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ORIGINAL
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An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA

József Geml^{1,2*}, Ina Timling², Clare H. Robinson³, Niall Lennon⁴, H. Chad Nusbaum⁴, Christian Brochmann⁵, Machiel E. Noordeloos¹ and D. Lee Taylor²

¹National Herbarium of the Netherlands, Netherlands Centre for Biodiversity Naturalis, Leiden University, PO Box 9514, Einsteinweg 2, 2300 RA Leiden, The Netherlands, ²Institute of Arctic Biology, University of Alaska Fairbanks, PO Box 757000, Fairbanks, AK 99775-7000, USA, ³School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Williamson Building, Oxford Road, Manchester, M13 9PL, UK, ⁴Genome Sequence and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, MA 02141, USA, ⁵National Centre for Biosystematics, Natural History Museum, University of Oslo, PO Box 1172 Blindern, NO-0318 Oslo, Norway

ABSTRACT

Aim Current evidence from temperate studies suggests that ectomycorrhizal (ECM) fungi require overland routes for migration because of their obligate symbiotic associations with woody plants. Despite their key roles in arctic ecosystems, the phylogenetic diversity and phylogeography of arctic ECM fungi remains little known. Here we assess the phylogenetic diversity of ECM communities in an isolated, formerly glaciated, high arctic archipelago, and provide explanations for their phylogeographic origins.

Location Svalbard.

Methods We generated and analysed internal transcribed spacer (ITS) nuclear ribosomal DNA sequences from both curated sporocarp collections (from Svalbard) and soil polymerase chain reaction (PCR) clone libraries (from Svalbard and the North American Arctic), compared these with publicly available sequences in GenBank, and estimated the phylogenetic diversity of ECM fungi in Svalbard. In addition, we conducted coalescent analyses to estimate migration rates in selected species.

Results Despite Svalbard's geographic isolation and arctic climate, its ECM fungi are surprisingly diverse, with at least 72 non-singleton operational taxonomic units (soil) and 109 phylogroups (soil + sporocarp). The most species-rich genera are *Thelephora/Tomentella*, *Cortinarius* and *Inocybe*, followed by *Hebeloma*, *Russula*, *Lactarius*, *Entoloma*, *Sebacina*, *Clavulina*, *Laccaria*, *Leccinum* and *Alnicola*. Despite the scarcity of available reference data from other arctic regions, the majority of the phylogroups (73.4%) were also found outside Svalbard. At the same time, all putative Svalbard 'endemics' were newly sequenced taxa from diverse genera with massive undocumented diversity. Overall, our results support long-distance dispersal more strongly than vicariance and glacial survival. However, because of the high variation in nucleotide substitution rates among fungi, allopatric persistence since the Pliocene, although unlikely, cannot be statistically rejected. Results from the coalescent analyses suggest recent gene flow among different arctic areas.

Main conclusions Our results indicate numerous recent colonization events and suggest that long-distance, transoceanic dispersal is widespread in arctic ECM fungi, which differs markedly from the currently prevailing view on the dispersal capabilities of ECM fungi. Our molecular evidence indicates that long-distance dispersal has probably played a major role in the phylogeographic history of some ECM fungi in the Northern Hemisphere. Our results may have implications for studies on the biodiversity, ecology and conservation of arctic fungi in general.

*Correspondence: József Geml, National Herbarium of the Netherlands, Netherlands Centre for Biodiversity Naturalis, Leiden University, P.O. Box 9514, Einsteinweg 2, 2300 RA Leiden, The Netherlands.
E-mail: geml@nhn.leidenuniv.nl

Keywords

Arctic, biodiversity, climate change, dispersal, fungi, gene flow, ITS rDNA, long-distance dispersal, migration, phylogeography.

INTRODUCTION

Mycorrhizal associations are abundant and widespread in almost all ecosystems, and c. 80% of land plant species form associations with mycorrhizal fungi (Trappe, 1987). In mycorrhizal symbioses, fungi support plants with mineral nutrients, water and other services, and the fungi in turn receive photosynthates from the autotrophic plants. They play a central role in the functioning of terrestrial arctic ecosystems, where, with the exception of a few graminoid species (*Carex* spp., *Eriophorum* spp.) and herbs of the Brassicaceae, arctic plants are highly dependent on mutualistic relationships with mycorrhizal fungi for survival in these nutrient-poor environments (Hobbie *et al.*, 2009). Ectomycorrhiza (ECM) is the predominant mycorrhiza type in arctic and alpine environments (Gardes & Dahlberg, 1996; Fujimura *et al.*, 2008; Bjorbækmo, 2009; Bjorbækmo *et al.*, 2010; D.L.T., unpublished data), and ECM fungi are crucial for the survival of arctic shrubs (e.g. *Betula*, *Dryas*, *Salix*). Reflecting our current state of knowledge, fungi have frequently been lumped with other soil microbes into a 'black box' in ecological studies, and fungal communities in arctic regions remain largely unknown (Callaghan *et al.*, 2004).

As a result of warming temperatures, shifts in land surface vegetation have already been observed (e.g. Chapin *et al.*, 1995; Bret-Harte *et al.*, 2002; Stow *et al.*, 2004). ECM fungi are expected to play an important role in arctic vegetation change, particularly in expansion of shrub cover. However, possible responses of ECM fungi to climate change and their potential roles in vegetation change are essentially unknown. The ability of fungi to cope with the changing arctic environment is likely to be related to their ability to follow shifts in regional climate, to colonize newly exposed, suitable habitats (e.g. following receding glaciers); and to exchange genes with populations inhabiting different geographic regions. The capacity to migrate is of particular importance because climate warming is expected to cause a northward shift in the distribution of many arctic species, and the long-distance dispersal capabilities of individual species will greatly influence the composition of future arctic communities (Alsos *et al.*, 2007).

There is considerable disagreement in the scientific community concerning the ability of fungi to disperse over long distances and become established (Kärnefelt, 1990; Galloway & Aptroot, 1995; Brown & Hovmöller, 2002; Moyersoen *et al.*, 2003; Feuerer & Hawksworth, 2007; Moncalvo & Buchanan, 2008; Printzen, 2008). Despite the fact that long-distance dispersal has been recorded in some fungi (e.g. Moyersoen *et al.*, 2003; Moncalvo & Buchanan, 2008), many boreal, temperate or tropical fungi show strong phylogeographic

patterns and limited dispersal, and there are rapidly accumulating examples of geographic endemism (Taylor *et al.*, 2006 and references therein; Geml *et al.*, 2008; Bergemann *et al.*, 2009). For example, morphological species complexes of fungi from the Northern Hemisphere have generally been shown to include two major lineages, one Eurasian and one North American (Shen *et al.*, 2002; Geml *et al.*, 2006; Taylor *et al.*, 2006). In most studied fungi, such allopatric phylogenetic clades inhabit similar environments in different continents, which implies a phylogenetic structure that has arisen as a result of the lack of intercontinental dispersal. Exceptions to this general trend come predominantly from fungi associated with humans, which are therefore more likely to be dispersed via shipment of goods: for example, plant pathogens of agricultural crops (e.g. Couch *et al.*, 2005); indoor fungi (e.g. Kauserud *et al.*, 2006); and fungi that are almost exclusively clonal and produce very high quantities of airborne mitospores (e.g. Rydholm *et al.*, 2006). Successful long-distance dispersal events and establishment are considered somewhat rare for fungi in nature (Brown & Hovmöller, 2002).

In ECM fungi, the majority of previous phylogeographic studies revealed patterns consistent with vicariance associated with breakup of the continents and/or with migration over land (Wu & Mueller, 1997; Wu *et al.*, 2000; Mueller *et al.*, 2001; Geml *et al.*, 2006, 2008; Nuytinck *et al.*, 2007; Halling *et al.*, 2008; Matheny *et al.*, 2009). Although long-distance dispersal has been reported in at least some ECM taxa in the Southern Hemisphere (Moyersoen *et al.*, 2003; Matheny *et al.*, 2009), transoceanic dispersal appears to be rare in basidiomycete ECM fungi and strong spatial genetic structure is emerging as the norm (Bergemann & Miller, 2002; Kretzer *et al.*, 2005; Grubisha *et al.*, 2007; Mueller & Schmit, 2007; Nuytinck *et al.*, 2007; Geml *et al.*, 2008; Halling *et al.*, 2008; Matheny *et al.*, 2009). Furthermore, Peay *et al.* (2010) showed that dispersal limitation is important for ECM fungal communities even on the landscape scale, with up to a 50% decrease in species diversity in tree islands from 0 to 1000 m distance from the forest edge.

Because most studies on ECM taxa focused on species inhabiting low- to mid-latitude forests, very little is known about the diversity and phylogeography of arctic ECM fungi. In addition, most studies of ECM ecology have focused on small-scale mechanisms that explain patterns of community composition, and there is a need for studies on large-scale, dispersal-related processes (Peay *et al.*, 2010). In this paper we address both of these understudied research areas by studying the importance of long-distance colonization in arctic ECM fungi. In addition to theoretical advancement of our knowledge on the role of long-distance dispersal in shaping ECM communities, studying migration has practical implications for

understanding the composition of past, present and future communities during shifts in species distributions due to climatic changes. In this study, our goal was to assess the capacity of arctic ECM fungi to disperse over large distances. Therefore we analysed the phylogenetic diversity of fungal communities on the high arctic archipelago of Svalbard.

The Svalbard Archipelago (a group of islands ranging from 74 to 81° N and 10 to 35° E) has been proposed as a good model system for studying long-distance dispersal in the Arctic, because of its remote location and glaciation history (Alsos *et al.*, 2007). Geologically, Svalbard shows a close affinity with north-eastern Greenland and the Canadian Archipelago, from which Svalbard has been separated since the Late Cretaceous (Eldholm & Thiede, 1980). Land bridges and island chains may have existed between Svalbard and north-eastern Greenland during parts of the Palaeocene–Eocene until the mid-Oligocene (*c.* 32 Ma), because the separating sea was shallow, becoming deep only later. There is some indication of a past land connection between Svalbard and mainland Eurasia in the Miocene (Rasmussen *et al.*, 2008), with Svalbard certainly having been isolated since at least the Pliocene, probably for longer (Jan Mangerud, University of Bergen, pers. comm.). During the Pleistocene, Svalbard was fully or almost fully glaciated repeatedly during the glacial cycles (Landvik *et al.*, 1998), with virtually no vegetation surviving *in situ* in the archipelago. Prior to using molecular markers, it had been debated whether Svalbard's flora had survived in local refugia, but genetic studies have indicated that colonization by plants occurred after the glacial retreat (Abbott & Brochmann, 2003; Brochmann *et al.*, 2003) with the possible exception of a few very hardy, non-ECM species (Westergaard *et al.*, 2010a,b). This is in agreement with reconstructions suggesting an extreme ice cover that excluded the local survival of most terrestrial plant species (Landvik *et al.*, 1998), and with palaeorecords that show evidence for arctic vegetation only after 10,000 years ago (Birks *et al.*, 1994).

Out of the five bioclimatic subzones (A–E, cold to warm) of the circumpolar Arctic, the coldest three (A–C) can be found in the ice-free areas of Svalbard (Walker *et al.*, 2005). Each of these subzones has its own climate and distinctive vegetation, 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, and 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 snow bed communities (Raynolds *et al.*, 2008). A more complete description of the typical vegetation found in each subzone is available in Walker *et al.* (2005).

While Svalbard is among the best studied arctic areas both in general terms and for mycological explorations, previous studies on Svalbard ECM fungi mainly comprised reports of mycorrhizal morphotypes, host plants and lists of morphological species (Väre *et al.*, 1992; Gulden & Torkelsen, 1996).

In the only molecular study to date, Bjorbækmo *et al.* (2010) investigated ECM colonization of *Dryas octopetala* along a latitudinal gradient from southern Norway to Svalbard. However, no comprehensive molecular study has been published on the biodiversity and phylogeographic origin of ECM species occurring on Svalbard.

Because many groups of fungi are poorly known with respect to taxonomy and phylogeography, the observation of previously unknown fungal species within these groups on Svalbard cannot presently be used to make strong inferences concerning colonization history. However, fungal groups that have been well studied with respect to molecular systematics at high latitudes provide opportunities to draw clear inferences. By focusing on previously described fungi with available data from multiple locations, examination of phylogenetic patterns can help us infer their possible biogeographic histories. For example, if long-distance dispersal has been rare, and Miocene or Pliocene vicariance, genetic isolation, *in situ* differentiation and survival in glacial refugia are responsible for a significant fraction of fungal diversity on Svalbard, we would expect to see the emergence of unique sequence types and/or phylogroups endemic to Svalbard in the majority of the lineages: under this scenario, phylogroups that occur both on Svalbard and elsewhere should comprise a low proportion of taxa within these groups. In contrast, if recent and/or continuing long-distance dispersal explains the bulk of fungal diversity on Svalbard, then the majority of phylogroups found in Svalbard are expected to occur elsewhere as well. In addition, the degree of sequence divergence within taxa, and the geographic distribution of haplotypes found on Svalbard and elsewhere, can be used to test for geographic differentiation and intercontinental migration rates under a coalescent model. A lack of statistically significant geographic differentiation would argue against vicariance and *in situ* speciation, and in favour of recent gene flow via long-distance dispersal. Admittedly, evidence for recent migration between Svalbard and other areas, and the presence of extant ECM taxa in Svalbard at the time of separation from other land masses, are not necessarily mutually exclusive scenarios (see below). Nonetheless, detecting patterns consistent with recent migration between arctic areas would still mean that these ECM fungi are capable of long-distance dispersal, regardless of how long Svalbard has been inhabited by them.

MATERIALS AND METHODS

Isolates and molecular work

Soil samples were taken between 27 and 30 July 2007 at three sites in Brøggerhalvøya, representing different vegetation types in the vicinity of Ny-Ålesund (78°55' N, 11°56' E) (Table 1; Fig. 1). At each site, we sampled soils from five non-sorted circles ('frost boils') and adjacent interboil areas. Due to the high spatial heterogeneity of soil fungal communities, we collected 20 cores, 1.8 cm in diameter and 5–10 cm deep, from each boil, and 20 cores from each interboil. Cores from the

Table 1 Description of soil sampling sites near Ny-Ålesund used in this study.

| Site | Bioclimatic subzone | Vegetation |
|---|---------------------|--|
| Site 1, Outer Kongsfjord; 78°56′09.6″ N, 11°48′03.7″ E | B | <i>Salix polaris</i> and <i>Dryas octopetala</i> tundra with <i>Minuartia stricta</i> , <i>Pedicularis lanata</i> , <i>Oxyria digyna</i> , <i>Saxifraga cernua</i> , <i>Cerastium arcticum</i> |
| Site 2, Inner Kongsfjord; 78°54′44.5″ N, 11°58′55.5″ E | C | <i>Cassiope tetragona</i> tundra with <i>Racomitrium</i> , <i>Pedicularis lanata</i> , <i>Cerastium arcticum</i> , <i>Papaver dahlianum</i> , <i>Silene acaulis</i> , <i>Sagina nivalis</i> , <i>Minuartia stricta</i> , <i>Draba</i> sp. |
| Site 3, Outer Kongsfjord; 78°55′16.5″ N, 11°51′21.9″ E | B | <i>Salix polaris</i> and <i>Dryas octopetala</i> tundra with <i>Oxyria digyna</i> , <i>Pedicularis lanata</i> , <i>Saxifraga nivalis</i> , <i>Saxifraga cernua</i> , <i>Cerastium arcticum</i> , <i>Cochlearia officinalis</i> , <i>Silene acaulis</i> |

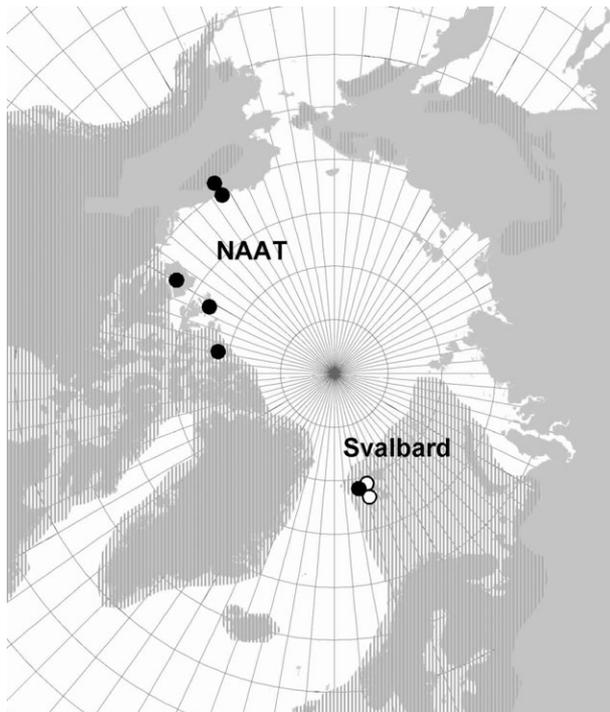


Figure 1 Map of the circumpolar Arctic, showing the geographic locations of soil (solid black circles) and sporocarp (solid white circles) samples collected in this study. Shading indicates areas that were glaciated during the Last Glacial Maximum. NAAT, North American Arctic Transect.

same boil or interboil were pooled and mixed, and frozen as quickly as possible, usually within 2 h of sampling. DNA was extracted separately for each frost boil and interboil, resulting in a total of 30 soil DNA extractions (from 600 cores). In addition, we generated DNA sequences from 132 curated specimens of ECM fungi (*Alnicola*, *Cortinarius*, *Hebeloma*, *Inocybe*, *Laccaria*, *Lactarius*, *Leccinum*, *Russula*, *Thelephora*) collected by M.E.N. and J.G. and deposited at the National Herbarium of the Netherlands (L), and from collections of the Natural History Museum of the University of Oslo (O). The majority of these samples had been collected in various areas of Svalbard, including the vicinities of Longyearbyen (Isfjorden) and Ny-Ålesund (Kongsfjorden). DNA was extracted from

small samples of dried specimens using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA).

The entire internal transcribed spacer (ITS) region of the nuclear ribosomal DNA repeat (rDNA) was polymerase chain reaction (PCR) amplified with forward and reverse primers ITS1F (Gardes & Bruns, 1993) and TW13 (Taylor & Bruns, 1999), respectively. PCR was performed using the following temperature programme: 95 °C for 2 min, 25 or 34 cycles of 95 °C for 30 s, 54 °C for 1 min, 72 °C for 2 min and 72 °C for 10 min. For each soil DNA extract, seven replicate PCRs were performed and pooled. To minimize chimera formation, 25 PCR cycles were performed for soil samples. 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 TOPO TA 4.0 vectors (Invitrogen, Carlsbad, CA, USA), then shipped transformed plasmids frozen to the Broad Institute of Massachusetts Institute of Technology and Harvard University, 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 are described in detail in Taylor *et al.* (2007, 2008, 2010) and Geml *et al.* (2009, 2010).

Delimitation of operational taxonomic units

Sequence data obtained for both strands were edited and assembled for each sporocarp or soil clone using ALIGNER 1.3.4 (CodonCode, Dedham, MA, USA). For this paper, sequences from frost boils and interboils were pooled, because our goal was to assess the overall diversity of the ECM communities. After discarding sequence reads with low base call quality scores from the initial 3072 soil clone ITS sequences, we retained 2810 ITS sequences of sufficiently high quality. Subsequently, we excluded 477 sequences that were suspected to be inter- or intragenomic chimeras. We regarded a sequence as an intergeneric chimera when it had > 90% sequence similarity to different genera in ITS1 and ITS2, while we considered sequences intragenomic chimeras when their ITS1 and ITS2 regions were 100% similar to different species.

We grouped the remaining 2333 soil clones into operational taxonomic units (OTUs) to reduce the sample size for the phylogenetic analyses. Such groupings, based on a specified

similarity cut-off, have been routinely used in ECM studies as a proxy for species, accounting for intraspecific variation and errors generated by PCR. Although there is no universal cut-off value for species delimitation in fungi, we used 97% ITS sequence similarity based on previous studies (e.g. O'Brien *et al.*, 2005; Arnold & Lutzoni, 2007; Higgins *et al.*, 2007; Geml *et al.*, 2009), because this cut-off value tends to provide a conservative, yet reasonably accurate, estimate of species diversity in the communities sampled. Pairwise sequence similarity-based groupings were estimated by CAP3 (Huang & Madan, 1999). Note that CAP3 uses a single-linkage clustering algorithm, meaning that sequences less than, for example, 97% similar will be grouped together if an intermediate sequence greater than 97% similar to both is found. We identified soil clone OTUs to genera, based on similarity searches using FASTA 3 (Pearson & Lipman, 1988) against a reference database containing all fungal ITS sequences from GenBank. We carried out subsequent analyses using only OTUs that were identified as members of ECM basidiomycete genera (e.g. *Alnicola*, *Clavulina*, *Cortinarius*, *Hebeloma*, *Inocybe*, *Laccaria*, *Lactarius*, *Leccinum*, *Russula*, *Sebacina*, *Tomentella*, *Thelephora*). Because sequencing errors can overestimate the diversity of 'rare taxa' (e.g. Kunin *et al.*, 2010), we opted to be conservative and excluded all singletons from the phylogenetic analyses. We realize that this may have resulted in the exclusion of some real taxa. However, in our preliminary analyses, almost all singletons grouped around the distinct OTUs, suggesting that our conservative approach did not significantly affect the final results. Because of the lack of reference sequences from arctic sites in publicly available databases, we compared the Svalbard soil clone sequences with our soil sequences (D.L.T., unpublished data) generated from sites along the North American Arctic Transect (NAAT), which spans more than 1000 km from the Brooks Range in Alaska to Ellef Ringnes Island in the Canadian Arctic (Fig. 1; <http://www.geobotany.uaf.edu/naat>). We included 53 soil clone sequences representing 97% ITS sequence identity OTUs that showed highest similarity to the Svalbard sequences. Sequences were deposited in GenBank (sporocarps: GU234009–GU234165 and JF304373–JF304420; Svalbard soil clone OTUs: HQ215749–HQ215833; NAAT soil clone OTUs: JF304320–JF304372).

Phylogenetic analysis

A randomly chosen clone was selected from each OTU for phylogenetic analyses. Sequences derived from sporocarps were included to link soil clones to taxa with voucher specimens. Because our goal was to identify the phylogenetic lineages present in Svalbard, the most similar publicly available sequences, as reported by the similarity searches discussed above, were downloaded from GenBank and UNITE (<http://unite.ut.ee>). From these, we selected non-identical representatives for the phylogenetic analyses. We generated multiple sequence alignments based on our Svalbard sequences and the most similar sequences in GenBank using CLUSTAL W 2.0.12 (Thompson *et al.*, 1997). The

alignments were subsequently refined using MUSCLE 3.7 (Edgar, 2004), and finally corrected manually if necessary. In *Thelephora/Tomentella*, we used an alignment previously published by Taylor & Bruns (1997) as a profile alignment, to which we added our congeneric sequences. Because our goal was not to reconstruct the phylogenies of these genera, but to identify taxa in our samples and to infer phylogeographic connections, we concentrated on lineages that were the most closely related to our sequences. Phylogenetic analyses were conducted using maximum-likelihood (ML) and Bayesian methods in GARLI 2.0 (Zwickl, 2006) and MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001), respectively. The best-fit evolutionary model was determined by comparing different nucleotide substitution models with varying values of base frequencies, substitution types, alpha-parameter for the gamma-distribution of variable sites, and proportion of invariable sites via the Akaike information criterion (AIC) using PAUP* 4.0 (Swofford, 2002) and MODELTEST 3.7 (Posada & Crandall, 1998). Gaps were scored as 'missing data'. Phylogroups were recognized as the smallest clades receiving > 0.95 Bayesian posterior probability support. It is worth noting that we do not claim any of the phylogroups to be true species, as species delimitation would require multi-gene analysis and morphological work (which is beyond the scope of this paper and impractical for soil-derived sequences). This may not be a crucial shortcoming, however, as dispersal patterns can be discerned from the phylograms regardless of species limits. Acknowledging our current limitations, recognizing the fundamental units of evaluation using the above statistical criteria seems the most sensible and uniformly applicable approach. By recognizing the smallest supported clades, we are in fact delimiting our units in a conservative manner, because the smaller the clade, the lower the chance its members will be found in multiple locations, thus the less supportive it is of the long-distance dispersal hypothesis. In rare cases with paraphyletic groupings, we distinguished both the more inclusive clade and the significantly supported derived clade (e.g. phylogroups 7 and 17 in *Tomentella*, 17 in *Cortinarius*, 4 and 5 in *Inocybe*, 2 in *Hebeloma*) to be consistent with our approach of applying statistical criteria for phylogroup delimitation. In addition, when 97% OTUs from the same soil samples were sister groups, we recognized each as a distinct phylogroup. This practice was based on our former studies on various basidiomycete genera, where 97% groupings almost always contained at least one, sometimes more, supported phylogroups, when multiple sequences per OTUs were included in the phylogenetic analyses (Geml *et al.*, 2008, 2009, 2010). The University of Alaska Life Sciences Informatics cluster portal (<http://biotech.inbre.alaska.edu>) was used for all analyses.

Coalescent analyses

In order to estimate recent gene flow among Svalbard and other arctic areas, we selected the only ECM species in our sample with a sufficient number of sequences available from

different geographic areas (Eurasia, North America and Svalbard): *Cortinarius favrei*. Available DNA sequences generated from specimens collected at various Eurasian and North American localities were obtained from GenBank and aligned with our sequences using CLUSTAL W. Identical sequences were collapsed into haplotypes using SNAP MAP (Aylor *et al.*, 2006) after recoding insertion or deletions (indels) and excluding infinite-sites violations. The analyses presented here assume an infinite sites model, under which a polymorphic site is caused by exactly one mutation and there can be no more than two segregating bases. Base substitutions were categorized as phylogenetically uninformative or informative, and as transitions or transversions. Site-compatibility matrices were generated from each haplotype dataset using SNAP CLADE and MATRIX (Markwordt *et al.*, 2003; Bowden *et al.*, 2008) to examine compatibility/incompatibility among all variable sites, with any resulting incompatible sites removed from the dataset. Genetic differentiation among geographic populations was analysed using SNAP MAP, SEQTOMATRIX and PERMTEST (Hudson *et al.*, 1992) implemented in SNAP WORKBENCH 2.0 (Price & Carbone, 2005). PERMTEST is a nonparametric permutation method based on Monte Carlo simulations that estimates Hudson's test statistics (K_{ST} , K_S and K_T) under the null hypothesis of no genetic differentiation. K_{ST} is equal to $1 - K_S/K_T$, where K_S is a weighted mean of K_1 and K_2 (mean number of differences between sequences in subpopulations 1 and 2, respectively) and K_T represents the mean number of differences between two sequences regardless of the subpopulation to which they belong. The null hypothesis of no genetic differentiation is rejected ($P < 0.05$) when K_S is small and K_{ST} is close to 1.

For this test, specimens were assigned to geographic groups (North America, Eurasia and Svalbard).

Analyses were performed on population pairs 'Svalbard' versus 'North America' and 'Svalbard' versus 'Eurasia' to estimate migration between the remote archipelago of Svalbard and the northern continents. We used MDIV (Nielsen & Wakeley, 2001), implemented in SNAP WORKBENCH (Price & Carbone, 2005), to determine if the diversity patterns in different geographic areas were the result of retention of ancestral polymorphism or recent gene flow. MDIV employs both likelihood and Bayesian methods using Markov chain Monte Carlo (MCMC) coalescent simulations to estimate the migration parameter (M) and the population mean mutation rate (θ). M is defined as the effective number of migrants exchanged between two populations each generation and it equals $2 \times$ the net effective population size (N_e) multiplied by the migration rate per generation, m . θ equals $2N_e$ multiplied by the mutation rate per generation, μ . For each dataset, the data were simulated assuming an infinite sites model with uniform prior. We used 2,000,000 steps in the chain for estimating the posterior probability distribution and an initial 500,000 steps to ensure that enough genealogies were simulated before approximating the posterior distribution.

RESULTS

We recovered 332 distinct non-singleton OTUs for all fungi from the Svalbard soil sequences based on a threshold of 97% ITS sequence similarity. Of these, basidiomycete ECM genera contained 72 OTUs, while the total number of 'soil +

Table 2 Number of Svalbard soil clone sequences, soil clone 97% internal transcribed spacer (ITS) nuclear ribosomal DNA sequence similarity operational taxonomic units (OTUs), sporocarp sequences, characteristics of the resulting multiple sequence alignments and phylograms, and number of phylogroups based on both soil and sporocarp sequence data.

| Genus | Svalbard soil clones | Svalbard soil clone OTUs | Sporocarps | Alignment (sequences/characters) | Best-fit evolutionary model† | ML score ($-\ln L$)‡ | Phylogroups |
|------------------------------|----------------------|--------------------------|------------|----------------------------------|------------------------------|------------------------|-------------|
| <i>Alnicola</i> | 6 | 1 | 2 | 38/648* | TVM+I+G* | 1757.0743* | 1 |
| <i>Clavulina</i> | 15 | 1 | 0 | 10/662 | GTR+I | 1510.8028 | 1 |
| <i>Cortinarius</i> | 486 | 12 | 53 | 105/730 | GTR+I+G | 4799.6324 | 19 |
| <i>Entoloma</i> | 11 | 3 | 4 | 19/661 | TrN+G | 2963.2452 | 4 |
| <i>Hebeloma</i> | 31 | 2 | 20 | 38/648* | TVM+I+G* | 1757.0743 | 7 |
| <i>Inocybe</i> | 356 | 17 | 30 | 148/983 | TVM+I+G | 25033.8544 | 27 |
| <i>Laccaria</i> | 23 | 2 | 7 | 16/659 | TVM+I | 1328.9822 | 2 |
| <i>Lactarius</i> | 2 | 1 | 10 | 38/720 | TVM+I+G | 2979.5695 | 6 |
| <i>Leccinum</i> | 0 | 0 | 1 | 19/596 | HKY+I+G | 1322.1423 | 1 |
| <i>Russula</i> | 24 | 2 | 18 | 48/710 | TrN+G | 4054.8889 | 8 |
| <i>Sebacina</i> | 24 | 3 | 0 | 18/663 | TVM+I+G | 2650.4979 | 3 |
| <i>Thelephora/Tomentella</i> | 323 | 30 | 1 | 130/713 | SYM+I+G | 11997.6717 | 30 |

*Because of their close phylogenetic relationship, *Alnicola* and *Hebeloma* species were included in the same alignment.

†HKY, Hasegawa–Kishino–Yano (Hasegawa *et al.*, 1985); GTR, general time reversible (Tavaré, 1986); SYM, symmetrical (Zharkikh, 1994); TrN, Tamura–Nei (Tamura & Nei, 1993); TVM, transversional (D. Posada, unpublished, except in MODELTEST 3.7 manual at <http://darwin.uvigo.es>); I, proportion of invariable sites; G, shape parameter of gamma distribution.

‡ML, maximum likelihood; $\ln L$, log likelihood.

sporocarp phylogroups was 109. The most diverse basidiomycete ECM genera in our samples were (with the number of soil clone OTUs and phylogroups in parentheses, respectively): *Thelephora/Tomentella* (30 OTUs, 30 phylogroups), *Inocybe* (17, 27), and *Cortinarius* (11, 19). Additional genera in the order of decreasing diversity were *Russula* (2, 8), *Hebeloma* (2, 7), *Lactarius* (1, 6), *Entoloma* (3, 4), *Sebacina* (3, 3), *Clavulina* (1, 1), *Laccaria* (2, 2), *Leccinum* (0, 1) and *Alnicola* (1, 1). The number of sequences and characters for each multiple sequence alignments based on both soil and sporocarp sequence data, the best-fit evolutionary models, the likelihood scores of the ML phylograms, and the number of phylogroups are shown in Table 2.

The extent to which we could successfully identify taxa to species varied greatly among genera, which is not surprising, given the largely unknown global diversity of most of these groups. Out of the 109 inferred phylogroups in total, we were able to identify 62 to known species or species complexes, while 47 remained unidentified (Table 3). For example, the highly diverse *Thelephora/Tomentella* group, with often inconspicuous sporocarps, is notoriously difficult to identify and poses serious taxonomic challenges, with many available reference sequences originating from ECM root samples (e.g. Bjorbækmo, 2009; Barrett *et al.*, 2010). In this lineage, 11 phylogroups were identified to species or species complexes, while five phylogroups represented previously sequenced but unidentified taxa, forming supported clades with publicly available sequences (*Thelephora/Tomentella* 6, 7, 13, 16 and 22). The remaining 14 phylogroups are not represented in public databases and may or may not represent novel taxa (see Appendix S1a in Supporting Information). Studies in virtually all other sampled ecosystems routinely uncover numerous new phylogroups in this lineage (Gardes & Bruns, 1993; Taylor & Bruns, 1997; Becerra *et al.*, 2005; Barrett *et al.*, 2010).

Among arctic fungi with agaric ('mushroom') sporocarps, the genus *Cortinarius* is considered the most diverse in arctic regions in general (Gulden & Torkelsen, 1996; Karatygin *et al.*, 1999; J.G., pers. obs.). This genus was indeed very diverse in our sample as well, although the genus *Inocybe* proved to be even more diverse. Of the 19 detected *Cortinarius* phylogroups, 15 were identified to species or species complexes, while the remaining four phylogroups are not represented in public databases (Table 3; Appendix S1b). Within *Cortinarius*, 16 out of 19 phylogroups were also represented by sequences from outside Svalbard. In *Inocybe*, we found 27 phylogroups, of which 16 remained unidentified (Appendix S1c). We found 18 of the 27 phylogroups in *Inocybe* with representation outside Svalbard, and nine without sequence matches. Given the rapid evolution of the ITS and massive undocumented diversity within this genus (Ryberg *et al.*, 2008), we do not find the nine previously unsequenced phylogroups at all surprising. In *Hebeloma*, we identified seven phylogroups to species that have been found outside Svalbard, and found one previously unsequenced taxon. *Alnicola*, a genus closely related to *Hebeloma*, was represented by a single species: *Alnicola tantilla*,

Table 3 Tentative identification of phylogroups of ectomycorrhizal fungi in Svalbard and their known distribution outside Svalbard.

| Phylogroup* | Identification† | Known distribution‡ |
|--------------------|--|---------------------|
| <i>Alnicola</i> | | |
| 1 | <i>Alnicola tantilla</i> | EU |
| <i>Clavulina</i> | | |
| 1 | <i>Clavulina rugosa</i> | NA, EU |
| <i>Cortinarius</i> | | |
| 1 | <i>Cortinarius</i> sp. | – |
| 2 | <i>Cortinarius obtusus</i> | NA |
| 3 | <i>Cortinarius olivaceofuscus</i> | EU |
| 4 | <i>Cortinarius delibutus</i> | EU |
| 5 | <i>Cortinarius flos-paludis</i> | NA |
| 6 | <i>Cortinarius saturninus</i> | EU |
| 7 | <i>Cortinarius</i> sp. | – |
| 8 | <i>Cortinarius parvannulatus</i> | NA, EU |
| 9 | <i>Cortinarius</i> aff. <i>flexipes</i> | NA |
| 10 | <i>Cortinarius</i> sp. | – |
| 11 | <i>Cortinarius saniosus</i> | NA, EU |
| 12 | <i>Cortinarius</i> aff. <i>saniosus</i> | – |
| 13 | <i>Cortinarius</i> aff. <i>erythrinus</i> | NA |
| 14 | <i>Cortinarius</i> sp. | NA |
| 15 | <i>Cortinarius</i> aff. <i>albonigrellus</i> | NA |
| 16 | <i>Cortinarius decipiens</i> | EU |
| 17 | <i>Cortinarius atrocoeruleus</i> | NA, EU |
| 18 | <i>Cortinarius fulvescens</i> | NA |
| 19 | <i>Cortinarius favrei</i> | NA, EU |
| <i>Entoloma</i> | | |
| 1 | <i>Entoloma caeruleopolitum</i> | EU |
| 2 | <i>Entoloma</i> sp. | – |
| 3 | <i>Entoloma</i> sp. | – |
| 4 | <i>Entoloma</i> sp. | – |
| <i>Hebeloma</i> | | |
| 1 | <i>Hebeloma mesophaeum</i> | NA, EU |
| 2 | <i>Hebeloma</i> aff. <i>mesophaeum</i> | NA |
| 3 | <i>Hebeloma</i> sp. | – |
| 4 | <i>Hebeloma velutipes</i> | EU |
| 5 | <i>Hebeloma incarnatulum</i> | EU |
| 6 | <i>Hebeloma helodes</i> | NA |
| 7 | <i>Hebeloma crustuliniforme</i> | NA, EU |
| <i>Inocybe</i> | | |
| 1 | <i>Inocybe dulcamara</i> | EU |
| 2 | <i>Inocybe leucoblema</i> | EU |
| 3 | <i>Inocybe</i> aff. <i>arthrocystis</i> | NA |
| 4 | <i>Inocybe</i> aff. <i>malenconii</i> | EU |
| 5 | <i>Inocybe</i> sp. | EU |
| 6 | <i>Inocybe</i> sp. | NA |
| 7 | <i>Inocybe</i> sp. | NA |
| 8 | <i>Inocybe</i> sp. | NA |
| 9 | <i>Inocybe bulbosissima</i> | EU |
| 10 | <i>Inocybe rimosa</i> | EU |
| 11 | <i>Inocybe flavella</i> | EU |
| 12 | <i>Inocybe</i> aff. <i>muricellata</i> | NA |
| 13 | <i>Inocybe</i> sp. | NA |
| 14 | <i>Inocybe</i> aff. <i>nematoloma</i> | NA, EU |
| 15 | <i>Inocybe</i> sp. | – |
| 16 | <i>Inocybe</i> sp. | – |

Table 3 Continued

| Phylogroup* | Identification† | Known distribution‡ |
|------------------------------|--|---------------------|
| 17 | <i>Inocybe</i> sp. | – |
| 18 | <i>Inocybe</i> sp. | NA |
| 19 | <i>Inocybe</i> sp. | – |
| 20 | <i>Inocybe</i> sp. | NA |
| 21 | <i>Inocybe</i> sp. | – |
| 22 | <i>Inocybe</i> sp. | – |
| 23 | <i>Inocybe hirculus</i> | NA, EU |
| 24 | <i>Inocybe oreina</i> | EU |
| 25 | <i>Inocybe</i> sp. | – |
| 26 | <i>Inocybe</i> sp. | NA |
| 27 | <i>Inocybe</i> sp. | – |
| <i>Laccaria</i> | | |
| 1 | <i>Laccaria</i> aff. <i>laccata</i> | NA |
| 2 | <i>Laccaria pumila</i> | NA |
| <i>Lactarius</i> | | |
| 1 | <i>Lactarius torminosus</i> | NA |
| 2 | <i>Lactarius</i> sp. | NA |
| 3 | <i>Lactarius glyciosmus</i> | NA, EU |
| 4 | <i>Lactarius</i> sp. | NA |
| 5 | <i>Lactarius dryadophilus</i> | NA |
| 6 | <i>Lactarius lanceolatus</i> | EU |
| <i>Leccinum</i> | | |
| 1 | <i>Leccinum rotundifoliae</i> | EU |
| <i>Russula</i> | | |
| 1 | <i>Russula nana</i> | NA, EU |
| 2 | <i>Russula</i> aff. <i>betularum</i> | NA |
| 3 | <i>Russula</i> sp. | NA, EU |
| 4 | <i>Russula pascua</i> | EU |
| 5 | <i>Russula xerampelina</i> | NA |
| 6 | <i>Russula</i> aff. <i>decolorans</i> | NA |
| 7 | <i>Russula</i> sp. | NA |
| 8 | <i>Russula brevipes</i> | NA |
| <i>Sebacina</i> | | |
| 1 | <i>Sebacina</i> aff. <i>epigaea</i> | – |
| 2 | <i>Sebacina</i> sp. | NA |
| 3 | <i>Sebacina</i> sp. | NA |
| <i>Thelephora/Tomentella</i> | | |
| 1 | <i>Tomentella</i> sp. | NA |
| 2 | <i>Tomentella</i> sp. | NA |
| 3 | <i>Tomentella</i> sp. | – |
| 4 | <i>Tomentella</i> sp. | NA |
| 5 | <i>Tomentella</i> sp. | – |
| 6 | <i>Tomentella</i> sp. | EU |
| 7 | <i>Tomentella</i> sp. | NA |
| 8 | <i>Tomentella</i> sp. | – |
| 9 | <i>Tomentella</i> aff. <i>pirolae</i> | – |
| 10 | <i>Tomentella</i> aff. <i>subtestacea</i> | NA |
| 11 | <i>Tomentella</i> sp. | – |
| 12 | <i>Tomentella</i> sp. | NA |
| 13 | <i>Tomentella</i> sp. | NA, EU |
| 14 | <i>Tomentella</i> sp. | – |
| 15 | <i>Tomentella</i> sp. | – |
| 16 | <i>Tomentella</i> sp. | NA |
| 17 | <i>Tomentella badia</i> | EU |
| 18 | <i>Tomentella</i> aff. <i>atramentaria</i> | NA |
| 19 | <i>Tomentella</i> sp. | NA |

Table 3 Continued

| Phylogroup* | Identification† | Known distribution‡ |
|-------------|--|---------------------|
| 20 | <i>Tomentella</i> aff. <i>stuposa</i> | NA |
| 21 | <i>Tomentella</i> sp. | – |
| 22 | <i>Tomentella</i> sp. | NA |
| 23 | <i>Tomentella</i> aff. <i>lapida</i> | NA |
| 24 | <i>Thelephora caryophyllea</i> | NA, EU |
| 25 | <i>Thelephora</i> aff. <i>anthocephala</i> | – |
| 26 | <i>Thelephora</i> aff. <i>anthocephala</i> | – |
| 27 | <i>Thelephora</i> aff. <i>anthocephala</i> | – |
| 28 | <i>Tomentella</i> sp. | NA |
| 29 | <i>Tomentella</i> sp. | – |
| 30 | <i>Tomentella</i> aff. <i>terrestris</i> | NA |

*Phylogroup designations correspond to those in Appendix S1.

†Although many species listed are known to have a wider distribution than indicated here, we include only locations with available sequence data.

‡NA, North America; EU, Eurasia.

also found outside Svalbard (Appendix S1d). The sampled taxa in the Russulaceae family included species in two genera: *Lactarius* and *Russula*, with four and six identified species, respectively, and two unidentified taxa in each genus (Appendix S1e,f). All Svalbard species in *Russula* and *Lactarius* had sequence matches from outside Svalbard. In *Clavulina*, we detected *Clavulina rugosa*, also found in mainland Europe and North America. The genus *Entoloma* was represented by *Entoloma caeruleopolitum* and three unidentified phylogroups. There were two *Laccaria* species in our samples, both with sequence matches from North America. In *Sebacina*, we detected *Sebacina* aff. *epigaea* and two unidentified *Sebacina* taxa. The *S.* aff. *epigaea* phylogroup from Svalbard occupied a long branch and its status is uncertain, while the two unidentified species from Svalbard had closest matching sequences among our soil clones from the North American Arctic. Boletes were represented by a single species: *Leccinum rotundifoliae* (Appendix S1g), also grouped with a sequence from mainland Europe and known to occur in the circumpolar Arctic (den Bakker *et al.*, 2007).

Of the 109 phylogroups in total, 80 are known to occur outside Svalbard. These include all previously sequenced species and numerous previously unsequenced lineages that were also found in our soil sequences from the North American Arctic Transect. In summary, for the majority of the phylogroups, that is, those that contain highly similar DNA sequences from multiple continents, our results support long-distance dispersal more strongly than vicariance.

Coalescent analyses

We estimated migration rates among the northern continents and Svalbard in *C. favrei*, for which sufficient sequence data were available from Eurasia, North America and Svalbard. The dataset consisted of 53 sequences and 610 characters, including

gaps, of which 37 were variable. Estimates of Hudson's test statistics (K_{ST} , K_S , K_T) using nonparametric permutation indicated no genetic differentiation among North American, Eurasian and Svalbard populations. The genetic differences within and between geographic areas were $K_S = 2.7840$, $K_T = 2.8229$, resulting in $K_{ST} = 0.0138$, $P = 0.168$. M_{div} showed evidence for several effective migrants per generation (with values between 1.5 and 2.5 receiving the highest likelihood scores, in general) exchanged between North American, Eurasian and Svalbard populations (Fig. 2). Similar migration values were considered to indicate an 'intermediate' level of gene flow in other organisms (Carbone *et al.*, 2004). In addition, M_{div} estimated no population divergence (T not significantly different from 0, data not shown). The relatively high Θ values indicated a sufficient number of polymorphisms in the data, therefore the non-significant divergence

time estimates do not appear to be artefacts caused by insufficient data.

DISCUSSION

Despite Svalbard's geographic isolation and high arctic climate, ECM fungal communities are surprisingly diverse. It is reassuring that we recovered in our samples most of the ECM taxa that Väre *et al.* (1992) had found in Svalbard using morphological techniques, and those in the species list of Gulden & Torkelsen (1996). In addition, we report many more, including a large number of species in genera (e.g. *Tomentella*, *Sebacina*) that are entirely missing from these earlier species lists of Svalbard fungi. It is very likely that increased future sampling will result in the discovery of additional lineages, particularly in very diverse and taxonom-

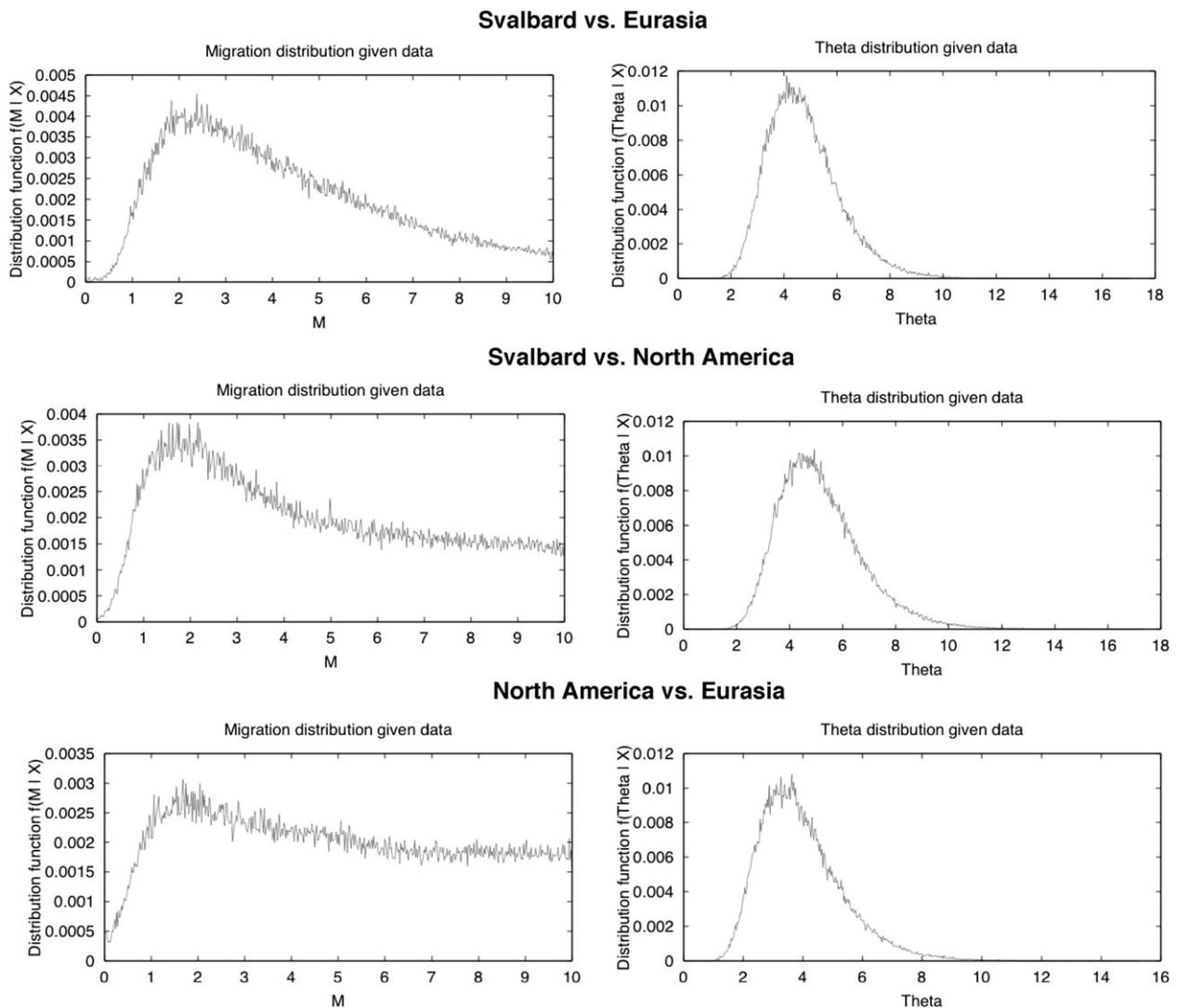


Figure 2 Posterior probability distributions of migration ($M = 2N_e m$) and population mean mutation rate (Θ) estimated between geographic population pairs of *Cortinarius favrei* using Markov chain Monte Carlo coalescent simulations in M_{div}. The M parameter is defined as the effective number of migrants exchanged between two populations each generation, where N_e is the effective population size and m is the migration rate per generation.

ically difficult genera such as *Tomentella*, *Cortinarius* and *Inocybe*. Because sequences generated from soil and sporocarp samples often give complementary views of phylogenetic diversity, our combined approach made it possible to detect more lineages than using either sampling method alone. In this regard, it is worth noting that fungi with presumably high biomass in the soil, but with rare and/or inconspicuous sporocarps (e.g. *Sebacina*, *Thelephora* and *Tomentella*), have been vastly underrepresented in sporocarp collections from Svalbard. This observation is in agreement with records of thelephoroid fungi from other biomes as well, where they are generally well represented as mycorrhizae in the soil, but are unrepresented in the above-ground fruiting record (Gardes & Bruns, 1996). On the other hand, some groups with generally abundant fruiting have been found in a low number of soil clones (e.g. *Lactarius* and *Russula*). These latter taxa may be present at relatively low biomass in arctic soils, contrary to observations in boreal regions (Geml *et al.*, 2009, 2010).

Due to the very scarce published data for reference, it is difficult to assess the geographic distribution of the taxa inhabiting Svalbard. Relying on our current knowledge, we can simply state that 19 of the 30 *Tomentella*/*Thelephora* phylogroups have been found outside Svalbard to date. In other genera, corresponding numbers of phylogroups are proven to be non-endemic: *Cortinarius* 16/19, *Inocybe* 18/27, *Hebeloma* 6/7, *Lactarius* 6/6, *Russula* 8/8, *Entoloma* 1/4, *Sebacina* 2/3, *Laccaria* 2/2, *Alnicola* 1/1, *Clavulina* 1/1 and *Leccinum* 1/1. On the other hand, we found several previously unreported taxa that are currently known only from Svalbard, including *Cortinarius* 1, *Cortinarius* 10, *Entoloma* 2, *Entoloma* 3, *Hebeloma* 3, *Inocybe* 22, *Inocybe* 25 and several phylogroups of *Tomentella*. The status and distribution of these phylogroups are unknown, as we provide the first record for them. These genera are famous for their diversity and taxonomic difficulties, and the lack of conspecific reference material from elsewhere may simply be due to the lack of knowledge and collecting efforts in arctic areas. In this regard, it is interesting to note that all species of the most conspicuous, best-studied genera (e.g. *Lactarius*, *Russula*) have been found in other arctic locations. Furthermore, many identical or nearly identical sequences in less well known genera (e.g. *Tomentella*) came from our own soil clone studies in Alaska and the Canadian Arctic (Geml *et al.*, 2009; D.L.T. *et al.*, unpublished data). Nonetheless, even with our limited knowledge and geographic coverage, we already have proof that the majority of the phylogroups (73.4%) occur outside Svalbard, often in both Europe and North America. Therefore, based on the phylogenetic patterns, we expect that most (if not all) phylogroups will also be found in the future at other arctic locations as the geographic coverage of field sampling is improved.

Taking into account Svalbard's geological isolation since approximately the early Pliocene (c. 4–5 Ma), phylogenetic patterns presented in our paper suggest that most ECM fungi in Svalbard have probably colonized the archipelago by long-distance dispersal events, as opposed to originating by vicariance and *in situ* divergence. There are possible exceptions

among taxa with no previous DNA sequence records, and thus with unknown geographic distributions outside Svalbard. Further sampling is needed in other arctic areas to reveal whether or not these species are true endemics. We argue that, for the majority of taxa, the low level of sequence divergence observed (regardless of species limits) is largely incompatible with the vicariance scenario, and suggest relatively recent migration to the archipelago. In many cases, the branch lengths between North American, European and Svalbard samples are 0–2 changes long. Furthermore, all successfully identified phylogroups represent species that had been described from outside Svalbard, hence proving the occurrence of certain species elsewhere. Admittedly, evidence demonstrating recent migration between Svalbard and the continental mainland areas does not automatically disprove the presence of extant ECM taxa in Svalbard at the time of separation from other land masses, although the vegetation in Svalbard must have been very different, given that the mid-Pliocene (c. 3 Ma) climate was 10–20 °C warmer than today in the circumpolar Arctic (Salzmann *et al.*, 2009). Although biologically unlikely, it is at least theoretically possible that Svalbard's extant ECM species were already present on the archipelago at the time of separation, and that their populations remained in contact with continental populations by intermittent or continuous long-distance gene flow, which would be indistinguishable from purely post-glacial gene flow. This limitation, however, is by no means crucial, as detecting recent migration between arctic areas still suggests that these ECM fungi do not depend on land routes to migrate, regardless of how long Svalbard has been inhabited by them. Similarly, it has to be noted that, due to the high variation in nucleotide substitution rates in ITS among fungi (Kasuga *et al.*, 2002), vicariant persistence since the early Pliocene for the putative endemic lineages, although biologically unlikely, cannot be statistically rejected. In summary, our data support recent long-distance dispersal of the majority of extant species more strongly than vicariance and *in situ* speciation on the archipelago.

ECM fungi are obligate symbionts of plants (mostly woody plants). Geological evidence shows that Svalbard was completely, or almost completely, glaciated in the Last Glacial Maximum (LGM) and during most of the other glacial maxima. Whether or not nunatak survival occurred in some very hardy arctic plants is still a matter of debate. Recent evidence suggests that the rare arctic–alpine pioneer plant *Arenaria humifusa* may have survived in eastern Greenland and/or Svalbard during the LGM (Westergaard *et al.*, 2010a). Nonetheless, ECM host plants (shrubs) are among the more thermophilous species, and molecular data suggest that they recolonized Svalbard post-glacially (Alsos *et al.*, 2007). In almost all ECM genera identified, we observed multiple lineages that are likely to have (re)colonized Svalbard in post-glacial times. On the other hand, although unlikely for most taxa, glacial survival of some ECM fungi cannot be entirely ruled out, given the possibility of nunatak survival for some arctic plants (Westergaard *et al.*, 2010a,b). It is important to note that we by no means imply that there had been no

ECM fungi on Svalbard before the current interglacial. Rather, it is very likely that ECM fungi had been present in Svalbard during many previous interglacials, but they were probably wiped out repeatedly by the glaciations, and then recolonized Svalbard again and again, as has also been shown in arctic plants (e.g. Eidesen *et al.*, 2007). The high current migration estimates for the only ECM species with available data suitable for coalescent analyses further support our view that long-distance dispersal is frequent in arctic ECM fungi.

Our findings for arctic fungi therefore contrast with general phylogeographic patterns seen in low- to mid-latitude fungi, as summarized in the Introduction. Former findings have suggested that typical ECM plant and fungus symbionts require overland routes for migration, possibly as a consequence of the obligate symbiotic habit of ECM associations (Malloch *et al.*, 1980; Halling, 2001; Chapela & Garbelotto, 2004). In theory, long-distance dispersal and establishment of either the mycorrhizal fungus or the host plant in isolation has been considered unlikely because the simultaneous arrival of fungal spore and host plant seed are presumed necessary (Moyersoen *et al.*, 2003). In addition, even for wind-dispersed ECM fungi, the vast majority (generally > 95%) of basidiospores have been shown to be deposited within 5 m of the parent basidiome (Li, 2005). As a result, there are numerous cases of marked ITS sequence divergence between sister lineages inhabiting different continents in various ECM species complexes in lower latitudes (Shen *et al.*, 2002; Geml *et al.*, 2006, 2008; Taylor *et al.*, 2006). There is little or no evidence for such patterns in our data.

Despite its geographic isolation from other land masses, the community of ECM fungi in Svalbard is by no means depauperate, as the observed diversity is similar to that found in other arctic areas: the majority of arctic ECM species (and all ECM genera) observed in the corresponding bioclimatic subzones in other parts of the Arctic are found in Svalbard (Gulden & Torkelsen, 1996; Karatygin *et al.*, 1999; J.G., pers. obs.). Therefore we hypothesize that dispersal capabilities of arctic ECM fungi are comparable with those of arctic plants, where it has been shown that Svalbard was colonized multiple times in the Holocene and that the number of dispersals is likely to be much higher than the number of successful establishments (Alsos *et al.*, 2007). The relatively large size and proximity of the islands comprising Svalbard (except Bjørnøya) may have contributed to the extent of colonization of the archipelago, although comparisons with small, isolated arctic islands with a similar range of habitats are needed to evaluate the influence of island size on colonization rates and biodiversity.

Due to the low number of reference DNA sequences publicly available for comparison, it is difficult to pinpoint source areas for the ECM colonization of Svalbard by ECM fungi. Sequences closely related to our Svalbard samples generally originated from specimens or environmental samples collected by a small group of research labs working on alpine, boreal and arctic fungi, for example in Norway, Sweden, Austria, Canada (British Columbia) and the USA (Alaska). In addition, because there is currently very little information on the distribution of arctic fungi, further projects with more complete geographic

sampling are needed to reveal their phylogeographic structures and to test whether or not dispersal patterns in arctic ECM fungi are similar to those observed in arctic plants, for which the Russian Arctic and eastern Greenland are the most important source areas for the colonization of Svalbard (Alsos *et al.*, 2007). To our knowledge, no specialized sporocarp adaptation to arctic environments has been reported (perhaps other than smaller sporocarp size in general). Therefore we hypothesize that wind dispersal is important, as expected for most fungi. The open arctic landscape, with strong winds and extensive snow and ice cover, seems to be particularly suitable for wind dispersal, as has also been suggested for arctic plants (Alsos *et al.*, 2007). Other possible means of dispersal include spores being carried by migratory animals, driftwood and drifting sea ice, which may also favour arctic fungi due to ocean currents and animal migrations (particularly birds) linking continents over shorter distances.

Besides the observed diversity of taxa, considering that some of the ECM fungi included in this paper have very narrow host ranges, we assume that basidiospores are probably dispersed regularly to Svalbard from remote areas. For example, *Leccinum rotundifoliae*, a circumpolar species, is specific to *Betula* hosts, of which only *Betula nana* (dwarf birch) is found in Svalbard. Furthermore, the distribution of *B. nana* in Svalbard is restricted to a very few localities with particularly suitable microclimatic conditions, namely Colesdalen and Adventdalen (Alsos *et al.*, 2002). The size of these *B. nana* populations is generally small and, unlike populations that are widespread in the circumpolar low arctic, this species apparently does not reproduce sexually in Svalbard (Alsos *et al.*, 2003). Even if we consider that the distribution of *B. nana* may have been somewhat wider in Svalbard during the Holocene Climatic Optimum (9000 to 5000 years ago), it almost certainly remained very localized and restricted to the Isfjorden region, and the reunion with its ECM symbionts (many of which occur nowhere else in Svalbard) after independent transoceanic journeys is particularly impressive.

This study reports the first comprehensive analyses of ECM communities in Svalbard, using combined soil and sporocarp data. Our evidence suggests that long-distance dispersal has probably played a major role in the phylogeographic history of many ECM fungi at high latitudes in the Northern Hemisphere, and our results may have implications for studies on the biodiversity, ecology and conservation of arctic fungi in general. It is very likely that many arctic fungi, particularly the widespread taxa with circumpolar distributions, have been selected for mobility during the glacial cycles, as has been suggested for plants (Brochmann & Brysting, 2008). In addition, we report numerous phylogroups that were not represented previously in public databases and that may or may not represent novel taxa. Further studies are needed to provide a solid taxonomic context for these taxa and to determine whether or not they represent species endemic to the Arctic. Improved knowledge of migration history, dispersal capacities and present-day genetic diversity is essential to predict how arctic species and communities will respond to global change.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Maximum-likelihood phylograms of (a) *Tomentella/Thelephora*, (b) *Cortinarius*, (c) *Inocybe*, (d) *Hebeloma*, (e) *Lactarius*, (f) *Russula*, (g) *Clavulina*, *Entoloma*, *Laccaria*, *Leccinum* and *Sebacina* taxa inferred from the ITS rDNA datasets showing the phylogenetic spread of soil clone operational taxonomic units (OTUs) and sporocarp sequences generated in this study (in bold) among representatives of congeneric taxa in GenBank.

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BIOSKETCHES

József Geml is interested in the biodiversity, phylogeography and evolutionary ecology of fungi and their historical and recent responses to changes in the landscape and the climate.

This paper is part of an overarching project, led by D. Lee Taylor, focusing on the diversity and functioning of arctic fungal communities on a circumpolar scale. D. Lee Taylor's group seeks improved understanding of the linkages among fungal biodiversity, species ecology and ecosystem function, particularly in boreal and arctic soils.

Author contributions: J.G. conceived the research idea for this paper, collected sporocarps in Svalbard with M.E.N., generated sequences from all sporocarps, completed phylogenetic analyses, and wrote the first draft and revision of the manuscript. I.T. collected soil samples, extracted soil DNA, generated PCR clone libraries, contributed part of the core alignment for the Thelephoraceae, and edited the manuscript. C.H.R. advised on the choice of field sites and logistics, assisted I.T. with soil sampling in the field, provided comments on the manuscript, and was a named collaborator on D.L.T.'s NSF proposal. C.N. and N.L. generated sequence data from the soil clone libraries. D.L.T., C.B. and M.E.N. provided financial and infrastructural support, intellectual guidance, and manuscript editing. In addition, D.L.T. provided part of the Thelephoraceae core alignment.

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