Taxonomy and population diversity of Botryosphaeriaceae associated with woody hosts in South Africa and Western Australia

by

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Dedicated to my dear friends from all around the World

Declaration

I, the undersigned, hereby declare that the thesis submitted herewith for the degree *Philosophiae Doctor* to the University of Pretoria contains my own independent work. This work has hitherto not been submitted for any degree at any other University.

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SUMMARY

The Botryosphaeriaceae (Ascomycetes), with more than 2000 species (http://www.indexfungorum.com), represents one of most widely distributed groups of fungal plant pathogens. These species are known to infect both economically important crops and native plants. In this study species of the Botryosphaeriaceae associated with native woody hosts in South Africa and Western Australia were investigated. Based on ITS rDNA sequence comparisons, combined with phenotypic characters and PCR-RFLP analyses, eight species were identified on native *Syzygium cordatum* in South Africa. These included *Neofusicoccum parvum, N. ribis, N. luteum, N. australe, N. mangiferae*, *Botryosphaeria dothidea, Lasiodiplodia gonubiensis* and *L. theobromae*. Three additional cryptic species were identified in the *N. parvum* / *N. ribis* complex from *S. cordatum* using five gene genealogies and the genealogical concordance phylogenetic species recognition (GCPSR). These are the first species of the Botryosphaeriaceae described using fixed single nucleotide polymorphisms (SNPs) as a defining character, and are described as *N. cordaticola*, *N. kwambonambiense* and *N. umdonicola*. The analysis of microsatellite marker data supported the distinction of these species. These data were also used to characterise the distribution of the latter three species and *N. parvum* on *S. cordatum*. Finding the same haplotypes of *N. parvum* on *S. cordatum* and closely related, planted *Eucalyptus* indicates movement of this pathogen between these hosts. Since all of the species recognised from *S. cordatum* were pathogenic to *Eucalyptus*, and the newly described species were more virulent than *N. parvum* and *N. ribis* on *S. cordatum*, their movement between hosts can pose a serious treat to both native and non-native plants. From Western Australia, molecular sequence data and morphological analyses revealed seven new species of the Botryosphaeriaceae from baobab and other native trees. These included *Dothiorella longicollis*, *Fusicoccum ramosum, Lasiodiplodia margaritacea*, *Neoscytalidium novaehollandiae*, *Pseudofusicoccum adansoniae*, *P. ardesiacum* and *P. kimberleyense*. In the literature review, which also considers work done in this thesis, the influence of molecular tools on the taxonomy of the Botryosphaeriaceae during the last decade, with a particular focus on cryptic species recognition, is considered. This study clearly showed that a polyphasic approach in species identification, as well as investigation of less well studied native flora, will reveal numerous new and cryptic species in the Botrysphaeriaceae and improve our knowledge of this group of important plant pathogens in the future.

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PREFACE

The Botryosphaeriaceae (Ascomycetes) with more than 2000 species (http://www.indexfungorum.com) represents one of the most widely distributed groups of fungal plant pathogens, occurring on a wide variety of economically important woody hosts. Some Botryosphaeriaceae species occur on both native and non-native plants. Their occurrence and diversity on native hosts is, however, often much less well understood than it is on planted crops where they have been more closely studied. In order to answer questions regarding their identity, distribution, diversity, ecology and pathogenicity, the project on which this thesis is based was initiated specifically to focus on the Botryosphaeriaceae associated with native, environmentally important woody hosts.

The experimental work making up this thesis is mostly based on isolates collected from native *Syzygium cordatum* trees across their distribution in South Africa between 2001 and 2003. In Chapter 1, isolates resembling species of the Botryosphaeriaceae were identified and characterised using a single gene approach based on ITS rDNA sequence comparisons, combined with phenotypic characters and PCR-RFLP analyses. Furthermore, the pathogenicity of the identified species from native *S. cordatum* was tested on *S. cordatum* and *Eucalyptus* in greenhouse trials. (**Pavlic D, Slippers B, Coutinho TA, Wingfield MJ. 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. Plant Pathology 56:624–636**)

The most abundant species identified from *S. cordatum* in South Africa were *N. parvum* and *N. ribis*. In many studies, these species have been treated as the *N. parvum* / *N. ribis* species complex. Substantial variation was observed in ITS sequence data and in conidial morphology for isolates in this complex that were collected from *S. cordatum*. This raised the question whether one or more species exist in the *N. parvum* / *N. ribis* complex on *S. cordatum* in South Africa. This question is addressed in Chapter 2, based on the genealogical concordance phylogenetic species recognition (GCPSR), a form of phylogenetic species concept (PSC), using five gene genealogies. In Chapter 2, three undescribed, cryptic phylogenetic species, as well as *N. parvum,* were identified among thirty isolates of the *N. parvum* / *N. ribis* complex from *S. cordatum*. (**Pavlic D, Slippers B, Coutinho TA, Wingfield MJ. 2009. Multiple gene genealogies and phenotypic data reveal cryptic species of the Botryosphaeriaceae: A case study on the *Neofusicoccum parvum* / *N. ribis* complex. Molecular Phylogenetics and Evolution 51:259–268**)

In Chapter 3, sequence comparisons for the RNA polymerase II subunit (RPB2) together with a conidial morphology, is used to clarify identity of a total 114 isolates within the *N. parvum* / *N. ribis* complex. Based on these data, the three phylogenetically recognized taxa in the *N. parvum* / *N. ribis* complex from *S. cordatum* are described as novel species. The newly described species, as well as *N. parvum* and *N. ribis*, are also tested for pathogenicity on *S. cordatum* under greenhouse conditions. (**Pavlic D, Slippers B, Coutinho TA, Wingfield MJ. 2009. Molecular and phenotypic characterisation of three phylogenetic species discovered within the *Neofusicoccum parvum* / *N. ribis* complex. Mycologia, in press**)

Simple Sequence Repeat (SSR) or microsatellite markers can be useful for cryptic species distinction and determination of their population structures. SSR markers have previously been developed for Botryosphaeriaceae including some *Neofusicoccum* species. In Chapter 4, these markers are used to test the GCPSR hypothesis regarding co-existence of four species in the *N. parvum* / *N. ribis* complex on *S. cordatum* in South Africa. These data are also used to investigate inter- and intra-species genetic diversity and structure amongst 114 isolates in the *N. parvum* / *N. ribis* complex from across the distribution of *S. cordatum* in South Africa. A particular focus of this chapter is to compare the diversity and species composition of these *Neofusicoccum* spp. on *S. cordatum* in natural areas, and human disturbed (planted or urban) areas (**Pavlic D, Wingfield MJ, Coutinho TA, Slippers B. 2009. Cryptic diversity and distribution of species in the *Neofusicoccum parvum* / *N. ribis* complex as revealed by microsatellite markers. Molecular Ecology, submitted**)

In line with the previous chapters that considered the diversity of Botryosphaeriaceae on native woody host in South Africa, a matching study was conducted in Western Australia. In Chapter 5, species of this fungal family on baobab (*Adansonia gibbosa*), the only *Adansonia* species endemic to Australia, are characterized. To better understand their host specificity and distribution I also identify them from the twenty-six species of surrounding native trees. These characterizations are accomplished based on anamorph morphology combined with DNA sequences of two loci the ITS and elongation factor 1α (EF-1α). (**Pavlic D, Wingfield MJ, Barber P, Slippers B, Hardy GEStJ, Burgess TI. 2008. Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. Mycologia 100:851–866**)

Accurate identification of species represents the first step as well as a foundation for future biological studies. Subsequent to the establishment of *Botryosphaeria*, by Cesati & de Notaris in 1863, identification and classification systems of these fungi have undergone vast

change. This has come about due to the appearance of new methods that can be used for improved delimitation of species boundaries. The most important of the changes in the taxonomy of the Botryosphaeriaceae have occurred during the last decade with the employment of DNA based tools for species delimitation and identification. This has led to the recognition of numerous cryptic species, to major changes in defining genera in the family Botryosphaeriaceae and to the establishment of order Botryosphaeriales to accommodate these groups. In the contemporary literature review, Chapter 6, presented at the end of this thesis, I summarise the influence of molecular tools on taxonomy of the Botryosphaeriaceae during the last ten years, with a particular focus on cryptic species recognition.