



Convergent evolution unites the population genetics of *Protea*-associated ophiostomatoid fungi

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ABSTRACT

Knoxdaviesia and *Sporothrix* species occupy the flower heads of some *Protea* plants in southern Africa. *Knoxdaviesia* species display exceptional genetic diversity within the Core Cape Subregion (CCR) and are readily dispersed across large distances. This study aimed to determine whether overlapping ecologies have led to a similar population genetic structure in *Sporothrix splendens*. Two DNA sequence markers, β -tubulin and a microsatellite region, were amplified in 97 *S. splendens* strains from eight populations that span its host distribution. Genetic diversity was low in a geographically isolated population, but high elsewhere. CCR populations were closely related, showing isolation by distance with populations at the eastern edge of the sampling range. Like *Knoxdaviesia* species, long-distance dispersal of *S. splendens* spores is prevalent, although likely affected by patchy host populations. This study is the first to consider populations of a non-clinical *Sporothrix* species, providing insights into the population attributes of a naturally distributed species.

1. Introduction

Ophiostomatoid fungi are a polyphyletic group, united by convergent evolution of their sexual structures that make them ideally suited to dispersal by arthropods (Malloch and Blackwell 1993; Spatafora and Blackwell 1994). They have been well-studied globally, primarily because they include many tree pathogens (Wingfield et al. 2017), as well as some pathogens of crops and mammals (Barros et al. 2011; De Beer et al. 2014). The latter are members of the genus *Sporothrix*, commonly known for causing sporotrichosis. This skin infection results from contact with an infected host or through traumatic skin contact with a causal species occurring naturally in plant and soil matter (Chakrabarti et al. 2014).

Only a few non-pathogenic members of the ophiostomatoid group have received attention. One of these less well-known groups is the ophiostomatoids that are associated with the African endemic plant genus *Protea* (Aylward et al., 2023; Roets et al. 2013). The flower heads of various serotinous *Protea* species are occupied by two distinct lineages

of ophiostomatoid fungi characterised by species in the genera *Knoxdaviesia* (Microascales) as well as *Sporothrix* (Ophiostomatales; De Beer et al., 2013, 2022; Wingfield et al. 1999). These fungi are saprotrophs that do not harm their *Protea* hosts, but rather benefit them by excluding generalist decomposing fungi from the infructescences (Mukwevho et al. 2021).

Sporothrix is the most diverse of the two ophiostomatoid lineages associated with *Protea* plants, with 11 species reported from *Protea* flower heads in South Africa (Aylward et al., 2023). Four species were described from hosts within the Core Cape Subregion (CCR) biodiversity hotspot (Marais and Wingfield 1994; Roets et al. 2006, 2008) and seven occur on non-CCR hosts (Marais and Wingfield 2001; Ngubane et al. 2018; Roets et al. 2006, 2010). *Sporothrix* species from *Protea* are not monophyletic, but intersperse among other species isolated from soil, plants or clinical settings (De Beer et al. 2016; Ngubane et al. 2018). This indicates that members of the *Sporothrix* genus have colonised the *Protea* niche more than once, with subsequent diversification in some instances (Roets et al. 2009b, 2013).

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The *Protea*-associated ophiostomatoid fungi have an intriguing vectored dispersal system that includes mites, beetles and birds. Mites serve as the primary spore vectors (Roets et al. 2007, 2011) and disperse the fungi between *Protea* plants through phoresy on nectar-feeding beetles (Roets et al. 2009a). More recently, Theron-De Bruin et al. (2018) confirmed that smaller mite vectors are also phoretic on larger mites and that mites are not only carried by beetles, but also by the orange-breasted sunbirds and Cape sugarbirds that visit *Protea* inflorescences. The relationship between *Sporothrix* fungi and some of their mite vectors, such as *Trichouropoda* species, is mutualistic. These mites can feed and reproduce on a diet consisting solely of their fungal partner and have specialised mycangia on their integuments, to transport fungal spores (Roets et al. 2007). The involvement of mites and beetles in dispersing ophiostomatoid fungi is a well-known phenomenon, extending to many other systems (Wingfield et al. 2017). In contrast, bird-mediated dispersal of ophiostomatoid fungi is an unusual occurrence that has thus far only been documented in the CCR *Protea* system.

The dispersal of the two *Knoxdaviesia* species found in *Protea* infructescences has been tracked using microsatellite markers (Aylward et al. 2014b, 2015b, 2017). These studies identified exceptional genetic diversity in both *K. capensis* and *K. proteae* and indicated that the fungi disperse over large distances, maintaining near panmictic populations across >200 km, despite geographical barriers. It is hypothesised that the population structure of *Sporothrix* species with similar ecology and vectors would mirror that of the *Knoxdaviesia* species. However, the population genetics of *Protea*-associated *Sporothrix* species has not been studied.

Ascomata of ophiostomatoid fungi are abundant within *Protea* infructescences, suggesting that sexual reproduction is common (Marais and Wingfield 2001; Roets et al. 2013). The two *Knoxdaviesia* species have a heterothallic mating system, requiring compatible *MAT1-1* and *MAT1-2* individuals for sexual reproduction to occur (Aylward et al. 2016). This obligatory outcrossing between two genetically distinct individuals explains why *Protea*-associated *Knoxdaviesia* species have such high genetic diversity. The two clinical and one entomopathogenic *Sporothrix* species that have been investigated also have a heterothallic mating system (Shang et al. 2016; Teixeira et al. 2015). Although they have not been analysed, the genomes of *Protea*-associated *S. phasma*, *S. protearum* and *S. varicibatus* are available on GenBank and BLAST searches suggest that they are also heterothallic. Consequently, the population biology of *Protea*-associated *Sporothrix* would most likely mirror that of the *Knoxdaviesia* species.

Sporothrix splendens is the ideal *Sporothrix* representative in which to investigate long-range dispersal and to compare the population structures of the two *Protea*-associated ophiostomatoid lineages. Although phylogenetically distant from *K. proteae* (De Beer et al. 2013), both *K. proteae* and *S. splendens* are primarily restricted to one host, i.e. *P. repens* (Aylward et al. 2015a; Roets et al. 2009b; Theron-De Bruin et al. 2018). *Protea repens* is one of the most widely distributed CCR *Protea* species, occurring from the Northern Cape Province, around Nieuwoudtville, throughout the CCR in the Western Cape Province and extending into the Eastern Cape Province, around Grahamstown (Rebelo 2001). The two fungi have also been isolated from the same mite vectors (Roets et al. 2011; Theron-De Bruin et al. 2018).

Microsatellite loci were previously mined from the genome of *S. splendens* isolate CMW 23050 (Duong et al. unpublished) to develop genetic markers (Ngubane 2017). All tested microsatellites were, however, degraded and many had variable flanking regions, thus not adhering to classic microsatellite mutation models (Estoup et al. 2002). Slower-evolving regions were subsequently tested as alternative markers (Ngubane 2017), but the calmodulin (*CAL*) and elongation factor-1 alpha (*EF-1 α*) primers used in previous *Sporothrix* studies (Rangel-Gamboa et al. 2015; Rodrigues et al. 2013; Zhang et al. 2015) amplified with low success in *S. splendens* (De Beer et al. 2016). Therefore, the first aim of the present study was to evaluate the β -tubulin gene

region and a degraded microsatellite region identified by Ngubane (2017), for use as *S. splendens* population-level markers. Secondly, these were used to investigate the genetic diversity and population structure of *S. splendens* across the distribution range of its *P. repens* host.

2. Materials and methods

2.1. Sampling and fungal identification

Protea repens infructescences were collected from eight localities spanning its natural distribution range, from Nieuwoudtville in the Northern Cape Province to Grahamstown in the Eastern Cape Province (Fig. 1b; Rebelo 2001). Between 40 and 60 one-to-two-year-old infructescences were collected per population from as many different *P. repens* trees as possible, with no more than three infructescences collected per individual tree. All infructescences were stored in paper bags at 4 °C until fungi were isolated.

Fungal isolation, DNA extraction and species confirmation followed methods outlined in Aylward et al. (2014a). Ascospores from single sporulating *S. splendens* ascomata (Fig. 1c) were collected using a sterile needle and transferred to Ophiostomatales-selective Malt Extract Agar (MEA; Merck, Wadeville, South Africa) plates containing streptomycin (0.04 g/L, Sigma-Aldrich, Steinham, Germany) and cycloheximide (2.5 g/L) and kept in the dark to germinate (Roets et al. 2006). Primary isolates were transferred to water agar (15 g agar/L; Merck, Wadeville, South Africa) and pure cultures of a single isolate were obtained by transferring a hyphal tip from the water agar to MEA plates, followed by culturing at 24 °C in the dark. The objective was to collect 10 isolates per population, each isolate originating from a different infructescence.

DNA was extracted using the CTAB protocol (Möller et al. 1992), with slight modifications described by Aylward et al. (2014a). To confirm the species identity of a randomly selected subset of individuals, the Internal Transcribed Spacer (ITS) region was amplified with primers ITS-1F and ITS4 (Gardes and Bruns 1993; White et al. 1990), following the methods described by Ngubane et al. (2018). PCR products were cleaned and sequenced at the Central Analytical Facilities (CAF), Stellenbosch University. Using BLASTn (Altschul et al. 1990), sequences were compared to *Sporothrix* reference sequences on GenBank for species identity confirmation.

2.2. DNA markers for population analyses

Beta-tubulin and an anonymous microsatellite region, named m128, were amplified in a subset of individuals from all populations, specifically considering the ease of amplification and variability. Beta-tubulin was amplified using the primers T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995) and m128 with the primers m128F (5'-AGG CGG CAT AGG TCT GTA ATA G-3') and m128R (5'-TGG CAG TAT GAG CGA ATG AT-3'). The components of the β -tubulin PCR were the same as those for ITS (Ngubane et al. 2018). The 20 μ l reaction mixtures for the m128 marker consisted of 1.25 mM MgCl₂, 0.2 μ M of each primer, 10 μ l 2X KAPA Taq ReadyMix PCR Kit and approximately 100 ng template DNA. Reaction conditions for β -tubulin were 94 °C for 4 min, followed by 35 cycles of 94 °C for 60 s, 52.5 °C for 90 s, and 72 °C for 60 s, and a final elongation step at 72 °C for 7 min. Reaction conditions for m128 were 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s, and a final elongation step at 72 °C for 10 min. The β -tubulin PCR products were sequenced in both directions, while m128 was sequenced with primer m128F. After confirming ease of amplification and population-level variability, the two markers were amplified in all sampled individuals.

2.3. Analysis of population genetic data

The sequences of each marker were aligned and trimmed in CLC Genomics Workbench 20.0.3 (CLC Bio, Denmark). Tajima's D (Tajima,

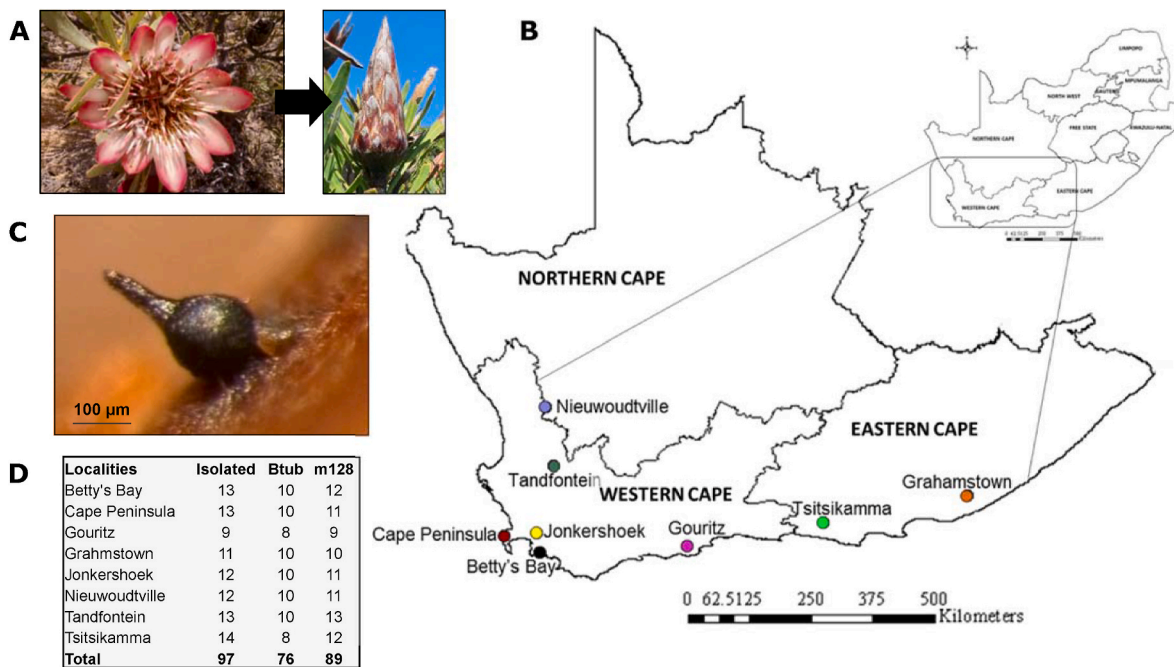


Fig. 1. Sampling of *Sporothrix splendens* from *Protea repens*. (A) *Protea repens* infructescences form after inflorescences have flowered. (B) Infructescences were collected from eight *P. repens* populations across the distribution of this host species in South Africa and (C) *S. splendens* was isolated from sporulating sexual structures found on the dead floral tissues. (D) The number of isolated strains, and the number of strains in which the two nuclear regions (beta-tubulin and m128) could be amplified, varied among the sampling localities.

1989) and Fu's F (Fu 1997), calculated in Arlequin 3.5.2.2 (Excoffier et al. 2005), were used to test whether the markers conform to the neutral theory of sequence evolution (Duret 2008). Potential linkage between the markers was assessed by calculating Linkage Disequilibrium in Genepop 4.7.5 (Rousset 2008). The presence of recombination within each marker was tested with GARD (A Genetic Algorithm for Recombination Detection; Kosakovsky Pond et al. 2006) and the pairwise homoplasy index (PHI; Bruen et al., 2006) implemented in SplitTree4 (Huson and Bryant 2005). Standard diversity indices were computed with DnaSP 6.12.03 (Rozas et al. 2017). These included the number of haplotypes (H), haplotype diversity (H_d ; Nei, 1987), the average number of nucleotide differences (K) and nucleotide diversity (π ; Nei, 1987). Haplotype networks were constructed using the Statistical Parsimony method (TCS; Templeton et al., 1992) in PopART (Leigh and Bryant 2015).

Population structure was investigated in R 4.2.0 (R Core Team 2020). The number of genetic clusters in the dataset was evaluated with k -means clustering and the multivariate Discriminant Analysis of Principal Components (DAPC) method, implemented in the package adegenet 1.2.5 (Jombart 2008, 2010). Analysis of Molecular Variance (AMOVA) was conducted using poppr 2.8.6 (Kamvar et al. 2014), applying 999 permutations for significance testing. F -statistics were computed according to Nei (1987) using hierfstat 0.5.10 (Goudet and Jombart 2021) and the various haplotype- and sequence-based gene flow (N_m) estimates implemented in DnaSP were determined. The presence of an isolation by distance (IBD; Wright, 1943) effect across the sampling distribution of *S. splendens* was investigated using dartR 2.0.3 (Gruber et al. 2018). Geographic distances between populations were calculated from the latitude and longitude of sampling localities using geosphere 1.5–14 (Hijmans 2021), log transformed, and compared to the pairwise Nei (1987) $F_{ST}/(1-F_{ST})$ values using a Mantel test in vegan 2.5–7 (Oksanen et al. 2020), applying Spearman's non-parametric rank correlation.

3. Results

3.1. Fungal sampling and marker amplification

Between nine and 14 *S. splendens* individuals were isolated from each of the eight localities to yield a total of 97 strains. BLASTn analysis of the sequenced ITS region against those of *Sporothrix* species on GenBank, confirmed their identity as *S. splendens*. Both markers amplified unproblematically in the test isolates and displayed inter-population variability and were, therefore, applied to the entire set of isolates. In the full dataset, m128 had the highest amplification success rate (91.8%), yielding at least nine sequences per locality (Fig. 1D). The β -tubulin marker amplified in at least eight isolates per locality (78.3% success rate). Both markers amplified in 68 isolates, whereas only a β -tubulin or m128 haplotype was recovered from eight and 21 isolates, respectively (Table S1).

3.2. Genetic diversity

Beta-tubulin yielded a nucleotide alignment length of 901, whereas the m128 alignment length was 511. For both markers, single-nucleotide polymorphisms (SNPs) were distributed across the length of the sequence, but were concentrated outside of the coding regions in β -tubulin (Fig. S1). Forty-eight β -tubulin and 36 m128 haplotypes were obtained. Both markers displayed high genetic diversity, with haplotypic (gene) diversity (H_d) > 95% and nucleotide diversity (π) $\geq 1\%$ (Table 1). The different estimators of the population parameter θ (Table 1), indicated a higher diversity in β -tubulin, compared to the m128 marker. The tests of neutrality did not indicate significant selection and the two markers were not linked ($\chi^2 = 10.44$, $P = 0.73$).

Evidence of recombination was detected within each marker. GARD detected one recombination breakpoint in both β -tubulin and m128, with support values of 0.95 and 0.30, respectively. Similarly, the PHI test confirmed significant recombination with P -values of 0.000 and 0.001, respectively. The reticulate structures within the MSN (Fig. 2), also implied the presence of recombination in both markers (Posada and

Table 1
DNA polymorphism of the beta-tubulin and m128 markers.

	β-tubulin	m128
Number of segregating sites (S)	66	44
Total number of mutations (Eta)	69	45
Number of Haplotypes (h)	48	36
Haplotype (gene) diversity (Hd)	0.98	0.96
Nucleotide diversity (π)	0.02	0.01
Theta estimators (per sequence) ^a		
θ _H	55.30 ± 17.46	25.45 ± 5.94
θ _S	14.08 ± 3.91	8.89 ± 2.53
θ _π	29.33 ± 14.37	10.43 ± 5.32
θ _k	54.92 (95% CI 34.71–87.55)	21.99 (95% CI 14.17–33.83)
Tajima's D	0.23 ^{ns}	-0.86 ^{ns}
Fu's Fs	-4.77 ^{ns}	-6.44 ^{ns}

ns = not significant.

^a The different theta estimators, calculated in Arlequin (Excoffier et al. 2005), are based on the observed homozygosity (H), number of segregating sites (S), the mean number of pairwise differences (π) and the number of alleles (k), respectively.

Crandall 2001).

The *S. splendens* populations were characterised by many rare haplotypes (Fig. 2), with >70% of detected haplotypes unique to a specific population. Of the few shared haplotypes, only one β-tubulin and one m128 haplotype were detected in more than two populations. Both markers indicated high haplotype (Hd; 78–100%) and nucleotide (π; 0.70–1.50%) diversity within populations, except Nieuwoudtville (Fig. 3). All Nieuwoudtville haplotypes were exclusive to this population and its low diversity was reflected by the three closely related β-tubulin haplotypes and a dominant m128 haplotype (Fig. 2). In contrast,

haplotypes from the other seven populations did not cluster according to their source populations. However, haplotypes from the Grahamstown and Tsitsikamma populations in the Eastern Cape tended to lie at the edges of the network.

Considering the combined β-tubulin and m128 genotype, the 68 isolates in which both markers amplified comprised 58 genotypes (Table S1). Only eight genotypes occurred more than once, of which six were obtained from individuals in the same populations. Three shared genotypes occurred in individuals from Nieuwoudtville (G41-43), one genotype from Tandfontein (G46), one from Tsitsikamma (G55) and one from Grahamstown (G28). The remaining two shared genotypes were recovered from more distantly separated individuals occurring in the Cape Peninsula and Grahamstown (G17), and Gouritz and Grahamstown (G21), respectively.

3.3. Population structure and differentiation

Based on the Bayesian Information Criterion (BIC) statistic of the k-means clustering algorithm, the collection of *S. splendens* individuals was best described by two genetic clusters (Fig. 4A). Cluster 1 comprised almost all *S. splendens* individuals in the dataset, whereas cluster 2 comprised 10 of the 12 individuals isolated from Nieuwoudtville. Visualising the DAPC scatter plot and the density plot based on a single discriminant function (Fig. 4B and C) illustrated the genetic distance of the Nieuwoudtville individuals from the other populations and indicated that the two Nieuwoudtville individuals, considered to be part of cluster 1, lie at the edge of this cluster. The plots furthermore suggested that some individuals in the easternmost Grahamstown population were more differentiated from the other individuals in cluster 1.

The AMOVA results (Table S2) were consistent between the two markers and indicated moderate, though highly significant, differentiation among the eight populations (F_{ST} = 0.09–0.20; P = 0.00). The variation among populations accounted for 8.50–20.25% of the total

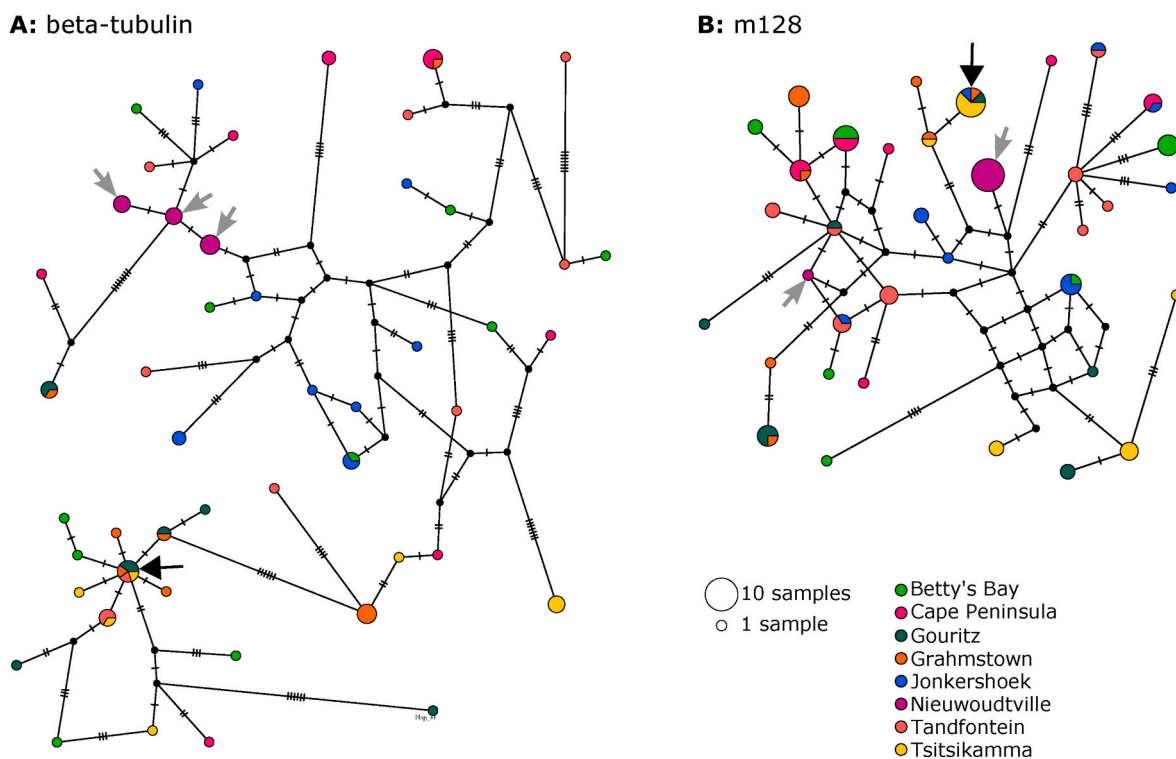


Fig. 2. Networks of the (A) beta-tubulin and (B) m128 haplotypes in the eight *Sporothrix splendens* populations, calculated based on the statistical parsimony method (Templeton et al., 1992). Sizes of circles indicate the relative abundance of each haplotype and colours correspond to population of origin. Small black circles represent hypothetical unsampled or ancestral haplotypes. Grey arrows highlight the unique Nieuwoudtville haplotypes, whereas black arrows indicate the two haplotypes present in multiple populations.

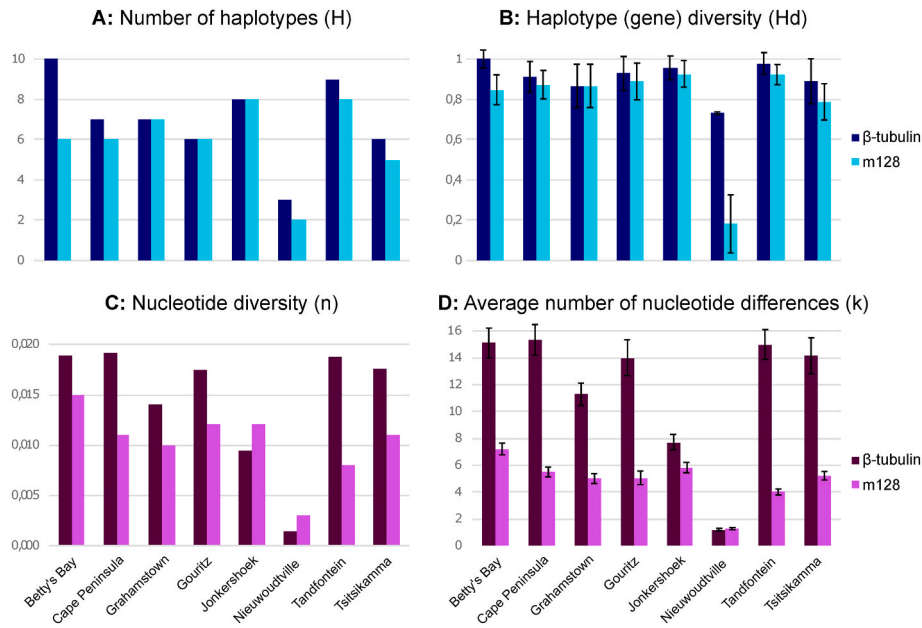


Fig. 3. (A–B) Haplotype and (C–D) nucleotide diversity of *Sporothrix splendens* beta-tubulin and m128 sequences obtained from eight different populations.

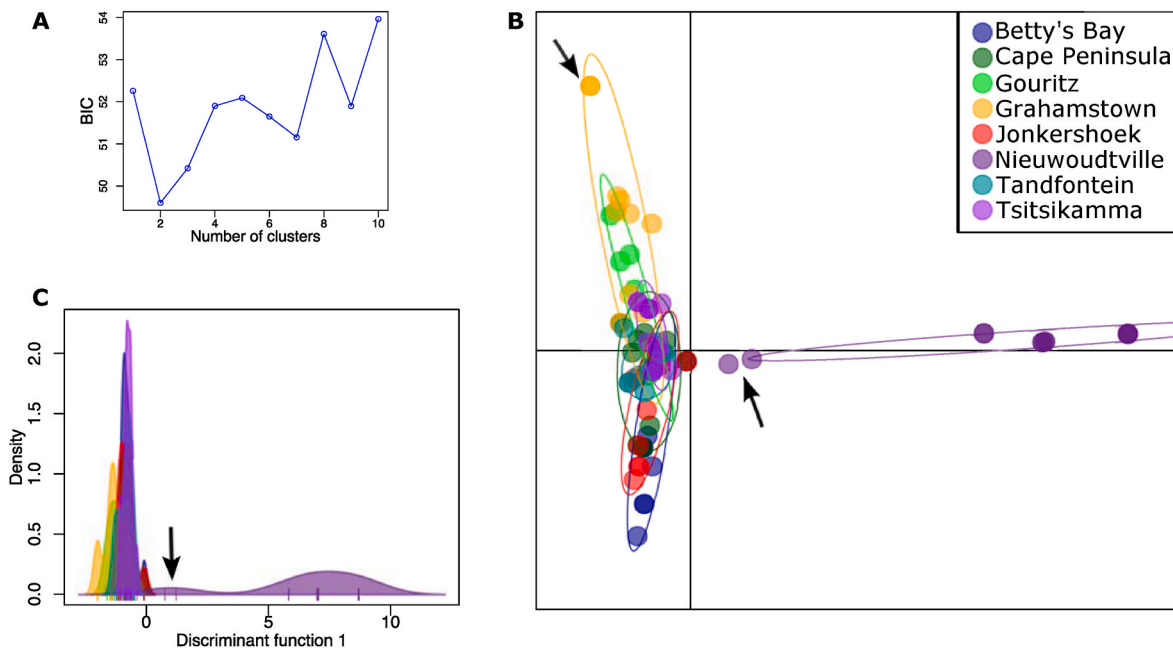


Fig. 4. Population structure of *Sporothrix splendens*. (A) According to *k*-means clustering and the Bayesian Information Criterion (BIC), the data is best described by two genetic clusters. Depiction of the eight populations in a (B) scatter plot and (C) density plot of the Discriminant Analysis of Principal Components (DAPC). Arrows indicate the Nieuwoudtville (purple) and Grahamstown (yellow) individuals assigned to cluster 1 that lay at the edge of this cluster.

diversity, whereas the bulk of diversity (79.74–91.50%) was attributed to variation within populations. Pairwise comparisons (Table 2) confirmed that the Nieuwoudtville population was isolated from the others, as it consistently showed the highest differentiation, with F_{ST} values ranging between 0.30 and 0.35. Excluding Nieuwoudtville from the AMOVA decreased overall population differentiation ($F_{ST} = 0.06–0.11$; 5.58%–11.22% among-population variation), although it remained significant ($P = 0.005$). Similarly, retaining only the five Western Cape populations yielded significant ($P = 0.001$), though low, differentiation ($F_{ST} = 0.04–0.09$; 3.80%–8.47%). The number of migrants (N_m) among the eight populations, according to the various measures of F_{ST} , was always greater than one (1.21–2.25) and increased

when excluding Nieuwoudtville.

Significant isolation by distance (IBD; Mantel statistic = 0.50, $P = 0.03$) was detected among the eight populations. However, according to the Coefficient of Determination (R^2 ; Fig. S2), IBD explained only 5.27% of the variation in genetic distance. Along with the isolated Nieuwoudtville population, the easternmost Grahamstown and Tsitsikamma populations contributed significantly to an IBD effect that was not detected among populations in the Western Cape (Mantel statistic = 0.68, $P = 0.11$). Including one distant population at a time strengthened the IBD correlation so that more of the variation was explained (25.4–52.2%). These results suggest an IBD effect between the central *S. splendens* populations within the Western Cape and the more distant

Table 2

Pairwise F_{ST} values among the eight *Sporothrix splendens* populations, calculated according to Nei (1987). The colour scale moves from green to yellow to red, as the F_{ST} values increase.

	BB	CP	GW	GE	JP	NWV	TF	T
Betty's Bay (BB)	-							
Cape Peninsula (CP)	0.06	-						
Gouritz (GW)	0.08	0.10	-					
Grahamstown (GE)	0.10	0.09	0.06	-				
Jonkershoek (JP)	0.05	0.08	0.07	0.09	-			
Nieuwoudtville (NWV)	0.31	0.33	0.32	0.34	0.30	-		
Tandfontein (TF)	0.06	0.08	0.05	0.09	0.04	0.29	-	
Tsitsikamma (T)	0.12	0.13	0.09	0.12	0.09	0.35	0.09	-

populations in the other two provinces.

4. Discussion

The *Protea*-associated fungus *S. splendens* is the first non-clinical species in this genus to be investigated at a population level. *Sporothrix splendens* is the most frequently encountered of the *Protea*-associated *Sporothrix* species in one of the most widespread *Protea* hosts in southern Africa (Aylward et al., 2023; Rebelo 2001). Consequently, its population structure is of interest, particularly in comparison to phylogenetically distant ophiostomatoid fungi that occupy the same niche. This study reveals the presence of diverse and yet closely related populations of *S. splendens* across the entire distribution range of its host. It also shows that the low level of genetic differentiation among populations is not entirely explained by geographic location. This implies that long-distance dispersal is an important component of *S. splendens* movement in a manner similar to that found for *Knoxdaviesia* species (Aylward et al. 2015b, 2017).

Two DNA sequences, a portion of the β -tubulin gene and the anonymous m128 microsatellite region, revealed high genetic diversity in *S. splendens*. This implies that some of the PCR failure (21.7% for β -tubulin and 8.2% for m128) could be due to polymorphism at the primer-binding sites. This level of diversity is consistent with that expected from a species in its native range (McDonald 1997). Almost maximal (>95%) genotypic diversity was previously reported in the *Protea*-associated *K. capensis* and *K. proteae* microsatellite-based studies (Aylward et al. 2014b, 2015b, 2017), but it is intriguing that a similar pattern could be detected in *S. splendens* based on analysis of a highly conserved barcoding gene (Einax and Voigt 2003). The β -tubulin gene, however, comprises several introns that are variable within *Sporothrix* (De Beer et al. 2016) and it is noteworthy that almost all polymorphisms detected in this gene lay within the intron (i.e. non-coding) sequences. Congruence between the results of the β -tubulin barcoding gene and the m128 microsatellite region increased confidence in their use as genetic markers in population-level analyses.

Nieuwoudtville in the Northern Cape was the most isolated locality, with regards to both its surrounding landscape and the genetic structure of *S. splendens*. Unlike the other populations, *S. splendens* in Nieuwoudtville had low variability and distinct genotypes, of which most were sampled more than once. This situation could have arisen because the sampled *P. repens* host plants were young and from a small, isolated population. Recolonization of young *P. repens* plants by *K. proteae* after fire depends on migration from neighbouring *P. repens* stands (Aylward et al., 2015b), which are less abundant in this region (Rebelo 2001). Assuming a similar dispersal mechanism to *K. proteae*, the isolated Nieuwoudtville population likely receives new *S. splendens* inoculants infrequently and from populations that were not sampled in this study. This would explain the limited number of *S. splendens* haplotypes identified in Nieuwoudtville and their genetic distance from the other

sampled populations.

Low, but significant, differentiation was detected in the seven remaining *S. splendens* populations and the populations in the Western and Eastern Cape displayed an isolation by distance (IBD) effect. This is congruent with the IBD reported for the *P. repens* host across its distribution, in which gene flow was largely limited to populations in close proximity (Prunier et al. 2017). The detected population structure in *S. splendens* was, however, only partially explained by geographic origin, since two instances of shared genotypes were also detected between the provinces where the *P. repens* host was sampled. One of these was shared between the westernmost Cape Peninsula population and the easternmost Grahamstown population. It was surprising that the Cape Peninsula population was not more isolated given the fact that it occurred in an area almost completely surrounded by the city of Cape Town. However, this might be explained by the fact that *P. repens* is widespread even in suburban areas and nectarivore birds, such as the secondary vectors of *S. splendens* (Theron-De Bruin et al. 2018), are known to fly long distances in search of flowering *Protea* stands (Harrison et al. 1997).

Although they were sufficient to reveal the broad population structure of *S. splendens*, the markers applied in this study provided information regarding genetic variability at only two genomic locations. In contrast, multiple microsatellite loci or genome-wide SNP markers would likely provide resolution at a finer scale (DeFaveri et al. 2013). We expect that application of markers with greater resolving power would highlight additional differentiation within cluster 1, specifically between the Western and Eastern Cape populations where IBD was detected. Analysis of recombination frequency was also not possible with the β -tubulin and m128 markers. Nonetheless, the evidence of recombination breakpoints, high genetic diversity and the fact that these two markers were unlinked suggests that recombination is frequent. Given that other ophiostomatoid fungi known from this system and all *Sporothrix* species investigated to date are heterothallic (Aylward et al. 2016; Shang et al. 2016; Teixeira et al. 2015), *S. splendens* is likely also an outcrossing species.

The genetically similar *S. splendens* individuals within the Western Cape populations of the fungus implies that there is regular gene flow among populations in the CCR. This is similar to the situation in *K. capensis* and *K. proteae* (Aylward et al. 2015b, 2017), in which populations at different locations are differentiated at only very low levels. Whereas sampling of *Knoxdaviesia* was limited to hosts within the CCR, the present study considered *S. splendens* over the entire distribution of *P. repens*. The observed IBD and increased differentiation when including the more distant *S. splendens* populations is congruent with IBD patterns in *P. repens* (Prunier et al. 2017) and can be explained by changing vegetation types outside of the CCR (Manning and Goldblatt 2012). Consequently, patchy *P. repens* populations would facilitate fewer migration events. While the CCR populations of *S. splendens* follow the near panmixia found in *Knoxdaviesia*, the Northern and Eastern Cape *S. splendens* populations conversely suggest that *Knoxdaviesia*

populations beyond the CCR are likely to be less cohesive.

The pattern of weakly differentiated populations revealed in this study highlight the importance of the secondary beetle and bird vectors in the long-distance dispersal of *Protea*-associated species. Effective spore dispersal of *S. splendens* has led to populations that are not strongly structured and become more cohesive with increasing abundance of their *P. repens* host in the CCR and decreasing geographic distances among populations. This is in direct contrast to the geographically structured populations reported for the mammalian pathogen *S. schenckii* and related species that cause sporotrichosis (Rangel-Gamboa et al. 2015; Rodrigues et al. 2013), but mirrors the population genetics of *Protea*-associated *Knoxdaviesia* species, as hypothesised. This study has confirmed that the population genetics of the two *Protea*-associated ophiostomatoid fungal lineages are united by their similar ecologies, which results from convergent evolution of the morphology of their sexual structures (Malloch and Blackwell 1993; Spatafora and Blackwell 1994). The results also provide important insights into the population attributes of a naturally distributed species in a genus previously studied primarily for its clinical significance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.funeco.2023.101242>.

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