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Low genetic diversity and strong geographic structure in introduced populations of the *Eucalyptus* foliar pathogen *Teratosphaeria destructans*

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Abstract

The aggressive *Eucalyptus* leaf pathogen, *Teratosphaeria destructans*, causes widespread damage in tropical and subtropical *Eucalyptus*-growing regions of Indonesia, China, Thailand, East Timor, Vietnam, Lao, and South Africa. Little is known regarding the origin, pathways of dispersal, or reproductive biology of this pathogen. The aim of this study was to investigate the genetic structure of a global collection of *T. destructans* isolates. This was achieved by developing and using polymorphic microsatellite markers. Low genotypic diversity and a limited number of private alleles were found in all investigated populations, with the highest maximum diversity of 10.7% in isolates from South Sumatra. This supports the hypothesis that *T. destructans* was introduced to these regions. High levels of clonality were common in all populations, especially in isolates sampled from the recent disease outbreak in South Africa, which were all identical. The global collection of isolates grouped into three distinct clusters, corresponding largely to their sampled regions. Low levels of genotypic diversity, high levels of clonality, and strong geographic structure suggest independent introductions into all the sampled areas from an unknown source. The results imply that strict biosecurity measures are needed to avoid introductions of additional genotypes in these areas.

Keywords

asexual reproduction, foliar pathogen, microsatellite markers, population genetics

1 Introduction

Worldwide, disease and insect pests pose increasing threats to forestry based on non-native *Eucalyptus* species (Paine *et al*., 2011). The growing need for *Eucalyptus*-derived products is accompanied by a rising demand for genetically superior planting stock, which in turn drives frequent movement of plant germplasm between countries (Burgess and Wingfield, 2017). In many cases, this process results in the accidental co-introduction of *Eucalyptus* pathogens (Burgess and Wingfield, 2017).

One of the most important pathogens to emerge and threaten *Eucalyptus* plantation forestry in recent years is the fungus *Teratosphaeria destructans* (Dothideomycetes: Teratosphaeriaceae). This pathogen causes severe shoot and leaf blight, which leads to defoliation and die-back of newly planted *Eucalyptus* trees cultivated outside of their native environment (Wingfield *et al*., 1996; Burgess *et al*., 2006). Asexual pycnidia of *T. destructans* form below *Eucalyptus* stomata under moist conditions and exude black cirri containing conidia (Wingfield *et al*., 1996). *Teratosphaeria* species do not have animal vectors (Aylward *et al*., 2019) and natural spore dispersal is probably limited to rain splash.

T. destructans was first discovered and described from northern Sumatra (Indonesia) in 1995 (Wingfield *et al*., 1996). It subsequently spread rapidly to other tropical and subtropical *Eucalyptus*-growing regions in South-East Asia, most probably with contaminated seed (Andjic *et al*., 2019). It was reported in Thailand in 1999 (Old *et al*., 2003), East Timor and Vietnam in 2002 (Old *et al*., 2003), in all Chinese *Eucalyptus*-growing regions in 2006 (Burgess *et al*., 2006; Dell *et al*., 2008; Burgess and Wingfield, 2017), and Lao in 2009 (Barber *et al*., 2012). In 2015, *T. destructans* was discovered causing serious damage to *Eucalyptus* plantations in South Africa (Greyling *et al*., 2016). This was the first case of the pathogen occurring outside South-East Asia.

The origin of *T. destructans* remains unknown, but is expected to be either Australia or East Timor (Andjic *et al*., 2019). The majority of *Eucalyptus* species are native to Australia, but after a six-year survey (Andjic *et al*., 2016), the presence of *T. destructans* in Australia remains unconfirmed. This is not unusual, with several species of *Teratosphaeria* causing leaf diseases of *Eucalyptus* first being discovered in non-native plantations before they were observed in Australia (Burgess and Wingfield, 2017). *T. destructans* has been reported on *E. urophylla* in East Timor (Old *et al*., 2003), where this tree is native (Ladiges *et al*., 2003), leading to the alternative view that this could be the area of origin of the pathogen (Andjic *et al*., 2019). However, it has not been possible to test this hypothesis, because cultures of the fungus are not currently available from that country.

T. destructans has a heterothallic mating system (Havenga *et al*., 2020), thus requiring two individuals of opposite mating type for sexual reproduction (Billiard *et al*., 2011). Both mating types of the pathogen occur in populations from Indonesia, China, and Thailand. Even so, only the asexual state of *T. destructans* has ever been found (Wingfield *et al*., 1996; Burgess *et al*., 2006).

All populations of *T. destructans* from South-East Asia have a single dominant mating type per population and the South African population only has one mating type (Havenga *et al*., 2020). Andjic *et al*. (2011) considered the population diversity of a collection of *T. destructans* isolates from various South-East Asian countries and found a low level of genetic diversity. However, their study was conducted with only two microsatellite markers and undertaken before the pathogen had spread outside South-East Asia.

The aim of this study was to investigate the genetic diversity and potential for recombination in a global collection of *T. destructans*. This was achieved using polymorphic microsatellite markers developed from recently generated whole genome sequences for the

pathogen (Wingfield *et al*., 2018; Havenga *et al*., 2020). More specifically, we sought to (a) assess the genotypic richness and diversity of six distinct populations from South-East Asia and South Africa; (b) determine the relatedness of individual isolates within and among populations; and (c) assess the genetic structuring of isolates in the global collection. These results enabled us to draw conclusions on the ability of the pathogen to reproduce sexually as well as its potential origin and pathways of dispersal between populations.

2 Materials and methods

2.1 Fungal isolates

For population analyses, 195 *T*. *destructans* isolates, representing six populations from five different countries, were sourced from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria (Figure 1; Table S1). A single isolate was obtained per tree, resulting in isolates from 195 trees. The collection included 33 isolates from five locations in North Sumatra, 25 isolates from a single location in South Sumatra, 62 isolates from three locations in South Africa, 43 isolates from a single location in the Zhanjiang region of China, 29 isolates from a single location in Thailand, and 3 isolates from Vietnam (Figure 1; Table S1). Isolates were cultured on malt extract agar (MEA; Merck) amended with 3 g yeast extract (MEA + Y; Oxoid) at 25°C. DNA was extracted following the protocol described by Havenga *et al*. (2020).

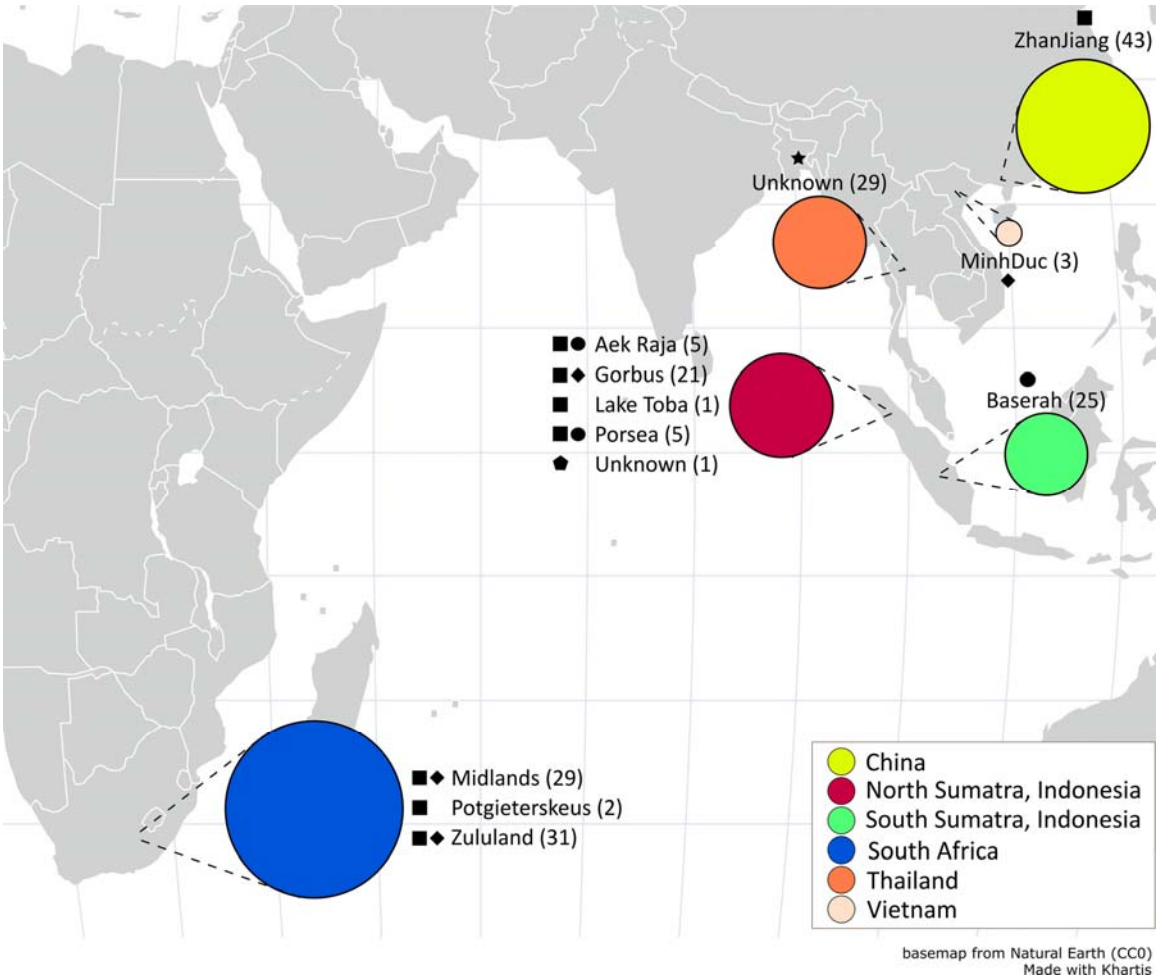

Figure 1 Sampling localities of *Eucalyptus* host species in South-East Asia and South Africa. Six *Teratosphaeria destructans* populations were collected. The size of the pie chart is proportional to the sample size. The location name, number of individuals (in brackets), and host species (symbols) are indicated next to each pie chart. Host species sampled were *Eucalyptus* sp. (⬟), *E. camaldulensis* (★), *E. grandis* (■), *E. grandis* × *E. pellita* (■●), *E. grandis* × *E. urophylla* (■◆), *E. pellita* (●), and *E. urophylla* (◆).

2.2 Polymorphic microsatellite loci for *T. destructans*

The sequenced *T. destructans* genomes of two Indonesian isolates CMW45661 (WBMM00000000; Havenga *et al*., 2020) and CMW45982 (WBMN00000000; Havenga *et al*., 2020), as well as the South African isolate CMW44962 (RIBY01000000; Wingfield *et al*., 2018) were used to select polymorphic microsatellites.

Microsatellites were mined from the genome of isolate CMW45661 using the Microsatellite Search and Building Database (MSDB; Du *et al*., 2013). The search for microsatellites was set to the default minimum motif repeat units (9 mono-, 7 di-, 5 tri-, 4 tetra-, 4 penta- and 4 hexanucleotide) and 500 bp upstream and downstream flanking sequence. Microsatellites and flanking sequences were extracted from MSDB and concatenated in Excel 2010 (Microsoft). Candidate microsatellite markers were mapped to each *T. destructans* genome in Geneious R11 (Biomatters Ltd) using the "mapped to reference" function.

The candidate microsatellite markers were filtered by discarding those that failed to map to the genomes and those situated within a gene or at the edge of a scaffold. Loci comprising unidentified nucleotides (NNN) in the microsatellite repeat or flanking sequences as well as markers with overlapping sequences were also discarded. Selections were made such that a single marker was present per scaffold for all genomes. Only microsatellite markers that displayed polymorphism in all three genomes were selected.

2.3 Primer selection, PCR amplification, and fragment analysis

Each of the putative microsatellite markers obtained from the genomes was aligned with MAFFT v. 7.388 (Katoh and Standley, 2013) using the L-INS-I method in Geneious. The alignments were used to identify conserved flanking regions in each microsatellite locus and primers were designed in these areas with PRIMER 3 v. 2.3.7 (Rozen and Skaletsky, 2000). Each primer was selected based on the following criteria: 18–24 bp primer size, 150–500 bp product size, 55%–60% GC content, 58–63°C melting temperature and, where possible, less than two A or T nucleotides in the last five 5′ and 3′ bases of the primer.

The specific targeted region for the remaining 30 candidate markers was confirmed by amplifying and sequencing them using DNA from the genome of the South African isolate

CMW44962. In a total reaction volume of 20 µl, the PCR mixture contained 150 ng DNA, 10 µl *Taq* DNA Polymerase Master Mix RED (Ampliqon), and 0.4 pmol/µl of each primer. Reaction conditions consisted of an initial denaturation step at 94°C for 5 min; followed by 35 cycles of 30 s at 94°C, 30 s at 56°C or 58°C (depending on the primer used), and 45 s at 72°C; and a final extension step at 72°C for 10 min. PCR products were sequenced at the Central Analytical Facility (CAF) at Stellenbosch University, South Africa.

Markers that successfully amplified target regions in isolate CMW44962 were further tested using DNA from three additional *T. destructans* isolates, CMW13349 (Thailand), CMW45649 (South Sumatra), and CMW47566 (China), by amplifying and sequencing all loci as described above. Microsatellite markers were divided into two panels for fragment analyses according to the optimal annealing temperatures and either the forward or reverse primer of each pair was labelled with an ABI fluorescent dye (Thermo Fisher Scientific) (Table 1).

Microsatellite markers were amplified for the test isolates in two multiplex PCRs (Table 1) using the *Taq* DNA Polymerase Master Mix RED and the PCR conditions described above. In a total reaction volume of 25 µl, the PCR contained 20 ng DNA, 12 µl Ampliqon master mix, 1 mM $MgCl_2$, and variable concentrations of primers (Table 1). The amplified products were resolved on a 96-capillary Applied Biosystems 3730xl DNA Analyser at CAF using a GeneScan 500 LIZ size standard (Applied Biosystems).

After successful amplification of the developed microsatellite markers in all test isolates, multiplex PCRs were performed with 12 microsatellite markers on a global collection of *T. destructans* isolates (Figure 1; Table S1) as described above. Each 96-well PCR plate contained a negative and positive (genome isolate CMW44962) control. Data scoring and allele calling were performed in Geneious.

Table 1. Polymorphic microsatellite markers and panel designs for *Teratosphaeria destructans*

Multiplex PCR	Locus	Motif	Primers	Primer sequence (5'-3')	Genbank accession	Fluorescent tag	Primer concentration (nm)	Amplicon size (bp)
Panel 1	A	TGG	Td7F	ATGGCAAGGCATCATCGGAT	MN991194	6-FAM	15	170-230
Ta = 56°C			Td7R	GCATTTGCGCTGTTTCCTCA		-	15	
	B	TGG	Td27F	CAGTGGCTTCGAAATCCGCT	MN991185	-	20	360-400
			Td27R	CCTCGGTACCTGTACCTGACTA		6-FAM	20	
	C	TG	Td3F	GCAATGGTGATGGTTCTCGC	MN991196	VIC	15	200-245
			Td3R	TCTCTCACCCGAGCTGATCA		-	15	
	D	CAC	Td24F	CCACCCAGTCCAATAACC	MN991187	-	20	280-310
			Td24R	TCTCCCCGTGACCCTATGAG		NED	20	
	E	TCC	Td5F	ACTCCTCYATCACCCTGACA	MN991195	PET	20	300-320
			Td5R	AGTAGCGCAGTGTTTCAGCT		-	20	
Panel 2	F	ACT	Td10F	TGCAGCGTATAGGAGGCATG	MN991192	6-FAM	20	300-340
Ta = 58°C			Td10R	GAGGCTGGCGTTTCACATTG		-	20	
	G	TG	Td9F	TGTCCATCTCCGTTCACTGC	MN991193	-	20	400-450
			Td9R	GTGTCTTCCGGTCTTCACGT		6-FAM	20	
	H	CCA	Td20F	CATCATCGGCAATCGGGAGA	MN991189	VIC	20	300-340
			Td20R	GTCTGCCTCATCATCGCTCA		-	20	
	I	ATC	Td23F	ATGCATCCTCTTGAGCCCAC	MN991188	-	20	420-460
			Td23R	TATGACTCTCGGGTGGCAGA		VIC	20	
	J	AG	Td14F	CCATGAGGAGTCGTTCGGTT	MN991190	-	20	360-390
			Td14R	AGCTACTACCGCCGCTACTA		NED	20	
	K	TCA	Td11F	AATCCTTCGAGGCGAGACAC	MN991191	NED	20	410-450
			Td11R	TTTCTGACCTCGTGCTCACC		-	20	
	L	ATC	Td25F	TTGGCAGACCCTGACCATC	MN991186	PET	20	390-410
			Td25R	TGTCGTGGTCAATGGGTACG		-	20	

2.4 Statistical analyses

2.4.1 Data preparation and verification

The full data set of all individuals and a clone-corrected data set, comprising only one of each unique multilocus genotype (MLG) per population, was prepared in GenAlEx 6v. .5.1 (Peakall and Smouse, 2006). Statistical analyses, unless otherwise stated, were conducted in R v. 3.6.0 (R Core Team, 2013) using the package POPPR v. 2.8.3 (Kamvar *et al*., 2014).

To verify that the 12 microsatellite loci are not linked, inherited independently, and could be analysed together, pairwise linkage disequilibrium for all loci was calculated for the clone-corrected data set. The index of association (I_A; Brown, 1980) and $\overline{r}_d$, a modified version that accounts for sample size (Agapow and Burt, 2001), was calculated. Each index was compared to a normal distribution of itself calculated for 999 simulated random data sets.

To assess whether the microsatellite loci applied in this study had sufficient variability to differentiate between unique individuals, a genotype accumulation curve was created. This curve plots the number of MLGs against the number of loci. A curve reaching a plateau indicates that the loci used would accurately represent the maximum MLGs present in the data set.

2.4.2 Genetic diversity

For each locus, the number of observed alleles, genetic diversity, and evenness were determined using the full data set. The genetic diversity of each locus was explored with Nei's unbiased expected heterozygosity (H_{exp}; Nei, 1978), which measures the frequency of alleles in the population. Evenness, calculated by Pielous' *E*.5 index (Pielou, 1975), measures how evenly alleles are distributed for each locus.

The number of unique MLGs, as well as the observed and expected frequency of each MLG, detected in the full data set and each population, was determined. The hypothesis that identical genotypes in a population arose independently through recombination was tested using MLGsim v. 2.0 (Stenberg *et al*., 2003). *P* values for testing the significance of P_{sex} statistics were calculated based on a simulation of 10,000 random permutations to determine significance at $p < .05$.

The expected MLG (eMLG) considers sample size and is equivalent to the genotypic richness of a population (Grünwald *et al*., 2003). The eMLG was calculated using the required minimum sample size of 10 individuals per population and estimated using a rarefaction curve. The isolates from Vietnam were therefore excluded from these calculations due to the small sample size (Figure 1; Table S1).

Three different indices were calculated to represent genotypic diversity: (a) Shannon–Wiener index (*H*; Shannon, 2001), (b) Stoddart and Taylor's index (*G*; Stoddard and Taylor, 1988), and (c) Simpson's corrected λ estimation ($\lambda \times N/[N - 1]$; Simpson, 1949). Unlike the *H* index, the *G* index incorporates sample size, which provides a better representation of population diversity. The percentage genotypic diversity was calculated using the *G* index results (McDonald *et al*., 1994). The relevant abundance of each MLG per population was calculated with *E*.5 and the number of private alleles (N_p) per population was calculated in GenAlEx. The null hypothesis of random recombination was tested for both the full and clone-corrected data set by calculating $\bar{r}_d$ with 999 permutations.

2.4.3 Population structure

To assess the relatedness of individuals across the different populations, a minimum spanning network (MSN) of all MLGs was constructed based on Bruvo's distance (Bruvo *et al*., 2004). Analysis of molecular variance (AMOVA), calculated in GenAlEx, was used to assess the

extent of population structure. STRUCTURE v. 2.3.4 (Pritchard *et al*., 2000; Falush *et al*., 2003) was subsequently used to assign individuals to clusters (*K*) according to their allele frequency using a Bayesian, model-based method. Twenty independent iterations of $K = 1–10$ were performed using the admixture model, with 50,000 burn-in iterations followed by 500,000 Markov chain Monte Carlo (MCMC) iterations. The optimal number of clusters was determined by calculating $L(K)$ and ΔK using the online CLUMPAK (Clustering Markov Packager Across K) platform (Kopelman *et al*., 2015) with best *K* function. Clustering was conducted in CLUMPAK using the calculated optimal number of clusters.

Population structure was further examined using the discriminant analysis of principal components (DAPC) in the Adegenet v. 2.0.0 package (Jombart, 2008). DAPC uses model selection and consecutive *K*-means to assume clusters among individuals. This approach maximizes between-population differences, while minimizing within-population differences, without presumptions of linkage disequilibrium or Hardy–Weinberg equilibrium (Jombart *et al*., 2010). The optimal number of clusters was calculated using the Bayesian information criteria (BIC) and compared to the optimal *K* value obtained from CLUMPAK. The optimal number of principal components (PCs) to retain was determined by running both the α-score and cross-validation tests. Scatter plots were created in Adegenet with the optimal *K* value and retained PCs.

3 Results

3.1 Identifying polymorphic microsatellite loci

Analysis of the genome of South Sumatra isolate CMW45661 using MSDB software identified a total of 3,396 microsatellite regions. The microsatellite density of this genome

was estimated at one region for every 8 kb. Tri- (2,045 regions), mono- (515 regions), and di-nucleotide (335 regions) repeats were the most abundant. Comparing all repeat regions, other than the mononucleotide repeats, for the remaining *T. destructans* genomes (isolates CMW44962 and CMW45982) revealed that only di- (19 regions) and trinucleotide (78 regions) repeats were polymorphic across all three genomes.

Further elimination based on the described marker and primer criteria yielded eight di- and 22 tri-nucleotide regions suitable for primer design. Twelve of the 30 potential markers amplified their specific target region in four *T. destructans* isolates and were confirmed as polymorphic. These markers were subsequently used to assess the genotypes of 195 *T. destructans* isolates from six populations. The linkage disequilibrium test revealed significant pairwise linkage for loci F and I at $p < .01$ (data not shown) and these loci were excluded from all downstream analyses. No missing data were present in the data set.

A total of 25 alleles were amplified across the 10 loci in the studied *T. destructans* isolates. There were between two and four alleles per locus (Table 2). Locus D had the most even distribution of alleles ($E.5 = 0.99$), with its most common allele present in all populations of *T. destructans* and its second allele present in the Chinese and Indonesian (South and North Sumatra; Table 2) populations. Locus K had the greatest number of observed alleles ($n = 4$), was the most diverse ($H_{exp} = 0.64$), and had a relatively even distribution of alleles ($E.5 = 0.82$; Table 2). Loci A, C, and E had the lowest diversity (0.11–0.12) and showed the most uneven allele distribution (0.49–0.51; Table 2), due to one allele at each locus occurring at low frequency.

Table 2. Diversity indices of ten *Teratosphaeria destructans* microsatellite markers

Locus	Observed alleles	Hexp[a]	E.5[b]
A	2.00	0.12	0.51
B	2.00	0.43	0.88
C	2.00	0.11	0.49
D	2.00	0.49	0.99
E	2.00	0.12	0.50
G	3.00	0.42	0.67
H	3.00	0.33	0.67
J	3.00	0.60	0.86
K	4.00	0.64	0.82
L	2.00	0.24	0.63
Mean	2.50	0.35	0.70

[a] Gene diversity (Nei, 1978); Hexp = (n/n – 1)[1 – Σpi2].

[b] Pielous' evenness index (Pielou, 1975); *E.5* = (*H'*/ log*S*).

3.2 Genetic diversity

Eighteen unique MLGs occurred in the six *T. destructans* populations and 11 of these occurred more than once (Figure 2). Only MLG9 occurred in multiple populations, comprising two individuals from North Sumatra, 24 from Thailand, and all individuals from South Africa and Vietnam (Figure 2). The North Sumatra population had the highest number of MLGs (nine) followed by those from South Sumatra (four), China (three), and Thailand (three) (Figure 2; Table 3).

Table 3 Diversity indices and assessment of random recombination calculated for *Teratosphaeria destructans* populations

Population	N[b]	MLG[cd]	eMLG ± SE[e]	H[f]	G[g]	Corrected λ[h]	$\hat{G}$ (%)[i]	$E.5$[j]	N_p[k]	Before clone correction[l]		After clone correction[l]	
										$\bar{r}_d$[m]	p	$\bar{r}_d$[m]	p
China	43	3	2.41 ± 0.61	0.30	1.15	0.14	2.67	0.44	3	0.661	0.001	0.280	0.076
Indonesia, North Sumatra	33	9	7.72 ± 0.91	1.49	2.76	0.66	8.36	0.51	1	0.239	0.001	−0.031	0.796
Indonesia, South Sumatra	25	4	4.00 ± 0.00	1.12	2.68	0.65	10.72	0.81	4	0.449	0.001	−0.014	0.646
South Africa	62	1	1.00 ± 0.00	0.00	1.00	0.00	1.61	N/A	0	—	—	—	—
Thailand	29	3	2.86 ± 0.35	0.55	1.42	0.31	4.90	0.58	0	-0.065	1.000	−0.500	1.00
Vietnam[a]	3	1	—	—	—	—	—	—	0	—	—	—	—
Total	195	21	7.33 ± 1.44	1.79	3.69	0.73	1.92	0.54	8	0.286	0.002	0.152	0.001

[a]Vietnam had three individuals and was excluded from diversity calculations.

[b]Number of isolates analysed.

[c]Number of multilocus genotypes (MLG) observed.

[d]Number of isolates analysed after clone correction.

[e]Number of expected multilocus genotypes (eMLG) and standard error at the smallest sample size of 25 individuals based on rarefaction.

[f]Shannon–Wiener index of MLG diversity (Shannon, 2001); $H' = -\Sigma(p_i)\,(\log_2 p_i)$.

[g]Stoddard and Taylor's index of MLG diversity (Stoddard and Taylor, 1988); $G = 1/\Sigma[f_x(x/n)^2]$.

[h]Corrected Simpson's index (Simpson, 1949); corrected $\lambda = (\lambda \times N/(N-1))$.

[i]Percentage genotypic diversity (McDonald *et al*., 1994); $\hat{G} = G/N \times 100$.

[j]Evenness, *E*.5 index (Pielou, 1975); $E.5 = (H'/\log S)$.

[k]Number of private alleles.

[l]Only populations with more than one MLG.

[m]Modified version of index of association I_A (Agapow and Burt, 2001).

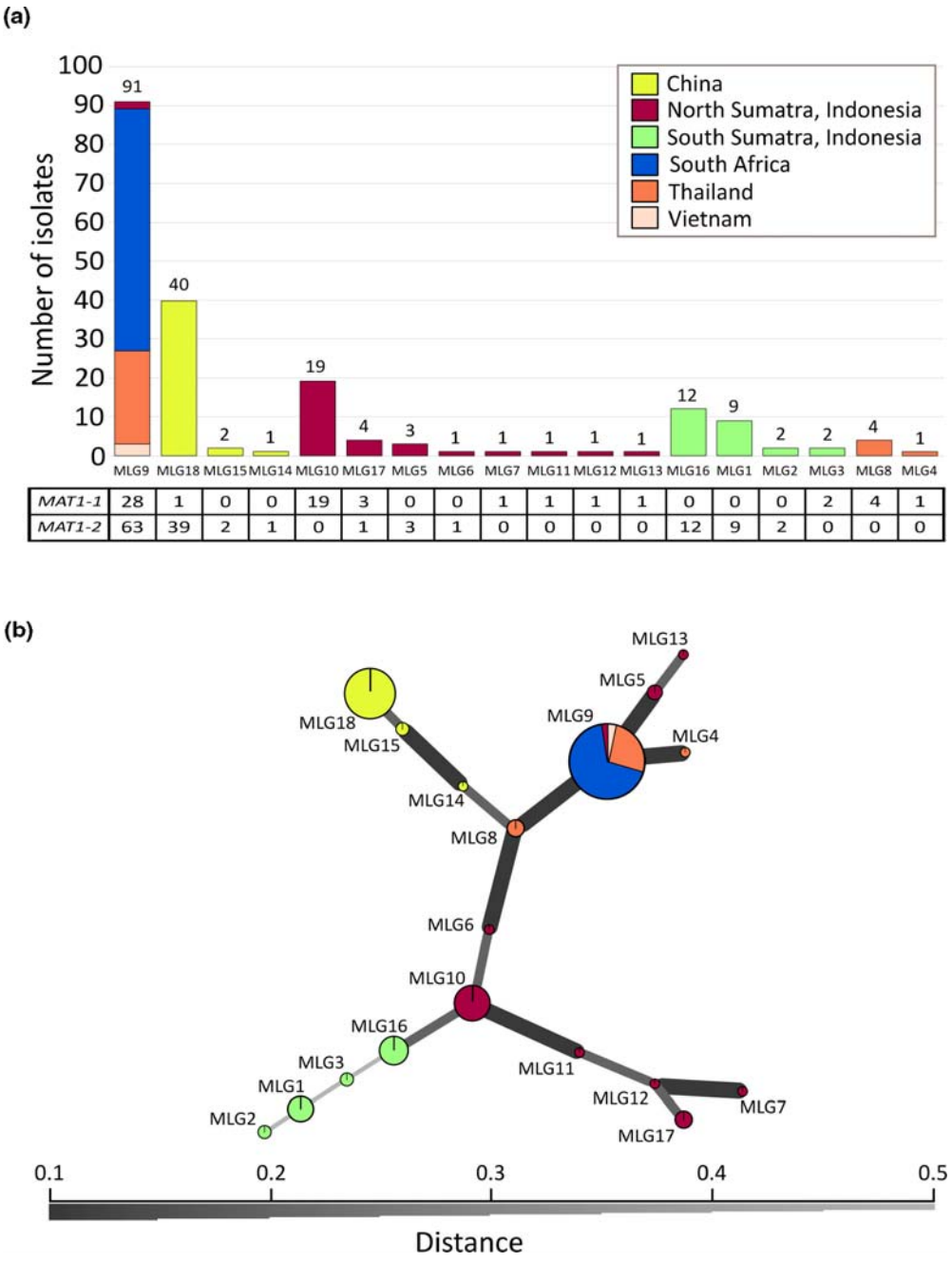

Figure 2 Multilocus genotypes (MLGs) found across six populations of *Teratosphaeria destructans*. (a) Eighteen genotypes identified in the full data set; the number of individuals belonging to each genotype is indicated above each bar and the mating type distribution below the graph. (b) Population structure on a minimum spanning network (MSN) calculated with Bruvo's genetic distance (Bruvo *et al*., 2004). Each node represents a single MLG (identity indicated next to node), consisting of 10 microsatellite markers, and the node size is proportional to sample size. Branch thickness and shading between genotypes represent genetic distance with branch thickness decreasing with increasing genetic distance.

The genotype accumulation curve estimated that the set of 10 *T. destructans* microsatellite markers were sufficient to detect 100% of the MLGs in the full data set (Figure S1). However, three genotypes (MLG9, MLG17, and MLG18) were associated with both *MAT1-1* and *MAT1-2* individuals (Figure 2a). All South African isolates and a single isolate from Thailand had the MLG9 genotype and the *MAT1-2* idiomorph (Table S1). The remaining MLG9 isolates from Thailand, Vietnam, and North Sumatra had the *MAT1-1* idiomorph (Table S1). Four North Sumatra isolates had the MLG17 genotype, of which three isolates were *MAT1-1* and one *MAT1-2* (Figure 2a). A single Chinese MLG18 isolate had a *MAT1-1* idiomorph, whereas the remaining MLG18 isolates were *MAT1-2* (Figure 2a). Havenga *et al*. (2020) sequenced the conserved *MAT* gene regions of the MLG17, MLG18, and Thai MLG9 outlier isolates and confirmed their identity. These results imply that MLG9, MLG17, and MLG18 could represent distinct genotypes.

Eight genotypes, including the three associated with different mating types, had P_{sex} values rejecting the hypothesis that they arose independently from sexual reproduction (Table S2). The hypothesis was supported ($p > .05$) for the remaining three genotypes. However, each of these genotypes was limited to a single sampling site and represented a single mating type (Figure 2a).

The genotypic richness of a population is proportional to its expected eMLGs. The sample size of the smallest population (South Sumatra; $N = 25$) was used as the minimum for calculating the eMLGs in each population (Figure S2). The North Sumatra population had the greatest genotypic richness (7.72) followed by those from South Sumatra (4.00), Thailand (2.86), China (2.41), and South Africa (1.00) (Figure S2; Table 3). The North Sumatra population also had the highest genotypic diversity according to the *H* (1.49), *G* (2.76), and corrected λ (0.66) indices (Table 3). Both North Sumatra sites, sampled in 1996 and 2000, from which only single isolates were available, had the common MLG9 genotype. The

remaining MLGs were unique to their sampling location within North Sumatra. The evenness of the North Sumatra population was relatively low ($E.5 = 0.51$), reflecting one or a few dominant MLGs, in this case MLG10 (Figure 2; Table 3).

The South Sumatra population had the most evenly distributed alleles ($E.5 = 0.81$). This population also had the second-highest overall genotypic richness and diversity and the highest percentage genotypic diversity $\hat{G}$ (10.72%; Table 3). The Chinese population had low levels of genotypic richness, diversity, and evenness (Table 3), due to the dominance of MLG18 (Figure 2). The South Africa population consisted of a single eMLG (Figure 2) and therefore had no genotypic richness or diversity ($G = 1$; $H = 0$; corrected $\lambda = 0$) (Table 3). Private alleles were identified only in the Indonesian and Chinese populations (Table 3). South Sumatra had the greatest number of private alleles ($N_p = 4$), followed by China ($N_p = 3$) and North Sumatra ($N_p = 1$). The private alleles occurred in three of the MLGs found in South Sumatra and China, respectively, and in five of the North Sumatra MLGs. When viewed collectively, the two Indonesian populations shared an additional three private alleles that occurred in four of their respective MLGs.

For the global population, the hypothesis of random mating was rejected for both the full and clone-corrected data sets at $p = .01$, indicating clonal reproduction (Table 3). For all individual populations, the null hypothesis was supported only after clone correction (Table 3). Thailand was the only population for which the null hypothesis was supported both before and after clone correction (Table 3), but the population consisted of only three isolates. For all populations, the clone-corrected data set represented only 10.3% of the full data set. A high level of clonality was found for all populations and all isolates from South Africa represented a single clone.

3.3 Population structure

The MSN (Figure 2) showed that genotypes were generally separated according to sampling areas. This was with the exception of the dominant MLG9 that was present in four of the populations. MLGs from the same populations grouped together and few interconnections were observed between genotypes occurring in different populations. The AMOVA revealed high molecular variation among populations (77%) compared to low variation within populations (23%) (Table 4). In North Sumatra, high molecular variation (79%) was also identified among the Aek Raja, Porsea, and Gorbus sampling sites (Table 4).

Table 4 Analysis of molecular variance (AMOVA) for the full data set of six *Teratosphaeria destructans* populations

	df	**Sum of squares**	**Mean squares**	**Estimate of variance**	**Total variation (%)**	***p***
Among populations	5	249.972	49.994	1.622	77	.001
Within populations	189	91.059	0.482	0.482	23	.001
Among locations within North Sumatra[a]	2	18.820	9.410	1.200	79	.001
Total[b]	194	341.031		2.103	100	

Note. Populations evaluated included China, North Sumatra (Indonesia), South Sumatra (Indonesia), South Africa, Thailand, and Vietnam.

[a]Only sampling sites in North Sumatra with more than one individual. Locations evaluated were Aek Raja, Porsea, and Gorbus.

[b]The total for among and within population results calculated for the entire data set.

The optimal number of clusters (ΔK) calculated for Bayesian modelling was three. STRUCTURE separated the data set into an Indonesian cluster (1), a South Africa-Thailand-

Vietnam cluster (2), and a Chinese cluster (3) (Figure S3). Cluster 2 also included some isolates from North Sumatra (Figure S3). Similarly, the DAPC calculations revealed three clusters and the same allocation of isolates to each cluster (Figure 3). These clusters correlated with geographical location. The *MAT* idiomorph, MLG identity, and DAPC cluster for each individual investigated in this study is presented in Table S1.

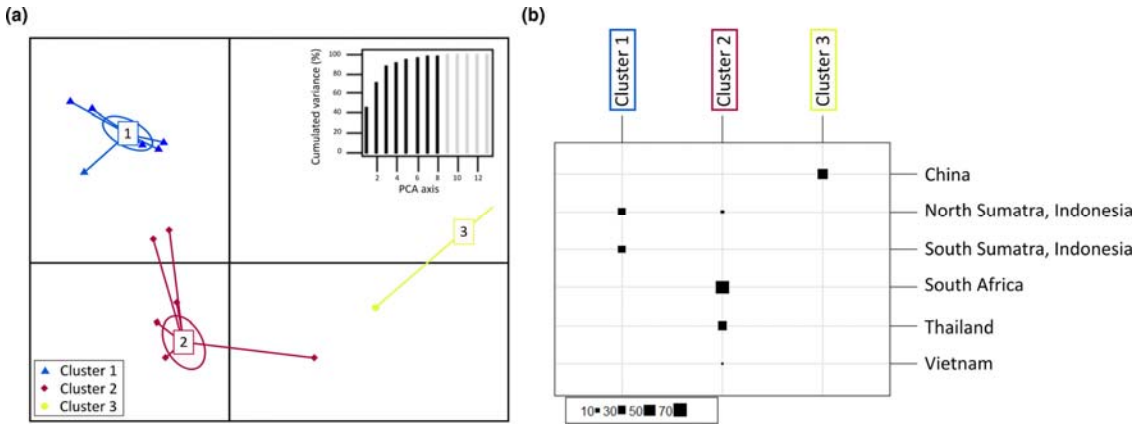

Figure 3 Discriminant analysis of principal components (DAPC) calculated for six populations of *Teratosphaeria destructans*. (a) A scatter plot of the DAPC with eight retained principle components (insert top right) comprised 98.6% of the conserved genetic variance. (b) Organization of the DAPC clusters, with rows corresponding to the populations sampled and columns corresponding to the inferred clusters.

4 Discussion

Twelve polymorphic microsatellite markers were developed using the full genome sequences for three isolates of *T. destructans*. Ten of these were effectively applied to investigate the genotypic diversity, richness, and structure of *T. destructans* isolates from six discrete populations of this important leaf and shoot pathogen collected in South-East Asia and South Africa. All investigated populations had low genotypic diversity, high levels of clonality, little to no evidence of recombination, and strong clustering among populations.

Consequently, the results support the hypothesis (Havenga *et al*., 2020) that *T. destructans* causing disease in *Eucalyptus* plantations propagates primarily via asexual reproduction. The fact that all South African isolates represented a single genotype is consistent with the view that the pathogen most probably entered the country via a single introduction, as suggested by Havenga *et al*. (2020).

The asexual state is known to be the dominant form in various *Eucalyptus* leaf- and shoot-infecting *Teratosphaeria* species, including *T. epicoccoides* and *T. cryptica* (Taole *et al*., 2015; Burgess and Wingfield, 2017), in both their native and introduced ranges. This is also true for the putatively heterothallic *T. pseudoeucalypti* and *T. eucalypti* (Andjic *et al*., 2019; Havenga *et al*., 2020). The high number of clones present in all *T. destructans* populations considered in this study supports the hypothesis that the asexual state also serves as the primary mode of reproduction and dispersal in *T. destructans*.

The highest maximum genotypic diversity in any of the *T. destructans* populations was only 10.7% and private alleles were restricted to China and Indonesia. Considering that native populations of fungal pathogens evolve over time and are expected to be more genetically diverse (McDonald, 1997), our results suggest that the studied populations had been introduced into the countries where they were collected. Similarly, populations of other *Eucalyptus* leaf-infecting *Teratosphaeria* species that have a known Australian origin had low genetic diversity in their introduced populations compared to their native origins (Pérez *et al*., 2012; Taole *et al*., 2015; Burgess and Wingfield, 2017). It is currently not known where *T. destructans* is native (Andjic *et al*., 2019) and the results of this study provide no further resolution regarding this question. The suggestion of Andjic *et al*. (2019) that the pathogen originated in East Timor could not be tested, because cultures are not available from that locality.

Indonesia has the longest history of leaf blight caused by *T. destructans*, having first been discovered by Wingfield *et al*. (1996) in North Sumatra. The age of a population affects its number of private alleles, genotypes, and level of genetic diversity (Linde *et al*., 2009). The results of this study support that view because the two Indonesian populations had the highest genotypic diversities and the highest number of combined private alleles. In contrast, isolates representing the most recent occurrence of the pathogen in South Africa (Greyling *et al*., 2016) were entirely clonal and had no private alleles. This was despite the fact that collections were from multiple sampling sites separated by up to 260 km.

The high level of variation among sampling sites within North Sumatra and unique genotypes limited to each sampling site suggest that there have been multiple introductions of *T. destructans* into North Sumatra. This could also explain the observed high level of genetic diversity observed for North Sumatra. This would be similar to observations for the related pathogens *T. epicoccoides* and *T. zuluensis* (Taole *et al*., 2015; Aylward *et al*., 2019). The genotypes within each of the populations from China, Thailand, and North and South Sumatra were closely related, suggesting that each population has evolved independently. The genotypic diversity of these populations could be a consequence of mutations in microsatellite loci after introduction into those regions, subsequently leading to unique and closely related genotypes in each population (Anmarkrud *et al*., 2008). Although very few unique genotypes remained in the clone-corrected dataset for these populations, there was some evidence of recombination. Both mating types also occurred in these populations (Havenga *et al*., 2020), indicating that sexual recombination is possible.

The North and South Sumatra populations were found to be genetically closely related. *T. destructans* was probably introduced into North and South Sumatra through the importation of seeds with latent infections (Andjic *et al*., 2019) as has been shown for *T. zuluensis* (Jimu *et al*., 2016). This would be consistent with the fact that Indonesia began to

establish plantations of *Eucalyptus* species in the early 1980s by importing plant material from countries with well-established *Eucalyptus* breeding programmes, especially Australia (Wingfield *et al*., 2013; Nambiar *et al*., 2018). Such introductions would have provided an easy source of host-specific pathogens such as *T. destructans*.

Andjic *et al*. (2011) hypothesized that Indonesia was the source population of the disease outbreak in Thailand, Vietnam, and China. In our study, the two earliest collected isolates from North Sumatra, one from the original 1995 disease outbreak (Wingfield *et al*., 1996), shared a genotype with isolates from Thailand, Vietnam, and South Africa, but not with China or South Sumatra. Consequently, it seems probable that North Sumatra would have been the source of disease outbreak in at least one of these countries. However, the genotype of the earliest collected isolates was associated with both mating types and could, therefore, represent distinct genotypes that were not resolved using the currently available microsatellite markers. Alternatively, size homoplasy may have occurred where identical genotypes arose from closely related genotypes of opposite mating type (Estoup *et al*., 2002). The South African population and a Thai isolate shared a mating type and genotype, which could suggest that the two have a common origin. The Chinese isolates of *T. destructans* shared no genotypes with other populations, had private alleles in all genotypes, and formed an independent genetic cluster. This suggests that *T. destructans* was introduced into China from a source different to that of the other populations. However, in the STRUCTURE plot, three isolates showed signs of admixture with the South Africa-Thailand-Vietnam cluster, indicating that they could have evolved from the same ancestors. Our study considered only a single sampling site in China, whereas *T. destructans* has been reported to cause widespread damage in all tropical and subtropical Chinese *Eucalyptus*-growing regions (Burgess *et al*., 2006; Dell *et al*., 2008; Burgess and Wingfield, 2017). An in-depth study, investigating

multiple sampling years and sites, would be required to understand the genetic diversity and movement of *T. destructans* throughout China.

Microsatellites have been widely used to determine the diversity and putative origin of pathogens in both native and exotic forest plantations (Burgess and Wingfield, 2017). The microsatellite markers developed in this study will be useful to investigate populations of *T. destructans* when it emerges in other parts of the world. The global collection of *T. destructans* isolates was characterized by low genetic diversity and strong genetic structure. A single genotype dominates different geographical areas, highlighting the strong influence of asexual reproduction in diseased *Eucalyptus* plantations.

The results of this study are congruent with what is known for non-native populations of other tree pathogens. These include *Teratosphaeria* leaf pathogens (Pérez *et al*., 2012; Taole *et al*., 2015; Burgess and Wingfield, 2017), exotic pine foliar pathogens, such as *Dothistroma septosporum* and *Lecanosticta acicola* (Barnes *et al*., 2014; Janoušek *et al*., 2016), exotic *Phytophthora alni* subsp. *uniformis* populations in Europe causing alder decline (Aguayo *et al*., 2013), and exotic *Chrysoporthe cubensis* on *Eucalyptus* in southern and eastern Africa (Nakabonge *et al*., 2007). They also provide important new knowledge regarding the probable pathways of introduction and inform strategies of disease management.

Discovery of the native range of *T. destructans* would enable a better understanding of its biology, including whether sexual reproduction is an important component of its lifecycle. The long-distance spread of *T. destructans* between countries is clearly a consequence of human-mediated movement of infected plant material (Burgess and Wingfield, 2017). This highlights the need for much stricter quarantine measures (Burgess

and Wingfield, 2002; McTaggart *et al*., 2016) when moving germplasm for plantation establishment.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon request. Primers for microsatellite markers: Genbank accession numbers MN991185–MN991196.

References

Agapow, P.M. and Burt, A. (2001) Indices of multilocus linkage disequilibrium. *Molecular*

Ecology Notes, 1, 101–102.

Aguayo, J., Adams, G.C., Halkett, F., Catal, M., Husson, C., Nagy, Z.A. *et al.* (2013) Strong genetic differentiation between North American and European populations of *Phytophthora alni* subsp. *uniformis*. *Phytopathology*, 10, 190–199.

Andjic, V., Dell, B., Barber, P.A., Hardy, G.E.St J., Wingfield, M.J. and Burgess, T. (2011) Plants for planting; indirect evidence for the movement of a serious forest pathogen, *Teratosphaeria destructans*, in Asia. *European Journal of Plant Pathology*, 131, 49–58.

Andjic, V., Maxwell, A., Hardy, G.E.St J. and Burgess, T.I. (2016) New cryptic species of *Teratosphaeria* on *Eucalyptus* in Australia. *IMA Fungus*, 7, 253–263.

Andjic, V., Carnegie, A.J., Pegg, G.S., Hardy, G.E.St J., Maxwell, A., Crous, P.W. *et al*. (2019) 23 years of research on *Teratosphaeria* leaf blight of *Eucalyptus*. *Forest Ecology and Management*, 443, 19–27.

Anmarkrud, J.A., Kleven, O., Bachmann, L. and Lifjeld, J.T. (2008) Microsatellite evolution: Mutations, sequence variation, and homoplasy in the hypervariable avian microsatellite locus HrU10. *BMC Evolutionary Biology*, 8, 138.

Aylward, J., Roets, F., Dreyer, L.L. and Wingfield, M.J. (2019) Teratosphaeria stem canker of *Eucalyptus*: two pathogens, one devastating disease. *Molecular Plant Pathology*, 20, 8–19.

Barber PA, Thu PQ, Hardy GE, Dell B. (2012) Emerging disease problems in Eucalypt plantations in LAO PDR. In: Mohammed C, Beadle C, Roux J and Rahayu S, (Eds.) *Proceeding of International Conferences on The Impacts of Climate Change to Forest*

Pests and Diseases in the Tropics. Yogyakarta, Indonesia: Faculty of Forestry, Universitas Gadjah Mada, pp. 79–84. Available online at: https://pdfs.semanticscholar.org/eb5c/a452b4640b4cbd325b921987615531bfe7f0.pdf [Accessed 22 June 2020].

Barnes, I., Wingfield, M.J., Carbone, I., Kirisits, T. and Wingfield, B.D. (2014) Population structure and diversity of an invasive pine needle pathogen reflects anthropogenic activity. *Ecology and Evolution*, 4, 3642–3661.

Billiard, S., Lopez-Villavicencio, M., Devier, B., Hood, M.E., Fairhead, C. and Giraud, T. (2011) Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. *Biological Reviews*, 86, 421–442.

Brown, A.H.D., Feldman, M.W. and Nevo, E. (1980) Multilocus structure of natural populations of *Hordeum spontaneum*. *Genetics*, 96, 523–536.

Bruvo, R., Michiels, N.K., D'Souza, T.G. and Schulenburg, H. (2004) A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology*, 13, 2101–2106.

Burgess, T. and Wingfield, M.J. (2002) Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. *International Forestry Review*, 4, 56–65.

Burgess, T.I. and Wingfield, M.J. (2017) Pathogens on the move: A 100-year global experiment with planted Eucalypts. *BioScience*, 67, 14–25.

Burgess, T.I., Andjic, V., Hardy, G.E.St J., Dell, B. and Xu, D. (2006) First report of *Phaeophleospora destructans* in China. *Journal of Tropical Forest Science*, 18, 144–

146.

Dell, B., Hardy, G.E.St J. and Burgess, T. (2008) Health and nutrition of plantation eucalypts in Asia. *Southern Forests*, 70, 131–138.

Du, L., Li, Y., Zhang, X. and Yue, B. (2013) MSDB: a user-friendly program for reporting distribution and building databases of microsatellites from genome sequences. *Journal of Heredity*, 104, 154–157.

Estoup, A., Jarne, P. and Cornuet, J. (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology*, 11, 1591–1604.

Falush, D., Stephens, M. and Pritchard, J.K. (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164, 1567–1587.

Greyling, I., Wingfield, M.J., Coetzee, M.P.A., Marincowitz, S. and Roux, J. (2016) The *Eucalyptus* shoot and leaf pathogen *Teratosphaeria destructans* recorded in South Africa. *Southern Forests*, 78, 123–129.

Grünwald, N.J., Goodwin, S.B., Milgroom, M.G. and Fry, W.E. (2003) Analysis of genotypic diversity data for populations of microorganisms. *Phytopathology*, 93, 738–746.

Havenga, M., Wingfield, B.D., Wingfield, M.J., Roets, F., Dreyer, L.L., Tatham, C.T. *et al*. (2020) Mating strategy and mating type distribution in six global populations of the *Eucalyptus* foliar pathogen *Teratosphaeria destructans*. *Fungal Genetics and Biology*, 137, 103350.

Janoušek, J., Wingfield, M.J., Monsivais, J.G., Jankovský, L., Stauffer, C., Konečný, A. *et al.*

(2016) Genetic analyses suggest separate introductions of the pine pathogen *Lecanosticta acicola* into Europe. *Phytopathology*, 106, 1413–1425.

Jimu, L., Kemler, M., Wingfield, M.J., Mwenje, E. and Roux, J. (2016) The *Eucalyptus* stem canker pathogen *Teratosphaeria zuluensis* detected in seed samples. *Forestry*, 89, 316–324.

Jombart, T. (2008) Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405.

Jombart, T., Devillard, S. and Balloux, F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, 11, 94.

Kamvar, Z.N., Tabima, J.F. and Grünwald, N.J. (2014) *Poppr*: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2, e281.

Katoh, K. and Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A. and Mayrose, I. (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, 5, 1179–1191.

Ladiges, P.Y., Udovicic, F. and Nelson, G. (2003) Australian biogeographical connections and the phylogeny of large genera in the plant family Myrtaceae. *Journal of Biogeography*, 30, 989–998.

Linde, C.C., Zala, M. and McDonald, B.A. (2009) Molecular evidence for recent founder populations and human-mediated migration in the barley scald pathogen *Rhynchosporium secalis*. *Molecular Phylogenetic Evolutions*, 54, 454–464.

McDonald, B.A. (1997) The population genetics of fungi: tools and techniques. *Phytopathology*, 87, 448–453.

McDonald, B.A., Miles, J., Nelson, L.R. and Pettway, R.E. (1994) Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. *Phytopathology*, 84, 250–255.

McTaggart, A.R., van der Nest, M.A., Steenkamp, E.T., Roux, J. Slippers, B. Shuey, L.S. *et al*. (2016) Fungal genomics challenges the dogma of name-based biosecurity. *PLoS Pathogens*, 12, e1005475.

Nakabonge, G., Gryzenhout, M., Wingfield, B.D., Wingfield, M.J. and Roux, J. (2007) Genetic diversity of *Chrysoporthe cubensis* in eastern and southern Africa. *South African Journal of Science*, 103, 261–264.

Nambiar, E.K.S., Harwood, C.E. and Mendham, D.S. (2018) Paths to sustainable wood supply to the pulp and paper industry in Indonesia after diseases have forced a change of species from acacia to eucalypts. *Australian Forestry*, 81, 146–161.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89, 583–590.

Old, K.M., Wingfield, M.J. and Yuan, Z.Q. (2003). *Phaeopleospora* leaf diseases. In: *A Manual of Diseases of Eucalypts in South-East Asia*. Indonesia: Center for International Forestry Research, pp. 25–31.

Paine, T.D., Steinbauer, M.J. and Lawson, S.A. (2011) Native and exotic pests of *Eucalyptus*: A worldwide perspective. *Annual Review of Entomology*, 56, 181–201.

Peakall, R. and Smouse, P.E. (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295.

Pérez, G., Slippers, B., Wingfield, M.J., Wingfield, B.D., Carnegie, A.J. and Burgess, T.I. (2012) Cryptic species, native populations and biological invasions by a eucalypt forest pathogen. *Molecular Ecology*, 21, 4452–4471.

Pielou, E.C. (1975) *Ecological Diversity*. New York: Wiley.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.

R Core Team (2013) R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, *Vienna*, *Austria*. Available at: http://www.R-project.org/ [Accessed 22 June 2020].

Rozen, S. and Skaletsky, H. (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S. and Misener, S. (Eds.) *Bioinformatics Methods and Protocols in the Series Methods in Molecular Biology*. Totowa, NJ, USA: Humana Press, pp. 365–386.

Shannon, C.E. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review*, 5, 3–55.

Simpson, E.H. (1949) Measurement of diversity. *Nature*, 163, 688.

Stenberg, P., Lundmark, M. and Saura, A. (2003) MLGsim: a program for detecting clones

using a simulation approach. *Molecular Ecology Resources*, 3, 329–331.

Stoddard, J.A. and Taylor, J.F. (1988) Genotypic diversity: estimation and prediction in samples. *Genetics*, 118, 705–711.

Taole, M., Bihon, W., Wingfield, B.D., Wingfield, M.J. and Burgess, T.I. (2015) Multiple introductions from multiple sources: invasion patterns for an important *Eucalyptus* leaf pathogen. *Ecology and Evolution*, 5, 4210–4220.

Wingfield, M.J., Crous, P.W. and Boden, D. (1996) *Kirramyces destructans* sp. nov., a serious leaf pathogen of *Eucalyptus* in Indonesia. *South African Journal of Botany*, 62, 325–327.

Wingfield, M.J., Roux, J., Slippers, B., Hurley, B.P., Garnas, J., Myburg, A.A. *et al*. (2013) Established and new technologies reduce increasing pest and pathogen threats to Eucalypt plantations. *Forest Ecology and Management*, 301, 35–42.

Wingfield, B.D., Kolarik, M., Menzies, J.G., Naidoo, K., Pochopski, O., Shoukouhi, P. *et al*. (2018) Nine draft genome sequences of *Claviceps purpurea s.lat*., including *C. arundinis*, *C. humidiphila*, and *C. cf. spartinae*, pseudomolecules for the pitch canker pathogen *Fusarium circinatum*, draft genome of *Davidsoniella eucalypti*, *Grosmannia galeiformis*, *Quambalaria eucalypti*, and *Teratosphaeria destructans*. *IMA Fungus*, 9, 401–418.