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Taxonomy and phylogeny of *Cryphonectria* and allied genera

by

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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.

Marieka Gryzenhout

June 2006

"Let him who boasts, boast in the Lord" (1 Cor. 1:31)

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

This Ph. D. thesis is, if I must say so myself, a rather unusual thesis. It is the product of serendipity, six years of work and co-operation between different people. It stretched a lot of boundaries. For me, the journey was worth much more than this thick book, this end-product, you are now holding in your hands. I have learnt much, and with this I do not only mean academically. Numerous times I have been looking forward to this moment where I could pin down in eternal words some of my impressions of the past few years. If the reader would indulge me for spilling a bit of my heart and not having a formal and functional list of thank you's, I would like to share my gratitude for the friendships formed over these years, and for the help I received from various people.

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SUMMARY

This thesis represents a critical taxonomical review of the fungal genus *Cryphonectria sensu lato*. An appropriate taxonomy for this group is of great importance because it includes many well known tree pathogens such as the chestnut blight fungus *Cryphonectria parasitica* and the *Eucalyptus* canker pathogen *Cryphonectria cubensis*. The many taxonomic changes introduced in studies presented in this thesis have largely arisen as a result of DNA sequence comparisons for *Cryphonectria* spp. that show that *Cryphonectria sensu lato* is comprised of different lineages, strongly supported by robust morphological characteristics. New taxa, of which many are pathogenic, have also been discovered. The expanded number of species of *Cryphonectria* and related genera as well as the consideration of large numbers of isolates has furthermore made it possible to establish a broad view of the group at the super-generic level.

The first part of the thesis deals with studies on *Cryphonectria cubensis*. A new genus *Chrysoporthe* is described for *C. cubensis sensu lato*. Two additional species are also described for phylogenetic sub-clades previously known as *C. cubensis*. These include *Chrysoporthe austroafricana*, representing all isolates from South Africa, and an anamorphic species described in the new genus *Chrysoporthella as Chrysop. hodgesiana*, which is currently only known from Colombia on native *Tibouchina* spp. Isolate collections from several new host genera for *Chr. cubensis* are also characterized. Collections from *Eucalyptus* in Cuba, now representing the epitype of *Chr. cubensis*, also define the type of *Chr. cubensis* as residing in the South American sub-clade. Another new species, *Chrysoporthe inopima* from *Tibouchina*

lepidota in Colombia is described as well as a new species *Chrysoporthe doradensis* for isolates from *Eucalyptus* spp. in Ecuador.

A new family *Cryphonectriaceae* is described in this thesis for *Cryphonectria*, *Chrysoporthe* and *Endothia*. Genera in this family are united by orange stromatic tissue, with the pigments colouring purple in 3% KOH and yellow in lactic acid. The existence of this new family confirms the close relationship of *Cryphonectria* and morphologically similar genera.

A proposal to conserve the name *Cryphonectria* against the new type *C. parasitica* is presented. This is required because *Cryphonectria gyrosa*, the currently accepted type, was erroneously used as type. The conservation of *Cryphonectria* against *C. parasitica* made it possible to describe the new genus *Amphilogia* for *C. gyrosa*. *Amphilogia* also includes a second species from New Zealand described as *Amphilogia major*, although no isolates currently exist for this species.

New genera for existing *Cryphonectria* spp., as well as newly discovered fungi are presented in this thesis. The new genus *Rostraureum* is established for a fungus pathogenic on *Terminalia ivorensis* in Ecuador. This fungus also represents a new species, *Rostraureum tropicale*. *Cryphonectria longirostris*, originating from Puerto Rico, Trinidad and Tabago, is also transferred to *Rostraureum*. A fungus morphologically similar to *Chrysoporthe* on native *Tibouchina*, *Miconia* and exotic *Eucalyptus* spp. in Colombia, is described as *Aurapex penicillata* gen. sp. nov. *Cryphonectria havanensis* is transferred to the new genus *Microthia*. *Cryphonectria coccolobae* also resides in this genus based on morphology, although its phylogenetic relationship to *C. havanensis* could not be confirmed due the absence of isolates. A new fungus was discovered during surveys for *C. coccolobae* on *Coccoloba uvifera* in Florida, which is described in the new genus *Ursicollum* as *U. fallax*. Phylogenetic

analyses in this study also clearly distinguish *Cryphonectria eucalypti* from *Cryphonectria*, and this fungus is thus transferred to the new genus *Holocryphia*.

A minireview is presented at the end of the thesis and discusses the new taxonomic concepts developed for *Cryphonectria* during this thesis, and recent studies by other authors. The review describes how this new taxonomic scheme has changed our view and understanding of the distribution and ecology of *Cryphonectria sensu stricto* from what it has traditionally been seen.

The final part of the thesis is written in the form of a monograph. It contains background information of all the species, including many pathogens, currently known in *Cryphonectria* and allied genera. The majority of these have recently been described, some in this thesis, and this chapter thus contains all recent information pertaining to them. It is intended that this monograph should be useful as a manual, enabling users to work with and isolate these fungi and to identify the different taxa based on morphology and phylogenetic relationships.

The studies presented in this thesis greatly change the taxonomy of *Cryphonectria sensu lato*, which is now seen as representing a large number of genera and species in a new family. Many would argue that *Cryphonectria* is still monophyletic, but the different lineages shown by DNA sequence comparisons are morphologically inordinately diverse, and clearly represent different genera. Studies presented in this thesis further suggest that additional genera await description from diverse geographical areas and ecological niches. The studies presented in this thesis will hopefully provide a foundation against which these new taxa can be compared and will improve our understanding of tree diseases.

PREFACE

Cryphonectria, in the broad sense, includes some of the most important pathogens of trees in the world. *Cryphonectria parasitica*, also known as the chestnut blight pathogen, caused an epidemic in North America that resulted in the death of vast areas of American chestnut (*Castanea dentata*), and it still negatively effects the this tree today. *Cryphonectria cubensis* is one of the most important pathogens of commercially planted *Eucalyptus* trees in tropical and sub-tropical areas of the world and its impact has shaped the composition of the *Eucalyptus* forestry industries, world wide.

The taxonomy of *Cryphonectria* has been seriously considered in the past. These studies were based on morphology and preceded the common application of DNA sequence comparisons. The taxonomy of this group of fungi was also confused because most works treated *Cryphonectria* as a synonym of the morphologically similar *Endothia*. Recent phylogenetic studies have clearly shown that the taxonomy of *Cryphonectria sensu lato* seriously needs to be reassessed.

This Ph. D. thesis is comprised of a suite of studies that reflects a radical revision of the taxonomy of *Cryphonectria* and allied genera. The thesis is presented in two sections. The first Section is comprised of several taxonomic studies presented in the first ten Chapters. These aim to determine the appropriate taxonomic positions of several *Cryphonectria* spp. and of new collections that generally represent newly discovered pathogens causing tree diseases. The second Section of this thesis represents a monograph treated as Chapter 11. In this monograph, all of the newly recognised genera and species are treated in a single document and all relevant literature pertaining to the taxonomy and

ecology of *Cryphonectria* and allied genera are presented. It also provided the opportunity to re-analyse DNA sequence data for all of the fungi in a single treatment and to compare results from past studies.

Chapters 1 to 3 involve taxonomic and ecological studies on *Cryphonectria cubensis*. Chapter 1 presents the description of a new genus *Chrysoporthe* for this fungus. The different phylogenetic sub-clades previously identified within *C. cubensis* based on DNA sequence data, are also studied further to determine whether they represent discrete species or not. A new sub-clade representing isolates from *Eucalyptus* spp. in Ecuador, is characterized in Chapter 2. Chapter 3 includes reports of several new host genera for *Chr. cubensis* and the description of a new species from Colombia, and it encompasses the epitypication of *Chr. cubensis* based on a collection from *Eucalyptus* in Cuba, the type location of *Chr. cubensis.*

Chapter 4 of this thesis presents studies on the family status of *Cryphonectria* and allied genera in the *Diaporthales*. The possible existence of a new family for *Cryphonectria* and *Endothia* had previously been recognized by earlier authors based on DNA sequences of the large subunit of the ribosomal operon. This warranted the description of a new family for *Cryphonectria*, *Chrysoporthe* and *Endothia*.

The studies presented in Chapter 5 reveal that *C. gyrosa* does not represent the true type of *Cryphonectria*. A proposal is consequently made that the name *Cryphonectria* is conserved against a new type, *C. parasitica.* *C. gyrosa* had been shown in previous studies to group in a distinct and undescribed genus including isolates from *Elaeocarpus* spp. in New Zealand. A new genus, *Amphilogia*, is described for this group in Chapter 6. The species, *Amphilogia major*, is also described in this chapter.

Chapters 7 to 10 of the thesis encompass several descriptions of new genera related to *Cryphonectria* that either represent existing *Cryphonectria* spp. or new species. In Chapter 7, a new fungus found on plantation-grown *Terminalia ivorensis* in Ecuador, is characterized. Its relatedness to *Cryphonectria longirostris*, a fungus that resembles it and known from Puerto Rico and Trinidad & Tabago, is also considered. During surveys for *Chr. cubensis* on native *Melastomataceae* in Colombia, a fungus morphologically similar to *Chrysoporthe* was found on native *Tibouchina*, *Miconia* and exotic *Eucalyptus* spp. This fungus is characterized in Chapter 8. Chapter 9 represents taxonomic studies on *Cryphonectria havanensis*, *Cryphonectria coccolobae* and *Cryphonectria eucalypti*. Surveys for *C. coccolobae* on *Coccoloba uvifera* in Florida yielded another fungus, which is also characterized in this chapter.

Studies presented in this thesis and by previous authors, have revealed that a new taxonomic scheme is needed for *Cryphonectria* and allied genera. The various taxonomic changes that are made in studies presented in this thesis impact on the understanding of the relatedness, ecology and pathology pertaining to this important group of fungi. Chapter 10 summarizes the recent changes made to the taxonomy of species broadly recognized as *Cryphonectria* and it treats the ecology, importance and distribution of these fungi.

Chapter 11 of this thesis is presented as a monograph of the newly recognised *Cryphonectriaceae*. Here I review all species that have been described and taxonomic schemes applied in this thesis. Studies by others relevant to the taxonomy of *Cryphonectria* and related fungi are also treated. The monograph provides information on the ecology and diseases caused by the species in the *Cryphonectriaceae*, morphological descriptions and keys, and phylogenetic trees including all known taxa.

The various chapters in this thesis were written as independent papers during the course of approximately six years. All of the papers, with the exception of the monograph, have been published or accepted for publication in recognised mycological journals. These publications represent part of an accumulating resource of taxonomic literature on the *Cryphonectriaceae* that has ultimately required a summary that is presented in a draft monograph in which all genera and species could be treated collectively. It is my hope that these studies will provide a strong foundation for subsequent studies on the taxonomy, ecology and distribution of this important group of fungi.

LIST OF ABBREVIATIONS

AB, AF, AY, DQ = sequence accession numbers for Genebank.

ATCC = American Type Culture Collection, Manassas, VA 20108, USA.

B = Herbarium, Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin, Berlin, Germany.

BPI = U. S. National Fungus Collections, Systematic Botany and Mycology, Beltsville, USA.

CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

CMW = Culture collection of Michael J. Wingfield, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

CRY = *Cryphonectria* culture collection of Michael J. Wingfield, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

CUP = Plant Pathology Herbarium, Cornell University, Ithaca, New York USA.

DAR = Plant Pathology Herbarium, Orange Agricultural Institute, Forest Road, Orange, N.S.W., Australia.

E = from the culture collection of Prof. R. J. Stipes (Department of Plant Pathology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia, USA) now housed in the culture collection (CMW) of FABI.

FLAS = Mycological Herbarium, Department of Plant Pathology, University of Florida, Gainesville, U.S.A.

IMI = Herbarium, CABI Bioscience, Egham, Surrey, U.K.

ITS = Internal transcribed spacer region of the ribosomal operon.

K = Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, England, U.K.

KB1, CD28, YM2 = isolates used in Liu *et al*. (2003).

KOH = potassium hydroxide.

LSU = Large subunit (28S) of the ribosomal operon.

MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

MEA = malt extract agar

MYA = malt yeast extract agar

NY = William and Lynda Steere Herbarium, New York Botanical Garden, Bronx, New York, USA.

OA = oats agar

PCR = polymerase chain reaction

PDA = potato dextrose agar

PDD = Landcare Research New Zealand Limited, Mt. Albert, Auckland, New Zealand.

PREM = National Collection of Fungi, Pretoria, South Africa.

RFLP = restriction fragment length polymorphism

s. l. = *sensu lato*

s. str. = *sensu stricto*

TFM:FPH = Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan, E or Ep refers to an isolate

TrN = Tamura Nei distance model

WA = water agar

PAD = Erbario Patavinum, Centro Interdipartimentale Musei Scientifici, Università degli Studi di Padova**,** Padova, Italy

G = Herbarium, Conservatoire et Jardin botaniques de la Ville de Genève**, Chambésy**/Genève, Switzerland

FH = Farlow Reference Library and Herbarium of Cryptogamic Botany, **Harvard University,** Cambridge, Massachusetts U.S.A.