

**Molecular phylogenetic studies on
species of
Cryphonectria and related fungi**

by

Henrietta Myburg

Submitted in partial fulfillment of the requirements for the degree of

Philosophiae Doctor

In the Faculty of Natural and Agricultural Science, Department of Genetics, Forestry and
Agricultural Biotechnology Institute, University of Pretoria, Pretoria.

May 2003

Promotor: Prof. Brenda D. Wingfield

Co-promotor: Prof. Michael J. Wingfield

DECLARATION

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Ph.D. to the University of Pretoria, contain my own independent work and have hitherto not been submitted for any degree at any other University.



Henrietta Myburg

May 2003



I dedicate this PhD to Marieka

..... a brilliant scientist,

.....a dear friend,

.....a true inspiration!

Thank you for all the prayers that should have gone into your PhD but has ended up in mine.

Thank you for reminding me that I'm taking a step in the right direction. Thank you for making my PhD come alive by sharing your knowledge and passion for fungi with me.

Thank you for being a guiding light through many chapters of darkness. Thank you for always being there, a smiling face with a word of encouragement. I was blessed to have had you on my team. I will treasure our time together for all eternity!

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
PREFACE	iii
CHAPTER 1 Literature review	1
The taxonomic position of <i>Cryphonectria</i> and <i>Endothia</i> within the order Diaporthales.	
CHAPTER 2	32
β -tubulin and Histone <i>H3</i> gene sequences distinguish <i>Cryphonectria cubensis</i> from South Africa, Asia and South America.	
CHAPTER 3	57
<i>Cryphonectria</i> canker on <i>Tibouchina</i> in South Africa.	
CHAPTER 4	86
Conspecificity of <i>Endothia eugeniae</i> and <i>Cryphonectria cubensis</i> : A re-evaluation based on morphology and DNA sequence data.	
CHAPTER 5	117
Phylogenetic relationships of <i>Cryphonectria</i> and <i>Endothia</i> based on DNA sequence data and morphology.	
CHAPTER 6	154
DNA sequence data and morphology define <i>Cryphonectria</i> species on Fagaceae in Europe and Asia.	



CHAPTER 7	203
Genera and species in the <i>Cryphonectria/Endothia</i> complex and their placement in the Diaporthales: A Molecular and Morphological synopsis.	
SUMMARY	277
APPENDIX 1	289
APPENDIX 2	296
APPENDIX 3	308
APPENDIX 4	328
APPENDIX 5	348



ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

My Heavenly Father and Friend

For blessing me with all the people mentioned below. For His unconditional love, a guiding light at all times.

“The best way to write a PhD is on your knees”

Marieka,

There are not enough words to thank you. I’m forever grateful for all your help. Thank you very much for all the line drawings and photoplates in my thesis. It has brightened up my PhD.

Mike and Brenda,

Thank you for guiding me back to my PhD and not losing faith in me. Thank you for having me in a group that is dynamic and where I could grow as a scientist. I will fondly think back of my time as a family member of FABI.

My family,

Mum and Dad, Zander, Chrisné, Rikkert, Berno, Velma and Marius. Thank you for all your love and prayers.

My friends,

FABI friends, thank you very much for being more than just colleagues during the past years. I will treasure the many wonderful memories of times shared in the lab and those from fieldtrips.

Non-FABI friends, thank you for making sure that I had a life outside the lab.

Others,

Prof. Michael Milgroom, thank you very much for spending some of your sabbatical leave on reading chapters from my PhD. Your critical reviews were most valuable and it improved my scientific writing skills a great deal.

Prof. Jay Stipes, thank you for donating your *Cryphonectria* and *Endothia* culture collection to FABI. This donation has led to a very insightful chapter in my PhD.

Institutions that were responsible for financial support of the studies presented in this thesis are acknowledged at the end of each chapter. Likewise, there are a number of individuals that were involved by contributing fungal cultures and specimens. These contributions led to many of the exciting discoveries presented in this PhD. They are also acknowledged at the end of each chapter.



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

PREFACE

PREFACE

The Diaporthales is a group of fungi that includes some important pathogens of agronomic crops as well as forest and plantation trees. The development of effective control strategies relies strongly on a thorough understanding of the taxonomic relationships of these pathogens as well as their relative pathogenicity. Taxonomic relationships have traditionally been based on morphological characteristics. More recently, comparisons of DNA sequence data also have been shown to provide a powerful basis for reconstructing phylogenetic relationships at all taxonomic levels. The use of both traditional and molecular characteristics in phylogenetic reconstructions, linked to biological data such as pathogenicity, provides a sound basis for studying pathogens in the Diaporthales.

Amongst the better-known genera in the Diaporthales are *Cryphonectria* and its close relative, *Endothia*. *Cryphonectria* is particularly important as it includes one of the most serious tree pathogens, i.e. *Cryphonectria*, discovered during the course of the last century. This fungus is commonly known as the chestnut-blight fungus and has caused serious damage to natural chestnut populations, especially those in North America. Another serious tree pathogen in this genus is *C. cubensis*, the causal agent of a serious canker disease affecting *Eucalyptus* spp. grown mainly in exotic plantations. *Endothia* is known for the pin oak blight fungus, *E. gyrosa*.

Conventional differentiation between species of *Cryphonectria* and *Endothia* has been based on morphological characteristics. Morphology-based taxonomy of these fungi is, however,

not decisive, as species of *Cryphonectria* and *Endothia* have many similar morphological characteristics. Little is known regarding the phylogeny of these genera and the species that they encompass. Phylogeny based on DNA sequence analyses in concert with morphology-based taxonomy presents a relatively unbiased means to assess relationships of the species in these genera.

This thesis presents a series of studies concerning the taxonomy of *Cryphonectria* and related fungi that have been conducted over a period of approximately five years. These studies have focused primarily on comparisons of DNA sequence data, but in many cases also include biology and morphological characteristics. The studies have been conducted sequentially and at the time of completing the thesis, many chapters had already been published or had advanced relatively far through the peer review process. Consequently, elements of redundancy have been unavoidable in certain parts of the document.

Chapter 1 of this thesis represents a literature study that considers the taxonomic position of *Cryphonectria* and *Endothia* within the Diaporthales. This study gives a brief introduction to the Diaporthales and discusses the morphological characteristics that define this order. The families in which *Cryphonectria* and *Endothia* resided have changed frequently with the consequence that the taxonomic position of these genera also been often re-evaluated. Most of these re-evaluations have been based on morphological characteristics and, to a lesser extent, on non-morphological criteria. In the literature review we also reflect on the available molecular and morphological information relating to the species of *Cryphonectria* and *Endothia* as well as the taxonomic challenges that pertain to these genera. Because

some chapters of the thesis have been published prior to completion of the larger document, the literature review might be seen as somewhat outdated. It should, however, be read as a prelude to the following chapters, the contents of which are not reflected in this review.

The ITS (Internal Transcribed Spacer) region of the rDNA operon has been used to differentiate many ascomycete species. However, in recent years, this region has been reported to lack adequate variation to differentiate species in some groups that are closely related. This was also the case where *C. cubensis* from South America and South Africa could not be distinguished at the intraspecific level, although certain biological differences exist. The use of alternative more variable gene regions, such as the protein coding genes, β -tubulin and histone *H3*, to resolve taxonomic and phylogenetic questions has been applied successfully in fungal systems. In **Chapter 2** we incorporate these gene regions to resolve the intraspecific phylogenetic relationships of *C. cubensis* and show how β -tubulin and histone *H3* gene sequences have contributed to our understanding of the phylogeny of *C. cubensis* in South America, South Africa and Southeast Asia. In this chapter the morphological characteristics of *C. cubensis* from these geographical areas are also considered.

Cryphonectria cubensis is well known as the causal agent of Cryphonectria canker of *Eucalyptus* species throughout the world. This fungus has also been shown to cause a canker disease to species other than *Eucalyptus*, but still residing in the Myrtaceae. The most recent host of *C. cubensis* that has been added to the list is *Tibouchina* in Colombia. This occurrence is unusual since *Tibouchina* spp. reside within the Melastomataceae and not

the Myrtaceae. In **Chapter 3** I report on the discovery of *C. cubensis* on *Tibouchina* spp. in South Africa. Identification and characterisation of this fungus was based on morphological comparisons and phylogenetic analyses of the ITS rDNA operon and two regions within the β -tubulin gene. The pathogenicity of the fungus isolated from South African *Tibouchina* species was tested on *T. granulosa* and *E. grandis*.

In **Chapter 4** of this thesis, I consider the relationships between *C. cubensis* and *Endothia eugeniae*, pathogens of *Eucalyptus* and clove species respectively. These fungi had been synonymised previously based on similar results from cross-inoculation studies, isozyme analyses, cultural studies and morphological comparisons. New collections from clove in Brazil and Indonesia have become available to us and provided the opportunity to re-evaluate the conspecificity of *C. cubensis* and *E. eugeniae* based on DNA sequence data. The morphology of the fungi from the new herbarium collections from clove were also studied and compared with previous collections and morphological descriptions of these fungi occurring on clove.

Chapter 5 focuses on the phylogenetic relationships of all described species of *Cryphonectria* and *Endothia* for which cultures are available. No studies exist that have considered the generic and specific relationships of these fungi at DNA level, collectively with morphological criteria. The molecular phylogeny produced was based on the sequence variation found in the ITS region of the rDNA operon and two regions in the β -tubulin gene. Morphological characteristics were also studied to define the different groups emerging from this phylogenetic study. This study also reflects on the usefulness of using both

molecular and morphological criteria to establish a taxonomic framework for the species residing in *Cryphonectria* and *Endothia*.

In **chapter 6**, I investigate the occurrence of *Cryphonectria* species on woody hosts in Europe and Asia. A collection of isolates and specimens from Greece, Japan and China, resembling *Cryphonectria* species, enabled me to conduct a study at both morphological and molecular levels. Identification and characterisation of these fungi was done by comparing morphological and DNA sequence data generated in this study with morphological and phylogenetic data previously published for *Cryphonectria* species.

Our collection of fungi resembling species of *Cryphonectria* has been obtained from a variety of host species from different areas of the world. Other than the recognised species included in chapters 2 to 6, additional isolates possibly representing new species, or established species isolated from new hosts, have been collected from around the world. **Chapter 7** presents a compilation including a broad morphological and molecular outline of all the fungi resembling *Cryphonectria* and *Endothia* that are currently available to us. The phylogenetic relationships of these fungi were determined from DNA sequence analyses of the ITS rDNA operon and two regions of the β -tubulin gene. The morphological data obtained in this study were used to support and explain the phylogenetic groups that emerged from the DNA sequence analyses and to compile a key to species based on morphology. In this study we also evaluate the taxonomic position of these fungi at the family level. This assessment was based on LSU DNA sequence comparisons with other families in the Diaporthales.

The seven chapters presented in this thesis represent independent units of which three have already appeared in peer reviewed scientific journals. A further two are reaching the end of the review process and should appear during the coming year. . Because the thesis has emerged from a series of consecutive studies, repetition between the different chapters has been unavoidable. Studies commenced more than five years prior to completion of this thesis and knowledge gained has increased our understanding of the taxonomy, phylogeny and geographical distribution of the species in *Cryphonectria* and *Endothia*, as well as related fungi. I am convinced that this study will be valuable to those who are interested in the taxonomy and phylogeny of this group of fungi and hope that it will provide an incentive for further studies on them.

SUMMARY

The Diaporthalean genera *Cryphonectria* and *Endothia* are of great importance to forest industries worldwide because they include pathogens that can cause serious damage to plantation trees. They also include devastating pathogens of native forest trees and present substantial threats to natural forest ecosystems. Accurate identification and characterisation of these fungal pathogens are, therefore, important and effective disease control and quarantine strategies depend on this ability. Conventional methods of identification and characterisation of fungi are based on morphological criteria. Advances in DNA based techniques has allowed for an objective means of identifying and describing fungal species.

The main objective of the studies making up this thesis was to gain a better understanding of the taxonomy and phylogeny of *Cryphonectria*, *Endothia* species and related fungal species. The conclusions reached have generally been based on morphological data, DNA sequence analyses and pathogenicity trials. The DNA sequence data used in the phylogenetic analyses making up this thesis were those from the ribosomal ITS region and either one of the protein coding genes, β -tubulin or histone *H3*. Morphological descriptions and comparisons supported most of the phylogenetic groups that have emerged from the DNA sequence analyses. The pathogenicity trials have promoted our understanding of the biology of these fungi.

In **Chapters 2 to 4** I have mainly focused on taxonomic, morphological and/or pathogenicity questions specifically pertaining to *C. cubensis*. A study on the phylogeny of

C. cubensis, based on DNA sequence analyses of only ITS sequence data, showed that this region in the fungal genome lacked sufficient variation to distinguish *C. cubensis* at the intraspecific level. In **Chapter 2** I considered the utility of more variable regions of the fungal genome to resolve some of the taxonomic questions not resolved by the ribosomal ITS sequence data. Two such regions are the protein coding genes, β -tubulin and histone *H3*. Results from the phylogenetic analyses showed that *C. cubensis* from South America, Southeast Asia and South Africa each grouped in distinct well-supported phylogenetic groups. Morphological comparisons, however, indicated that no obvious differences exist between *C. cubensis* from South America, Southeast Asia and South Africa and that differentiation between the different geographic groups will need to be based on DNA sequence data and biological information.

Cryphonectria cubensis is a pathogen of mainly *Eucalyptus* species but is also known to occur on clove species. This fungus has recently been shown to cause a canker disease on native South American *Tibouchina* spp. Similar disease symptoms were found on *Tibouchina* spp. in South Africa and the causal agent was identified as *C. cubensis*, the same fungus infecting *Eucalyptus* spp. in South Africa (**Chapter 3**). This conclusion was based on results of phylogenetic, morphological and pathological studies. These results also indicated that the *C. cubensis* on South African *Tibouchina* is different to the fungus of the same name found on *Tibouchina* spp. in South America. Pathogenicity tests showed that *C. cubensis* isolated from *Tibouchina* in South Africa is more pathogenic than *C. cubensis* from *Tibouchina* in Colombia.

Cryphonectria cubensis and *E. eugeniae*, pathogens of *Eucalyptus* and clove, respectively, have been shown to be conspecific. New isolates and specimens made from clove in Brazil and Indonesia gave us the opportunity not only to confirm the conspecificity of *C. cubensis* and *E. eugeniae* based on DNA sequence similarities, but also to document the occurrence of other fungi on clove that are morphologically similar to *Cryphonectria* species. Morphological and phylogenetic results presented in **Chapter 4** confirmed the conspecificity of *C. cubensis* and *E. eugeniae* and also showed that an unidentified species of *Cryphonectria* occurs together with *C. cubensis* on clove. I have, however, elected not to provide species descriptions for the undescribed fungus, as there are too few specimens available linked to cultures to justify doing this.

In **Chapter 5** I consider the paraphyly of *Cryphonectria* and *Endothia*. The phylogenetic tree emerging from studies in this chapter showed that *Cryphonectria* and *Endothia* species reside in two separate clades. *Cryphonectria* is represented by isolates of *Cryphonectria parasitica*, *C. macrospora*, *C. nitschkei*, *C. eucalypti* and *C. radicalis* (Europe) while *Endothia* included isolates of *E. gyrosa* and *E. singularis*. Morphological comparisons showed that the different species residing in these genera are defined by distinct morphological characteristics. Two phylogenetic clades, grouping separately from the clade representing *Cryphonectria*, emerged from the DNA sequence analyses. The one clade represented *C. cubensis sensu lato* while the other included fungi isolated from *Elaeocarpus dentatus* in New Zealand, respectively. Distinct morphological characteristics, based on anamorph morphology, stromatic structure and ascospore septation, supported their distinct arrangement in the phylogenetic tree. The phylogenetic and morphological data resulting

from this study will be useful to establish a taxonomic framework based on DNA sequence and morphological data, for *Cryphonectria*, *Endothia* and related fungi. This taxonomic framework should assist future researchers and aid in the identification of species residing in *Cryphonectria* and *Endothia* and those related to these genera.

The species in *Cryphonectria* include important tree pathogens as well as apparently innocuous saprophytes. These species, *C. parasitica*, *C. radicalis*, *C. havanensis*, *C. nitschkei* and *C. macrospora*, have a Northern Hemisphere origin and are mainly found on woody hosts such as *Castanea* and *Quercus* spp. These fungal species look similar and have spore sizes that overlap, thus complicating accurate identification. In **Chapter 6** I present the results of phylogenetic analyses and morphological comparisons done on a collection of *Cryphonectria*-like fungi from Greece, Japan and China. The results confirmed the presence of *C. parasitica* and *C. radicalis* occurring on *Quercus* and *Castanea* species as well as a new *Cryphonectria* species closely related to *C. nitschkei* and *C. macrospora*. This new species has been provided with the provisional name *C. clavata* prov. nom. The morphological and phylogenetic data presented will facilitate identification of these fungi occurring on *Quercus* and *Castanea* in Eurasia.

In **Chapter 7** I present a comprehensive synopsis including all the *Cryphonectria*-like fungi in the culture collection that has been available to me, using all the molecular and morphological information currently available for these fungi. Included in this collection of fungi were undescribed fungi resembling species of *Cryphonectria* and *Endothia* that have recently been collected from various hosts and originating from different parts of the world.

Phylogenetic analyses and morphological comparisons showed that, even though *Cryphonectria* and *Endothia* are still well defined by previously described species; new undescribed species residing in these genera exist. The phylogenetic and morphological data also indicate that some of the newly collected fungi resembling *Cryphonectria* spp. should in fact be considered as representing closely related, but separate genera. Morphological features such as anamorph form, stromatal structure and colour as well as ascospore morphology define each of the phylogenetic groups. I was, therefore, able to compile a key to genera and species, based on morphology.

A second aim in **Chapter 7** was to assess the taxonomic position of *Cryphonectria* and *Endothia* as well as the new proposed generic groups at family level. Conclusions were based on LSU rDNA sequence comparisons with other family members in the Diaporthales. Phylogenetic analyses showed that the representatives of *Cryphonectria* and *Endothia* as well as the proposed new generic relatives formed a distinct group within the greater Diaporthales in the LSU rDNA phylogenetic tree. This result provides ample evidence that the genera *Cryphonectria* and *Endothia* should be included in a separate family within the order Diaporthales.

This thesis contains a series of studies that attempt to resolve a large number of problems that have surrounded *Cryphonectria* and related fungal pathogens of trees. It includes a very large DNA sequencing effort and has provided a large number of sequences in GenBank that are already being used by colleagues worldwide. However, it by no means represents a completed story. There are many questions, both at the ordinal, family, generic and species

level that need to be resolved for this group of fungi. At this point, it is my hope that studies presented in this thesis will provide a foundation for further studies of *Cryphonectria* and *Endothia*. Furthermore, I hope that they will encourage an interest in these fungi and lead towards a more complete understanding of one of the most interesting groups of fungi known to me.

OPSOMMING

Die genera *Cryphonectria* en *Endothia* behoort tot die orde Diaporthales. Hierdie genera is baie belangrik vir die internasionale bosbou industrie aangesien dit swampatogene insluit wat ernstige skade kan aanrig aan boomspesies wat aangeplant word in plantasies. Hierdie genera sluit ook patogene in wat 'n bedreiging is vir boomspesies in natuurlike woude. Dit is dus baie belangrik dat effektiewe siektebeheer en kwarantynmaatreëls geformuleer moet word. Samestelling van sulke strategieë is egter afhanklik van korrekte identifikasie en karakterisering van hierdie swampatogene. Konvensionele identifikasie en karakterisering is gebaseer op morfologiese kriteria. Vooruitgang in DNS gebaseerde tegnieke het egter die weg gebaan vir objektiewe maniere om swamspesies te identifiseer en te beskryf.

Die hoofdoel van die studies in hierdie tesis, was om beter insig te bekom aangaande die taksonomie en filogenie van *Cryphonectria* en *Endothia* spesies en verwante swamspesies. Die gevolgtrekkings wat gemaak is, was gebaseer op morfologiese data, DNS volgorde analises en patogenisiteitstoetse. Die DNS volgorde data wat gebruik is in die filogenetiese analises in hierdie tesis, verteenwoordig die ITS gedeelte van die ribosomale DNS operon en een van twee proteïen koderende gene, naamlik β -tubulien of histoon *H3*. Morfologiese beskrywings en vergelykings het die meeste van die filogenetiese groepe ondersteun. Die patogenisiteitstoetse het ons insig aangaande die biologie van hierdie swamspesies bevorder.

In **Hoofstukke 2 tot 4** is daar hoofsaaklik gefokus op die taksonomiese, morfologiese en/of patogenisiteitsvrae aangaande *C. cubensis*. 'n Filogenetiese studie op *C. cubensis*, gebaseer

op die DNS volgorde analise van slegs die ribosomale ITS gedeelte, het gewys dat hierdie gedeelte van 'n swamgenoom nie genoegsame variasie insluit om *C. cubensis* op 'n intraspesifieke vlak te onderskei nie. In **Hoofstuk 2** word meer varieerbare gedeeltes van die swam genoom ge-evalueer om taksonomiese vrae, wat andersins nie met die ribosomale ITS data opgelos kon word nie, te beantwoord. Twee sulke gedeeltes is die proteïen koderende gene, β -tubulien and histoon *H3*. Resultate verkry vanaf die filogenetiese analises het gewys dat *C. cubensis* in Suid-Amerika, Suid-oos Asië en Suid-Afrika in aparte filogenetiese groepe rangskik. Hierdie groepering was in ooreenstemming met die ekologiese inligting betreffende *C. cubensis* in hierdie wêrelddele. Morfologiese vergelykings het egter getoon dat daar geen vanselfsprekende morfologiese verskille is wat gebruik kan word om tussen verskillende geografiese groepe van *C. cubensis* te kan onderskei nie. Dit is dus duidelik dat onderskeiding van *C. cubensis* in hierdie wêrelddele gebaseer moet word op DNS data en biologiese informasie.

Cryphonectria cubensis is 'n patogeen van hoofsaaklik *Eucalyptus* spesies, maar kom ook voor op naeltjie spesies. Hierdie swam is onlangs gekoppel as die oorsaak van 'n kankersiekte op inheemse *Tibouchina* spesies in Suid-Amerika. Soortgelyke siektesimptome is gevind op Suid-Afrikaanse *Tibouchina* spesies en die swam is geïdentifiseer as *C. cubensis*, dieselfde swam wat *Eucalyptus* spesies in Suid-Afrika infekteer (**Hoofstuk 3**). Hierdie afleiding is gemaak vanaf die resultate van die filogenetiese analises, morfologiese vergelykings en patogenisiteitstoetse. Die resultate het ook getoon dat *C. cubensis* op die Suid-Afrikaanse *Tibouchina* spesies verskil van die swam met dieselfde naam wat voorkom op *Tibouchina* spesies in Suid-Amerika.

Patogenisiteitstoetse het gewys dat *C. cubensis*, wat vanaf die Suid-Afrikaanse *Tibouchina* spesies geïsoleer is, meer virulent is as dié vanaf die Colombiaanse *Tibouchina* spesies.

Cryphonectria cubensis en *E. eugeniae* is patogene van onderskeidelik *Eucalyptus* en naeltjie spesies en studies het gewys dat hierdie swampatogene gelyksoortig is. Nuwe isolate en spesimens vanaf naeltjies in Brasilië en Indonesië het die geleentheid verskaf om die gelyksoortigheid van *C. cubensis* en *E. eugeniae* te re-evalueer (**Hoofstuk 4**). Resultate vanaf die filogenetiese analises en morfologiese vergelykings het nie net hierdie gelyksoortigheid ondersteun nie, maar ook die teenwoordigheid van twee nuwe swamspesies op naeltjies bevestig. Hierdie nuwe spesies kon egter nie in die huidige studie beskryf word nie aangesien daar geen spesimens beskikbaar is wat aan hierdie isolate gekoppel kan word nie.

In **Hoofstuk 5** oorweeg ek die parafilie van *Cryphonectria* en *Endothia*. Die filogenetiese boom het aangedui dat spesies in *Cryphonectria* en *Endothia* in twee taksonomiese groepe voorkom. *Cryphonectria* is verteenwoordig deur isolate van *C. parasitica*, *C. macrospora*, *C. nitschkei*, *C. eucalypti* en *C. radicalis* (Europa) terwyl *Endothia* deur isolate van *E. gyrosa* en *E. singularis* voorgestel is. Morfologiese vergelykings het getoon dat die spesies in hierdie genera gedefinieer word deur bepaalde morfologiese kenmerke. Vanuit die DNS volgorde analises het ook twee filogenetiese groepe, wat apart groepeer van *Cryphonectria*, uitgekam. Die een groep verteenwoordig *C. cubensis sensu lato* terwyl die ander een swamme insluit wat van *Elaeocarpus dentatus* in New Zealand geïsoleer is. Kenmerkende morfologiese eieskappe, gebaseer op anamorf morfologie, stromatiese struktuur en

askospoor septasie, het hierdie aparte groepering ondesteun. Die filogenetiese en morfologiese inligting wat in hierdie studie gegeneer is, kan nuttig gebruik word om 'n taksonomiese raamwerk saam te stel vir *Cryphonectria*, *Endothia* en verwante spesies. Hierdie taksonomiese raamwerk sal dan toekomstige navorsers help om korrekte identifikasies te maak van spesies wat in *Cryphonectria* en *Endothia* ingesluit is asook die wat naverwant is aan hierdie genera.

Spesies in *Cryphonectria* verteenwoordig baie belangrike boompatogene asook onskadelike saprofiete. Dit sluit in *C. parasitica*, *C. radicalis*, *C. havanensis*, *C. nitschkei* en *C. macrospora*, swam spesies met 'n Noordelike Halfrond oorsprong en wat hoofsaaklik op houtagtige gashere, soos *Castanea* en *Quercus* spp., voorkom. Hierdie swam spesies lyk baie dieselfde en spoor groottes stem baie ooreen. Hierdie ooreenstemmings maak akkurate identifikasie baie moeilik. In **Hoofstuk 6** word resultate voorgelê wat gegeneer is vanaf filogenetiese analises en morfologiese vergelykings van 'n versameling van swam spesies wat baie lyk soos dié in *Cryphonectria*. Hierdie versameling is geïsoleer vanaf *Castanea* en *Quercus* spp. vanuit Griekeland, Japan en China. Resultate vanuit hierdie studie bevestig dat *C. parasitica* en *C. radicalis* voorkom op *Castanea* en *Quercus* spp. Resultate het ook aangedui dat 'n nuwe *Cryphonectria* spesie op hierdie gashere voorkom. Hierdie nuwe spesie is beskryf as *C. clavata* *prov. nom.* en is filogeneties naby verwant aan *C. nitschkei* en *C. macrospora*. Die morfologiese en filogenetiese data vanuit hierdie studie sal toekomstige identifikasie van hierdie swam spesies op *Quercus* en *Castanea* in Eurasia, vergemaklik.

In **Hoofstuk 7** word 'n alomvattende oorsig voorgelê wat al die *Cryphonectria*-soortgelyke swamme in ons kultuurversameling insluit. Hierdie oorsig sluit in al die molekulêre en morfologiese inligting wat ons tans het oor hierdie fungi. Ingesluit in hierdie versameling was onbeskryfde swamme wat onlangs vanaf verskeie gashere vanuit verskillende werêlddele geïsoleer is. Filogenetiese en morfologiese analyses het getoon dat, alhoewel *Cryphonectria* en *Endothia* nogsteeds deur reeds beskryfde spesies gedefinieer word, nuwe spesies bestaan wat in hierdie genera ingesluit moet word. Hierdie analyses het ook getoon dat van die fungi in hierdie versameling in nuwe genera beskryf moet word. Hierdie genera is naverwant aan *Cryphonectria*. Morfologiese kenmerke soos anamorf vorm, stromatiese struktuur en kleur asook askospoor septasie het elk van hierdie nuwe filogenetiese groepe ondersteun. Dit was dus moontlik om 'n morfologiese sleutel saam te stel vir die genera en spesies wat in hierdie studie geïdentifiseer is.

Die tweede objektief van **Hoofstuk 7** was om die taksonomiese posisie van *Cryphonectria* en *Endothia*, asook die nuwe voorgestelde genera, op familie vlak te evalueer. Gevolgtrekkings was gebaseer op LSU rDNA volgorde vergelykings met ander familieleden in die Diaporthales. Filogenetiese analyses het getoon dat verteenwoordigers van *Cryphonectria* en *Endothia* asook die nuwe genera 'n kenmerkende familie binne die Diaporthales verteenwoordig.

Hierdie tesis bestaan uit 'n reeks van studies wat poog om 'n aantal vraagstukke aangaande *Cryphonectria* en verwante boompatagene spesies op te los. Hierdie studies is gebaseer op aansienlike DNA volgorde analiserings en hierdie inligting verteenwoordig 'n groot aantal

van die DNS volgordes in GenBank. Hierdie inligting in GenBank word reeds deur verskeie kollegas oor die wêreld heen gebruik. Daar moet egter ook besef word dat hierdie tesis nie finale studies insluit nie. Daar is nog verskeie vrae wat op alle taksonomiese vlakke gevra word en wat net in verdere studies beantwoord kan word. Op hierdie oomblik is dit my hoop dat die inligting vervat in hierdie tesis 'n fondament sal verskaf vir verdere studies op *Cryphonectria* en *Endothia*. Verder is dit ook my wens dat hierdie tesis 'n belangstelling in hierdie groep fungi tot gevolg sal hê.