Genetic uniformity characterizes the invasive spread of *Neofusicoccum parvum* and *Diplodia sapinea* in the Western Balkans

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Summary

In the past decade trees and shrubs in the Western Balkans region have been damaged by canker and die-back disease caused by Botryosphaeriaceae species. These pathogens include *Neofusicoccum parvum* and *Diplodia sapinea*. In this study, we determine genetic diversity and structure between populations of *N. parvum* and *D. sapinea* from Serbia and Montenegro (Western Balkans) using DNA sequence data of the internal transcribed spacer (ITS) rDNA, translation elongation factor 1-alpha (TEF 1-α), β-tubulin-2 (BT2) and microsatellite markers. The relationship of both pathogens was compared for populations from the Continental (CR) and Mediterranean (MR) regions and for isolates of *D. sapinea* from *Cedrus* spp. and *Pinus* spp. *Neofusicoccum parvum* and *D. sapinea* were shown to have a low gene and genotypic diversity across the regions and hosts. All genotypes of *D. sapinea* found on *Pinus* spp. were also present on *Cedrus* spp. The CR and MR populations of both species were found to be only slightly separated from one another by a geographical barrier. Low genetic diversity and dominance of *N. parvum* and *D. sapinea* on non-native trees suggests that these species have most likely been introduced into Western Balkans, possibly through the movement of infected plants.

1 Introduction

Biological invasion by ''alien'' plant pathogens represents an important driver of tree disease epidemics worldwide (Desprez-Loustau et al., 2007; Brasier, 2008; Santini et al., 2013; Wingfield et al., 2015). Classic examples include the chestnut blight epidemic on *Castanea* spp. in USA and Europe caused by *Cryphonectria parasitica* (Murr.) Bar (Anagnostakis, 2001); Dutch elm disease epidemics on American and European *Ulmus* spp. caused by *Ophiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* (Brasier) (Brasier and Buck, 2001); canker stain disease of plane in Europe on *Platanus* spp. caused by *Ceratocystis platani* (Walter) Engelbrecht & Harrington (Tsopelas et al., 2017), spread of the pitch canker disease caused by *Fusarium circinatum* Nirenberg & O'Donnell from its origin in Central America (Wingfield et al., 2008), *Phytophthora* spp. outbreaks in USA and Europe (Gruenwald et al., 2012;

Hardham and Blackman, 2018) and the recent emergence of the ash die-back pathogen *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya in Europe (Pautasso et al., 2013; Keča et al., 2017; Milenković et al., 2017).

Increased international trade and travel is considered to be the main driver of invasive alien fungi (Desprez-Loustau, 2008; Vannini et al., 2012; Ghelardini et al., 2016; Wingfield et al., 2015). Trade in plants and plant products are considered amongst the most common means for the introduction of alien fungal pathogens (Liebhold et al., 2012; Vannini et al., 2012; Migliorini et al., 2015; Ghelardini et al., 2016). Amongst these, rough wood packaging and large mature specimen trees ("instant trees") traded with bark, root balls and soil attached are considered to be the most important risk pathways for invasive pathogens (Brasier, 2008; Santini et al., 2013; Ghelardini et al., 2016).

Pathogens that move with their hosts as "hitchhikers" as saprophytes, endophytes or as resting spores in the soil are especially difficult to detect and to prevent their introduction (Santini et al., 2013; Migliorini et al., 2015; Burgess et al., 2016; Crous et al., 2016; Ghelardini et al., 2016). A typical example is fungi in the Botryosphaeriaceae, which are known as endophytes that remain latent in asymptomatic plant tissue for long periods of time. Some of these endophytes may become important pathogens where they are introduced (Slippers and Wingfield, 2007; Wingfield et al., 2015; Burgess and Wingfield, 2017).

Risks of host range expansion of introduced pathogen are considered greatest in urban areas with gardens and parks comprising dense assemblages of exotic and native plants. Moreover, urban trees are grown in "locally heated islands", experience stress from i.e. pollution, soil compaction and such conditions may predispose trees to pathogen attack, increasing the likelihood that an alien pathogen will successfully establish (Walther et al., 2009; Santini et al., 2013). After establishment, invasive pathogens can cause severe ecological, social and economic impacts and destabilize entire ecosystems by affecting i.e. hydrology, recreation, carbon and nitrogen cycles (Brasier, 2008; Stenlid et al., 2011; Mitchell et al., 2014). Invasive pathogens are also affected by other drivers of global change, such as climate change, which may increase their invasion potential (Walther et al., 2009; Ghelardini et al., 2016; Ramsfield et al., 2016).

Despite various phytosanitary measures and regulations (e.g. plant passport, ISPM-15, 36, "EU black list", EPPO A1 and A2 lists, sentinel plantings), the problem of invasive pathogens is especially evident in Europe. Santini et al. (2013) reported 60 alien invasive forest pathogens in Europe compared to only 17 found in the USA by Aukema et al. (2010). A long history of colonialism and extensive planting of non-native trees are important factors

considered to be responsible for the high number of alien pathogens being established on this continent (Brasier, 2008; Santini et al., 2013).

Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips and *Diplodia sapinea* (Fr.) Fuckel (syn. *Diplodia pinea* (Desm.) Kickx., *Sphaeropsis sapinea* (Fr.: Fr.) Dyko & Sutton) are plant pathogens that commonly occur in temperate, Mediterranean and tropical climates worldwide (Slippers and Wingfield, 2007; Phillips et al., 2013; Slippers et al., 2017). *Neofusicoccum parvum* has been found associated with a wide range of tree species, including grapevine, fruit and forest trees, whereas *D. sapinea* is known as a pathogen of conifers, mostly pines, but it also infects spruces and firs (Swart and Wingfield, 1991; Slippers et al., 2017; Zlatković et al., 2017). These fungi are latent pathogens, causing disease when trees are subjected to stresses (e.g. climate extremes) (Slippers and Wingfield, 2007; Mehl et al., 2013). *Diplodia sapinea* is thought to be native in North America and Eurasia and it has been introduced into countries of the Southern Hemisphere where pines are exotic (Smith et al., 2000; Burgess et al., 2001a, 2004; Bihon et al., 2012). *Neofusicoccum parvum* has been speculated to be native to Southern Africa, where populations exhibit high genetic diversity (Sakalidis et al., 2013).

Neofusicoccum parvum and *D. sapinea* have recently been shown responsible for disease on various trees and shrubs in the Western Balkans region (Zlatković et al., 2016a, b, 2017, 2018), but nothing is known about the population genetics or biology of these species in this region. In this study, we determined genetic diversity and structure between different populations of *N. parvum* and *D. sapinea*. The relationships between isolates from the Continental (CR) and Mediterranean (MR) regions and between isolates of *D. sapinea* from *Cedrus* spp. and *Pinus* spp. were also considered. This was achieved using the DNA sequence data for the internal transcribed spacer (ITS) rDNA, translation elongation factor 1-alpha (TEF 1-α), β-tubulin-2 (BT2), RNA polymerase II gene (RPB2) and microsatellite markers.

2 Materials and methods

2.1 Fungal isolates

The isolates used in this study were collected during a survey of trees showing disease symptoms such as die-back, cankers and resin exudation as previously described (Zlatković et al., 2016a) in Serbia and Montenegro between 2009 and 2014. Symptomatic samples were mostly collected in the cities, but also in plantations, forest stands,

nurseries of ornamental plants and two isolates of *D. sapinea* were collected from *Pinus radiata* D. Don trees grown on Mt. Athos in Greece (Table S1, Zlatković et al., 2016a, b, 2017, 2018).

From each tree, isolations were made from symptomatic and asymptomatic tissues as described previously by Zlatković et al. (2016a). Fifty six isolates of *N. parvum* and 87 isolates of *D. sapinea* were identified in previous studies based on the DNA sequence data of the ITS region, TEF-1-α, β-tubulin, large subunit (LSU) rRNA and RNA polymerase II gene (RPB2) (Zlatković et al., 2016a, b, 2017, 2018). Haploid cultures established from hyphal-tip transfers used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

2.2 DNA extraction, microsatellite-PCR amplification and genotyping

Microsatellite analyses were conducted on a subset of *N. parvum* and *D. sapinea* isolates identified in the previous studies, including 46 isolates of *N. parvum* and 85 isolates of *D. sapinea* (Zlatković et al., 2016a, b, 2017, 2018). Fungal cultures were grown on malt extract agar (MEA) in Petri dishes for seven days and DNA was extracted from the mycelium as previously described (Zlatković et al., 2016a). Six of the seven fluorescently-labelled primer pairs previously designed for species of *Neofusicoccum* spp. (Slippers et al., 2004) successfully amplified isolates of *N. parvum*. Primer pairs BOT21 and BOT22 could not amplify these isolates and were excluded from subsequent analyses. Primer pairs BOT15 and BOT16 produced numerous stutter peaks that would hamper the reliable interpretation of the genotypes and were also excluded from further analyses. Thirteen microsatellite loci of *D. sapinea* were amplified using primer pairs specifically designed for this fungus by Burgess et al. (2001b) and Bihon et al. (2011). However, primer pairs TB19 and TB 20-2, TB37 and TB38 and WB1-a and WB1-b were discarded from the analyses due to the excessive stuttering (Table S2).

The 25 µl PCR reaction mixtures contained 2.5 µl of 10 mM PCR buffer (PCR buffer with $MgCl_2$), 1 µl of 100 mM of each dNTPs, 0.25 µl of 10 mM of each primer, 2 ng of genomic DNA, 0.2 µl (1U) of Fast Start Taq polymerase (Roche Molecular Biochemicals, Indianapolis) and 18.8 µl of sterile distilled Sabax water. In most cases, the PCR reaction mixture contained an additional 1-3 µl of 25 mM $MgCl_2$ (data not shown). PCR reactions were performed using the following protocol: 95°C for 3-6 min initial denaturation followed by ten cycles of 95°C for 30 s, 52-65°C for 45 s, 72°C for 1 min; then by 20-30 cycles of 95°C for 30 s, 52-65°C for 45 s, 72°C for 1 min +0.05s/cycle increase and 60°C for 30 min final extension (to reduce stutters in the microsatellite peaks). Where the

above-mentioned PCR protocol failed to amplify in some isolates, PCR reactions were performed using touchdown thermal cycling programs (Don et al., 1991) with a six to ten °C span of annealing temperatures, ranging from 68 to 62 °C or 65 to 55 °C. Amplifications were carried out alongside a control containing sterile water in place of the DNA. Details of the cycling parameters and PCR conditions for each primer set are shown in Table S2.

PCR products were separated by electrophoresis on 2 % agarose gels, stained with GelRed (Biotium, Hayward, California, USA) and visualized under ultraviolet light. Sizes of PCR products were estimated by comparison with a DNA molecular weight marker (Gene Ruler ^{TM} 100 bp DNA Ladder, Fermentas). Fluorescently labelled PCR products were multiplexed for each species separately and 1 µl of these multiplexed PCRs were separated on ABI Prism 3500 Genetic analyzer. Allele sizes of labeled microsatellite-PCR products were compared against a LIZ-500 internal size standard and analyzed with the GeneScan 2.1 and GeneMapper 3.7 softwares (Applied Biosystems).

2.3 Microsatellite analyses

The multilocus genotypes (MLGs) for each isolate were generated by coding the alleles at different loci using alphabetical letters for each individual allele (e.g. AAAAAABBAA). Prior to analyses, data for isolates of each species were separated into two collections representing the climatic region from which they originated (Continental (CR) and Mediterranean (MR)). Additionally, data for isolates of *D. sapinea* were separated into two host populations that originated either from *Cedrus* spp. or *Pinus* spp. Allele frequencies were determined for the entire populations, as well as for predefined geographic and host sub-populations (Table 1). For each population, the observed number of alleles, number of unique alleles, the observed number of multilocus genotypes (MLGs, g) and percentage of polymorphic loci (P) were evaluated. *Diplodia sapinea* datasets were clone-corrected by removing the duplicate MLGs, and microsatellite analyses were conducted on both original and clone-corrected data sets. Population genetic parameters for the *N. parvum* population were calculated only on the original data sets because the number of individuals after clone-correction was less than ten.

Table 1. Allele size (bp) and frequency at five loci for *Neofusicoccum parvum* and at ten loci for *Diplodia sapinea*.

Locus	Allele (bp)	Pop 1 [1] (Continental)	Pop 2 [1,2] (Mediterranean)	Pop 3 [3] (*Cedrus* spp.)	Pop 4 [3] (*Pinus* spp.)	All [4]
			Neofusicoccum parvum			
BotF11	429	1.000	+			1.000
	232	1.000	+			0.935
BotF17	248	−	+			0.043
	260	−	+			0.022
	244	−	+			0.022
BotF18	245	1.000	+			0.913
	247	−	+			0.022
	250	−	+			0.043
BotF23	423	1.000	+			0.065
	424	−	+			0.065
BotF35	223	1.000	+			1.000
			Diplodia sapinea			
SS1	411	1.000	1.000	1.000	1.000	1.000
SS2	195	1.000	1.000	1.000	1.000	1.000
SS7	384	1.000	1.000	1.000	1.000	1.000
SS8	280	1.000	1.000	1.000	1.000	1.000
SS10	313	1.000	1.000	1.000	1.000	1.000
SS11	173	1.000	1.000	1.000	1.000	1.000
	149	0.078	−	0.091	0.040	0.059
SS13	151	0.094	−	0.023	0.160	0.071
	157	0.828	1.000	0.886	0.800	0.129
	149	0.125	−	0.091	0.040	0.094
	161	0.797	0.762	0.795	0.160	0.788
SS14	164	0.047	0.238	0.120	0.091	0.094
	171	0.031	−	0.023	−	0.024
	68	0.938	0.714	0.795	0.040	0.882
SS15	71	1.000	1.000	0.205	0.040	1.000
SS16	108	1.000	1.000	1.000	1.000	1.000

[1] Populations 1 and 2=geographically defined populations of *N. parvum* and *D. sapinea* originating from Continental and Mediterranean climate type region, [2] Allele frequencies for population 2 of *N. parvum* were not calculated because of the small sample size, [3] Populations 3 and 4= isolates of *D. sapinea* originating from *Cedrus* spp. and *Pinus* spp. [4] All isolates of *N. parvum*/*D. sapinea*, + Allele was present in a population.

Gene diversity of each population was estimated by calculating the Nei's unbiased gene diversity (corrected for sample size) (Hexp; Nei, 1978). Genotypic diversity was evaluated using the Shannon's diversity

index of MLG diversity (H), which was calculated with H=∑PilnPi and corrected for differences in isolate numbers with H'= H/ln(g), where Pi is the frequency of the ith MLG in a given host population and g is the number of MLGs observed in each population (Shannon, 2001; Grünwald et al., 2003). Stoddart and Taylor's index of MLG diversity (G) was estimated using the equation G=1/∑Pi^2 and corrected for unequal sample sizes with G'= G/g where Pi is the observed frequency of the ith genotype in the population and g is the observed number of MLGs in the population (Stoddart and Taylor, 1988; Grünwald et al., 2003). Values for H' and G' range from 0 (single genotype present) to 1 (each isolate represents a different genotype). Genotypic evenness (E$_5$) was calculated with E$_5$ = (G-1)/(e$^{H'}$-1) (Grünwald et al., 2003). Values of E can range from 0 to 1, with lower values indicating that a certain genotype dominates in the collection of isolates from a particular host population. Clone corrections, estimations of gene diversity, genotypic diversity and genotypic evenness were performed in R v. 3.2.3 (R Core Team, 2015) using population genetics package "poppr" v. 2.1.0 (Kamvar et al., 2014). Allelic richness (Ar) was computed in R using package "hierfstat" v. 004-22 (Goudet and Jombart, 2015). The package implements a rarefaction approach allowing for comparison of the values for a standardized sample size corresponding to the smallest sample size across populations. Other analyses were not conducted because of the clonal structure and small sample sizes of each population. Values of the genetic diversity for the *N. parvum* and *D. sapinea* populations were compared using z-test (Pocock, 2006) at the 5 % significance level (z > 1.96, p < 0.05).

2.4 DNA sequence haplotype networks and phylogeographic relationships with haplotypes outside Western Balkans

DNA sequence haplotype analyses were conducted using all isolates of *N. parvum* and *D. sapinea* identified in the previous studies ie 56 isolates of *N. parvum* and 87 isolates of *D. sapinea* (Zlatković et al., 2016a, b, 2017, 2018). Datasets containing sequences of ITS, TEF-1-α, β-tubulin and RPB2 for isolates of *N. parvum* and ITS, TEF-1-α and β-tubulin for isolates of *D. sapinea* were aligned separately as described in Zlatković et al. (2016a). Because incongruence between the studied genes was not detected following the partition homogeneity test (Farris et al., 1995) using PAUP v. 4 (Swofford, 2003), combined datasets for each species were included in the subsequent analyses. Haplotypes were determined from the aligned data in DnaSP 5.10.1 (Librado and Rozas, 2009). Unique haplotypes were then pooled with unique haplotypes of *N. parvum* and *D. sapinea* obtained from GenBank covering a large portion of the known geographic range of each species. Relationships between the haplotypes for each gene

region separately and for the combined datasets were inferred via a median-joining network calculated using NETWORK 4.6.1.2 (http://www.fluxus-engineering.com, Table S3).

3 Results

3.1 Genetic and genotypic diversity of *Neofusicoccum parvum*

All five microsatellite loci were monomorphic for the *N. parvum* CR population and three loci were polymorphic in the MR population (Tables 1, 2). For the entire dataset of 46 *N. parvum* isolates, a total of 11 alleles were observed across five loci examined. There were five alleles detected in the CR population and 11 alleles were found in the MR population. Five alleles were shared among the two populations and there were six private alleles in the MR population. Low gene diversity was observed across in the total *N. parvum* population (Hexp= 0.084). The CR population was completely clonal.

Among the 46 isolates of *N. parvum* examined, a total of six microsatellite MLGs were found (Fig. 1, Table 2). Of these, one MLG was detected in the CR population and five MLGs were detected in the MR population. No MLGs were shared between the two populations. Corrected Shannon-Wiener genotypic diversity (H') and Stoddart and Taylor genotypic diversity (G') values were low for the entire data set of *N. parvum* isolates (0.33, 0.22).

The MLG S1 was shared among the highest number of hosts (ten), MLG S4 was shared among the two hosts and all the remaining MLGs were associated with a single host species. *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Prunus laurocerasus* L. and *Eucalyptus globulus* Labill. each harboured two MLGs and ten other hosts harboured a single MLG. With the exception of *Pittosporum tobira* (Thunb.) W.T.Aiton, all the hosts had one MLG shared with at least one other host. Ten hosts shared a MLG with two other hosts and two hosts shared a MLG with one other host (Fig. S1, Table S4). Six MLGs were found on hosts in urban areas and one was also detected on seedlings in ornamental plant nurseries (Table S4, Fig. S2).

Table 2. Measures of genetic diversity based on the analysis of five microsatellite loci for *Neofusicoccum parvum* and ten loci for *Diplodia sapinea*.

	Neofusicoccum parvum			*Diplodia sapinea*				
	Pop 1 (Continental)[1]	Pop 2 (Mediterranean)[2]	Total isolates	Pop 1 (Continental)[1]	Pop 2 (Mediterranean)[2]	Pop 3 (*Cedrus*)[3]	Pop 4 (*Pinus*)[4]	Total isolates
No. of isolates	40	6	46	64	21	44	25	85
No. of alleles	5	11	11	16	12	16	15	16
No. of private alleles	0	6	N/A	4[5]	0	1[6]	0	N/A
g	1	5	6	7	3	7	6	7
P (%)	0	60	60	30	20	30	30	30
Ar	2[7]		2.7[7]	2.33[8]	2.2[8]	2.38[9]	2.33[9]	2.33[8]/2.37[9]
Ar(c)				2.6[8]	2.2[8]	2.6[9]	2.5[9]	2.6[8,9]
Hexp	0		0.084	0.078	0.081	0.09	0.072	0.081
Hexp(c)				0.152	0.133	0.152	0.153	0.152
H	0		0.591	1.43	1.05	1.5	1.24	1.47
H(c)				1.95	1.1	1.95	1.79	1.95
H'	0		0.33	0.73	0.96	0.77	0.69	0.76
H'(c)				1	1.78	1	1	1
G	1		1.32	2.82	2.74	3.38	2.47	3
G(c)				7	3	7	6	7
G'	0		0.22	0.4	0.91	0.48	0.41	0.43
G'(c)				1	1	1	1	1
E_5			0.392	0.57	0.93	0.68	0.6	0.6
E_5(c)				1	1	1	1	1

[1] Isolates collected in the Continental climate-type region, [2] Isolates collected in the Mediterranean climate-type region, [3] Isolates obtained from *Cedrus* spp., [4] Isolates obtained from *Pinus* spp., [5] Compared to isolate collection from the Mediterranean region, [6] Compared to isolate collection from *Pinus* spp., [7] Standardized for 40 isolates, [8] Standardized for 21 isolates, [9] Standardized for 25 isolates.

g. Observed number of multilocus genotypes (MLGs)

P. Number of polymorphic loci

Ar. Allelic richness using non clone-corrected data, Ar (c). Allelic richness using clone-corrected data

Hexp. Nei's unbiased gene diversity using non clone corrected data (Nei, 1978), Hexp (c). Nei's unbiased gene diversity using clone corrected data

H. Shannon-Wiener index of MLG diversity using non clone-corrected data (Shannon, 2001), H(c). Shannon-Wiener index of MLG diversity using clone-corrected data

H'. Corrected Shannon-Wiener index of MLG diversity using non clone-corrected data (Grünwald et al., 2003), H'(c). Corrected Shannon-Wiener index of MLG diversity using clone-corrected data

G. Stoddart and Taylor's index of MLG diversity using non clone-corrected data (Stoddart and Taylor, 1988), G(c). Stoddart and Taylor's index of MLG diversity using clone-corrected data

G'(c). Corrected Stoddart and Taylor's index of MLG diversity using non clone-corrected data (Grünwald et al., 2003), G(c). Corrected Stoddart and Taylor's index of MLG diversity using non clone-corrected data

E_5. Genotypic evenness using non clone-corrected data (Grünwald et al., 2003), E_5(c). Genotypic evenness using clone-corrected data

Comparison of the measures of genetic diversity of population1 of *N. parvum* and *N. parvum* entire isolate collection, and *D. sapinea* populations 1 and 2, 3 and 4, 2 and *D. sapinea* entire isolate collection was not significant at $p < 0.05$ (z-test). Population genetic parameters for the *N. parvum* population were calculated only on the original data sets because the number of individuals after clone correction was less than ten. Some of the population genetic parameters for the *N. parvum* population were not calculated due to a small sample size.
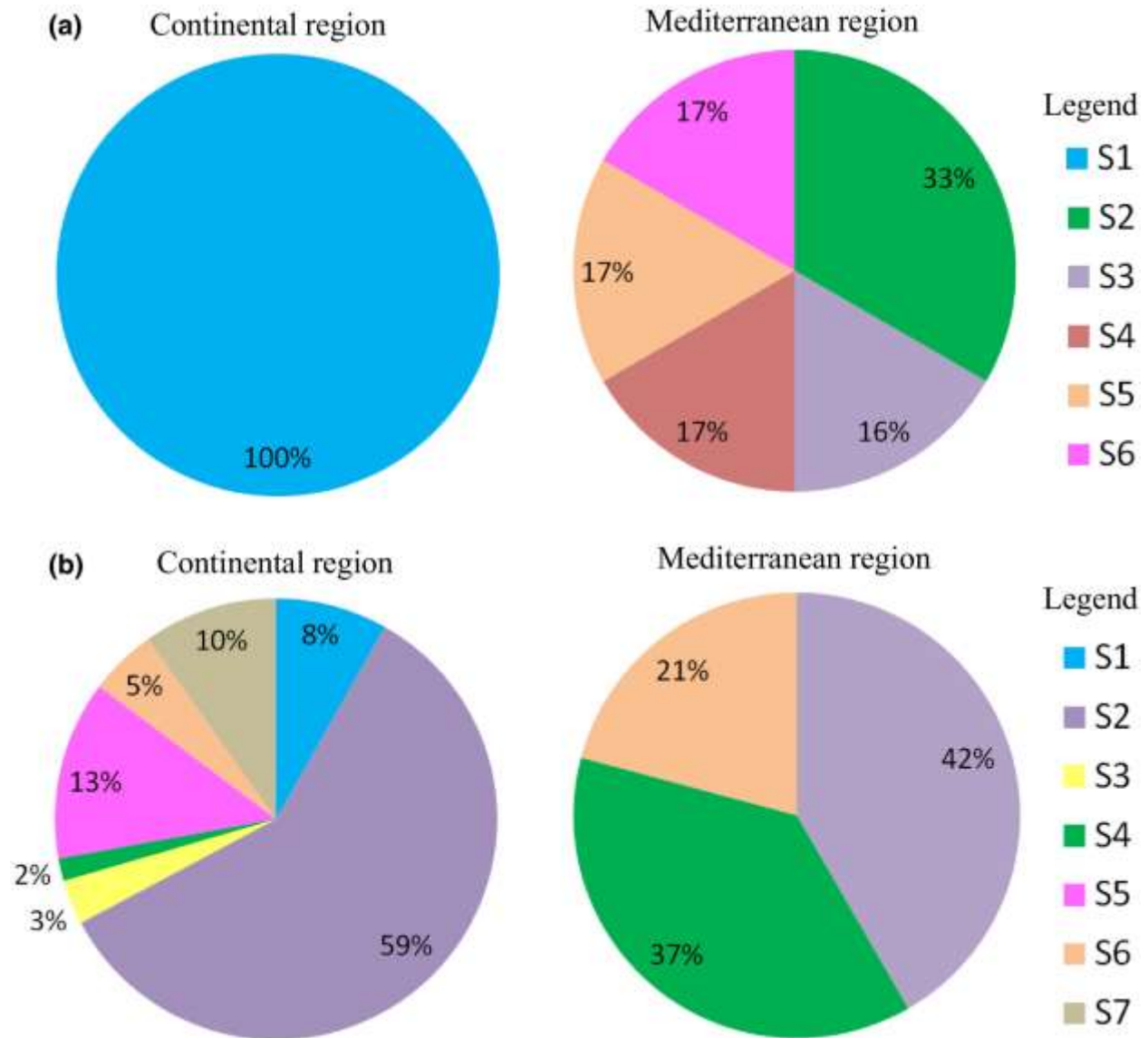
Figure 1. Pie charts representing genotypic diversity of *N. parvum* and *D. sapinea* populations from the Continental climate-type region and Mediterranean climate-type region along the Adriatic cost. (a) *Neofusicoccum parvum*; (b) *Diplodia sapinea*. Different multilocus genotypes are indicated as S1-S6 for *N. parvum* and S1-S7 for *D. sapinea*.

3.2 Genetic and genotypic diversity of *Diplodia sapinea*

Two of the loci were polymorphic in the MR population of *D. sapinea* and three of the loci were polymorphic in the remaining analyzed populations (Table 2). For the *D. sapinea* dataset of 85 isolates, a total of 23 alleles were observed across ten loci examined (Table 1). Sixteen alleles were detected in the CR population, in the population from *Cedrus* spp. and for the entire dataset. Fifteen alleles were identified in the population from *Pinus* spp. and 12 alleles were detected in the MR population. Twelve alleles were shared among the two geographically defined

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populations and 15 alleles were shared among the populations from *Cedrus* spp. and *Pinus* spp. There was one private allele in the CR population and four in the *D. sapinea* population from *Cedrus* spp. The corresponding allelic richness (Ar) was low for the total *D. sapinea* population (2.2-2.38, Table 2). Low gene diversity was observed across all isolates of *D. sapinea*, as well as across geographically and host defined *D. sapinea* populations (Hexp= 0.072-0.09).

Seven MLGs were observed among 85 isolates of *D. sapinea* from the CR and three MLGs were identified among isolates from the MR. Seven MLGs were found among isolates from *Cedrus* spp. and six MLGs were detected among isolates from *Pinus* spp. (Figs. 1, 2; Table 2). Three MLGs were shared between populations from the CR and MR and six MLGs were shared between populations from *Cedrus* spp. and *Pinus* spp. Corrected Shannon-Wiener genotypic diversity (H') and Stoddart and Taylor genotypic diversity (G') values did not differ significantly among *D. sapinea* populations. As reflected by the E_5 values, the frequencies of the MLGs in all isolate collections were not evenly distributed. For example, in a population from the CR, MLG S2 comprised 60 % of the population (E_5= 0.57). The only exception was the population from MR that was characterized by high E_5 value (E_5= 0.93), suggestive of a more even within-population distribution of MLGs.
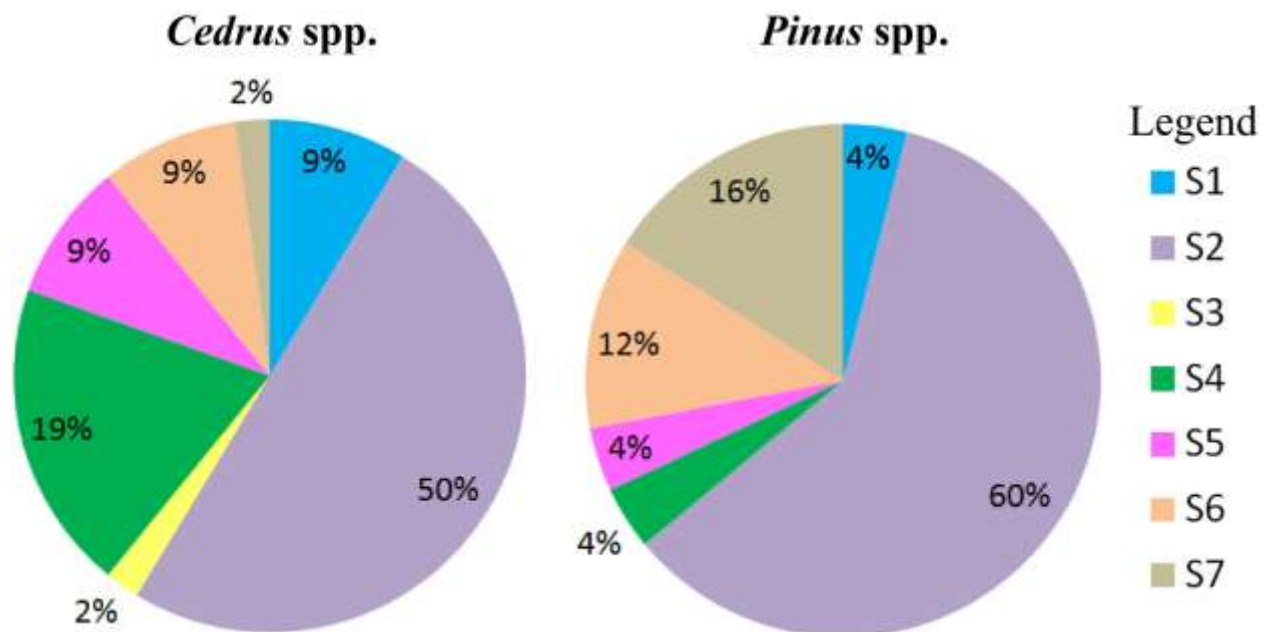

Figure 2. Pie charts representing genotypic diversity of the *Diplodia sapinea* populations from *Cedrus* spp. and *Pinus* spp. Different multilocus genotypes are indicated as S1-S7.

The MLG S2 was shared among the highest number of hosts (11); MLG S7 was shared among four hosts, MLGs S4, S5 and S6 were shared among three hosts, whereas MLGs S1 and S3 were shared among the two hosts. *Cedrus atlantica* harboured the greatest number of MLGs (7), *Picea pungens* Engelm., *Pinus nigra* J. F. Arnold and *Pinus sylvestris* L. each harboured three MLGs, *Picea omorika* (Pančić) Purk. and *Pinus halepensis* Miller harboured two MLGs and five other hosts harboured a single MLG. Each host had at least one MLG shared with at least one other host. *Cedrus atlantica* shared all seven MLGs with other hosts, three hosts shared three MLGs with other hosts, two hosts shared two MLGs with other hosts and the rest of the hosts had a single MLG shared with other host species. *Cedrus atlantica* shared MLGs with all other hosts (15), ten hosts shared MLGs with five or six other hosts and five hosts shared MLGs with two or one other host (Fig. S1; Table S4). Seven MLGs were found on hosts in urban areas. Among them, one MLG was also detected in both pine plantations and forest stands and two other MLGs were shared with either pine plantations or forest stands (Table S4, Fig. S3). Up to three *D. sapinea* genotypes were found coexisting in the same host tree and in the same lesion or tree part. All but one of the trees and tree parts had genotype S2 coexisting with other genotypes (Table S5).

3.3 DNA sequence haplotype networks

A total of five multilocus haplotypes were detected in the *N. parvum* population (Fig. 3, Table S1). Haplotype 1 (H1) was the most common, being present in 52 of 56 isolates. H1 was the only haplotype detected in the CR and all five haplotypes were found in the MR. Three of five MR haplotypes were separated from one another by a single point mutation giving a haplotype network a chain-like pattern. H1 was found on 14 of 15 hosts (Table S1).

Five multilocus haplotypes were found in the *D. sapinea* population (Fig. 3, Table S1). Among them, three haplotypes were found in the MR and four haplotypes were detected in the CR. Two haplotypes were shared among the regions. H1 was the most common and it was found in 76 of 87 isolates. This haplotype was shared among the greatest number of hosts (14 of 16) (Table S1).
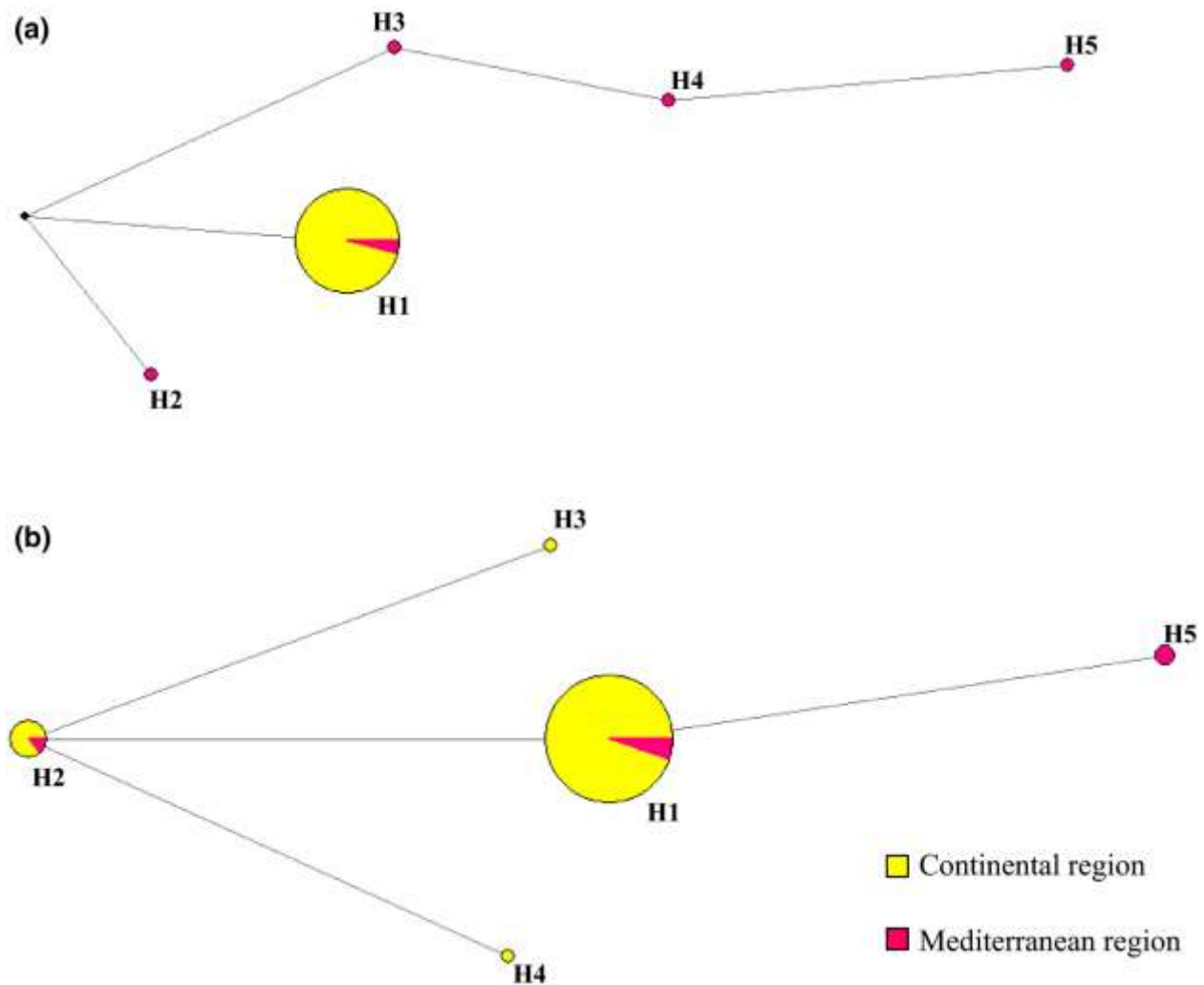
12

Figure 3. Median joining network for the multilocus haplotypes of (a) ITS, TEF-1-α, β-tubulin and RPB2 for *Neofusicoccum parvum*; (b) ITS, TEF-1-α and β-tubulin for *Diplodia sapinea*. Each circle represents a haplotype and circle size is shown proportional to haplotype frequency. Colours indicate the geographic origin of haplotypes. Median vectors (small black dots) represent missing or not sampled haplotypes. Branch lengths are approximately equal to inferred mutational steps. Haplotype codes according to those are represented in Table S1.

3.4 Phylogeographic relationships with haplotypes outside Western Balkans

Neofusicoccum parvum haplotype networks showed a star-like pattern with 1-3 dominating haplotypes and multiple less frequent haplotypes with mostly short branches and only one or few longer branches (Fig. 4). In the ITS network isolates from Serbia and Montenegro belonged to H9, which was the most common haplotype found in 18 countries and on four continents (Fig. 4a). In the TEF-1-α network, isolates from Serbia and Montenegro represented haplotype H19 and its closest relative was haplotype H13, which was found in isolates from Iran, Kenya

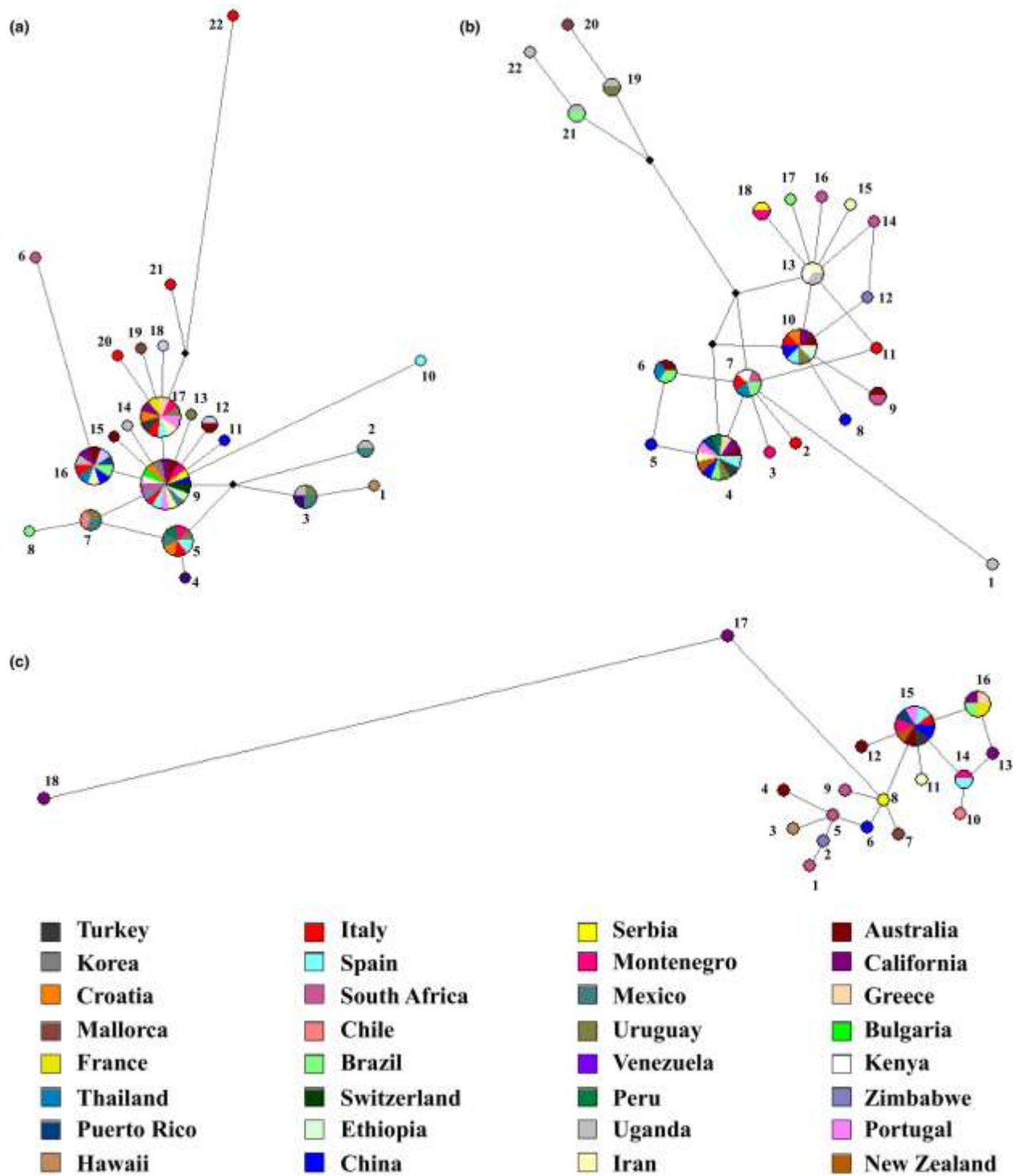**Figure 4.** Median joining networks for the multilocus haplotypes of (a) ITS; (b) TEF-1-α; (c) β-tubulin for *Neofusicoccum parvum*. Each circle represents a haplotype and circle size is shown proportional to haplotype frequency. Colours indicate the geographic origin of haplotypes. Median vectors (small black dots) represent missing or not sampled haplotypes. Branch lengths are approximately equal to inferred mutational steps.

and Uganda (Fig. 4b). In the network based on β-tubulin haplotypes, isolates from Serbia belonged to descendant haplotype H8, which was related to dominant haplotype H15. Isolates from Montenegro belonged to dominant haplotype H15, which was found in nine countries and on four continents and to haplotype H14 that was also found in Spain. Haplotype H14 was in close proximity to haplotype H10 which was found in Chile, and its close relative was H13 that was found in California (Fig. 4c).

There was no apparent geographical structure in the relationships among the *D. sapinea* haplotypes (Fig. 5). With the exception of TEF-1-α network, the *D. sapinea* haplotype networks showed star-like shape with multiple descendant haplotypes arising from the single dominating haplotype. In the ITS network, isolates from Serbia and Montenegro belonged to H5 which was a descendant haplotype and its closest relative was the most frequent haplotype H13, which was found in 21 countries and on five continents. Haplotype H5 was in close proximity to haplotypes H4 and H3 which were found in isolates from Central Europe. In the TEF-1-α network isolates from Serbia and Montenegro belonged to haplotype H5 which was also found in isolates from Iran. The closest relatives of this haplotype were, i.e. haplotype H4 which was found in isolates from the Netherlands, Belgium, Italy and South Africa and haplotype H6 that was found in isolates from the USA. In the β-tubulin network, isolates from Serbia and Montenegro belonged to H3, which was the most frequent and dominant haplotype being found in nine countries and on three continents.

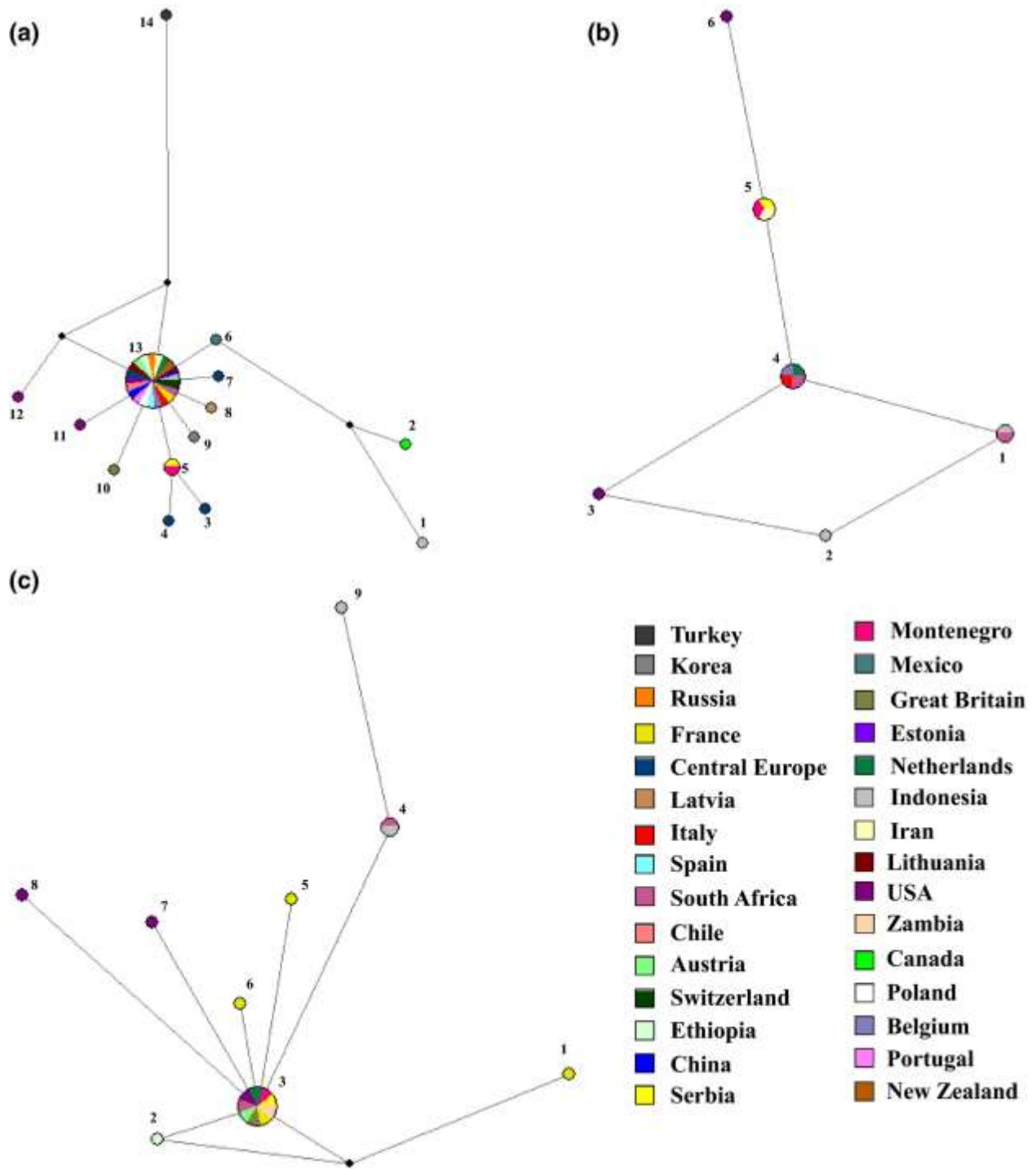**Figure 5.** Median joining network for the multilocus haplotypes of (a) ITS; (b) TEF-1-α; (c) β-tubulin for *Diplodia sapinea*. Each circle represents a haplotype and circle size is shown proportional to haplotype frequency. Colours indicate the geographic origin of haplotypes. Median vectors (small black dots) represent missing or not sampled haplotypes. Branch lengths are approximately equal to inferred mutational steps.

4 Discussion

This study represents the first attempt to determine genetic diversity and structure of *N. parvum* and *D. sapinea* in the Western Balkans. It is also the first study to consider genetic diversity and structure of *N. parvum* in Europe. Microsatellite markers revealed low gene and genotypic diversity for these fungi in the region. The low diversity across the region and on a diversity of trees suggests that *N. parvum* and *D. sapinea* have probably been introduced. Shared genotypes between native and introduced tree species suggested that *N. parvum* and *D. sapinea* can move between them, and this is especially true for *D. sapinea* populations on *Cedrus* spp. and *Pinus* spp. The results also showed that there are shared genotypes between trees in urban areas, pine plantations, forest stands and nurseries. Moreover, multiple genotypes of *D. sapinea* were shown to exist on a single infected tree and interestingly within a single lesion.

The allelic richness, number of private alleles, number of MLGs and percent polymorphic loci reflected a low level of gene and genotypic diversity for *D. sapinea* in the Western Balkans. Nei's (1978) unbiased gene diversity (Hexp) for the geographically and host defined populations for this fungus was low, ranging from 0.072 to 0.09. Similarly, there was low genotypic diversity in all of the populations of *D. sapinea*, ranging from 0.4 to 0.91. Likewise, Burgess et al. (2004) reported only 1.45 genotypic diversity of *D. sapinea* populations collected in forests and plantations in France and Switzerland. These authors also found little diversity in *D. sapinea* populations from North America and Southern Hemisphere. Moreover, Luchi et al. (2014) using DAMD-PCR markers found an almost clonal population of *D. sapinea* in Italy. In contrast, in the study of Bihon et al. (2012) in populations of *D. sapinea* from South Africa, Ethiopia and Argentina the genetic diversity was moderate to high, indicating extensive introductions most likely linked to the trade of living plants or plant tissues.

Low levels of gene and genotypic diversity were found in all populations of *N. parvum* from this study. Gene and genotypic diversity ranged from 0 to 0.084 and 0 to 0.33, respectively. Likewise, Baskarathevan et al. (2012) using UP-PCR markers found low genetic diversity of *N. parvum* from grapevine in Australia, South Africa and California. In contrast, high levels of genetic diversity were detected in populations of *N. parvum* from various hosts in New Zealand, China, Colombia, Hawaii, Australia and South Africa. In these studies, high genetic diversity of *N. parvum* was explained by introductions of multiple genotypes over time and movement of this pathogen between native and non-native hosts (Sakalidis et al., 2013, Pavlic-Zupanc et al., 2015, Mehl et al., 2017a).

The low gene and genotypic diversity of *N. parvum* and *D. sapinea* is not surprising as these fungi are believed to mostly reproduce asexually (Slippers and Wingfield, 2007; Bihon et al., 2012; Mehl et al., 2013; Slippers et al., 2017). Although larger founding populations and multiple introductions from distinct genetic sources can increase genetic diversity of asexual populations, they are typically expected to have low genotypic diversity (Dlugosch and Parker, 2008; Gladieux et al., 2015). In this study, evidence for clonal reproduction was observed in the form of identical MLGs among several isolates, especially those collected from the same tree. Likewise, Bihon et al. (2011) using microsatellite and VCG markers found high genotypic diversity of *D. sapinea* within individual asymptomatic trees in South Africa. Similarly, Pavlic-Zupanc et al. (2015) reported the existence of numerous *N. parvum* isolates with identical multilocus haplotypes in different populations of *N. parvum* in the same country.

Low genetic diversity across the region and on a diversity of trees suggests that *D. sapinea* has probably been introduced to Western Balkans. Low diversity of this pathogen could be explained by founder effects where a reduced number of individuals carrying a fraction of the diversity of the original population, establish a new population in a new area (Dlugosch and Parker, 2008; Barres et al., 2008). Similarly, Burgess at al. (2004) suggested that a single source of the genotype MS1 could explain its predominance and low genetic variation of *D. sapinea* in France and Switzerland. The possibility of introduction of *D. sapinea* to the region is further supported by the star-like topology of the DNA sequence haplotype networks depicted by the *D. sapinea* haplotypes indicating a pattern of large-scale dispersal of this fungal species (Posada and Crandall, 2001). Moreover, isolates of *D. sapinea* from Serbia and Montenegro belonged to dominant haplotypes or their closest relatives shared with many countries and continents.

The lack of structure and low genetic diversity of *N. parvum* indicated that this pathogen has probably been introduced to the region. This is expected considering the wide host range, broad geographical distribution of *N. parvum* haplotypes and the lack of structure amongst a global collection of *N. parvum* isolates (Sakalidis et al., 2013; Slippers et al., 2017; Zlatkovic et al., 2017). The broad geographical distribution of *N. parvum* haplotypes was confirmed in this study and DNA sequence haplotype networks showed a star-like topology with isolates of *N. parvum* from Serbia and Montenegro belonging to dominant haplotypes shared with many countries and continents. Low levels of genetic diversity and the lack of structure have also been found in other Botryosphaeriaceae species that are reported to have been moving globally. For example, Ma et al. (2001) reported a highly clonal population of *B. dothidea* from pistachio in California. Moreover, Marsberg et al. (2017) reported a lack of phylogeographic

structure for the global collection of *B. dothidea* isolates and dominance of identical multilocus haplotypes on distant continents. Mehl et al. (2017b) showed the lack of structure in *L. theobromae* isolates obtained from a large number of hosts and in many countries of the world.

The shared multilocus haplotypes in *N. parvum* and *D. sapinea* from Serbia and Montenegro with many countries and continents suggest assisted dispersal and introduction of these fungi into the region. Spores of these fungi are thought to be predominantly dispersed by wind and rain and are not expected to be naturally spread over large distances, including continents (Swartand Wingfield, 1991; Mehl et al., 2013, 2017b). Therefore, their the introduction into the region could be facilitated by human-associated global trade in plants and plant products (Santini et al., 2013; Wingfield et al., 2015; Burgess et al., 2016). In this regard, the majority of the plant hosts from which isolates of *N. parvum* and *D. sapinea* in this study were obtained are traded globally as ornamentals (e.g. *Chamaecyparis* spp., *Thuja occidentalis*, *Cedrus atlantica*, *Pinus* spp.). Moreover, these fungi are well known as endophytes in plants and endophytic infections of Botryosphaeriaceae are symptomless and would be easily overlooked by phytosanitary systems (Burgess et al., 2016; Crous et al., 2016). Likewise, Burgess et al. (2004) suggested that *D. sapinea* movement across continents is probably assisted by human activities. Sakalidis et al. (2013) concluded that the world-wide dispersal of *N. parvum* is probably due to repeated introductions of plant material into new growing areas. Pavlic-Zupanc et al. (2015) showed that *N. parvum* is a more effective invader in human-disturbed environments, such as plantations, orchards, and urban environments.

Microsatellite data and DNA sequence haplotype networks displayed only slight geographic separation between populations of *N. parvum* and *D. sapinea* from the CR and MR. This result was expected given the wide geographic distribution of these pathogens and increase in global trade (Sakalidis et al., 2013; Santini et al., 2013; Slippers et al., 2017). However, it is in contrast to studies of organisms other than fungi for which almost 3000m high Dinaric Alps have shown to act as an effective natural geographic barrier that causes reduction in gene flow and environmental isolation in natural populations (e.g. Temunović et al., 2012; Lacković et al., 2015). Given the limited number of isolates obtained from the MR, the lack of diversity for *N. parvum* and *D. sapinea* and small number of molecular markers employed for *N. parvum* population in this study, it is possible that genetic diversity was underestimated and additional sampling would be required to draw a valid conclusion.

The similarity in gene frequency and shared genotypes between the *D. sapinea* isolates from *Cedrus* spp. and *Pinus* spp. indicates that the pathogen spreads between these two hosts and between native and non-native trees.

This is not unexpected and a similar pattern has been observed in other Botryosphaeriaceae. For example, Mehl et al. (2017a) found significant gene flow between populations of *N. parvum* on native marula and non-native mango trees in South Africa. The authors suggested that the ability to infect multiple hosts and to migrate amongst them facilitates the establishment and spread of *N. parvum* and other Botryosphaeriaceae in new areas. Moreover, *C. atlantica* trees are often found in close proximity to pine trees in urban areas and could have served as inoculum reservoirs for the infections of *Pinus* spp. and *vice versa* (Zlatković et al., 2017). In addition, because in urban environments *Cedru*s and *Pinus* trees are surrounded by various other known hosts for *D. sapinea*, such as firs, spruces and junipers (Zlatkovic et al., 2017), these conifers could have provided a source of this fungus, but the opposite situation could also have applied.

The results of this study support the suggestion (Slippers and Wingfield, 2007; Wingfield et al., 2015; Burgess and Wingfield, 2017) that plant trade is an important source for the spread of Botryosphaeriaceae species. The international plant health regulations, including Serbian plant health policies rely mostly on visual inspection of plants (Slippers and Wingfield, 2007; Law on Plant Health of the Republic of Serbia, 2009; Santini et al., 2013; Crous et al., 2016). This would allow latent pathogens, such as species in the Botryosphaeriaceae to be imported with asymptomatic plants. The lack of host specificity for *N. parvum* and *D. sapinea* was confirmed, with the same genotypes found on different host species. Multilocus sequencing analyses and microsatellite markers suggest that these fungi were most likely introduced into the Balkan region and together with data from previous studies (Zlatković et al., 2016b, 2017, 2018) this suggests that aggressive genotypes of *N. parvum* and *D. sapinea* have been spreading in the Western Balkans. The present study also emphasizes the need for precautionary measures where plants and plant parts are traded globally.

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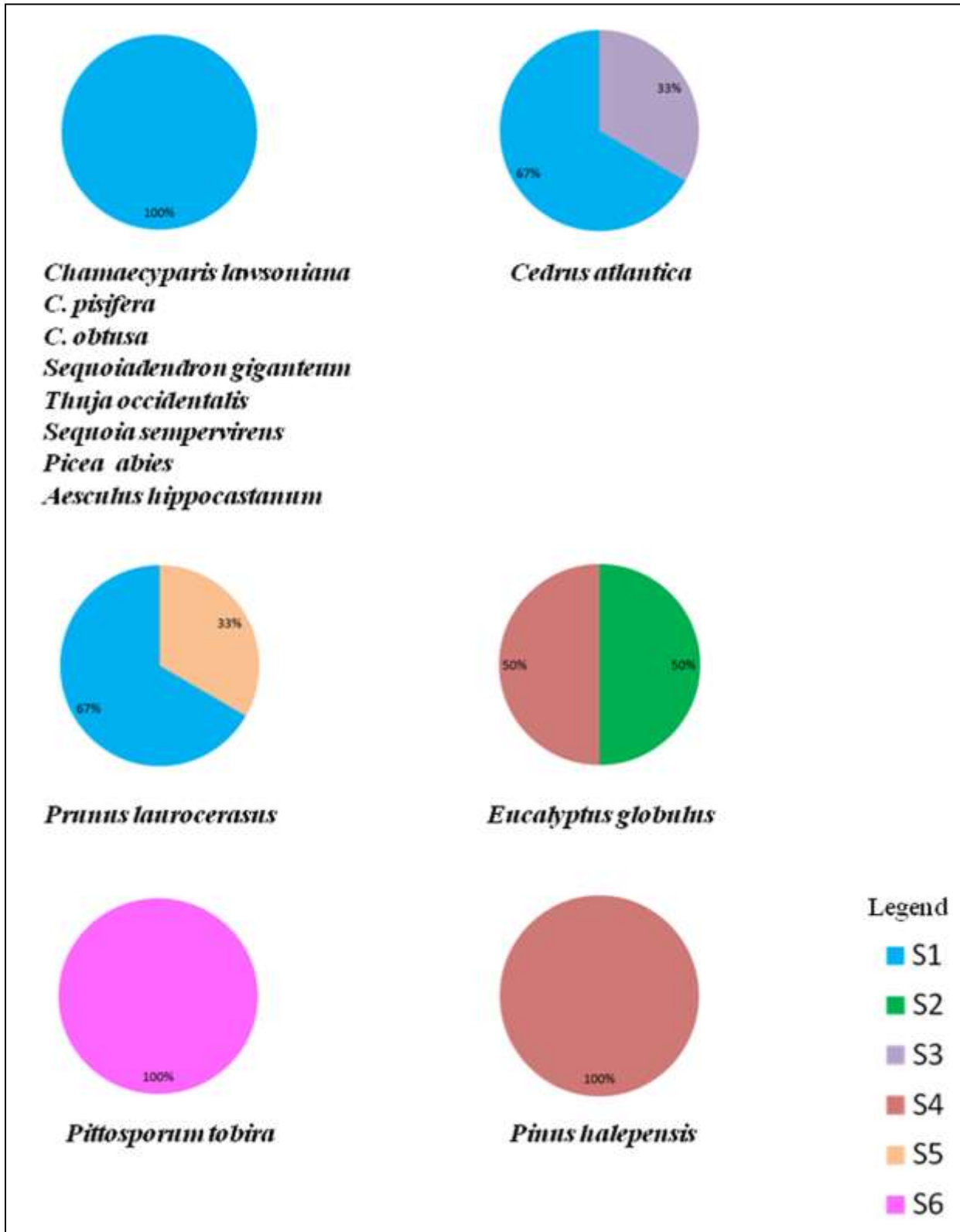**Figure S1.** Multilocus genotypes associated with different hosts of *Neofusicoccum parvum*.
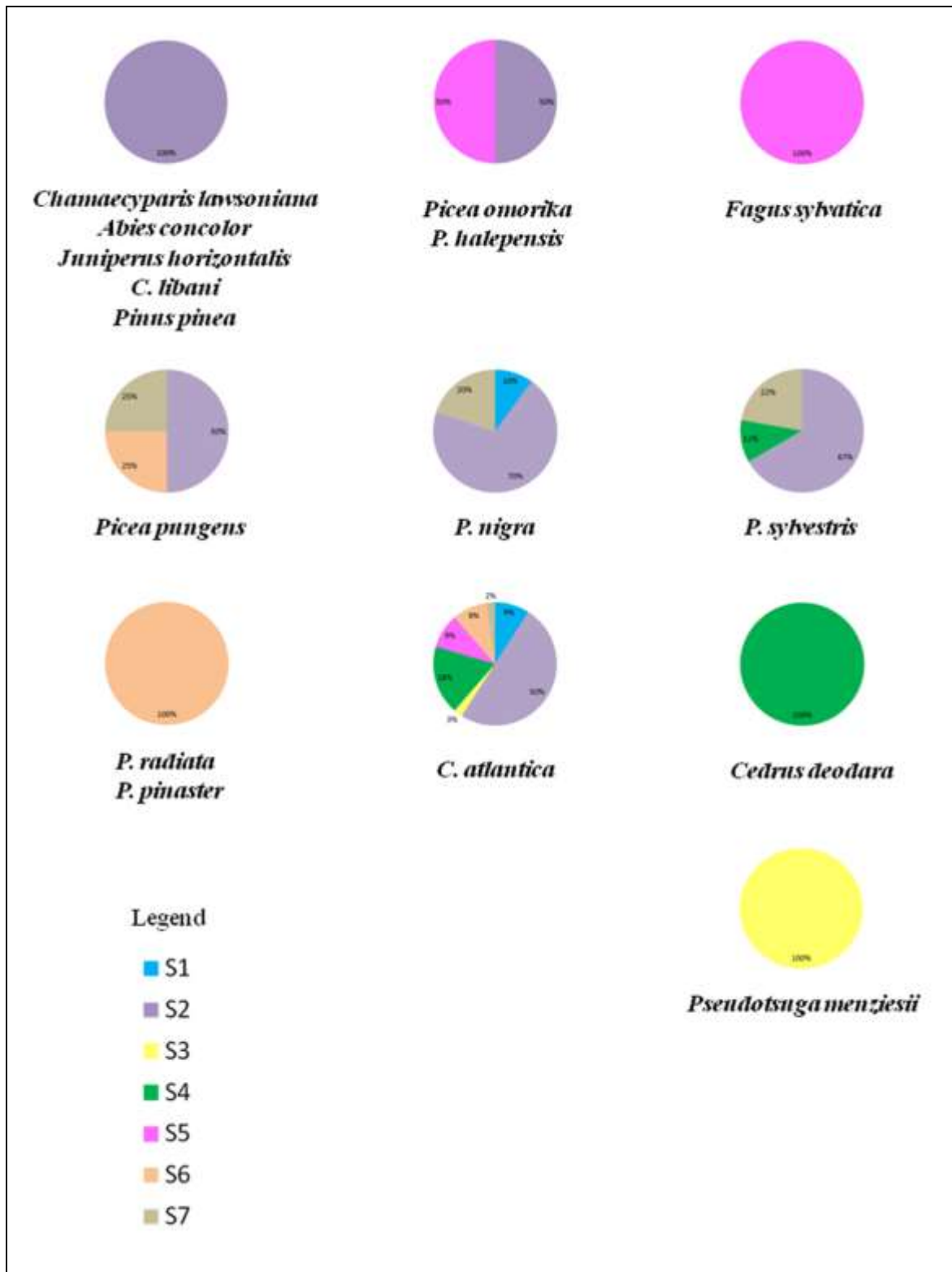
Figure S2. Multilocus genotypes associated with different hosts of *Diplodia sapinea.*
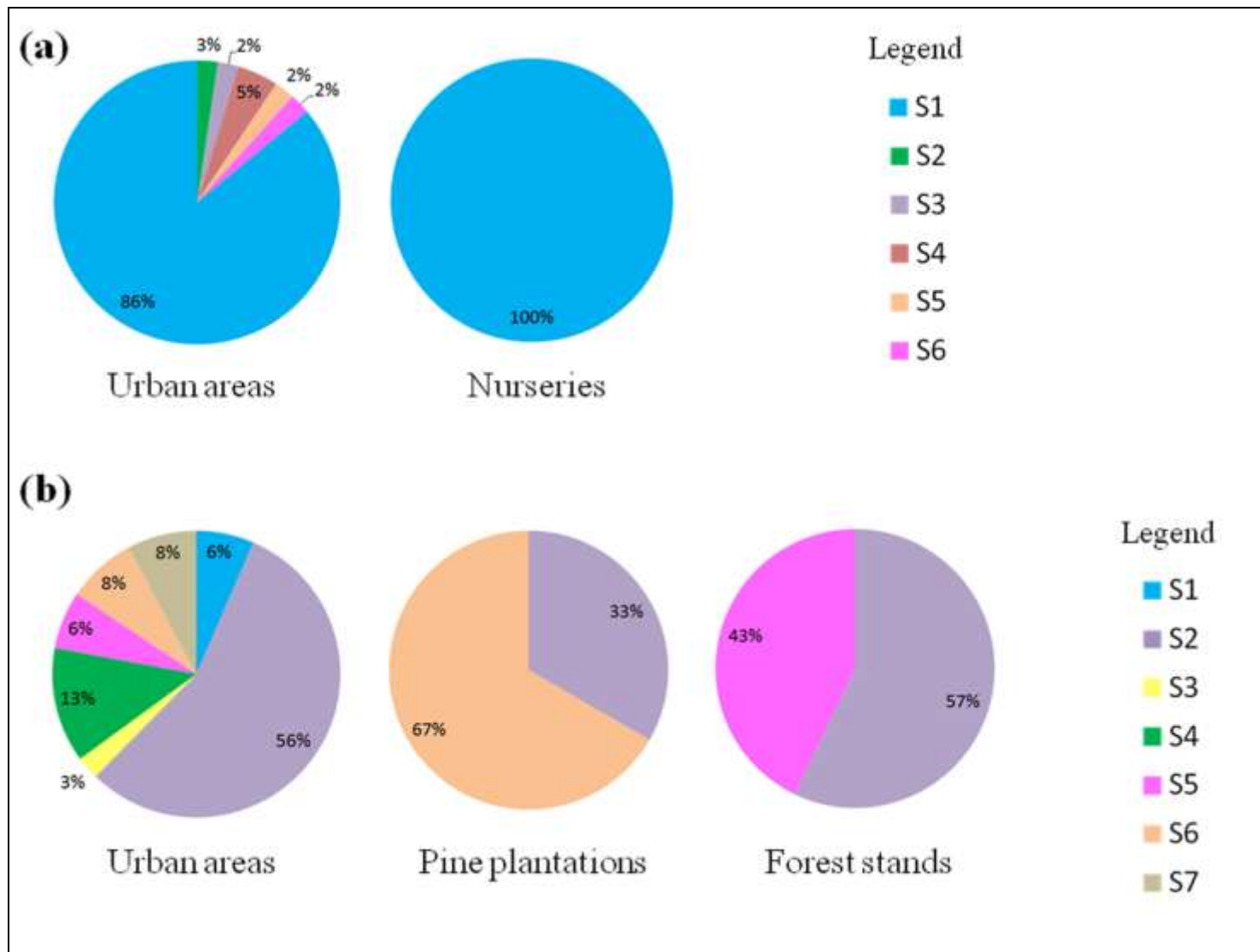
Figure S3. Multilocus genotypes of (a) *Neofusicoccum parvum* associated with hosts in urban areas and nurseries; (b) *Diplodia sapinea* associated with hosts in urban areas, pine plantations and forest stands.

Table S1. Isolates of *Diplodia sapinea* and *Neofusicoccum parvum* analyzed in this study.

Isolate	Identity	Host	Location	Collector	ITS	EF1-α	β-tubulin	LSU	RPB2	Haplotype
							GenBank Accession No.			
CMW 39341	*D. sapinea*	*Cedrus deodara*	Podgorica, Montenegro	M. Zlatković	KF574998	KF575028	KF575094	KF575062	-	H1
CMW 39338	*D. sapinea*	*Cedrus atlantica*	Belgrade, Serbia	M. Zlatković	KF574999	KF575029	KF575095	KF575063	-	H2
CMW 39346	*D. sapinea*	*Picea omorika*	Belgrade, Serbia	M. Zlatković	KF575000	KF575030	KF575096	KF575064	-	H1
CMW 39335	*D. sapinea*	*Pinus halepensis*	Belgrade, Serbia	M. Zlatković	KF729170	KF729404	-	-	-	H1
BOT 229	*D. sapinea*	*Picea pungens*	Belgrade, Serbia	M. Zlatković	KF729171	KF729405	-	-	-	H1
BOT 33	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729172	KF729406	-	-	-	H1
CMW 39330	*D. sapinea*	*Juniperus horizontalis*	Belgrade, Serbia	M. Zlatković	KF729173	KF729407	-	-	-	H1
BOT 204	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729174	KF729408	-	-	-	H1
BOT 157	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729175	KF729409	-	-	-	H1
BOT 60	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729176	KF729410	-	-	-	H1
CMW 39329	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729177	KF729411	-	-	-	H1
BOT 63	*D. sapinea*	*J. horizontalis*	Belgrade, Serbia	M. Zlatković	KF729178	KF729412	-	-	-	H1
BOT 169	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729179	KF729413	-	-	-	H1
BOT 248	*D. sapinea*	*P. pungens*	Belgrade, Serbia	M. Zlatković	KF729180	KF729414	-	-	-	H1
BOT 242	*D. sapinea*	*C. atlantica*	Niš, Serbia	M. Zlatković	KF729181	KF729415	-	-	-	H1
BOT 220	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729182	KF729416	-	-	-	H1
BOT 253	*D. sapinea*	*C. atlantica*	Nikšić, Montenegro	M. Zlatković	KF729183	KF729417	-	-	-	H1
BOT 147	*D. sapinea*	*Pinus nigra*	Deliblato sands, Serbia [1]	D. Karadžić	KF729184	KF729418	-	-	-	H1
BOT 277	*D. sapinea*	*Pinus sylvestris*	Belgrade, Serbia	M. Zlatković	KF729185	KF729419	-	-	-	H1
BOT 268	*D. sapinea*	*P. sylvestris*	Bar, Montenegro	M. Zlatković	KF729186	KF729420	-	-	-	H1
BOT 100	*D. sapinea*	*P. nigra*	Belgrade, Serbia	M. Zlatković	KF729187	KF729421	-	-	-	H1
CMW 39345	*D. sapinea*	*Pinus pinea*	Budva, Montenegro	M. Zlatković	KF729188	KF729422	-	-	-	H1
BOT 212	*D. sapinea*	*Abies concolor*	Belgrade, Serbia	M. Zlatković	KF729189	KF729423	-	-	-	H1
CMW 39344	*D. sapinea*	*P. pungens*	Niš, Serbia	M. Zlatković	KF729190	KF729424	-	-	-	H1
BOT 251	*D. sapinea*	*C. atlantica*	Nikšić, Montenegro	M. Zlatković	KF729191	KF729425	-	-	-	H1
BOT 285	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729192	KF729426	-	-	-	H1
BOT 196	*D. sapinea*	*C. atlantica*	Niš, Serbia	M. Zlatković	KF729193	KF729427	-	-	-	H1
BOT 266	*D. sapinea*	*P. nigra*	Belgrade, Serbia	M. Zlatković	KF729194	KF729428	-	-	-	H1
CMW 39343	*D. sapinea*	*P. sylvestris*	Niš, Serbia	M. Zlatković	KF729195	KF729429	-	-	-	H1
BOT 222	*D. sapinea*	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729196	KF729430	-	-	-	H1
BOT 115	*D. sapinea*	*P. sylvestris*	Niš, Serbia	M. Zlatković	KF729197	KF729431	-	-	-	H1
CMW 44981	*D. sapinea*	*P. omorika*	Mt. Tara, Serbia [2]	D. Karadžić	KF729198	KF729432	-	-	-	H1

Isolate	Identity	Host	Location	Collector	GenBank Accession No.					Haplotype
					ITS	EF1-α	β-tubulin	LSU	RPB2	
CMW 39342	*D. sapinea*	*A. concolor*	Belgrade, Serbia	M. Zlatković	KF729199	KF729433	-	-	-	H1
CMW 39340	*D. sapinea*	*P. omorika*	Mt. Tara, Serbia [2]	D. Karadžić	KF729200	KF729434	-	-	-	H1
BOT 228	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729201	KF729435	-	-	-	H1
BOT 10	*D. sapinea*	*P. nigra*	Mt. Goč, Serbia [2]	D. Karadžić	KF729202	KF729436	-	-	-	H1
BOT 101	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729203	KF729437	-	-	-	H1
BOT 153	*D. sapinea*	*P. nigra*	Mt. Goč., Serbia [2]	D. Karadžić	KF729204	KF729438	-	-	-	H1
CMW 39339	*D. sapinea*	*Pinus pinaster*	Miločer, Montenegro	M. Zlatković	KF729205	KF729439	-	-	-	H1
BOT 104	*D. sapinea*	*P. nigra*	Niš, Serbia	M. Zlatković	KF729206	KF729440	-	-	-	H1
BOT 97	*D. sapinea*	*P. sylvestris*	Belgrade, Serbia	M. Zlatković	KF729207	KF729441	-	-	-	H1
BOT 119	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729208	KF729442	-	-	-	H1
BOT 137	*D. sapinea*	*P. nigra*	Mt. Goč, Serbia [2]	D. Karadžić	KF729209	KF729443	-	-	-	H1
BOT 133	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729210	KF729444	-	-	-	H1
BOT 201	*D. sapinea*	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729211	KF729445	-	-	-	H1
BOT 130	*D. sapinea*	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729212	KF729446	-	-	-	H1
BOT 184	*D. sapinea*	*P. sylvestris*	Bar, Montenegro	M. Zlatković	KF729213	KF729447	-	-	-	H1
BOT 148	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729214	KF729478	-	-	-	H1
BOT 241	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729215	KF729449	-	-	-	H1
BOT 198	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729216	KF729450	-	-	-	H1
BOT 207	*D. sapinea*	*P. halepensis*	Herceg Novi, Monenegro	M. Zlatković	KF729217	KF729451	-	-	-	H1
BOT 194	*D. sapinea*	*P. sylvestris*	Bar, Montenegro	M. Zlatković	KF729218	KF729452	-	-	-	H1
BOT 199	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729219	KF729453	-	-	-	H1
BOT 183	*D. sapinea*	*P. nigra*	Niš, Serbia	M. Zlatković	KF729220	KF729454	-	-	-	H1
BOT 230	*D. sapinea*	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729221	KF729455	-	-	-	H1
BOT 162	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729222	KF729456	-	-	-	H1
BOT 182	*D. sapinea*	*P. nigra*	Mt. Goč, Serbia [2]	D. Karadžić	KF729223	KF729457	-	-	-	H1
BOT 211	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729224	KF729458	-	-	-	H1
BOT 138	*D. sapinea*	*P. pungens*	Belgrade, Serbia	M. Zlatković	KF729225	KF729459	-	-	-	H1
BOT 171	*D. sapinea*	*C. atlantica*	Podgorica, Montenegro	M. Zlatković	KF729226	KF729440	-	-	-	H1
BOT 128	*D. sapinea*	*P. sylvestris*	Belgrade, Serbia	M. Zlatković	KF729227	KF729461	-	-	-	H1
BOT 126	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729228	KF729462	-	-	-	H1
BOT 200	*D. sapinea*	*C. atlantica*	Niš, Serbia	M. Zlatković	KF729229	KF729463	-	-	-	H1
BOT 174	*D. sapinea*	*P. sylvestris*	Budva, Montenegro	M. Zlatković	KF729230	KF729464	-	-	-	H1
BOT 179	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729231	KF729465	-	-	-	H1

Isolate	Identity	Host	Location	Collector	GenBank Accession No.					Haplotype
					ITS	EF1-α	β-tubulin	LSU	RPB2	
BOT 203	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729232	KF729466	-	-	-	H1
CMW 39334	*D. sapinea*	*Pseudotsuga menziesii*	Belgrade, Serbia	M. Zlatković	KF729233	KF729467	-	-	-	H1
BOT 20	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729234	KF729468	-	-	-	H1
CMW 39332	*D. sapinea*	*P. nigra*	Pirot, Serbia	M. Zlatković	KF729235	KF729469	-	-	-	H1
CMW 39331	*D. sapinea*	*Cedrus libani*	Podgorica, Montenegro	M. Zlatković	KF729236	KF729470	-	-	-	H1
BOT 205	*D. sapinea*	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729237	KF729471	-	-	-	H1
BOT 280	*D. sapinea*	*Chamaecyparis lawsoniana*	Herceg Novi, Monenegro	M. Zlatković	KF729238	KF729472	-	-	-	H5
CMW 39333	*D. sapinea*	*C. lawsoniana*	Herceg Novi, Montenegro	M. Zlatković	KF729239	KF729473	-	-	-	H5
BOT 150	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729240	KF729474	-	-	-	H1
BOT 223	*D. sapinea*	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729241	KF729475	-	-	-	H1
BOT 170	*D. sapinea*	*P. omorika*	Belgrade, Serbia	M. Zlatković	KF729242	KF729476	-	-	-	H2
BOT 152	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729243	KF729477	-	-	-	H3
BOT 237	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729244	KF729478	-	-	-	H2
BOT 227	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729245	KF729479	-	-	-	H2
CMW 39337	*D. sapinea*	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729246	KF729480	-	-	-	H2
CMW 39336	*D. sapinea*	*Fagus sylvatica*	Mt. Rudnik, Serbia [2]	N. Keča	KF729247	KF729481	-	-	-	H2
BOT 245	*D. sapinea*	*C. atlantica*	Nikšić, Montenegro	M. Zlatković	KF729248	KF729482	-	-	-	H4
BOT 246	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729249	KF729483	-	-	-	H2
CMW 39347	*D. sapinea*	*Pinus radiata*	Mt. Athos, Greece [1]	D. Karadžić	KT749856	KT749858	-	-	-	H1
BOT 239	*D. sapinea*	*P. radiata*	Mt. Athos, Greece [1]	D. Karadžić	KT749857	KT749859	-	-	-	H1
BOT294	*D. sapinea*	*P. menziesii*	Prokuplje, Serbia [1]	I. Milenković	KT749860	-	-	-	-	H1
BOT295	*D. sapinea*	*P. menziesii*	Prokuplje, Serbia [1]	I. Milenković	KT749861	-	-	-	-	H1
CMW 39328	*N. parvum*	*Pittosporum tobira*	Herceg Novi, Monenegro	M. Zlatković	KF575017	KF575041	KF575113	KF575080	KF729318	H5
CMW 39321	*N. parvum*	*Prunus laurocerasus*	Budva, Montenegro	M. Zlatković	KF575018	KF575042	KF575114	KF575081	KF729319	H3
CMW 39326	*N. parvum*	*Eucalyptus globulus*	Herceg Novi, Montenegro	M. Zlatković	KF575019	KF575043	KF575115	KF575082	KF729320	H4
CMW 39317	*N. parvum*	*E. globulus*	Bar, Montenegro	M. Zlatković	KF575020	KF575044	KF575116	KF575083	KF729321	H1
CMW 39325	*N. parvum*	*Aesculus hippocastanum*	Belgrade, Serbia	I. Milenković	KF575021	KF575045	KF575117	KF575084	KF729322	H1

Isolate	Identity	Host	Location	Collector	GenBank Accession No.					Haplotype
					ITS	EF1-α	β-tubulin	LSU	RPB2	
CMW 39318	*N. parvum*	*C. lawsoniana*	Belgrade, Serbia	N. Keča/ M. Zlatković	KF575022	KF575046	KF575118	KF575085	KF729323	H1
BOT 107	*N. parvum*	*Sequoiadendron giganteum*	Belgrade, Serbia	M. Zlatković	KF729034	KF729364	KF729324	-	KF729277	H1
BOT 3	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729035	KF729365	KF729325	-	KF729278	H1
BOT 15	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729036	KF729366	KF729326	-	KF729279	H1
BOT 49	*N. parvum*	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729037	KF729367	KF729327	-	KF729280	H1
BOT 79	*N. parvum*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729038	KF729368	KF729328	-	KF729281	H1
BOT 6	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	I. Milenković	KF729039	KF729369	KF729329	-	KF729282	H1
BOT 275	*N. parvum*	*P. laurocerasus*	Belgrade, Serbia	M. Zlatković	KF729040	KF729370	KF729330	-	KF729283	H1
CMW 39323	*N. parvum*	*Chamaecyparis obtusa*	Kanjiža, Serbia	N. Keča	KF729041	KF729371	KF729331	-	KF729284	H1
BOT 267	*N. parvum*	*A. hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF729042	KF729372	KF729332	-	KF729285	H1
CMW 39320	*N. parvum*	*Sequoia sempervirens*	Belgrade, Serbia	M. Zlatković	KF729043	KF729373	KF729333	-	KF729286	H1
BOT 87	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729044	KF729374	KF729334	-	KF729287	H1
CMW 39324	*N. parvum*	*Chamaecyparis pisifera*	Belgrade, Serbia [3]	M. Zlatković	KF729045	KF729375	KF729335	-	KF729288	H1
BOT 90	*N. parvum*	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729046	KF729376	KF729336	-	KF729289	H1
CMW 39322	*N. parvum*	*Picea abies*	Niš, Serbia	M. Zlatković	KF729047	KF729377	KF729337	-	KF729290	H1
BOT 43	*N. parvum*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729048	KF729378	KF729338	-	KF729291	H1
BOT 82	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729049	KF729379	KF729339		KF729292	H1
CMW 39327	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729050	KF729380	KF729340	-	KF729293	H1
BOT 42	*N. parvum*	*Thuja occidentalis*	Belgrade, Serbia [3]	M. Zlatković	KF729051	KF729381	KF729341	-	KF729294	H1
BOT 59	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729052	KF729382	KF729342	-	KF729295	H1
BOT 136	*N. parvum*	*C. atlantica*	Herceg Novi, Montenegro	M. Zlatković	KF729053	KF729383	KF729343	-	KF729296	H2
CMW 39319	*N. parvum*	*T. occidentalis*	Belgrade, Serbia [3]	M. Zlatković	KF729054	KF729384	KF729344	-	KF729297	H1
BOT 281	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KF729055	KF729385	KF729345	-	KF729298	H1
BOT 64	*N. parvum*	*S. giganteum*	Kumane, Serbia	N. Keča	KF729056	KF729386	KF729346	-	KF729299	H1
BOT 112	*N. parvum*	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729057	KF729387	KF729347	-	KF729300	H1
BOT 113	*N. parvum*	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729058	KF729388	KF729348	-	KF729301	H1
BOT 214	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729059	KF729389	KF729349		KF729302	H1
BOT 17	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729060	KF729390	KF729350	-	KF729303	H1
BOT 16	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729062	KF729392	KF729352	-	KF729305	H1
CMW 39316	*N. parvum*	*P. halepensis*	Herceg Novi, Montenegro	M. Zlatković	KF729061	KF729391	KF729351	-	KF729304	H1

Isolate	Identity	Host	Location	Collector	ITS	EF1-α	β-tubulin	LSU	RPB2	Haplotype
						GenBank Accession No.				
BOT 160	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729063	KF729393	KF729353	-	KF729306	H1
BOT 26	*N. parvum*	*A. hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF729064	KF729394	KF729354	-	KF729307	H1
BOT 1	*N. parvum*	*S. giganteum*	Kumane, Serbia	N. Keča	KF729065	KF729395	KF729355	-	KF729308	H1
BOT 39	*N. parvum*	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729066	KF729396	KF729356	-	KF729309	H1
BOT 27	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729067	KF729397	KF729357	-	KF729310	H1
BOT 131	*N. parvum*	*P. laurocerasus*	Belgrade, Serbia	M. Zlatković	KF729068	KF729398	KF729358	-	KF729311	H1
BOT 30	*N. parvum*	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729070	KF729399	KF729359	-	KF729313	H1
BOT 8	*N. parvum*	*S. giganteum*	Kumane, Serbia	N. Keča	KF729071	KF729400	KF729360	-	KF729314	H1
BOT 11	*N. parvum*	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729072	KF729401	KF729361	-	KF729315	H1
BOT 286	*N. parvum*	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729073	KF729402	KF729362	-	KF729316	H1
BOT 14	*N. parvum*	*S. giganteum*	Kumane, Serbia	N. Keča	KF729074	KF729403	KF729363	-	KF729317	H1
BOT 13	*N. parvum*	*S. giganteum*	Kumane, Serbia	N. Keča	KF729069	KF767529	KF729484	-	KF729312	H1
CMW 45090	*N. parvum*	*Populus nigra* var. italica	Vršac, Serbia	N. Keča	KT964325	-	-	-	-	H1
CMW 45093	*N. parvum*	*Q. rubra*	Belgrade, Serbia	S. Milanović	KT964326	-	-	-	-	H1
BOT 288	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964327	-	-	-	-	H1
BOT 289	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964328	-	-	-	-	H1
BOT 290	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964329	-	-	-	-	H1
BOT 291	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964332	-	-	-	-	H1
BOT 292	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964330	-	-	-	-	H1
BOT 293	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964333	-	-	-	-	H1
BOT 301	*N. parvum*	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KT964331	-	-	-	-	H1

Culture collections: CMW: FABI, University of Pretoria, South Africa. [1] Plantation. [2] Forest stand. [3] Nursery.

Table S2. Primers and PCR conditions used in this work.

Locus	Primer pair	Primer sequence (5'-3')	No. of cycles	Additional Mg (mM)	Initial denaturation	Annealing temperature	TD
					Neofusicoccum parvum		
BotF11	BOT 11, 12	F: CGGCATGGTCTGCCGCTCC R: GCATCTCCGGCTACCAACCG	30-35	−	95°C for 3 min	60/62/64°C	TD1/TD2
BotF17	BOT 17, 18	F: GGCGCAATCTCGATTCGAGC R: CCACGATGTCCGTTCATCG	35-40	−/ 2.5	95°C for 3 min	54/60°C	TD1
BotF18	BOT 19, 20	F: GGCGGTCGCAGATGCGGTC R: GCCCTATTCTGCGTGCCTCC	30-40	−	95°C for 3 min	64°C	TD1/TD2/ TD3
BotF23	BOT 23, 24	F: CATCGCACAGGAGCCGATTCT R: CATACATCGAGCTTTCTTGAGGG	40	−	95°C for 3 min	54°C for 45 s	−
BotF35	BOT 35, 36	F: CTCCATCCTGATCCAGGGTCC R: GACGAATCAAGCGGGCTGCCC	40	−	95°C for 3 min	60/62°C	−
					Diplodia sapinea		
SS1	TB 1, 2-2	F: CAT GCA TCG ATC CTG TAG AGC R: CCA AGT GAT GAC CCT ATA GAG	38-40	1/2	95°C for 3 min/ 95°C for 5 min (TD1)	58°C	TD1
SS2	TB 5, 6	F: TGT GGT GAG AGA CTA CTG GAC R: CGC TCA TTT GCT GGA ACT TGG	30	−/ 1	95°C for 3 min	52°C	−
SS7	TB 23, 24	F: GAC AGA CAT CTA GGC CCT GC R: GAT CAG TCG GTC GAG ACG AG	30-35	−/ 1	95°C for 3 min	60°C	−
SS8	TB 35-2, 36	F: CCA CGA ATA ACG CCC CCA CC R: GCA TGG CAT CAG TGT CTG GC	30	−	95°C for 3 min	62°C	−
SS10	TB 41, 42	F: GCC AAC CCT AAT GCT TCC ATG R: CAG CGG CGA TTG CGG TAT GG	40	−/ 1	95°C for 3 min	−	TD1
SS11	TB 43, 44	F: GTA ACA TTT CCC CAC GTC AGC R: GGA AGT ACT ACA TGG TCT TCG	35	1	95°C for 3 min	50°C	−
SS13	WB 2a, b	F: GGC GTG TGT GAT GAG ATG AG R: GTC CTT TGT GTG TTG GGT TG	35-40	1/2	95°C for 3 min	55°C	−
SS14	WB 4a, b	F: CAC CAC CAC CAA CAC CTT G R: CGT GTT GGA AGC GAC GAC	30-40	−/ 1/1.5	95°C for 3 min	52°C/55°C	−
SS15	WB 7a, b	F: GAA TCA CTG GCC GGT TTG R: GAG TCC AGC CTT TCC TCC TC	30-40	−/ 1-3	95°C for 3-6 min	55°C	−
SS16	WB 8a, b	F: GGG GAA AAG ACG TGT TGT TGT R: CAG CAT CGT CGT CCC ATT AG	40	−/ 3	95°C for 3/5 min	−	TD1

TD=Touch Down thermal cycling programs (Don et al. 1991): TD1- the first 10 cycles: annealing temperature of 65°C with a decrease of 1°C per cycle followed by 25 to 30 cycles of 55°C; TD2: the first 20 cycles: annealing temperature of 65°C with a decrease of 1°C every second cycle followed by 15-20 cycles of 55°C; TD3: the first 12 cycles: annealing temperature of 68°C with a decrease of 1°C every second cycle followed by 28 cycles of 62°C.

Materials and methods- Molecular cloning

In the microsatellite data analyses of *N. parvum* two alleles of similar size were constantly present at locus BotF18 and the given locus was thus cloned to obtain single allele sequences. PCR products were first purified using Sephadex G-50 columns (Sigma, Steinheim, Germany) following the manufacturer's instructions. Purified PCR products were then cloned into the vector pGEM-T-Easy and transformed into *Escherichia coli* JM109 Competent Cells by using the pGEM-T-Easy Vector System II (Promega, Madison, USA) cloning kit, following the manufacturer's instructions. Several positive clones were verified by colony PCR and sequencing using T6 and SP7 primer set (Invitrogen, Life technologies, Johannesburg, SA). Colony PCR reaction mixture and amplification conditions were similar to those described in Liu et al. (2015). The sequencing PCR mixture and sequencing conditions were similar to those described by Begoude et al. (2010). The products were purified with Sephadex and separated with an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems). Sequencing was done in both directions, with the same primers used for the colony PCR reactions.

Single allele sequences showed that bigger allele had additional 12 bp DNA fragment that corresponded to the 3' end of the forward primer sequence and was thus considered to represent an allele-like artefact generated by PCR. The higher peak was therefore recognized as a true allele and included in the subsequent analyses (Table 1).

Table S3. Isolates of *Diplodia sapinea* and *Neofusicoccum parvum* retrieved from GenBank and used for haplotype network construction.

Isolate	Identity	Host	Location	ITS	EF1-α	β-tubulin
					GenBank	
UCR 1566	*N. parvum*	*Ficus microcarpa*	USA, California	JN543669	−	JQ080552
PD 17	*N. parvum*	*Prunus dulcis*	USA, California	GU251143	GU251275	GU251803
1L 83	*N. parvum*	*Juglans regia*	USA, California	KF778854	KF779044	KF778949
CDFA B139	*N. parvum*	*Vaccinium corymbosum*	USA, California	KJ126850	KJ126847	−
PD 106	*N. parvum*	*Prunus dulcis*	USA, California	GU251139	GU251271	GU251799
PD 39	*N. parvum*	*Prunus dulcis*	USA, California	GU251144	GU251276	GU251804
UCR 735	*N. parvum*	*Prunus americana*	USA, California	HQ529766	−	HQ529735
UCR 295	*N. parvum*	*P. americana*	USA, California	HQ529765	−	HQ529734
CMW 9081	*N. parvum*	*Populus nigra*	New Zealand	AY236943	AY236888	AY236917
CMW 9077	*N. parvum*	*Actinidia deliciosa*	New Zealand	AY236939	AY236884	AY236913
CMW 994	*N. parvum*	*Malus sylvestris*	New Zealand	AF243395	AY236883	AY236912
CMM 1845	*N. parvum*	*Mangifera indica*	Brasil	KC507814	KC507811	KC507808
CMM 1291	*N. parvum*	*M. indica*	Brasil	JX513633	JX513613	KC794029
N 46	*N. parvum*	*Psidium guajava*	Brasil	KC621070	KM349776	KC621058
CMM 3944	*N. parvum*	*M. indica*	Brasil	JX513636	JX513616	KC794028
BV 23	*N. parvum*	*Vitis vinifera*	Brasil	JX521862	−	−
CMM 1846	*N. parvum*	*M. indica*	Brasil	KC507812	KC507809	KC507806
PD 62	*N. parvum*	*Pistacia vera*	Greece	GU251133	GU251265	GU251793
2E 29	*N. parvum*	*P. vera*	Greece	KF955752	KF955851	KF955950
CMW 1130	*N. parvum*	*Sequoiadendron giganteum*	South Africa	AY236945	AY236890	AY236919
CMW 26714	*N. parvum*	*Terminalia catappa*	South Africa	FJ900610	FJ900656	FJ900637
CMW 10122	*N. parvum*	*Eucalyptus grandis*	South Africa	AF283681	AY236882	AY236911
PD 251	*N. parvum*	*Eucalyptus* sp.	South Africa	GU251124	GU251256	GU251784
CPC 23297	*N. parvum*	*M. indica*	South Africa	KJ193657	KJ193701	−
A 4	*N. parvum*	-	Peru	FJ528596	FJ528597	−
SCCDJF 01S	*N. parvum*	*V. vinifera*	China	JX275796	JX462299	JX462272
AH-3-1-01s	*N. parvum*	*V. vinifera*	China	−	JX462283	JX462257
R1	*N. parvum*	*Rhododendron decorum*	China	KJ657709	−	−
CMW 27135	*N. parvum*	*Eucalyptus dunnii*	China	HQ332205	HQ332221	−
MPT 1	*N. parvum*	*Vitis heyneana*	China	KJ599627	KM921768	−
SDAU 08-55	*N. parvum*	*Populus* sp.	China	FJ214103	FJ238524	GU997687
SDAU 07-16	*N. parvum*	*Populus* sp.	China	GU997688	FJ238526	FJ238525

Isolate	Identity	Host	Location	GenBank		

				ITS	EF1-α	β-tubulin
NF-37	*N. parvum*	*M. indica*	Italy	N814442	JN814485	−
BA 20	*N. parvum*	*Quercus robur*	Italy	HQ893535	HQ893537	−
NF-10	*N. parvum*	*M. indica*	Italy	JN814454	JN814497	−
PVFnP 28	*N. parvum*	*V. vinifera*	Italy	GU188007	GU188037	−
NF-62	*N. parvum*	*M. indica*	Italy	JN814450	JN814493	−
NF-82	*N. parvum*	*M. indica*	Italy	JN814456	JN814499	−
F 49	*N. parvum*	*Quercus suber*	Italy	DQ487157	DQ487158	−
B 93	*N. parvum*	*V. vinifera*	Italy	KM675764	KM822736	−
NF-5	*N. parvum*	*M. indica*	Italy	JN814430	JN814473	−
DB 05112010	*N. parvum*	*Rhododendon* sp.	Italy	HQ589259	−	−
N 12	*N. parvum*	*V. vinifera*	Italy	KJ946457	KJ946459	KJ946461
UCD 577S	*N. parvum*	*V. vinifera*	Spain	DQ356358	GU294747	DQ356365
CMW 10089	*N. parvum*	*Eucalyptus* sp.	Ethiopia	AY210477	AY210483	−
5B 602	*N. parvum*	*Juglans regia*	Spain	KF778863	KF779053	KF778958
BN 66	*N. parvum*	*Eryobotrya japonica*	Spain	KT240279	KT240253	−
F 49	*N. parvum*	*Quercus suber*	Spain	DQ487158	DQ487157	−
EFA 183	*N. parvum*	*V. vinifera*	Spain	JQ974953	JQ974954	−
6.1	*N. parvum*	*Vaccinium* sp.	Spain	KC556960	KC556961	−
KC 15	*N. parvum*	*M. indica*	Puerto Rico	KC631662	KC631658	KC631654
Npa 1	*N. parvum*	*Prunus dulcis*	Mallorca (Spain)	JF330779	−	JN191296
MUCC 673	*N. parvum*	*Eucalyptus globulus*	Australia	EU339553	EU339520	EU339483
CMW 9071	*N. parvum*	*Ribes* sp.	Australia	AY236938	AY236880	AY236909
MUCC 124	*N. parvum*	*E. dunnii*	Australia	EU339544	EU339518	EU339481
CMW 6235	*N. parvum*	*Tibouchina lepidota*	Australia	AY615136	AY615128	AY615120
WAC 13381	*N. parvum*	*Araucaria heterophylla*	Australia	HM545147	HM545143	HM545151
MUCC 211	*N. parvum*	*Corymbia torreliana*	Australia	EU301017	EU339517	EU339480
IRN 1	*N. parvum*	*V. vinifera*	Iran	GU121891	GU121863	GU121836
IRAN 1528C	*N. parvum*	*Juglans regia*	Iran	JQ772031	JQ772068	−
IRAN 1535C	*N. parvum*	*Salix* sp.	Iran	JQ772045	JQ772082	−
CJA 8	*N. parvum*	*J. regia*	Iran	JQ772040	JQ772077	−
CJA 56	*N.parvum*	*Actinidia deliciosa*	Iran	JQ772036	JQ772073	−
CBS 110301	*N. parvum*	*V. vinifera*	Portugal	AY259098	AY573221	EU673095
CAA 126	*N. parvum*	*Juniperus communis*	Portugal	JX878535	−	−

Isolate	Identity	Host	Location	ITS	GenBank EF1-α	β-tubulin
NpSV	*N. parvum*	*V. vinifera*	France	KP190147	KP190149	KP190148
SO 334	*N. parvum*	*V. vinifera*	Croatia	KF296318	KF296319	−
HPP 121	*N. parvum*	*V. vinifera*	Croatia	KF923329	KF923331	−
HPP 110	*N. parvum*	*V. vinifera*	Croatia	KF923328	KF923330	−
APEC 1210	*N. parvum*	*Juglans sinensis*	Korea	KC818612	−	−
CRM 36	*N. parvum*	*Vaccinium corymbosum*	Mexico	JQ647905	−	−
CPONa 3	*N. parvum*	*M. indica*	Mexico	JQ619648	−	−
CIAD0 2111	*N. parvum*	*Persea Americana*	Mexico	JN203129	−	−
CRM 152	*N. parvum*	*V. corymbosum*	Mexico	JQ647912	−	−
FM	*N. parvum*	*E. globulus*	Mexico	KC479185	−	−
i53	*N. parvum*	*V. vinifera*	Turkey	KJ921842	KP721665	KP721703
MBA i27AG	*N. parvum*	*V. vinifera*	Turkey	KF182330	−	−
3852	*N. parvum*	*Solanum melongena*	Bulgaria	KT966746	−	−
A4B	*N. parvum*	*Eucalyptus* sp.	Uganda	−	GU064944	−
830	*N. parvum*	*Eucalyptus* sp.	Uganda	JQ772545	JQ982036	−
P3	*N. parvum*	*Eucalyptus* sp.	Uganda	JQ981975	JQ982027	−
MT8B	*N. parvum*	*Eucalyptus* sp.	Uganda	−	GU130536	−
20819	*N. parvum*	*Grevillea robusta*	Uganda	GQ922509	−	−
P301	*N. parvum*	*Eucalyptus* sp.	Uganda	JQ981987	JQ982028	−
822	*N. parvum*	*Eucalyptus* sp.	Uganda	JQ772544	JQ982021	−
EG36	*N. parvum*	*Eucalyptus grandis*	Kenia	FJ904816	FJ904894	−
GRF42	*N. parvum*	*G. robusta*	Kenia	−	FJ904895	−
CMW 25468	*N. parvum*	*G. robusta*	Kenia	−	FJ904895	−
WASWS 1394	*N. parvum*	*Platanus acerifolia*	Switzerland	KR978445	−	−
CMW 38724	*N. parvum*	*Eucalyptus* sp.	Zimbabwe	KF923244	KF923277	KF923265
CPC 22752	*N. parvum*	*Prunus cerasoides*	Thailand	KM006430	KM006461	−
CPC 22757	*N. parvum*	*Eucalyptus obliqua*	Thailand	KM006435	KM006466	−
CMW 7885	*N. parvum*	*Eucalyptus* sp.	Hawaii (USA)	AY236944	AY236889	AY236918
CMW 13350	*N. parvum*	*Psidium guajava*	Venezuela	EF118036	−	−
CMW 13555	*N. parvum*	*Eucalyptus urophylla*	Venezuela	EF118035	−	−
B 168	*N. parvum*	*Malus* sp.	Uruguay	KJ499744	KJ499657	−
UY 231	*N. parvum*	*Eucalyptus* sp.	Uruguay	EU080917	EU863164	−

Isolate	Identity	Host	Location	ITS	GenBank	
					EF1-α	β-tubulin
B 171	*N. parvum*	-	Uruguay	KJ499745	KJ499658	−
UY 37	*N. parvum*	-	Uruguay	EU080909	EU863161	−
V 2	*N. parvum*	*V. vinifera*	Uruguay	JX271830	−	−
VID 1560	*N. parvum*	*V. vinifera*	Chile	KM870226	−	KP762485
DP 001	*D. sapinea*	*Pseudotsuga menziesii*	Turkey	KF372874	−	−
CBS 393.84	*D. sapinea*	*Pinus nigra*	Netherlands	DQ458895	DQ458880	DQ458863
CBS 109727	*D. sapinea*	*Pinus radiata*	South Africa	DQ458897	DQ458882	DQ458865
CBS 109725	*D. sapinea*	*Pinus patula*	South Africa	DQ458896	DQ458881	DQ458864
CMW 4898	*D. sapinea*	*Pinus greggii*	South Africa	AY253293	−	−
CMW 190	*D. sapinea*	*Pinus banksiana*	USA	KF766159	AY624251	AY624256
CMW 8745	*D. sapinea*	*P. menziesii*	USA	EU220435	−	EU220471
PD 23	*D. sapinea*	*Malus* sp.	USA	GU251110	GU251242	GU251770
1031	*D. sapinea*	*P. nigra*	USA	AY156719	−	−
BOT 275	*D. sapinea*	*P. patula*	Indonesia	AF283689	−	−
CMW 4876	*D. sapinea*	*P. patula*	Indonesia	AY253294	AY624252	AY624257
CBS109943	*D. sapinea*	*P. patula*	Indonesia	DQ458898	DQ458883	DQ458866
CMW 14656	*D. sapinea*	*Pinus merkusii*	Indonesia	EU220447	−	EU220483
DpEST 1	*D. sapinea*	*P. nigra*	Estonia	EU330229	−	−
WA 19144	*D. sapinea*	-	Poland	JX981458	−	−
IRAN 2208c	*D. sapinea*	*Pinus* sp.	Iran	KF890210	KF890192	−
Dp 1992	*D. sapinea*	*P. sylvestris*	Russia	KJ401036	−	−
CMW 11250	*D. sapinea*	*P. patula*	Ethiopia	AY244402	−	−
CMW 11252	*D. sapinea*	*P. patula*	Ethiopia	AY244403	−	−
910843	*D. sapinea*	-	Canada	AF110814	−	−
CAP 166	*D. sapinea*	*Olea europaea*	Italy	EU392284	EU392261	−
11Do37	*D. sapinea*	-	Korea	KF717040	−	−
CJK 2	*D. sapinea*	*P. sylvestris*	Austria	JX431883	−	JX431879
ZP 31	*D. sapinea*	*P. sylvestris*	China	HQ845048	−	−
VL 150	*D. sapinea*	*Pinus mugo*	Lithuania	JF440618	−	−
CI-63	*D. sapinea*	*P. radiata*	Chile	EF506938	−	−
CAA 025	*D. sapinea*	*Thuja plicata*	Portugal	JX878530	−	−
NZFS 2992	*D. sapinea*	*P. radiata*	New Zealand	JQ922534	−	−
CMW 12513	*D. sapinea*	*Larix* sp.	France	EU22044	−	EU220477
Isolate	Identity	Host	Location		GenBank	

				ITS	EF1-α	β-tubulin
BEI 06	*D. sapinea*	*V. vinifera*	France	KT595692		KT595693
BEI 39	*D. sapinea*	*V. vinifera*	France	KT954169	−	KT954170
CMW 13234	*D. sapinea*	*Cedrus deodara*	France	EU220440	−	EU220476
CMW 8750	*D. sapinea*	*P. menziesii*	Great Britain	EU220436	−	EU220472
KUP 5.2.1.2	*D. sapinea*	*Pinus contorta*	Latvia	KP698189	−	−
CAP 339	*D. sapinea*	*Pinus* sp.	Belgium	GQ923875	GQ923843	−
CMW 30129	*D. sapinea*	*Pinus oocarpa*	Zambia	−	−	FJ858720
5 F	*D. sapinea*	-	Central Europe	GQ336491	−	−
BR 4	*D. sapinea*	-	Central Europe	GQ336502	−	−
2 B	*D. sapinea*	-	Central Europe	GQ336489	−	−
BL 1	*D. sapinea*	-	Central Europe	GQ336496	−	−
DIP-15	*D. sapinea*	*Dryocoetes autographus*	Spain	DQ674377	−	−
UASWS 1111	*D. sapinea*	*Pinus* sp.	Switzerland	KM280038	−	−

Culture collections: CMW: FABI, University of Pretoria, South Africa. [1] Pine plantation. [2] Forest stand. [3] Nursery.

Table S4. Overlap of multilocus genotypes (MLGs) associated with different hosts of *Neofusicoccum parvum* and *Diplodia sapinea*

N. parvum	Ng	Ni	Ns	N_h	LSD	*D. sapinea*	Ng	Ni	Ns	N_h	LSD
Host						Host					
Chamaecyparis lawsoniana	1	4	1	2		*C. lawsoniana*	1	2	1	5	A [1]
Cedrus atlantica	2	3	1	2		*C. atlantica*	7	42	7	15	B
Sequoiadendron giganteum	1	21	1	2		*Abies concolor*	1	2	1	5	A
Thuja occidentalis	1	2	1	2		*P. halepensis*	2	2	2	5	A
Pinus halepensis	1	1	1	1		*Juniperus horizontalis*	1	2	1	5	A
Sequoia sempervirens	1	1	1	2		*P. nigra*	3	10	3	5	A
C. pisifera	1	1	1	2		*P. sylvestris*	3	9	3	6	A
C. obtusa	1	1	1	2		*C. deodara*	1	1	1	2	A
Picea abies	1	1	1	2		*C. libani*	1	1	1	5	A
Aesculus hippocastanum	1	5	1	2		*P. pungens*	3	4	3	6	A
Prunus laurocerasus	2	3	1	2		*P. omorika*	2	4	2	6	A
Pittosporum tobira	1	1	0	0		*P. radiata*	1	2	1	2	A
Eucalyptus globulus	2	2	1	1		*P. pinea*	1	1	1	5	A
						P. pinaster	1	1	1	2	A
						Fagus sylvatica	1	1	1	2	A
						Pseudotsuga menziesii	1	1	1	1	A
Urban areas	6	43	1			Urban areas	7	75	3		
Nurseries	1	3	1			Pine plantations	2	3	2		
						Forest stands	2	7	2		
MLGs						MLGs					
S1					A [2]	S1					A [2]
S2					B	S2					B
S3					B	S3					A
S4					B	S4					A
S5					B	S5					A
S6					B	S6					A
						S7					A

Ng-number of MLGs, Ni-number of isolates, Ns-number of MLGs shared with other hosts/environments, N_h- number of other hosts that harbour the same MLG. [1] Hosts with the same letter did not differ significantly in the number of MLGs shared with other hosts using the LSD test at α= 0.05, [2] MLGs with the same letter did not differ significantly in the number of hosts they were shared with. *Neofusicoccum parvum* hosts did not differ significantly in the number of MLGs shared with other hosts using the LSD test at α= 0.05. The effects of shared MLGs between urban areas and nurseries for *N. parvum* and urban areas, pine plantations and forest stands for *D. sapinea* were not statistically analysed due to the differences in sample sizes.

Table S5. Multiple *D. sapinea* genotypes coexisting in the same host tree (A), in the same tree part/lesion (B).

Tree species (A)	Isolate	Genotype	Tree species (B)	Isolate	Genotype	Tree part/lesion
	BOT 98, BOT 33, BOT 227	S2		BOT 227	S2	necrotic stem
	BOT 101, BOT 148	S5		BOT 101, BOT 148	S5	
	BOT 150	S1		BOT 150	S1	
	BOT 157, BOT 133	S2	*C. atlantica*	BOT 187	S4	resinous stem
	BOT 197	S4		BOT 201	S6	
Cedrus atlantica	BOT 187	S4		BOT 20	S1	resinous branch
	BOT 201	S6		BOT 203	S2	
	BOT 20	S1				
	BOT 203	S2		BOT 128, BOT 97	S2	pycnidia on cones
			P. sylvestris	BOT 277	S7	
	BOT 128, BOT 97	S2		BOT 115	S2	pycnidia at the base of diseased needles
	BOT 277	S7		BOT 95	S7	
Pinus sylvestris	BOT 115	S2				
	BOT 95	S7				
P. nigra	BOT 266, BOT 199	S2				
	BOT 104	S7				