of *Lactarius* was much higher in either type of boreal forest than in the arctic tundra, supporting one of the oldest and most widely recognized patterns in ecology, that is, the decrease in species richness with increasing latitude. Moreover, all of the detected arctic *Lactarius* soil clone sequences were obtained from the southernmost subzone (subzone E), while no congeneric clones were identified in subzones D and C. This is somewhat surprising, because some *Lactarius* species are able to grow and fruit at least as far north as subzone C, although in low abundance (Geml, personal observation). The inferred low diversity and abundance of *Lactarius* in the Arctic is in agreement with findings of Timling *et al*. (unpublished data) based on ectomycorrhizal root tips of arctic dwarf shrubs. Within the boreal forest, most forest types and subtypes had comparable 97% OTU diversity with generally six to eight OTUs detected in every habitat (Table 4). The sole exception were the non-acidic, wet black spruce sites (BNW), where a total of only three OTUs were detected, all in the organic soil horizon. The reasons for the relative distinctiveness of BNW sites compared to other black spruce sites both in UniFrac analyses and in OTU diversity are unknown, although, unlike other black spruce sites, the wet subtype of non-acidic black spruce occurs in minerotrophic areas, indicating fens rather than bogs, and it is often codominated by Alaska larch (*Larix laricina* [Du Roi] K. Koch) (Hollingsworth *et al*. 2006).

The mechanisms responsible for the observed diversity and community patterns of *Lactarius* at the sampled sites are presently unclear. According to Dahlberg (2002), the two main hypotheses to explain high diversity of ectomycorrhizal communities are: (i) niche-based differentiation; and (ii) nonequilibrium models based on competitive ability. An example for the first would be a predictable

Table 4 Distribution of *Lactarius* soil clones among different habitats and soil horizons based on 97% ITS sequence similarity OTUs putatively identified by phylogroups as shown in Fig. 2. Groups containing only sporocarp sequences, OTUs represented by only a single clone sequence, and site/soil horizon combinations with no *Lactarius* clone sequence are not shown

		Habitat	BAD		BAW		BND		BNW	UP1		UP2		UP3		ARC E	
97% OTU	Identification (phylogroup)	Horizon	M	O	M	O	M	O	O	M	O	M	O	M	O	n/a	Total
Contig1	*L.* sp. (10)												4	4	2		10
Contig2	*L. rufus* (8)		108	66	10	103	3	1				2					293
Contig5	*L. trivialis* (29)												3				3
Contig6	*L.* cf. *deterrimus* (20)			29		3	92	35									159
Contig7	*L.* cf. *deliciosus* (22, 23)			1					28								29
Contig9	*L.* cf. *uvidus* (31, 32)							1				1		8			10
Contig10	*L. scoticus* (15)									8	29						37
Contig12	*L.* sp. (38, 39, 40, 41)		1	2		8		3			1	3	7	8	16	12	61
Contig13	*L.* cf. *scrobiculatus* (2, 3, 6, 7)			3		3	1	1	1		1	4	16	1	8		39
Contig14	*L.* cf. *torminosus* (17)										4	5	5		1		15
Contig16	*L. repraesentaneus* (34)						1	11									12
Contig17	*L.* sp. (42)									5							5
Contig18	*L.* sp. (28)		1			2	4	5	3								15
Contig19	*L.* cf. *glyciosmus* (25, 26, 27)				10	7				1		4		3		7	32
Contig20	*L.* cf. *necator* (36, 37)				6	22								5			33
Number of OTUs per habitat and soil horizon			3	5	3	7	5	7	3	3	4	5	6	5	5	2	
Number of OTUs per habitat			6		7		7		3	6		8		7		2	

Labels: BAD, black spruce, acidic, dry; BAW, black spruce, acidic, wet; BND, black spruce, non-acidic, dry; BNW, black spruce, non-acidic, wet; UP1, early successional upland birch-aspen-white spruce; UP2, mid-successional upland birch-aspen-white spruce; UP3, late successional upland birch-aspen-white spruce; ARC E, arctic tundra, subzone E; M, mineral soil horizon; O, organic soil horizon.

succession of species following disturbance, while the second assigns dominant role to stochastic events, such as dispersal and local abundance. Our data tend to support the niche-based differentiation hypothesis, as most phylogroups predictably occurred in specific habitats, regardless of location. However, even within a habitat type, the occurrence of particular *Lactarius* taxa was patchy (Table 4), suggesting a stochastic component as well.

The implications of our results are not restricted to *Lactarius*, but are important for informing approaches to biodiversity studies and conservation of ectomycorrhizae in general, and for assessing fungal resilience and future responses to climate change. The major differences in *Lactarius* communities between black spruce and upland mixed forests are particularly important in the view of current global warming trends. For example, white spruce forests are already showing decreased growth response to temperature increase in the growing season due to warming-induced drought stress (Barber *et al*. 2000) and spruce budworm (*Choristoneura* spp.) outbreaks (Juday & Marler 1997). Projections suggest that continued warming will result in zero net annual growth, decreased fire interval, and eventually in the expansion of birch- and aspen-dominated forests at the expense of white spruce (Calef *et al*. 2005; Chapin *et al*. 2006). Similar processes may also take place in black spruce sites, although black spruce may be somewhat buffered from drought, as it tends to grow in poorly drained areas and on north slopes that are underlain by permafrost. Nonetheless, increased fire frequency and the degradation of permafrost due to warming may facilitate the invasion of deciduous forests into areas formerly inhabited by black spruce (Calef *et al*. 2005). Such changes will undoubtedly alter ectomycorrhizal communities, and the distribution and abundance of some taxa, including members of the genus *Lactarius*.

Similar to boreal regions, there are serious concerns among researchers and the public alike related to the future of arctic biodiversity, particularly because of the threats represented by accelerating climate change scenarios and the growing human impact in arctic areas. The Arctic is uniquely sensitive to both of these changes. Understanding the causes, patterns, and consequences of such changes is critical to protecting biodiversity. Recent warming and increases in winter precipitation have already resulted in shrub expansion and the advancement of tree limit in many areas (Tape *et al*. 2006). Ectomycorrhizal fungi most certainly play an important, yet unknown role in these processes. Our study shows that even though *Dryas* and *Salix*, the most important ectomycorrhizal partners of *Lactarius* in the Arctic, are found in all three bioclimate subzones, the southernmost subzone harbours by far the greatest abundance and diversity, which suggests that

temperature may be the primary limiting factor for *Lactarius*, either directly or indirectly (e.g. affecting nutrient availability). The importance of soil temperature is also supported by the observation that the closest relatives of most arctic lineages in our phylogram were those found in black spruce sites, which are typically underlain by permafrost and have the lowest soil temperatures in the boreal region (Viereck *et al*. 1992; Osterkamp & Romanovsky 1999; Hollingsworth *et al*. 2006). With the increase in shrub cover, the abundance of *Lactarius* is also expected to increase, particularly in the low arctic tundra.

In addition to estimating biodiversity of boreal fungi, and thus providing baseline information for future in-depth fungal systematic studies, our results have important implications for ecological studies focusing on northern ecosystems. Triggered by recent climatic changes, arctic and boreal regions are on the brink of significant changes both in composition and function that, in turn, could substantially alter the global climate system (Chapin *et al*. 2006). Because mycorrhizal fungi play key ecological roles in the decomposition, mineralization, immobilization, and the transfer of nutrients to plants, documenting and preserving fungal diversity may be crucial for preserving overall functional biodiversity.

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The Taylor lab, in general, is seeking to better understand the diversity, community structure and function of fungi in boreal and arctic soils. Specific interests are as follows, J.G.: biodiversity, phylogeography, and evolutionary ecology of arctic and boreal fungi and their historical and recent responses to changes in the landscape and the climate; G.L.: systematics of macrofungi of Alaska; I.T.: diversity and ecology of arctic ectomycorrhizae; J.M.: functional diversity of soil microorganisms in terrestrial forest ecosystems, particularly the reciprocal influences between plant and fungal communities in response to environmental change; M.B.: ecology of ectomycorrhizae; N.L. and C.N.: genome projects and new sequencing technologies.
