# INVESTIGATION AND ANALYSIS OF TAXONOMIC IRREGULARITIES WITHIN THE BOTRYOSPHAERIACEAE

By Monique Louise Sakalidis (BSc Hons)

This thesis is presented for the fulfilment of the requirements for the degree of Doctor of Philosophy School of Biological Science and Biotechnologies

Murdoch University, Perth Western Australia, March 2011

## Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Monique Sakalidis

## Acknowledgements

I am honoured by the opportunities I have been given throughout the development of this thesis. I am particularly grateful for being given the opportunity to undertake research that I am passionate in and still, find absolutely fascinating.

I owe my deepest gratitude to my two supervisors Dr. Treena Burgess and Associate Professor Giles Hardy for their gentle guidance and wisdom throughout this process. In particular Treena, thank you for sharing your wisdom, ideas and friendship with me and Giles for his endless enthusiasm and his fine attention to detail.

There are many people that have shaped my life since the commencement of this thesis. Friends, family and work colleagues (from Murdoch University, Western Australia and the Forestry and Biotechnology Institute (FABI), South Africa) all have provided support that made my work easier.

To my work colleagues, whom I also consider my friends, particularly Diane White, Vera Andjic, Kate Taylor and Fahimeh Jami thank you for your advice and camaraderie. You have made my work environment a place that I continually enjoyed coming too.

To my coffee buddies over the years, Kerry Ramsay, Linda Maccarone, Stacey Hand and Kylie Ireland I have appreciated your perspective, your friendship and your laughter.

Thank you to Adam Bazinet (Chapter Four), Bernard Slippers (Chapter Four and Five), Brenda Wingfield (Chapter Two, Four and Five), Chyreene Wingfield (Chapter Five), Diane White (Chapter Three), Didier Begoude (Chapter Six), Greg Johnson (Chapter Three ), James Cunnington (Chapter Eight), James Mehl (Chapter Six), Jane Ray

(Chapter Two and Three), Leila Eshraghi (Chapter Three), Mike Wingfield (Chapter Two, Six and Seven) and Vincent Lanoiselet (Chapter Three).

Additionally I would like to thank the staff from the Darwin Botanical Gardens for providing assistance (Chapter Two), Dr. Thomas Jung for his help with the latin description and microscope work (Chapter Four) and the helpful and extensive recommendations of anonymous reviewers towards manuscripts generated from this thesis.

I am grateful for the Australian Post Graduate award and to the Australian Federation of University Women (WA Chapter) for their financial assistance in the form of the Jill Bradshaw bursary which funded my studies. Chapter Two was financially supported by ARC Discovery Project DP0664334 and thank you to Professor Mike Wingfield for providing financial support to fund my time spent at FABI in South Africa.

To my mum Judy Sakalidis, thanks for providing a roof over my head. To my sisters Vanessa and Alexandra, my brother Nicholas and my dad George Sakalidis, his wife Jackie Sakalidis and my grandparents thank you for your support and yes I have finally finished!

Finally I would like to thank my partner Randall Lynton, (for his assistance with the figures in this thesis), he has supported me since the beginning, despite being in a completely contrasting work field- art and design, he understands enough of my work to insist he loves every strand of my DNA. Thank you for your kindness and patience over the years and for never doubting in me.

A PhD is a milestone in life, and the people that have been there throughout it serve as a constant reminder that the PhD is part of a life journey, which will continue once the PhD is finished.

## Abstract

Many members of Botryosphaeriaceae live as endophytes with a latent phase that can cause disease in native and non-native plant hosts around the world. The main Botryosphaeriaceae examined in this thesis included species in the *Lasiodiplodia theobromae* species complex, *Neofusicoccum parvum-ribis* species complex and *Neofusicoccum australe.* A combination of traditional morphology, pathogenicity trials, multiple gene phylogenies and microsatellite analyses were used to probe between and within species.

Within native bushland in the Kimberley, Western Australia, 13 taxa of the Botryosphaeriaceae were identified; *Lasiodiplodia mahajangana* was the most common species and was confirmed as a potentially significant pathogen of *Adansonia gregorii*. These fungi also colonised non-native *Mangifera indica* in the same region possibly displacing the exotic microflora of *M. indica*. Pathogenicity tests resulted in lesion development of mango fruit and excised stems.

Isolates in the *N. parvum- ribis* complex collected from eucalypt cankers in eastern Australia exhibited overlapping morphology and pathogenicity. Phylogenetic analysis of four gene regions and application of the Genealogical Sorting Index to the same data set supported two new species. Consequently, the description of *Neofusicoccum occulatum* is presented. *Neofusicoccum parvum* has been recorded in 71 host species across six continents and 21 countries. Population data analysis of *N. parvum* populations reflects admixture and repeat introductions of new genetic material. No specific host associations were observed.

Evaluation of EF1-α molecular data amongst members of *L. theobromae* species complex suggests there are an additional four taxa and two potential hybrids. The 19 *Lasiodiplodia* taxa have been recorded in 56 host species, across six continents and 23 countries. Nine *Lasiodiplodia* taxa and one hybrid have been identified in Australia. Population analysis suggests the Kimberley populations are sexually reproducing with no discernable host restriction and display moderate genetic diversity.

*Neofusicoccum australe* is found across nine countries and 46 host species. Phylogenetic analysis of the ITSrDNA sequence identified a single dominant ITS haplotype found in most locations and another 12 rare to moderately rare haplotypes found in one to two locations. Using microsatellite markers, populations of *N. australe* were found to be highly diverse and there was no discernable host or habitat restriction. The dominance of *N. australe* in native forest throughout the southwest of Western Australia suggests that this species is endemic to this area.

The species studied in this thesis appear to be capable latent pathogens with no obvious restriction to host colonisation or habitat. Multiple species and multiple genotypes of one species can colonise small sections of a single host. Cryptic sympatric speciation is common despite no observable telomorphs. These species appear to be highly competitive and their endophytic life strategy appears to provide effective means for dissemination via asymptomatic host tissue, which could complicate quarantine efforts that typically rely on the visual presence of disease symptoms.

## Publications arising from the thesis

Sakalidis ML, Hardy GEStJ, Burgess TI, 2011. Endophytes as potential pathogens of the baobab species *Adansonia gregorii*: a focus on the Botryosphaeriaceae. *Fungal Ecology* 4, 1-14.

Sakalidis ML, Ray JD, Lasnoiselet V, Hardy GEStJ, Burgess TI, 2011. Pathogenic Botryosphaeriaceae associated with *Mangifera indica* in the Kimberley Region of Western Australia. *European Journal of Plant Pathology* 130, 379-391.

Sakalidis ML, Hardy GEStJ, Burgess TI, 2011. Use of the Genealogical Sorting Index (GSI) to delineate species boundaries in the *Neofusicoccum parvum- N. ribis* species complex. *Molecular Phylogenetics and Evolution* 60, 333-344.

Sakalidis ML, Hardy GEStJ, Burgess TI, 2011. Class III endophytes, clandestine movement amongst hosts and habitats and their potential for disease; a focus on *Neofusicoccum australe*. *Australasian Plant Pathology* 40, 510-521.

### TreeBase Files

**Chapter Two:** http://purl.org/phylo/treebase/phylows/study/TB2:S10433

**ChapterThree:** http://purl.org/phylo/treebase/phylows/study/TB2:S10464?x-access-code=5e54f63d86f98ac8bcec9cd150571b19&format=html

**Chapter Four:** http://purl.org/phylo/treebase/phylows/study/TB2:S10644?x-access-code=4093d079287d20ecd3ea80519b09821a&format=html

**Chapter Six:** http://purl.org/phylo/treebase/phylows/study/TB2:S11150?x-access-code=96cb33bb7d7704ffedb8e5ee6a92adcc&format=html.

# List of fungal species

*Amphisphaeriaceae* species
*Aplosporella yalgorensis*
*Aureobasidium pullulans*
*Batrachochytrium dendrobatidis*
*Botryosphaeria australis*
*Botryosphaeria corticis*
*Botryosphaeria dothidea*
*Botryosphaeria larincina*
*Botryosphaeria lutea*
*Botryosphaeria rhodina*
*Botryosphaeria ribis*
*Candida* spp.
*Colletotrichum acutatum*
*Colletotrichum gloeosporioides*
*Colletotrichum magna*
*Cryptodiaporthe melanocraspeda*
*Curvularia* sp.
*Cytospora eucalypticola*
*Daldinia eschscholzii*
*Diplodia pinea*
*Diplodia scrobiculata*
*Diplodia seriata*
*Dothideomycete species*
*Dothidotthia sarmentorum*
*Dothiorella longicollis*
*Dothiorella moneti*
*Dothiorella santalui*
*Erysiphe necator*
*Fusicoccum luteum*
*Fusicoccum ramosum*
*Geosmithia* sp.
*Gibberella moniliformis*
*Guignardia larincina*
*Lasiodiplodia citricola*
*Lasiodiplodia crassispora*
*Lasiodiplodia gilanensis*
*Lasiodiplodia gonubiensis*
*Lasiodiplodia hormozganensis*
*Lasiodiplodia* hybrid 1
*Lasiodiplodia* hybrid 2
*Lasiodiplodia iraniensis*
*Lasiodiplodia mahajangana*
*Lasiodiplodia margaritaceae*
*Lasiodiplodia parva*
*Lasiodiplodia plurivora*
*Lasiodiplodia pseudotheobromae*
*Lasiodiplodia rubropurpurea*
*Lasiodiplodia* sp. 1
*Lasiodiplodia* sp. 2
*Lasiodiplodia sp. cryptotheobromae*
*Lasiodiplodia theobromae*
*Lasiodiplodia venezuelensis*
*Lophodermium piceae*
*Magnaporthe oryzae*
*Neofusicoccum australe*
*Neofusicoccum batangarum*
*Neofusicoccum cordaticola*
*Neofusicoccum kwambonambiense*
*Neofusicoccum mangiferae*
*Neofusicoccum occulatum*
*Neofusicoccum parvum*
*Neofusicoccum pennatisporum*
*Neofusicoccum ribis*
*Neofusicoccum umdonicola*
*Neoscytalidium dimidiatum*
*Neoscytalidium novaehollandiae*
*Neurospora cerealis*
*Nigrospora sphaerica*
*Pseudofusicoccum kimberleyense*
*Rhynchosporium secalis*
*Phoma* spp.
*Phytophthora cinnamomi*
*Phytophthora* spp.
*Pseudofusicoccum adansoniae*
*Pseudofusicoccum ardesiacum*
*Rhytidhysteron* sp.
*Sclerostagonospora* sp.
*Sordariomycete* species
*Spencermartinsia uruguayensis*
*Venturia inaequalis*
*Xylariaceae* species

# List of plant species

*Acacia cochlearis*
*Acacia mangium*
*Acacia mearnsii*
*Acacia rostellifera*
*Acacia synchronicia*
*Actinidia deliciosa*
*Adansonia digitata*
*Adansonia grandidieri*
*Adansonia gregorii*
*Adansonia kilima*
*Adansonia madagascariensis*
*Adansonia perrieri*
*Adansonia rubrostipa*
*Adansonia suarezensis*
*Adansonia za*
*Agonis flexuosa*
*Allocasuarina fraseriana*
*Araucaria cunninghami*
*Araucaria heterophylla*
*Araucaria* sp.
*Artemisia* sp.
*Azadirachta indica*
*Balansia*
*Banksia caleyi*
*Banksia coccinea*
*Banksia grandis*
*Blepharocalyx salicifolius*
*Bombax* sp.
*Bruguiera gymnorhiza*
*Bruguiera sexangula*
*Callitris preissii*
*Calytrix* sp.
*Camptotheca acuminata*
*Casuarina cunninghamiana*
*Cinnamomum camphora*
*Citrullus lanatus*
*Citrus aurantium*
*Citrus sinensis*
*Citrus* sp.
*Cocos nucifera*
*Coffea arabica*
*Coffea* sp.
*Corymbia citriodora*
*Corymbia* sp.
*Corymbia torelliana*
*Crotalaria medicaginea*
*Cryptomeria japonica*
*Cupressus funebris*
*Cytisus scoparius*
*Dimocarpus longan* L.
*Diospyros* sp.
*Elaeocarpus holopetalus*
*Epichloë*
*Eucalyptus camaldulensis*
*Eucalyptus citriodora*
*Eucalyptus cloeziana*
*Eucalyptus diversicolor*
*Eucalyptus dorrigoensis*
*Eucalyptus dunnii*
*Eucalyptus filicifolia*
*Eucalyptus globulus*
*Eucalyptus gomphocephala*
*Eucalyptus grandis*
*Eucalyptus grandis* × *Eucalyptus camaldulensis*
*Eucalyptus involucrata*
*Eucalyptus marginata*
*Eucalyptus microcorys*
*Eucalyptus nicholii*
*Eucalyptus ovata*
*Eucalyptus pellita*
*Eucalyptus phylacis*
*Eucalyptus robusta*
*Eucalyptus saligna*
*Eucalyptus scoparia*
*Eucalyptus smithii*
*Eucalyptus* sp.
*Eucalyptus tereticornis*
*Eucalyptus urophylla*
*Eucalyptus urophylla x Eucalyptus camaldulensis*
*Eucalyptus urophylla x Eucalyptus grandis*
*Eugenia repanda*
*Eugenia uruguayensis*
*Ficus opposita*
*Fraxinus augustifolia*
*Gmelina arborea*
*Grevillea agrifolia*
*Grevillea robusta*
*Grevillea* sp.
*Heteropyxis natalensis*
*Hexachlamis edulis*
*Juglans* sp.
*Kolkwitzia amabilis*
*Larix kaempferi*
*Larix* sp.
*Lilium lancifolium*
*Lysiphyllum cunninghamii*
*Malus domestica*
*Malus* sp.
*Malus sylvestris*
*Mangifera indica*
*Maytenus hookeri*
*Melaleuca* sp.
*Musa spientum*
*Myrcianthes pungens*
*Myrciaria tenella*
*Myrrhinium atropurpureum var octandrum*
*Nothapodytes nimmoniana*
*Olea africana*

List of plant species (continued)

*Olea europaea*
*Persea americana*
*Phoenix canariensis*
*Pinus jeffreyi*
*Pinus patula*
*Pinus ponderosa*
*Pinus radiata*
*Pinus* sp.
*Pistacia* sp.
*Pistacia vera*
*Platycladus orientalis*
*Populus nigra*
*Populus* sp.
*Protea cynaroides*
*Protea* sp.
*Prunus ameniaca*
*Prunus domestica*
*Prunus dulcis*
*Prunus persica*
*Prunus salicina*
*Psidium pubifolium*
*Pterocarpus angolensis*
*Punica granatum*
*Pyrus communis*
*Ribes* sp.
*Robinia pseudoacacia*
*Rosa* sp.
*Rubus* sp.
*Salix* sp.
*Salvadora persica*
*Santalum acuminatum*
*Senna siamea*
*Sequoia* sp.
*Sequoiadendron giganteum*
*Sesbania formosa*
*Sonneratia apetala*
*Syzygium cordatum*
*Syzygium guinesee*
*Syzygium paniculatum*
*Terminalia sambesiaca*
*Terminalia* sp.
*Terminalia catappa*
*Terminalia pterocarya*
*Theobromae cacao*
*Tibochina lepidota*
*Tibouchina* sp.
*Tibouchina urvilleana*
*Vaccinium* sp.
*Vitex donniana*
*Vitis* spp.
*Vitis verlandieria x V. rupestris*
*Vitis vinifera*
*Widdringtonia nodiflora*
*Wollemia nobilis*
*Zea mays*

## Table of Contents