Biodiversity and molecular ecology of *Russula* and *Lactarius* in Alaska based on soil and sporocarp DNA sequences

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Abstract. – Although critical for the functioning of ecosystems, fungi are poorly known in high-latitude regions. This paper summarizes the results of the first genetic diversity assessments of Russula and Lactarius, two of the most diverse and abundant fungal genera in Alaska. LSU rDNA sequences from both curated sporocarp collections and soil PCR clone libraries sampled in various types of boreal forests of Alaska were subjected to phylogenetic and statistical ecological analyses. Our diversity assessments suggest that the genus Russula and Lactarius are highly diverse in Alaska. Some of these taxa were identified to known species, while others either matched unidentified sequences in reference databases or belonged to novel, previously unsequenced groups. Taxa in both genera showed strong habitat preference to one of the two major forest types in the sampled regions (black spruce forests and birch-aspen-white spruce forests), as supported by statistical tests. Our results demonstrate high diversity and strong ecological partitioning in two important ectomycorrhizal genera within a relatively small geographic region, but with implications to the expansive boreal forests. The pronounced differences in community composition between various forest types are particularly relevant to climate change studies, as a diverse set of Russula and Lactarius species undoubtedly play an important role in the predicted expansion of deciduous forests in the boreal and low arctic regions.

Résumé. – Biodiversité et écologie moléculaire de *Russula* et *Lactarius* en Alaska, sur base de séquences ADN de sol et de sporocarpes. Quoique très importants pour le fonctionnement des écosystèmes, les champignons des régions de haute latitude restent mal connus. Cet article résume les résultats des premières évaluations de la diversité génétique de *Russula* and *Lactarius*, deux des genres les plus diversifiés et abondants en Alaska. Des séquences LSU rDNA provenant aussi bien des carpophores conservés en herbier que de la bibliothèque de clone PCR des sols récoltés dans divers types de forêts boréales d'Alaska, ont été soumis à des analyses écologiques phylogénétiques et statistiques. Nos évaluations de diversité suggèrent que les genres *Russula* et *Lactarius* sont très diversifiés en Alaska. Certains de ces taxons ont été rapportés à des espèces connues, alors que d'autres soit correspondaient à des séquences non identifiées dans des bases de données de référence soit appartenaient à des groupes nouveaux, non encore séquencés. Les taxons des deux genres ont montré une forte préférence écologique pour l'un des deux grands types de forêts existant dans les régions échantillonnées (forêts de *Picea mariana* et forêts de *Betula neoalaskana*, *Populus tremuloides* et *Picea glauca*), ainsi que le montrent les tests statistiques. Nos résultats démontrent une large diversité et un fort partitionnement écologique chez deux genres ectomycorhiziens importants au sein d'une région géographique relativement restreinte, mais avec des implications vers les forêts boréales expansives. Les différences marquées dans la composition des communautés entre les différents types de forêts sont particulièrement pertinentes

pour les études sur les modifications climatiques, étant donné qu'un ensemble différent d'espèces de *Russula* et *Lactarius* jouent indubitablement un rôle important dans l'expansion prévue des forêts feuillues dans les régions boréales et le sud des régions arctiques.

Key Words. – Alaska, fungi, ribosomal large subunit gene, soil microbes

INTRODUCTION

Despite our constantly improving understanding, we still know very little about the true diversity of life and our lack of knowledge severely compromises our ability to recognize and to respond intelligently to recent and future environmental changes (Donoghue et al. 2009). Currently, the majority of biodiversity studies and conservation efforts are focused on vascular plants, vertebrates, and to some extent on insects (Elvebakk 2005), while soils remain a relatively unexplored, yet presumably significant source of biodiversity (Usher et al. 2005, Buée et al. 2009, Geml et al. 2009, Chu et al. 2010).

Fungi play central roles in the functioning of terrestrial ecosystems due to their activities as mycorrhizal symbionts and decomposers, and are also sensitive indicators of habitat quality. Approximately 100,000 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). Hence, we currently know less than 10% of total fungal species. Macrofungi, e.g. mushroom-forming fungi, are among the most studied groups of fungi and have the longest history of diversity studies. Nonetheless, even for macrofungi, basic questions about the number of species at a given location or differences in species richness among vegetation types have generally remained unanswered due to taxonomic problems and the scarcity of long-term sporocarp-monitoring projects (Lodge et al. 2004).

In recent years, DNA-based studies of soil fungal communities have provided valuable insights into the biodiversity and ecology of fungi as well as evidence that there is a large component of fungal diversity in soils that is still unknown (Schadt et al. 2003, O'Brien et al. 2005, Lynch & Thorn 2006, Porter et al. 2008, Buée et al. 2009). Unfortunately, both methods have limitations. Production of sporocarps (upon which current surveys for long-term monitoring are based) by any particular species is an unpredictable phenomenon and many species produce nearly invisible sporocarps or are asexual. On the other hand, the identification of lineages by DNA profiling is limited by the available reference sequences from known taxa. Moreover, novel and/or previously unsequenced lineages are often known only from their DNA sequences, and without any data on their morphology their systematic classification is very difficult or impossible.

It has been shown that sporocarp and soil DNA sampling often give complementary views of biodiversity, with a relatively high number of taxa detected only by one of the two methods (Porter et al. 2008, Geml et al. 2009). Therefore, utilizing their complementary strengths, we apply both sporocarp surveys and DNA-based diversity assessments of fungal communities for our fungal biodiversity studies. Large-scale projects, such as our work presented here, have immense potential to augment our current knowledge of fungal diversity.

The boreal forest is the largest terrestrial biome, including ca. 33% of all remaining forests in the World (Bradshaw et al. 2009). It covers an area of approximately 18.5 million km^2 and forms a continuous northern circumpolar zone (Bonan & Shugart 1989, Bradshaw et al. 2009). Ectomycorrhizal (ECM) fungi are among the most important and most abundant fungi in boreal ecosystems (Dahlberg 2002, Taylor et al. 2007, 2010), and form associations with all major tree genera found in this region (e.g. *Picea, Betula, Populus, Alnus*, etc.) (Molina et al. 1992, Smith & Read 1997). Such mycor-

rhizal associations can form on more than 95% of these trees' fine roots and participate in the nutrient and carbon transfer between soil and the host plant (Smith & Read 1997).

Our fungal diversity assessments in this paper are focused on *Lactarius* and *Russula* species in the boreal forests of Interior Alaska. Both *Lactarius* and *Russula* are very diverse genera, with approximately 350 and 750 described species worldwide, respectively (Kirk et al. 2001), and are among the most speciose and abundant groups of boreal ECM fungi. Based on their sheer abundance and wide distribution, these fungi presumably have great ecological importance as mycorrhizal partners of boreal trees and shrubs in Alaska. For example, based on our soil clone libraries including all fungi, russuloid species are among the most abundant taxa in boreal forest soils (Taylor et al. 2010) and there are many species that fruit regularly and abundantly in various forest types in Alaska (pers. obs.). In addition to assessing their biodiversity, we analysed how their assemblages vary among different plant communities.

MATERIALS AND METHODS

The study region

The Intermontane Boreal Forest ecoregion (Nowacki et al. 2001) of Interior Alaska is bordered by the Alaska Range to the south, the arctic treeline in the Brooks Range to the north and climatic treeline to the west. This ecoregion represents the westernmost end of the boreal belt spanning the North American continent. Interior Alaska is an area of discontinuous permafrost, of which approximately 75-80% is underlain by permafrost, with the exception of most south-facing slopes (Osterkamp & Romanovsky 1999). Climate in this region is strongly continental 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 black spruce (*Picea mariana* [Mill] Britton, Sterns & 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).

Soil samples were taken at sites monitored by the Bonanza Creek Long Term Ecological Research program (BNZ LTER, http://www. lter.uaf.edu/), representing multiple vegetation types and successional stages of forest development in Interior Alaska (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 (ca. 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).

Molecular work

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. In each subtype, three replicate plots (50 ´ 100 m) were sampled. In each plot, 50 soil cores were taken that were later sepa-

Table 1 – Habitat, clone library names, plot numbers, and locations (with 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. This sampling was carried out in 2004 and repeated in 2005. Abbreviations of dominant tree species are as follows: Bene: *Betula neoalaskana*, Lala: *Larix laricina*, Pigl: *Picea glauca*, Pima: *Picea mariana*, Poba: *Populus balsamifera*, Potr: *Populus tremuloides*.

Habitat and clone library	Plot number	Stand age (years)	Dominant tree species	Location (Latitude, Longitude)
Early successional upland mixed forest UP1 (2004) and UP4 (2005)	UP1 A	23-25	Bene: 77% Potr: 23%	Bonanza Creek LTER site, Parks Hwy. (64.73473541 - 148.2976791)
	UP1 B	23-25	Bene: 94% Pigl: 0.5% Potr: 5.5%	Bonanza Creek LTER site, Parks Hwy. (64.73195762 - 148.2974016)
	UP1 C	23-25	Bene: 3% Poba: 1% Potr: 96%	Bonanza Creek LTER site, Parks Hwy. (64.73195762 - 148.2974016)
Mid-successional upland mixed forest UP2 (2004) and UP5 (2005)	UP2 A	93-98	Bene: 51% Pigl: 46% Poba: 3%	Bonanza Creek LTER site, Parks Hwy. (64.69390269 - 148.3537914)
	UP2 B	93-98	Bene: 54% Pigl: 22.5% Poba: 12.5% Potr: 11%	Bonanza Creek LTER site, Parks Hwy. (64.68945831 - 148.3599027)
	UP2 C	93-98	Bene: 0.5% Pigl: 10.5% Potr: 89%	Bonanza Creek LTER site, Parks Hwy. (64.68862521 - 148.3790685)
Late successional upland mixed forest UP3 (2004) and UP6 (2005)	UP3 A	225-230	Bene: 32% Pigl: 62% Potr: 6%	Bonanza Creek LTER site, Parks Hwy. (64.7666796 - 148.2740661)
	UP3 B	225-230	Bene: 39% Pigl: 59.5% Potr: 1.5%	Bonanza Creek LTER site, Parks Hwy. (64.75973477 - 148.2446238)
	UP3 C	225-230	Bene: 30% Pigl: 66% Potr: 4%	Bonanza Creek LTER site, Parks Hwy. (64.72445795 - 148.324901)
Black spruce, acidic, dry TKN7 (2004) and TKN11 (2005)	TKN-0012	200-210	Bene: 1% Pima: 99%	Washington Creek, Elliott Hwy. (65.16721667 - 147.8941333)
	TKN-0122	90-100	Pima: 100%	Delta Junction, Alaskan Hwy. (63.90620584 - 145.3711972)
	TKN-0001	95-100	Bene: 1% Pima: 99%	Bonanza Creek LTER site, Parks Hwy. (64.76572442 - 148.295527)
Black spruce, acidic, wet TKN8 (2004) and TKN12 (2005)	TKN-0015	170-180	Pima: 100%	Washington Creek, Elliott Hwy. (65.15451667 - 147.8631667)
	TKN-0022	150-180	Pima: 100%	Babe Creek, Elliott Hwy. (64.99653333 - 147.65305
	TKN-0109	90-104	Pima: 100%	Caribou Poker Creek Research Watershed, Steese Hwy. (65.16166012 - 147.4878514)

Table 1, continuation

Habitat and clone library	Plot number	Stand age (years)	Dominant tree species	Location (Latitude, Longitude)
Black spruce, non-acidic, dry TKN9 (2004) and TKN13 (2005)	TKN-0039	130-190	Pima: 100%	Ballaine Rd., Fairbanks (64.91003773 - 147.8218001)
	TKN-0123	97-100	Pima: 100%	Delta Junction, Alaskan Hwy. (63.92459496 - 145.3167362)
	TKN-0126	120-130	Pima: 100%	Delta Junction, Alaskan Hwy. (63.84655372 - 145.7208714)
Black spruce, non-acidic, wet TKN10 (2004) and TKN14 (2005)	TKN-0051	68-91	Lala: 3% Pigl: 3% Pima: 94%	UAF Arboretum, Fairbanks (64.86588323 - 147.8736745)
	TKN-0119	280-320	Pima: 100%	Delta Junction, Alaskan Hwy. (63.81379184 - 144.9532331)
	TKN-0040	179-216	Lala: 3% Pigl: 1% Pima: 96%	Ballaine Rd., Fairbanks (64.9148493 - 147.8297945)

rated into organic and mineral horizons. For every plot, the 50 cores sampled 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 the following year, resulting in a total of 84 DNA extractions. In addition, 383 *Lactarius* and 799 *Russula* specimens were collected from different forested regions of Alaska over a 35-year period. All collections are publicly available from the University of Washington Herbarium (WTU) at the Burke Museum. Of these collections, fifty-five and eighteen specimens, representing the diversity of morphological groups and geographic areas, were selected for molecular work, respectively.

The entire ITS and partial LSU regions were PCR amplified from soil samples and sporocarps. For each soil DNA extract, seven replicate PCRs were performed and pooled. We utilized a molecular tagging strategy to mark PCR products from various sources with DNA tags, which can then be pooled prior to library sequencing (Taylor et al. 2008). We cloned the resulting PCR products into the Invitrogen TOPO TA 4.0 vectors, then shipped transformed plasmids frozen to the Broad Institute of MIT and Harvard, where plating, colony picking, Templiphi reactions and sequencing were carried out on automated equipment. DNA extraction, PCR, and sequencing methods for both soil and sporocarp samples have been described in detail in Taylor et al. (2007, 2010) and Geml et al. (2006, 2009, 2010).

Bioinformatic work

Sequence data obtained for both strands were edited and assembled for each sporocarp or soil clone using Aligner v. 1.3.4 (CodonCode Inc., Dedham, MA). We identified soil clone sequences to genera, based on similarity searches using FASTA (Pearson & Lipman 1988) against a reference database containing all fungal ITS sequences from GenBank. Similarity searches identified 6943 Russulaceae-affiliated sequences in all clone libraries. These were further screened to reduce the number of identical sequences from the same clone library. The resulting 533 LSU sequences selected for phylogenetic analyses were deposited in Genbank (EU712097–EU712629). To make phylogenetic analyses more manageable, we further reduced the number of sequences by selecting representatives of quasi-identical clones for each major plant community type (i.e. upland mixed forest vs. lowland black spruce). This

allowed us to maintain a representative phylogenetic diversity and habitat-association of the lineages, while making the results more easily interpretable. Thus, in the final LSU alignment, there were 92 soil clone and 50 sporocarp sequences from the boreal forests of Alaska. To obtain a picture of the diversity of Russulaceae in our samples, homologous sequences of other taxa within Russulaceae were downloaded from GenBank. A family-wide LSU sequence alignment was initiated by Clustal W (Thompson et al. 1997) using M3101 alignment deposited in TreeBase by Miller et al. (2006), respectively, as profile alignment. The alignment was subsequently corrected manually. Phylogenetic analyses were conducted using maximum-likelihood (ML) method in Garli 0.94 (Zwickl 2006), Bayesian method in MrBayes (Huelsenbeck & Ronquist 2001), and maximum-parsimony (MP) method in PAUP* 4b10 (Swofford 2002) with the maximum number of saved trees set to 100 in this latter. To compare different tree topologies, Shimodaira-Hasegawa tests were used (Shimodaira & Hasegawa 1999). The Life Sciences Informatics cluster portal (http://biotech.inbre.alaska.edu/) was used for all analyses.

Comparing Russulaceae communities among vegetation types

We used LSU sequences generated from soil clone libraries to test for statistical differences among vegetation types. A multiple sequence alignment containing only soil clone sequences was constructed as described above. The alignment was subsequently corrected manually and was subject to maximum-parsimony (MP) analyses in PAUP* with the maximum number of saved trees set to 100. The strict consensus tree (not shown) 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 test for differences among Russulaceae communities of the sampled vegetation type plots. The UniFrac distance is calculated as the percent 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 versus the others. The P test compares the number of parsimony changes (when environment of origin is considered a character state) to the distribution of the number of character state changes in a set of trees with randomly generated topologies. PCoA is a multivariate statistical technique used 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.
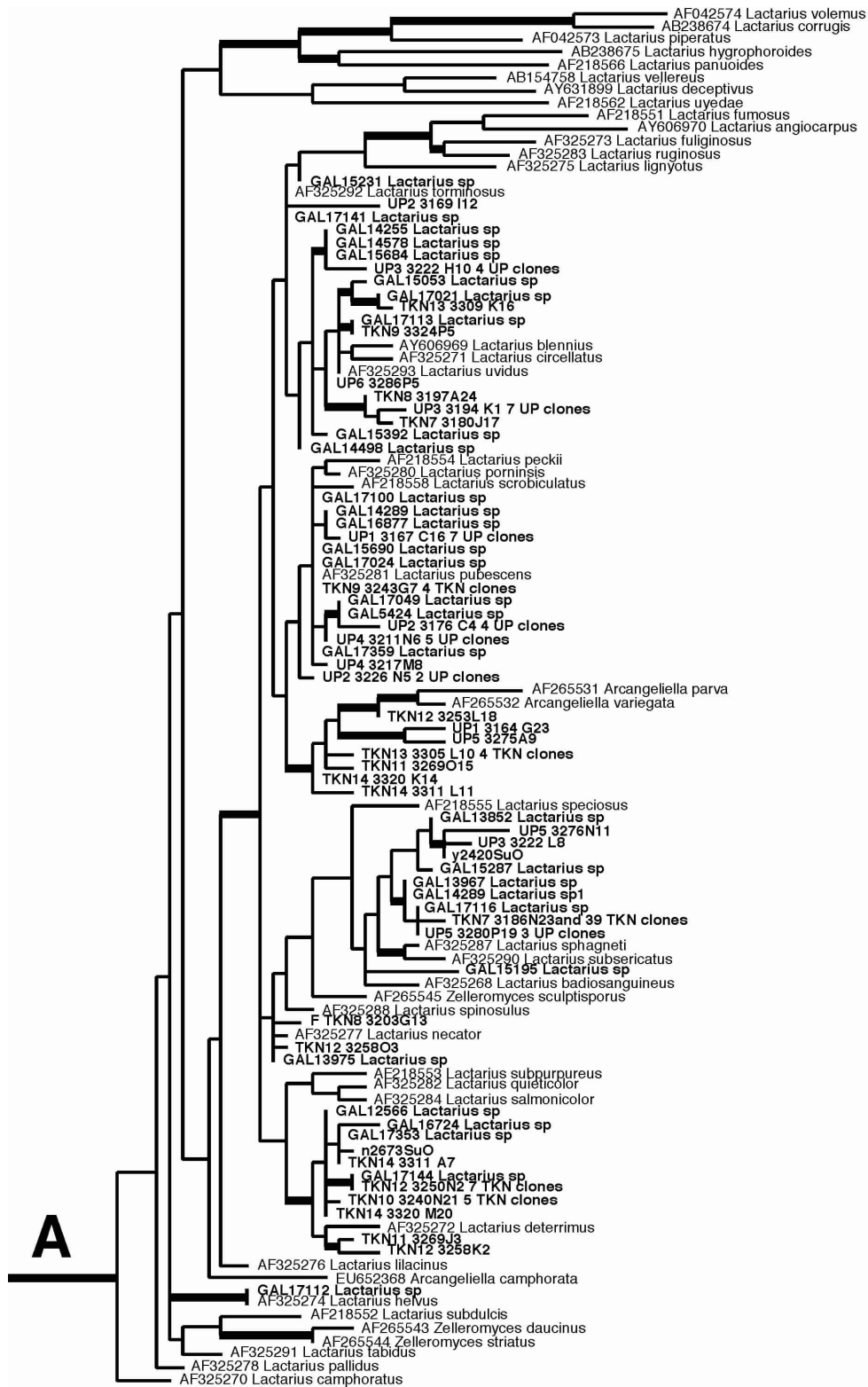
RESULTS

The final LSU alignment contained 276 sequences and 694 characters, of which 206 were parsimony-informative. Shimodaira-Hasegawa tests revealed that the MP and ML trees were not significantly different (values ranging from $P = 0.131$ to $P = 0.169$). One of 100 equally parsimonious trees is shown in figure 1 with branches supported by 0.95 or greater Bayesian posterior probability thickened. Alaskan sequences are widely distributed on the tree and grouped with several genera and with major infrageneric groups. The detected taxa fell into two major genera: *Lactarius* and *Russula*. Several taxa showed affinity to members of several small, secotioid genera embedded in either *Lactarius* or *Russula* (e.g. *Arcangeliella, Cystangium, Gymnomyces, Macowanites*). As expected based on earlier studies, such as Miller et al. (2006), the genus *Lactarius* formed a monophyletic group within the paraphyletic genus *Russula*.
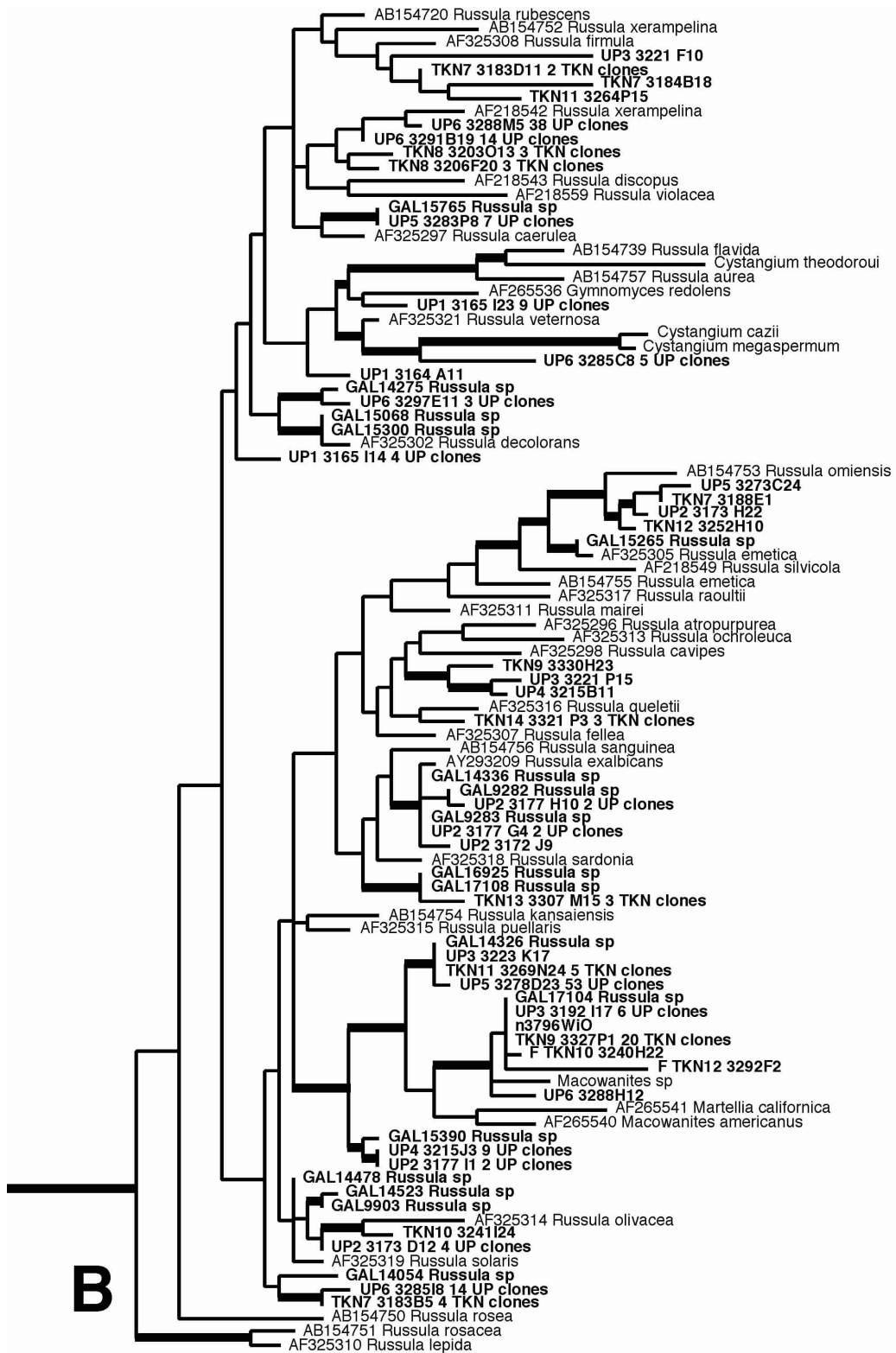
In many cases, Alaskan sequences grouped together with previously published sequences with known identity, such as *Lactarius deterrimus* Gröger, *L. helvus* (Fr.) Fr., *L. necator* (Bull.) Pers., *L. pubescens* (Fr.) Fr., *L. scrobiculatus* (Scop.) Fr., *L. torminosus* (Schaeff.) Pers., *L. uvidus* (Fr.) Fr., *Russula adusta* (Pers.) Fr., *R. brevipes* Peck, *R. decolorans* (Fr.) Fr., *R.*

Figure 1 – A maximum-parsimony phylogram showing the phylogenetic spread of boreal Alaskan russuloid sequences (in bold) generated in this study among representatives of Russulaceae taxa in GenBank. Sequences with GAL numbers were derived from herbarium specimens, while UP and TKN sequences in bold are from soil clone libraries of upland and lowland boreal forests, respectively. Branches with Bayesian posterior probability support [3] 0.95 are thickened. GenBank sequences with no name attached are from unidentified environmental samples. Clades marked by "A" and "B" are shown in details in expansions 1b and 1c, respectively.

AF042574 Lactarius volemus
AB238674 Lactarius corrugis
AF042573 Lactarius piperatus
AB238675 Lactarius hygrophoroides
AF218566 Lactarius panuoides
AB154758 Lactarius vellereus
AY631899 Lactarius deceptivus
AF218562 Lactarius uyedae
AF218551 Lactarius fumosus
AY606970 Lactarius angiocarpus
AF325273 Lactarius fuliginosus
AF325283 Lactarius ruginosus
AF325275 Lactarius lignyotus
GAL15231 Lactarius sp
AF325292 Lactarius torminosus
UP2 3169 I12
GAL17141 Lactarius sp
GAL14255 Lactarius sp
GAL14578 Lactarius sp
GAL15684 Lactarius sp
UP3 3222 H10 4 UP clones
GAL15053 Lactarius sp
GAL17021 Lactarius sp
TKN13 3309 K16
GAL17113 Lactarius sp
TKN9 3324P5
AY606969 Lactarius blennius
AF325271 Lactarius circellatus
AF325293 Lactarius uvidus
UP6 3286P5
TKN8 3197A24
UP3 3194 K1 7 UP clones
TKN7 3180J17
GAL15392 Lactarius sp
GAL14498 Lactarius sp
AF218554 Lactarius peckii
AF325280 Lactarius porninsis
AF218558 Lactarius scrobiculatus
GAL17100 Lactarius sp
GAL14289 Lactarius sp
GAL16877 Lactarius sp
UP1 3167 C16 7 UP clones
GAL15690 Lactarius sp
GAL17024 Lactarius sp
AF325281 Lactarius pubescens
TKN9 3243G7 4 TKN clones
GAL17049 Lactarius sp
GAL5424 Lactarius sp
UP2 3176 C4 4 UP clones
UP4 3211N6 5 UP clones
GAL17359 Lactarius sp
UP4 3217M8
UP2 3226 N5 2 UP clones
AF265531 Arcangeliella parva
AF265532 Arcangeliella variegata
TKN12 3253L18
UP1 3164 G23
UP5 3275A9
TKN13 3305 L10 4 TKN clones
TKN11 3269O15
TKN14 3320 K14
TKN14 3311 L11
AF218555 Lactarius speciosus
GAL13852 Lactarius sp
UP5 3276N11
UP3 3222 L8
y2420SuO
GAL15287 Lactarius sp
GAL13967 Lactarius sp
GAL14289 Lactarius sp1
GAL17116 Lactarius sp
TKN7 3186N23and 39 TKN clones
UP5 3280P19 3 UP clones
AF325287 Lactarius sphagneti
AF325290 Lactarius subsericatus
GAL15195 Lactarius sp
AF325268 Lactarius badiosanguineus
AF265545 Zelleromyces sculptisporus
AF325288 Lactarius spinosulus
F TKN8 3203G13
AF325277 Lactarius necator
TKN12 3258O3
GAL13975 Lactarius sp
AF218553 Lactarius subpurpureus
AF325282 Lactarius quieticolor
AF325284 Lactarius salmonicolor
GAL12566 Lactarius sp
GAL16724 Lactarius sp
GAL17353 Lactarius sp
n2673SuO
TKN14 3311 A7
GAL17144 Lactarius sp
TKN12 3250N2 7 TKN clones
TKN10 3240N21 5 TKN clones
TKN14 3320 M20
AF325272 Lactarius deterrimus
TKN11 3269J3
TKN12 3258K2
AF325276 Lactarius lilacinus
EU652368 Arcangeliella camphorata
GAL17112 Lactarius sp
AF325274 Lactarius helvus
AF218552 Lactarius subdulcis
AF265543 Zelleromyces daucinus
AF265544 Zelleromyces striatus
AF325291 Lactarius tabidus
AF325278 Lactarius pallidus
AF325270 Lactarius camphoratus

A

emetica (Schaeff.) Pers., *R. exalbicans* (Pers.) Melzer & Zvára, and *R. xerampelina* (Schaeff.) Fr. Among, the sequestrate taxa, *Arcangeliella parva* Thiers*, A. variegata* Thiers*, C. megaspermum* (Rodway) T. Lebel & Castellano*,* and an unidentified *Macowanites* sp. were found to be grouped with Alaskan soil clones with significant Bayesian posterior probability values. In many other cases, Alaskan sequences formed unique, unidentified clades that have formerly not been sequenced, with or without sequenced close relatives.

UniFrac analyses revealed that the *Russulaceae* communities significantly differed between the two major types: black spruce and mixed birch-aspen-white spruce forests (UniFrac metric: *P* < 0.01 ; P test: *P* < 0.01). 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 supported by PCoA (fig. 2), 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. Here we summarize results that assess the phylogenetic diversity of *Russula* and *Lactarius* in the boreal region of Alaska. Boreal 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 fungal diversity assessments suggest that ectomycorrhizal fungi are diverse in Alaska and confirm previous findings of Taylor et al. (2007), i.e. fungal communities in boreal regions appear to be species rich.

Based on the phylogenetic breadth of our sequences, most, if not all, major phylogenetic clades of *Russula* and *Lactarius*, as published in Miller et al. (2006), Nuytinck & Verbeken (2005) and Nuytinck et al. (2006), are represented in Alaska. Some of the Alaskan sequences clearly matched known species and some were unique with or without known close relatives. These taxa of unknown identity may or may not represent newly discovered species, the formal description of which is beyond the scope of this paper.

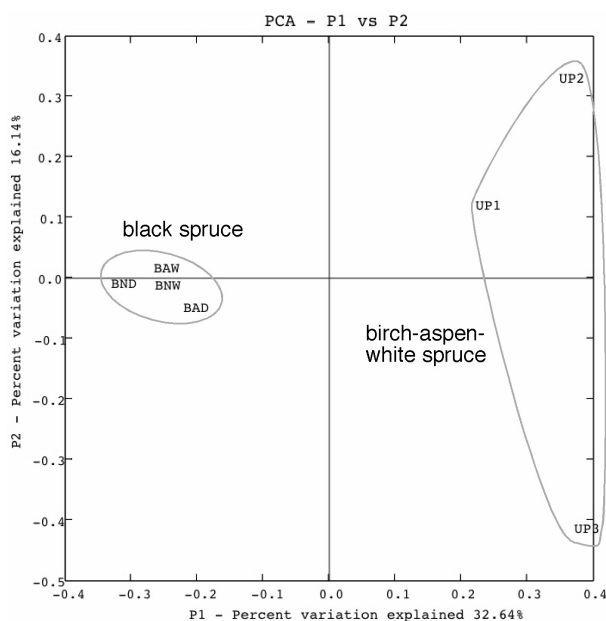It is important to note that the identification of the phylogroups have to be considered

Figure 2 – Principal coordinates analysis (PCoA) ordination plot for the Russulaceae communities from black spruce (*Picea mariana*) and upland birch-aspen-white spruce (*Betula neoalaskana, Picea glauca, Populus tremuloides*) forest types. The percent 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-white spruce; UP2: mid successional upland birch-aspen-white spruce; UP3: late successional upland birch-aspen-white spruce.

approximate and merely provide indication of affiliations to publicly available reference sequences. Consequently, more detailed taxonomic analyses are needed for the in-depth taxonomic treatment of Alaskan taxa and to compare them to other North American and Eurasian species. In the case of circumpolar species, the names applied here are more likely to be correct, while for species that are only known from Europe, the phylogroups may, in reality, refer to sister taxa. On the other hand, in the view of the biogeographic history of Beringia, it will not be surprising if some *Russula* and *Lactarius* species currently considered endemic to Eurasia will indeed be proven to occur in Alaska, but not in other parts of North America. Such distribution patterns have already been shown for sister species in some other groups, e.g. the *Amanita muscaria* complex (Geml et al. 2008).

Phylogenetic groups across the Russulaceae showed strong habitat preference to one of the two major forest types, i.e. black spruce vs. upland birch-aspen-white spruce forests as shown by the UniFrac analyses of the family-wide LSU tree. In our example, the vegetation types compared overlap with respect to some host tree and shrub genera. For example, *Picea*, *Populus, Alnus, Betula,* and *Salix* can be found in both major boreal forest types. Therefore, the specificity of most russuloid taxa 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 presence or absence of permafrost is probably the most important threshold regulating the structure and functioning of Alaskan boreal forests (Chapin et al. 2006). Our data suggest that, similar to plants, the presence or absence of permafrost and associated soil characteristics (hydrological features, nutrient levels etc.) may have a major influence on the species composition of the sampled ECM communities, even when congeneric hosts (e.g. in *Betula* and *Picea*) occur in both major vegetation types. However, further research is needed to test this hypothesis.

In addition to estimating biodiversity of boreal fungi, and thus providing baseline in-

formation for future in-depth fungal systematic studies, our results have important implications for ecological studies focusing on boreal ecosystems. Triggered by recent climatic changes, the boreal forest is on the brink of a significant change in both composition and function that, in turn, could substantially alter the global climate system (Chapin et al. 2006). In Alaska, projections suggest that temperature-induced drought stress, increased fire frequency, more frequent insect outbreaks, and changes in drainage due to the degradation of permafrost will facilitate the expansion of deciduous forests (predominantly birch and aspen) at the expense of spruce, particularly white spruce (Barber et al. 2000, Calef et al. 2005, Chapin et al. 2006). Such changes will undoubtedly alter ectomycorrhizal communities, and some taxa that are predominantly found in late-successional upland stands (especially those restricted to old-growth white spruce forests) could become more restricted in their distributions.

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