# The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the Neofusicoccum parvum–N. ribis species complex

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#### Abstract

*Aim:* Cryptic species in the Neofusicoccum parvum–N. ribis species complex have only recently been described, invalidating previous interpretations on host and geographical distribution. This study aimed to characterize the diversity and distribution of these species and to understand the patterns of host associ- ation, likely origins and their patterns of spread.

*Location:* Australia, Brazil, Cameroon, Chile, China, Colombia, Ethiopia, France, Greece, India, Indonesia, Iran, Italy, Japan, Kenya, Mexico, New Zealand, Panama, Portugal, Puerto Rico, South Africa, South Korea, Spain, Swaziland, Taiwan, Thailand, Uganda, United States of America, Uruguay, Zambia and Zimbabwe.

*Methods:* Using the unique polymorphisms that separate species within the complex, we evaluated sequence search results available in public and in our own databases. In addition, the global distribution of diversity of N. parvum was analysed using seven microsatellite markers.

*Results:* Neofusicoccum parvum is found in 90 hosts across six continents and 29 countries. Neofusicoccum kwambonambiense is found on four continents, six countries and on 14 hosts; N. occulatum is found on four continents, four countries and on 11 hosts; N. umdonicola is found on two continents, countries and hosts; N. cordaticola is found on three continents, countries and hosts; N. batangarum is found on two continents, three countries and three hosts; and N. ribis is found on e host in one country. Population genetic analysis of the global N. parvum population reflects admixture and repeat introductions.

*Main conclusions:* This study illustrates the unfettered and frequent movement of latent pathogens across international borders. Amongst the species in the N. parvum–N. ribis complex, N. parvum is the most widespread and has been reported on the majority of the hosts studied. The current dispersal of N. par- vum and its sister species is probably due to repeated introductions of plant material into new growing areas, with Eucalyptus and Vitis vinifera being two prominent candidates for material transfer.

#### Keywords

Botryosphaeriaceae, Latent pathogen, Neofusicoccum cordaticola and N. batanga- rum, N. kwambonambiense, N. occulatum, N. umdonicola, N. parvum–N. ribis species complex.

# Introduction

Invasion and establishment pathways of fungi and Oomycetes are less understood than that of plants and animals. The effects following establishment of serious pathogens is, however, all too evident, for example, the ecological disasters caused by Jarrah Dieback in Western Australia (Shearer & Smith, 2000) and Chestnut Blight in North America and Europe (Dutech *et al.*, 2012). The cryptic nature of fungi and the difficulty in distinguishing species hampers efforts to monitor their presence, define their origin and determine their host and geographical range. This in turn, seriously affects our ability to proactively manage potential invasion pathways, and the processes required to reduce the chances of further catastrophes. The problem is further exacerbated for latent pathogens where, due to their asymptomatic life stage (as an endophyte), disease symptoms may not be apparent on infected plants. In essence, most 'healthy' plant material moving around the world could be colonized by latent pathogens. This problem is highlighted in the *Neofusicoccum parvum–N. ribis* species complex, a group of related latent tree pathogens. In this study, we attempt to unravel the questions around their identity, distribution and host range, to better understand the frequency and effect of anthropogenic movement.

*Neofusicoccum* species (previously known as *Botryosphaeria* species with *Fusicoccum* anamorphs) are commonly associated with numerous woody hosts world-wide (von Arx, 1987). Of these, *N. parvum* Pennycook and Samuels (Pennycook & Samuels, 1985) has the widest distribution, host range and proven ability to cause disease (Slippers & Wingfield, 2007). For example, isolations made from diseased material and subsequent pathogenicity trials have found *N. parvum* to cause lesions on stems of a range of hardwood plantation tree species, native tree species and horticultural plant species (van Niekerk *et al.*, 2004; Sakalidis, 2004; Pavlic *et al.*, 2009; Pérez *et al.*, 2010; Golzar & Burgess, 2011).

Disease and host reports of *N. parvum* prior to the taxonomic revision of *N. parvum*, *N. ribis* and *B. dothidea* are unreliable (Slippers *et al.*, 2004b). *Neofusicoccum parvum* has been frequently confused with *N. ribis* Grossenb. and Duggar and *B. dothidea* (Moug. Fr.) Ces. and De Not. (Slippers *et al.*, 2004b,c). *Neofusicoccum ribis* was described as a species separate from *B. dothidea* by Grossenbacher and Duggar in 1911, however, in 1954, von Arx and Muller synonymized these two species due to the lack of distinct morphological characters and overlapping host ranges. Consequently, for approximately 45 years, many diseases of woody hosts were attributed to *B. dothidea*. A combination of RAPD marker analysis, ISSR fingerprinting, sequence data and detailed morphological work provided support for the separation of *N. ribis* and *B. dothidea* and for the distinction of these two species from *N. parvum* (Smith & Stanosz, 1997, 2001; Jacobs & Rehner, 1998; Zhou *et al.*, 2001; Slippers *et al.*, 2004b). Slippers *et al.* (2004b) selected an epitype for *B. dothidea* and an ex-type culture for *N. ribis* from isolates collected from the location of the holotype. Therefore, only records collected since 2004 with corresponding molecular data, or isolates collected earlier but for which sequence data is available, can be considered reliable.

Since 2004, the use of multiple gene phylogenies to delineate species boundaries has led to the recognition of five additional species within the *N. parvum–N. ribis* species complex, namely *N. umdonicola*, *N. cordaticola*, *N. kwambonambiense* (Pavlic *et al.*, 2009), *N. batangarum* (Begoude *et al.*, 2010) and *N. occulatum* (Sakalidis *et al.*, 2011c). These recent descriptions, however, also invalidate the identities assigned to many isolates in GenBank. This misinformation hampers a clear understanding of the host and geographical distribution of the cryptic species, which is crucial for understanding their potential origin,

pattern of spread around the world and impact on various ecologically and commercially important hosts.

In this study, all sequence data for the *N. parvum–N. ribis* complex available from GenBank and private databases available to us are reinterpreted in the light of a current phylogenetic framework for this species complex. The aim is to characterize the host and geographical distribution of the individual species. Additionally, polymorphic microsatellite markers are used to characterize the distribution of the diversity in a world-wide collection of the most common species in the complex, *N. parvum*.

# Methods

# **Global species distribution**

Isolate information from the population study presented here was pooled with GenBank searches to determine the current known distribution and host range of each species in the *N. parvum–N. ribis* complex. Representative sequences for each species in the *N. parvum–N. ribis* complex (Pavlic *et al.*, 2009; Begoude *et al.*, 2010; Sakalidis *et al.*, 2011c) were used to perform a BLAST search in GenBank with maximum sequences limited to 500; matches of > 99% homology were aligned with type species in Bioedit v 7.09 (Hall, 1999) using the ClustalW multiple alignment function and default options. This was repeated for up to four gene regions: internal transcribed spacer of the rDNA (ITS),  $\beta$ -tubulin (BT), translation elongation factor 1-  $\alpha$  (EF) and RNA polymerase subunit II RPB2 depending upon which loci contained unique polymorphisms that could be used to distinguish the different species (Pavlic *et al.*, 2009; Sakalidis *et al.*, 2011c). *Neofusicoccum parvum* can be separated from all other species based on unique polymorphisms in the ITS region. For *N. parvum*, maximum sequences were limited to 1000; matches of >97% homology (374) sequences were aligned. In some cases, only the genus, for example, *Eucalyptus* sp. is provided for a host on GenBank.

# Neofusicoccum parvum population analysis

# Origin and identity of isolates

Data from three separate microsatellite studies (Slippers, 2003; Sakalidis, 2004; Pavlic, 2009) conducted prior to the formal description of many cryptic species within the *N. parvum*–*N. ribis* species complex were compiled, isolates that are now recognised as species other than *N. parvum* were removed. These account for some of the unevenness in sampling amongst hosts and countries. Isolates were identified to species level using a combination of available sequence data, RFLP data and unique alleles. Of the 169 verified *N. parvum* isolates from this collection, 38 were isolated from Australia, two from Chile, six from China, 16 from Colombia, 14 from Hawaii, two from Indonesia, 14 from New Zealand and 77 from South Africa. The majority of isolates (81) came from *Eucalyptus* species, 50 isolates came from *Syzygium cordatum* and 38 came from other woody hosts (*Actinidia deliciosa, Araucaria* sp., *Cinnamonum camphora, Malus sylvestris, M. indica, Populus nigra, Ribis* sp. and *Tibochina lepidota*) (Table 1). Cultures of all isolates are maintained on half strength potato dextrose agar (19.5 g BBL<sup>TM</sup> PDA, 7.5 g agar and 1 L distilled water) at Murdoch University (MUCC) or in the CMW collection of the Forestry and Agriculture Biotechnology Institute, University of Pretoria, South Africa.

Sample	Host	No. isolates	No. Haplotypes	P	Primar y		Secondary		у	Tertiary					
-				1	2	x	1	2	3	x A	B	С	D	Е	x
	Eucalyptus sp.	27	18	3	24		3		24	3		8	16		
Australia	Mangifera indica	3	3		3				3			3			
	Tibochina lepidota	6	5		6				6			1	5		
	Cinnamomum camphora	1	1		1				1				1		
	Ribis sp.	1	1		1			1						1	
	Total Australia	38	28	3	35		3	1	34	3		12	22	1	
Chile	Eucalyptus sp.	2	2	2		2	2			2					
China	Eucalyptus sp.	6	4		6				6				6		
Colombia	Eucalyptus sp.	16	9	16	,		16			16					
Hawaii	Eucalyptus sp.	14	12		12	2 2	2		11	1		3	8		3
Indonesia	Eucalyptus sp.	2	2		2				2			1	1		
	Malus sylvestris	1	1		1				1				1		
	Populus nigra	2	2		2				2				2		
New Zeeland	Araucaria sp.	2	2		2				2				2		
New Zealand	Actinidia deliciosa	3	3		3			1	2			1	1	1	
	T. lepidota	6	4		6				6			6			
	Total New Zealand	14	12		14			1	13			7	6	1	
	Eucalyptus sp.	14	5		14		1	13				3		11	
South Africa	Syzygium cordatum	50	18	21	29	2	21	25	4	11	10	4		25	
South Africa	M. indica	13	10	6	7	(	5		7	6		7			
	Total South Africa	77	33	27	50		28	38	11	17	10	14		36	1
Total		169	102	48	119	2 :	51	40	77	1 38	10	37	43	38	3

**Table 1**. Number of haplotypes of *Neofusicoccum parvum* assigned to each group in primary, secondary and tertiary analysis in structure (*x* denotes isolates that could not be assigned to any group)

### DNA extraction, SSR amplification and visualization

Single conidial isolates were grown on half strength PDA plates for approximately 1 week at 20 °C. The mycelial mass was harvested and placed into 1.5-mL sterile Eppendorf tubes. A modified method from (Raeder & Broda, 1985) was used to extract the DNA (Taylor *et al.*, 2005). DNA was purified using the Ultrabind DNA purification kit in accordance with the manufacturer's instructions (MO BIO Laboratories, Solana Beach, CA, USA).

Seven polymorphic loci that contain SSR sequences were used to amplify DNA from 169 isolates using primers and protocols developed previously (Slippers *et al.*, 2004a). PCR were run on a programme of 94 °C for 2 min then 10 cycles of: 94 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min then 25 cycles of: 94 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min, with a final extension of 72°C for 5 min.

All primer pairs were run at the above conditions with the following exceptions; Expand High<sup>TM</sup> Fidelity *Taq* polymerase (Roche Biochemicals, Alameda, CA, USA) and Expand PCR buffer (2 mM Tris–HCl, pH 7.5; 1.5 mM MgCl<sub>2</sub>; 10 mM KCl) were used to amplify locus *BotF11* and locus *BotF21* was amplified at an annealing temperature of 56 °C instead of 60 °C.

Amplified products that exhibited non-overlapping base pair sizes were combined into two groups, the first containing 0.2 ng of product/ $\mu$ l of amplicons from *BotF21*, *BotF17*, *BotF37* and *BotF11*, the second with amplicons from *BotF35*, *BotF23* and *BotF15*. One  $\mu$ l of diluted product was added to 2 ml of formamide containing a ROX-labelled GeneScan-500 size standard (Applied Biosystems, Warrington, UK) (14  $\mu$ l ROX ml<sup>-1</sup> formamide). These were then run on an ABI PRISM 3100 autosequencer (Perkin-Elmer Applied Biosystems, Warrington, UK). Results were analysed using Gene Mapper software (Perkin-Elmer Applied Biosystems).

### **Population genetic analyses**

Gene diversity, distance analysis, population differentiation, gene flow and mode of reproduction were determined on seven loci of populations of *N. parvum* as outlined in Sakalidis *et al.* (2011b). Chi-squared tests were conducted to determine whether isolates from different hosts within the same region could be combined.

#### **Global population structure**

The program structure v 2.2 (Pritchard *et al.*, 2000) was used to assign the global collection of *N. parvum* isolates to distinct populations. structure determines the most likely number of genetic groups (*K*) using a Bayesian Monte Carlo Markov Chain (MCMC) clustering algorithm. A total of 250000 iterations were performed after a burn-in period of 70000 where *K* values ranged from 1 to 15. The 'admixture model' was chosen as ancestry model, the correlated allele frequency model for setting the parameter  $\lambda$  (Pritchard *et al.*, 2000). For the best choice of *K*, the Log likelihood values of *K*, *L* (*K*) (Pritchard *et al.*, 2000) and  $\Delta K$  values (Evanno *et al.*, 2005) were plotted against the ranging *K* values (Fig. 1). Isolates were assigned to populations using structure v 2.2 (Pritchard *et al.*, 2000) once the best value of *K* was determined. CLUMPP (Jakobsson & Rosenberg, 2007) was used to align multiple runs of Structure at the optimum K value using the Greedy algorithm. Distruct (Rosenberg, 2004) was used to generate a visual interpretation of the population assignments of each individual.



**Figure 1.** Log likelihood values of  $\Delta K$  values against the ranging *K* values for the *Neofusicoccum parvum* population.

# Results

## **Global species distribution**

GenBank BLAST search of the ITS sequence of *N. parvum* identified 374 sequence deposits that could be identified as *N. parvum*. Isolates were labelled on GenBank as *N. parvum*, *N. ribis*, *B. parva*, *B. dothidea*, *Botryosphaeria* sp., *Guignardia cryptomeriae*, *Neofusicoccum* sp., fungal endophyte, fungal sp. and uncultured fungus. They were collected from 90 mainly woody plant species, across six continents from 29 countries: Australia; Africa: Ethiopia, Kenya, South Africa, Swaziland, Uganda, Zambia and Zimbabwe; Asia: China, India, Indonesia, Iran, Japan, South Korea, Taiwan and Thailand; Europe: France, Greece, Italy, Portugal and Spain; South America: Brazil, Chile, Colombia and Uruguay; New Zealand; North America: Mexico, USA and Puerto Rico. *N. parvum* has been collected from *Eucalyptus* spp. in 13 countries and from *V. vinifera* in 11 countries (see Table S1 in Supporting Information).

Blast searches of *N. kwambonambiense* sequence matched isolates that were identified on GenBank as *N. parvum*, *N. ribis*, *Botryosphaeria* sp. and *Neofusicoccum* sp. Isolates were collected from six countries: Australia, China, Mexico, South Africa, Uganda, the USA and from 14 host species (see Table S1).

*Neofusicoccum occulatum* sequences were identified on GenBank as *Botryosphaeria* sp., *B. parva, B. ribis, Neofusicoccum* sp., *N. parvum* and *N. ribis.* Isolates were collected from four countries: Australia, Uganda, Uruguay and the USA, and from 11 host species (see Table S1).

In addition to the type isolates of *N. umdonicola* obtained from *S. cordatum* in South Africa (Pavlic *et al.*, 2009), one sequence was identified as a '*Fungal* sp.' collected from ungerminated seed sampled from Colorado Island in Panama (see Table S1). The only isolates of *N. batangarum* are those collected on *T. catappa* in Cameroon, which were used in the original species description (Begoude *et al.*, 2010), a single isolate collected from *Ficus insipida* seed in Colorado Island in Panama (Kluger *et al.*, 2008) and one collected from *Schinus terebinthifolius* seed in the USA (Shetty *et al.*, 2011). The ex-type isolates of *N. cordaticola* sequence matched isolates that were identified on GenBank as *N. parvum* collected from Italy and Australia from two host species (see Table S1). The only isolates of *N. ribis* are those collected on *Ribes* sp. in North America (Slippers *et al.*, 2004b).

### Neofusicoccum parvum population analysis

### **Genetic diversity**

Due to the low sample number in some populations, estimates of genetic diversity may be underestimated. However, our focus was on migration pathways of *N. parvum*, and every data point was thus considered valuable and is included. For genetic diversity estimates, please refer to Appendix S1 in Supporting Information.

Of the 169 samples collected, there were a total of 93 multilocus haplotypes. One haplotype was shared between Australia, New Zealand and South Africa. Australia shared another



**Figure 2.** Distance tree and STRUCTURE bar plot of *Neofusicoccum parvum* isolates from Australia (AUST), China (CHN), Chile (CHI), Colombia (COL), Hawaii (HAW), Indonesia (INDO) and South Africa (SA). Colours correspond to groups generated in STRUCTURE. Isolates in bold and italics were collected from *Eucalyptus* species. Isolates without any shading were not assigned to any population.

haplotype with New Zealand, whilst South Africa shared a different haplotype with New Zealand. Hawaii and Indonesia shared one haplotype (Table 1).

STRUCTURE detected three levels of subdivision; isolates were grouped into two (primary division), three (secondary division) and then five groups (tertiary division) (Fig. 2, Table 1). South African and Australian isolates were represented in all groups at all levels of subdivision except in the tertiary division, in group B, there were only South African isolates, whilst in group D, there were no South African isolates (Fig. 2, Table 1). Colombian and Chilean isolates consistently grouped together with South African isolates (although members from Australia, and Hawaii were also present in some cases) (Group A, Table 1). Chinese, Indonesian and New Zealand isolates tended to cluster together in a group with the Australian isolates (Group C and D, Table 1).

*Eucalyptus* species were sampled 81 times (48% of total samples) in seven countries. Of these, the eight samples taken from *Eucalyptus* species in Chile and Colombia clustered together (Group A, Table 1). Group C contained 30% of Australian *Eucalyptus* samples, 21% of Hawaiian *Eucalyptus* samples, 50% of Indonesian *Eucalyptus* samples and 21% of South African *Eucalyptus* samples (Table 1).

Group D contained 57% of samples taken from *Eucalyptus* species in Hawaii, 50% of Indonesian *Eucalyptus* isolates, 59% of Australian *Eucalyptus* isolates and 100% of Chinese isolates (Table 1). The majority (78%) of South African *Eucalyptus* isolates grouped together in Group E (Table 1).

#### **Distance analysis**

Distance analysis resolved 12 lineages (Fig. 2). Isolates identified in groups C and D in the tertiary subdivision by structure were not clearly resolved in the distance analysis. Group A formed five lineages (1, 2, 3, 11 and 12), four of which (1, 3, 11 and 12) contained only members from Group A, and one lineage (2) also contained members from Group B. Group B formed one lineage (2) with members from Group A. Group C formed three lineages (4, 5 and 8) all mixed with members from Group D. Group D formed six lineages (4, 5, 6, 8, 9 and 10); three lineages (4, 5 and 8) were mixed with members from Group C; two lineages (9 and 10) were mixed with members from Group E; and one lineage (6) contained only members from Group D. Group D formed six lineages 7 containing only members from Group E; whilst lineages 9 and 10 also contained members from Group D.

### Population differentiation and gene flow

Chi-squared tests indicate no significant differences (P > 0.05) in gene diversity between isolates grouped by host or geographical region from each country, indicating these isolates can be placed into populations according to country of collection. Theta values ( $\theta$ ) between populations comparing Australia and New Zealand and New Zealand and Hawaii were not significant, indicating that there was no population differentiation between Australia and New Zealand and New Zealand and Hawaii. Theta values between all other populations were significant (P < 0.05), indicating various levels of population differentiation between these countries (Table 2). A low degree of differentiation was exhibited when comparing South Africa with Australia, Colombia, Hawaii and New Zealand, and when comparing Australia with Hawaii. Moderate to high levels of differentiation occurred between all other populations, particularly when comparing China with Colombia and Hawaii. This may also be a reflection of small sample size in some of these countries.

**Table 2**. Pairwise comparisons of population differentiation ( $\theta$ ) (above the diagonal) and gene flow (below the diagonal) among *N. parvum* populations from Australia (AUST), China (CHN), Colombia (COL), Hawaii (HAW), New Zealand (NZ) and South Africa (RSA). Values obtained are for clone-corrected populations

	,	· /			1 1
AUST	CHN	COL	HAW	NZ	SA
For $\theta$ values, sta	ars and NS indica	te significance (*	*P < 0.05, **P <	$0.001, ^{\text{NS}} P > 0.$	05-not significant).
-	0.309**	0.442**	0.127**	$0.007^{NS}$	0.216**
1.118	-	0.690**	0.521**	0.412*	0.400**
0.631	0.225	-	0.408**	0.407**	0.153**
3.437	0.460	0.725	-	$0.055^{NS}$	0.123*
70.928	0.714	0.728	8.591	-	0.148**
1.815	0.750	2.768	3.565	2.878	-
	AUST For θ values, sta - 1.118 0.631 3.437 70.928 1.815	AUST CHN   For θ values, stars and NS indica   - 0.309**   1.118 -   0.631 0.225   3.437 0.460   70.928 0.714   1.815 0.750	AUST CHN COL   For θ values, stars and NS indicate significance (* - 0.309** 0.442**   1.118 - 0.690** 0.690**   0.631 0.225 - 3.437 0.460 0.725   70.928 0.714 0.728 1.815 0.750 2.768	AUSTCHNCOLHAWFor $\theta$ values, stars and NS indicate significance (*P < 0.05, ** P <	AUSTCHNCOLHAWNZFor $\theta$ values, stars and NS indicate significance (* $P < 0.05$ , ** $P < 0.001$ , <sup>NS</sup> $P > 0$ 0.309**0.442**0.127**0.007^NS1.118-0.690**0.521**0.412*0.6310.225-0.408**0.407**3.4370.4600.725-0.055^NS70.9280.7140.7288.591-1.8150.7502.7683.5652.878

#### Mode of reproduction

For Australia, China, Colombia, Hawaii and New Zealand, the  $I_A$  of the observed data of individual populations fell within the values produced from the randomized data sets supporting the null hypothesis that alleles are freely recombining (Table 3). The  $I_A$  of the observed data of South Africa was greater than that of the values produced from the randomized data sets, indicating predominantly clonal reproduction in this population. Additionally, a significant *P*-value was produced for the South African and Australian populations, suggesting association between alleles.

**Table 3.** Index of association for individual data sets from Australia (AUST), China (CHN), Colombia (COL),Hawaii (HAW), New Zealand (NZ) and South Africa (SA)

#### Observed (original data set) Range (randomized data sets) P-value

1. Values obtained are for clone-corrected populations.

AUST	0.647	-0.436 to 0.702	0.001
CHN	0.261	-0.783 to 1.304	0.360
COL	-0.034	-0.487 to 1.000	0.570
HAW	0.251	-0.651 to 1.153	0.179
NZ	0.260	-0.477 to 0.712	0.093
SA	0.495	-0.308 to 0.366	< 0.001

### Discussion

This study shows how widely a generalist, latent pathogen such as *Neofusicoccum parvum* can be distributed across the globe. The re-evaluation of data from GenBank, in conjunction with data from isolates collected as part of this study, confirmed the presence of closely related populations of *N. parvum* in temperate, mediterranean to subtropical climates on 90 different host species. The hosts are mainly woody angiosperms, but isolations were also made from four conifer species and a single monocot species (a lily). Microsatellite analysis suggests two major exchange routes of *N. parvum*; firstly between South Africa, Chile and Colombia and secondly between Australia, China, Indonesia, Hawaii and New Zealand. These routes are not exclusive, and there is evidence of minor incursions between other countries such as South Africa and New Zealand. It is likely that the current dispersal of

diverse populations of *N. parvum* throughout the world is due to repeat introductions of plant material used for agricultural, plantation or horticultural industries, with widely distributed and common hosts of *N. parvum*, such as *Eucalyptus* and *V. vinifera* being two prominent candidates for transfer.

A re-evaluation of GenBank sequence data has significantly expanded the reported host and geographical range of *N. parvum.* This pathogen has previously been reported to cause disease on a range of economically important agricultural, horticultural and forestry plants (van Niekerk *et al.*, 2004; Sakalidis, 2004; Zea-Bonilla *et al.*, 2007; Javier-Alva *et al.*, 2009; Pavlic *et al.*, 2009; Pérez *et al.*, 2010; Golzar & Burgess, 2011). GenBank sequences were associated with fungal samples taken from canker and die-back of stems, various fruit disfigurement and rot, inflorescence abortion and (once) from ungerminated seed. Most GenBank sequences have been deposited from studies involving exotic plants and/or native plants that have commercial value and are grown under human management. The study shows that *N. parvum* has been isolated from 18 fruit and nut crops and numerous tree and shrub species used for wood production, medicinal or ornamental purposes. It should, however, be noted that isolation of *N. parvum* from diseased material alone does not constitute proof of its involvement in causing the symptoms, and further trials are thus required to verify pathogenicity on specific hosts.

The pattern of distribution seen in the N. parvum-N. ribis complex would appear to reflect the occupation of wild ecosystems and the introduction into managed ecosystems, most likely via human-mediated transfer of germplasm. This is apparent due to the same fungal species occurring on hosts such as V. vinifera and Eucalyptus spp. in different countries (GenBank). Transfer of Eucalyptus spp. has been implicated in the movement of Teratosphaeria destructans throughout Southeast Asia (Andjic et al., 2011). Neofusicoccum occulatum (Sakalidis et al., 2011c) was described from Eucalyptus spp. and W. nobilis in Australia but has also been collected from V. vinifera in Australia, a Eucalyptus sp. in Hawaii, native Myrtaceae species and exotic *E. grandis* in Uruguay and a *Grevillea* sp. and a *Eucalyptus* sp. in Uganda. Similarly, the existence of *N. occulatum* and *N. parvum* on exotic *Eucalyptus* spp. and adjacent native Myrtaceae in Uruguay (Pérez et al., 2010) suggests they have been transferred on Eucalyptus germplasm. Neofusicoccum kwambonambiense, provides another example of a pathogen that has only been described from native vegetation in South Africa (Pavlic et al., 2009) and China (Table S1), but it has appeared as a serious pathogen on a commercial host elsewhere [i.e. commercial blueberry fields in Florida (Wright & Harmon, 2010) and Mexico (Table S1)]. Neofusicoccum cordaticola is present in South Africa (S. cordatum) (Pavlic et al., 2009), Australia (Eucalyptus) and Italy (Vitis) (GenBank), which possibly reflects an endemic range (South Africa) and migration via the plant trade industry.

Extensive sampling of native vegetation in eastern Australia may provide further evidence of when or if *N. parvum* was introduced to Australia; it is known that *N. parvum* has been in eastern Australia for at least 28 years on horticultural crops (Cunnington *et al.*, 2007). Recently, *N. parvum* has been isolated from declining *Araucaria heterophylla* (Norfolk pine-endemic to Norfolk Island) in metropolitan Perth, Western Australia (Golzar & Burgess, 2011). This sudden decline of Norfolk pine might be due to a recent introduction of *N. parvum*, as prior to this report only one record of *N. parvum* had been made in Western Australia despite extensive sampling (Barber *et al.*, 2005; Burgess *et al.*, 2005, 2006; Taylor *et al.*, 2005, 2009; Dakin *et al.*, 2009; Sakalidis *et al.*, 2011d). The single record had been collected from an exotic *E. globulus* species in a plantation (Burgess *et al.*, 2005). This suggests that although Western Australia provides a suitable climate for *N. parvum*, its

geographical isolation and strict quarantine measures has provided an effective barrier to exotic fungi. It is also possible that introduced *N. parvum* is out-competed by the endemic species, *N. australe* (Sakalidis *et al.*, 2011b).

Despite the close genetic relationship of species in the *N. parvum–N. ribis* complex and the ability for some species to colonize the same hosts (Sakalidis *et al.*, 2011a; Spagnolo *et al.*, 2011), except for *N. parvum*, all the species from the complex exhibit a relatively restricted geographical range and a preference for certain host species. This is particularly evident for *N. ribis* that have not been recorded beyond the country and host associated with its original description.

The lack of host specificity of many members of the Botryosphaeriaceae (Slippers & Wingfield, 2007), suggests range expansion and speciation within this group are influenced by other environment factors. For example, climate is suggested as a limiting factor in Botryosphaeriaceae colonization of V. vinifera in Mexico (Úrbez-Torres et al., 2008), Spain (Úrbez-Torres et al., 2006) and South Australia and New South Wales (Pitt et al., 2010). It is also possible that the dominance of a single species, despite the introduction of multiple species via imported germplasm, is influenced by locally adapted Botryosphaeriaceae already present and environmental factors acting on the host and subsequently on the endophyte. This may account for the dominance of different species of Botryosphaeriaceae collected from T. catappa in three different countries; N. parvum was dominant in South Africa, L. pseudotheobromae was dominant in Cameroon and L. mahajangana was dominant in Madagascar (Begoude et al., 2010). Furthermore, in a recent study of M. indica in tropical conditions, N. parvum was not observed, yet it is often associated with this host in other climates (Slippers et al., 2005). In addition, M. indica trees occurring in the Kimberley Region, Western Australia, were found to harbour endemic Botryosphaeriaceae (and not N. parvum) (Sakalidis et al., 2011a), suggesting that the microflora in the surrounding environment determines the internal microflora of the exotic host in an environment.

One interpretation of the population genetic analysis (structure and geneflow analyses) of the microsatellite data suggest that a native or naturalized South African N. parvum population has moved from native to non-native plant species in South Africa (or vice-versa) and has also been introduced from South Africa to other countries in which N. parvum is reported. The founder population within a species' endemic range is generally expected to portray a higher genetic diversity than introduced populations (Nei et al., 1975; Dlugosch & Parker, 2008). Of the seven species in the N. parvum-N. ribis species complex three have been described from the native S. cordatum in South Africa and N. parvum is also commonly collected in this host (Pavlic, 2009; Pavlic et al., 2009). Furthermore, isolates of N. parvum from both native and non-native plants in South Africa were genetically diverse. They also contained a high number of unique alleles and haplotypes, suggesting South Africa as a possible candidate for the origin of N. parvum. However, the lack of detectable sexual reproduction amongst South African strains may conflict with the South African-origin hypothesis. Isolates found in Colombia and Chile grouped with isolates from South Africa in distance and structure analyses, and there was also a low level of genetic differentiation between isolates from these three countries, suggesting South Africa as the source of the populations in South America.

A second interpretation from the population genetic analysis is that *N. parvum* has moved between Australia, New Zealand, China and Hawaii. Isolates from these countries consistently grouped together in the distance and structure analyses and exhibited high levels

of gene flow between them. Also, high genetic diversity in the region may reflect an initial large introduction or multiple introductions, as was evident from the relatively high genetic diversity despite a small number of samples collected in New Zealand.

The genetic composition of *N. parvum* collections in Hawaii and New Zealand probably reflect introductions from both Australia and South Africa. Isolates from Hawaii and New Zealand grouped with isolates from South Africa and also Australia in the distance and structure analyses. In addition to this, despite no significant geneflow between Australia and South Africa, one Australian haplotype and one South African haplotype were identical to one found in New Zealand, and another single haplotype was shared between these three countries.

In most populations, the genetic diversity of *N. parvum* reflects a combined sexual/asexual mating strategy. Mixed reproduction has been observed in the field, as both the teleomorph and anamorph life stages of *N. parvum* were described in New Zealand in 1985 (Pennycook & Samuels, 1985) from introduced *Actinidia deliciosa* (kiwifruit). Most populations exhibited an independent assortment of alleles as expected with sexual recombination (I<sub>A</sub> test). Furthermore, recent work by Baskarathevan *et al.* 2011) has demonstrated anastomosis between *N. parvum* strains (outcrossing via hyphal tips) providing another source of genetic variation. Field observations of asexual reproduction, identical haplotypes occurring multiple times within all populations (not accessible in Indonesia as there were only two samples), and significant linkage disequilibrium in the Australian and South Africa populations suggests clonal reproduction in *N. parvum*. The significant linkage disequilibrium occurring in the South African population was surprising considering the high level of genetic diversity in this population. It is possible that sexual reproduction is occurring in South Africa, but our sampling strategy was insufficient to detect it.

The genetic diversity reflected both within and between the *N. parvum* populations may be attributed to a number of factors. Firstly, the genetic composition of a population is a reflection of the original immigrant population introduced to an area, as N. parvum populations are genetically diverse, the original immigrant population was probably genetically diverse and may have come from different and/or multiple sources (Goodwin et al., 1994; Dlugosch & Parker, 2008). Considering both the Australian and South African populations are genetically diverse, share haplotypes with other populations, may have mixed reproduction strategies and multiple infections can co-occur in a single host; immigration events from these two countries may well compose a genetically diverse population. Secondly, there may have been singular or repeat introductions (migration events) over time (Milgroom, 1996). There is evidence for extensive geneflow between Australia and New Zealand, also between New Zealand and Hawaii, and the initial establishment of Eucalyptus plantations may have involved of the movement of Eucalyptus germplasm between countries as has been shown for Southeast Asia (Andjic et al., 2011). Thirdly, variation in wellestablished populations may be also due to accumulation of mutations over a period of time, although these are expected to play a small role over the time periods of relevance here (Goodwin et al., 1994; Zhan & McDonald, 2004).

This study has used molecular identification techniques to confirm the broad range of hosts that *N. parvum* has been isolated from. The mixed reproduction strategy of *N. parvum* allows for increased genetic variation (sexual reproduction) and rapid dissemination of advantageous genotypes (asexual reproduction) (Zhan & McDonald, 2004; Sakalidis *et al.*, 2011a). These reproductive characteristics when combined with the inherent lack of host specificity

increases the chance of successful establishment when introduced to a new area. This success would also depend on the host health and the ability of *N. parvum* to compete with the native fungal microflora normally present within the host and surrounding environment. The lack of host specificity, common endophytic life stage and reputation as a latent pathogen are a potent combination for potential plant disease; especially when a host is stressed. Stressed hosts may have a reduced ability to combat fungal infection (Schulz *et al.*, 1999; Arnold & Engelbrecht, 2007), and endemic fungi present within the host may be antagonistic to other exotic fungi (Idnurm & Howlett, 2001; Redman *et al.*, 2001).

The ability of *N. parvum* to exist asymptomatically as an endophyte and to colonize a wide range of hosts is likely to explain its movement to many locations. It is likely that the current dispersal of *N. parvum* throughout the world is due to repeat introductions of plant material into regions used for agricultural, plantation or horticultural industries, with widely distributed and common hosts of *N. parvum* likely candidates for transfer.

# Acknowledgements

The authors would like to thank Chyreene Wingfield for her assistance in extraction of DNA, Draginja Pavlic for the use of some of the microsatellite data, the Forestry and Agricultural Biotechnology Institute, University of Pretoria for the use of facilities for the microsatellite analysis and the Australian Federation of University Women (AFUWA) for the financial assistance in the form of the Jill Bradshaw Bursary.

# **Biosketch**

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Author's contributions: MLS, BS and TIB collected the microsatellite data, MLS collected, analysed and interpreted the remaining data and led the writing, MLS, BS, BW, GH and TIB conceived the ideas and contributed towards the writing.

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using inter simple or short sequence repeat (ISSR) fingerprinting. Mycological Resources, 105, 919–926.

#### **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

Table S1. Neofusicoccum parvum-N. ribis sequence information as downloaded from GenBank (where possible Australian states are included NSW= New South Wales, QLD= Queensland, VIC= Victoria, WA= Western Australia),- indicates no information was provided in the GenBank record.

Table S2. Allele size (bp) and frequency at seven loci (BOTF11, 15,17, 21, 23, 35, and 37) for N. parvum populations collected from Australia (AUST), China (CHN), Colombia (COL), Hawaii (HAW), Indonesia (INDO), New Zealand (NZ) and South Africa (SA).

Table S3. Gene diversity (H) and contingency ?2 tests for differences in allele frequencies for the seven polymorphic BOTF loci across clone corrected populations of Neofusicoccum parvum from Australia (AUST), China (CHN), Colombia (COL), Hawaii (HAW), New Zealand (NZ) and South Africa (SA).

Appendix S1. Genetic Diversity.

Table S1 *Neofusicoccum parvum-N. ribis* sequence information as downloaded from GenBank (where possible Australian states are included NSW= New South Wales, QLD= Queensland, VIC= Victoria, WA= Western Australia),- indicates no information was provided in the GenBank record. In some cases data has been retrieved from the associated published manuscript (indicated by an asterisk \*).

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
Neofusicoccum parvum	WAC13360	N. parvum	Araucaria heterophylla	WA, Australia	HM545150
N. parvum	WAC13381	N. parvum	A. heterophylla	WA, Australia	HM545147
N. parvum	WAC13382	N. parvum	A. heterophylla	WA, Australia	HM545148
N. parvum	WAC13383	N. parvum	A. heterophylla	WA, Australia	HM545149
N. parvum	STE-U 3036	N. ribis	Buckinghamia sp.*	Australia*	AF452519
N. parvum	CMW6814 <sup>1</sup>	N/A	Cinnamomum camphora	Australia	N/A
N. parvum	DAR65081	Botryosphaeria parva	Citrus sinensis	NSW, Australia	EF173922
N. parvum	MUCC277	N. parvum	Corymbia citriodora	QLD, Australia	EU339536
N. parvum	MUCC211	N. parvum	Corymbia torreliana	QLD, Australia	EU301017
N. parvum	MUCC239	N. parvum	Eucalyptus camaldulensis	QLD, Australia	EU301023
N. parvum	MUCC240	N. parvum	E. camaldulensis	QLD, Australia	EU301024
N. parvum	MUCC591	N. parvum	E. camaldulensis	QLD, Australia	EU301021
N. parvum	MUCC119	N. parvum	Eucalyptus dunnii	QLD, Australia	EU339540
N. parvum	MUCC124	N. parvum	E. dunnii	QLD, Australia	EU339544
N. parvum	MUCC138	N. parvum	E. dunnii	QLD, Australia	EU339541
N. parvum	MUCC149	N. parvum	E. dunnii	QLD, Australia	EU339539
N. parvum	MUCC150	N. parvum	E. dunnii	QLD, Australia	EU339542
N. parvum	MUCC155	N. parvum	E. dunnii	QLD, Australia	EU339543
N. parvum	MUCC680	Neofusicoccum sp.	E. dunnii	QLD, Australia	EU339549
N. parvum	MUCC145	N. parvum	Eucalyptus globulus	QLD, Australia	EU339548
N. parvum	MUCC673	N. parvum	E. globulus	QLD, Australia	EU339553
N. parvum	CMW15950	B. parva	E. globulus	WA, Australia	DQ093193
N. parvum	MUCC151	Neofusicoccum sp.	Eucalyptus grandis	QLD, Australia	EU339551
N. parvum	MUCC681	N. parvum	E. grandis	QLD, Australia	EU339550
N. parvum	MUCC282	N. parvum	Eucalyptus pellita	QLD, Australia	EU339538
N. parvum	WAC12397	B. parva	E. pellita	QLD, Australia	AY744370
N. parvum	MUCC220	N. parvum	E. pellita	QLD, Australia	EU301022
N. parvum	MUCC312	N. parvum	E. pellita	QLD, Australia	EU301025
N. parvum	MUCC313	N. parvum	E. pellita	QLD, Australia	EU301026

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	DAR80006	N. parvum	Vitis vinifera	NSW, Australia	GU944798
N. parvum	DAR77821	N. parvum	V. vinifera	NSW, Australia	GU944792
N. parvum	DAR77823	N. parvum	V. vinifera	NSW, Australia	GU944800
N. parvum	DAR80003	N. parvum	V. vinifera	NSW, Australia	GU944791
N. parvum	DAR80004	N. parvum	V. vinifera	NSW, Australia	GU944796
N. parvum	DAR80005	N. parvum	V. vinifera	NSW, Australia	GU944797
N. parvum	DAR80008	N. parvum	V. vinifera	NSW, Australia	GU944801
N. parvum	DAR77822	N. parvum	V. vinifera	NSW, Australia	GU944806
N. parvum	AC45c	N. parvum	V. vinifera	NSW, Australia	GU944794
N. parvum	A212a	N. parvum	V. vinifera	NSW, Australia	GU944793
N. parvum	B14	N. parvum	V. vinifera	NSW, Australia	GU944795
N. parvum	C41	N. parvum	V. vinifera	NSW, Australia	GU944799
N. parvum	121	N. parvum	V. vinifera	NSW, Australia	GU944807
N. parvum	H342	N. parvum	V. vinifera	NSW, Australia	GU944805
N. parvum	G32	N. parvum	V. vinifera	NSW, Australia	GU944802
N. parvum	G33	N. parvum	V. vinifera	NSW, Australia	GU944803
N. parvum	DAR48983	N. parvum	Kolkwitzia amabilis	NSW, Australia	EF173923
N. parvum	CMW7025	B. parva	Mangifera indica	Australia	AY615181
N. parvum	CMW7026	B. parva	M. indica	Australia	AY615182
N. parvum	CMW7798	B. parva	M. indica	Australia	AY615183
N. parvum	BRIP19684	N. parvum	M. indica	Australia	EF585513
N. parvum	DAR41726	B. parva	Olea africana	NSW, Australia	EF173924
N. parvum	CMW7799	B. parva	Persea americana	Australia	AY615184
N. parvum	DAR45915	B. parva	P. americana	NSW, Australia	EF173925
N. parvum	BRIP19486	N. parvum	P. americana	QLD, Australia	EF585515
N. parvum	VPRI32689	B. parva	Pistacia vera	NSW, Australia	EF173926
N. parvum	DAR49440	B. parva	Prunus persica	NSW, Australia	EF173927
N. parvum	CMW9071	N. parvum	<i>Ribis</i> sp.	Australia	EU339552
N. parvum	CMW6967	B. parva	<i>Tibouchina</i> sp.	Australia*	AY194473
N. parvum	CMW6236	B. parva	<i>Tibouchina</i> sp.	Australia*	AY194472
N. parvum	CMW6235	B. parva	Tibouchina lepidota	VIC, Australia*	AY615136
N. parvum	CMW6237	B. parva	Tibouchina urvilleana	VIC, Australia*	AY615137
N. parvum	DAR75339	B. parva	V. vinifera	NSW, Australia*	EF173928
N. parvum	CSU-07-WP-B7A	N. parvum	V. vinifera	NSW, Australia*	EU919700
N. parvum	CSU-07-WP-B12	N. parvum	V. vinifera	NSW, Australia*	EU919694

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	CSU-07-WP-B19A	N. parvum	V. vinifera	NSW, Australia*	EU919695
N. parvum	CSU-07-WP-B19B	N. parvum	V. vinifera	Australia*	EU919696
N. parvum	CSU-07-WP-B7B	N. parvum	V. vinifera	NSW, Australia*	EU919701
N. parvum	CSU-07-WP-CB1	N. parvum	V. vinifera	NSW, Australia*	EU919704
N. parvum	CSU-07-WP-B30	N. parvum	V. vinifera	Australia*	EU919697
N. parvum	CSU-07-WP-B31A	N. parvum	V. vinifera	Australia*	EU919698
N. parvum	CSU-07-WP-B31B	N. parvum	V. vinifera	Australia*	EU919699
N. parvum	CSU-07-WP-TS17	N. parvum	V. vinifera	Australia*	EU919706
N. parvum	CSU-07-WP-TS24	N. parvum	V. vinifera	Australia*	EU919705
N. parvum	CSU-07-WP-C9	N. parvum	V. vinifera	Australia*	EU919703
N. parvum	MUCC675	N. parvum	Wollemia nobilis	Australia	EU339535
N. parvum	MUCC674	N. parvum	W. nobilis	Australia	EU339534
N. parvum	MUCC676	N. parvum	W. nobilis	Australia	EU339545
N. parvum	MUCC677	N. parvum	W. nobilis	Australia	EU339546
N. parvum	MUCC678	N. parvum	W. nobilis	Australia	EU339547
N. parvum	MUCC679	N. parvum	W. nobilis	Australia	EU339537
N. parvum	CMM1317	B. parva	M. indica	Brazil	EU938333
N. parvum	CMM1271	B. parva	M. indica	Brazil	EU938335
N. parvum	CMM1276	B. parva	M. indica	Brazil	EU938334
N. parvum	PD299	N. ribis	Vaccinium corymbosum	Chile	GU251147
N. parvum	PD300	N. ribis	V. corymbosum	Chile	GU251148
N. parvum	B02-07	N. parvum	V. corymbosum	Chile	EU833984
N. parvum	B4.2-06	B. parva	V. corymbosum	Chile	EU812205
N. parvum	B1-06	N. parvum	V. corymbosum	Chile	EU856063
N. parvum	CMW10549 <sup>1</sup>	N/A	<i>Eucalyptus</i> sp.	Chile	N/A
N. parvum	HMUC-104(Neo-PD)	N. parvum	V. vinifera	Chile	JF273631
N. parvum	HMUC-105(Neo-PD)	N. parvum	V. vinifera	Chile	JF273632
N. parvum	Fungal endophyte sp. AiS1	fungal endophyte	Artemisia sp.	China	EU054413
N. parvum	clone 35nian-1041	Uncultured fungus	Aquilaria sinensis	China	FN667934
N. parvum	clone 35nian-1007	Uncultured fungus	Aquilaria sinensis	China	FN667928
N. parvum	hsy101	B. parva	Bruguiera sexangula	China	FJ441608
N. parvum	LH107	Neofusicoccum sp.	Camellia sinensis		HQ832811
N. parvum	SXZ-08	Botryosphaeria sp.	Camptotheca acuminata	China	DQ145727
N. parvum	CYN99	N. parvum	Cupressus funebris	China	FJ842961
N. parvum	CYN100	N. parvum	Cupressus funebris	China	FJ842960

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	MUCC688	N. parvum	E. globulus	China	EU675676
N. parvum	MUCC689	N. parvum	E. globulus	China	EU675677
N. parvum	MUCC690	N. parvum	E. globulus	China	EU675678
N. parvum	CZ112B	B. parva	<i>Eucalyptus</i> sp.	China	FJ755241
N. parvum	CMW24704	N. parvum	<i>Eucalyptus</i> sp.	China	HQ332201
N. parvum	CMW27110	N. parvum	<i>Eucalyptus</i> sp.	China	HQ332202
N. parvum	CMW27111	N. parvum	<i>Eucalyptus</i> sp.	China	HQ332203
N. parvum	CMW27125	N. parvum	<i>Eucalyptus</i> sp.	China	HQ332204
N. parvum	CMW27135	N. parvum	<i>Eucalyptus</i> sp.	China	HQ332205
N. parvum	MHF-2008	<i>Botryosphaeria</i> sp.	Maytenus hookeri	China	EU523117
N. parvum	SDAU 7-125	N. parvum	P. persica	China	GQ855789
N. parvum	SDAU07-128	N. parvum	P. persica	China	GQ855790
N. parvum	SDAU08-52	B. parva	Punica granatum	China	FJ214100
N. parvum	SDAU07-168	N. parvum	P. granatum	China	GU997684
N. parvum	SDAU08-54	B. parva	P. granatum	China	FJ214102
N. parvum	SDAU07-111	N. parvum	P. granatum	China	GU723470
N. parvum	SDAU07-167	N. parvum	P. granatum	China	GU997685
N. parvum	SDAU08-55	B. parva	Populus sp.	China	FJ214103
N. parvum	SDAU07-16	N. parvum	Populus sp.	China	GU997688
N. parvum	SDAU07-103	N. parvum	Populus sp.	China	GQ865690
N. parvum	SDAU07-118	N. parvum	Populus sp.	China	GQ865691
N. parvum	1	N. parvum	Taxus chinensis var. mairei	China	JN198393
N. parvum	VT-04	N. parvum	Vernicia fordii	China	HQ844226
N. parvum	FG43 18S	N. parvum	V. vinifera	China	EU030352
N. parvum	CDZ1-1s1	N. parvum	V. vinifera	China	HQ840416
N. parvum	CDZ1-1s2	N. parvum	V. vinifera	China	HQ859951
N. parvum	CDZ1-1s3	N. parvum	V. vinifera	China	HQ859952
N. parvum	NW727	N. parvum	-	China	EU520124
N. parvum	T149	N. parvum	-	China	FJ462749
N. parvum	Mht-16	N. parvum	-	China	JF439214
N. parvum	Mht-17	N. parvum	-	China	JN662928
N. parvum	Vega385	Botryosphaeria sp.	Coffea arabica	Colombia	EF672311
N. parvum	CMW8937 <sup>1</sup>	N/A	<i>Eucalyptus</i> sp.	Colombia	N/A
N. parvum	CMW10089	B. parva	<i>Eucalyptus</i> sp.	Ethiopia	AY210477
N. parvum	CMW10094	B. parva	E. saligna*	Ethiopia	AY210478

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N. parvum	CMW10095	B. parva	E. grandis*	Ethiopia	AY210479
N. parvum	CMW11060	B. parva	E. citriodora*	Ethiopia	AY210474
N. parvum	CMW11062	B. parva	E. citriodora*	Ethiopia	AY210475
N. parvum	CMW11064	B. parva	E. citriodora*	Ethiopia	AY210476
N. parvum	CMW11246	B. parva	Pinus patula	Ethiopia	AY210486
N. parvum	CMW11253	B. parva	P. patula	Ethiopia	AY210487
N. parvum	STE-U 4584	B. parva	V. vinifera	France*	AY343471
N. parvum	IGS	B. parva	Actinidia deliciosa	Greece	FN640477
N. parvum	PD6	N. parvum	P. vera	Greece	GU251129
N. parvum	PD62	N. parvum	P. vera	Greece	GU251133
N. parvum	-	N. parvum	P. persica	Greece	JN135282
N. parvum	UAS015	B. parva	Nothapodytes nimmoniana	India	FJ158131
N. parvum	MUCC687	N. parvum	E. grandis	Indonesia	EU675675
N. parvum	CMW4049	B. parva	<i>Eucalyptus</i> sp.	Indonesia	AY236937
N. parvum	IRN1	N. parvum	V. vinifera	Iran	GU121891
N. parvum	IRN10	N. parvum	V. vinifera	Iran	GU121900
N. parvum	IRN11	N. parvum	V. vinifera	Iran	GU121901
N. parvum	IRN12	N. parvum	V. vinifera	Iran	GU121902
N. parvum	IRN2	N. parvum	V. vinifera	Iran	GU121892
N. parvum	IRN3	N. parvum	V. vinifera	Iran	GU121893
N. parvum	IRN4	N. parvum	V. vinifera	Iran	GU121894
N. parvum	IRN5	N. parvum	V. vinifera	Iran	GU121895
N. parvum	IRN6	N. parvum	V. vinifera	Iran	GU121896
N. parvum	IRN7	N. parvum	V. vinifera	Iran	GU121897
N. parvum	IRN8	N. parvum	V. vinifera	Iran	GU121898
N. parvum	IRN9	N. parvum	V. vinifera	Iran	GU121899
N. parvum	CAP247	N. parvum	Olea europaea	Italy	EF638786
N. parvum	BA20	N. parvum	Quercus robur	Italy	HQ893535
N. parvum	DB 05112010	N. parvum	Rhododendron sp.	Italy	HQ589259
N. parvum	B18	N. parvum	V. vinifera	Italy	FJ481577
N. parvum	B25	N. parvum	V. vinifera	Italy	FJ481578
N. parvum	B27	N. parvum	V. vinifera	Italy	FJ481579
N. parvum	B29	N. parvum	V. vinifera	Italy	FJ481580
N. parvum	B3	N. parvum	V. vinifera	Italy	FJ481576
N. parvum	B30	N. parvum	V. vinifera	Italy	FJ481581

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	B31	N. parvum	V. vinifera	Italy	FJ481582
N. parvum	B44	N. parvum	V. vinifera	Italy	FJ481583
N. parvum	B46	N. parvum	V. vinifera	Italy	FJ481584
N. parvum	PVFi-Np3	N. parvum	V. vinifera	Italy	GU187982
N. parvum	PVFi-Np4	N. parvum	V. vinifera	Italy	GU187983
N. parvum	PVFi-Np5	N. parvum	V. vinifera	Italy	GU187984
N. parvum	PVFi-Np9	N. parvum	V. vinifera	Italy	GU187988
N. parvum	PVFi-Np11	N. parvum	V. vinifera	Italy	GU187990
N. parvum	PVFi-Np12	N. parvum	V. vinifera	Italy	GU187991
N. parvum	PVFi-Np14	N. parvum	V. vinifera	Italy	GU187993
N. parvum	PVFi-Np15	N. parvum	V. vinifera	Italy	GU187994
N. parvum	PVFi-Np16	N. parvum	V. vinifera	Italy	GU187995
N. parvum	PVFi-Np17	N. parvum	V. vinifera	Italy	GU187996
N. parvum	PVFi-Np18	N. parvum	V. vinifera	Italy	GU187997
N. parvum	PVFi-Np19	N. parvum	V. vinifera	Italy	GU187998
N. parvum	PVFi-Np20	N. parvum	V. vinifera	Italy	GU187999
N. parvum	PVFi-Np21	N. parvum	V. vinifera	Italy	GU188000
N. parvum	PVFi-Np30	N. parvum	V. vinifera	Italy	GU188009
N. parvum	PVFi-Np31	N. parvum	V. vinifera	Italy	GU188010
N. parvum	PVFi-Np2	N. parvum	V. vinifera	Italy	GU187981
N. parvum	PVFi-Np29	N. parvum	V. vinifera	Italy	GU188008
N. parvum	Botryosphaeria sp. 67	<i>Botryosphaeria</i> sp.	V. vinifera	Japan	AB034815
N. parvum	Guignardia cryptomeriae	Guignardia cryptomeriae	Cryptomeria japonica	Japan	AB454305
N. parvum	Botryosphaeria sp. 74	<i>Botryosphaeria</i> sp.	Pyrus communis	Japan	AB034818
N. parvum	Botryosphaeria sp. 76	<i>Botryosphaeria</i> sp.	P. communis	Japan	AB034819
N. parvum	Botryosphaeria sp. 77	<i>Botryosphaeria</i> sp.	A. deliciosa	Japan	AB034820
N. parvum	Botryosphaeria sp. 78	<i>Botryosphaeria</i> sp.	Diospyrus kaki	Japan	AB034821
N. parvum	GrF42	N. parvum	Grevillea robusta	Kenya	FJ904817
N. parvum	GrS4	N. parvum	G. robusta	Kenya	FJ904915
N. parvum	Eg36	B. parva	G. robusta	Kenya	FJ904816
N. parvum	Gr78	N. parvum	G. robusta	Kenya	FJ904846
N. parvum	GrY95-7	B. parva	G. robusta	Kenya	FJ904818
N. parvum	Ss57	B. parva	Senna siamea	Kenya	FJ904819
N. parvum	CMW25475/Ec36	Botryosphaeria sp.	E. camaldulensis	Kenya	FJ904820
N. parvum	CRM-152	N. parvum	V. corymbosum	Mexico	JQ647912

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N. parvum	CRM-146	N. parvum	V. corymbosum	Mexico	JQ647911
N. parvum	CMW9077	B. parva	A. deliciosa	New Zealand	AY236939
N. parvum	CMW9078	B. parva	A. deliciosa	New Zealand	AY236940
N. parvum	CMW9079	B. parva	A. deliciosa	New Zealand	AY236941
N. parvum	CMW10120	B. parva	A. heterophyla	New Zealand	AY615160
N. parvum	CMW10121	B. parva	A. heterophyla	New Zealand	AY615161
N. parvum	96-29	B. parva	<i>Malus</i> sp.	New Zealand	AF243395
N. parvum	CMW994	B. parva	Malus sylvestris	New Zealand	AY236883
N. parvum	PD286	N. parvum	<i>Populus</i> sp.	New Zealand	GU251125
N. parvum	CMW9080	B. parva	Populus nigra	New Zealand	AY236942
N. parvum	CMW9081	B. parva	P. nigra	New Zealand	AY236943
N. parvum	10847	N. parvum	Pseudopanax laetus	New Zealand	JN595856
N. parvum	NZFS3125	N. parvum	Rhododendron niveum	New Zealand	JN017921
N. parvum	CMW9952	B. parva	<i>Tibouchina</i> sp.	New Zealand*	AY194471
N. parvum	CMW9945	B. parva	<i>Tibouchina</i> sp.	New Zealand*	AY194470
N. parvum	CMW10117 <sup>1</sup>	N/A	T. lepidota	New Zealand	N/A
N. parvum	STE-U 5253	B. parva	V. vinifera	Portugal*	AY343477
N. parvum	STE-U 5035	B. parva	V. vinifera	Portugal*	AY343473
N. parvum	CBS110301	B. parva	V. vinifera	Portugal*	AY259098
N. parvum	221.IV	N. parvum	Dimocarpus longan L.	Puerto Rico	GU797368
N. parvum	231.I	N. parvum	D. longan L.	Puerto Rico	GU797369
N. parvum	86Q	N. parvum	mango flower	Puerto Rico	GU968434
N. parvum	PD250	N. parvum	<i>Eucalyptus</i> sp.	South Africa	GU251123
N. parvum	PD251	N. parvum	<i>Eucalyptus</i> sp.	South Africa	GU251124
N. parvum	CMW20792	N. parvum	Eucalyptus dorrigoensis	South Africa	FJ752736
N. parvum	BOT21	B. dothidea	E. grandis	South Africa	AF283681
N. parvum	BOT30	B. dothidea	E. grandis	South Africa	AF283682
N. parvum	BOT7	B. dothidea	E. grandis	South Africa	AF283678
N. parvum	CMW20722	N. parvum	Eucalyptus microcorys	South Africa	FJ752727
N. parvum	CMW20727	N. parvum	E. microcorys	South Africa	FJ752735
N. parvum	CMW20735	N. parvum	Eucalyptus nicholii	South Africa	FJ752733
N. parvum	CMW20719	N. parvum	Eucalyptus ovata	South Africa	FJ752724
N. parvum	CMW20726	N. parvum	Eucalyptus robusta	South Africa	FJ752729
N. parvum	CMW20736	N. parvum	E. robusta	South Africa	FJ752730
N. parvum	CMW20720	N. parvum	Eucalyptus saligna	South Africa	FJ752728

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	CMW20724	N. parvum	E. saligna	South Africa	FJ752726
N. parvum	CMW20725	N. parvum	Eucalyptus scoparia	South Africa	FJ752725
N. parvum	BOT19	B. dothidea	Eucalyptus smithii	South Africa	AF283683
N. parvum	CMW20730	N. parvum	Eucalyptus tereticornis	South Africa	FJ752731
N. parvum	CMW20733	N. parvum	E. tereticornis	South Africa	FJ752734
N. parvum	CMW20734	N. parvum	E. tereticornis	South Africa	FJ752732
N. parvum	STE-U 4417	B. parva	V. vinifera	South Africa*	AY343461
N. parvum	STE-U 4420	B. parva	V. vinifera	South Africa*	AY343462
N. parvum	STE-U 4424	B. parva	V. vinifera	South Africa*	AY343463
N. parvum	STE-U 4431	B. parva	V. vinifera	South Africa*	AY343464
N. parvum	STE-U 4437	B. parva	V. vinifera	South Africa*	AY343466
N. parvum	STE-U 4438	B. parva	V. vinifera	South Africa*	AY343467
N. parvum	STE-U 4439	B. parva	V. vinifera	South Africa*	AY343468
N. parvum	STE-U 4530	B. parva	V. vinifera	South Africa*	AY343469
N. parvum	STE-U 4534	B. parva	V. vinifera	South Africa*	AY343465
N. parvum	STE-U 4540	B. parva	V. vinifera	South Africa*	AY343470
N. parvum	STE-U 4589	B. parva	V. vinifera	South Africa*	AY343472
N. parvum	STE-U 5049	B. parva	V. vinifera	South Africa*	AY343474
N. parvum	STE-U 5130	B. parva	V. vinifera	South Africa*	AY343475
N. parvum	STE-U 5142	B. parva	V. vinifera	South Africa*	AY343476
N. parvum	STEU 7036	N. parvum	V. vinifera	South Africa*	JQ038880
N. parvum	STEU 7037	N. parvum	V. vinifera	South Africa*	JQ038881
N. parvum	BOT681	B. dothidea	Heteropyxis natalensis	South Africa	AF283676
N. parvum	CMW10494	N. parvum	Syzygium cordatum	South Africa	EU821912
N. parvum	CMW14029	B. parva	S. cordatum	South Africa	EU821902
N. parvum	CMW14029	N. parvum	S. cordatum	South Africa	EU821902
N. parvum	CMW14030	B. parva	S. cordatum	South Africa	DQ316077
N. parvum	CMW14080	N. parvum	S. cordatum	South Africa	EU821916
N. parvum	CMW14082	N. parvum	S. cordatum	South Africa	EU821907
N. parvum	CMW14085	N. parvum	S. cordatum	South Africa	EU821908
N. parvum	CMW14087	N. parvum	S. cordatum	South Africa	EU821909
N. parvum	CMW14088	N. parvum	S. cordatum	South Africa	EU821910
N. parvum	CMW14089	N. parvum	S. cordatum	South Africa	EU821911
N. parvum	CMW14097	N. parvum	S. cordatum	South Africa	EU821914
N. parvum	CMW14121	N. parvum	S. cordatum	South Africa	EU821917

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	CMW14135	N. parvum	S. cordatum	South Africa	EU821918
N. parvum	CMW14141	N. parvum	S. cordatum	South Africa	EU821920
N. parvum	CMW14143	N. parvum	S. cordatum	South Africa	EU821921
N. parvum	CMW27901	N. parvum	S. cordatum	South Africa	EU821927
N. parvum	BOT683	B. dothidea	S. cordatum	South Africa	AF283677
N. parvum	BOT682	B. dothidea	Syzygium guinesee	South Africa	AF283680
N. parvum	CMW26714	N. parvum	Terminalia catappa	South Africa	FJ900610
N. parvum	CMW 10337	B. parva	Tibouchina sp.	South Africa*	AY194469
N. parvum	CMW26717	N. parvum	T. catappa	South Africa	FJ900611
N. parvum	CMW26718	N. parvum	T. catappa	South Africa	FJ900612
N. parvum	CMW26720	N. parvum	T. catappa	South Africa	FJ900613
N. parvum	CMW26690	N. parvum	Terminalia sericea	South Africa	GQ471815
N. parvum	CMW26842	N. parvum	T. sericea	South Africa	GQ471821
N. parvum	CMW26844	N. parvum	T. sericea	South Africa	GQ471819
N. parvum	CMW26846	N. parvum	T. sericea	South Africa	GQ471818
N. parvum	CMW26848	N. parvum	T. sericea	South Africa	GQ471816
N. parvum	CMW26851	N. parvum	T. sericea	South Africa	GQ471817
N. parvum	CMW26864	N. parvum	T. sericea	South Africa	GQ471820
N. parvum	Sunchang-P1	Neofusicoccum sp.	Vaccinium sp.	South Korea	HQ384218
N. parvum	Sunchang-P3	Neofusicoccum sp.	Vaccinium sp.	South Korea	HQ384220
N. parvum	Sunchang-P5	Neofusicoccum sp.	<i>Vaccinium</i> sp.	South Korea South Korea: Yellow	HQ384219
N. parvum	MF-20(2)	Neofusicoccum sp.	-	Sea	AB563197
N. parvum	CMW36774	N. parvum	<i>Eucalyptus</i> sp.	Spain	JN119284
N. parvum	CMW37773	N. parvum	<i>Eucalyptus</i> sp.	Spain	JN119283
N. parvum	BON065	N. parvum	Juglans regia	Spain	GU292640
N. parvum	BON066	N. parvum	Juglans regia	Spain	GU292640
N. parvum	IFAPA-CH415	B. parva	P. americana	Spain	AM410966
N. parvum	IFAPA-CH441	B. parva	P. americana	Spain	AM410965
N. parvum	Npa1	N. parvum	Prunus dulcis	Spain	JF330779
N. parvum	UCD578Spa	B. parva	V. vinifera	Spain	DQ356359
N. parvum	CBS121486	N. parvum	V. vinifera	Spain*	EU650672
N. parvum	BOT25	B. dothidea	E. grandis	Swaziland	AF283679
N. parvum	B1001	Neofusicoccum sp.	M. indica	Taiwan	GQ861435
N. parvum	B1174	Neofusicoccum sp.	P. americana	Taiwan	HM015908

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	B1118	Neofusicoccum sp.	P. americana	Taiwan	HM015907
N. parvum	TDARES33	N. parvum	Pyrus pyrifolia	Taiwan	GU395186
N. parvum	TDARES37	N. parvum	Pyrus pyrifolia	Taiwan	GU395187
N. parvum	TWFS10	N. parvum	-	Taiwan	JQ408390
N. parvum	CMU37	Fungal sp.	-	Thailand	HM473075
N. parvum	CMW7236	B. parva	E. grandis	Uganda	AY228100
N. parvum	CMW7238	B. parva	E. grandis	Uganda	AY228097
N. parvum	CMW7500	B. parva	E. grandis	Uganda	AY228095
N. parvum	CMW8045	B. parva	E. grandis	Uganda	AY228096
N. parvum	208/1/9/Grevillea	N. parvum	<i>Grevillea</i> sp.	Uganda	GQ922509
N. parvum	196/1/9/Grevillea	N. parvum	<i>Grevillea</i> sp.	Uganda	GQ922512
N. parvum	UY16	N. parvum	Blepharocalyx salcifolius	Uruguay	EU080908
N. parvum	UY37	N. parvum	E. grandis	Uruguay	EU080909
N. parvum	UY129	N. parvum	Myrrhinium atropurpureum var. octandrum	Uruguay	EU860379
N. parvum	UY193	N. parvum	Psidium pubifolium	Uruguay	EU860381
N. parvum	UY1267	N. parvum	B. salcifolius	Uruguay	EU860385
N. parvum	UY1313	N. parvum	Myrciaria tenella	Uruguay	EU860387
N. parvum	UY1325	N. parvum	Myrcianthes cisplatensis	Uruguay	EU860389
N. parvum	UY1609	N. parvum	Eucalyptus cinerea	Uruguay	EU860395
N. parvum	UY1706	N. parvum	E. robusta	Uruguay	EU860398
N. parvum	PD106	N. parvum	P. dulcis	USA	GU251139
N. parvum	PD140	N. parvum	P. dulcis	USA	GU251140
N. parvum	PD142	N. parvum	P. dulcis	USA	GU251141
N. parvum	PD148	N. parvum	P. dulcis	USA	GU251142
N. parvum	PD17	N. parvum	P. dulcis	USA	GU251143
N. parvum	PD39	N. parvum	P. dulcis	USA	GU251144
N. parvum	PD43	N. parvum	P. dulcis	USA	GU251132
N. parvum	PD57	N. parvum	P. dulcis	USA	GU251145
N. parvum	PD59	N. parvum	P. dulcis	USA	GU251146
N. parvum	PD65	N. parvum	P. dulcis	USA	GU251134
N. parvum	PD81	N. parvum	P. dulcis	USA	GU251135
N. parvum	PD92	N. parvum	P. dulcis	USA	GU251136
N. parvum	PD93	N. parvum	P. dulcis	USA	GU251137
N. parvum	PD94	N. parvum	P. dulcis	USA	GU251138
N. parvum	UCD2393TX	N. parvum	V. vinifera	Texas, USA	FJ790824

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	1	B. parva	Lilium lancifolium	Georgia, USA	DQ499154
N. parvum	Ш	B. parva	L. lancifolium	Georgia, USA	DQ499155
N. parvum	UCR1566	N. parvum	Ficus microcarpa var. nitida	California, USA	JN543669
N. parvum	UCR1166	N. parvum	citrus	California, USA	JF271762
N. parvum	UCR273	N. parvum	<i>P. americana</i> Mill.	California, USA	HQ529768
N. parvum	UCR295	N. parvum	P. americana Mill.	California, USA	HQ529765
N. parvum	UCR531	N. parvum	P. americana Mill.	California, USA	GQ857661
N. parvum	UCR735	N. parvum	<i>P. americana</i> Mill.	California, USA	HQ529766
N. parvum	UCR736	N. parvum	<i>P. americana</i> Mill.	California, USA	HQ529767
N. parvum	UCR737	N. parvum	P. americana Mill.	California, USA	GQ857660
N. parvum	UCD1125Na	B. parva	V. vinifera	California, USA	DQ233612
N. parvum	UCD1349So	B. parva	V. vinifera	California, USA	DQ008330
N. parvum	UCD642So	B. parva	V. vinifera	California, USA	DQ008328
N. parvum	UCD646So	B. parva	V. vinifera	California, USA	DQ008329
N. parvum	UCD577Spa	B. parva	V. vinifera	California, USA	DQ356358
N. parvum	UCD759St	B. parva	V. vinifera	California, USA	DQ233611
N. parvum	STE-U 2051	N. ribis	Leucospermum sp.*	Hawaii, USA*	AF452526
N. parvum	STE-U 2057	N. ribis	Protea cynaroides*	Hawaii, USA*	AF452524
N. parvum	STE-U 2100	N. ribis	<i>Telopea</i> sp.*	Hawaii, USA*	AF452521
N. parvum	STE-U 4371	N. ribis	Protea cynaroides*	Hawaii, USA*	AF452518
N. parvum	STE-U 4376	N. ribis	P. cynaroides*	Hawaii, USA*	AF452527
N. parvum	CMW7883 <sup>1</sup>	N/A	<i>Eucalyptus</i> sp.	Hawaii, USA	N/A
N. parvum	STE-U 2055	N. ribis	Leucadendron salignum x L. laureolum*	Florida, USA*	AF452523
N. parvum	96-8	B. ribis	Rhizophora mangle*	Florida, USA*	AF243394
N. parvum	KJ 94.11	B. ribis	R. mangle*	Florida, USA*	AF027744
N. parvum	PD18	N. parvum	Juglans regia	USA	GU251130
N. parvum	PD299	N. parvum	J. regia	USA	GU251131
N. parvum	CMW30144	N. parvum	Eucalyptus cloeziana	Zambia	FJ826610
N. parvum	CMW30143	N. parvum	E. grandis	Zambia	FJ826609
N. parvum	CMW30142	N. parvum	E. grandis	Zambia	FJ826608
N. parvum	STE-U 4381	N. ribis	Protea cynaroides*	Zimbabwe*	AF452522
N. parvum	STE-U 4382	N. ribis	Protea cynaroides*	Zimbabwe*	AF452520
N. parvum	CMW10328	B. parva	Tibouchina sp.	-	AY194468
N. parvum	WF160	Botryosphaeria sp.	Warburgia ugandensis	-	HQ130715
N. parvum	1314	Fungal endophyte	Dendrobium sp.		FJ450043

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. parvum	ATCC 58191	B. parva	-	-	FJ545228
N. parvum	Po66	B. parva	-	-	AY206460
N. parvum	SYJM20	N. parvum	-	-	JN222970
N. batangarum	CMW28315	N. batangarum	T. catappa	Cameroon	FJ900606
N. batangarum	CMW28363	N. batangarum	T. catappa	Cameroon	FJ900607
N. batangarum	CMW28320	N. batangarum	T. catappa	Cameroon	FJ900608
N. batangarum	CMW28637	N. batangarum	T. catappa	Cameroon Panama: Barro	FJ900609
N. batangarum	222c3b	Fungal sp.	Ficus insipida	Colorado Island	EU563590
N. batangarum	AR 4676	N. batangarum	Schinus terebinthifolius	USA	HM357636
N. cordaticola	MUCC297	N. parvum	E. pellita	QLD, Australia	EU301020
N. cordaticola	B318s	N. parvum	V. vinifera	Italy	FJ481576
N. cordaticola	CMW13992	N. cordaticola	S. cordatum	South Africa	EU821898
N. cordaticola	CMW14056	N. cordaticola	S. cordatum	South Africa	EU821903
N. cordaticola	CMW14054	N. cordaticola	S. cordatum	South Africa	EU821906
N. cordaticola	CMW14151	N. cordaticola	S. cordatum	South Africa	EU821922
N. kwambonambiense	MUCC206	N. parvum	Corymbia torreliana x C. citradora	QLD, Australia	EU301018
N. kwambonambiense	MUCC210	N. parvum	Corymbia torreliana x C. citradora	QLD, Australia	EU301016
N. kwambonambiense	MUCC209	N. parvum	Corymbia torreliana x C. citradora	QLD, Australia	EU301015
N. kwambonambiense	MUCC140	Neofusicoccum sp.	E. dunnii	Australia	EU339521
N. kwambonambiense	MUCC157	Neofusicoccum sp.	E. dunnii	Australia	EU339522
N. kwambonambiense	MUCC291	N. parvum	E. pellita	QLD, Australia	EU301019
N. kwambonambiense	WAC12727	N. ribis	Sesbania formosa	NW Australia	EF585518
N. kwambonambiense	Mky19	N. parvum	Bruguiera gymnorhiza	China	FJ441610
N. kwambonambiense	ZJ9-4L	<i>Botryosphaeria</i> sp.	Mangrove sp.	China	FJ487915
N. kwambonambiense	ZH4-E1	Neofusicoccum sp.	Mangrove sp.	China	FJ037734
N. kwambonambiense	CRM-35	N. parvum	V. corymbosum	Mexico	JN088050
N. kwambonambiense	CRM-36	N. parvum	V. corymbosum	Mexico	JQ647905
N. kwambonambiense	CRM-20	N. parvum	V. corymbosum	Mexico	JQ647906
N. kwambonambiense	CRM-125	N. parvum	V. corymbosum	Mexico	JQ647907
N. kwambonambiense	CRM-126	N. parvum	V. corymbosum	Mexico	JQ647908
N. kwambonambiense	CRM-18	N. parvum	V. corymbosum	Mexico	JQ647909
N. kwambonambiense	CRM-129	N. parvum	V. corymbosum	Mexico	JQ647910
N. kwambonambiense	CMW7231	Botryospaheria sp.	E. grandis	Uganda	AY228105
N. kwambonambiense	CMW8286	<i>Botryospaheria</i> sp.	E. grandis	Uganda	AY228102

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. kwambonambiense	CMW8041	Botryospaheria sp.	E. grandis	Uganda	AY228101
N. kwambonambiense	CMW7230	Botryospaheria sp.	E. grandis	Uganda	AY228098
N. kwambonambiense	CMW8036	<i>Botryospaheria</i> sp	E. grandis	Uganda	AY228099
N. kwambonambiense	T4B1/16/1Eucalyptus	N. parvum	<i>Eucalyptus</i> sp.	Uganda	GQ922510
N. kwambonambiense	108/2/6/Grevillea	N. parvum	G. robusta	Uganda	GQ999854
N. kwambonambiense	GAC1	N. ribis	Vaccinium darrowii	Florida, USA	GQ336828
N. kwambonambiense	WRS1	N. ribis	V. darrowii	Florida, USA	GQ336829
N. kwambonambiense	WWC38	N. ribis	V. darrowii	Florida, USA	GQ336830
N. kwambonambiense	MixSuC4	N. ribis	V. darrowii	Florida, USA	GQ845092
N. kwambonambiense	UFO440	N. ribis	V. darrowii	Florida, USA	FJ877139
N. kwambonambiense	WFF9	N. ribis	V. darrowii	Florida, USA	GQ845089
N. kwambonambiense	ARS	N. ribis	Vaccinium sp.	Florida, USA	GQ845088
N. kwambonambiense	RBE2	N. ribis	<i>Vaccinium</i> sp.	Florida, USA	GQ845087
N. kwambonambiense	CMW26856	N. kwambonambiense	Terminalia sericea	South Africa	GQ471843
N. kwambonambiense	CMW26860	N. kwambonambiense	Terminalia sericea	South Africa	GQ471844
N. kwambonambiense	CMW26865	N. kwambonambiense	Terminalia sericea	South Africa	GQ471845
N. kwambonambiense	CMW26850	N. kwambonambiense	Terminalia sericea	South Africa	GQ471846
N. kwambonambiense	CMW26853	N. kwambonambiense	Terminalia sericea	South Africa	GQ471847
N. kwambonambiense	CMW26855	N. kwambonambiense	Terminalia sericea	South Africa	GQ471848
N. kwambonambiense	CMW14025	N. ribis/N. cordaticola	S. cordatum	South Africa	EU821901
N. kwambonambiense	CMW14031	N. kwambonambiense	S. cordatum	South Africa	DQ316076
N. kwambonambiense	CMW14023	N. kwambonambiense	S. cordatum	South Africa	EU821900
N. kwambonambiense	CMW14123	N. kwambonambiense	S. cordatum	South Africa	EU821924
N. kwambonambiense	CMW14140	N. kwambonambiense	S. cordatum	South Africa	EU821919
N. kwambonambiense	CMW14155	N. kwambonambiense	S. cordatum	South Africa	EU821923
N. occulatum	CMW3388	Botryosphaeria sp.	A. cunninghamii	QLD, Australia	AY615154
N. occulatum	MUCC158	Neofusicoccum sp.	E. dunnii	QLD, Australia*	EU339532
N. occulatum	MUCC270	B. ribis	E. grandis	QLD, Australia*	EU339529
N. occulatum	MUCC225	Neofusicoccum sp.	<i>E. grandis</i> hybrid	QLD, Australia*	EU339530
N. occulatum	MUCC227	N. ribis	<i>E. grandis</i> hybrid	QLD, Australia*	EU339509
N. occulatum	MUCC286	N. ribis	E. pellita	QLD, Australia*	EU736947
N. occulatum	MUCC296	N. ribis	E. pellita	QLD, Australia	EU301034
N. occulatum	MUCC317	Neofusicoccum sp.	E. pellita	QLD, Australia	EU339533
N. occulatum	MUCC683	Neofusicoccum sp.	<i>Eucalyptus</i> sp.	QLD, Australia	EU339531

Correct ID	Isolate Code	GenBank ID	Host	Location	Genbank No.
N. occulatum	PD289	N. ribis	<i>Eucalyptus</i> sp.	Australia	GU251788
N. occulatum	PD288	N. ribis	<i>Eucalyptus</i> sp.	Australia	GU251259
N. occulatum	MUCC232	N. ribis	Eucalyptus urophylla x E. camaldulensis	QLD, Australia	EU301031
N. occulatum	H73-1	N. ribis	V. Vinifera	Australia	HQ392733
N. occulatum	W45-3-2	N. ribis	V. Vinifera	Australia	HQ392765
N. occulatum	CMW3389	Botryosphaeria sp.	W. nobilis	QLD, Australia*	AY615155
N. occulatum	CMW9070	Botryosphaeria sp.	W. nobilis	E Australia*	AY615164
N. occulatum	CMW7885	B. parva	<i>Eucalyptus</i> sp.	Hawaii, USA	AY236944
N. occulatum	107/2/6/Grevillea	B. parva	<i>Grevillea</i> sp.	Uganda	GU130544
N. occulatum	MT9B2/32/2/Masaka2	N. parvum	<i>Eucalyptus</i> sp.	Uganda	GU130543
N. occulatum	UY52	N. parvum	E. grandis	Uruguay	EU080912
N. occulatum	UY231	N. parvum	Blepharocalyx salicifolius	Uruguay	EU863164
N. umdonicola	Fungal sp. 222c3b	Fungal sp.	seed	Panama	EU563590
N. umdonicola	CMW13990	B. ribis	S. cordatum	South Africa	DQ316074
N. umdonicola	CMW14011	B. ribis	S. cordatum	South Africa	DQ316072
N. umdonicola	CMW14012	B. ribis	S. cordatum	South Africa	DQ316073
N. umdonicola	CMW14058	N. umdonicola	S. cordatum	South Africa	EU821904
N. umdonicola	CMW14060	N. umdonicola	S. cordatum	South Africa	EU821905
N. umdonicola	CMW14096	N. umdonicola	S. cordatum	South Africa	EU821913
N. umdonicola	CMW14079	N. umdonicola	S. cordatum	South Africa	EU821915
N. umdonicola	CMW14106	N. cordaticola	S. cordatum	South Africa	EU821899
N. umdonicola	CMW14127	N. umdonicola	S. cordatum	South Africa	EU821926
N. ribis	CMW7772	N. ribis	Ribes sp.	New York, USA	AY236935
N. ribis	CMW7773	N. ribis	Ribes sp.	New York, USA	AY236936
N. ribis	CMW7054	N. ribis	Ribes rubrum	New York, USA	AF241177

<sup>1</sup>There is no associated DNA sequence data for this isolate; instead it was identified in the population analysis.

**Table S2** Allele size (bp) and frequency at seven loci (BOTF11, 15,17, 21, 23, 35, and 37) for *N. parvum* populations collected from Australia (AUST), China (CHN), Colombia (COL), Hawaii (HAW), Indonesia (INDO), New Zealand (NZ) and South Africa (SA).

Locus	Allele	AUST	CHN	COL	HAW	INDO	NZ	SA
BotF11	421 426 427 428 432 434 Null	- 0.026 0.895 0.053 0.026 -	- 0.167 0.834 - -	- - 0.750 0.250 - -	0.143 - - 0.714 0.143 - -	- - 0.500 0.500 - - -	- 0.071 - 0.929 - - -	- - 0.675 0.026 0.026 0.273
BotF15	365 374 377 378 387 389 390 395	- 0.868 - 0.105 - - 0.026	- - - 1.000 - -	- 1.000 - - - -	- 1.000 - - - -	- 1.000 - - - -	- 1.000 - - - -	0.117 0.013 0.779 0.013 - 0.052 0.026 -
BotF17	230 232 236 240 242 244 246 248 250 252 256 259 Null	0.105 0.026 0.079 0.632 - 0.026 0.026 - - 0.079 - - 0.079 - 0.026	1.000 - - - - - - - - - - - - - - -	- 0.938 - - - - - - - - - - - - - - - - - 0.062	- 0.571 0.071 - 0.214 - 0.143 -	- - 1.000 - - - - - - - - - - - -	- 0.643 0.071 - 0.143 0.143 - - - -	- 0.143 - 0.013 - 0.065 0.143 0.208 0.299 - 0.065 0.065 -
BotF21	196 199 203 204 207 208 209 217 219 231 Null	0.026 0.026 0.316 - 0.263 0.132 - - 0.210 - 0.220	- 0.667 - - - - 0.167 - 0.167	- - - 0.938 0.062 - - - - -	- 0.143 - - 0.357 - - - 0.071 0.214 0.214	0.500 - - - - - - - 0.500 - -	- 0.286 - 0.357 0.071 - - 0.214 - 0.071	- - 0.039 0.260 0.065 0.078 0.039 0.520 - -
BotF23	415 420 422 423 424 425 425 426	- 0.921 0.079 - -	- 0.500 0.500 - -	- - 0.375 0.062 0.500 0.062	- - 0.857 - - 0.143 -	- - 1.000 - - -	0.071 0.071 0.857 - - -	- 0.649 0.013 0.026 0.221 0.091

Locus	Allele	AUST	CHN	COL	HAW	INDO	NZ	SA
BotF35	221 224 227 230 237 241	0.737 0.026 0.105 - 0.105 -	1.000 - - - -	- - - - 0.062	0.500 0.286 0.143 0.071 - -	1.000 - - - -	0.357 0.071 0.509 - - -	0.156 0.480 0.078 - 0.091 -
BotF35	244 247 261 263 265 Null	 - - 0.026 - -	- - - -	0.812 0.125 - - -	- - - -	- - - -	- 0.071 - - -	0.130 - 0.026 - 0.013 0.026
BotF37	310 311 312 314	0.053 0.447 0.474 0.026	- 1.000 - -	- - 1.000 -	- - 1.000 -	- - 1.000 -	- 0.357 0.643 -	- 0.026 0.948 0.026
No. Isolates		38	6	16	14	2	14	77
No. Haplotypes		25	4	9	11	2	12	33
Unique Haplotypes		23	4	9	10	1	9	31
No. Alleles		31	10	14	19	9	20	38
Null Alleles		2	0	2	1	0	1	2
Unique Alleles		5	0	1	3	0	3	13
Polymorphic loci		7	3	5	5	2	6	7

**Table S3** Gene diversity (H) and contingency  $\chi^2$  tests for differences in allele frequencies for the seven polymorphic BOTF loci across clone corrected populations of *Neofusicoccum parvum* from Australia (AUST), China (CHN), Colombia (COL), Hawaii (HAW), New Zealand (NZ) and South Africa (SA). Stars and NS indicate significance level (\* P<0.001, <sup>NS</sup>P>0.05) numbers in superscript indicate standard deviation.

Locus	Gene Diversity							df
	AUST	CHN	COL	HAW	NZ	SA		
BotF11	0.221	0.560	0.444	0.430	0.153	0.291	43.4 <sup>NS</sup>	30
BotF15	0.218	0.320	0.000	0.000	0.000	0.572	90.3*	35
BotF17	0.625	0.320	0.000	0.546	0.597	0.834	171.5*	60
BotF21	0.749	0.625	0.198	0.735	0.694	0.680	87.1*	50
BotF23	0.147	0.320	0.667	0.165	0.292	0.709	73.8*	30
BotF35	0.510	0.320	0.531	0.678	0.639	0.813	106.4*	45
BotF37	0.536	0.320	0.000	0.000	0.486	0.117	52.7*	15
MEAN	0.432 <sup>+/-</sup> 0.235	<b>0.398</b> <sup>+/-</sup> 0.134	0.263 <sup>+/-</sup> 0.283	0.365 <sup>+/-</sup> 0.310	0.409 <sup>+/-</sup> 0.265	0.574 <sup>+/-</sup> 0.272		

#### Appendix S1

#### **Genetic Diversity**

The seven primer pairs produced 58 alleles across seven loci; amplifying fragments ranging from 196 - 434 bp (Table 2). *BotF11* and *BotF21* were polymorphic in all populations. *BotF15* and was *BotF37* were monomorphic in six and four populations, respectively. *BotF17* and *BotF35* were fixed in two populations, whilst *BotF15* was fixed in one (Table S2).

Isolates from the South African and the Australian populations contained the most alleles (38 and 31, respectively), whilst those from the Indonesian and Chinese populations contained the least (9 and 10, respectively). Isolates from the remaining countries contained 14- 20 alleles (Table S2).

All geographically defined populations (populations verified in the chi-squared analysis) exhibited high haplotype diversity; the lowest was exhibited by South Africa (43 % of isolates exhibited a different haplotype). The highest haplotype diversity was exhibited in New Zealand (85 % of isolates exhibited a different haplotype) (Table 1).

Isolates from Indonesia and Chile were not considered as there were only two samples from each of these countries. Chi-squared tests across all populations and all loci indicated that at all loci frequency of alleles were significantly ( $X^2=43.4$ , p>0.05) different for all populations except at *BotF11*. The mean total gene diversity across all loci was  $0.585^{+/-0.212}$ . This was similar to the gene diversity of South Africa (0.574); lower gene diversities was exhibited by all remaining countries (Table S3). Some populations had a small sample number and gene diversity values must be treated with caution as they would probably be an underestimate of the true gene diversity present in these populations.