For. Path. 37 (2007) 192–216
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Phylogeographic patterns of *Armillaria ostoyae* in the western United States

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Summary

Nuclear ribosomal DNA regions (i.e. large subunit, internal transcribed spacer, 5.8S and intergenic spacer) were sequenced using a direct-polymerase chain reaction method from *Armillaria ostoyae* genets collected from the western USA. Many of the *A. ostoyae* genets contained heterogeneity among rDNA repeats, indicating intragenomic variation and likely intraspecific hybridization. Intragenomic variation was verified by visually editing base-sequence offsets in regions with insertions/deletions, and using sequence-specific internal primers to resequence heterogeneous regions. Phylogenetic analyses with Bayesian Inference methods were used to define groups within *A. ostoyae*. Analysis of *A. ostoyae* from outside the western USA indicated the presence of a Circumboreal group of *A. ostoyae* that also occurs in Utah; two other phylogeographic groups were associated with the Rocky Mountain and Pacific Northwest regions of the USA. Mixed sequence types, an indication of intraspecific hybrids, were common in some geographic regions. Hybridization events may have influenced species evolution, contributing to variation in pathogenicity and virulence. The occurrence of these groups and intraspecific hybrids also indicates that paleogeography and paleoclimate may have influenced the phylogeography of *A. ostoyae*. In addition, other *Armillaria* species were examined for evolutionary relationships with the groups of *A. ostoyae*. These findings will provide a basis for future research relating ecological function to genetic diversity within *A. ostoyae*.

1 Introduction

Throughout its circumboreal distribution, *Armillaria ostoyae* (Romag.) Herink is the principal cause of Armillaria root disease on conifers (Guillaumin et al. 1989; Morrison and Pellow 2002). *Armillaria ostoyae* is widely distributed in coniferous forests of north-western, interior south-western, north-central and north-eastern USA (Hanna 2005). Within the western USA, *A. ostoyae* has been commonly found in the Pacific Northwest (northern Idaho, western Montana, Oregon and Washington) and the Colorado Plateau (Hanna 2005). At present, no reports are available that conclusively demonstrate the occurrence of *A. ostoyae* in western Arizona, California, southern and central Idaho, Nevada, western Utah and western Wyoming.

Armillaria ostoyae adversely impacts commercial timber production by causing significant tree mortality and a reduction in tree growth (Williams et al. 1986). The effects of root disease in general are often underestimated, and losses caused by *A. ostoyae* are often difficult to detect because signs of infection may not be readily observable (Partridge et al. 1977; Cruickshank 2000). For these reasons, total losses caused by *A. ostoyae* across western North America are largely unknown; however, studies have shown volume loss as high as 40% over 4–8 years in a 18-year-old Douglas-fir [*Pseudotsuga menziezii* (Mirb.) Franco] plantation (Cruickshank 2000).

Received: 26.1.2006; accepted: 11.12.2006; editor: C. G. Shaw

An individual genet of *A. ostoyae* can range in size from a small patch occurring on a single tree to one of the largest organisms on Earth (SMITH et al. 1992). In north-eastern Oregon, one individual (genet) was identified that occupied nearly 900 ha, with an estimated age between 1900 and 8650 years old (FERGUSON et al. 2003). The advanced age of this individual shows an ability to survive several forest generations that likely included diverse species compositions. Other studies have shown that *A. ostoyae* genets can exhibit a range in pathogenicity (OMDAL et al. 1995), virulence (OMDAL et al. 1995; MORRISON and PELLOW 2002) and ecological behaviour (MCDONALD et al. 1998). While *A. ostoyae* is generally considered highly pathogenic, one study reported most isolates of *A. ostoyae* behaved primarily as saprophytes, with only occasional mild pathogenicity on declining trees (BÉRUBÉ and DESSUREAULT 1988). Moreover, distinct epidemiological differences in *A. ostoyae* have also been noted among coastal and interior populations of western North America (MCDONALD 1990; GOHEEN and OTROSINA 1998; MORRISON and PELLOW 2002).

Within *A. ostoyae*, successful mating of compatible, basidospore-derived mycelia produces a mycelium that is transiently dikaryotic (LARSEN et al. 1992); however, long-term growth of vegetative mycelia occurs in the diploid state (KIM et al. 2000). *Armillaria ostoyae* is typically identified by *in vitro* mating tests (KORHONEN 1978; LARSEN et al. 1992). Using these tests, *A. ostoyae* previously classified as European Biological Species C (EBS C) and North American Biological Species I (NABS I), can be distinguished from four other *Armillaria* species in Europe, nine other *Armillaria* species in North America and nine other *Armillaria* species in Asia (KORHONEN 1978; ANDERSON and ULLRICH 1979; OTA et al. 1998). Methods based on polymerase chain reaction (PCR) are now commonly used to discern ribosomal DNA (rDNA) sequence differences among *Armillaria* species (ANDERSON and STASOVSKI 1992; HARRINGTON and WINGFIELD 1995; BANIK et al. 1996; VOLK et al. 1996; WHITE et al. 1998; KIM et al. 2000, 2006; KEČA et al. 2006; and others as reviewed by PEREZ-SIERRA et al. 2000). These differences in DNA can be used for identification, phylogenetic analyses and assessments of genetic variability.

Genetic variation within *A. ostoyae* from various geographic locations has been shown to exist within multiple regions of the rDNA. Genetic variation has been observed within the intergenic spacer region 1 (IGS-1) of North American (ANDERSON and STASOVSKI 1992; KIM et al. 2006), European (SICOLI et al. 2003; KEČA et al. 2006) and Asian (TERASHIMA et al. 1998) isolates of *A. ostoyae*. Genetic variation has also been observed within the internal transcribed spacers (ITS-1 and ITS-2) of North American (KIM et al. 2006) and European (CHILLALI et al. 1998) isolates, and also within the large subunit (LSU) of North American (KIM et al. 2006) isolates. However, most previous studies on genetic variability of *A. ostoyae* have only examined a relatively small number of isolates. Genetic variation was identified in the IGS-2 among 24 Canadian isolates and two European isolates of *A. ostoyae* (WHITE et al. 1998). In addition, random amplified polymorphic DNA (RAPD) analysis showed high genetic variability among 20 European *A. ostoyae* isolates (SCHULZE et al. 1997), and anonymous nucleotide sequences provided evidence of genetic variability among North American *A. ostoyae* genets (PIERCEY-NORMORE et al. 1998).

DNA-based techniques have been primarily aimed at distinguishing *Armillaria* species. Recently, KIM et al. (2006) used nuclear rDNA (LSU, ITS, 5.8S and IGS-1) sequences and amplified fragment length polymorphisms to analyse genetic relationships among NABS of *Armillaria*, including *A. ostoyae*. Although genetic variability has been observed in *A. ostoyae*, variation has not been studied in depth. Studies of other *Armillaria* species have shown a high degree of intraspecific genetic variability (COETZEE et al. 2000; DUNNE et al. 2002). Phylogeographic relationships have also been investigated within *Armillaria* species, including *A. mellea sensu stricto* (Vahl:Fr.), a species having circumboreal distribution (COETZEE et al. 2000), and *A. luteobubalina* Watling & Kile, a species with partial circumaustral distribution that occurs in Australia and South America, but not in Africa (COETZEE et al. 2003). The goals of this study are to identify genetic differences among

diploid genets of *A. ostoyae* from the western USA, and examine intraspecific and interspecific phylogeographic relationships based upon rDNA-derived genetrees. Investigations of these differences are important for understanding (i) varying levels of pathogenicity and virulence within *A. ostoyae*, (ii) phylogeographic relationships among *A. ostoyae* genets and genets of other *Armillaria* species and (iii) adaptation to diverse environmental factors.

2 Materials and methods

2.1 Genet selection

Representative genets of *A. ostoyae* from the western USA (Oregon, Washington, Idaho, Montana, Utah and New Mexico; Table 1) and several genets as geographic outgroups (Russia, Finland, eastern USA and Mexico; Table 1) and other *Armillaria* species from the Northern Hemisphere (Table 2) were obtained from an archived collection at the USDA Forest Service, Rocky Mountain Research Station, Forestry Sciences Laboratory (Moscow, ID, USA). Somatic incompatibility pairing tests had previously been used to differentiate isolates into unique genets (McDonald and Martin 1988; Guillaumin et al. 1991; Wu et al. 1996), which represent a single genotype (Guillaumin et al. 1996; Worrall 1997; Dettman and van der Kamp 2001). These genets were further identified to species using haploid × haploid mating, haploid × diploid pairing tests, diploid × diploid paring tests (Korhonen 1978; McDonald and Martin 1988; Mallett et al. 1989) and/or restriction fragment length polymorphic (RFLP) analysis of the IGS-1 region of rDNA (Harrington and Wingfield 1995; White et al. 1998; Kim et al. 2000). Species identification was verified by sequence similarity within GenBank.

2.2 PCR and DNA sequencing

Genets were grown on malt-agar medium (0.75% malt extract, 0.75% dextrose, 0.5% peptone and 1.5% agar) at 21°C in the dark for 2 weeks. PCR products from nuclear rDNA [IGS-1, ITS 1 and ITS 2, including the 5.8S (ITS + 5.8S) and LSU] were obtained by a direct-PCR method (i.e. mycelium was scraped from pure culture and added directly to the PCR mixture to serve as DNA template), primer sets were used for initial amplification of the following specified rDNA regions: (i) LSU (5′/D-domain end proximal to ITS-2) : 5.8SR and LR7 (Moncalvo et al. 2000); (ii) ITS + 5.8S : ITS-1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) and (iii) IGS-1 : LR12R (Veldman et al. 1981) paired with O-1 (Duchesne and Anderson 1990) and/or A5SR1 (5′-AAC CAC AGC ACC CAG GAT T-3′), a primer specifically designed for this project based on the 5S rRNA gene of 29 Basidiomycotina species (Hwang and Kim 1995). Each reaction mixture included 2.5 units Ampli*Taq*® DNA polymerase (Applied Biosystems, Inc., Foster City, CA, USA) along with 200 μM dNTPs, 4 mM $MgCl_2$, 5 μl 10X PCR buffer and 0.5 μM of each primer for a final reaction volume of 50 μl, and incubated in a MJ PTC-200 peltier thermal cycler (Bio-Rad Laboratories, Waltham, MA, USA) under the following conditions for specified rDNA regions: (i) LSU: 94°C for 3 min, followed by 35 cycles of 94°C for 60 s, 55°C for 30 s and 72°C for 2 min followed by 5 min at 72°C; (ii) ITS + 5.8S: 94°C for 2 min 30 s, followed by 36 cycles of 94°C for 60 s, 48°C for 60 s and 72°C for 1 min 30 s, followed by 10 min at 72 C or (iii) IGS-1: 95°C for 1 min 35 s, followed by 35 cycles of 90°C for 30 s, 60°C for 1 min and 72°C for 2 min, followed by 10 min at 72°C. PCR products were prepared for sequencing using ExoSAP-IT™ (USB Corporation, Cleveland, OH, USA) and

Table 1. ***Armillaria ostoyae*** **isolates used in this study**

Isolate	Alternate number(s)	Origin	Collector(s) or reference	LSU sequence type(s)	ITS + 5.8S sequence type(s)	IGS sequence type(s)
BC18F		Washington, USA	M.-S. Kim	LSUOS7, LSUOS10	ITSOS6	IGSOS3
FF4	91058/T1	Eastern Finland	unknown	LSUOS1	ITSOS35[1], ITSOS36[1]	IGSOS32[1], IGSOS33[1]
MNF4	118	Oregon, USA	Ferguson et al. 2003	LSUOS10	ITSOS6[1], ITSOS8[1]	IGSOS9, IGSOS15
NA142		Washington, USA	G. I. McDonald	LSUOS10, LSUOS11	ITSOS15	IGSOS16
NA144		Washington, USA	G. I. McDonald	LSUOS25[1]	ITSOS21, ITSOS22	IGSOS10, IGSOS17
NA150		Washington, USA	G. I. McDonald	LSUOS8, LSUOS10	ITSOS26[1], ITSOS27[1]	IGSOS2
NA212		Washington, USA	G. I. McDonald	LSUOS10, LSUOS11	ITSOS8	IGSOS2, IGSOS15
NA254		Washington, USA	G. I. McDonald	LSUOS22[1]	ITSOS10, ITSOS11	IGSOS2, IGSOS3
NA260		Washington, USA	G. I. McDonald	LSUOS6, LSUOS10	ITSOS14	IGSOS2
NC53		Idaho, USA	IFTNC[2]	LSUOS10, LSUOS11	ITSOS10, ITSOS11	IGSOS3, IGSOS16
NC94		Washington, USA	IFTNC[2]	LSUOS10	ITSOS6	IGSOS2
NC164		Idaho, USA	IFTNC[2]	LSUOS7, LSUOS10	ITSOS26[1]	IGSOS2, IGSOS3
NC436		Idaho, USA	IFTNC[2]	LSUOS9, LSUOS10	ITSOS6	IGSOS5, IGSOS15
NC491		Washington, USA	IFTNC[2]	LSUOS6, LSUOS10	ITSOS37[1]	IGSOS2, IGSOS6
NC580		Washington, USA	IFTNC[2]	LSUOS10	ITSOS6	IGSOS2, IGSOS4
NC671		Idaho, USA	IFTNC[2]	LSUOS7, LSUOS10	ITSOS12, ITSOS13	IGSOS3, IGSOS16
NC765		Washington, USA	IFTNC[2]	LSUOS6, LSUOS10	ITSOS34[1]	IGSOS2
NC837		Washington, USA	IFTNC[2]	LSUOS19[1]	ITSOS28[1], ITSOS29[1]	IGSOS9, IGSOS23
NC863		Idaho, USA	IFTNC[2]	LSUOS10	ITSOS16	IGSOS3, IGSOS15
NC887		Idaho, USA	IFTNC[2]	LSUOS10	ITSOS10, ITSOS11	IGSOS2, IGSOS5
NC895		Idaho, USA	IFTNC[2]	LSUOS10	ITSOS6	IGSOS2, IGSOS11
NC905		Washington, USA	IFTNC[2]	LSUOS6	ITSOS24, ITSOS25	IGSOS2
NC911		Washington, USA	IFTNC[2]	LSUOS10, LSUOS11	ITSOS38[1], ITSOS39[1]	IGSOS2, IGSOS16
NC1070		Idaho, USA	IFTNC[2]	LSUOS23[1], LSUOS24[1]	ITSOS40[1], ITSOS41[1]	IGSOS2, IGSOS7
NC1091		Idaho, USA	IFTNC[2]	LSUOS10	ITSOS62[1], ITSOS63[1]	IGSOS2
NC1126		Idaho, USA	IFTNC[2]	LSUOS11	ITSOS6	IGSOS16
NC1180		Idaho, USA	IFTNC[2]	LSUOS19[1]	ITSOS28[1], ITSOS29[1]	IGSOS2, IGSOS23
NC1187		Idaho, USA	IFTNC[2]	LSUOS10, LSUOS11	ITSOS10, ITSOS23	IGSOS2, IGSOS16
NC1245		Idaho, USA	IFTNC[2]	LSUOS10, LSUOS11	ITSOS6	IGSOS2, IGSOS16
NM115		New Mexico, USA	G. I. McDonald	LSUOS3	ITSOS42[1]	IGSOS18
NM120		New Mexico, USA	G. I. McDonald	LSUOS3	ITSOS2	IGSOS18, IGSOS19
NM235	BS2	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18, IGSOS22
NM236	PP1	New Mexico, USA	Omdal et al. 1995	LSUOS14[1]	ITSOS2, ITSOS4	IGSOS26[1]

Table 1. **Continued**

Isolate	Alternate number(s)	Origin	Collector(s) or reference	LSU sequence type(s)	ITS + 5.8S sequence type(s)	IGS sequence type(s)
NM238	BS1	New Mexico, USA	Omdal et al. 1995	LSUOS1, LSUOS3	ITSOS2, ITSOS3	IGSOS27[1]
NM239	WF2	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18
NM241	DF1	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18
NM242	A2	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS43[1]	IGSOS18
NM244	A3	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18
NM245	WP2	New Mexico, USA	Omdal et al. 1995	LSUOS12[1]	ITSOS44[1]	IGSOS26[1]
NM246	WF1	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS30[1]	IGSOS18
NM248	PP3	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS30[1]	IGSOS18
NM249	A4	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS31[1]	IGSOS18, IGSOS21
NM250	PP4	New Mexico, USA	Omdal et al. 1995	LSUOS13[1]	ITSOS2, ITSOS3	IGSOS26[1]
OR10		Oregon, USA	G. I. McDonald	LSUOS15[1]	ITSOS45[1], ITSOS46[1]	IGSOS2
OR22		Oregon, USA	G. I. McDonald	LSUOS6	ITSOS59[1], ITSOS61[1]	IGSOS2
P255		Idaho, USA	G. I. McDonald	LSUOS3	ITSOS5	IGSOS18, IGSOS20
P1401		Idaho, USA	G. I. McDonald	LSUOS6	ITSOS24	IGSOS2
P1404		Idaho, USA	G. I. McDonald	LSUOS6	ITSOS24	IGSOS2
P2003		Idaho, USA	G. I. McDonald	LSUOS10	ITSOS6	IGSOS2
P4352		Idaho, USA	G. I. McDonald	LSUOS10	ITSOS12	IGSOS16
P4661		Idaho, USA	G. I. McDonald	LSUOS10	ITSOS12	IGSOS16
PC514		Idaho, USA	G. I. McDonald	LSUOS16[1]	ITSOS56[1], ITSOS57[1]	IGSOS2, IGSOS18
R957		Washington, USA	G. I. McDonald	LSUOS6, LSUOS10	ITSOS17	IGSOS2
R959		Washington, USA	G. I. McDonald	LSUOS10	ITSOS18	IGSOS2
R1075		Idaho, USA	G. I. McDonald	LSUOS18[1]	ITSO32[1], ITSOS33[1]	IGSOS24[1], IGSOS25[1]
R1083		Idaho, USA	G. I. McDonald	LSUOS10	ITSOS6	IGSOS2, IGSOS12
R1140		Oregon, USA	G. I. McDonald	LSUOS10	ITSOS6	IGSOS2, IGSOS14
R1202		Oregon, USA	G. I. McDonald	LSUOS8	ITSOS14	IGSOS2
R1237		Washington, USA	G. I. McDonald	LSUOS6, LSUOS10	ITSO58[1], ITSOS60[1]	IGSOS2, IGSOS13
R1283		Idaho, USA	G. I. McDonald	LSUOS10, LSUOS11	ITSO47[1], ITSOS48[1]	IGSOS8, IGSOS16
R1329		Montana, USA	G. I. McDonald	LSUOS10, LSUOS11	ITSOS6	IGSOS2, IGSOS16
R1334		Montana, USA	G. I. McDonald	LSUOS10	ITSOS6	IGSOS2, IGSOS16
R1348		Montana, USA	G. I. McDonald	LSUOS6, LSUOS10	ITSOS34[1]	IGSOS2
R1362		Montana, USA	G. I. McDonald	LSUOS4, LSUOS5	ITSOS2	IGSOS23
R1366		Montana, USA	G. I. McDonald	LSUOS10	ITSOS6, ITSOS9	IGSOS2, IGSOS15
R1374		Montana, USA	G. I. McDonald	LSUOS20[1]	ITSOS49[1], ITSOS50[1]	IGSOS16, IGSOS23

Table 1. **Continued**

Isolate	Alternate number(s)	Origin	Collector(s) or reference	LSU sequence type(s)	ITS + 5.8S sequence type(s)	IGS sequence type(s)
R1424		Oregon, USA	G. I. McDonald	LSUOS21[1]	ITSOS51[1], ITSOS52[1]	IGSOS28[1], IGSOS29[1]
SSF4		Montana, USA	B. A. Ferguson	LSUOS10, LSUOS11	ITSOS6, ITSOS7	IGSOS2, IGSOS16
SSF6		Montana, USA	B. A. Ferguson	LSUOS10	ITSOS19, ITSOS20	IGSOS3, IGSOS16
ST1		New Hampshire, USA	T. C. Harrington	LSUOS1	ITSOS1	IGSOS1
ST2		Washington, USA	J. F. Ammirati	LSUOS10	ITSOS6, ITSOS7	IGSOS2, IGSOS16
TS7		Chihuahua, Mexico	C. G. Shaw	LSUOS3	ITSOS2	LSUOS18
U5		Utah, USA	G. I. McDonald	LSUOS1	ITSOS53[1]	IGSOS34[1], IGSOS35[1]
U16		Utah, USA	G. I. McDonald	LSUOS1	ITSOS54[1]	IGSOS34[1], IGSOS35[1]
U73		Utah, USA	G. I. McDonald	LSUOS3	ITSOS31[1]	LSUOS18
USSR		Primorye, Russia	G. M. Filip	LSUOS1, LSUOS2	ITSOS55[1]	IGSOS30[1], IGSOS31[1]
WA6		Washington, USA	G. I. McDonald	LSUOS17[1]	ITSOS32[1], ITSOS33[1]	LSUOS2, LSUOS23

[1]Represents a heterogeneous sequence type not included in analysis.
[2]Intermountain Forest Tree Nutrition Cooperative.

Table 2. ***Armillaria*** **isolates used in this study**

Species	Isolate[1]	Alternate number	Origin	Collector	LSU sequence type(s)
A. calvescens	ST3	JB56A	Quebec, Canada	J. A. Bérubé	LSUCA3
	ST17	PR-3	Michigan, USA	M. T. Banik	LSUCA2
	ST18	FFC-7	Michigan, USA	M. T. Banik	LSUCA1
A. cepistipes	M110	SP82-14	British Columbia, Canada	D. J. Morrison	LSUCE2
	S20	SP83-07	British Columbia, Canada	D. J. Morrison	LSUCE1, LSUCE2
A. gallica	M70	SP81-29	British Columbia, Canada	D. J. Morrison	LSUGA1
	ST22	EL-1	Michigan, USA	M. T. Banik	LSUGA1, LSUCE2
	ST23	MA-1	Wisconsin, USA	M. T. Banik	LSUGA3, LSUGA4
A. gemina	ST8[2]	JJW153	New York, USA	J. J. Worrall	LSUGE1
	ST9[3]	JJW64	New York, USA	J. J. Worrall	LSUGE1
	ST11[4]	MIELKE	West Virginia, USA	M. E. Mielke	LSUGE1, LSUGE2
A. mellea	ST5	GB934	Virginia, USA	G. F. Bills	LSUME4[5]
	ST20	A3	Wisconsin, USA	M. T. Banik	LSUME1, LSUME2
	ST21	TCH-2	New Hampshire, USA	T. C. Harrington	LSUME1, LSUME3
A. nabsnona	C21		Idaho, USA	G. I. McDonald	LSUNA1
	M90		British Columbia, Canada	D. J. Morrison	LSUNA1
	ST16	SHAW,C	Alaska, USA	C. G. Shaw	LSUNA2
A. sinapina	M50	SP81-1	British Columbia, Canada	D. J. Morrison	LSUSI2
	ST12	AMM9065	Washington, USA	J. F. Ammirati	LSUSI1
	ST13	CF-2	Michigan, USA	M. T. Banik	LSUSI1, LSUSI2
A. tabescens	AtMuS2		South Carolina, USA	G. Schnabel	LSUTA1, LSUTA2
	OOi99		Georgia, USA	G. Schnabel	LSUTA1
	OOi210		Georgia, USA	G. Schnabel	LSUTA1
NABS[6] X	837		Idaho, USA	G. I. McDonald	LSUX1, LSUX2
	D82		Idaho, USA	G. I. McDonald	LSUX1, LSUX3
	POR100		Idaho, USA	G. I. McDonald	LSUX1, LSUX4

[1]Each isolate represents a distinct genet.
[2]Also represents sequence types ITSGE1, IGSGE3 and IGSGE4.
[3]Also represents sequence types ITSGE2[5], ITSGE3[5], IGSGE5 and IGSGE6.
[4]Also represents sequence types ITSGE1, IGSGE1 and IGSGE2.
[5]Represents a heterogeneous sequence type not included in analysis.
[6]North American Biological Species.

sequenced at Davis Sequencing, Inc. (Davis, CA, USA). IGS-1 and ITS + 5.8S regions were sequenced with the same primers used for initial amplification, while the LSU region was sequenced using the LR0R, LR5 and LR15 primers (Vilgalys and Sun 1994; Moncalvo et al. 2000).

2.3 Sequence editing

The sequence chromatograms were visually edited with BIOEDIT software (HALL 1999) by two separate researchers to minimize errors. While some chromatograms indicated homogeneous rDNA repeats (one peak for each nucleotide position), most showed heterogeneity among the rDNA repeats. Careful attention was given to these chromatograms because heterogeneous rDNA can represent interspecific and/or intraspecific genetic variation within an individual. In this study, heterogeneity was detected when a chromatogram contained either one or more single nucleotide polymorphism(s) (SNP; represented by a double peak occurring at a single nucleotide position) or a 'frame-shift' (overlapping peaks because of length variation among rDNA repeat; Fig. 1). When possible, heterogeneous sequences were deciphered into homogeneous sequence representations by one of the three methods illustrated in Fig. 1 before phylogenetic analyses. The first method involves visual editing of a frame-shift. The second method is similar to that of the mismatch amplification mutation assay method (CHA et al. 1992; RAUSCHER et al. 2002) and was used within the IGS-1, for which reverse complementary primers were created and applied to products shown to contain a SNP at base pair position 683 of the IGS-1 region. Primers applied to this position were AOHR1T (5′-TGC CGT TCA AAA-3′), AOHR1G (5′-TGC CGT TCA AAC-3′) and AOHR1C (5′-TGC CGT TCA AAG-3′). The third method simply splits a chromatogram containing a single SNP into two predicted sequences. If predicted sequences deciphered by these three methods showed heterogeneity

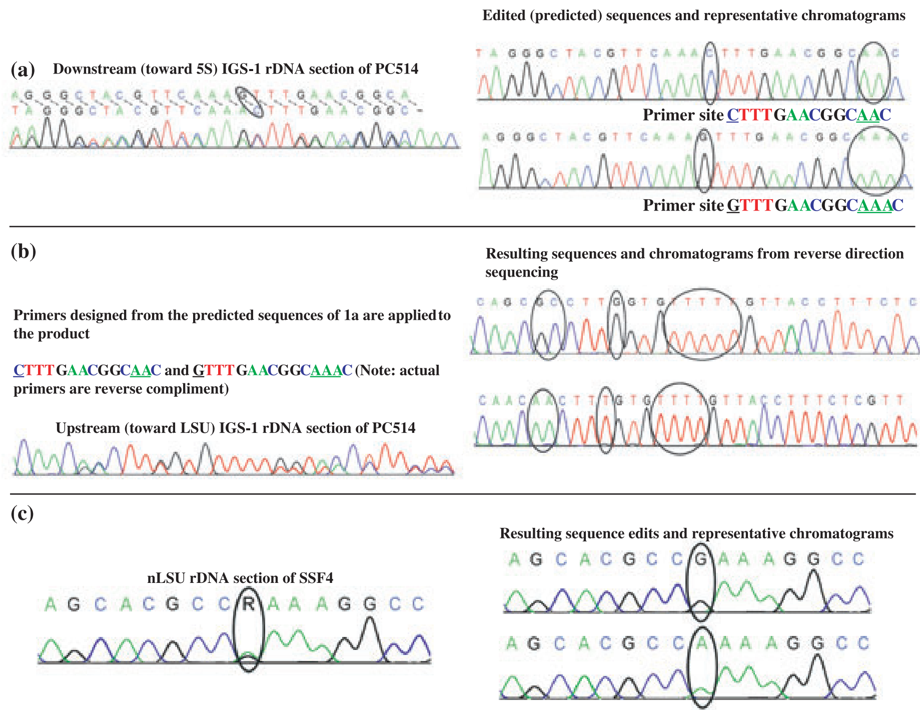

Fig. 1. Several methods for editing heterogeneous product into homogenous sequence types (a) editing of a 'frame-shift'; (b) application of specific primers on heterogeneous polymerase chain reaction product; (c) splitting a single single nucleotide polymorphism

among rDNA repeats from a single genet, the different sequences were assigned a letter code (e.g. A or B) after the genet names. Remaining polymorphisms were coded with the IUPAC codes for ambiguous nucleotides; however, these polymorphisms were not regarded as ambiguous but rather the result of heterogeneous product. Sequences with two or more ambiguous sites were eliminated from further analyses to minimize uncertainty. All sequences used in analysis have been deposited into GenBank (accession numbers: AY996615–AY996722, AY973655–AY973752, AY968085–AY968203 and DQ011902–DQ011905).

2.4 Sequence alignments

Sequences were manually aligned for each of three regions (LSU – D-domain, proximal to ITS-2; ITS + 5.8S and IGS-1). Duplicate sequences were eliminated from the alignments so that only unique sequence types were compared. For the LSU region, genets from both Tables 1 and 2 were included in the alignment for interspecific comparison, while sequence alignments for ITS + 5.8S and IGS-1 regions contained only genets in Table 1 for intraspecific comparisons.

2.5 Phylogenetic analyses

Phylogenetic analyses were performed for each data set using neighbor-joining (Saitou and Nei 1987), parsimony and Bayesian analysis methods. Any gaps in the alignments were treated as missing and coded using a simple gap-coding method (Simmons and Ochoterena 2000). Neighbor-joining analysis was performed using the Tamura-Nei model for estimation of evolutionary distance in mega (version 2.1, Kumar et al. 2001) and relative support for nodes in resulting trees was generated using 1000 bootstrap replicates (Felsenstein 1985). Parsimony analysis was performed using PAUP* (4.0b10) (Swofford 2001). Multistate taxa were interpreted as polymorphisms, starting trees were obtained via stepwise addition with random addition sequence of 10 replicates, one tree was held at each step during stepwise addition, tree-bisection-reconnection was used, and the steepest descent option was not in effect. The analysis of the LSU was performed with MaxTrees set to auto increase, whereas the ITS + 5.8S and IGS-1 regions were set to a maximum of 10 000 trees. A bootstrap method with heuristic search was used with 1000 bootstrap replicates on each data set to obtain 50% bootstrap majority-rule consensus trees (Felsenstein 1985).

Bayesian analysis was performed by mrbayes v3.0B4 (Huelsenbeck and Ronquist 2001). Bayesian inference of phylogeny calculates the posterior probability of phylogenetic trees. To select appropriate evolutionary models for use in Bayesian analysis, mrmodeltest 1.0b (Nylander 2003) was used. Four chains were run for 3×10^6 generations generating files with 30 001 trees, the first 6000 of these trees were discarded as the 'burnin' of the chains. The remaining 24 001 trees were used to make 90% majority-rule consensus trees using PAUP* (4.0b10). The trees generated from Bayesian inference analyses of LSU, ITS + 5.8S and IGS-1 sequences were deposited into TreeBASE (study accession number S1643).

3 Results

3.1 Heterogeneity (intraindividual variation)

Heterogeneous rDNA products, an indication of intraspecific and intragenomic variation, were common in all regions analysed. Many of these heterogeneous rDNA products were deciphered into two predicted sequences using the methods illustrated in Fig. 1. In this

situation, the deciphering process could result in two sequence types per individual for a single rDNA region. When heterogeneous rDNA products remained undecipherable, the remaining heterogeneous rDNA product was represented by two or more ambiguously-coded nucleotide positions per individual within a single rDNA region (Tables 1 and 2). Sequences containing ambiguous nucleotides at multiple positions were excluded from analyses. Of 77 genets of *A. ostoyae*, heterogeneity was detected in 37 (48%) individuals within the LSU region, 45 (58%) within the ITS + 5.8S and 46 (60%) within the IGS-1. Only 14 (16%) of the 77 genets were homogeneous in all three rDNA regions analysed. Using sequence editing techniques (Fig. 1), we were able to decipher heterogeneous product into homogenous sequence representations to be used in phylogenetic analyses for 23 (30% of total) individuals of the LSU, 14 (18% of total) of the ITS + 5.8S and 36 (47% of total) of the IGS-1.

3.2 Sequence data

3.2.1 LSU

The LSU data set produced 11 unique sequence types of *A. ostoyae* (LSUOS1–LSUOS11) for comparison with sequences representing the nine other North American *Armillaria* species (Table 2). Each sequence contained 976 characters including simple-gap coded indels. Of these characters, 920 were constant, 16 variable characters were parsimony uninformative, and 40 characters were parsimony informative. A single most-parsimonious tree was found with optimality criterion set to parsimony in PAUP* (4.0b10). This tree yielded a total length of 60 steps, consistency index (CI) = 0.950, retention index (RI) = 0.967, rescaled consistency index (RC) = 0.919, homoplasy index (HI) = 0.050 and a G-fit score of −39.250.

3.2.2 ITS + 5.8S

The ITS + 5.8S data set consisted of 25 unique sequence types of *A. ostoyae* (ITSOS1–ITSOS25) and one *A. gemina* outgroup sequence (ITSGE1). Each sequence contained 793 characters including simple-gap coded indels. Of these characters, 770 were constant, 13 variable characters were parsimony uninformative, and 10 characters were parsimony informative. With optimality criterion set to parsimony, 120 equally parsimonious trees were revealed. An optimum sample tree from the heuristic search yielded a total length of 27 steps, CI = 0.852, RI = 0.857, RC = 0.730, HI = 0.148 and a G-fit score of −9.000.

3.2.3 IGS-1

The IGS-1 data set consisted of 23 unique sequence types of *A. ostoyae* (IGSOS1–IGSOS23), and six sequences of the *A. gemina* (IGSOS1–IGSOS6) were used as an outgroup. Each sequence contained 583 characters including simple-gap coded indels. Of these characters, 537 were constant, 15 variable characters were parsimony uninformative and 31 characters were parsimony informative. With optimality criterion set to parsimony, 805 equally parsimonious trees were revealed. An optimum sample tree from the heuristic search yielded a total length of 54 steps, CI = 0.889, RI = 0.955, RC = 0.848, HI = 0.111 and a G-fit score of −29.600.

3.3 Phylogeographic analyses

For LSU, ITS + 5.8S and IGS-1 data sets, nearly identical congruency was shown among the 50% majority-rule bootstrap-consensus trees from the parsimony analysis, the 50%

majority-rule consensus trees from neighbor-joining analysis (data not shown), and the 90% majority-rule consensus trees created for the Bayesian analysis (Figs 2, 4 and 6).

3.3.1 LSU

Phylogenetic trees of the LSU region using three different inference methods showed three phylogeographically distinct groups of *A. ostoyae*, as shown in the Bayesian radial 90% majority-rule consensus tree (Fig. 2) and corresponding map (Fig. 3). These groups are referred to as the Circumboreal, Rockies and Pacific Northwest groups. A group with circumboreal distribution consisted of two clades represented by sequence types LSUOS1 and LSUOS2, which were derived from genets collected in Utah (USA), New Hampshire (USA), Russia and Finland. The Rockies group consisted of three sequence types, which were distributed among genets from Idaho (USA), Montana (USA), Utah (USA) and New Mexico (USA). The Pacific Northwest group consisted of six sequence types, with associated genets collected from Idaho (USA), Montana (USA), Oregon (USA) and Washington (USA). A hybrid individual from New Mexico (USA) was found to contain both Circumboreal (LSUOS1) and Rockies (LSUOS3) sequence types. Rockies and Pacific Northwest groups were separated from the root of the Circumboreal groups with posterior probabilities of 93% and 92%, respectively.

The LSU region was also used to compare relationships of North American *Armillaria* species to *A. ostoyae* groups, as the LSU was the only rDNA region studied that allowed unambiguous alignment of *A. mellea sensu stricto* and *A. tabescens* sequences with sequences of *A. ostoyae* and the other seven North American *Armillaria* species. The Circumboreal (LSUOS1) sequence type was identical to and shared a terminal node with sequence types of five other *Armillaria* species (*A. calvescens*, *A. cepistipes*, *A. gemina*, *A. sinapina* and NABS X; Fig. 2). This branch forms a 'starburst-like' structure (polytomy) with other sequence types of *A. gemina* (LSUGE2), *A. ostoyae* (LSUOS2) and NABS X (LSUX2, LSUX3 and LSUX4). The branches of this polytomy are rooted by an ancestral node from which several of the North American *Armillaria* species (i.e. *A. calvescens*, *A. cepistipes*, *A. gallica*, *A. gemina*, *A. nabsnona*, *A. ostoyae*, *A. sinapina* and NABS XX) may have been derived. *Armillaria mellea* and *A. tabescens* were separated from this root by a Bayesian posterior probability of 94% and separated from each other by a posterior probability of 100%. *Armillaria nabsnona* was another species showing sequence similarity to *A. ostoyae*, with two sequence types (LSUNA1 and LSUNA2) showing close relationship to (forms a multifurcation with) the sequence type LSUOS3 of the *A. ostoyae* Rockies group.

3.3.2 ITS + 5.8S

Phylogenetic analyses of the ITS + 5.8S region using three different inference methods showed two phylogeographically distinct groups (Rockies/New Hampshire and Pacific Northwest) of *A. ostoyae*, as shown in the Bayesian radial 90% majority-rule consensus tree (Fig. 4) and corresponding map (Fig. 5). The two groups were separated by a posterior probability of 100%. The Pacific Northwest group consisted of a large polytomy containing 16 sequence types and two subgroups; each subgroup was composed of two sequence types that radiated from the nodal root of the polytomy with posterior probabilities of 90% (ITSOS22 and ITSOS23) and 99% (ITSOS24 and ITSOS25). Although phylogenetic analyses of ITS + 5.8S region clearly show that these Pacific Northwest subgroups are distinct from the large polytomy, non-hybrid genets containing these sequence types are co-defined by their LSU sequence types that show a monophyletic Pacific Northwest group (Fig. 2). The *A. gemina* outgroup, represented by a single sequence type (ITSGE1), grouped with that of the *A. ostoyae* Rockies/New Hampshire group (ITSOS1–ITSOS5).

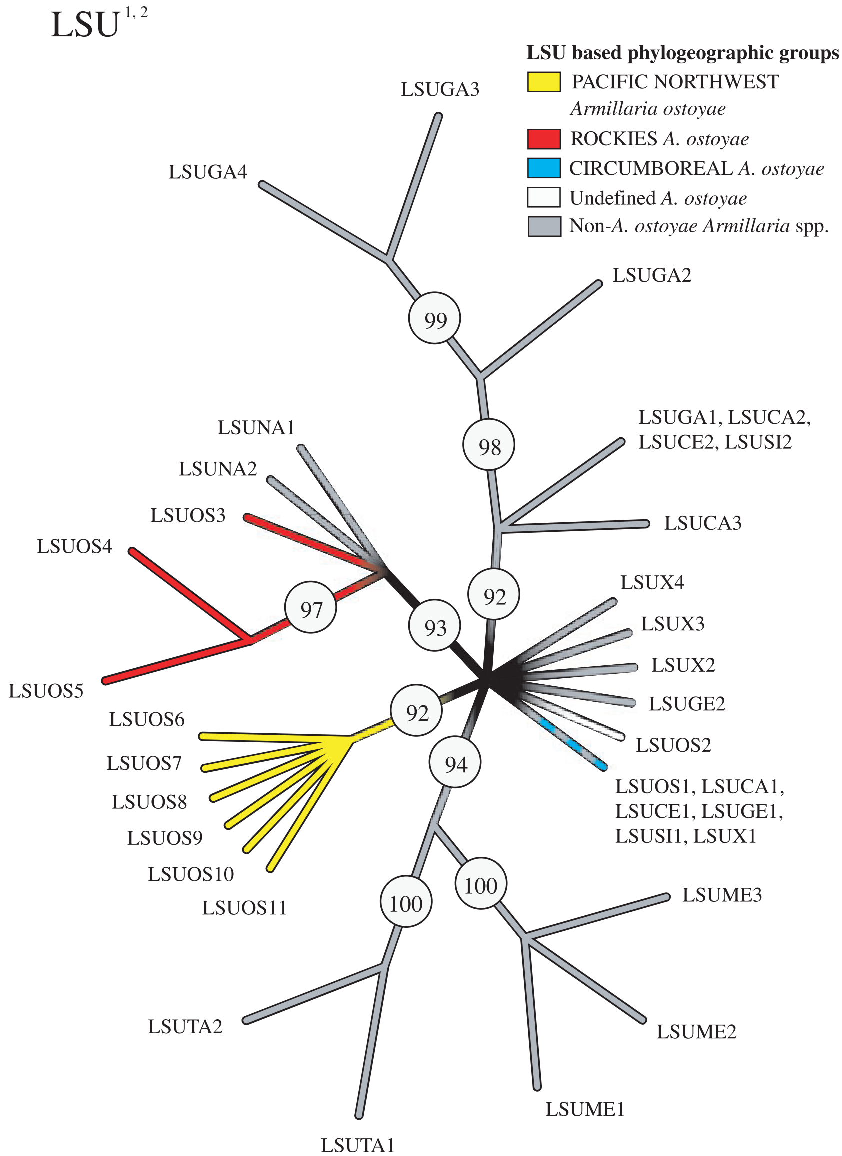

Fig. 2. Radial 90% majority-rule consensus tree of *Armillaria* species based on 24 000 trees from Bayesian inference analysis of the nuclear large ribosomal subunit (LSU) region. Numbers between clades indicate estimated posterior probability. [1]*A. calvescens* (LSUCA1–LSUCA3), *A. cepistipes* (LSUCE1 and LSUCE2), *A. gallica* (LSUGA1–LSUGA4), *A. gemina* (LSUGE1 and LSUGE2), *A. mellea* (LSUME1–LSUME3), *A. nabsnona* (LSUNA1 and LSUNA2), *A. ostoyae* (LSUOS1–LSUOS11), *A. sinapina* (LSUSI1 and LSUSI2), *A. tabescens* (LSUTA1 and LSUTA2) and North American Biological Species X (LSUX1–LSUX4). [2]Ambiguous sequences from 14 heterogeneous *Armillaria* isolates were excluded from analysis

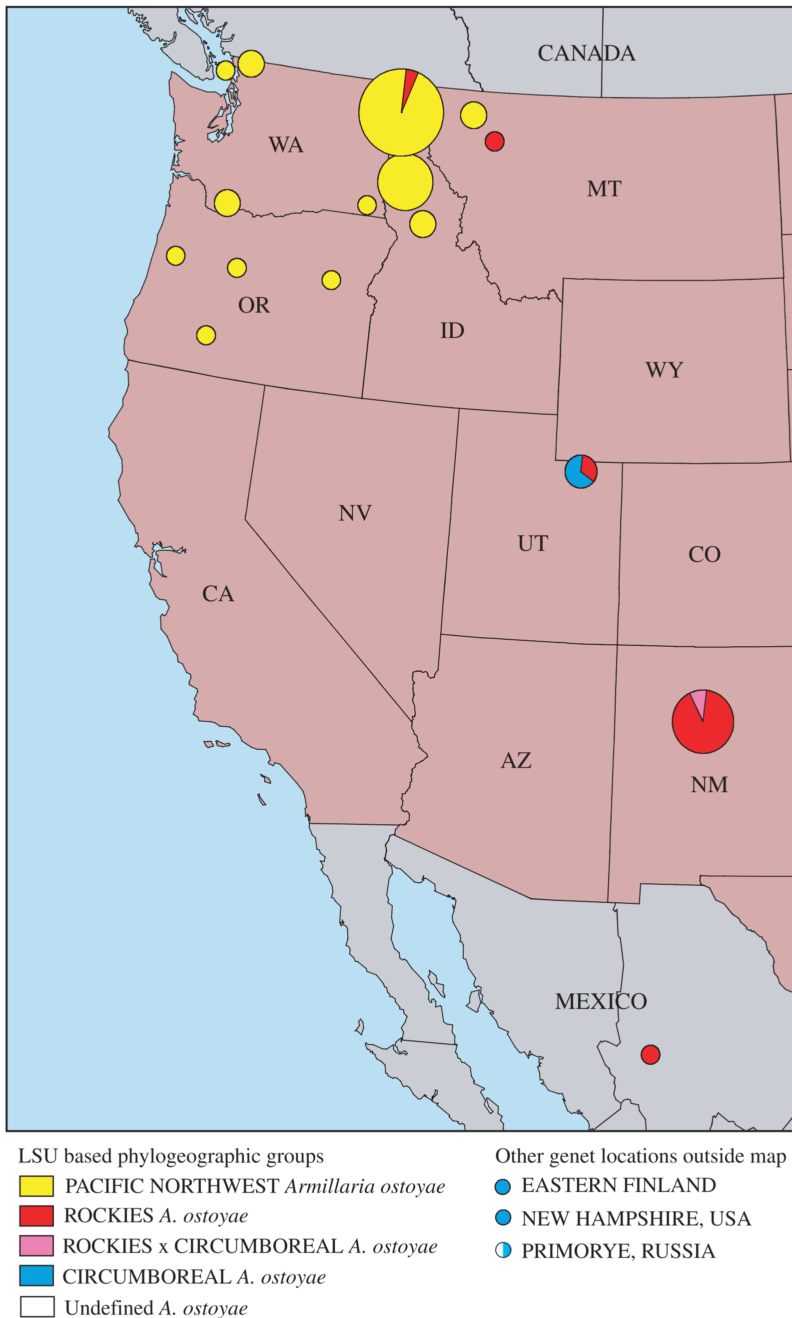

Fig. 3. Phylogeographic distribution of *Armillaria ostoyae* genets based on major clades from Bayesian inference analysis of the nuclear large ribosomal subunit DNA (LSU). Circle area is proportional to the number of genets analysed, with the smallest area representing a single genet

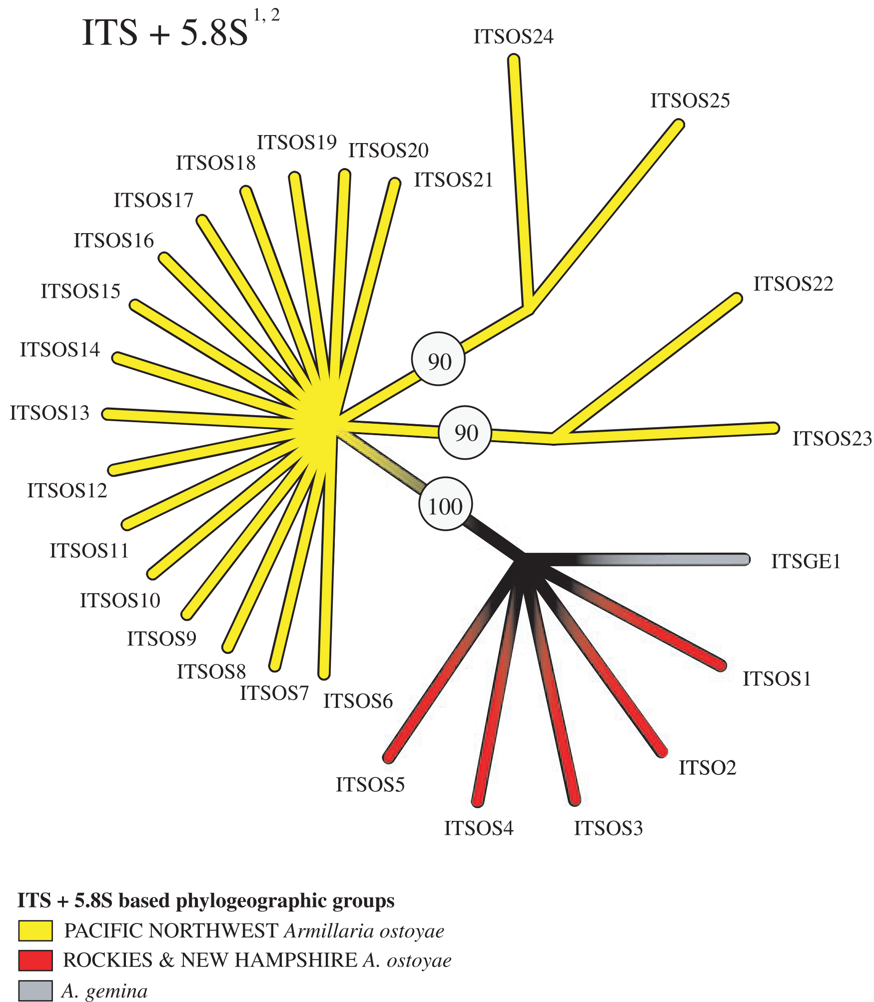

Fig. 4. Radial 90% majority-rule consensus tree of *Armillaria* species based on 24 000 trees from Bayesian inference analysis of the internal transcribed spacer and 5.8S rDNA (ITS + 5.8S) region. Numbers between clades indicate estimated posterior probability. [1]*A. gemina* (ITSGE1) and *A. ostoyae* (ITSOS1–ITSOS25). [2]Ambiguous sequences from 31 heterogeneous *Armillaria* isolates were excluded from analysis

3.3.3 IGS-1

Phylogenetic analyses of the IGS-1 region using three different inference methods showed two phylogeographically distinct groups (Rockies and Pacific Northwest/New Hampshire) of *A. ostoyae* as shown in the Bayesian radial 90% majority-rule consensus tree (Fig. 6) and corresponding map (Fig. 7). The Rockies group (IGSOS18–IGSOS23) was separated from the 14 polytomous Pacific Northwest/New Hampshire group sequences by a posterior probability of 100%. Several Pacific Northwest sequence types branched from the internal node of the Pacific Northwest/New Hampshire polytomy. The first of these sequence types (IGSOS15) split from this internal node with a posterior probability of

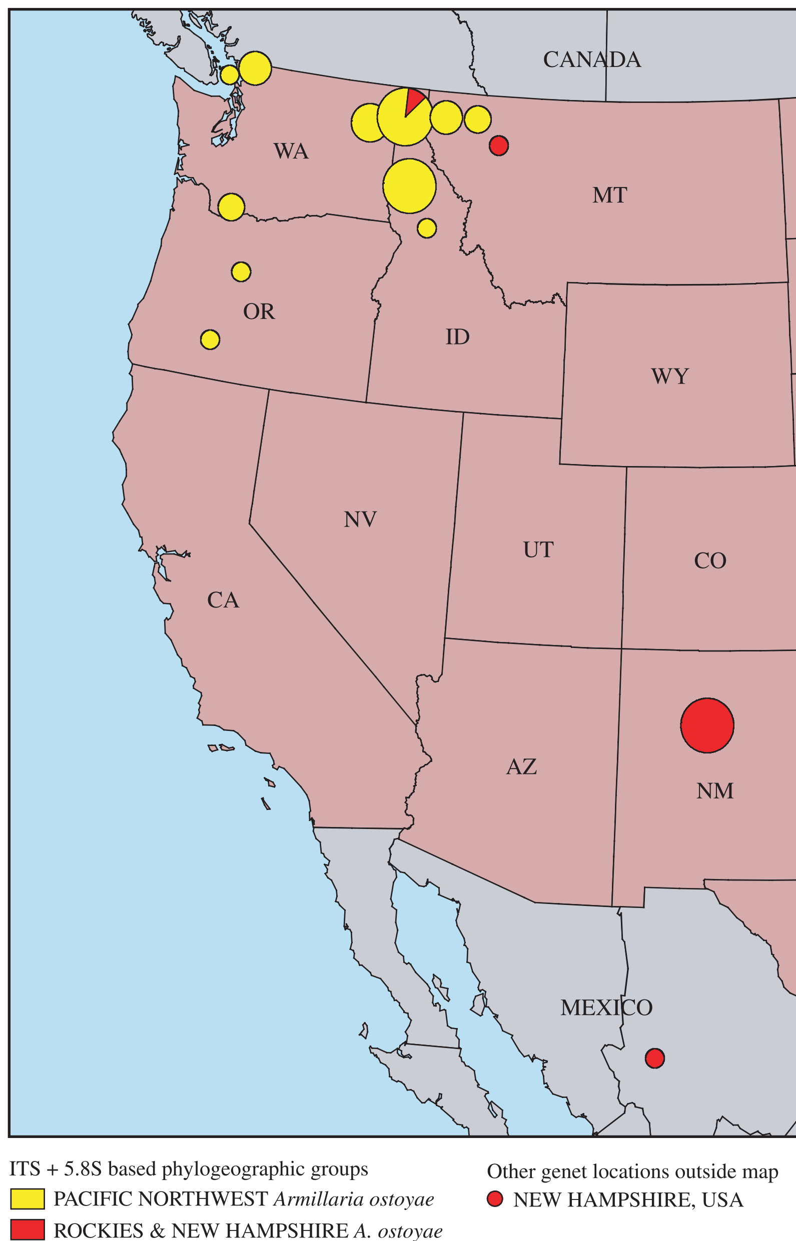

Fig. 5. Phylogeographic distribution of *Armillaria ostoyae* genets based on major clades from Bayesian inference analysis of the internal transcribed spacer and 5.8S rDNA (ITS + 5.8S). Circle area is proportional to the number of genets analysed, with the smallest area representing a single genet

96%, whereas two other sequence types (IGSOS16 and IGSOS17) split from the basal node of the former by a posterior probability of 99% ending in bifurcation. Similar to the ITS + 5.8S region, non-hybrid genets with these sequence types are co-defined to the

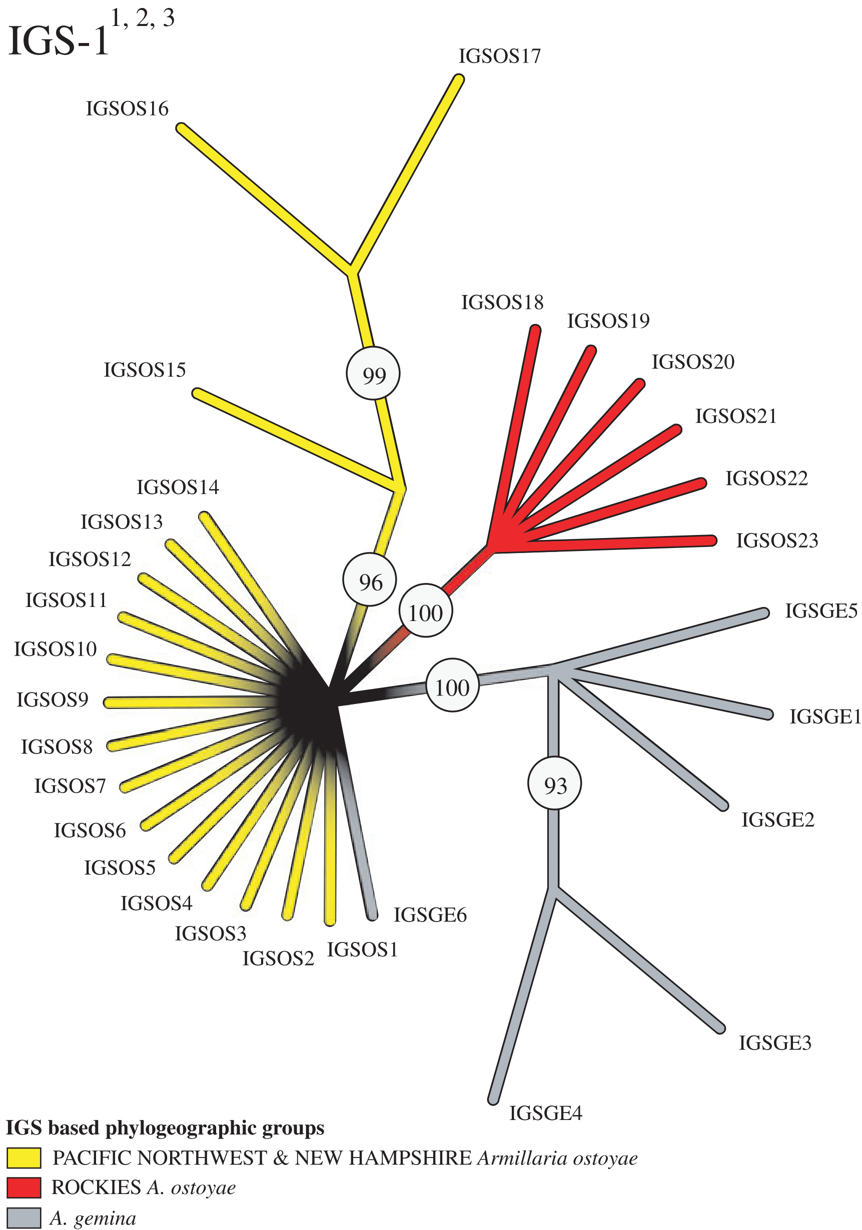

Fig. 6. Radial 90% majority-rule consensus tree of *Armillaria* species based on 24 000 trees from Bayesian inference analysis of the intergenic spacer 1 rDNA (IGS-1) region. Numbers between clades indicate estimated posterior probability. [1]*A. gemina* (IGSGE1–IGSGE6) and *A. ostoyae* (IGSOS1–ITSOS23). [2]Ambiguous sequences from 10 heterogeneous *Armillaria* isolates were excluded from analysis. [3]IGSGE5 and IGSGE6 sequence types represent a single individual of *A. gemina* having hybrid sequence types between *A. ostoyae* and *A. gemina* phylogenetic groups in the IGS-1 region

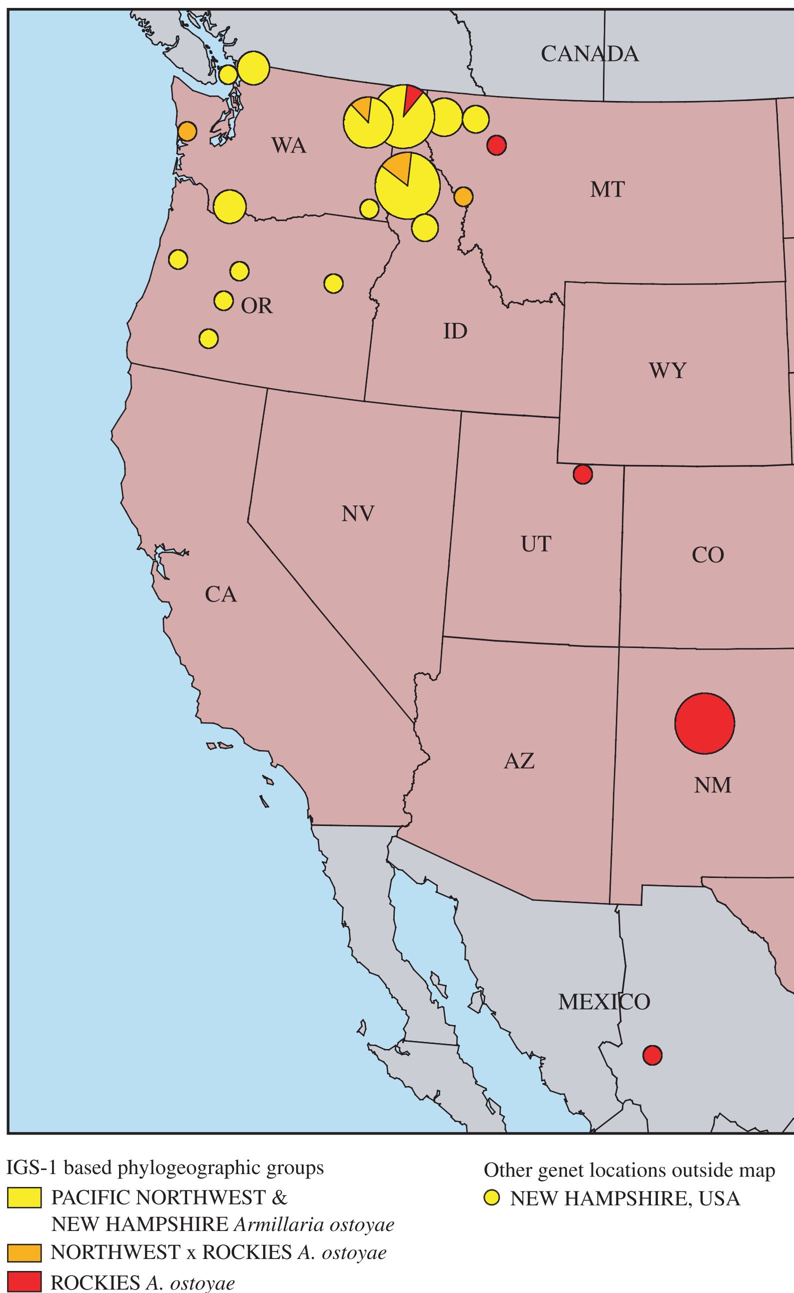

Fig. 7. Phylogeographic distribution of *Armillaria ostoyae* genets based on major clades from Bayesian inference analysis of the intergenic spacer 1 rDNA (IGS-1). Circle area is proportional to the number of genets analysed, with the smallest area representing a single genet

Pacific Northwest group by the LSU analysis. Five *A. gemina* sequence types were separated from the Pacific Northwest/New Hampshire group by a 100% posterior probability; however, one *A. gemina* sequence type (IGSGE6) grouped with that of the Pacific Northwest/New Hampshire polytomy. One genet (ST9) of *A. gemina* was found to harbour both IGSGE5 and IGSGE6 sequence types. This individual may represent an interspecific hybrid between *A. ostoyae* and *A. gemina*, as previously suggested by Kim et al. (2006).

3.3.4 Phylogeographic congruency of rDNA regions

The Rockies and Pacific Northwest groups were observed in all three rDNA regions analysed. The only inconsistency is the representative sequences from the New Hampshire isolate (ST1), which grouped with the Circumboreal sequence types for the LSU, the Rockies sequence types for the ITS + 5.8S and the Pacific Northwest sequence types for the IGS-1. The three other Circumboreal group representatives from the LSU analysis could not be included in the ITS + 5.8S or IGS-1 analysis due to heterogeneity in their PCR products. The genet (USSR) from Primorye, Russia, consisted of two different sequence types (Fig. 3) in the LSU region suggesting that a greater sample size in regions outside the western USA may yield more regionally defined groups of *A. ostoyae* throughout the Northern Hemisphere.

4 Discussion

4.1 Phylogeographic patterns

This study was successful in showing genetic differences among diploid genets of *A. osotoyae* in the western United States. It also establishes a baseline of general phylogeographic patterns. These preliminary baseline findings will aid in the design of future phylogenetic studies that address populations, genetic drift and/or hybrid zones.

4.2 Circumboreal group

It has been hypothesized that the origin of Southern Hemisphere *Armillaria* species, *A. novae-zelandiae* (G.Stev.) Herink and *A. luteo bubalina*, may precede the breakup of the supercontinent Gondwanaland (Coetzee et al. 2003). This concept raises considerations about the influence of historical paleogeographic and paleoclimatic events on modern day distribution of phylogeographically distinct groups of *A. ostoyae* and other *Armillaria* species of the Northern Hemisphere. Such global factors have well-known influences on population distributions of diverse flora and fauna (Swenson and Howard 2005). In the Northern Hemisphere, the Circumboreal group of *A. ostoyae* occurs on three continents, and this group shares identical LSU sequence types with several other *Armillaria* species. This pattern may indicate sequence conservation since the Jurassic period, with an origin that may precede Pangea. Although this postulated date of origin is earlier than current estimates for the divergence of *Armillaria* (Piercey-Normore et al. 1998), current trends in the estimation of fungal divergence times have pushed back earlier estimates (Taylor 2004).

4.3 Rockies group

This study provides evidence that the Rockies group of *A. ostoyae* may be ancestral to *A. nabsnona*. *Armillaria nabsnona* sequence types were similar to *A. ostoyae* Rockies

sequence types in the LSU. As shown by the Circumboreal *A. ostoyae* group, relationships based on the LSU may date back hundreds of millions of years. A similar relationship between *A. nabsnona* and *A. ostoyae* was not observed for the more variable rDNA repeat regions. These relative differences may reflect different evolutionary rates for various rDNA regions. Similarly, the single *A. ostoyae* isolate from eastern USA used in this study clusters with either the Rockies group using ITS + 5.8S data or the Pacific Northwest group using IGS-1 data. This discrepancy perhaps represents an anomaly derived from the small sample size from the eastern USA. However, it is possible that these 'non-coding' rDNA regions may evolve at different rates and/or may have been subjected to selection pressure from differences in local ecological factors. This premise opposes neutral theory (Kimura 1983), and is supported by evidence that evolutionary rates are not always constant across lineages (Britten 1986; Avise 1994; Li 1997; Sanderson 1997). Although function of these non-coding regions remains cryptic, growing evidence suggests they may have influence on growth rates (Elser et al. 2000; Gorokhova et al. 2002).

4.4 Pacific Northwest group

The distribution of the Pacific Northwest group of *A. ostoyae* is similar to a well-known distribution pattern shared by over 100 species known as the mesic forest disjunct (Brunsfeld et al. 2001). The present study does not provide definitive evidence as to when this group diverged from the Rockies and Circumboreal groups, but it does show significant variation and relatively large polytomies for each of the three rDNA regions analysed. These polytomies show sequence types having equal interrelatedness, which can result from a sample size that is too small to resolve differences (soft polytomy) or from adaptive radiation (hard polytomy; Maddison 1989). Adaptive radiation occurs when a single lineage produces descendants with a wide variety of adaptive forms. The history of the Pacific Northwest is filled with events that may have favoured adaptive radiation which include insect outbreaks (Speer et al. 2001), catastrophic fires (Agee 1993), volcanic activity (Townsend and Figge 2002), glacial events (Whitlock 1992) and some of the largest floods ever documented (O'Conner and Costa 2004). Heterogeneous environments created by such events may have favoured diverse genotypes within this group. Further study of this group with molecular techniques with greater resolution and a larger sample size may reveal phylogenies related to these events.

4.5 Heterogeneity (intragenomic variation)

Direct-PCR has been shown to detect 90% of the heterogeneous rDNA products in an individual and the relative peak height seems to reflect relative copy number (Rauscher et al. 2002). In this study, heterogeneous products indicating intraspecific and intragenomic variation within *A. ostoyae* were common in all regions analysed.

Several sequence type arrangements are possible when two or more ambiguously coded nucleotide positions exist for an individual, adding uncertainty to genotyping (Presa et al. 2002). Often these heterogeneous individuals appear to be hybrids among known sequence types, having ambiguous nucleotide positions at locations that are polymorphic among known sequence types. Including these putative hybrids not only adds uncertainty of sequence types, but also can potentially reduce phylogenetic signal by collapsing clades between parental origins.

The process of concerted evolution is thought to homogenize rDNA repeats throughout the genome of most individual eukaryotes (Elder and Turner 1995). However in *Armillaria* species, heterogeneity within rDNA repeats seems to be the rule rather than the exception. Over time, concerted evolution may homogenize rDNA repeats; however, Kim et al. (2001) demonstrated that individuals of *Armillaria* spp. with divergent rDNA

sequence types can mate in culture. The direct-PCR results of this study suggest that individuals with divergent rDNA sequence types may be naturally derived from intraspecific hybridization. Additional studies could determine if intraspecific hybridization among *A. ostoyae* reflects well-defined suture zones that were established by ancient climatic, geologic and/or other influences (SWENSON and HOWARD 2005). The rate and mechanisms that control homogenization within *Armillaria* species remain unknown. Further mating tests coupled with sequencing that probe for differences among rDNA repeats may help address these questions. Phylogenetic analysis based on sequences derived from few to several rDNA clones may produce erroneous results because scores of clones may be needed to sufficiently detect variation among rDNA repeats (RAUSCHER et al. 2002). Although we were successful in showing heterogeneity within individuals, our deciphered sequence types may represent a consensus of repeat types, each containing minor variations. If a single individual retains rare ancestral sequence types because of incomplete homogenization from concerted evolution, it is possible that heterogeneous sequences from individuals can be used to produce phylogenies that show parental evolutionary history of those individuals.

4.6 Protein coding *vs*. rDNA genes

Current trends in evolutionary studies of fungi have encouraged many researchers to examine DNA regions that encode protein, such as β-tubulin and elongation factor 1-α (BRUNS and SHEFFERSON 2004). These protein-coding sequences have been favoured because they are easier to align. However, variation among repeat types can provide significant phylogenetic insights that are not available with single-copy genes, if highly ambiguous alignments (i.e. alignments of ITS + 5.8S and IGS-1 regions of *A. mellea sensu stricto* with those of *A. ostoyae*) or ambiguous sequences are excluded from analysis. Through vigilant sequence editing, techniques to decipher heterogeneity within an individual (Fig. 1), and elimination of ambiguity within the data set, we were able to show rDNA genes can harbour phylogenetic information that has previously been unavailable. With this approach, rDNA sequences may provide more powerful information towards understanding evolutionary events, because the divergent parental histories should be represented within heterogeneous rDNA from a single organism.

4.7 Implications of hybridization

Although once thought to be very rare, hybridization has now been recognized in diverse fungal phyla (BRASIER 2000; SCHARDL and CRAVEN 2003) and families (DE SOUZA et al. 2004). Furthermore, most individuals in this study showed hybridization at some level. Three different levels of hybridization are observed in this study: (i) interspecific hybridization between two species (e.g. *A. gemina* × *A. ostoyae* within the IGS-1), (ii) intraspecific hybridization between divergent groups of the same species (e.g. Rockies *A. ostoyae* × Circumboreal *A. ostoyae* in the LSU) and (iii) intraspecific hybridization within the same group of the same species (detected in all three regions analysed). In theory, a hybrid may have greater adaptability to diverse environmental niches than either parent, thereby allowing hybrids to occupy new or changing niches from which speciation events may subsequently occur (FOWLER and LEVIN 1984; RIESEBERG et al. 1990, 2003; RIESEBERG 1991; ARNOLD 1997; GOLDMAN et al. 2004). In addition, hybrids often show increased vigour and ability to exploit resources (INGVARSSON and WHITLOCK 2000; EBERT et al. 2002; KAYE and LAWRENCE 2003). Differences in virulence, pathogenicity and epidemiology are often most notable among hybrid fungal pathogens; however, fungal mutualists with increased vigour may better adapt and exploit resources for host plants as well (SCHARDL and CRAVEN 2003).

4.8 Future studies

Continued studies are underway at the USDA Forest Service – RMRS, Forestry Sciences Laboratory in Moscow, Idaho, USA to: (i) understand rates and mechanisms of concerted evolution within *Armillaria* species; (ii) understand relationships among *Armillaria* species, groups and individuals; (iii) analyse possible relationships of phylogeographically distinct groups to differences in pathogenicity and epidemiology; (iv) determine phylogenetic relationships to historical paleogeographic and paleoclimatic events and (v) use phylogeography to predict potentially invasive *Armillaria* biotypes.

Acknowledgements

This work was supported by the USDA Forest Service, RMRS-4552 (Microbial processes as ecosystem regulators in western forests) research unit in Moscow, ID, USA, USDA Forest Service, Forest Health Protection, Coeur d'Alene Field Office (John Schwandt), Research Joint Venture Agreement 03-JV-11222062-288 (Genetic variation of *Armillaria ostoyae* from the Pacific Northwest), and the Sustainable Forestry component of Agenda 2020 collaborative project (Tools to predict and manage Armillaria root disease), a joint effort of the USDA Forest Service Research & Development and the American Forest & Paper Association with Forest Capital (formerly BOISE corporation) as a research partner. We thank Dr Karen S. Humes, Jane E. Stewart and Bryce A. Richardson for their comments on an earlier version of this manuscript. We also thank the members of the Intermountain Forest Tree Nutrition Cooperative (Dr Mariann Garrison Johnston, Terry Shaw, Mark Kimsey, Bob Atwood and others who contributed to *Armillaria* collections); Dr Steven J. Brunsfeld for his expertise in phylogenetic analysis; James B. Donley for maintaining fungal archives and sequence editing; Dr Phil Cannon and Phil Anderson for their assistance with *Armillaria* collections; Dr Paul J. Zambino, Dr Leonard Johnson, Bonnie G. Hanna and Raini C. Rippy for general advice and support; and Dr Joe Ammirati, Dr Greg Filip, Dr Tom C. Harrington, Dr Charles G. (Terry) Shaw III and Brennan Ferguson for providing collections and information on *A. ostoyae*. Use of trade names does not constitute endorsement by the USDA Forest Service.

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