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Frequent circumarctic and rare transequatorial dispersals in the lichenised agaric genus *Lichenomphalia* (*Hygrophoraceae*, *Basidiomycota*)

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ABSTRACT

Species of the genus *Lichenomphalia* are mostly restricted to arctic-alpine environments with the exception of *Lichenomphalia umbellifera* which is also common in northern forests. Although *Lichenomphalia* species inhabit vast regions in several continents, no information is available on their genetic variation across geographic regions and the underlying population-phylogenetic patterns. We collected samples from arctic and subarctic regions, as well as from newly discovered subantarctic localities for the genus. Phylogenetic, nonparametric permutation methods, and coalescent analyses were used to assess phylogeny and population divergence and to estimate the extent and direction of gene flow among distinct geographic populations. All known species formed monophyletic groups, supporting their morphology-based delimitation. In addition, we found two subantarctic phylogenetic species (*Lichenomphalia* sp. and *Lichenomphalia* aff. *umbellifera*), of which the latter formed a well-supported sister group to *L. umbellifera*. We found no significant genetic differentiation among conspecific North American and Eurasian populations in *Lichenomphalia*. We detected high intercontinental gene flow within the northern polar region, suggesting rapid (re)colonisation of suitable habitats in response to climatic fluctuations and preventing pronounced genetic differentiation. On the other hand, our phylogenetic analyses suggest that dispersal between northern circumpolar and subantarctic areas likely happened very rarely and led to the establishment and subsequent divergence of lineages. Due to limited sampling in the Southern Hemisphere, it is currently uncertain whether the northern lineages occur in Gondwanan regions. On the other hand, our results strongly suggest that the southern lineages do not occur in the circumpolar north. Although rare transequatorial dispersal and subsequent isolation may explain the

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emergence of at least two subantarctic phylogenetic species lineages in *Lichenomphalia*, more samples from the Southern Hemisphere are needed to better understand the phylogeographic history of the genus.

Introduction

One of the key questions in fungal phylogeography and biodiversity studies is whether disjunct populations inhabiting different continents belong to the same species. Beside the possible theoretical advancement in our knowledge regarding long-distance dispersal, studying intercontinental gene flow has practical implications for understanding the composition of past, present, and future communities during shifts in species distributions due to climatic changes. The capacity of a certain taxon for transoceanic dispersal will obviously have a profound effect on its intercontinental population structure and the potential emergence of divergent lineages.

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 & Hovmøller 2002; Moyersoen *et al.* 2003; Feuerer & Hawksworth 2007; Moncalvo & Buchanan 2008; Printzen 2008). In recent years, molecular tools have revealed several examples of distinct phylogeographic lineages or cryptic species within fungal species complexes that were previously treated as single morphological species. The majority of boreal, temperate or tropical fungi subjected to genetic studies show strong phylogeographical patterns and limited dispersal, and there is an increasing amount of geographical endemism being discovered (e.g., Taylor *et al.* 2006; Geml *et al.* 2008; Bergemann *et al.* 2009 and references therein). In most studied fungi, the observed phylogenetic structures likely have arisen as a result of the lack of intercontinental dispersal, because allopatric clades often inhabit similar environments on different continents (e.g., Shen *et al.* 2002; Taylor *et al.* 2006; Geml *et al.* 2008). Exceptions to this general trend mostly come from fungi associated with humans, which are, therefore, more likely to be dispersed *via* shipment of goods: e.g., 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).

Similar studies on high-latitude agarics are virtually nonexistent. Apart from our recent study (Geml *et al.* 2011), we are not aware of any studies published on the phylogeography of arctic basidiomycetes, not to mention possible bipolar connections. This is unfortunate, because fungi play critical roles in the functioning of high-latitude ecosystems (Callaghan *et al.* 2004; Printzen 2008). Studying dispersal and migration in high-latitude fungi, i.e., the degree to which they are able to exchange genes with populations inhabiting different geographical regions and to colonize suitable habitats, is relevant not only for the theoretical advancement in our knowledge regarding the dispersal abilities and evolutionary dynamics of fungi, but also has practical implications for climate change studies. Climate warming is expected to cause a pole-ward shift in the distribution of many high-latitude species, and the dispersal capability of individual species will greatly influence the composition of future polar communities (Alsos *et al.* 2007).

Lichenomphalia Redhead, Lutzoni, Moncalvo, and Vilgalys is a basidiomycete genus with omphalinoid fruiting bodies. The genus contains eight lichenised taxa that form symbioses with the unicellular green algal photobiont *Coccomyxa* (Zoller & Lutzoni 2003). The genus has been shown to be monophyletic, with *Lichenomphalia umbellifera* as sister to the remaining *Lichenomphalia* species (Lutzoni 1997). Although more recent studies have questioned the monophyly of the genus (Lawrey *et al.* 2009), there is no unequivocal evidence *pro or contra*. *Lichenomphalia* species are generally restricted to arctic-alpine environments with the notable exception of *L. umbellifera*, which is also found in boreal and northern temperate rain forests and is considered to be the most broadly distributed and ecologically most plastic species in the genus (Kranner & Lutzoni 1999; Redhead *et al.* 2002). In this study, we sampled populations across the northern circumpolar distribution of three species that are widespread in the arctic regions: *Lichenomphalia alpina*, *Lichenomphalia hudsoniana*, and *L. umbellifera*. In addition, we also included samples from newly discovered subantarctic populations on Campbell Island tentatively identified in the field as *Lichenomphalia* sp. and *Lichenomphalia* aff. *umbellifera* based on morphological characteristics of the basidiomes. We examined genetic diversity and phylogeographic structure in *Lichenomphalia* in an effort to answer the following questions: (1) Is there phylogeographic structure in the Northern Hemisphere similar to the continental endemism seen in most temperate and boreal agaric species; (2) Is the phylogeographic history of northern and southern lineages different from bipolar patterns seen in other fungi?

Materials and methods

Materials and molecular work

Specimens were collected in various locations in Europe, Asia, North America, and one subantarctic island of New Zealand, or were obtained through herbarium loans (Table 1). DNA was extracted from small samples of dried specimens using the DNeasy® Plant Mini Kit (QIAGEN, Inc., Valencia, CA, U.S.A.). Sequences of the nuclear internal transcribed spacer (ITS1 + 5.8S + ITS2) and the large subunit (LSU) region of the ribosomal DNA repeat were generated, supplemented by translation elongation factor 1-alpha gene (EF1) sequences for a subset of the *Lichenomphalia umbellifera* specimens. The primers and PCR and sequencing protocols have been described previously (Geml *et al.* 2005, 2006). Sequences were deposited in GenBank (Table 1). Available homologous

Table 1 – Geographical origin and GenBank accession numbers of *Lichenomphalia* specimens included in this study.

Isolate code	Origin	GenBank accession number		
		ITS	LSU	EF1
L. alpina				
FL930816-8	Iqaluit, Baffin Island, Nunavut, Canada	U66447*	U66447*	–
GAL1264	Barrow, AK, U.S.A.	GU810972	GU811048	–
GAL1550	Barrow, AK, U.S.A.	GU810973	GU811049	–
GAL2126	Barrow, AK, U.S.A.	GU810974	GU811050	–
GAL2689	Longyearbyen, Svalbard, Norway	GU810975	GU811051	–
GAL2712	Longyearbyen, Svalbard, Norway	GU810976	GU811052	–
GAL4855	Denali National Park, AK, U.S.A.	GU810977	GU811053	–
L. grisella				
FL930822-6	Schefferville, Québec, Canada	U66443*	U66443*	–
L. hudsoniana				
FL920728-4a	Mont Albert, Québec, Canada	U66446*	U66446*	–
FL930724-3	Nuuk, Greenland	AY293950*	–	–
FL930822-3	Schefferville, Québec, Canada	AY293951*	–	–
FL930724-6	Nuuk, Greenland	AY293952*	–	–
FL930811-6	Disko Island, Greenland	AY293953*	–	–
FL930805-6	Myvatn, Iceland	AY293954*	–	–
GAL1209	Barrow, AK, U.S.A.	GU810978	GU811054	–
GAL2128	Barrow, AK, U.S.A.	GU810979	GU811055	–
GAL3265	Atqasuk, AK, U.S.A.	GU810980	GU811056	–
GAL4558	Denali National Park, AK, U.S.A.	GU810981	GU811057	–
GAL7548	Nome, AK, U.S.A.	GU810982	GU811058	–
GAL14648	Imnavait Creek, AK, U.S.A.	GU810983	GU811059	–
GAL14655	Toolik Lake LTER site, AK, U.S.A.	GU810984	GU811060	–
GAL18249	Barrow, AK, U.S.A.	JQ065873	JQ065875	–
Gulden247/86	Ny-Ålesund, Svalbard, Norway	JQ065874	–	–
L. lobata				
Palice2327	Ecuador	AY542866*	AY542866*	–
Palice3275	Ecuador	AY542867*	AY542867*	–
L. umbellifera				
Dlaber 5/7/1989	Schwarzwald, Baden-Wurtenberg, Germany	GU810951	–	–
DU0011853	Iqaluit, Baffin Island, Nunavut, Canada	GU810943	GU811028	–
DU0011863	Iqaluit, Baffin Island, Nunavut, Canada	GU810942	GU811027	–
DU0011879	Abisko, Lappland, Sweden	GU810945	GU811030	–
FL930724-1	Nuuk, Greenland	AY293958*	–	–
FL930724-2	Nuuk, Greenland	AY293959*	–	–
FL930805	Myvatn, Iceland	AY293961*	–	–
FL930810-2	Disko Island, Greenland	AY293955*	–	–
FL930817-2	Iqaluit, Baffin Island, Nunavut, Canada	U66445*	–	–
FL930822-2	Schefferville, Québec, Canada	AY293956*	–	–
FL930822-4	Schefferville, Québec, Canada	AY293960*	–	–
FL930822-8	Schefferville, Québec, Canada	AY293957*	–	–
GAL2616	Longyearbyen, Svalbard, Norway	GU810944	GU811029	–
GAL2667	Longyearbyen, Svalbard, Norway	GU810962	GU811038	–
GAL2687	Longyearbyen, Svalbard, Norway	GU810964	GU811040	–
GAL2690	Longyearbyen, Svalbard, Norway	GU810963	GU811039	–
GAL5374	Columbia Glacier, AK, U.S.A.	GU810934	GU811019	GU810993
GAL7544	Nome, AK, U.S.A.	GU810927	GU811012	GU810986
GAL8441	Kenai Lakes, AK, U.S.A.	GU810932	GU811017	GU810991
GAL8933	Denali National Park, AK, U.S.A.	GU810933	GU811018	GU810992
GAL9836	Kobuk National Park, AK, U.S.A.	GU810928	GU811013	GU810987
GAL12138	Adak Island, AK, U.S.A.	GU810936	GU811021	GU810995
GAL12214	Amchitka Island, AK, U.S.A.	GU810937	GU811022	GU810996
GAL12224	Amchitka Island, AK, U.S.A.	GU810938	GU811023	GU810997
GAL12274	Amchitka Island, AK, U.S.A.	GU810939	GU811024	GU810998
GAL12717	Kobuk National Park, AK, U.S.A.	GU810931	GU811016	GU810990
GAL14811	Imnavait Creek, AK, U.S.A.	GU810935	GU811020	GU810994
GAL14845	Toolik Lake LTER site, AK, U.S.A.	GU810930	GU811015	GU810989
GAL15152	Sitka, AK, U.S.A.	GU810926	GU811011	GU810985
GAL15669	Bonanza Creek LTER site, AK, U.S.A.	GU810929	GU811014	GU810988
GAL18192	Fairbanks, AK, U.S.A.	GU810940	GU811025	GU810999

Table 1 – (*continued*)

Isolate code	Origin	GenBank accession number		
		ITS	LSU	EF1
GAL18247	Barrow, AK, U.S.A.	GU810941	GU811026	GU811000
Gulden 302/86	Ny-Ålesund, Svalbard, Norway	GU810961	GU811037	GU811007
Gulden 393/86	Ny-Ålesund, Svalbard, Norway	GU810960	GU811036	GU811006
HN4615	Longyearbyen, Svalbard, Norway	GU810965	GU811041	–
HN4616	Bjørndalen, Svalbard, Norway	GU810966	GU811042	–
L 3865	Pyasino Gulf, Taymyr Autonomous Okrug, Russia	GU810956	–	–
L 3915	Lake Baikal, Irkutsk Oblast, Russia	GU810958	–	–
L 201124	Khibiny Mtns., Province of Murmansk, Russia	GU810957	–	–
L 203055	Valaam, Republic of Karelia, Russia	GU810954	–	–
L 208257	Bolshevik Island, Severnaya Zemlya, Russia	GU810953	–	–
L 215201	Lisino-Korpus, Province of Leningrad, Russia	GU810959	–	–
L 215343	Lebedevka, Province of Leningrad, Russia	GU810955	–	–
LU113	Pechora, Republic of Komi, Russia	GU810952		–
O 64705	Ålesund, Møre og Romsdal, Norway	GU810948	GU811033	GU811003
O 66530	Nøtteroy, Vestfold, Norway	GU810950	GU811035	GU811005
O 72207	Dovre, Oppland, Norway	GU810949	GU811034	GU811004
O 72224	Hemnes, Nordland, Norway	GU810946	GU811031	GU811001
O 73818	Ulvik, Hordaland, Norway	GU810947	GU811032	GU811002
L. aff. *umbellifera*				
GAL9512	Campbell Island, New Zealand	GU810967	GU811043	GU811008
GAL9517	Campbell Island, New Zealand	GU810968	GU811044	GU811009
GAL9547	Campbell Island, New Zealand	GU810969	GU811045	GU811010
L. velutina				
FL930812-1	Disko Island, Greenland	U66454*	U66454*	–
L. sp.				
GAL9540	Campbell Island, New Zealand	GU810971	GU811047	–
GAL9541	Campbell Island, New Zealand	GU810970	GU811046	–

Asterisks (*) refer to previously published data.

Lichenomphalia sequences were downloaded from GenBank and included in the analyses. DNA sequences were analysed for 80 *Lichenomphalia* samples: *L. umbellifera* ($n = 49$), *Lichenomphalia alpina* ($n = 7$), *Lichenomphalia hudsoniana* ($n = 15$), *Lichenomphalia grisella* ($n = 1$), *Lichenomphalia lobata* ($n = 2$), *Lichenomphalia velutina* ($n = 1$), and the subantarctic *Lichenomphalia* sp. ($n = 2$) and *Lichenomphalia* aff. *umbellifera* ($n = 3$).

Phylogenetic analysis

Sequence data obtained for both strands of each locus were edited and assembled for each isolate using Aligner v. 1.3.4 (CodonCode Inc., Dedham, MA, U.S.A.) or Sequencher 4.5 (GeneCodes, Ann Arbor, MI, U.S.A.). We constructed two multiple sequence alignments using MUSCLE (Edgar 2004) that were subsequently corrected manually. The first was a genus-wide ITS and LSU alignment, while the second included ITS, LSU, and EF1 sequences of the *Lichenomphalia umbellifera* complex (*L. umbellifera* and *Lichenomphalia* aff. *umbellifera*). We recognized genetically isolated groups on the basis of concordance of multiple gene genealogies, applying phylogenetic species recognition as outlined in Taylor *et al.* (2000). To determine if DNA sequence data from different loci were phylogenetically congruent, we conducted a maximum likelihood (ML) bootstrap analysis on each locus separately using PAUP* 4b10 (Swofford 2002). Topological conflict was recognized as significant when members of a monophyletic group received bootstrap values ≥70 % based on one locus and were shown to be significantly not monophyletic with data from a different locus (Mason-Gamer & Kellogg 1996). Because we did not detect significant conflicts among loci, we carried out heuristic searches on the combined datasets under the ML criterion, using PAUP*. For each dataset, the best-fit evolutionary model was determined by comparing different evolutionary models with varying values of base frequencies, substitution types, alpha-parameter of the gamma-distribution of variable sites, and proportion of invariable sites *via* the Akaike information criterion (AIC) using PAUP* and Modeltest 3.7 (Posada & Crandall 1998). Trees including multiple *Lichenomphalia* species were rooted using *Arrhenia* species based on Lutzoni (1997). Phylogenetic analyses restricted to the *L. umbellifera* complex were midpoint-rooted. The bootstrap tests (Felsenstein 1985) were used with 1000 replicates, with 'fast' stepwise-addition. The High Performance Computing cluster maintained by the University of Alaska Fairbanks Life Sciences Informatics Core (http://biotech.inbre.alaska.edu/) was used to run MUSCLE and PAUP*.

Coalescent analyses

We detected no or very little intraspecific variation in *Lichenomphalia alpina* s.str. (i.e., from the Arctic) and *Lichenomphalia*

hudsoniana, for which only 1 and 2 ITS sequence types were detected, respectively. Therefore, coalescent analyses were carried out only for the northern circumpolar *Lichenomphalia umbellifera*. Identical ITS sequences were collapsed into haplotypes, with retaining information on their observed frequencies in the populations, using SNAP Map (Aylor *et al.* 2006) after excluding insertion or deletions (indels) and infinite-sites violations. Though well represented across our samples, we did not use LSU data due to much lower levels of sequence variation relative to ITS. 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 informative or uninformative, 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) and no incompatibility was detected among all variable sites. Genetic differentiation among geographical populations was analysed using SNAP Map, Seqtomatrix, and Permtest (Hudson *et al.* 1992) implemented in SNAP Workbench (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 $K1$ and $K2$ (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 these tests, specimens were assigned to groups depending on the geographical scale of the research question. For estimating intercontinental gene flow in the Northern Hemisphere, we assigned groups according to continents (North America or Eurasia, excluding southern specimens), while, to estimate the level of genetic differentiation between arctic and subantarctic populations, sequences were grouped according to the hemispheres of origin.

Subsequently, coalescent methods were used to determine whether there was any evidence of transoceanic migration between pairs of populations inhabiting different continents. Because the permutation tests indicated significant geographic structure and the phylogenetic analyses indicated reciprocal monophyly for the northern and southern lineages, both of which imply no current gene flow, we conducted coalescent analyses only for the arctic and boreal populations to estimate migration between North America and Eurasia. We used MDIV (Nielsen & Wakeley 2001), implemented in SNAP Workbench (Price & Carbone 2005), employing both likelihood and Bayesian methods using Markov chain Monte Carlo (MCMC) coalescent simulations to determine if the diversity patterns in different geographic areas were the result of retention of ancestral polymorphism or recent gene flow. We estimated the migration parameter (M) and the divergence time (T). M is defined as the effective number of migrants exchanged between two populations each generation and it equals 2× the net effective population size (N_e) multiplied by m (migration rate), while T is measured in coalescent units of $2N_e$ generations. Data were simulated assuming an infinite sites model with uniform prior. We used 2 000 000 iterations in the chain for estimating the posterior probability distribution and an initial 500 000 iterations to ensure that sufficient genealogies were simulated before approximating the posterior distribution. Subsequently, we reconstructed the genealogy with the highest root probability, the ages of mutations, and the time to the most recent common ancestor of the sample using coalescent simulations in Genetree v. 9.0 (Griffiths & Tavaré 1994).

Results

Phylogenetic analyses

The combined ITS and LSU dataset consisted of 1566 characters, including gaps. There were 614 variable sites, of which 502 were parsimony-informative. The General-Time-Reversible model, with calculated proportion of invariable sites ($I = 0.233$) and estimated alpha-parameter (=0.481) of gamma-distribution (GTR + I + G), was selected as the best-fit evolutionary model. The phylogram with the highest likelihood value ($-\ln L = 7076.8495$) is shown in Fig 1A. All sampled known species formed distinct, well-supported monophyletic groups. In northern species, where samples were available from multiple geographic areas, we did not find any genetic partitioning corresponding to the geographic origin of the samples. In addition, the subantarctic samples grouped in two distinct phylogenetic species. One of these (*Lichenomphalia* sp.) was quite different from any other species, while the other (*Lichenomphalia* aff. *umbellifera*) formed a well-supported sister group to *Lichenomphalia umbellifera*.

In the *L. umbellifera* complex, the ITS, LSU, EF1, and the combined datasets consisted of 616, 610, 563, and 1789 characters, respectively, including gaps. The General-Time-Reversible model, without calculated proportion of invariable sites and estimated alpha-parameter (GTR), was selected as the best-fit evolutionary model. There were 74, 13, 64, and 151 variable positions, respectively. The phylogram with the highest likelihood value ($-\ln L = 3387.6449$) is shown in Fig 1B. All three genes supported the existence of two phylogenetic species lineages corresponding to populations in the Northern and Southern Hemispheres: *L. umbellifera* and *L.* aff. *umbellifera*, respectively. Apart from the clades resulting from the bipolar genetic differentiation, there were no other well-supported clades.

Coalescent analyses

Estimates of Hudson's test statistics (K_{ST}, K_S, and K_T) using nonparametric permutation method indicated no significant genetic differentiation among North American and Eurasian populations of *Lichenomphalia umbellifera*. The genetic differences within and between continents were $K_S = 3.076$, $K_T = 3.053$, resulting in $K_{ST} = -0.008$, $P = 0.726$. In contrast, we detected strong genetic structure among northern and subantarctic populations, where the values were $K_S = 3.007$, $K_T = 7.873$, $K_{ST} = 0.618$, $P < 0.001$. After removing indels and infinite-sites violations from the original ITS datasets, there were 21 ITS haplotypes in northern populations of *L. umbellifera* (Table 2). MDIV showed evidence for high gene flow between North American and Eurasian populations

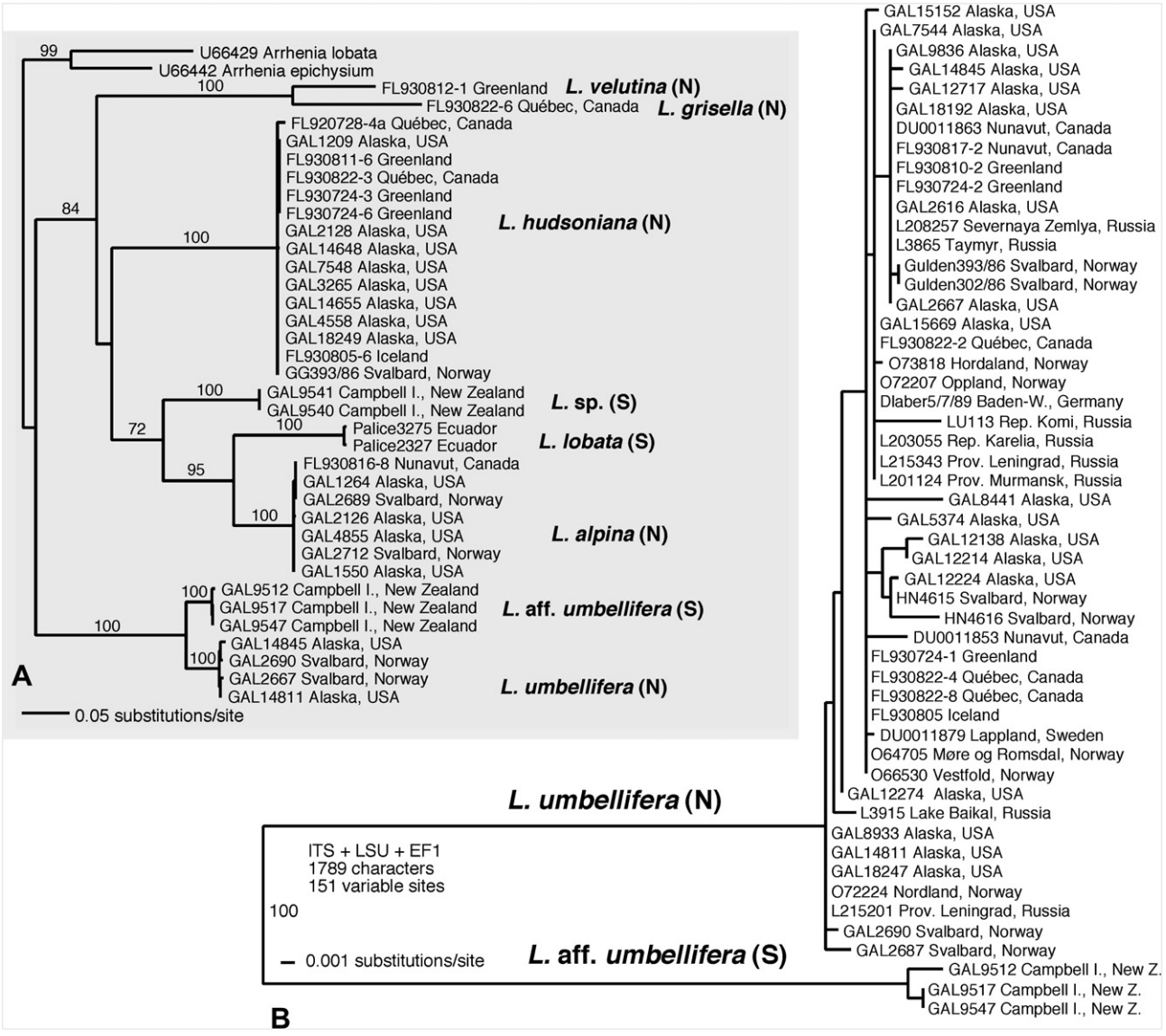

Fig 1 – (A) ML phylogram of *Lichenomphalia* species (−lnL = 7076.8495) inferred from the combined ITS + LSU rDNA dataset. The tree was rooted using *Arrhenia* based on phylogenetic results from a study with broader taxon sampling (Lutzoni 1997). Bootstrap values greater than 70 % are shown above the branches. Geographic distribution of the supported clades is marked by N ('northern') and S ('southern'). (B) ML phylogram of *L. umbellifera* (−lnL = 3387.6449) inferred from the combined ITS + LSU + EF dataset. The tree is midpoint-rooted. The only bootstrap value greater than 70 % is shown on the longest branch.

(Fig 2) and estimated no population divergence (*T* not significantly different from 0). In simulations using Genetree, we assumed a moderately high level of migration ($M = 0.1$) among northern populations. As expected, the coalescent-based genealogy did not show any historical population division in the Northern Hemisphere and was informative with respect to inferring the mutational history and variation between and within geographical regions (Fig 3).

Discussion

Biogeographers have long been fascinated by species that have disjunct distributions, among which bipolar species or species pairs have received particular attention (e.g., Darwin 1859; Du Rietz 1940; Galloway & Aptroot 1995 and the references therein). Long-distance dispersal in cryptogams has remained controversial, particularly regarding the ability of cold-climate species to cross the tropical belt (Van Zanten & Pócs 1981; Galloway & Aptroot 1995). Based on our results, the phylogeographic structure of *Lichenomphalia* appears to have been shaped by extensive dispersals within one hemisphere combined with rare transequatorial dispersals that is different from previously observed patterns in other fungi. On one hand, we observed a high level of intercontinental gene flow in the Northern Hemisphere, which is markedly different from the general patterns observed for boreal and temperate agarics, for which the lack of intercontinental gene flow generally results in the divergence of often morphologically cryptic species pairs on different continents. On the

Table 2 – Polymorphic sites in the ITS haplotypes of arctic and boreal *L. umbellifera* collapsed after removing indels and infinite-sites violations from the original ITS dataset for the subsequent coalescent analyses. Haplotype designations, position, site number, and designation of the given mutation are as shown in Fig 3. Position refers to that in the original alignment, site type refers either transition (t), transversion (v), deletion (–) change with regard to the consensus sequence, while character type designation indicates whether the site is parsimony-informative (i) or not (–).

Position	1111222233334444445555555556
	4477890222123803381456670122334579O
	4461341314875563396789015760427515 86
Site number	1111111111222222222233333333
	1234567890123456789012345678901234 56
Site Type	ttttttttttttvtvvtvttttttttttvtvvttt
Character Type	-----i--------i---i---i-ii-------i--
Consensus	GTCTCTGTTCTCGTGGTGGGTGCGGCTGGACTTTCC
Haplotypes (Frequency)	
A (1)	.....A.....
B (1)	ACT...A.....
C (1)	...CT.....C.....A.C...T....T
D (18)	.....C.....
E (1)	.....C.C.....
F (1)	.....C........A.....
G (1)	.....C........A..A.....
H (2)	.....C.....A.....
I (1)	.....C.....A....
J (1)	.....CT.....T..T.....C..
K (1)	.....T.....A.....
L (1)	.....A.....C...T..T.....C..
M (1)	.....G.TG..A.....C.....
N (2)	.....C...T..T.....C..
O (1)	.....C.C.T..T.....GC..
P (1)	.....A.....
Q (3)	.....T..T.....C..
R (7)	.....
S (1)	.....A.....
T (1)	.....T.....C..
U (2)	.....A.....T.

other hand, on the global scale, despite our limited sampling from the Southern Hemisphere, we did observe some geographic endemism, primarily at the hemispheric level. This, in turn, differs from patterns reported for other bipolar genera, predominantly ascolichens (e.g., Thell *et al.* 2002; Myllys *et al.* 2003; Seymour *et al.* 2007), where such bipolar genetic divergence has not been reported.

Intercontinental gene flow in Northern Hemisphere

The phylogeographic structure of arctic *Lichenomphalia* seems very different from mid-latitude agarics, as a pattern of multiple phylogenetic lineages with nonoverlapping geographic distributions was not observed in the Northern Hemisphere. Instead, arctic populations of *Lichenomphalia* species for which we had samples from distant geographical regions were not genetically distinct. Of course, we acknowledge that three loci are insufficient to rule out significant genetic structure in other areas of the genome. Previously, Redhead & Kuyper (1987) had noted that *Lichenomphalia hudsoniana* (cited as *Botrydina viridis*) predominantly produced yellowish basidiomes in western North America, more orangish pilei in eastern North America, and only in Europe sometimes had lilac tints on their stipes. Nevertheless, the phylogeographic differentiation reported for mid-latitude agarics has also been based upon a limited number of loci, sometimes only on ITS. Our results suggest that, in response to climatic fluctuations, *Lichenomphalia umbellifera* has been able to migrate over considerable distances due to effective dispersal. The considerable genetic diversity observed in the Arctic indicates long-term survival at northern high latitudes, and that large and diverse populations have served as sources for migrants. The estimated migration rates and the absence of geographical population structure suggest continuing gene flow between northern continents that has prevented pronounced genetic differentiation. This was also supported by the lack of polymorphism in *Lichenomphalia alpina* and *L. hudsoniana* among all sampled populations, despite the highly elevated nucleotide substitution rate in the genus in general (Lutzoni & Pagel 1997). Similar patterns of circumpolar genetic diversity have recently been detected in arctic ectomycorrhizal fungi (Geml *et al.* 2011) and some other arctic organisms, for example in highly mobile animals such as the arctic fox, *Alopex lagopus* (Dalén *et al.* 2005) and the snowy owl, *Bubo scandiacus* (Marthinsen *et al.* 2008), and in the arctic-alpine lineage of the bog blueberry *Vaccinium uliginosum* (Alsos *et al.* 2005), as well as in the arctic-alpine lichens *Flavocetraria cucullata* and *Flavocetraria nivalis* (Geml *et al.* 2010).

The lichenised nature may make *Lichenomphalia* particularly suitable for intercontinental dispersal. The small globules of the crustose thallus detach easily and can act as vegetative propagules (Kranner & Lutzoni 1999), allowing vertical transmission of the alga from generation to generation and providing more autonomy than basidiospores. Moreover, *Lichenomphalia* taxa often colonize disturbed soils that are likely to be exposed above snow level and subjected to strong winter

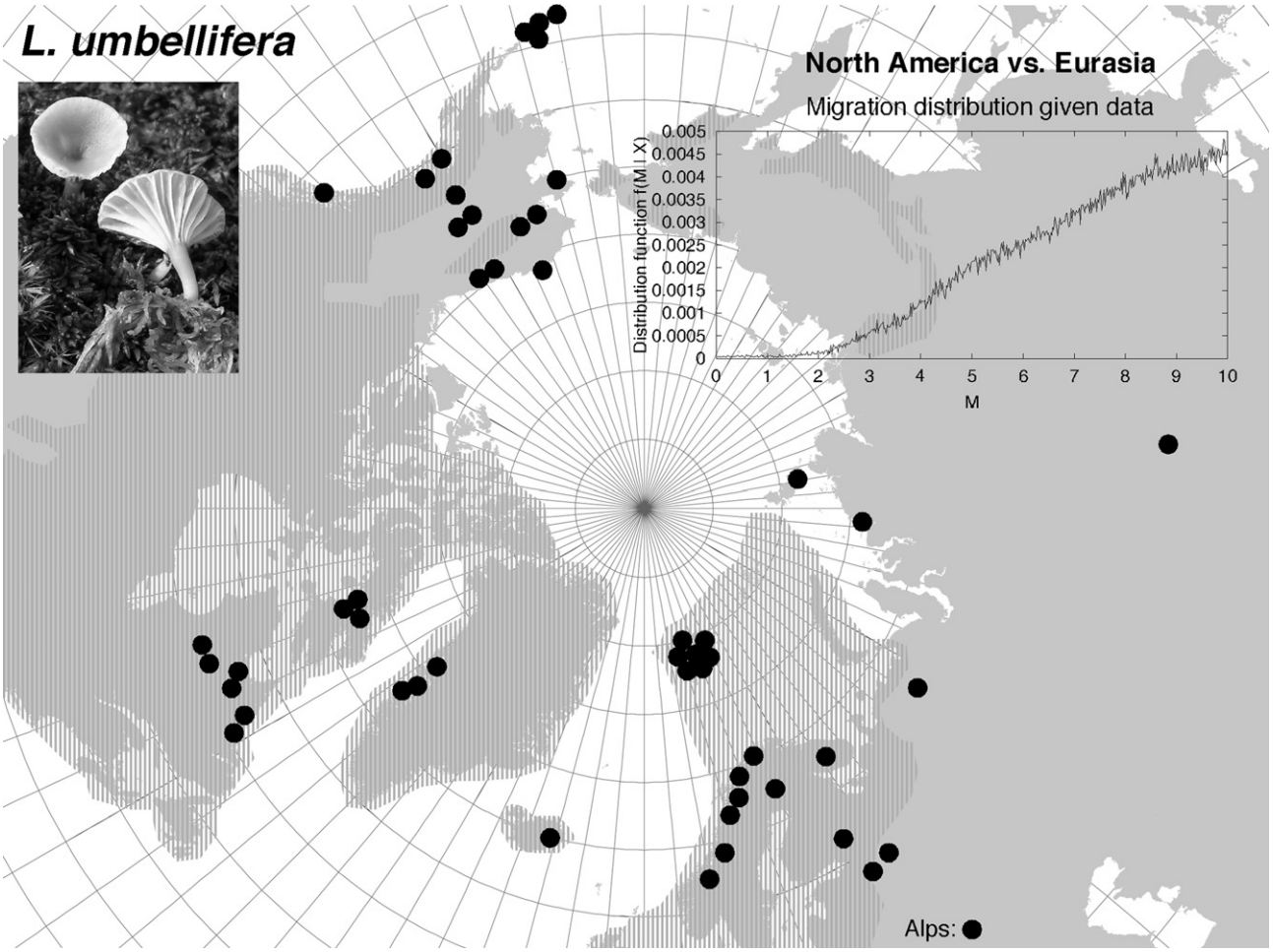

Fig 2 – Geographical locations of *L. umbellifera* samples in the Northern Hemisphere and intercontinental migration estimates. Shading indicates areas that were glaciated during the Last Glacial Maximum. Inserts show sporocarps of *L. umbellifera* and the posterior probability distribution of migration ($M = 2N_em$) estimated between Eurasia and North America using MCMC coalescent simulations in MDIV. For each dataset, the data were simulated assuming an infinite sites model, using 2 000 000 iterations in the chain, and an initial 500 000 iterations to ensure that a sufficient number of genealogies were simulated before approximating the posterior distribution.

winds. For example, *L. umbellifera* is often found on top of cryogenic earth hummocks and is particularly abundant when the soil of the hummocks has been exposed due to erosion or other perturbations. Finally, most mushrooms have mycelia that grow within the substrate and reproduce mostly *via* spores produced by ephemeral fruiting bodies, whereas the mycelium of these lichen-forming fungi occurs above ground, forming a symbiotic thallus to allow photosynthesis by the algal partner. These symbiotic crustose-globular thalli are also long-lived, offering year-round dispersal opportunities of the fungal and algal partners. The thalli are also preadapted to tolerate desiccation and exposure to UV light (Kranner & Lutzoni 1999; Zoller & Lutzoni 2003) which would benefit high altitude aerial dispersal, while preadaption to freezing would facilitate winter dispersal at the ground level (Savile 1972).

Besides the dispersal of thallus fragments, dispersal of basidiospores probably is important, as expected for many fungi. However, the symbiotic nature of *Lichenomphalia* means that after dispersal of its basidiospores, the fungus and the photobiont have to reestablish the lichenised state, a slow and complex process (Zoller & Lutzoni 2003). Wind dispersal of spores and thallus fragments should be particularly effective in the Arctic as a result of open landscapes, strong winds, and extensive snow and ice cover, as has also been suggested for arctic fungi, plants, and lichenised ascomycetes (Savile 1962, 1972, 1982; Alsos *et al.* 2007; Geml *et al.* 2010, 2011). In this regard, sea ice may be of particular importance for intercontinental dispersal, as it provides a frozen surface bridging the continents and archipelagos. Besides wind, other possible means of dispersal include spores and thallus fragments being carried by migratory animals, driftwood, and drifting sea ice. Despite the very low number of phylogeographic studies on arctic fungi, the gradually emerging picture indicates that arctic fungi may differ substantially from their low- and mid-latitude relatives regarding the extent of intercontinental migration, which has important implications for studies on the biodiversity, ecology, and conservation of arctic fungi in general.

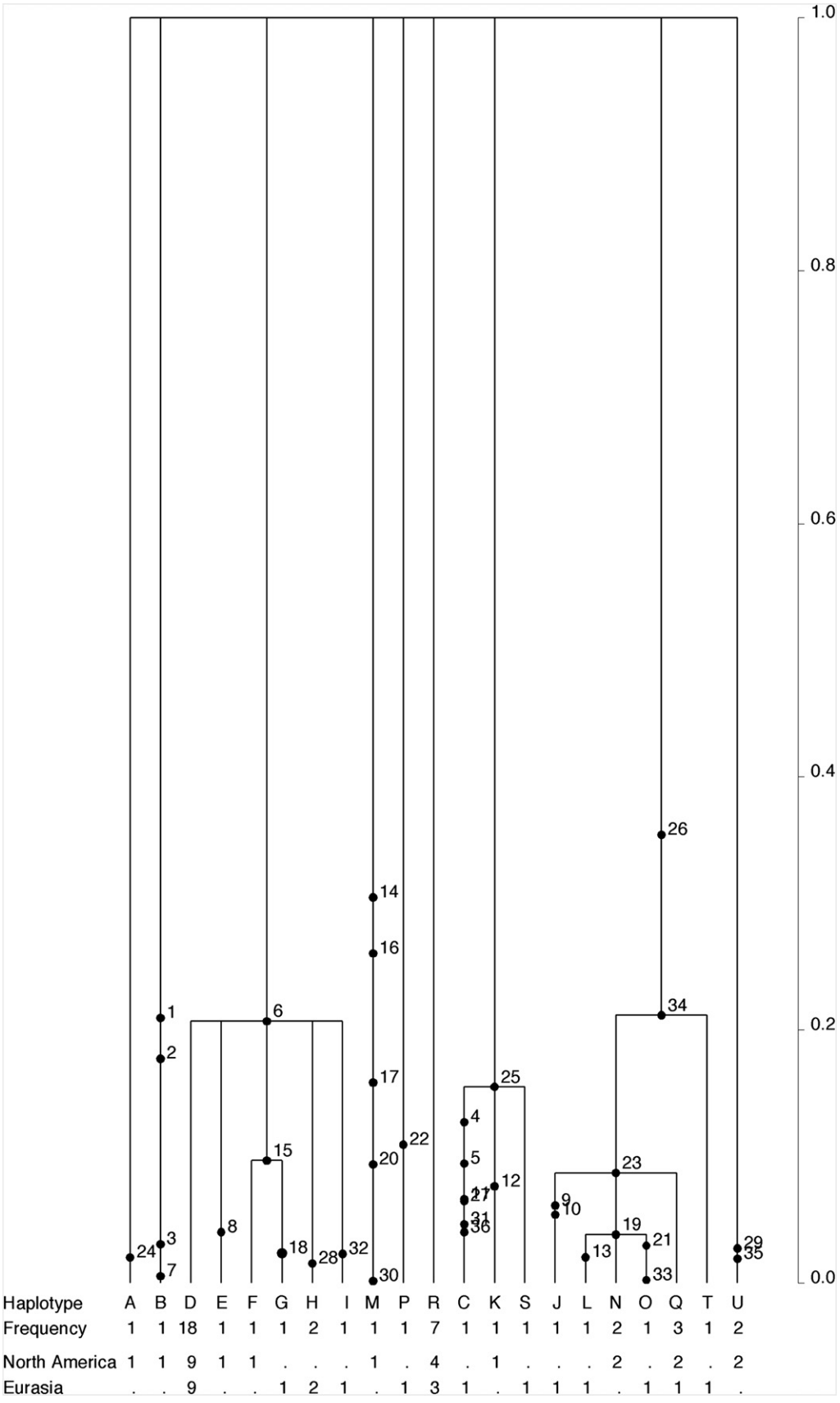

Fig 3 — Coalescent-based genealogy of haplotypes found in arctic and boreal *L. umbellifera* samples with the highest root probabilities (likelihood scores: $L = 9.9356 \times 10^{-52}$, $SD = 2.1275 \times 10^{-49}$) showing the distribution of mutations for the ITS region. The inferred genealogy is based on 2 000 000 simulations of the coalescent with a Watterson's (1975) estimate of *Theta* = 3.2. The time scale is in coalescent units of $2N_e$, where N_e is the effective population size. Mutations and bifurcations are time ordered from the top (past) to the bottom (present). Mutation designations correspond to the site numbers in Table 2. Numbers below the tree designate the distinct haplotypes, their observed frequencies in total and on the different northern continents.

Northern vs. *southern lineages*

Among fungi, many biogeographic studies comparing populations in the Northern *vs*. Southern Hemispheres have focused on bipolar ascolichens that form a ubiquitous component of high-latitude vegetation. In general, bipolar lichen species tend to be circumpolar in the north and only known from scattered localities in the Southern Hemisphere, although there are exceptions (Printzen 2008). The unequal distributions of landmasses and sampling efforts are likely to have contributed to this pattern.

In contrast to the high intercontinental gene flow observed in northern high latitudes, we found no phylogenetic species shared between the polar regions. Our phylogenetic analyses indicate pronounced genetic divergence between the sampled northern and southern lineages. Due to limited sampling in the Southern Hemisphere, it is currently uncertain whether the northern lineages occur in Gondwanan regions. On the other hand, our results strongly suggest that the southern lineages do not occur in the circumpolar north. The genealogical concordance suggests that the diverging arctic and subantarctic sister lineages (e.g., *Lichenomphalia umbellifera vs*. *Lichenomphalia* aff. *umbellifera*) have or are still undergoing phylogenetic speciation. This pronounced genetic differentiation between the polar regions is different from patterns seen in bipolar species of fungi for which comparable data have been obtained. Despite their moderate to high intraspecific nucleotide diversity, these latter species generally exhibit no genetic differentiation between southern and northern populations that are thought to have originated from very recent dispersal between the polar regions (Thell *et al*. 2002; Myllys *et al*. 2003; Seymour *et al*. 2007; Geml *et al*. 2010). In the majority of these studies, southward colonisations were inferred based on phylogeographic structure and comparisons of genetic diversity, with the exception of the lichen *Usnea sphacelata* (Seymour *et al*. 2007). Although our data are compatible with the hypothesis of colonisation of the Southern Hemisphere from northern populations in *Lichenomphalia*, more data from southern populations are needed to test this hypothesis. The molecularly sampled southern lineages are currently only represented by two species from one subantarctic island. It is, therefore, possible that these lineages are distributed over a larger area in the Southern Hemisphere and that they may have colonized southern islands from southern mid-latitude mainland areas or *vice versa*. Strong wind currents over the southern oceans have been repeatedly shown to play a major role in the long-distance dispersal of fungi and other cryptogams (e.g., Brown & Hovmøller 2002; Moyersoen *et al*. 2003; Moncalvo & Buchanan 2008) and migrating birds are suspected of transporting organisms as well (Savile 1972). Similarly, in another agaric, *Galerina patagonica*, specimens from Campbell Island and South America had identical ITS sequences, suggesting very recent transoceanic dispersal (Lee Taylor, Gary Laursen, Egon Horak unpubl. data).

Estimating divergence time is difficult in fungi because of the scarcity of fossils suitable for calibration and because of the substantial nucleotide substitution rate heterogeneity across lineages (Berbee & Taylor 2001; Taylor & Berbee 2006). Extrapolating from divergence time estimates published for other groups is particularly difficult in *Lichenomphalia*, because the genus has been shown to have significantly higher mutation rates than closely related, nonlichenised genera, possibly associated with the transition from free-living to mutualist lifestyle requiring exposure of the mycelium to ultraviolet light (Lutzoni & Pagel 1997; Zoller & Lutzoni 2003). Therefore, we cannot estimate the divergence times between the northern and southern phylogenetic species lineages with high confidence. On the other hand, our current knowledge is sufficient to distinguish between alternative scenarios that have been represented by two major schools of thought regarding the distribution of bipolar taxa. The first explains bipolar distribution with migration across the tropics either by 'mountain-hopping' during glacial periods (Darwin 1859; Kristiansen & Vigna 1996) or *via* recent long-distance dispersal, presumably by migrating animals or air currents (Galloway & Aptroot 1995). According to the second, presently disjunct populations are thought to be the remnants of formerly continuous populations that were broken up by vicariance events mainly as a result of continental drift and/or climatic changes (e.g., Du Rietz 1940; Kärnefelt 1990; Thomson 1995). Proponents of this latter view have claimed that many bipolar species are old and probably originated in the early Cenozoic (Kärnefelt 1990; Thomson 1995).

Based on our results, it is very likely that the northern and southern lineages diverged relatively recently and cannot represent the remnants of formerly continuous populations that were broken up by vicariance events in the early Cenozoic. A much more plausible explanation is migration across the tropics either by 'mountain-hopping' and/or *via* direct long-distance dispersal, although additional samples from the southern continents, especially from South America, are needed to test this hypothesis. Anecdotal evidence for taxa morphologically similar to *L. umbellifera* and *Lichenomphalia alpina* in Colombia and Venezuela (Jesús Hernández, pers. comm.) supports this argument. On the other hand, *Lichenomphalia aurantiaca*, a taxon morphologically similar to *L. alpina*, was described from Colombia (Singer 1970; Redhead & Kuyper 1987) and it is, therefore, possible that the name *L. alpina* is misapplied for South American collections. The possible presence of *L. umbellifera* in Gondwanan regions is similarly uncertain, because a morphologically similar taxon, *Lichenomphalia chromacea*, is a Southern Hemisphere species known from Southeast and Southwest Australia (Cleland 1924; Fuhrer 1985, 2005; Redhead & Kuyper 1987, 1988; Grgurinovic 1997; May & Wood 1997; Grey & Grey 2001; Anon 2011a) and this taxon may or may not be conspecific with the South American specimens labelled as *L. umbellifera*. However, another undoubted Australian *Lichenomphalia* identified as '*Omphalina ericetorum*' is illustrated and differentiated from *L. chromacea* in the books by Fuhrer (1985, 2005). Additionally, there are many scattered records of various synonyms of *L. umbellifera* from Australia (May & Wood 1997), some doubtful records from New Zealand (Horak 1971), voucher specimens of taxa under the names *L. alpina* and *L. umbellifera* from New Zealand's main islands (Anon 2011b), records of '*Omphalia umbellifera*' from New Zealand's subantarctic islands (Chilton 1909; Horak 1982), including South Georgia (Pegler *et al*. 1981; Horak 1982; Redhead 1989).

Therefore, southern reports of *L. alpina* and *L. umbellifera* are suspected until verified *via* molecular and thallar comparisons. Similarly, at least some of the records by Grgurinovic (1997) are suspected because she reports the presence of clamp connections. Clamp connections are absent in previously documented *Lichenomphalia* including specimens of *L. chromacea* examined by Redhead & Kuyper (1987, 1988). If present it would be novel. However, at least one other southern *Lichenomphalia* is definitely known from Tierra del Fuego, Argentina, under the name *Omphalina defibulata* Singer (Singer 1952; Horak 1979; Redhead *et al.* 2002). Redhead *et al.* (2002) examined an isotype and confirmed it was a *Lichenomphalia* and placed it in the *Lichenomphalia grisella–velutina* complex characterized by narrow thallar hyphae. Molecular data from this lineage from the south are not available. The only other reports of *Lichenomphalia* from South America are of *Lichenomphalia lobata* from Colombia and Ecuador (Singer 1970 [as *Gerronema hudsonianum*]; Redhead & Kuyper 1987; Palice *et al.* 2005). Two samples from Ecuador (Palice *et al.* 2005) are included in our analyses. It is possible that our southern phylogenetic species (*L.* sp. and *L.* aff. *umbellifera*) correspond in part to *L. aurantiaca* and *L. chromacea* and possibly a third taxon based on the morphological similarities and differences mentioned above. Unfortunately, no publicly available genetic data exist on *L. aurantiaca* and *L. chromacea* for comparison. Therefore, future studies should clarify the status and phylogenetic position of these species and whether the unidentified southern lineages in our analyses correspond to any of these taxa.

Based on their recent, but pronounced divergence, it is possible that northern *vs.* southern species pairs, particularly *L. umbellifera* and *L.* aff. *umbellifera*, separated during the glacial cycles of the Pleistocene, when northern tundra and boreal forest areas were geographically closer to their southern cold-climate equivalents, making transequatorial dispersal more likely. Such a scenario is currently the most accepted biogeographical hypothesis for plant bipolar disjunctions: i.e., dispersal during the cold periods of the Pleistocene, when polar regions both hemispheres, either by long-distance dispersal, presumably by migrating animals or air currents (Galloway & Aptroot 1995), or *via* 'mountain-hopping', particularly along the north–south mountain chains (Van Steenis 1962; Raven 1963; Kristiansen & Vigna 1996; Vollan *et al.* 2006; Escudero *et al.* 2010). Animals may play a particularly important role in occasional transequatorial dispersal of spores and/or thallus fragments, because many migratory bird species travel between the polar regions. In the bipolar crowberries (*Empetrum*), which have fleshy bird-dispersed fruits, a fossil-calibrated relaxed molecular clock has recently been used to model the sequence evolution in two nuclear low-copy genes and two plastid DNA regions (Popp *et al.* 2011). The median estimates of the time to the most recent common ancestor for Northern and Southern Hemisphere *Empetrum* were 0.56–0.93 Ma, and 0.26–0.59 Ma for the Southern Hemisphere plants only. The southern clade was imbedded in a large and widespread northern clade, with Northwestern American *Empetrum* consistently identified as sister to the southern clade. This implies that a single mid-Pleistocene long-distance dispersal event, possibly *via* birds migrating from Alaska to southernmost South America, could explain the extreme bipolar disjunction.

Despite the above uncertainties, it is clear that Campbell Island was almost certainly colonized by at least two *Lichenomphalia* lineages *via* transoceanic dispersal either from another southern landmass or perhaps directly from the Northern Hemisphere. Although the times of colonisation of Campbell Island could substantially differ according to the alternative scenarios, all scenarios support our argument: i.e., the frequency of dispersal appears to be scale-dependent: subject to the limitations of our markers, intercontinental gene flow appears to be high in the same climatic belt (e.g., the northern circumpolar), while transequatorial dispersal is rare, ultimately leading to allopatric speciation, a pattern also demonstrated in *Empetrum* (Popp *et al.* 2011). Not surprisingly, our sampling efforts likely do not cover the entire distribution ranges of the studied *Lichenomphalia* species, partly because the complete ranges are unknown and because of the logistical difficulties of obtaining specimens spanning different continents and hemispheres. Therefore, future works incorporating collections from additional geographic areas will likely improve our current understanding of the phylogeography of the genus.

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