Botryosphaeriaceae on *Syzygium cordatum* across a latitudinal gradient in South Africa

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Abstract

The Botryosphaeriaceae is a family of endophytic fungi, many of which are latent pathogens of woody plants. Although extensively sampled in some parts of the world, little is known regarding their occurrence across different environmental conditions. This study considered the presence of the Botryosphaeriaceae on *Syzygium cordatum* trees across a latitudinal gradient. We examined the relative importance of different environmental factors on the presence of the Botryosphaeriaceae across this latitudinal gradient. Specifically, Botryosphaeriaceae community composition and species richness were analysed. The optimal growth temperature of the most common Botryosphaeriaceae isolates and its relation to isolate origin was also tested in culture. We identified 14 Botryosphaeriaceae species including seven each of *Lasiodiplodia* and *Neofusicoccum* species. The maximum historical temperature emerged as the environmental factor that best predicted the presence of Botryosphaeriaceae species in *S. cordatum* trees. Specifically, maximum historical temperature influenced Botryosphaeriaceae community composition on these trees, but not species richness. For all the Botryosphaeriaceae species studied *in vitro*, temperature strongly influenced mycelial growth and they all had an optimal growth temperature of 25 ºC. Contrary to our hypothesis, the optimal growth temperature was not related to isolate origin. These results contribute to understanding the presence of the Botryosphaeriaceae in trees and our ability to detect these latent pathogens.

Keywords

Lasiodiplodia, *Neofusicoccum*, environmental variables, climate, *in vitro* mycelial growth.

1. Introduction

The Botryosphaeriaceae is a taxonomically diverse family of endophytic fungi with a broad host plant range and wide geographic distribution. The family accommodates 23 genera including important pathogens such as species of *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Macrophomina* and *Neofusicoccum*. All the genera have been delineated based on comparisons of DNA sequence data in combination with morphological characters (Crous et al., 2015; Phillips et al., 2013; Slippers et al., 2017).

The Botryosphaeriaceae are common on commercially cultivated and native tree species (Mehl et al., 2013; Slippers and Wingfield, 2007). Many of these fungi are latent and opportunistic plant pathogens, predominantly of woody plants (Mehl et al., 2013; Slippers and Wingfield, 2007). They infect trees via wounds or natural openings, causing disease when their hosts are stressed (Slippers and Wingfield, 2007). Diseases attributed to these fungi span all the life stages of plants including seedling damping-off and blight, stem and branch cankers and die-back, blue-stain, root rot, fruit rots, seed capsule abortion, and death of mature trees (Slippers and Wingfield, 2007).

The damage caused by pathogenic Botryosphaeriaceae has stimulated surveys for these fungi, mainly focused on commercially cultivated plants (Bihon et al., 2012; Chen et al., 2011; Trakunyingcharoen et al., 2014; Úrbez-Torres, 2011). However, these studies have not considered how abiotic variables could affect the Botryosphaeriaceae. Recently, Burgess et al. (2019) published a study examining the distribution (location and climate) and host range of these taxa in Australia. This is an important knowelege gap because environmental variables can influence fungal endophyte community composition and diversity in plant species (Coince et al., 2014; Cordier et al., 2012; Giauque and Hawkes, 2016; Peršoh, 2015). For example, tree

endophyte composition differs along a gradient of elevation and the variations are correlated with changes in climatic variables in forests dominated by *Fagus sylvatica* (Coince et al., 2014; Cordier et al., 2012). Similarly, endophyte diversity decreases from cooler and wetter sites to warmer and drier sites in *Panicum hallii* (Giauque and Hawkes, 2016). Especially for the Botryosphaeriaceae, temperature is known to influence the life-cycles of these fungi, hindering or promoting spore production (Swart and Wingfleld, 1991). Consequently, environmental variables are expected to influence species presence of these fungi in natural environments.

The Botryosphaeriaceae have been extensively sampled in *Syzygium cordatum,* a species of Myrtaceae native to South Africa (Pavlic et al., 2009, 2007, 2004; Pavlic-Zupanc et al., 2015; Pillay et al., 2013). These studies have been prompted due to the relatedness of *S. cordatum* to *Eucalyptus* that are planted commercially in the country. Importantly, nothing is known regarding the influence of environmental variables on the presence of Botryosphaeriaceae on this tree.

Syzygium cordatum occurs along a latitudinal gradient on the east coast of South Africa. (Palgrave, 2002). Its distribution stretches from warm sub-tropical areas in the north to cooler temperate areas in the south. Trees grow in close proximity to plantations of commercially propagated non-native *Eucalyptus* species (Myrtaceae) that are known to be alternative hosts for several groups of important pathogens such as the Cryphonectriaceae (Mausse-Sitoe et al., 2016) but also for the Botryosphaeriaceae (Pavlic et al., 2007). Consequently, *S. cordatum* provides a unique opportunity for a case study to interrogate and understand the presence of the Botryosphaeriaceae across a latitudinal gradient.

The objectives of this study were to: i) identify the species of Botryosphaeriaceae present on

S. cordatum trees along a latitudinal gradient on the east coast of South Africa, ii) consider the possible effect of different environmental conditions (historical-climate and current-weather) across this gradient on the presence of the Botryosphaeriaceae on these trees, and iii) determine the optimal temperature for *in vitro* mycelial growth of the collected Botryosphaeriaceae, in order to consider whether this might be related to isolate origin across the latitudinal gradient.

2. Materials and methods

2.1. Study site and field sampling

Samples from apparently healthy *S. cordatum* trees were collected across a latitudinal gradient in South Africa. This latitudinal gradient made it possible to examine different environmental conditions on the presence of Botryosphaeriaceae (Fig 1; Table S1). In March 2013, eleven areas where *S. cordatum* was present were defined. Ten trees per area, where possible, were arbitrarily selected for sampling. Three branches facing different directions were collected from the middle portion of each of these trees (11 areas × 10 trees × 3 branches).

2.2. Fungal isolations

To identify the species of Botryosphaeriaceae present on *S. cordatum* trees, isolations were made from each branch sampled, as soon as possible after collection. Branch sections were surface disinfested (Pavlic et al., 2004), cut in half and both pieces placed face-down onto the surface of 2% malt extract agar (MEA, Biolab, Midrand, South Africa) in Petri dishes. Isolates were purified and those with morphological characteristics typical of the Botryosphaeriaceae such as fluffy white or grey aerial mycelium were selected. These isolates were grouped according to the trees from which they had been collected, as well as culture morphotype. A maximum of five isolates per group (tree and morphotype identity) were selected for further study. Selected isolates were purified by transferring single hyphal tips to clean culture plates

as described by Mehl et al. (2011). Isolates used in the study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, Pretoria, South Africa.

2.3. Fungal identification

DNA was extracted from isolates using the method described by Wright et al. (2010). DNA pellets were suspended in 50 µl TE buffer. DNA extracts were quantified using a NanoDrop® ND-1000 and accompanying software (NanoDrop Technologies, DuPont Agricultural Genomics Laboratories, Delaware).

The ITS rDNA (including the ITS1, 5.8S rRNA, and ITS2), translation elongation factor 1α (*tef1α*), and β-tubulin-2 (*tub2*) loci were amplified using PCR and the primers (i) ITS1, ITS1F, and ITS4 (Gardes and Bruns, 1993; White et al., 1990), (ii) EF1-728F and EF1-986R (Carbone and Kohn, 1999), EF1F and EF2R (Jacobs et al., 2004), and (iii) Bt2a and Bt2b (Glass and Donaldson, 1995) for the three loci, respectively. For PCR amplification, ~20 ng template DNA was used in combination with one of two mixes to amplify loci for DNA sequencing. The first mix consisted of 1 × KAPA Taq Buffer A (KAPA Biosystems, Cape Town, South Africa), 0.4 µM of each primer, 2.5 µM of each dNTP, and 1 U KAPA Taq (KAPA Biosystems). The second mix consisted of 1 × MyTaq Reaction Buffer (Bioline GmbH, Germany), 0.2 µM of each primer, and 0.5 U MyTaq DNA Polymerase (Bioline). Mixes were adjusted to 25 µl per reaction by adding sterile Sabax water (Adcock Ingram, Johannesburg, South Africa). PCR cycling conditions were the same as those used by Mehl et al. (2014). Products from PCR amplifications were stained with Gel-Red (Biotium, Hayward, US) and visualized on 2 % agarose gels run in 1 × TAE buffer. Product sizes were estimated using a Lambda DNA/*Eco*RI + *Hind*III marker 3 (Fermentas Life Sciences, USA).

PCR products were purified and sequenced following the methods described by Mehl et al. (2011). Sequences were examined visually and edited using MEGA5.2 (Tamura et al., 2011). Consensus sequences were subjected to BLASTn analyses to establish preliminary identities. To confirm identities, additional sequences from the ex-type strain and one ex-paratype strain for each species were obtained from GenBank and used to construct sequence datasets for each locus.

Sequences were aligned in MAFFT 6 (Katoh and Toh, 2008) using the G-INS-i algorithm and checked visually. Both, maximum likelihood (ML) and maximum parsimony (MP) phylogenetic analyses were performed on sequence datasets for each locus, as well as the combined dataset for the ITS and *tef1α* regions. For ML analyses, the best nucleotide substitution model for each dataset was determined by parsing the dataset through jModelTest 2.1.3 (Darriba et al., 2012) and selecting the optimal Akaike Information Criterion. Each of the datasets was then analyzed using PhyML v3.0.1 (Guindon et al., 2010) with the model parameters selected by jModelTest. For MP analyses, the same settings as those used by Mehl et al. (2014) were implemented in PAUP* (Phylogenetic Analysis Using Parsimony) v4.0b10 (Swofford, 2003). Robustness of trees from both analyses was determined using 1000 bootstrap analyses. TreeGraph 2 (Stöver and Müller, 2010) was used to visualize trees resulting from both analyses. Sequences generated in the study were deposited in GenBank (Table S2).

2.4. Environmental data along the latitudinal gradient

Samples collected along a latitudinal gradient made it possible to examine areas with different climate and weather conditions. For this purpose, a list of climatic (historical) and weather (current) data for each sampling area was compiled (Table 1 and Table S1). Climate data for

each of the areas were obtained from WorldClim global climate dataset (30-year, 1970-2000, Table S1) (Fick and Hijmans, 2017). Meteorological variables were obtained for the year 2012 from the South African Weather Service (SAWS) (Table S1). For each area sampled, daily meteorological data were sourced from the weather station closest to the collection sites and averages for the year were calculated for each data set.

2.5. Statistical analyses

To study the effect of environmental conditions along the latitudinal gradient on the presence of the *S. cordatum*-associated Botryosphaeriaceae, Botryosphaeriaceae community composition and richness were analysed. For this purpose, the environmental variables that best correlated with Botryosphaeriaceae presence were initially selected. Due to the high dimensionality and possible collinearity of the environmental variables included (Table 1), we used the *bioenv* function of the software R 3.6.2 (R Core Team, 2020). This function selects the best subset of environmental variables such that the Euclidean distances of scaled environmental variables have the maximum Spearman correlation with community dissimilarities (Clarke and Ainsworth, 1993).

The environmental variables chosen by the *bioenv* function were used to conduct a constrained ordination analysis. Differences among community composition of Botryosphaeriaceae and the contribution of environmental conditions (*Tmax_worldclim*) were visualized using distance-based redundancy analysis (dbRDA) (Anderson and Willis, 2003; Legendre and Anderson, 1999). dbRDA was performed on a presence-absence matrix applying Jaccard distance with the *capscale* function of the software R 4.0.0 (Oksanen et al., 2019; R Core Team, 2020). To assess the statistical significance of environmental variables that best correlated with Botryosphaeriaceae community composition, a permutational multivariate analysis of variance

(PERMANOVA) was applied using the *adonis* function of the software R 4.0.0 (Anderson, 2017).

Botryosphaeriaceae species richness was estimated as the total number of species observed per tree. The effect of the environmental variable (*Tmax_worldclim*), that best correlated with Botryosphaeriaceae presence, on species richness was analysed using a generalized linear model (GLM). The environmental variable was included as the explanatory variable and the dependent variable, species richness, was fitted assuming a Poisson error distribution. Statistical analyses were made using the "vegan" package of the software R 4.0.0 (Oksanen et al., 2019; R Core Team, 2020).

2.6. *In vitro* isolate growth at different temperatures

To determine the optimal temperature for growth of the Botryosphaeriaceae collected in this study, *in vitro* mycelial growth of the most commonly occurring species was assessed at different temperatures. The most common Botryosphaeriaceae were defined as those species isolated more than 10 times. These species, seven in total, were not isolated from all areas sampled and a different number of isolates were found for each of the species.

Mycelial plugs (10 mm diameter) were taken from the actively growing margins of each isolate and placed, mycelial surface facing downwards, at the centres of 90 mm MEA plates. Six plates per isolate were incubated in growth chambers at four temperatures ranging from 15 ºC to 30 ºC at 5 ºC intervals. Two measurements of colony diameter perpendicular to each other were made for each temperature after five days of incubation. Culture growth was calculated by subtracting 10 mm from the diameters measured to account for the size of the plug of inoculum and averages were computed. The influence of the temperature on the *in vitro* mycelial growth

of isolates of the seven Botryosphaeriaceae species was analysed using ANOVAs. Statistical analyses were conducted using the "agricolae" package of the software R 4.0.0 (R Core Team, 2020).

3. Results

3.1. Isolate collections, phylogenetic analyses and species identifications

Of the total 104 *S. cordatum* trees sampled, 80 trees yielded positive isolations (159 Botryosphaeriaceae isolates). The ITS dataset consisted of 510 characters (75 parsimony informative, 414 constant, 21 parsimony uninformative), and yielded 60 equally most parsimonious trees (TL = 109, CI = 0.826, RI = 0.986, RC = 0.814). The model selected for ML analysis was TIM1ef (γ = 0.254). The *tef1α* dataset consisted of 312 characters (145 parsimony informative, 155 constant, 12 parsimony uninformative), and yielded 5386 equally most parsimonious trees (TL = 288, CI = 0.74, RI = 0.967, RC = 0.715). The model selected for ML analysis was K80 (ti/tv = 1.376, γ = 0.552). The *tub2* dataset consisted of 377 characters (72 parsimony informative, 298 constant, seven parsimony uninformative), and yielded eight equally most parsimonious trees (TL = 104, CI = 0.808, RI = 0.981, RC = 0.793). The model selected for ML analysis was TrNef (γ = 0.342). The combined analysis of ITS and *tef1α* consisted of 822 characters (220 parsimony informative, 569 constant, 33 parsimony uninformative), and yielded 5702 equally most parsimonious trees (TL = 405, CI = 0.748, RI = 0.972, RC = 0.727). The model selected for ML analysis was HKY (ti/tv = 1.541, γ = 0.293). The PHT value was 0.079.

Isolates collected in this study grouped with known species of the Botryosphaeriaceae in two genera. These included seven species each of *Lasiodiplodia* and *Neofusicoccum* (Fig. 1; Table S1). Five species (*L. gilanensis*, *L. iraniensis*, *L. laeliocattleyae*, *L. rubropurpurea*, and *N.*

cryptoaustrale) were found on *S. cordatum* for the first time, and three species (*L. gilanensis*, *L. laeliocattleyae*, and *L. rubropurpurea*) are reported from South Africa for the first time.

Tree topologies emerging from the MP and ML analyses were similar for each analysis. Differences occurred where some species-linked clades collapsed for a single gene phylogeny, but were evident when analyzing phylogenies for the other loci. For example, *L. theobromae* and *L. laeliocattleyae* had identical ITS sequences (Cruywagen et al., 2017), but were distinct based on *tef1α* and *tub2*. Similarly, species in the *N. parvum-N. ribis* complex (*N. cordaticola*, *N. kwambonambiense*, *N. parvum*, and *N. umdonicola*) were poorly resolved in the *tef1α* phylogeny, but were delineated as distinct species in the single gene phylogenies for ITS and *tub2*. To determine species identities, sequence datasets for the ITS and *tef1α* loci were concatenated and combined (Fig. 2). The single gene phylogenies are included as supplementary data (Fig. S1-3).

3.2. Effects of the environmental variables on the presence of Botryosphaeriaceae

The environmental variable that best correlated with Botryosphaeriaceae presence along the latitudinal gradient was the historical maximum temperature (*Tmax_worldclim*). The dbRDA showed that the Botryosphaeriaceae community composition differed between areas, with dissimilarity increasing with increasing difference in *Tmax_worldclim* (Fig. 3). There was a clear distinction between species related to colder areas and warmer areas (Fig. 3). The PERMANOVA confirmed that *Tmax_worldclim* was a significant factor determining the variation of Botryosphaeriaceae community composition along the latitudinal gradient (R^2 = 0.252, $P < 0.01$). However, Botryosphaeriaceae species richness on *S. cordatum* (number of species per tree) was not significantly influenced by *Tmax_worldclim* ($P = 0.065$) along the latitudinal gradient.

3.3 *In vitro* mycelial growth of Botryosphaeriaceae

The most common Botryosphaeriaceae species (and the number of isolates) included in the *in vitro* experiment were *Lasiodiplodia gonubiensis* (n = 5), *Neofusicoccum cordaticola* (n = 13), *N. cryptoaustrale* (n = 7), *N. luteum* (n = 12), *N. mangiferae* (n = 10), *N. parvum* (n = 14), and *N. umdonicola* (n = 14). The optimal temperature for mycelial growth of all these species was 25 ºC (Fig. S4a). The individual analyses per species showed that temperature, the different isolates of the species and their interaction influenced mycelial growth (Table S3 and Fig. S4b-h). However, the influence of the isolates on mycelial growth showed no relation to isolate origin (Fig. S4b-h).

4. Discussion

Fourteen species of Botryosphaeriaceae were identified from *S. cordatum* along a latitudinal gradient in South Africa. Nine of these species have previously been isolated from this host (Pavlic et al., 2009, 2007; Pillay et al., 2013). Five species were found on *S. cordatum* for the first time, and three species are reported from South Africa for the first time. These results are consistent with previous studies, considering not only *S. cordatum* (Osorio et al., 2017; Pavlic et al., 2009, 2007) showing that species of *Lasiodiplodia* and *Neofusicoccum* are dominant along the eastern coast of South Africa. This is in contrast to species in other genera of the Botryosphaeriaceae (e.g. *Botryosphaeria dothidea*, *Diplodia* species, *Dothiorella* species) that occur more commonly on trees in the interior and western areas of the country (Jami et al., 2017; Mehl et al., 2011; Slippers et al., 2014).

The results of this study showed that maximum historical temperature was the environmental variable that best correlated with Botryosphaeriaceae presence along the latitudinal gradient.

Maximum historical temperature influenced Botryosphaeriaceae community composition on *S. cordatum*, but not Botryosphaeriaceae species richness. Specifically, species isolated from the northern and warmer areas differed from those isolated from the southern and cooler areas.

Consistent with the results of this study, temperature is known to be an important factor in the biology of fungi, especially for their growth rate and survival (Desprez-Loustau et al., 2007; Laine, 2008). In the Botryosphaeriaceae, temperature and humidity has also been shown to influence its distribution in Australia (Burgess et al., 2019). Temperature is also known to influence differences in fungal community composition (Coince et al., 2014; Zimmerman and Vitousek, 2012). Moreover, some studies have revealed that historical climate, rather than short-term annual conditions (weather data), influence fungal assemblages along climatic gradients (e.g. Cordier et al., 2012; Giauque and Hawkes, 2016). Understanding the community composition of Botryopshaeriaceae under such different historical temperatures is clearly relevant to understand and predict Botryosphaeriaceae distributions.

Maximum historical temperature did not affect Botryosphaeriaceae species richness. This suggests that the number of Botryosphaeriaceae species was similar along the latitudinal gradient sampled. We expected different species richness in northern and warmer areas than in southern and cooler areas. As has been demonstrated in previous studies, temperature is normally one of the main factors that explains the richness of fungi in different niches (Talley et al., 2002). For example, the grass *Panicum hallii* had fewer endophyte taxa in historically drier and warmer sites as compared with wetter and cooler sites (Giauque and Hawkes, 2016). Similarly, Arnold and Lutzoni (2007) showed that richness of foliar fungal endophytes from a diverse group of plant communities, gradually increase from high latitudes to low latitudes. We hypothesize that the range of maximum historical temperature in our study (max. 27.5 ºC

- min. 22.3 ºC) might not be sufficiently wide to have shown differences in species richness.

Analysis of *in vitro* mycelial growth for the most commonly isolated Botryosphaeriaceae species showed that all species grew optimally at 25 ºC, but different isolates of the tested species differed in their growth at different temperatures. Although isolates of the same species showed different growth, we expected patterns of phenotypic changes across the latitudinal gradient. Specifically, temperature has been shown to influence mycelial growth of isolates from warm areas growing more rapidly at higher temperatures and those from cool areas performing better at lower temperatures (Robin et al., 2017; Zhan and McDonald, 2011). However, no relationships were observed between optimum temperatures for growth of the most common Botryosphaeriaceae sampled and temperatures across the latitudinal distribution of *S. cordatum*. Future studies should consider whether rare Botryosphaeriaceae species or a wider range of environmental temperatures might influence relationships between temperature for growth of the isolates in culture and environmental temperatures where these fungi occur.

This is the first study to consider the influence of environmental variables on the presence of species of the Botryosphaeriaceae along a latitudinal gradient in South Africa. We showed that maximum historical temperature in which the host trees of these fungi grow, has a clear influence on the community composition of Botryosphaeriaceae species. Optimal temperature for mycelial growth in culture was, however, not related to isolate origin. Broadly, the results of this study provide a foundation to consider environmental factors that influence the presence of the Botryosphaeriaceae in their woody hosts.

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Table 1. List of environmental variables for each sampling area.

Abbreviation	Description
Climatic variables	
Tmax_worldclim	Maximum temperature obtained from WorldClim dataset[a]
Tmin_ worldclim	Minimum temperature obtained from WorldClim dataset
Tmean_ worldclim	Mean temperature obtained from WorldClim dataset
Pmean_ worldclim	Mean precipitation obtained from WorldClim dataset
Weather variables	
Tmax_SAWS	Maximum temperature obtained from the South African Weather Service[b]
Tmin_ SAWS	Minimum temperature obtained from the South African Weather Service
Tmean_ SAWS	Mean temperature obtained from the South African Weather Service
Pmean_ SAWS	Mean precipitation obtained from the South African Weather Service
Wspeed_ SAWS	Wind speed obtained from the South African Weather Service
Hmean_ SAWS	Mean humidity obtained from the South African Weather Service

[a]WorldClim global climate dataset. Averages for the years 1970-2000.

[b]South African Weather Service. Averages for the year 2012.

Fig. 1. Map of the 11 areas sampled, located along a latitudinal gradient in South Africa. Pattern squares represent the species isolated at the areas sampled. Area codes: MBA = Mbazwana, CHA = Charters Creek, MTU = Mtubatuba, RB = Richards Bay, MTZ = Mtunzini, MAN = Mandini, DUR = Durban, MAR = Margate, PED = Port Edward, EL = East London, and PEL = Port Elizabeth.

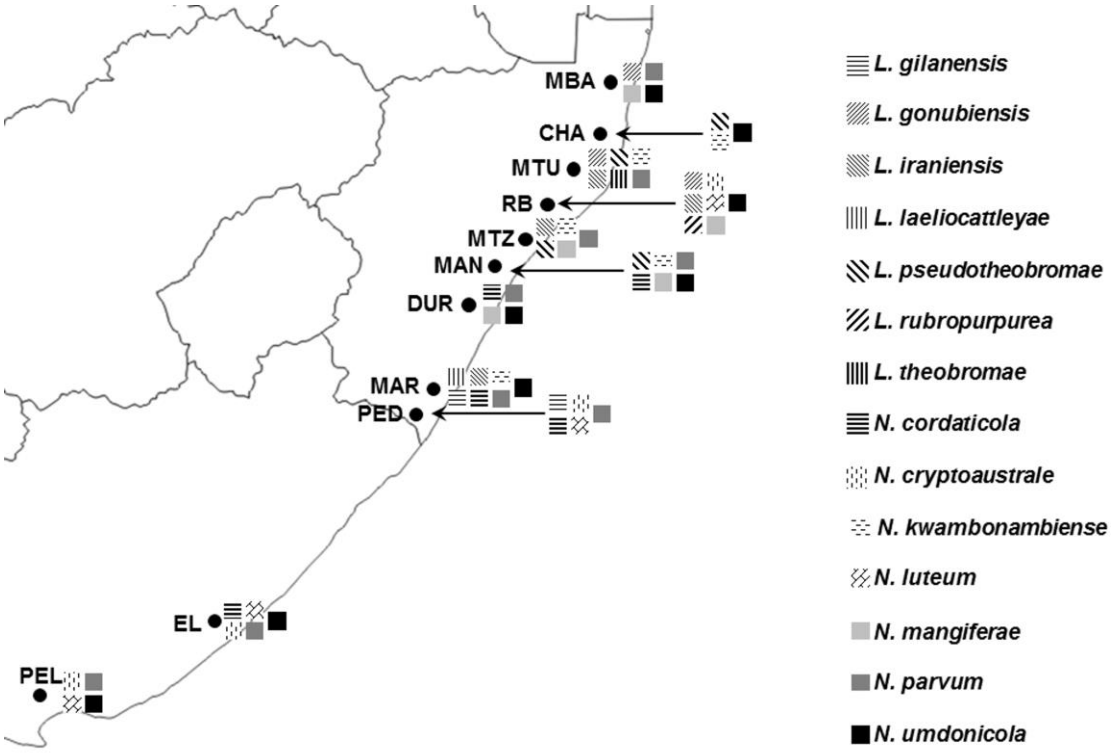

Fig. 2. Maximum likelihood (ML) tree resulting from analysis of the combined ITS and *tef1α* dataset. The tree is rooted in two isolates of *Botryosphaeria dothidea* (CMW7780, CMW8000). Bootstrap values above 70 % for the ML analysis (normal) and maximum parsimony analyses (italicized) appear at the relevant nodes. A bold T after an isolate designates an ex-type isolate for the respective species. Isolates in bold were obtained during this study. Pattern squares illustrate from which areas species were isolated.

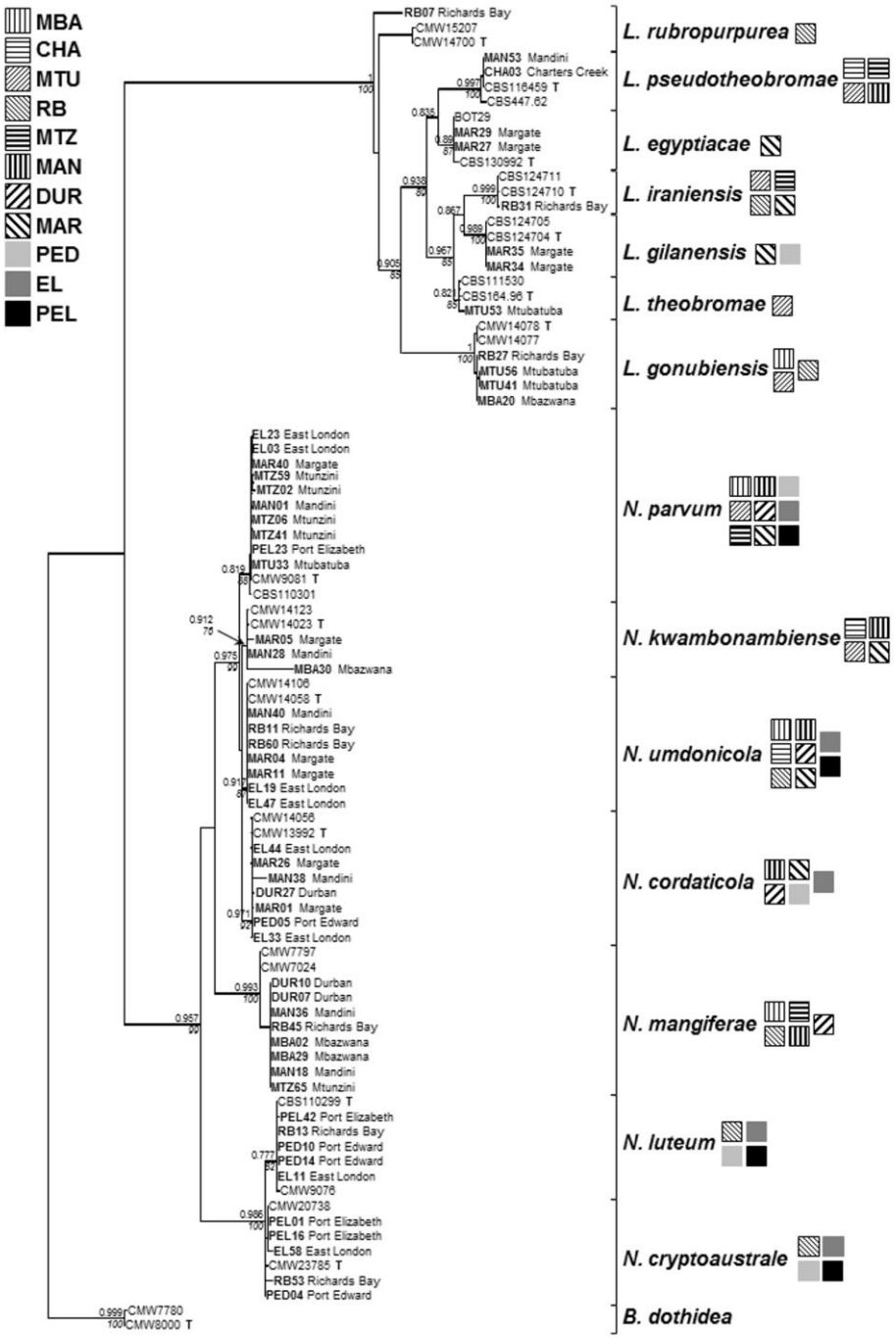

Fig. 3. Botryosphaeriaceae species on *Syzygium cordatum* represented by distance based redundancy analysis (dbRDA). The plot shows the two first components of the dbRDA and the percentages of variation explained by each axis. Botryosphaeriaceae species are labelled in black. Areas sampled are coloured labelled according to its latitudinal gradient (humid sub-tropical climate in the north in red and temperate oceanic climate in the south in blue). The blue arrow indicates the environmental factors best correlated with the presence of Botryosphaeriaceae species on *S. cordatum*. The direction of the arrow indicates the direction of a positive correlation with the species and areas.

Species code: L.lae = *Lasiodiplodia laeliocattleyae*, L.gil = *L. gilanensis*, L.gon = *L. gonubiensis*, L.ira = *L. iraniensis*, L.pse = *L. pseudotheobromae*, L.rub = *L. rubropurpurea*, L.the = *L. theobromae*, N.cor = *Neofusicoccum cordaticola*, N.cry = *N. cryptoaustrale*, N.kwa = *N. kwambonambiense*, N.lut = *N. luteum*, N.man = *N. mangiferae*, N.par = *N. parvum*, N.um = *N. umdonicola*. Area codes: MBA = Mbazwana, CHA = Charters Creek, MTU = Mtubatuba, RB = Richards Bay, MTZ = Mtunzini, MAN = Mandini, DUR = Durban, MAR = Margate, PED = Port Edward, EL = East London, and PEL = Port Elizabeth.