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A phylogeographic survey of a circumboreal polypore indicates introgression among ecologically differentiated cryptic lineages

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ABSTRACT

Gloeoporus taxicola is a widespread saprotrophic polypore that occurs on a variety of coniferous substrata in the Northern Hemisphere. In this study a multi-locus sequencing approach was used, on an extensive worldwide sample, to investigate the phylogeography of *G. taxicola* with respect to substratum affinity. DNA sequences from two nuclear and one haploid mitochondrial marker gave a complex phylogeographic pattern that roughly divided the specimens into two evolutionary lineages, but some admixed and highly heterozygous sequences appeared as well in the diplophase data. To increase the resolution, cloning was performed and haplophase sequences obtained from the nuclear markers. This revealed three main clusters of haplotypes, one representing a European lineage associated with pine, while the other two had more northern circumboreal distributions, occurring on a wide number of substrata. Some specimens contained two highly divergent haplophase sequences, probably reflecting hybridization and further introgression between the separate evolutionary lineages. Despite the saprobic status of the fungus, there was a strong indication of different host affinity between the two main evolutionary lineages.

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Introduction

Multiple cryptic species are commonly found in phylogenetic and phylogeographic studies of fungal morphospecies (Hibbett et al. 1995; Cruse et al. 2002; Dettman et al. 2006; Geml et al. 2006; Le Gac et al. 2007; Kauserud et al. 2007b; Carlsen et al.

2011). In most eukaryote taxa nomenclature and species descriptions are mainly based on morphological characters. However, in fungi, the phenotype often seems to evolve slower than intrinsic reproductive barriers, resulting in numerous cryptic lineages (Taylor et al. 2006). The occurrence of cryptic species adds an extra layer of complexity to

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phylogeographic analyses of fungal morphospecies. Another complicating factor is gene flow and hybridization among cryptic lineages (Kausserud et al. 2007c; Linzer et al. 2008). Hybrids may have lower fitness than their parents when they are competing on the same substratum (Garbelotto et al. 2007). However, in some instances hybridization can result in the formation of a 'superspecies' which combines the host range of its parents (Brasier 1994) or even develops the ability to exploit a new niche (Newcombe et al. 2000).

A common view of microorganisms (including fungi) has been that 'everything is everywhere' (Finlay 2002), meaning that an organisms' propagules have the ability to spread worldwide. However, several studies have revealed that fungal populations on different continents are often genetically highly differentiated (O'Donnell et al. 1998; Johannesson & Stenlid 2003; Kuehne et al. 2007), suggesting that speciation through differentiation in allopatry may be as important in fungi as in other organism groups (Kohn 2005). Moreover, significant geographic structuring has recently been observed in many fungal taxa even at intracontinental geographic scales (Dai et al. 2002, 2003; Dettman et al. 2003; Geml et al. 2008).

Ecological adaptation through host shifts and substratum specialization is likely to be an important mode of speciation in fungi (Peever 2007). Divergence within a species due to exploitation of different niches is a process that may reduce gene flow and lead to speciation (Schluter 2000). Such ecologically based divergence has been observed both in the field (Feder et al. 1994) and in the laboratory (Dettman et al. 2007). In theory, a gene with a bearing on fitness associated with the choice of hosts may lead to ecological speciation in sympatry through disruptive selection (Smith 1966) as proposed in fungi by Stukenbrock et al. (2007). Fungal species, described upon morphology as generalists on several hosts, have often turned out to consist of multiple phylogenetic (cryptic) species specialized on various hosts (Garbelotto et al. 1996; Le Gac et al. 2007). Such host-specificity appears to be common in pathogenic fungi, in which two symbionts interact with each other. For saprobes, true host-specialization seems to be much rarer, however (Zhou & Hyde 2001).

In this study, we analyze the circumboreal phylogeographic structure of the bracket fungus *Gloeoporus taxicola*. *G. taxicola* is a saprotrophic polypore with a northern boreal distribution, but it is also reported from Australia and New Zealand (Ryvarden & Gilbertson 1993). It occurs on dead wood from a number of coniferous hosts and infrequently also on hardwood trees. Like most other basidiomycetes, it is presumed that *G. taxicola* has a dominant dikaryotic vegetative stage ($n + n$). Compared to many pathogenic fungi, which are affected by human agriculture and settlements, *G. taxicola* may be little influenced by human activities. Accordingly, the species may provide an example of a more natural phylogeographic pattern, compared to those seen in many other fungi. In an earlier study of *G. taxicola* (Kausserud et al. 2007a) it was shown that two differentiated lineages with divergent substratum requirements occur in Norway. One lineage occurs in Norwegian highland regions, mostly on *Picea abies*, while another resides exclusively in lowland regions on *Pinus sylvestris*.

The aims of this study were to: (1) analyze the phylogeographic pattern of *G. taxicola* on a global scale; (2) investigate

whether the various lineages of *G. taxicola* differ in host tree substratum affiliation; (3) test whether hybridization, as defined by Arnold (1997), is occurring among lineages. To answer these questions, a worldwide collection of *G. taxicola* specimens was analyzed using multi-locus sequence data.

Materials and methods

Material

We analyzed a total of 215 herbarium specimens and 18 live cultures of *G. taxicola* in this study. For details see [Supplementary Information Table S1](#). Where available, information about host substrata (tree genus) is included.

Molecular analyses

Small pieces of the herbarium specimens (basidiocarps) were cut off using a sterile blade and frozen to -80°C in 600 μl hexadecyltrimethylammonium bromide (CTAB). From the cultures, mycelia were scraped off potato dextrose agar plates, transferred to an eppendorf tube containing the same amount of CTAB, and frozen. Two sterile tungsten carbide beads were used to crush the material on a Retsch[®] MM301 machine (Anders Pihl AS, Dale, Norway) for 4 min with the frequency of 20 Hz. DNA extraction was completed following a 2 % CTAB protocol (Murray & Thompson 1980) with a few modifications as described in Kausserud et al. (2007a).

DNA sequences were obtained from three different regions: ITS (the nuclear ribosomal internal transcribed spacer region), *tef* (a part of the translation elongation factor 1- α gene) and mtLSU (the mitochondrial ribosomal large subunit). ITS was amplified with the primers ITS4 and ITS5 (White et al. 1990), *tef* was amplified with the primers EF595F and EF1160R (Kausserud & Schumacher 2001) and mtLSU was amplified with the primers ML5 and ML6 (White et al. 1990). PCR was performed in 25 μl reactions using puReTaq[™] Ready-To-Go[™] PCR Beads (GE healthcare, Buckinghamshire, UK) with 5 μl of 50X diluted DNA, 2.5 μl of each 5 μM primer and 15 μM sterilized H_2O . For both ITS and *tef* the PCR program was as follows: denaturation at 94°C for 4 min followed by 37 cycles of denaturation at 94°C for 25 s, annealing at 54°C for 30 s and extension at 72°C for 35 s followed by a final elongation step at 72°C for 10 min. The conditions for mtLSU were: 94°C for 5 min followed by 30 cycles at 94°C for 20 s, 54°C for 20 s, 72°C for 45 s followed by a final step at 72°C for 7 min. In cases of initial amplification failure, we rinsed the DNA with columns from E.Z.N.A.[®] DNA Blood Kit (Omega Bio-Tek, Doraville, GA, USA) and ran a second PCR reaction using 5 μl of undiluted DNA. DNA was successfully isolated from 10 more specimens in that way. All PCR products were sequenced in both directions using the ABI BigDye Terminator sequencing buffer and v3.1 Cycle Sequencing kit on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

A total of 232 ITS, 172 *tef* and 135 mtLSU sequences were obtained through direct sequencing of amplicons derived from dikaryotic mycelia, hereafter referred to as dikaryophase sequences. The ITS region was amplified more successfully

than mtLSU and *tef* from old herbarium specimens (see Table S1 for successfully obtained sequences by specimen).

In many ITS and *tef* dikaryophase sequences, double peaks occurred in several positions, reflecting heterozygosity. Special emphasis was put on the putative hybrid sequences and dikaryophase sequences with three or more heterozygous sites; these were cloned to separate the underlying allelic haplophase sequences. Sequences containing one heterozygous site were manually split into two haplotypes while those containing two heterozygous sites (less likely to represent hybrids) were not analyzed by cloning and discarded from the haplophase datasets. For these specimens, PCR reactions were re-run using the fidelity enzyme DyNAzyme EXT (Finnzymes Oy, Espoo, Finland) in combination with prolonged steps in the PCR, to reduce the amount of artificial mutations and chimeric sequences. The program was as follows: 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 45 s and extension at 72 °C for 90 s followed by an ending elongation step at 72 °C for 10 min. Cloning was done with the TOPO TA Cloning Kit (Invitrogen Corporation, Carlsbad, CA, USA) using blue-white screening according to the manufacturer's manual. Eight positive colonies per amplicon were subjected to direct PCR with the universal M13R and T7 primers using the same PCR conditions as above. The cloned fragments were sequenced in one direction with the M13R primer and used to infer the two haplophase sequences. Only those specimens from where two or more readable cloned sequences were obtained were included since the appearance of artificial PCR mediated mutations is a risk when performing cloning. For each specimen, sub-alignments were constructed including the original dikaryophase sequence together with the cloned haplophase sequences. Mutations appearing in the cloned haplophase sequences but not in the original dikaryophase sequence were interpreted as PCR mediated mutations and discarded from further analysis (see Popp & Oxelman 2001 for a more thorough explanation). Two allelic haplophase sequences were obtained from the sub-alignment of each specimen.

All sequence chromatograms were examined manually and sequences were aligned in BIOEDIT (Hall 1999). Haplophase and dikaryophase sequence alignments were established for both ITS and *tef*, and one haplophase dataset for the mitochondrial marker, i.e. five datasets. All sequences have been accessioned in GenBank (accession nos. JX875956–JX876487) (see Supplementary information Table S1 for details).

Analyses of dikaryophase data

In addition to the high number of heterozygous sites appearing in the dikaryophase sequences, indels at two different positions in either of the nuclei in some of the *tef* sequences occurred (four and two bp). This caused unreadable sequences downstream of the indel (but full length sequences were obtained by sequencing in both directions). In such heterozygous sequences, these sites were coded as XXXX (CTTC/—) and XX (GT/—) in the dikaryophase alignment. To keep information from these sites in the phylogenetic analyses, the indelsites were treated as single characters and recoded (e.g. GT = G, — = A, XX = R), enabling their inclusion in the phylogenetic analyses. Only one indel occurred in the ITS dataset and was

recoded as an A (the other sequences had a T in that position). Phylogenetic analyses of alignments including multiple heterozygous sites are problematic. We chose to do NeighborNet network analyses on the dikaryophase datasets in SplitsTree 4 (Huson & Bryant 2006), as networks give graphical outputs that place heterozygous sequences at an intermediate position between two divergent homozygous sequences. Mid-point rooting was used in all phylogenetic presentations, since no applicable outgroups to *G. taxicola* are known. For example, *Gloeoporus dichrous* is distantly related to *G. taxicola* (data not shown). The number of variable and parsimony informative sites were calculated in MEGA version 4 (Tamura et al. 2007).

Analyses of haplophase data

Haplophase data were obtained by splitting up the heterozygous sequences into the two underlying haplophase sequences (see above). To visualise the degree of reticulation among the haplotypes, median joining networks were made in ARLEQUIN version 3.1 (Excoffier et al. 2005). The indels were coded as separate single characters as in the dikaryophase analyses.

To evaluate whether the different genetic lineages of *G. taxicola* had substratum preferences (*Pinus* versus *Picea*) in Fennoscandia, chi-square tests were conducted in R (R Development Core Team 2006).

Results

Dikaryophase data

The ITS alignment included 568 characters, of which seven were variable (1.2 %) and four (0.7 %) were parsimony informative. The *tef* alignment included 487 characters, of which seven were variable (1.4 %) and five (1.0 %) parsimony informative. Sixty-eight unique sequences appeared in the ITS dataset while 28 were found in the *tef* dataset. A relatively high proportion of the sequences included multiple (up to five) polymorphic sites (see Appendix), putatively reflecting heterozygosity. To make full use of the information in dikaryophase sequences, including multiple heterozygous sites, NeighborNet networks were obtained (Fig 1). Both the ITS and *tef* networks divided the samples into various sub-clusters (see Fig 1). One mainly European group, mostly growing on *Pinus* (hereafter referred to as PG), comprised specimens from Continental Europe and Fennoscandia (82 ITS sequences, 93 *tef* sequences). Further, there appeared two closely related widespread groups (designated WG). The two WG sub-groups, detected on numerous substrata, comprised specimens from Quebec through the Russian taiga to Fennoscandia and the Alps (120 ITS sequences and 70 *tef* sequences in total). In both the ITS and *tef* networks some highly heterozygous sequences (up to five polymorphic sites) appeared between the PG and the WG groups (Fig 1). Most of the heterozygous sequences in the *tef* network clustered within the WG group (i.e. between the two WG sub-groups). The heterozygous sequences appearing in an intermediate position in the network (designated as Hybrid group in Fig 1) were from Russia, Canada, Austria, The Czech Republic, Finland, Sweden and Norway (31 ITS sequences, 13 *tef* sequences).

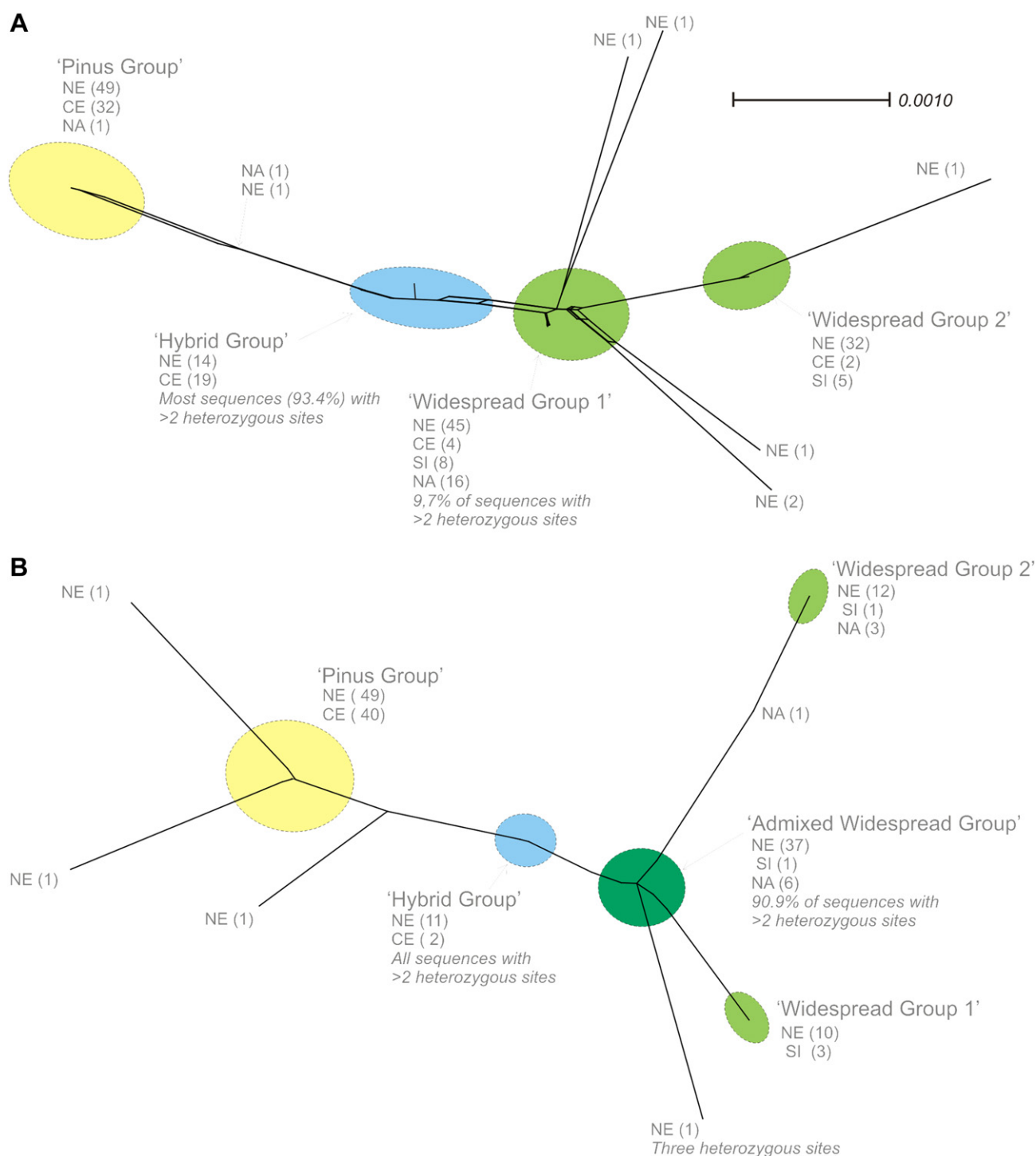


Fig 1 – NeighborNet analysis of the unique dikaryophase sequences of (A) ITS and (B) *tef*. The number of identical sequences from each region is indicated. Two main groups were detected, the widespread group (WG) depicted in blue and the Pinus group (PG) in yellow. Some putative admixed (hybrid) sequences appeared as well (blue), clustering at an intermediate position. The highly heterozygous sequences within the WG group in the *tef* gene are interpreted as admixed specimens including alleles from the different sub-groups within WG. Abbreviations: Continental Europe (CE), North America (NA), Northern Europe (NE), Siberia (SI).

Haplophase data

Three haplophase sequence data sets were obtained (see [Material and methods](#)), including 372 ITS sequences (derived from 186 specimens), 296 *tef* sequences (representing 148

specimens) and 135 mtLSU sequences. Forty unique ITS haplotypes, 15 *tef* haplotypes and only two mtLSU haplotypes were found. Both the resulting ITS and *tef* haplotype-networks revealed three main haplotype clusters ([Fig 2](#)), one representing the PG group and the two others corresponding to the

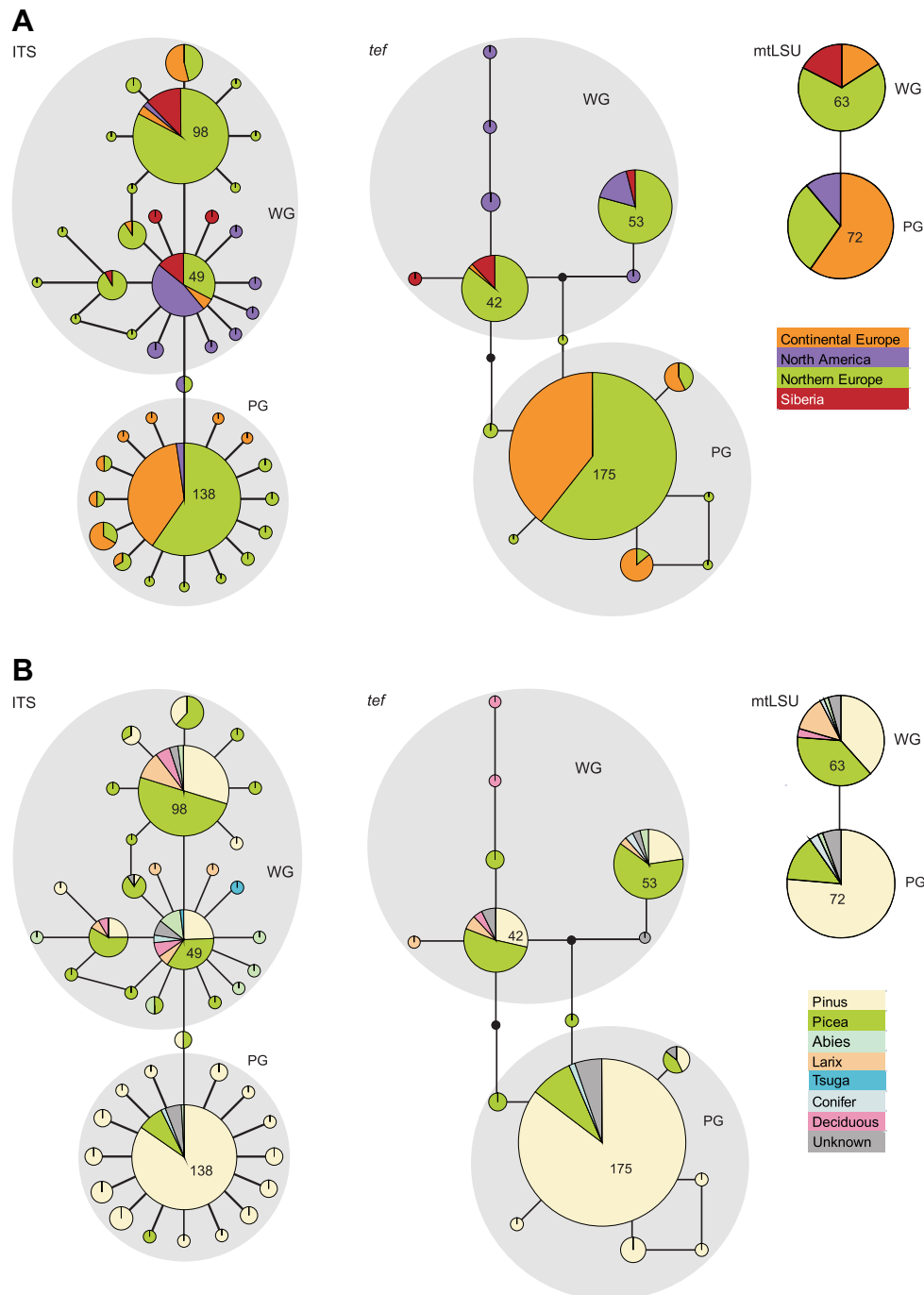


Fig 2 – Haplotype-networks for ITS, *tef* and mtLSU with their distributions according to (A) geography and (B) substratum are indicated. Each circle represents a unique haplotype and the size of circles represent the relative frequencies of the haplotypes, ranging from 1 to 175. Numbers of the three most frequent haplotypes are indicated. There is one mutational step between each circle. Black circles indicate inferred haplotypes.

WG group in the dikaryophase analysis. The mtLSU haplotypes only occurred in two states, one representing the PG group and the other the WG group (but see below). Noteworthy, largely the same specimens appeared in the PG and WG groups across the ITS and *tef* networks; only 10 specimens had ITS haplotypes in the PG group and *tef* haplotypes in the WG group, or vice versa. These specimens were from Norway,

Finland, Czech Republic and Austria. However, many specimens switched between the two WG haplotype clusters across ITS and *tef*, indicating recombination within WG. The mtLSU haplotype network further supported the grouping into two main lineages but there were some inconsistencies between the nuclear and the mitochondrial markers; eight specimens from Austria, Bulgaria, Czech Republic, Germany, Finland and

Sweden had a mitochondrial PG haplotype when a WG type was expected or *vice versa*. Furthermore, all the mtLSU haplotypes from North America belonged to the PG type, while these specimens almost exclusively belonged to the WG type in the nuclear markers. The geographic distributions of the three haplotype main groups, as indicated in Fig 2, are shown in Fig 3.

The haplophase datasets revealed that 12 of the highly heterozygous dikaryophase sequences (with three or more heterozygous sites) included one haplotype from the PG group and another from the WG group. Furthermore, some highly heterozygous dikaryophase sequences included haplotypes from both WG clusters (five in the ITS dataset and 23 in the *tef* dataset). A higher level of molecular variation was found in the WG group, both in ITS ($\pi = 0.00185$, $k = 1.05$) and *tef* ($\pi = 0.0023$, $k = 1.06$), compared to PG ($\pi = 0.00055$, $k = 0.31$ for ITS and $\pi = 0.0005$, $k = 0.23$ for *tef*). Since only one polymorphism was observed in the mtLSU dataset it was discarded from the latter analyses.

Substrate affiliation

The substratum affiliation of the haplotypes is shown in Fig 2B. Although none of the most frequent haplotypes were restricted to a specific host, there were some clear trends. The PG haplotypes were most frequently detected on *Pinus* (85 % in ITS and *tef*, 75 % in mtLSU). Noteworthy, when a PG haplotype was found on a different host it often occurred in individuals that also possessed a WG haplotype. The WG haplotypes were detected on a variety of substrata and more often on *Picea*, *Larix*, *Tsuga*, *Abies* and deciduous trees than the PG haplotypes. A more detailed comparison of the substratum affiliation (*Pinus* versus *Picea*) of PG and WG haplotypes from Norway and Finland was performed as we had large sample sizes ($n = 125$) from both of these areas. The distribution of the PG and WG haplotypes on the two substrata differed significantly from even (chi-square test, $p < 0.05$), with the PG haplotypes being more common on *Pinus* and the WG haplotypes on *Picea*. Interestingly, there was a more distinct host differentiation of the PG and WG groups in Norway compared to Finland (Table 1).

Discussion

Biogeography

Phylogenetic analyses of the three loci, ITS, *tef* and mtLSU revealed that the morphospecies *G. taxicola* consists of two major evolutionary lineages in the Northern Hemisphere; one almost exclusively European (PG) associated with *Pinus* and another more northern circumboreal (WG) occurring on a variety of substrata. The WG lineage included two sub-groups. Despite the few parsimony informative sites, high congruence was observed across the three loci in delimiting specimens into the two main groups (PG and WG), indicating that these two groups to a large extent can be seen as independent evolutionary units. The division is in accordance with what was observed in a smaller sample from Norway (Kausserud et al. 2007a). Specimens belonging to the two WG sub-groups were not consistent across the two analyzed loci;

some specimens belonged to one sub-group in ITS and the other in *tef* and *vice versa*, probably due to extensive recombination within WG. Hence, WG was considered as one main lineage. The finding of only two mtLSU haplotypes supports the existence of two main lineages in *G. taxicola*.

The WG lineage has a wide circumboreal distribution from Scandinavia throughout Eurasia to eastern Canada. Wide-spread circumboreal distributions of lineages, like in WG, have been observed in several basidiomycetes, including *Amanita muscaria* (Geml et al. 2006) and *Coniophora puteana* (Kausserud et al. 2007c). Although recombination is apparent between the two haplotype sub-groups occurring in WG, there seems to be some geographic sub-structuring among them, since almost all the North American haplotypes belonged to one of the sub-groups in both ITS and *tef*. The two WG sub-groups could represent lineages that have diverged in allopatry, followed by range expansions leading to secondary contact, interbreeding and recombination as observed in, for example, the orchid *Dactylorhiza majalis* (Nordström & Hedrén 2008). Notably, most of the highly heterozygous sequences in the *tef* dataset (including three or more heterozygous sites) contained divergent haplotypes from within WG, which might reflect admixture of previously differentiated lineages (see Fig 1).

Compared to the widespread WG lineage, PG has a more narrow distribution and host range, occurring from Bulgaria to Fennoscandia. It occurs almost exclusively on *P. sylvestris*, which is thought to have recolonized Europe after the last glaciation from a few patchy refugia in southern Europe (Cheddadi et al. 2006). Like its main host (Soranzo et al. 2000), the PG lineage has one predominant haplotype, both in ITS and *tef*, spread all over Europe. PG nucleic haplotypes were not recorded outside Europe, except for two Canadian specimens from Saskatchewan and Ontario. These could represent human mediated introduction events, which is a common phenomenon in fungi (Coetzee et al. 2001; James et al. 2001; Slippers et al. 2001; Kausserud et al. 2004; Kausserud et al. 2006). It seems unlikely that *G. taxicola* has spread naturally from Europe to Canada by wind, especially since *G. taxicola* is an outcrossing species where the germinating spore apparently must fuse with another compatible monokaryon to complete its lifecycle.

Hybridization

Some specimens contained nucleic haplotypes from both PG and WG. These putative hybrids were from Fennoscandia and the Alps, where the two lineages occur in sympatry. These are also the areas where the eight mitochondrial sequences conflicting with the nucleic sequences with respect to affinity with the PG or WG groups were derived from, indicating gene flow between the two main lineages. Hybrid zones in these areas are known from other organisms, including *Ursus arctos* (Taberlet & Bouvet 1994), *Corvus* (Haas et al. 2009), *Quercus* (Ferris et al. 1998) and *Abies alba* (Konnert & Bergmann 1995). Hybridization is likely to occur between PG and WG because their ecological niches overlap fully in these areas, thus making intertaxon crossing as likely as normal intrataxon crosses (Burnett 2003). Hybridization among lineages occurring in sympatry is also known from other wood decomposers such as *Heterobasidion annosum* s.l. (Garbelotto et al. 1996).

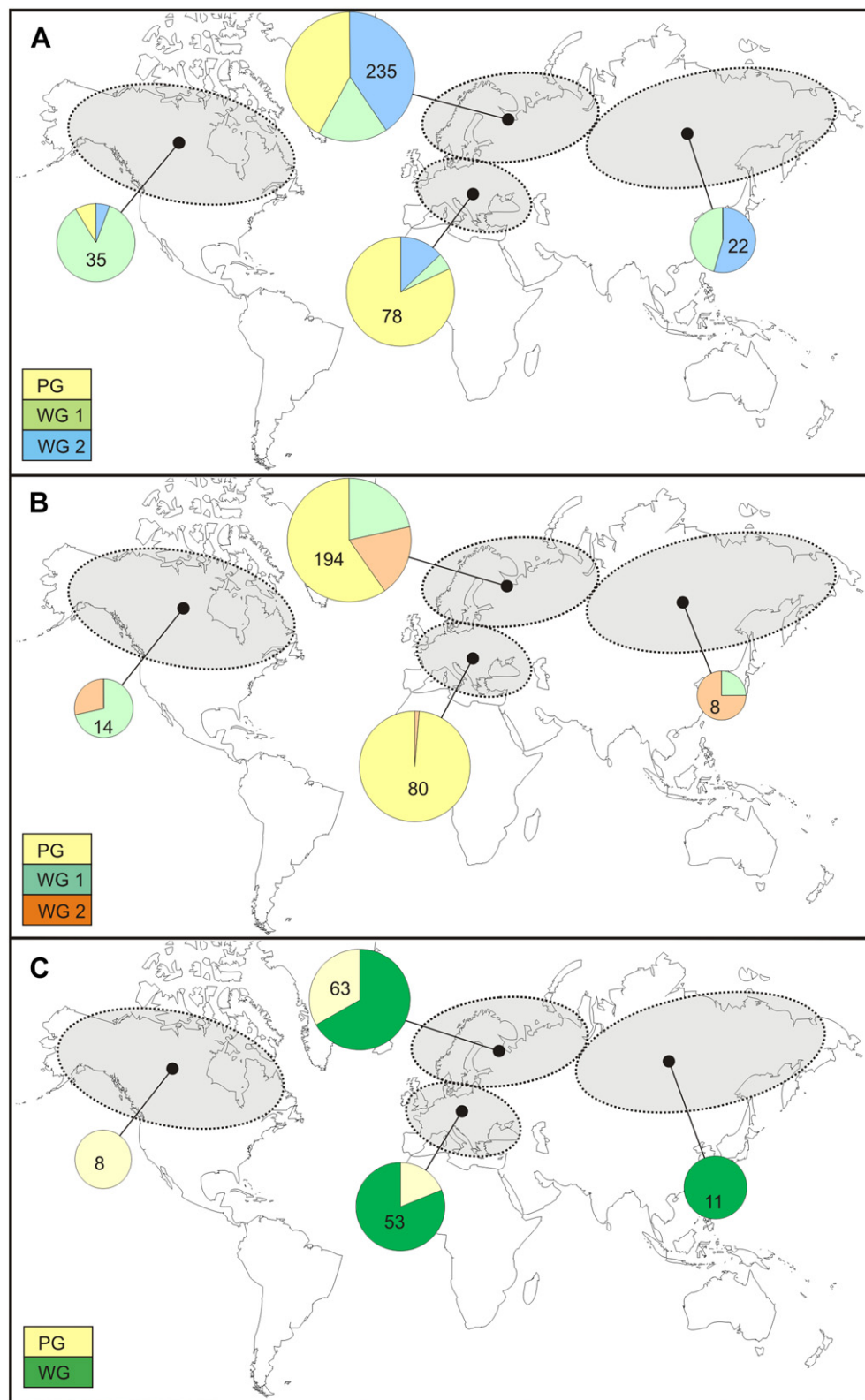


Fig 3 – The geographical distribution of the three haplotype main groups, as revealed in Fig 2 for (A) ITS, (B) *tef* and (C) *mtLSU*. Two ITS haplotypes having an intermediate position between PG and WG are not shown. The sample is for simplicity divided into four large geographical regions and the different colours in the two WG-lineages in ITS and *tef* indicates that they do not correspond to each other.

Table 1 – The p-values of chi-square test of the WG and PG lineages occurring on *Pinus* and *Picea* in Norway and Finland, assuming that the distributions of the two genera are similar in both countries. The mtLSU dataset was not included due to low sample. Cut off was set to $p < 0.05$. Non-significant value is given in bold

Region	Locus	Group				χ^2 – test	
		WG		PG		χ^2	p
		<i>Pinus</i>	<i>Picea</i>	<i>Pinus</i>	<i>Picea</i>		
Both	ITS	34	84	78	9	74.8	***
	<i>tef</i>	23	51	93	21	54.8	***
Norway	ITS	5	51	64	3	92.9	***
	<i>tef</i>	7	36	67	12	54.8	***
Finland	ITS	29	33	11	3	4.6	*
	<i>tef</i>	16	15	12	7	0.6	–

Significance level: $-p > 0.10$, $*p < 0.05$, $***p < 0.001$.

Another example from Europe is the Dutch elm disease pathogens *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* (Brasier & Buck 2001). The putative hybrid zones between the WG and PG lineages are likely to have formed after the ice retreated, i.e. not more than 9 000 yr ago (Barton & Hewitt 1985).

Selection on substrata could be a barrier to gene flow between PG and WG, leading to reinforcement (Dobzhansky 1937). Garbelotto et al. (2007) demonstrated experimentally that different lineages of *Heterobasidion* were able to mate and that the fitness of the hybrid, measured as ability to decompose, was reduced. Controlled mating studies on different substrata are needed to test this hypothesis in *G. taxicola*.

Interestingly, several of the *G. taxicola* specimens displayed haplotypes from PG and WG together at one locus, but only six individuals had an admixed genotype in both loci from PG and WG. This indicates that the majority of the admixed specimens are not direct hybrid dikaryons (F1 generation) but rather F2 or later generation backcrosses, suggesting that introgression may occur. More extensive sampling in the putative hybrid zones is necessary to estimate the shape of the hybrid zone and rate of introgression.

Substratum affiliation

The WG lineage was found on a variety of substrata, including deciduous trees, throughout its range and one of the most frequent WG haplotypes was collected from as many as 13 different substrata. Another widespread (cosmopolitan) lineage of a saprotrophic basidiomycete, occurring on numerous substrata, is known from *Schizophyllum commune* (James et al. 2001). Hence, WG seems to be more of a generalist compared to PG, where more than 83 % of the specimens were found on *Pinus*. Notably, when a PG haplotype was found on substrata other than *Pinus*, it was usually in pair with a WG haplotype, likely caused by hybridization. Only a single specimen (from Sweden) possessing all four haplotypes from the PG lineage occurred on a substratum other than *Pinus*, namely on *Picea*. In Fennoscandia, the WG type was found mainly on *Picea*. Interestingly, in Russia east of the Ural Mountains, WG

was detected mainly on *Larix*, in the Alps mainly on *Pinus* and in Canada mainly on *Abies*, despite the occurrence of *Picea* in all of these areas. This pattern indicates that WG to some extent has adapted to various types of substrata in different regions and that it possesses a robust and plastic enzymatic apparatus, or locally adaptations in ways of establishment, able to shift between substrata. A similar pattern has been observed in the polypore *Fomitopsis pinicola* (Ryvarden & Gilbertson 2003). This also indicates that WG probably has not been confined to the refugium of a single specific host tree during glacial periods. The pattern parallels, to some degree, what was observed in the S and P intersterility groups of *H. annosum* s.l. in Europe (Johannesson & Stenlid 2003): while PG and P occur mostly on *Pinus*, WG and S occur on *Picea* and *Abies*.

Even at smaller geographic scales (within Fennoscandia), a difference in substratum affiliation was detected in the WG lineage (Table 1). In Norway, WG is almost exclusively recorded from *Picea*, but in Finland it is commonly found on *Pinus*, as well. The pattern could be related to the time span the two lineages have been in sympatry. Finland is thought to have been rapidly colonized by both *Pinus* and *Picea* after the last glacial maximum (Giesecke & Bennett 2004) while Norway was colonized by *Picea* at a comparably later stage, which means that more gene flow could have happened between the lineages in Finland compared to Norway. We hypothesize that this has led to a breakdown in substratum affiliation, as partly observed in Finland. In Finland, only 19 % ($n = 84$) of the total ITS haplotypes were designated to the PG lineage, compared to 54 % ($n = 122$) in Norway. Despite the few PG haplotypes in Finland, 25 % of them were in pair with a WG haplotype, compared to 4.5 % in Norway, consistent with a higher degree of recombination and, thus, breakdown in substratum affiliation in Finland. Alternatively, the WG lineage could be about to outcompete the PG lineage in Finland, as possibly reflected by the few PG haplotypes observed. The hybridization between the two lineages could also be enforced by the lack of potential mates for the PG specimens in Finland as observed in other taxa, e.g. *Canis lupus* (Boitani & Mech 2003).

The PG lineage had a narrower host range combined with lower molecular variation compared with the WG lineage. This could reflect that the former has gone through a bottleneck as a consequence of re-colonization from a smaller population or fast re-colonization, following the leading edge model (Hewitt 2000).

Zhou & Hyde (2001) proposed that enzyme systems of the saprobe and/or unique characteristics presented by the host tree are what make the fungus a specialist or generalist. Some wood-inhabiting fungi occur on a variety of substrata, e.g. *S. commune* (Cooke 1961), while others are reported only from one substratum, e.g. *Rosellinia herpotrichoides* that occurs only on *Tsuga canadensis* (Huhndorf & Lodge 1997). *H. annosum* s.l. is known to have several hosts in its saprobic stage, but is only capable of attacking one host in its parasitic stage (Otrosina et al. 1993), thus illustrating the dissimilarities between parasitic and saprobic fungi regarding host affinity. No parasitic stage is known in *G. taxicola*, but the PG lineage, at least in Fennoscandia, typically occurs on dying branches, still attached to living *Pinus*, while WG is restricted to decaying logs (field observations). Hence, the higher host recurrence of the

PG lineage on *Pinus* could be because some defence mechanisms remain that it has to cope with, requiring specificity to exploit the substratum.

Conclusion

The results of this study indicate that two main lineages of *G. taxicola*, with divergent distributions and substratum affinity, occur in the Northern Hemisphere, with some hybridization occurring among them. Furthermore, two sub-groups seem to be present in one of the lineages but with frequent recombination between them. This pattern might be due to earlier divergence in allopatry during colder periods, followed by range expansions leading to overlapping ranges during warmer periods. In this study both diplo- and haplophase sequence datasets were obtained and analyzed, which was necessary due to the large amounts of heterozygous sequences. In most other phylogeographic (or phylogenetic) studies of basidiomycetes only diplophase datasets are analyzed and heterozygous sites largely ignored. This study demonstrates that splitting the diplophase sequences into its two haplophase sequences can be necessary to unveil the underlying population structure.

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Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funeco.2012.09.001>.

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