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

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.

H. Myburg

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!

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ACKNOWLEDGEMENTS

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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 aangaandie 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 protein 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 protein 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 geidentifiseer as *C. cubensis*, dieselfde swam wat *Eucalyptus* spesies in Suid-Afrika infekteer (**Hoofstuk 3**). Hierdie afleidings 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 geisoleer 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*, uitgekom. 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 gegenereer 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 gegenereer 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 analises 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 analises 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 mootlik 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 rDNS volgorde vergelykings met ander familielede in die Diaporthales. Filogenetiese analises 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 boompatogene spesies op te los. Hierdie studies is gebaseer op aansienlike DNS 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.

CHAPTER 1

Literature Review

The taxonomic position of *Cryphonectria* and *Endothia* in the Diaporthales.

THE TAXONOMIC POSITION OF *CRYPHONECTRIA* AND *ENDOTHIA* IN THE DIAPORTHALES

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1. THE DIAPORTHALES

1.1. Introduction

The Diaporthales includes many well-known plant pathogenic fungi. Taxa in this group are causal agents of a number of diseases on economically important fruit and vegetable crops (Farr et al. 2002), while others are known to produce secondary metabolites that lead to toxicoses in animals (Williamson et al. 1994). Some members of this order are also causal agents of serious tree diseases. Of these, the best known is *Cryphonectria parasitica* (Murrill) Barr (Merkel 1905). This fungus causes the devastating tree disease known as chestnut blight that has resulted in the virtual elimination of the American chestnut [*Castanea dentata* (Marsh.) Borkh.] from natural ecosystems in eastern parts of North America (Anagnostakis 1987, Griffin 1986).

1.2. Morphological characterisation

The Diaporthales is delineated by well-defined morphological characteristics (Barr 1978, Cannon 1988). The most distinctive characteristic is the *Diaporthe*-type centrum (Lutrell 1951), which, in the early stages of development, is characterised by thin-walled pseudoparenchymatous tissue and the absence of paraphyses (Alexopoulus and Mims 1978).

Asci develop between collapsing pseudoparenchymatous tissue (Alexopoulus and Mims 1978). Characteristics such as perithecia with long necks situated in pseudostromata and thick-walled asci are also linked with the *Diaporthe*-type centrum (Alexopoulus and Mims 1978). Furthermore, asci have refractive apical rings (Barr 1978).

Anamorphs of the Diaporthales are usually coelomycetous (Barr 1978, Castlebury et al. 2002, Farr et al. 2001, Hawksworth et al. 1995). Fruiting bodies are either acervuli or pycnidia characterised by the presence or absence of well-developed stromatic tissue (Barr 1978, Castlebury et al. 2002, Farr et al. 2001). Conidiogenous cells are phialidic or annellidic (Barr 1978, Castlebury et al. 2002, Farr et al. 2001). Examples of anamorph genera in the Diaporthales are *Phomopsis* (Sacc.) Bubák and *Cytospora* Ehrenb.:Fr.

1.3. Molecular characterisation

A number of studies have focused on family delineation in the Diaporthales (Barr 1978, Barr 1990, Cannon 1988, Castlebury et al. 2002, Farr et al. 2001, Wehmeyer 1975, Zhang and Blackwell 2001). Families that have been recognised in this order in the past include the Diaporthaceae Höhn. ex Wehm., Gnomoniaceae G. Winter, Melanconidaceae G. Winter, Pseudovalsaceae M.E. Barr and the Valsaceae Tul. & C. Tul. (Zhang and Blackwell 2001). Hawksworth et al. (1995) recognised two families, i.e. the Valsaceae and the Melanconidaceae, accommodating all the members of the Diaporthales. Eriksson et al. (2001) recently presented a web-based outline of the Ascomycota and recognised a third family Vialaeaceae P.F. Cannon in the order.

As opposed to the taxonomy at the morphological level, the taxonomy of the group based on DNA is less well-defined. Examples of DNA-based taxonomic studies that have included Diaporthalean fungi are those of Spatafora and Blackwell (1993) and Rehner and Uecker (1994). Spatafora and Blackwell (1993) focused their study on the ordinal level of perithecial ascomycetes including two species of Diaporthales. Rehner and Uecker (1994) considered the phylogeny of *Phomopsis* anamorphs of *Diaporthe*. These studies, however, did not include representatives for all the families necessary to make definitive and comprehensive conclusions regarding the taxonomy of the order as a whole.

A DNA-based phylogenetic study that included a large assemblage of Diaporthalean species was that of Zhang and Blackwell (2001). The primary purpose of this study was to determine the taxonomic position of the mitotic fungus that causes dogwood anthracnose (*Discula Destructiva* Redlin), but family relationships of other diaporthalean species also were evaluated. This assessment was based on the phylogenetic analyses of sequence data from large (LSU) and small (SSU) subunit rDNA as well as RNA polymerase II (RPB2) gene. The phylogenetic results supported the placement of *D. destructiva* in the Diaporthales.

The twenty diaporthalean species included in the study of Zhang and Blackwell (2001) grouped in three major clades in the Diaporthales based on LSU and SSU sequence analyses. Zhang and Blackwell (2001) concluded that the Valsaceae, as defined by Wehmeyer (1975) and Barr (1990), was paraphyletic because it included species with

allantoid ascospores as well as non-allantoid ascospores. Similarly, the Melanconidaceae of Barr (1978, 1990) and Hawksworth (1995) were also found to be paraphyletic. Species representing the Diaporthaceae and Gnomoniaceae were polyphyletic and the characters that defined them were distributed in more than one clade (Zhang and Blackwell 2001).

Representatives of the Magnaporthaceae P.F. Cannon (Cannon 1994), previously considered in the Diaporthales (Alexopoulos et al. 1996), were also studied by Zhang and Blackwell (2001). Based on similarities in pathogenicity, fungi in this family were considered to reside in the Diaporthales (Cannon 1994). The SSU sequence data of Zhang and Blackwell (2001) showed that the Magnaporthaceae might be connected to the Ophiostomatales or the Sordariales rather than the Diaporthales. These conclusions did, however, not have strong statistical support. The authors could, thus, not recommend an ordinal placement for the Magnaporthaceae with any level of confidence.

The most recent and comprehensive phylogenetic study of Diaporthalean fungi was that of Castlebury et al. (2002). In this study, 69 members of the Diaporthales were compared based on LSU nrDNA sequence data. It was concluded that there are at least six lineages in the Diaporthales and not two (Hawksworth 1995) or three (Eriksson et al. 2001). Four of the six lineages have previously been defined. These are the Melanconidaceae (Barr 1978, 1990, Hawksworth et al. 1995), the Gnomoniaceae (Barr 1978, 1990, Wehmeyer 1975), the Valsaceae (Barr 1978, 1990, Hawksworth et al. 1995) and the Diaporthaceae (Wehmeyer 1975).

Two of the six Diaporthalean lineages proposed by Castlebury et al. (2002) have not previously been recognised as distinct families. These lineages are designated as the *Schizoparme* complex and the *Cryphonectria/Endothia* complex. The *Schizoparme* complex also included the anamorph genera *Coniella* and *Pilidiella* (Castlebury et al. 2002), which were previously accommodated in the Melanconidaceae (Samuels et al. 1993). Castlebury et al. (2002) suggested that the *Schizoparme* complex and *Cryphonectria/Endothia* complex, previously incorporated in the Valsaceae (Hawksworth et al. 1995), might ultimately be distinguished in their own families.

DNA sequence analyses have highlighted the fact that families in the Diaporthales require revision (Castlebury et al. 2002). A number of taxa included in the phylogenetic analyses of Castlebury et al. (2002) did not associate with any of the other lineages and likely represent new families or genera. These taxa represent *Wuestneia* and its *Harkenessia* anamorphs as well as the anamorph species *Greeneria uvicola*. Castlebury et al. (2002) suggested that, as their study did not include all members of the Diaporthales, additional families might emerge in future studies on the Diaporthales.

Castlebury et al. (2002) did not include members of the Vialaeaceae and the Sydowiellaceae in their study due to the lack of representative species. The authors, therefore, excluded both these families from their study. Phylogenetic studies of Zhang and Blackwell (2001) and Farr et al (2001) also lacked adequate representatives of the Sydowiellaceae and thus precluded the opportunity to draw conclusions regarding the ordinal position of this family. The ordinal placement of the Vialaeaceae and the Sydowiellaceae, therefore, remain

uncertain and will only be conclusively resolved once appropriate representatives are found and included in phylogenetic comparisons.

The taxonomic relationships of the families in the Diaporthales require further characterisation. Studies such as those of Zhang and Blackwell (2001), Farr et al. (2001), Eriksson et al. (2001) and Castlebury et al. (2002) suggest that the taxonomy of the Diaporthalean lineages will need additional revision and consideration in future. However, these studies already provide a taxonomic framework, based on morphology and phylogenetic data, which provide a firm foundation for future studies.

2. *CRYPHONECTRIA* AND *ENDOTHIA*

2.1. Introduction

Endothia and *Cryphonectria* were established in the 1800's, with *Endothia* the older of the two names. This genus was established in 1849 by Fries. Fries (1849) separated *Endothia* from *Sphaeria* Haller and based the description of the newly established *Endothia* on the tubular, red to tawny stromata, pale perithecia and evanescent asci of the *Sphaeria gyrosa* Schw. specimens he had received from Schweinitz. *Endothia gyrosa* was designated as the type species.

Cryphonectria was first established as a sub-genus of *Nectria* in 1883 (Saccardo 1883). Saccardo (1905) gave *Cryphonectria* full generic status (Barr 1978, Kobayashi 1970). In

1909, Von Höhnel synonymised *Cryphonectria* with *Endothia*. *Cryphonectria gyrosa* (Berk. & Br.) Sacc. was considered as the type of *Cryphonectria* by Von Höhnel as it was the first species listed in Saccardo's description of *Cryphonectria* (Shear et al. 1917), was thus transferred to *Endothia*. *Cryphonectria gyrosa* was given a new name, *E. tropicalis* Shear & Stevens, as the older name *E. gyrosa* had been used to designate the type of *Endothia* (Barr 1978, Shear et al. 1917).

2.2. Morphology of *Cryphonectria* and *Endothia*

Species of *Cryphonectria* and *Endothia* have superficially similar morphology, both with ascomata in well developed, yellow to orange or orange red stromata, with stromatal wall pigments that turn purple in 3% KOH and yellow in lactic acid (Castlebury et al. 2002). These colour reactions can also be observed in culture (Castlebury et al. 2002). Another morphological feature common to these genera is the dark brown to black perithecial walls (Kobayashi 1970, Shear et al. 1917).

Cryphonectria and *Endothia* species share similar *Endothiella* anamorphs (Barr 1978, Kobayashi 1970, Roane 1986a, Walker et al. 1985). Asexual structures are uni- to multilocular stromata and conidia are minute and aseptate (Kobayashi 1970, Roane 1986a, Shear et al. 1917). Differentiation of *Cryphonectria* and *Endothia* species has been based on ascospore and conidial sizes (Barr 1978, Kobayashi 1970, Roane 1986a), but ranges of spore size usually overlap (Kobayashi 1970, Roane 1986a), complicating conclusive identification and differentiation among the species.

Barr (1978) used stromatal and ascospore morphology to distinguish between *Cryphonectria* and *Endothia*. *Endothia* species were characterised by strongly developed, widely erumpent stromata consisting mainly of pseudoparenchymatous tissue (Barr 1978, Micales and Stipes 1987) with perithecia usually arranged in a diatrypoid fashion (Barr 1978, Micales and Stipes 1987). Species of *Cryphonectria* were characterised by less developed, semi-immersed stromata. The stromatic tissue is mainly prosenchymatous with perithecia arranged in a valsoid fashion (Barr 1978, Micales and Stipes 1987). *Cryphonectria* species were those having one-septate, fusoid to ellipsoid ascospores, while *Endothia* was reserved for species with non-septate, allantoid to cylindrical ascospores (Barr 1978, Micales and Stipes 1987).

Venter et al. (2002) showed that stromatal morphology, rather than ascospore shape and septation is the more useful characteristic to separate *Cryphonectria* from *Endothia*. Ascospore characteristics were found to be an unreliable feature when used to exclusively differentiate between *Cryphonectria* and *Endothia*. In their study, Venter et al. (2002) found that the ascospores of a fungus previously identified as *E. gyrosa* in South Africa and Australia were typical to those of *Endothia*, i.e. unicellular and allantoid (Roane 1986a, Shear et al. 1917) while the stromatal morphology resembled that of *Cryphonectria*. DNA sequence analyses showed that the South African and Australian fungus were the same, should reside in *Cryphonectria* and was described as a new species, *C. eucalypti* Venter and M.J. Wingfield.

2.3. Non-morphological differentiation between *Cryphonectria* and *Endothia*

A number of non-morphological methods have been used to distinguish between *Cryphonectria* and *Endothia* species. These methods include differences in pigment production (Micales et al. 1987, Roane and Stipes 1978), polyacrylamide gel electrophoresis of buffer-soluble proteins (Micales et al. 1987), fungitoxicant tolerance (Micales and Stipes 1986), optimal growth temperatures (Hodges et al. 1986, Stipes and Ratliff 1973) and isozyme analysis (Hodges et al. 1986, Micales et al. 1987). These non-morphological methods were mainly used to differentiate between *C. cubensis*, *C. parasitica* and *E. gyrosa* and could not be used to distinguish between all the species of *Cryphonectria* and *Endothia*. Hodges et al. (1986) also used non-morphological methods to show the conspecificity of *C. cubensis* and *E. eugeniae*.

A number of factors have influenced the use of non-morphological methods to make unambiguous conclusions regarding the taxonomic and phylogenetic relationships of species in *Cryphonectria* and *Endothia*. Hodges et al. (1986) showed that pigment production in culture is variable and is dependant on the formation of pycnidia. Similarly, isozyme metabolism of these fungi changes when they are maintained in culture as opposed to those growing on host material (Micales et al. 1987). Polyacrylamide gel electrophoresis of buffer-soluble proteins and fungitoxicant tolerance allow only for a rapid estimate of the similarity among isolates (Micales et al. 1986) and they allow us only to determine whether the fungal species reside in *Cryphonectria* or *Endothia*.

2.4. Family status of *Cryphonectria* and *Endothia*

Barr (1978) transferred *Endothia* from the Diaporthaceae to the Gnomoniaceae. Differences in ascospore morphology for species in the Diaporthales influenced this decision. The genus *Cryphonectria* was resurrected and accommodated in the Valsaceae (Barr 1978, Micales and Stipes 1987). However, in a subsequent study, Barr (1990) placed greater emphasis on ascospore morphology in these genera and treated *Endothia* in the Valsaceae and *Cryphonectria* in the Gnomoniaceae. Other authors disputed the placement of these genera in two separate families as they were thought too closely related (Chen et al. 1996, Walker et al. 1985). The Gnomoniaceae was reduced to the Valsaceae but the name "Gnomoniaceae" was given *nomen conservandum* status (Hawksworth et al. 1995). Thus *Cryphonectria* and *Endothia* currently reside in the Valsaceae.

Cryphonectria and *Endothia* are closely related. Vasilyeva (1998) recognised this close relationship by placing these genera in the tribe Endothiae M.E. Barr. Castlebury et al. (2002) suggested that *Cryphonectria* and *Endothia* should be considered in a separate family in the Diaporthales. Their results were based in LSU sequence analyses showing that *Cryphonectria* and *Endothia* reside in a discrete lineage. These conclusions were, however, based on the inclusion of only four *Cryphonectria* species (i.e. *C. macrospora*, *C. nitschkei*, *C. parasitica*, *C. cubensis*), one isolate designated as *Endothiella gyrosa* (Castlebury et al. 2002), that probably represented the genus *Endothia*, and an isolate of *E. eugeniae*. Zhang and Blackwell (2001) used LSU and SSU nrDNA sequences to compare species of

Cryphonectria and *Endothia* (i.e. *C. parasitica* and *E. eugeniae*) and also supported the relationship between these genera.

Representatives of two species, not residing in *Cryphonectria* or *Endothia*, were associated with the *Cryphonectria/Endothia* complex designated in Castlebury et al. (2002). These were *Chromendothia citrina* Lar. N. Vasiljeva and *Cryptodiaporthe corni* (Wehm.) Petr. The association of *C. corni* with the *Cryphonectria-Endothia* complex was surprising as *C. corni* grouped separately from the type species of *Cryptodiaporthe*, *C. aesculi*, residing in the Gnomoniaceae. These authors, therefore, suggested that *C. corni* either resides in *Cryphonectria* or *Endothia*.

2.5. Species of *Cryphonectria* and *Endothia*

Cryphonectria and *Endothia* include a number of important fungal pathogens of forest trees as well as less important saprophytic species. Well-documented examples of such pathogens are the chestnut blight fungus *C. parasitica* (Anagnostakis 1987, Merkel 1905, Murrill 1906, Roane 1986a), the *Eucalyptus* canker fungus *C. cubensis* (Bruner) Hodges (Boerboom and Maas 1970, Davison and Coates 1991, Florence et al. 1986, Gibson 1981, Hodges and Reis 1974, Hodges et al. 1979, Sharma et al. 1985a, b, Wingfield et al. 1989) and the pin oak blight fungus, *Endothia gyrosa* (Schw.: Fr.) Fr. (Appel and Stipes 1986, Roane et al. 1974, Stipes and Phipps 1971). The most recent addition to *Cryphonectria* is *C.*

eucalypti M. Venter & M.J. Wingf., a fungal pathogen of *Eucalyptus* spp. in Australia and South Africa (Venter et al. 2001, Venter et al. 2002).

Remaining species of *Endothia* and *Cryphonectria* are considered as saprophytes of woody species in various parts of the world (Roane 1986b). These species include *C. radicalis* (Schw.: Fr.) Barr (Shear et al. 1917), *C. havanensis* (Bruner) Barr (Bruner 1916), *C. gyrosa* (Berk. & Br.) Sacc. (type species) (Berkeley and Broome 1875, Shear et al. 1917), *C. longirostris* (Earle) Micales & Stipes (Earle 1909), *C. coccolobii* (Vizioli) Micales & Stipes (Vizioli 1923), *E. viridistroma* Wehmeyer (Wehmeyer 1936) and *E. singularis* (H. & B. Syd.) Shear & Stevens (Shear et al. 1917).

2.6. Taxonomic challenges relating to *Cryphonectria* and *Endothia* and particularly *C. cubensis*.

Knowledge relating to species of *Cryphonectria* and *Endothia* has increased substantially during the course of the past few years. Acceptance of *Cryphonectria* and *Endothia* as valid genera is now supported by morphological and phylogenetic data (Myburg et al. 1999, Venter et al. 2001, Venter et al. 2002). A firm understanding of the taxonomy of these genera is important, as some of the members are serious pathogens of economically valuable tree species. Knowledge of the taxonomic associations of the species residing in *Cryphonectria* and *Endothia* is essential for the development of disease control strategies and effective identification protocols for this group of fungi. One of the most important of

these is *C. cubensis* and this fungus is the primary topic of the thesis for which this review of the literature is presented.

Cryphonectria cubensis is found in mainly tropical and subtropical areas of the world (Boerboom and Maas 1970, Florence et al. 1986, Hodges and Reis 1974, Wingfield et al. 1989) affecting plantation *Eucalyptus* spp. *Eucalyptus* spp. are propagated in these areas as a source of solid wood products and pulp (Turnbul 2000). These products are important commodities and are valuable to the economic well being of the countries producing them. Damage due to disease is thus recognised as an important threat to the economies of countries propagating exotic plantation species such as *Eucalyptus*.

Cryphonectria cubensis was discovered in South Africa in the late 1980's (Wingfield et al. 1989). At that stage, Cryphonectria canker was considered to be the same as the disease known in South America and Southeast Asia. However, there were significant differences in the symptoms found in South Africa and the rest of the world. In South Africa, infection by *C. cubensis* leads to the formation of only basal cankers on young (1 to 2 year-old) *Eucalyptus* trees (Wingfield et al. 1989, Wingfield 2003). In contrast, in South America and Southeast Asia, cankers develop at the base of trees but also higher up on the stems of *Eucalyptus* trees (Florence et al. 1986, Hodges et al. 1979, Sharma et al. 1985).

Another difference between Cryphonectria canker in South Africa and that in other parts of the world, concerns the states of the fungus found on cankers. In South Africa, cankers are covered with asexual fruiting structures (pycnidia) and sexual fruiting structures (perithecia)

are seldom present (Wingfield et al. 1989). This is different to cankers in South America and Southeast Asia where both pycnidia and/or perithecia are found in abundance on cankers (Van Heerden et al. 1997, Van Heerden and Wingfield 2001, Wingfield et al. 1997). The only time that perithecia have been found on cankers in South Africa was at the time of the discovery of this pathogen in South Africa (Wingfield et al. 1989). These structures were found on a single tree and at the base of a canker below soil level. This difference between the incidence of sexual and asexual fruiting structures on the cankers suggests that sexual reproduction is the common means of reproduction of *C. cubensis* in South America and Southeast Asia while in South Africa, *C. cubensis* reproduces primarily through asexual reproduction.

Studies on *C. cubensis* in South Africa, South America and Southeast Asia were initiated to investigate the differences in disease symptoms in these countries. The first study considering this issue was a DNA based study by Myburg et al. (1999). This phylogenetic study, based on sequence analyses of the ITS region of the rDNA operon, included *C. cubensis* from South America, Southeast Asia and South Africa. The *C. cubensis* isolates from South America and South Africa resolved into one phylogenetic group, separate from those representing *C. cubensis* in Southeast Asia. Based on these results, Myburg et al. (1999) hypothesised that *C. cubensis* in South Africa might have been introduced from South America. These results were puzzling considering the fact that the differences in disease symptoms suggested that the fungus in South Africa was different from the fungus of the same name in South America.

Careful consideration was needed before conclusions could be made regarding the origin of *C. cubensis* in South America, Southeast Asia and South Africa. Myburg et al. (1999) suggested that inconsistencies between the phylogenetic data and the biological data might be the result of a lack of sequence variation in the ITS region of the ribosomal DNA operon. A similar situation has been reported in ITS based phylogenetic studies done on other fungi. For example, studies on *Fusarium* spp. have resulted in researchers largely abandoning the use of ITS sequence data to differentiate between *Fusarium* species, as this region lacked sufficient sequence variation for species in this genus (O'Donnell et al. 1998, O'Donnell et al. 2000, Steenkamp et al. 1999, Steenkamp et al. 2000, Steenkamp et al. 2002). This lack of variation has been resolved by the discovery of more variable regions in the fungal genome such as the β-tubulin (Glass and Donaldson 1995, Donaldson et al. 1995), histone *H3* (Glass and Donaldson 1995, Donaldson et al. 1995 Steenkamp et al. 1999, Steenkamp et al. 2000, Steenkamp et al. 2002), elongation factor EF 1α (Carbone and Kohn 1999) and calmodulin gene regions (Carbone and Kohn 1999). These regions have been used effectively as a discriminating tool for *Fusarium* spp. (O'Donnell et al. 1998, O'Donnell et al. 2000, Steenkamp et al. 1999, Steenkamp et al. 2000, Steenkamp et al. 2002) and offer a solution to resolve the inconsistencies seen with the intraspecific differentiation of *C. cubensis*.

Despite the fact that the ITS region has been abandoned as a taxonomic delineator in *Fusarium* (O'Donnell et al. 1998, O'Donnell et al. 2000, Steenkamp et al. 1999, Steenkamp et al. 2000, Steenkamp et al. 2002), this region is still used successfully in other fungal systems. An example of this is the taxonomic impact ITS sequence analyses have had on

the Hypocrealean genus, *Trichoderma* Pers. The ITS region has proven useful to characterize strains of *Trichoderma* to sectional and sometimes species level (Lieckfeldt and Seifert 2000). This region has also been successfully implemented in the delineation of new *Trichoderma* species (Samuels et al. 1999) and the description of neotypes of previously known *Trichoderma* spp. (Lieckfeldt et al. 1998). It is, therefore, worthwhile to initially include the ITS region of the rDNA operon in phylogenetic analyses before considering alternative gene regions.

The occurrence of *C. cubensis* in countries on different continents raises the issue of the origin of this fungus. A possible origin for *C. cubensis* was suggested by Hodges et al. (1986) who showed that *E. eugeniae* (Nutman & Roberts) Reid & Booth, associated with die-back on clove species in Indonesia, and *C. cubensis* are conspecific (Hodges et al. 1986). Micales et al. (1987) also confirmed this conspecificity. Hodges et al. (1986) suggested that *C. cubensis* could have had an origin in Indonesia where clove is native and that it has spread via the spice trade since the Middle Ages (Gibbs 1909). The fungus would then have cross-infected *Eucalyptus* spp., which was propagated in close proximity to clove. Although this hypothesis might be feasible to explain the occurrence of *C. cubensis* in Southeast Asian countries, DNA sequence data (Myburg et al. 1999) have suggested that an alternative origin should be considered for *C. cubensis* in South American countries.

An origin for *C. cubensis* in South America has recently been proposed. In a study by Wingfield et al. (2001), phylogenetic analyses, pathogenicity trials and morphological comparisons showed that *C. cubensis* causes a canker disease on native *Tibouchina* spp. in

Colombia. In this study, the authors suggested that *C. cubensis* in South America might have originated from native South American *Tibouchina* spp. and other Melastomataceae, rather than having an Indonesian origin. The idea of a South American origin is also supported by a population study on Brazilian *C. cubensis*, suggesting that *C. cubensis* has been present in South America for a long time (Van Zyl et al. 1998).

The study of Wingfield et al. (2001) reporting the occurrence of *C. cubensis* on *Tibouchina* spp. in Colombia was also the first report of *C. cubensis* occurring on host species that resided in a family (Melastomataceae) other than that of *Eucalyptus* (Myrtaceae). What is important in this regard is the fact that the Melastomataceae and Myrtaceae are members of the Myrtales and are now recognised to be relatively closely related (Conti et al. 1996, Conti et al. 1997, Dahlgren and Thorne 1984). The dual occurrence of *C. cubensis* on native trees and exotic plantation tree species in South America further suggest that *C. cubensis* in this country might have originated from native Melastomataceae. In addition, the possibility that *C. cubensis* has passed from native Melastomataceae to clove species or that the fungus has crossed from clove to Melastomataceae will need to be considered in future studies.

The origin of *C. cubensis* in South Africa has been the subject of considerable study and consideration (Van der Merwe 2000, Van Heerden 1999, Van Heerden et al. 2001). Studies in this thesis and some others associated with it have been conducted to address the question of differences between *C. cubensis* in South Africa, South America and Southeast Asia Because of these differences and the fact that origins have been proposed to explain *C.*

cubensis in South America and Southeast Asia, an origin for the South African *C. cubensis* needs to be considered.

In a study of Venter et al (2001), although aimed at the molecular characterisation of *E. gyrosa* in South Africa and Australia, preliminary observations were made regarding the taxonomic position of *C. cubensis* in *Cryphonectria*. Based on the phylogenetic results, the authors proposed that *C. cubensis* might be segregated from *Cryphonectria* in future. The suggestion of separating *C. cubensis* from *Cryphonectria* clearly required investigation as Venter et al. (2001) included only two taxa representing *C. cubensis*, and limited species of *Cryphonectria*. Investigations pertaining to this question have been included in chapters of the thesis for which this review is presented.

Venter et al. (2002) demonstrated the value of using morphological together with DNA-based phylogenetic data when assessing taxonomic relationships in *Cryphonectria sensu lato*. These combined data sets provide a means to re-consider taxonomic relationships at DNA level and to re-evaluate morphological characteristics that have been used to describe the fungal groups in *Cryphonectria* and *Endothia*. No comprehensive study has as yet been done that includes *C. cubensis* and all available *Cryphonectria* and *Endothia* species. The information from these data sets will also be useful as a reliable identification tool to distinguish currently known and new species in *Cryphonectria* and related genera.

CONCLUSIONS

The Diaporthales represents an important group of fungi that includes a number of important plant pathogenic fungal genera. Examples of such genera are *Cryphonectria* and *Endothia*. These genera and others in the Diaporthales have been described based on distinct morphological characteristics. Advances in DNA sequence comparisons and the phylogenetic species concept have provided taxonomists, morphologists and plant pathologists with new and objective characteristics to identify fungi, including those in the Diaporthales. Using traditional morphological criteria in combination with DNA sequence data has already been useful and will certainly be most valuable in the future.

This thesis presents a series of studies relating to questions regarding the taxonomy of the Diaporthales and more specifically species of *Cryphonectria* that are tree pathogens. Some of the key questions that will be considered are as follows:

- Is *Cryphonectria cubensis* in South Africa different from the fungus of the same name found in other parts of the world? (**Chapter 2**)
- Is *Cryphonectria cubensis* present on South African *Tibouchina* spp? (**Chapter 3**)
- Is the conspecificity of *C. cubensis* and *E. eugeniae* valid? (**Chapter 4**)
- What are the phylogenetic relationships between all described species of *Cryphonectria* and *Endothia*? (**Chapter 5**)
- What species of *Cryphonectria* are present on Fagaceous hosts in Europe and Asia? (**Chapter 6**)

- What are the taxonomic positions of *Cryphonectria* and *Endothia*, as well as other undescribed taxa closely related to these genera, within the Diaporthales? (**Chapter 7**)

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CHAPTER 2

β-Tubulin and histone *H3* gene sequences distinguish *Cryphonectria cubensis* from South Africa, Asia and South America.

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β-TUBULIN AND HISTONE *H3* GENE SEQUENCES DISTINGUISH *CRYPHONECTRIA CUBENSIS* FROM SOUTH AFRICA, ASIA AND SOUTH AMERICA.

ABSTRACT

Cryphonectria cubensis is the causal agent of an important stem canker disease of *Eucalyptus*. Previous phylogenetic studies based on sequence data have shown that *C. cubensis* is distinct from other species of *Cryphonectria*, and that *C. cubensis* isolates reside in two distinct groups, consistent with geographical origin. Thus, isolates of *C. cubensis* from South America and South Africa grouped together but apart from those originating from South East Asia and Australia. These results were in contrast to the symptoms of Cryphonectria canker in South Africa, which are different to those observed elsewhere in the world. The aim of this study was to use more variable regions of the fungal genome to test whether South African isolates of *C. cubensis* are genetically distinct from those from other parts of the world. For this comparison, β-tubulin and histone *H3* gene sequences were used. Specimens from South America, Southeast Asia, Australia and South Africa were also compared morphologically. The phylogram emerging from the analysis indicated that South American and Southeast Asian/Australian isolates resided in two, well resolved but closely related clades. However, isolates from South Africa were distinct from other groups. This is

consistent with ecological aspects of the South African fungus, although no obvious morphological differences between the fungi from the various regions could be found. Our results suggest that the South African fungus represents a species distinct from *C. cubensis* occurring elsewhere in the world.

INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is an important stem canker pathogen of *Eucalyptus* trees in plantations. It causes a disease known as Cryphonectria canker that is common in many tropical and sub-tropical parts of the world where *Eucalyptus* spp. are propagated commercially (Boerboom and Maas 1970, Davison and Coates 1991, Florence et al. 1986, Gibson 1981, Hodges and Reis 1974, Hodges et al. 1979, Sharma et al. 1985a, b, Wingfield et al. 1989). *Eucalyptus* species are primarily cultivated as a source of fibre to produce pulp and to a lesser extent, solid wood products. In South Africa, *Eucalyptus* species are widely grown in plantations and Cryphonectria canker is of major concern to local forestry companies (Wingfield et al. 1989).

Cryphonectria cubensis was first observed in South Africa in 1988 (Wingfield et al. 1989). Surveys for this fungus in the late 1970's did not detect the pathogen, and it thus appeared that the pathogen had been introduced recently into the country. Consistent with this view, Van Heerden and Wingfield (2001) showed that *C. cubensis* in South Africa is represented by a relatively low number of genets and thus, a narrow genetic base. This is in contrast to

the high level of genetic variation that is found in *C. cubensis* populations elsewhere (Van Heerden et al. 1997).

Cryphonectria canker in South Africa is different from the disease occurring elsewhere in the world. While cankers in South America and Southeast Asia are generally at various heights on stems, those in South Africa form exclusively at the bases of trees (Conradie et al. 1990). Another unusual aspect of the disease in South Africa is that only asexual fruiting structures are found on cankers. This is in contrast to those in Southeast Asia and South America where sexual and/or asexual fruiting bodies are the predominant structures present on cankers (Van Heerden and Wingfield 2001).

In a previous phylogenetic study, *C. cubensis* isolates from various parts of the world were compared using ribosomal RNA gene sequence data (Myburg et al. 1999). Results showed that isolates from South Africa and South America group together and separately from those originating in Southeast Asia and Australia. This result was inconsistent with pathological data, which suggested that *C. cubensis* from South Africa might be different from the fungus occurring elsewhere in the world.

Previous studies have shown that β-tubulin and histone *H3* gene regions are polymorphic (Donaldson et al. 1995, Glass and Donaldson 1995, Steenkamp et al. 1999, Steenkamp et al. 2000) and useful in phylogenetic studies on fungi. The aim of this study was, therefore, to compare isolates of *C. cubensis* from South Africa with those from other parts of the world

using DNA sequences likely to have higher resolution than the ITS region of the ribosomal RNA operon. Morphological comparisons of asexual fruiting structures were also made to determine whether specimens from South Africa could be distinguished from those originating in Southeast Asia and South America.

MATERIALS AND METHODS

Fungal isolates

Isolates used in this study included *Cryphonectria parasitica* (Murr.) Barr, the causal agent of chestnut blight (Elliston, 1981), and *C. cubensis* isolates from Southeast Asia, Australia, South America and South Africa (Table 1). *Diaporthe ambigua* Nits., a canker pathogen of stone and pome fruit trees (Smit et al. 1996, Smit et al. 1997) was included as outgroup. All isolates used in this study are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

DNA isolation and amplification

DNA isolations were performed as described by Myburg et al. (1999). PCR amplifications were performed as described by Glass and Donaldson (1995). Amplification reactions of the β-tubulin gene and the histone *H3* gene were done using primer pairs Bt1a/1b, Bt2a/2b and H3 1a/1b respectively. Each 50µl amplification reaction consisted of the following: 1mM dNTPs (0.25mM of each), 1× reaction buffer (supplied with the enzyme), 2.5mM $MgCl_2$,

0.1μM of each primer, 5 units of Expand *Taq* polymerase (Roche Biochemicals, Mannheim, Germany) and DNA template. Amplifications were done on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied BioSystems, Inc., Foster City, California). Amplification of the histone *H3* gene was done using the following reaction conditions: an initial denaturing step at 94 °C (1 min), followed by 30 cycles of denaturing at 94 °C (1 min), annealing at 68 °C (1 min), and elongation at 72 °C (1 min). Amplification of the Bt1a/1b region of the β-tubulin gene was done using the same reaction conditions except that the annealing temperature was adjusted to 60 °C. Amplification of the Bt2a/2b region was done over a range of annealing temperatures (55 °C-68 °C) because the Bt2a/2b primers annealed at different temperatures for the respective isolates used in this study. PCR products were visualised on 1% agarose (Promega, Madison, Wisconsin) gels containing ethidium bromide.

DNA sequencing

PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). The PCR products were sequenced in both directions using the same primers used in the amplification reactions. Sequencing reactions were conducted using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, United Kingdom). DNA sequences were determined using an ABI PRISM 377™ automated DNA sequencer.

Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) was used to translate the β-tubulin and histone *H3* DNA sequences into putative amino-acid sequences. The amino-acid sequence of the respective β-tubulin gene was compared with β-tubulin amino-acid sequence of *Neurospora crassa* Shear and B.O. Dodge (Genbank accession no. M13630, Orbach et al. 1986). The amino-acid sequence of the histone *H3* gene was compared to that of *N. crassa* (Genbank accession no. CAA25761, Woudt et al. 1983, Glass and Donaldson 1995). Conserved exon and variable intron sites were determined for *C. cubensis*, *C. parasitica* and *D. ambigua*. DNA sequences were aligned using CLUSTAL W (Thompson et al. 1997) and the alignment was checked manually.

Phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford, 1998). A partition-homogeneity test was performed on a combined data set including β-tubulin and histone *H3* gene sequences. Analyses were done using heuristic searches with no branch swapping and MULTREES (saving all optimal trees) options effective. Gaps were treated as fifth characters (NEWSTATE) in the heuristic searches. The confidence levels of the branching points were determined by a bootstrap analysis (1000 replications). *Diaporthe ambigua* was used as outgroup taxon to root trees. Sequences were deposited in Genbank and accession numbers are listed in Table 1.

Morphological comparisons

Anamorph structures of *C. cubensis* occurring on *E. grandis* bark from South Africa, were examined microscopically. These specimens, PREM 49377, PREM 49378, PREM 49379 (Table 2), were those deposited as part of the study by Wingfield et al. (1989). Specimens of bark with pycnidia from Colombia, Mexico, Vietnam, Indonesia and Hong Kong, were also examined (Table 2). Pycnidia were sectioned in length using a Leitz 1310K freezing microtome and KRYOMAT 1700 generator, following a method described by Venter et al. (2001a). Sections of pycnidia, as well as other anamorph structures, were subsequently examined using a Zeiss Axioskop compound microscope. Measurements were presented as (min-)(x bar -sd) - (x bar +sd)(-max).

RESULTS

DNA amplification and sequencing

The β-tubulin gene fragments (1a/1b and 2a/2b) were both approximately 550 bp (base pairs) in size. Amplification of the histone *H3* gene generated a 550 bp fragment. Manual alignment of the β-tubulin gene and histone *H3* gene sequence data resulted in a total of 1365 characters (Appendix 1). Positions of introns and exons of the β-tubulin and histone *H3* genes amplified in this study, were the same as those of *N. crassa* (Genbank accession no. M13630 and CAA25761 respectively). The coding regions were highly conserved with

sequence variation limited to the third codon position. No insertions or deletions of coding regions were observed in the gene regions considered in this study.

The partition-homogeneity test generated a P-value of 0.01. This indicated that the β-tubulin gene and the histone *H3* gene sequence data sets could be combined into one phylogenetic analysis. The aligned β-tubulin and histone *H3* gene sequences were, therefore, analysed as one data set. For each taxon 1365 characters were included in the heuristic search. Among these, 966 characters were constant and 136 characters parsimony-uninformative. One most parsimonious tree (length of tree = 499 steps, consistency index (CI) = 0.9579 and retention index (RI) = 0.9651) was produced from 263 parsimony-informative characters (Fig. 1).

The phylogenetic tree (Fig. 1) generated from the combined β-tubulin and histone *H3* gene sequence data showed that all of the *C. cubensis* isolates grouped within one clade (bootstrap = 100%), separately from the *C. parasitica* isolates and the outgroup, *D. ambigua*. Within the greater *C. cubensis* clade, three distinct groups were obvious. These included a South American (bootstrap = 90%), a Southeast Asian/Australian (bootstrap = 80%) and a South African sub-clade (bootstrap = 95%). The South American and Southeast Asian/Australian clades were more closely related to each other (bootstrap value = 55%) than they were to the South African clade (Fig. 1).

Morphological comparisons

Pycnidia from the South African specimens differed slightly in morphology from structures collected in South America and Southeast Asia. Pycnidia from South Africa had an obvious eustromatic appearance where the layer of cells giving rise to the conidiophores was convoluted (Fig. 2a) and tissues in the pycnidial walls were prosenchymatous. Furthermore, sections through the edge of the pycnidia often revealed more than one cavity (Fig. 2b), while those near the middle revealed a single cavity (Fig. 2a). In contrast, pycnidial cavities from South American and Southeast Asian collections (Fig. 2c, 2d) were seldom as strongly convoluted, and longitudinal sections rarely revealed more than one pycnidial cavity. Where additional conidial cavities were observed, these were usually small (Fig. 2d).

Conidia from specimens representing different geographical areas were similar in size and shape (Fig. 2e-h). Conidia from Southeast Asian were 3-4(-4.5) x 1-1.5 µm (Fig. 2e), South American conidia were 3-4(-4.5) x 1-1.5 µm (Fig. 2f), and conidia from South Africa were (3-)3.5-4.5(-5) x 1-1.5 µm (Fig. 2g). Although rarely so, some conidia from the South African collections had papillate apices (Fig. 2h). These have not previously been noted in *C. cubensis* and were not found in the specimens from other parts of the world.

DISCUSSION

Using comparisons of histone *H3* and β-tubulin gene sequences, we have been able to show conclusively that *C. cubensis* from South Africa is phylogenetically distinct from the fungus

of the same name occurring in Southeast Asia/Australia and South America. This finding is consistent with the fact that the fungus in South Africa is associated with different symptoms from those found in the latter areas. Our results suggest that the South African fungus has an origin different from that of *C. cubensis* from other parts of the world.

In a previous study, Myburg et al. (1999) examined the relationships between *C. cubensis* and other *Cryphonectria* spp. as well as between *C. cubensis* from different hosts and areas. Results of the present study confirm results from Myburg et al. (1999) that the fungus from Southeast Asia/Australia and South America forms two distinct, yet closely related groups. However, ITS sequences presented by Myburg et al. (1999), indicated that South African isolates were most closely related to the fungus from South America. However, the sequences from the ITS1/ITS2 regions are insufficiently variable to resolve the taxonomic questions relating to *C. cubensis sensu lato.*

Cryphonectria cubensis cankers in South America and Southeast Asia are typically covered with perithecia. Pycnidia are present but rare (M.J. Wingfield, data not shown). This is in contrast to the situation in South Africa where structures thought to be perithecia of *C. cubensis* have been observed only once (Wingfield et al. 1989) and pycnidia are the dominant structures on cankers (Van Heerden and Wingfield 2001). These differences, and the unique nature of cankers in South Africa, are consistent with findings in this study, showing that the South African fungus is unique.

Cankers caused by *C. cubensis* in Australia have only been reported once (Davison and Coates 1991) and are different from cankers observed in Southeast Asia. These cankers on *E. marginata* Sm. were on the roots and only pycnidia were observed (Davison and Coates 1991). This could indicate that the Australian fungus is also different. Molecular data reported in the present study, however, clearly show that the Australian fungus is part of the Southeast Asian subclade.

The overall morphology of pycnidia found on cankers in South Africa, Southeast Asia and South America is very similar. In this study, however, slight differences between pycnidia from South Africa and those from elsewhere in the world have been detected. Small differences were also noted in conidial shape, although the papillate apices of a small number of South African conidia are an insufficiently consistent feature to note with any confidence.

Previous descriptions of *C. cubensis* have treated the anamorph fruiting structures as pycnidia (Bruner 1917, Hodges et al. 1976, Hodges 1980). This is possibly due to the fact that the anamorph has the typical shape and appearance of a pycnidium when viewed on the bark. However, when sectioned, the anamorph structures closely resemble convoluted eustromata (Hawksworth et al. 1996). We suggest that the term eustroma should be used in future to describe the anamorph structure of *C. cubensis.*

Although not an objective of the present study, our results confirm those of previous studies (Myburg et al. 1999, Venter et al. 2001b) that *C. cubensis* is very distantly related to *C. parasitica.* This is also consistent with the fact that the two fungi can be distinguished by a number of clear morphological characteristics. The most obvious of these are that *C. cubensis* has loosely aggregated perithecia embedded in a weakly developed basal stroma. This is different from *C. parasitica* where perithecial bases are embedded in a well developed stroma. These differences have led Venter et al. (2001b) to conclude that the fungi probably reside in distinct genera. This conclusion is supported by SSU and LSU sequence data, where *C. cubensis* (= *E. eugeniae*) does not group in the same clade as *C. parasitica* (Zhang and Blackwell 2001). Results of the present study have also shown that β-tubulin and histone *H3* gene sequence data should be useful in future investigations aimed at providing better resolution to differentiate between various species of *Cryphonectria* and related fungi.

Substantial sequence data are now available to support the view that *C. cubensis* in South Africa is different from the fungus with the same name occurring elsewhere in the world. Available data also suggest that the South African fungus and *C. cubensis* elsewhere in the world have different origins. One commonly held hypothesis is that *C. cubensis* originated from native clove trees (*Syzigium aromaticum* (L.) Merr. and Perry, Myrtaceae) in Indonesia (Hodges et al. 1986). There is equally strong evidence to suggest that *C. cubensis* originated on *Tibouchina* trees (Melastomataceae) in South America (Wingfield et al. 2001). This raises the intriguing question of where the South African fungus might have originated.

Based on results of this study, the origin of the South African fungus is likely to be different from the origin of the fungus in South America, Southeast Asia and Australia. Resolution of this question is likely to emerge from collections of fungi similar to *C. cubensis* on native Myrtaceae and Melastomataceae, both in Africa and elsewhere in the world.

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Table 1. Taxa used in this study.

Species	Culture no*	Origin	Genbank accession number
Cryphonectria cubensis	CMW 2113	South Africa	AF273067[¶], AF273462[¶], AF281805[#]
Cryphonectria cubensis	CMW 62	South Africa	AF273063[¶], AF273458[¶], AF281806[#]
Cryphonectria cubensis	CMW 8755	South Africa	AF273064[¶], AF273459[¶], AF281807[#]
Cryphonectria cubensis	CMW 8757	Venezuela	AF273069[¶], AF273464[¶], AF281810[#]
Cryphonectria cubensis	CMW 8758	Venezuela	AF273068[¶], AF273463[¶], AF281243[#]
Cryphonectria cubensis	CMW 1853	Brazil	AF273070[¶], AF273465[¶], AF281808[#]
Cryphonectria cubensis	CMW 8756	Indonesia	AF273077[¶], AF375606[¶], AF285165[#]
Cryphonectria cubensis	CMW 1840	China	AF273071[¶], AF273466[¶], AF281814[#]
Cryphonectria cubensis	CMW 2632	Australia	AF273078[¶], AF375607[¶], AF466697[¶]
Cryphonectria parasitica	CMW 1652	USA	AF273468[§], AF273075[§], AF281802[#]
Cryphonectria parasitica	CMW 7047	USA	AF273469[§], AF273073[§], AF281803[#]
Cryphonectria parasitica	CMW 7048	USA	AF273470[§], AF273076[§], AF281804[#]
Diaporthe ambigua	CMW 2498	Netherlands	AF273471[§], AF273072[§], AF281815[#]

* Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.

[§] β-tubulin 1a/1b and 2a/2b sequence data obtained from Venter et al. (2001a).

[¶] β-tubulin 1a/1b and 2a/2b sequence data generated in this study.

[#] Histone *H3* sequence data generated in this study.

Table 2. Specimens used in morphological comparisons.

Herbarium no.*	Identity	Host	Origin	Date	Collector
PREM 49379	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	1988	M.J. Wingfield
PREM 49377	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	1986	M.J. Wingfield
PREM 49378	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	1987	M.J. Wingfield
PREM 57293	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	2001	M. Venter
PREM 57294	*Cryphonectria cubensis*	*Eucalyptus grandis*	Colombia	2000	M.J. Wingfield
PREM 57295	*Cryphonectria cubensis*	*Eucalyptus* sp.	Mexico	2000	M.J. Wingfield
IMI 263717	*Cryphonectria cubensis*	*Eucalyptus* sp.	Hong Kong	1981	C.S. Hodges
PREM 57296	*Cryphonectria cubensis*	*Eucalyptus grandis* X *urophylla* clone	Vietnam	2000	M.J. Wingfield
PREM 57297	*Cryphonectria cubensis*	*Eucalyptus* sp.	Indonesia	2001	M.J. Wingfield

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

* **IMI**, Herbarium, CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, UK.

Fig. 1. One most parsimonious tree (tree length = 499 steps, CI = 0.9579, RI = 0.9651) generated from sequence variation within a combined β-tubulin and histone *H3* gene sequence data set. Bootstrap values (1000 replicates) are indicated.

Tree length = 499 steps
CI = 0.9579
RI = 0.9651

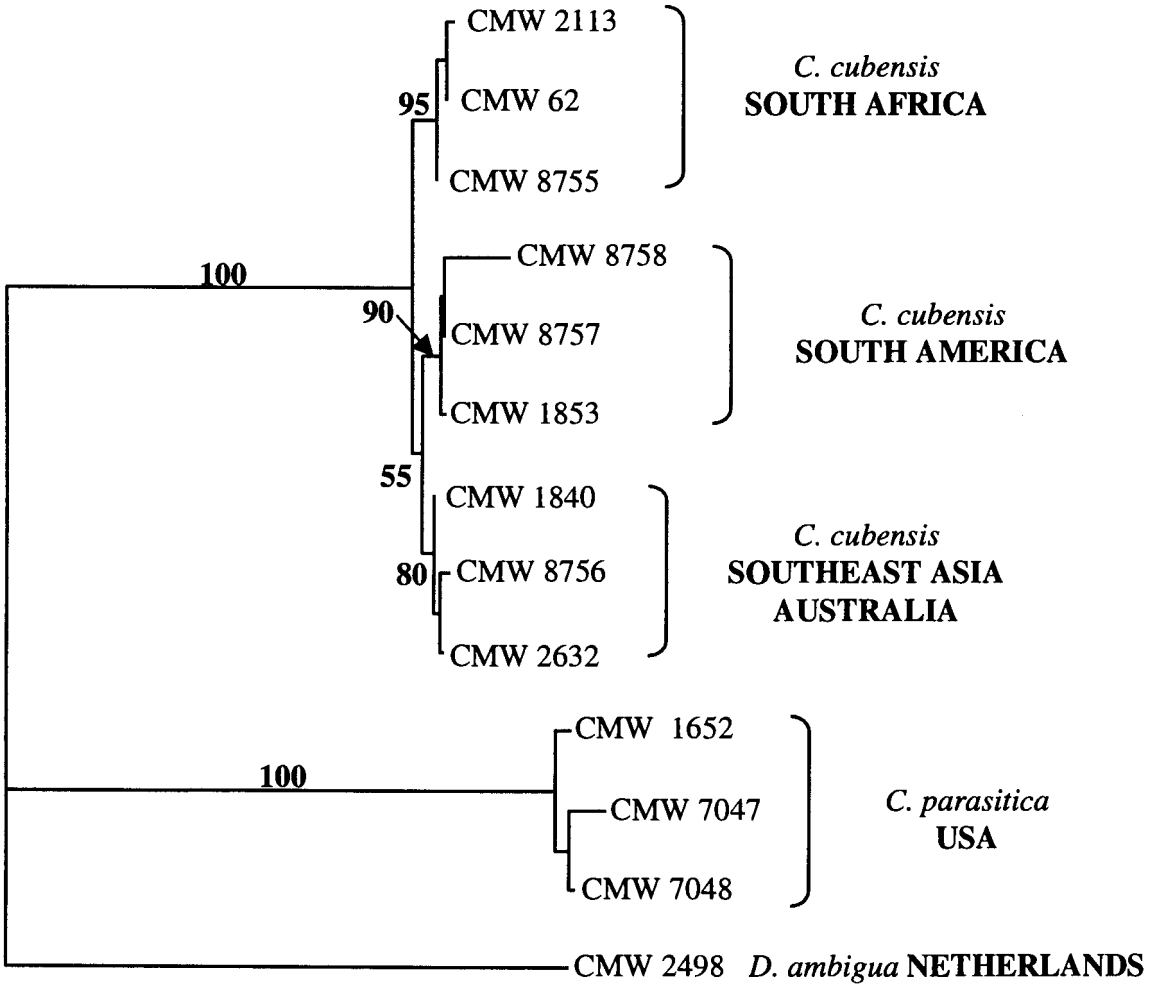
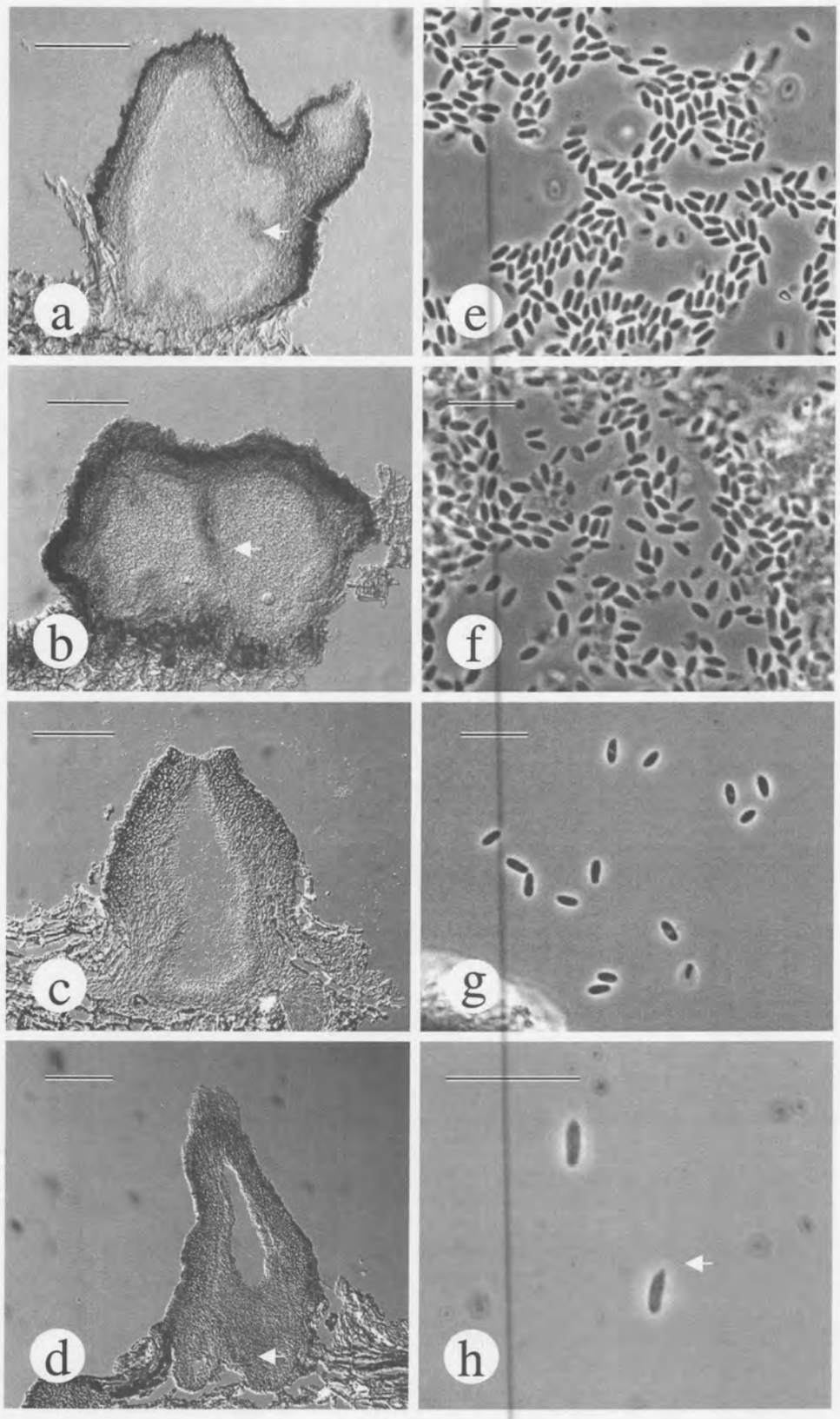


Fig. 2. Conidiomata and conidia of *Cryphonectria cubensis* from various parts of the world. **a**, **b**. Cross section of a conidiomata from South Africa showing convoluted basal layer (arrow in **a**) and cross wall (arrow in **b**) (bar = 100 μm). **c**. Cross section of a conidioma from Hong Kong (bar = 100 μm). **d**. Cross section of a conidioma from Colombia with second, small cavity (arrow) (bar = 100 μm). **e**, **f**, **g**. Conidia from Hong Kong, Mexico and South Africa (bars = 10 μm). **h**. Papillate apex (arrow) on a conidium from South Africa (bar = 10 μm).



CHAPTER 3

Cryphonectria canker on *Tibouchina* in South Africa.

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CRYPHONECTRIA CANKER ON *TIBOUCHINA* IN SOUTH AFRICA.

ABSTRACT

Cryphonectria cubensis is an important canker pathogen of plantation *Eucalyptus* spp. in tropical and sub-tropical areas of the world, including South Africa. It is best known on *Eucalyptus* spp., but it also occurs on *Syzigium aromaticum* (clove). In 1998, *C. cubensis* was found to cause cankers on the non-myrtaceous hosts *Tibouchina urvilleana* and *T. lepidota* in Colombia. In this study, we report on a similar canker disease that has recently been found in South Africa on *T. granulosa*, commonly grown as an ornamental tree. The identity of the pathogen was determined through morphological comparisons and phylogenetic analyses of ITS and β-tubulin gene sequences. The pathogenicity of the fungus was also tested on *T. granulosa* and *E. grandis*. Morphological as well as DNA sequence comparisons showed that the fungus on *T. granulosa* is the same as *C. cubensis* occurring on *Eucalyptus* spp. in South Africa. Pathogenicity tests on *T. granulosa* and *E. grandis* clones showed that the fungus from *T. granulosa* is able to cause cankers on both hosts.

INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is one of the most important pathogens in *Eucalyptus* plantations in tropical and sub-tropical areas of the world (Boerboom and Maas 1970, Hodges 1980, Wingfield et al. 1989). High temperatures and rainfall favor the occurrence of this disease (Hodges et al. 1976, Hodges, Geary and Cordell 1979, Sharma et al. 1985a, b). Reduction of losses due to Cryphonectria canker is usually achieved by the vegetative propagation of selected disease tolerant *Eucalyptus* clones and hybrids (Alfenas et al. 1983, Conradie et al. 1990).

Cryphonectria cubensis is known to occur naturally on two host genera other than *Eucalyptus*. In 1986, the fungus was reported from clove [*Syzigium aromaticum* (L.) Merr. & Perry] (Hodges et al. 1986) in Brazil. Subsequently the opportunistic pathogen of clove, *Endothia eugeniae* (Nutman & Roberts) Reid & Booth, was reduced to synonymy with *C. cubensis*. Micales et al. (1987) confirmed the synonymy of *C. cubensis* and *E. eugeniae* using isozyme analysis, total protein banding patterns and fungal pigments. More recently, *C. cubensis* was reported on native *Tibouchina urvilleana* Cogn. and *T. lepidota* Baill. in Colombia (Wingfield et al. 2001). This was an intriguing discovery because it was the first time that the fungus had been found on trees outside the Myrtaceae.

Species of *Tibouchina* are widely grown in the warmer parts of South Africa as ornamentals in gardens, parks and along roadsides. In January 1999, a fungus had been found on *T. granulosa* Cogn and *T. granulosa* var. *rosea* in KwaMbonambi, South Africa. This disease

resulted in the death of branches and die-back of mature trees. Fruiting bodies on the surface of cankers resembled those of the anamorph of *C. cubensis* found on *Eucalyptus* spp. in South Africa (Wingfield et al. 1989). The aim of this study was to identify the causal agent of the disease found on *T. granulosa* in South Africa using morphological and molecular tools, and to test its pathogenicity.

MATERIALS AND METHODS

Disease symptoms and collection of samples

In 1999, *T. granulosa* trees showing branch die-back with girdling cankers were identified in the town of KwaMbonambi, South Africa (28°22'S 32°19'E). Bark samples from the surface of cankers bearing fruiting bodies were incubated in moist chambers for 2-3 days to induce production of spores. Single conidial isolations were made onto 2% Malt Extract Agar (MEA) (20 g Biolab Malt Extract, 15g Biolab Agar, 1L water) and incubated at 25 °C. These cultures are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Morphological comparisons

Bark specimens from *T. granulosa*, with fruiting structures of the fungus, were collected from natural cankers and deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM) (Table 1). Bark material from field pathogenicity trials (see

Pathogenicity section), was also examined (Table 1). These specimens were compared with specimens of *C. cubensis* from *Eucalyptus* spp. used in a previous study (Wingfield et al. 1989) (Table 1). Comparisons were made using standard light microscopic techniques.

Stems (~10-15 mm diam) of an unknown *E. grandis* W. Hill ex Maiden clone were inoculated with isolate CMW 9343 from *T. granulosa* and isolate CMW 2113 from *E. grandis*. This was done to induce the formation of fresh fruiting structures. These stems were cut into segments (5-8 cm long) and the ends sealed with melted paraffin wax. Bark plugs 6 mm in diam were removed from the segments with a cork borer and replaced with mycelial plugs of the same size. Wounds were covered with parafilm and incubated in a moisture chamber until fruiting structures were produced.

Fruiting structures were sectioned using a Leitz 1310K freezing microtome with a KRYOMAT 1700 generator (Setpoint Technologies, Johannesburg) as described in Venter et al. (2002). Sections were executed at –30 °C and were 14-20 μm thick. Ten measurements each was taken of relevant structures and are presented as (min-)(mean - sd) - (mean + sd)(-max). For stromata, a range was obtained from three structures. Colour notations described by Rayner (1970) were used to standardise colour annotations.

DNA isolation and amplification

Isolates used to confirm the identity of the fungus from *T. granulosa* are listed in Table 2. DNA was isolated as described by Myburg et al. (1999). The internal transcribed spacer

(ITS) regions of the ribosomal RNA operon were amplified using the primer sets ITS1 and ITS4 (White et al. 1990). Amplification of two β-tubulin gene regions was done using primer pairs Bt1a/Bt1b and Bt2a/Bt2b (Glass and Donaldson 1995). Amplification of the ITS1 and ITS2 and the two β-tubulin gene regions were as described by Myburg et al. (1999) and Myburg et al. (2002) respectively. Amplification reactions were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Inc. Foster City, California). PCR products were visualised on a 1 % agarose-ethidium bromide gel using an ultraviolet light source.

Sequencing and analysis of sequence data

PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The respective DNA fragments were sequenced in both directions with the same primer pairs used in the amplification reactions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington) was used for sequencing. DNA sequences were determined with an ABI PRISM 377™ automated DNA sequencer. Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Foster City, CA) was used to translate the DNA sequences to amino-acid sequences. The DNA sequences were aligned using CLUSTAL W (Thompson et al. 1997) and verified manually.

Phylogenetic analyses were performed using PAUP* version 4.0b (Swofford 1998). Heuristic searches with branch swapping (no swapping) and MULTREES (saving all

optimal trees) effective were used in parsimony analyses. Gaps inserted during sequence alignment were treated as fifth characters (NEWSTATE). A partition-homogeneity test (PHT) was conducted (500 replicates, heuristic search type) to assess the combinability of the ITS and β-tubulin data sets. Bootstrap analyses (1000 replications) were done to determine the confidence levels of the tree branching points. Previously published sequences for *Cryphonectria parasitica*, the causal agent of chestnut blight (Elliston 1981), were included in the study for comparative purposes. *Diaporthe ambigua* Nitschke was used as the outgroup taxon to root the phylogenetic tree. All sequences obtained in this study have been deposited in GenBank (accession nos. are listed in Table 2).

Pathogenicity

Tibouchina granulosa trees (~10-15 mm diam) were kept under greenhouse conditions for two weeks to acclimatise prior to inoculation. The greenhouse was subjected to natural day/night conditions and a temperature setting of ~25 °C. Mycelial plugs (9 mm diam) were taken from the edges of actively growing cultures and placed, mycelium facing the cambium, into wounds made with a cork borer (9 mm diam). Wounds were sealed with parafilm to protect the inoculated fungus and cambium from desiccation. Ten trees were inoculated for each of the ten selected isolates. Sterile plugs were used for control inoculations on ten *Tibouchina* trees. To determine the relative pathogenicity, lesion lengths were measured on each tree after four weeks and the averages computed. The most virulent isolate was selected and used in a subsequent inoculation study on *T. granulosa* and *E. grandis*.

To assess reciprocal pathogenicity of *Tibouchina* and *Eucalyptus* isolates, the most virulent isolate (CMW 9343) from *Tibouchina* and a previously identified virulent isolate (CMW 2113) of *C. cubensis* from *E. grandis* (Van Zyl and Wingfield 1999), were inoculated on 20 *T. granulosa* and 20 *E. grandis* (clone ZG 14) trees (~10 mm diam) respectively. The same inoculation technique as described above was used and results based on lesion length were assessed after 4 weeks. Ten *E. grandis* and ten *T. granulosa* trees were inoculated with sterile MEA plugs to serve as controls. This trial was repeated once. To determine the variance between isolates and among trees, the inoculation data were subjected to analysis of variance using the General Linear Model procedure of SAS (SAS/STAT Users guide, Version 6).

To investigate the pathogenicity of the *Tibouchina* and *Eucalyptus* isolates under field conditions, inoculations were performed on trees (~1 yr old) of an established *E. grandis x E. camaldulensis* clone (GC 747). These inoculations were performed at KwaMbonambi (KwaZulu-Natal) (28°22'S 32°19'E) where *C. cubensis* is known to occur in *Eucalyptus* plantations. Twenty trees (100-150 mm diam) each were inoculated with the three most virulent isolates from *Tibouchina* (CMW 9343, CMW 9346, CMW 9358) and one *C. cubensis* isolate from *E. grandis* (CMW 2113). Twenty trees were also inoculated with sterile MEA plugs to serve as controls. Results based on lesion length were measured after 6 months and the entire trial was repeated once. Statistical significance of results was determined as described above.

RESULTS

Disease symptoms and collection of samples

During the first disease survey of *T. granulosa* trees, ten trees showing branch die-back were found in KwaMbonambi. One infected tree was also found in Richards Bay during a preliminary survey. Disease symptoms included branch die-back, cracking of the bark (Fig. 1, Fig. 2), and the development of girdling stem cankers. Fruiting bodies of a fungus resembling the anamorph of *C. cubensis* were found between the cracks and on dead areas of the stem.

Morphological comparisons

Anamorph structures of the fungus occurring on *T. granulosa* were superficial to slightly immersed on the bark, pyriform to clavate (Fig. 3a). Longitudinal sections revealed a unilocular to occasionally multilocular, convoluted eustroma (Fig. 3b) similar to those observed in Myburg et al. (2002). Structures were blackened with a luteous (colour 17) interior, 192-310 μm wide; base 248-477 μm long, base above the bark surface 167-477 μm long (Fig. 3a). One to four necks, either originating from a single locule or from more than one locule, occurred on a single superficial eustroma, and were up to 240 μm long, 105-124 μm wide. Conidiophores were hyaline, septate, with or without branching underneath the septum, 9.5-14(-15.5) x 1.5 μm (Fig. 3c). Conidia were exuded as bright luteous (colour 17) spore tendrils or drops (Fig. 3a). Conidia were hyaline, non-septate, cylindrical to oblong to oval, 3-4.5(-5.5) x (1.5-)1-1.5 μm (Fig. 3d).

Perithecia were rarely seen, and formed either below the conidioma (Fig. 3e) or separately (Fig. 3f). When perithecia developed on their own, they were semi-immersed and surrounded with weakly developed, orange (colour 15), predominantly prosenchymatous stromatic tissue, which was often slightly erumpent (Fig. 3f). When the perithecia were produced below the blackened eustroma of the conidioma, the same type of stromatic tissue as that observed for the single ascomata was produced around the bases of the perithecial necks (Fig. 3e). This tissue was different from that of the conidiomata, which was umber (colour 15m) and composed of *textura globulosa*. Perithecia were black, globose, 267-310 μm diam. Black, periphysate perithecial necks protruded through the stromatal surface for up to 500 μm. Necks were 62-105 μm wide and covered in brown, *textura porrecta* tissue as it extends beyond the stroma, with the width of the extended parts being 112-200 μm. Ascospores were hyaline, septate, fusoid to oblong, sometimes slightly curved, (5-)5.5-7.5(-8) μm long, 1.5-2 μm wide (Fig. 3g). No asci were seen in the available material.

The anamorph of the fungus from *T. granulosa* is similar to *C. cubensis* from *Eucalyptus* spp. in South Africa (Myburg et al. 2002, Wingfield et al. 1989). The presence of the teleomorph of this fungus in South Africa has been reported only once (Wingfield et al. 1989). The teleomorph material observed in this study conforms to the description provided by these authors. The anamorph and teleomorph of the fungus on *T. granulosa* were also similar to those described for *C. cubensis* from other parts of the world (Hodges et al. 1976, Hodges et al. 1979, Hodges 1980, Wingfield et al. 2001).

Identification of the fungus on *T. granulosa* as *C. cubensis* is supported by the morphology of the structures produced on plants used in the inoculation studies. Fruiting structures formed by isolates from *T. granulosa* (PREM 57362, PREM 57363, PREM 57364, PREM 57365, PREM 57367) on the *Eucalyptus* clone (GC 747) were similar to those formed by isolate CMW 2113 (isolated from *E. grandis*) on the same host (PREM 57366). Only anamorph structures were produced in these inoculations.

Morphology of conidia from conidiomata formed on the artificially inoculated stems and field inoculations (PREM 57362, PREM 57363, PREM 57364, PREM 57365, PREM 57366, PREM 57367) (Table 1) was variable. Conidia produced by isolates CMW 9343 and CMW 2113 on the *Eucalyptus* stems varied from clavate to cylindrical to allantoid (Fig. 3h). The conidia were also variable in size and longer (3·5-)3.5-5.5(-8) x 1-1.5(-2) μm, than those found on material collected from natural infections. Such variation in spore morphology was not observed for pycnidia from natural infections. Many of the conidia in the inoculated material also had papillate apices (Fig. 3h), although these were rarely seen in the natural material on *T. granulosa*. The papillate apices on conidia of South African material have been noted before, but were thought inordinately rare and not taxonomically significant (Myburg et al. 2002).

Sequencing and analysis of sequence data

A PHT value of P = 0.2 showed that the data partitions (ITS1, 5.8S, ITS2, Bt1a/1b and Bt2a/2b) could be combined as one data set in the phylogenetic analyses. Manual alignment

of the combined sequences resulted in a data set of 1530 characters (Appendix 2), consisting of 996 constant characters, 338 parsimony informative characters and 196 variable characters that were parsimony uninformative. A strict consensus tree (50% majority rule) (tree length = 671 steps, consistency index/CI = 0.96 and retention index/RI = 0.95) was generated from the 196 variable characters (Fig. 4).

The phylogenetic tree based on the combined DNA sequences (Fig. 3a) showed that the fungi from *T. granulosa* and *Eucalyptus* in South Africa, South America and Southeast Asia/Australia resided in separate groups to *C. parasitica* and the outgroup *D. ambigua* (bootstrap support = 100%). The *C. cubensis/Tibouchina* clade is subdivided into sub-groups, consistent with geographical origins of the isolates. The South African isolates thus formed a clade separate (bootstrap support = 93%) from the Southeast Asian and South American isolates. The South American *C. cubensis* strains isolated from *Tibouchina* spp. grouped together with the South American *C. cubensis* isolates from *Eucalyptus* spp. The *C. cubensis* isolates from Southeast Asia/Australia grouped together (bootstrap support = 63%). The bootstrap support for the branch node separating the South American and Southeast Asian/Australian groups is 64%, suggesting that these two groups are closely related.

Pathogenicity

Greenhouse inoculation of *T. granulosa* seedlings and the *E. grandis* clone (ZG 14) resulted in the formation of extensive lesions on both the hosts within 4 wks (Table 3). Some of the seedlings were already dropping their leaves, dying and producing epicormic shoots on the

stems within this time period. The control inoculations showed no lesions. Both isolates from *Tibouchina* and *Eucalyptus* were more pathogenic to *Eucalyptus* than to *Tibouchina* (P> 0·0001) (Table 3).

Field inoculations resulted in the formation of girdling cankers, cracking bark and fungal sporulation around the lesions. The same symptoms were seen on the naturally infected *Tibouchina* trees (Fig. 1, Fig. 2). The analysis of data from the field inoculations, however, showed no significant differences in pathogenicity between isolates (P>0·1464).

DISCUSSION

This study represents the first report of a serious canker and die-back disease of ornamental *Tibouchina* trees in South Africa. To the best of our knowledge, this is the first disease to be recorded on this important ornamental tree in South Africa. Our results clearly show that the disease is caused by the well-known *Eucalyptus* canker pathogen, *C. cubensis*. The fungus has recently been reported as a serious pathogen of native *Tibouchina* spp. in Colombia (Wingfield et al. 2001) and the current study represents the first report of the disease on a species of *Tibouchina* outside South America.

In South Africa, *C. cubensis* on *Eucalyptus* is mostly characterised by the occurrence of the asexual fruiting structures. The sexual state on *Eucalyptus* has been found only once (Wingfield et al. 1989, Van Heerden et al. 1997). It was, therefore interesting that perithecia of *C. cubensis* were occasionally found on *T. granulosa* in South Africa. This is in contrast

to the situation in Colombia where only asexual fruiting structures were found on *T. urvilleana* and *T. lepidota* (Wingfield et al. 2001), while the sexual state is the dominant form on *Eucalyptus* spp. in that country (Van der Merwe et al. 2001). Wingfield et al. (2001) suggested that the differences in occurrence of the sexual state could be the result of differences in biology of *C. cubensis* on different hosts. This hypothesis is supported by our data, where the South African *C. cubensis* rarely produces sexual structures on *Eucalyptus*.

Sequence data generated in this study confirm the fact that the fungus from *T. granulosa* in South Africa is *C. cubensis*. Our results further support a recent report that *C. cubensis* isolates from South Africa reside in a well resolved and strongly supported group different from that accommodating South American and Southeast Asian *C. cubensis* isolates (Myburg et al. 2002). Isolates from *T. granulosa* in South Africa cluster together with the South African *C. cubensis* isolates, separate from the *Tibouchina* isolates in Colombia. These data suggest that South African *C. cubensis* on *Tibouchina* and *Eucalyptus* has a similar, but probably different origin to *C. cubensis* found in other parts of the world.

Greenhouse inoculation trials conducted in this study suggest that the *Eucalyptus* clone used is more susceptible to infection by the South African *C. cubensis* than is *T. granulosa*. After four weeks, both tree species were producing epicormic shoots and had begun to die. This is in contrast to Colombian trials (Wingfield et al. 2001), where none of the *Eucalyptus* trees died and the *Tibouchina* trees were dead or dying after four months. It also appears that the South African *C. cubensis* isolates are more virulent than *C. cubensis* from *Tibouchina* in Colombia, since trees had begun to die within four weeks of inoculation.

At the present time, the origin of the South African form of *C. cubensis*, now known to occur on *Eucalyptus* and *Tibouchina*, is uncertain. Although morphologically almost identical to the fungus from Asia and South America (Myburg et al. 2002), biological and molecular evidence provide robust support for the notion that the South African fungus represents a discrete taxon. This leads us to speculate that the fungus on *Eucalyptus* and *Tibouchina* in South Africa will have an origin different to that of the fungus in Asia and South America. A likely origin of the fungus would be on native Myrtaceae in South Africa and surveys have been initiated to consider this hypothesis.

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Table 1. Specimens of *Cryphonectria cubensis* used in morphological comparisons.

Reference no.[a]	Host	Origin	Date	Collector
PREM 49379	*Eucalyptus grandis*	South Africa	1988	M.J. Wingfield
PREM 49377	*E. grandis*	South Africa	1986	M.J. Wingfield
PREM 49378	*E. grandis*	South Africa	1987	M.J. Wingfield
PREM 57357	*Tibouchina granulosa*	South Africa	1999	J. Roux
PREM 57358	*T. granulosa*	South Africa	1999	J. Roux
PREM 57359	*T. granulosa*	South Africa	1999	J. Roux
PREM 57360	*T. granulosa*	South Africa	2000	J. Roux, R. Heath and L. Lombaard
PREM 57361	*T. granulosa*	South Africa	2000	J. Roux, R. Heath and L. Lombaard
PREM 57362	CMW 9343 inoculation of GC 747[b]	South Africa	2000	J. Roux and R. Heath
PREM 57363	CMW 9343 inoculation of GC 747[b]	South Africa	2001	M. Gryzenhout and R. Heath
PREM 57364	CMW 9346 inoculation of GC 747[b]	South Africa	2001	M. Gryzenhout and R. Heath
PREM 57365	CMW 9358 inoculation of GC 747[b]	South Africa	2001	M. Gryzenhout and R. Heath
PREM 57366	CMW 2113 inoculation of GC 747[b]	South Africa	2001	M. Gryzenhout and R. Heath
PREM 57367	CMW 9343 stick inoculation[c]	South Africa	2002	R. Heath

[a] **PREM**, National Collection of Fungi, Pretoria, South Africa.

[b] An *E. grandis* X *camaldulensis* clone (GC747) inoculated with *C. cubensis* isolate in the field.

[c] Cut stems of an unknown *E. grandis* clone inoculated in the lab.

Table 2. Isolates used for molecular comparison and pathogenicity trials[a].

Isolate no.	Isolate identity	Host	Origin	GenBank accession numbers
CMW 8757	*Cryphonectria cubensis*	*Eucalyptus grandis*	Venezuela	AF046897[b], AF273069[d], AF273464[d]
CMW 1853	*C. cubensis*	*Eugenia caryophyllus*	Brazil	AF046891[b], AF273070[d], AF273465[d]
CMW 9927	*C. cubensis*	*Tibouchina urvilleana*	Colombia	AF265653[c], AF292034[f], AF292037[g]
CMW 9928	*C. cubensis*	*T. urvilleana*	Colombia	AF265654[c], AF292036[f], AF292039[g]
CMW 9929	*C. cubensis*	*T. urvilleana*	Colombia	AF265655[c], AF292035[f], AF292038[g]
CMW 2113	*C. cubensis*	*Eucalyptus grandis*	South Africa	AF046892[b], AF273067[d], AF273462[d]
CMW 62	*C. cubensis*	*E. grandis*	South Africa	AF292041[d], AF273063[d], AF273458[d]
CMW 8755	*C. cubensis*	*E. grandis*	South Africa	AF292040[d], AF273064[d], AF273459[d]
CMW 9932	*C. cubensis*	*T. granulosa*	South Africa	AF273472[e], AF273062[f], AF273457[g]
CMW 9327	*C. cubensis*	*T. granulosa*	South Africa	AF273473[e], AF273060[f], AF273455[g]
CMW 9328	*C. cubensis*	*T. granulosa*	South Africa	AF273474[e], AF273061[f], AF273456[g]
CMW 8756	*C. cubensis*	*E. grandis*	Indonesia	AF046896[b], AF273077[d], AF285165[d]
CMW 9903	*C. cubensis*	*Eucalyptus* sp.	Kalimantan	AF292044[e], AF273066[f], AF273461[g]
CMW 9906	*C. cubensis*	*Eucalyptus* sp.	Kalimantan	AF292045[e], AF273065[f], AF273460[g]
CMW 1651	*C. parasitica*	*Castanea dentata*	USA	AF046901[b], AF273074[d], AF273467[d]
CMW 1652	*C. parasitica*	*C. dentata*	USA	AF046902[b], AF273075[d], AF273468[d]
CMW 2498	*Diaporthe ambigua*	*Malus sylvestris*	Netherlands	AF046906[b], AF273072[d], AF273471[d]
CMW 9343	*C. cubensis*	*T. granulosa*	South Africa	
CMW 9346	*C. cubensis*	*T. granulosa*	South Africa	
CMW 9358	*C. cubensis*	*T. granulosa*	South Africa	

[a] Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. CMW refer to the general and *Cryphonectria* culture collections.

[b] Ribosomal DNA sequences obtained from Myburg et al. (1999).

[c] Ribosomal DNA sequences obtained from Wingfield et al. (2001).

[d] Sequence data obtained from Myburg *et al.* (2002).

[e] Ribosomal DNA sequences generated in this study.

[f] Beta-tubulin 1a/1b sequences generated in this study.

[g] Beta-tubulin 2a/2b sequences generated in this study.

Table 3. Lesion lengths on 1 yr-old *Eucalyptus grandis* (clone ZG 14) and *Tibouchina granulosa* four wks after inoculation in the greenhouse.

Isolate	**[a]Lesion length (mm) (1st trial)**		**[a]Lesion length (mm) (2nd trial)**		**[a]Lesion length (mm) (3rd trial)**	
	Tibouchina	**ZG 14**	***Tibouchina***	**ZG 14**	***Tibouchina***	**ZG 14**
CMW 2113	49.9	146.5	65.6	975	867	155.5
CMW 9343	108.9	133.0	34.2	104.1	90.9	129.3
Control	10	10	9.7	9.6	10	10

[a] Each value is the average of 20 measurements for each isolate.

$P > 0.0001$

CV = 43.23666

R-Square = 0.806038

F = 7.45

Table 4. Lesion length on 18 month-old GC 747 trees grown in the plantation after inoculation with *Cryphonectria cubensis* isolates from *Tibouchina* and *Eucalyptus.*

Isolate	**[a]Lesion length (mm) (1st trial)**	**[a]Lesion length (mm) (2nd trial)**
CMW 2113	77.9	56.1
CMW 9343	83.4	45.9
CMW 9346	50.8	92.5
CMW 9358	68	54
Control	10	10

[a] Each value is the average of 20 measurements for each isolate.

$P > 0.1464$

CV = 77.84968

R-Square = 0.555225

F = 1.24

Figs. 1. Disease symptoms. **a.** *Tibouchina* tree showing branch die-back caused by *C. cubensis*. **b**. Girdling canker on the main stem of a *Tibouchina* tree infected by *C. cubensis*.

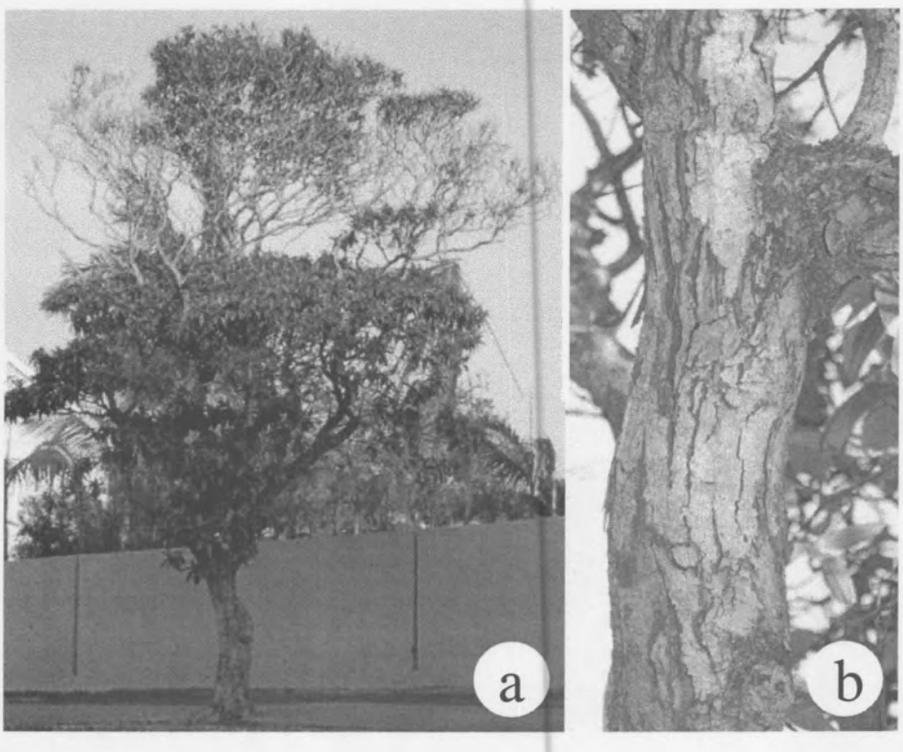


Fig. 2. Light micrographs of the anamorph and teleomorph of *Cryphonectria cubensis* from *Tibouchina granulosa* in South Africa. **a.** Conidioma on bark. **b.** Vertical section through conidioma. **c.** Conidiophores. **d.** Conidia. **e.** Perithecia (pe) with stromatic tissue (arrow) below a conidioma (co). **f.** Ascoma. **g.** Ascospores. **h.** Conidia produced by isolate CMW 9343 after inoculation into a cut stem of an unknown *Eucalyptus* clone. Bars Figs. **a**, **b**, **e**, **f** = 100 μm; Figs. **c**, **d**, **g**, **h** = 10 μm.

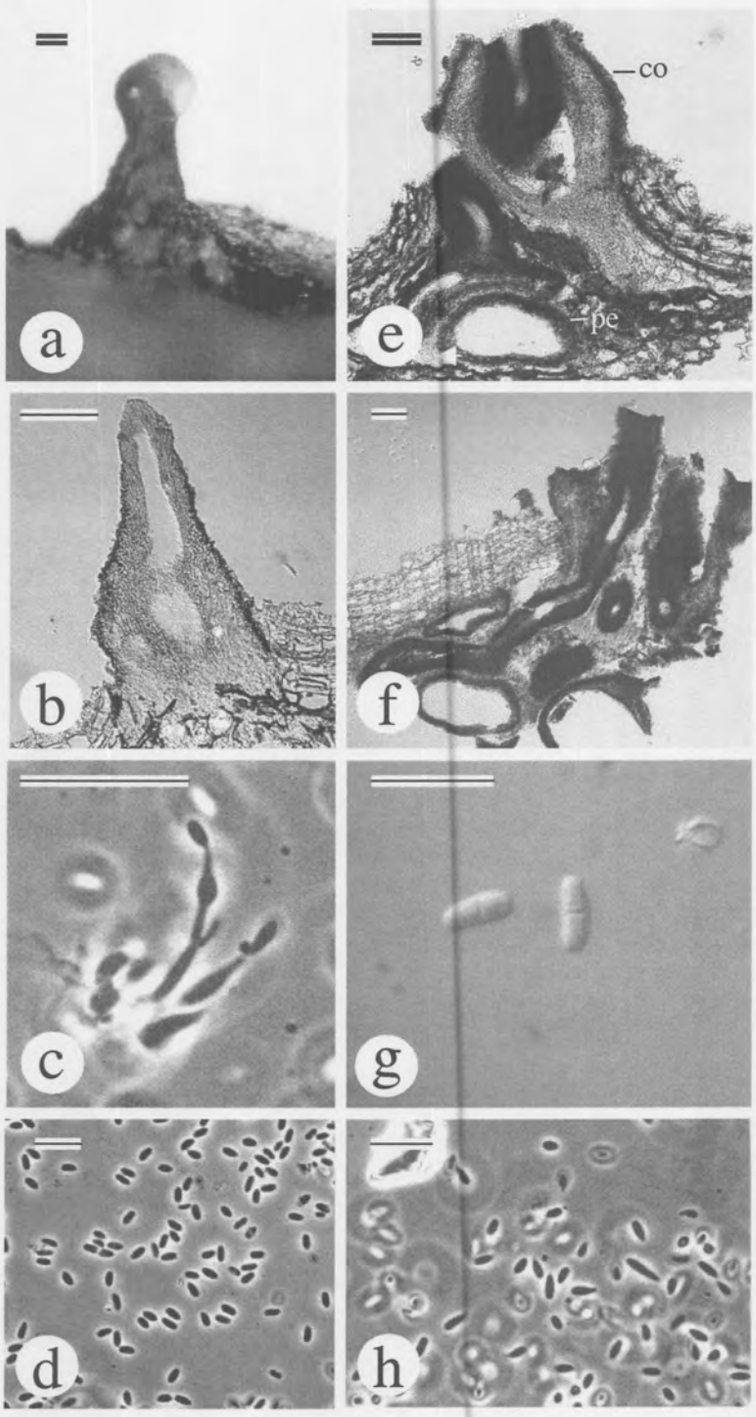
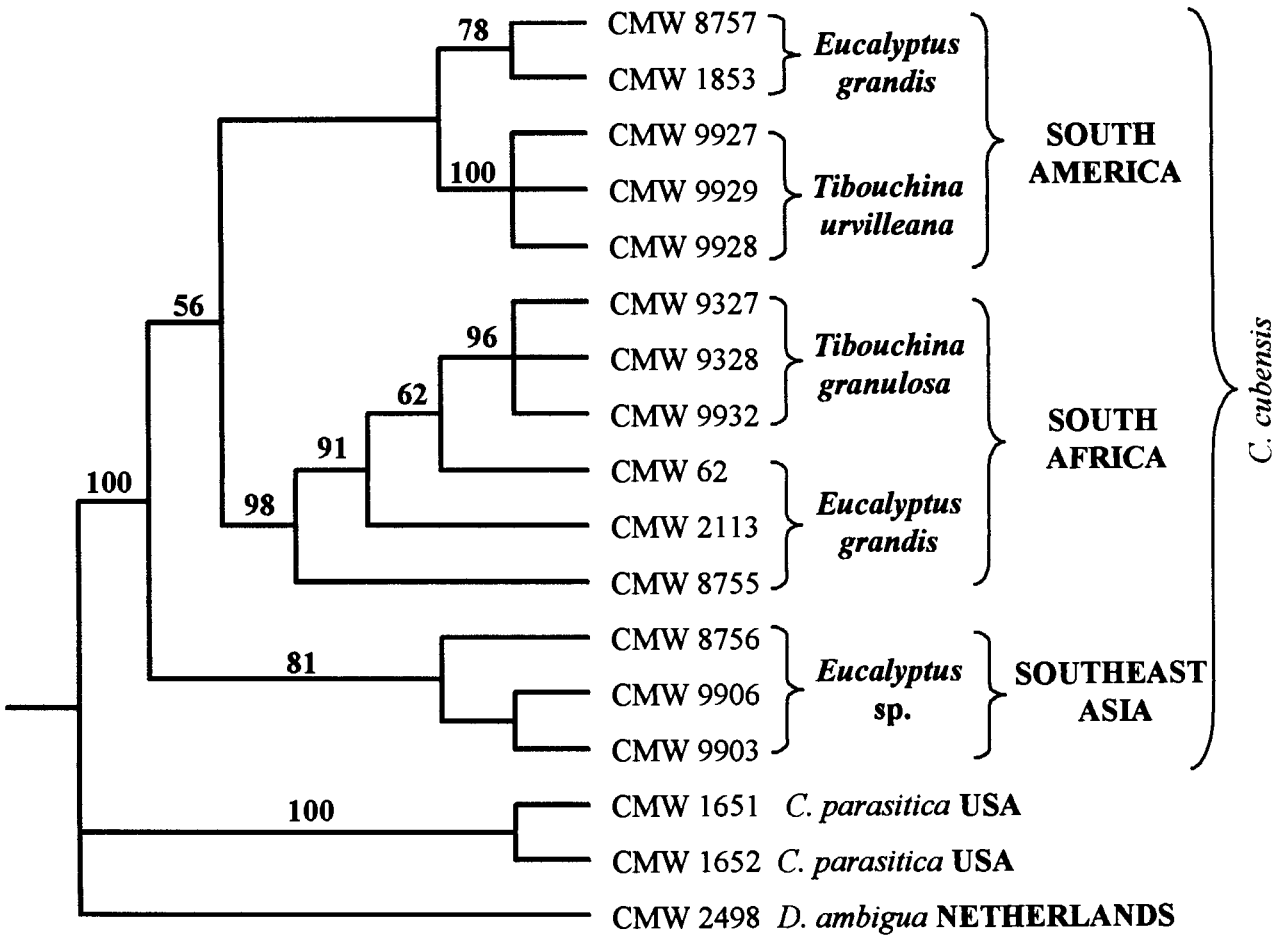


Fig. 3. Phylogenetic tree generated from a combined data set including ribosomal DNA (ITS1/ITS2) and β-tubulin gene sequence data. One strict consensus tree (tree length = 671 steps, CI = 0.96 and RI = 0.95) was generated from heuristic searches performed on the combined data set. Bootstrap values (1000 replicates) are indicated above the branches and those lower than 50 % are not shown. *Diaporthe ambigua* was used to root the tree.

Tree length = 671 steps
CI = 0.96
RI = 0.95



CHAPTER 4

Conspecificity of *Endothia eugeniae* and *Cryphonectria cubensis*: A re-evaluation based on morphology and DNA sequence data.

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CONSPECIFICITY OF *ENDOTHIA EUGENIAE* AND *CRYPHONECTRIA CUBENSIS*: A RE-EVALUATION BASED ON MORPHOLOGY AND DNA SEQUENCE DATA.

ABSTRACT

Cryphonectria cubensis and *Endothia eugeniae* are fungal pathogens of *Eucalyptus* and clove respectively that were reduced to synonymy based on the results of cross-inoculation studies, isozyme analysis, cultural studies and morphology. A previous phylogenetic study on *Cryphonectria*, based on sequence variation in the ITS region of the ribosomal RNA operon, also supported the conspecificity of *C. cubensis* and *E. eugeniae,* but was based on only one *E. eugeniae* isolate. New collections from clove in Brazil and Indonesia have become available, providing the opportunity to reconsider the conspecificity of *C. cubensis* and *E. eugeniae*. The occurrence of *C. cubensis* on clove was confirmed based on morphological comparisons and phylogenetic analyses of ribosomal DNA and β-tubulin gene sequence data. In addition to *C. cubensis*, other fungi morphologically similar to *Cryphonectria* species based on their orange stromata were also present on some clove specimens. These isolates, for which no herbarium material exists, grouped separately from the *C. cubensis* clade and closer to the *Cryphonectria* clade. The presence of more than one closely related fungus on clove raises questions relating to the legitimacy of the synonymy of *E. eugeniae* and *C. cubensis*. However, based on the presence of *C. cubensis* on the type specimen of *E. eugeniae*, we retain the synonymy of the two fungi but provide evidence that other fungi, closer related to *Cryphonectria* spp. than to *C. cubensis*, are present on clove.

INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is a well-known and important canker pathogen of *Eucalyptus* species (Boerboom and Maas 1970, Hodges 1980, Wingfield et al. 1989). The fungus is present in tropical and subtropical areas of the world, where high temperatures and rainfall favor infection and disease development (Alfenas et al. 1982). Management of Cryphonectria canker is primarily achieved by the vegetative propagation of disease-tolerant *Eucalyptus* clones and *Eucalyptus* hybrids (Alfenas et al. 1983, Van Zyl and Wingfield 1999).

Endothia eugeniae (Nutman & Roberts) Reid & Booth was first reported from Zanzibar, Tanzania, causing acute die-back of clove [*Syzygium aromaticum* (L.) Merr. & Perry] (Nutman and Roberts 1952). The pathogen infected trees through wounds and caused die-back of branches or death of whole trees by girdling of trunks. At the point of infection, the wood was stained reddish-brown (Nutman and Roberts 1952). The disease has also been reported from Malaysia (Anonymous 1954, Heath 1956, Reid and Booth 1969), which is the region of native cloves (Hodges et al. 1986).

The clove pathogen, now known as *C. cubensis*, was first described as *Cryptosporella eugeniae* Nutman & Roberts (1952), but was later transferred to the genus *Endothia* (Reid and Booth 1969). Hodges et al. (1986) reduced *E. eugeniae* to synonymy with *C. cubensis*. This synonymy was based on morphological comparisons, cultural characteristics, inoculation studies as well as analysis of isozyme banding patterns. Micales et al. (1987) confirmed this synonymy using additional isozyme analyses, general protein patterns and pigment identification.

Previous descriptions of *E. eugeniae* describe a fungus with brown pycnidia, immersed in the bark and emerging through the periderm, to assume a flattened conical shape (Nutman and Roberts 1952). Reid and Booth (1969) and Booth and Gibson (1973) describe immersed, becoming erumpent, conical and orange to rust brown stromata containing more than one convoluted to irregular conidial cavity. This is in contrast to *C. cubensis* that has superficial to slightly immersed, cylindrical to pyriform pycnidia-like eustromata with attenuated necks (Bruner 1917, Hodges 1980, Myburg et al. 2002a). These pycnidia are reddish-brown when young, but turn black with age (Bruner 1917, Hodges et al. 1979, Hodges 1980). These inconsistencies in the descriptions of the two fungi continue to raise questions pertaining to the validity of their synonymy.

Descriptions suggest that a fungus, morphologically similar but different to *C. cubensis*, could be present on clove. The possibility thus exists that the second fungus on clove, and not *C. cubensis*, might represent the originally described *E. eugeniae*. A phylogenetic study of isolates of *C. cubensis* based on sequence variation within the internal transcribed spacer (ITS) regions of the ribosomal RNA operon (Myburg et al. 1999), provided support for the synonymy of *C. cubensis* and *E. eugeniae*. These authors, however, noted that their conclusion was based on a single isolate of *E. eugeniae* and that this question should be addressed more closely using additional isolates from clove. Recently, a larger collection of isolates from clove has become available to us. The objective of the present study was, therefore, to reconsider the conspecificity of *E. eugeniae* with *C. cubensis,* based on DNA sequence data from two different gene regions. In addition, a comprehensive morphological study was

undertaken on the original herbarium specimens from clove, as well as newly obtained, fresh specimens from this host.

MATERIALS AND METHODS

Fungal isolates

Isolates used in this study were obtained from culture collections, supplied by colleagues or collected during field studies by the last author (Table 1). These include *C. cubensis* isolated from *Eucalyptus* and *S. aromaticum* from various parts of the world. Sequence data generated for other members of *Cryphonectria* (Myburg et al. 2002b, Venter et al. 2002), i.e. *C. parasitica* (Murr.) Barr, *C. macrospora* (Kobayashi & Ito) Barr, *C. nitschkei* (Otth.) Barr and *C. radicalis* (Schw.: Fr.) Barr, were also included in this study. *Diaporthe ambigua* Nitschke, the causal agent of stem cankers on stone fruit trees (Smit et al. 1996, 1997), was used as the outgroup taxon to root the phylogenetic trees (Table 1). Cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, 0002.

Morphological comparisons

Herbarium specimens from clove listed in the original descriptions (Nutman and Roberts 1952, Reid and Booth 1969) were studied (Table 2). These specimens originated from Zanzibar and Malaysia. New clove material was also collected from Sulawesi, Indonesia (Table 2) and has been deposited in the herbarium of the National Collection of Fungi,

Pretoria, South Africa (PREM). Isolates CMW 8649, CMW 8650 and CMW 8651 (Table 1) originated from these specimens. Specimens of *C. cubensis* from *Eucalyptus* spp., used in the study of Myburg et al. (2002a), were also included (Table 2).

Some isolates originating from clove (CMW 10779, CMW 10780, CMW 10781) had culture morphology different from clove isolates that were thought to represent *C. cubensis*. The cultures were buff (colour 19''f) to hazel (colour 11'k) in contrast to those of *C. cubensis* that were creamy white with cinnamon (colour 15'') patches. Unfortunately no vouchered specimens exist for these isolates.

Fruiting structures of *Cryphonectria* spp. are infrequently produced in culture and are not representative of fruiting structures occurring naturally on bark. Isolate CMW 10781 from clove was, therefore, inoculated into wax-sealed sticks of another member of the Myrtaceae, *Eucalyptus grandis* W. Hill: Maiden clone (ZG 14), to gain additional information on its morphology. Isolates CMW 8649, CMW 8650 and CMW 8651 from clove, known to be *C. cubensis*, were also inoculated into *E. grandis* sticks for comparative purposes. These inoculations were done using the technique described by Van Heerden and Wingfield (2001). Specimens resulting from these inoculations (Table 2) have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Hodges et al. (1986) performed inoculations on *E. saligna* and clove sticks using *C. cubensis* isolates from *Eucalyptus* and clove. The aim of that study was to consider the effect of clove and *Eucalyptus* bark on the morphology of the infecting fungus. The

specimens from these inoculations were made available to us by Dr. C.S. Hodges, Department of Plant Pathology, North Carolina State University, Raleigh, USA. These specimens (Table 2) have also been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Structures for morphological study were mounted in Leica mountant (Setpoint Premier, Johannesburg, South Africa) after boiling in water for 1 minute. Specimens were sectioned 12–16 μm using a Leica CM1100 cryostat (Setpoint Premier) at –20°C. Sections were mounted in lacto-phenol and examined microscopically. Ten measurements were taken for conidia and ascospores and are presented as (min-) (mean - SD) - (mean + SD) (-max). The colour notations of Rayner (1970) were used throughout this study.

DNA isolations and PCR

DNA was isolated as previously described by Myburg et al. (1999). Amplification of the ITS region of the ribosomal RNA operon, as well as the two regions within the β-tubulin gene were carried out as described in Myburg et al. (1999) and Myburg et al. (2002a) respectively. The primer pairs used to amplify the two β-tubulin regions were Bt1a with Bt1b and Bt2a with Bt2b (Glass and Donaldson 1995), while ITS 1 and ITS 4 (White et al. 1990) were used to amplify the ITS 1 and ITS 2 region of the ribosomal RNA operon. PCR products were separated on 1% agarose (Promega, Madison, Wisconsin) gels containing ethidium bromide and visualised using an UV light.

Sequencing

PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). The PCR products were sequenced in both directions using the same primers mentioned above. Sequencing reactions were carried out using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, United Kingdom). DNA sequences were determined using an ABI PRISM 3100™ automated DNA sequencer. DNA sequences were verified with Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) and aligned using CLUSTAL X (Thompson et al. 1997). The resulting alignment was checked manually.

A Templeton nonparametric Wilcoxon Signed Ranked (WSR) test (Kellogg et al. 1996) was done on a combined sequence data set including aligned β-tubulin and ITS sequences. Results from this test indicated that the data sets could be combined and considered as one data set in subsequent to phylogenetic analyses.

A heuristic search was executed on the aligned data set using PAUP* version 4.0b (Swofford 1998). The TBR (tree-bisection-reconnection) algorithm of the heuristic search (MulTrees option effective, saving all optimal trees) was chosen. Seventeen trees were generated and a strict consensus tree was computed. Gaps were treated as fifth characters (Newstate) and characters were unordered and equally weighted. A bootstrap analysis of 1000 replicates was done to assess the confidence levels of the internodes. The consensus tree was rooted with the two *D. ambigua* isolates. Sequences generated in this study were deposited in GenBank and accession numbers are listed in Table 1.

Sequence alignments are available from TreeBase (Study accession number = S874, Matrix accession number = M1419). Accession numbers of sequence data obtained from previous studies (Myburg et al. 1999, Myburg et al. 2002a ,b, Roux et al. 2003) are also listed in Table 1.

RESULTS

Morphological comparisons

More than one fungus residing in *Cryphonectria* was observed on the various clove specimens included in this study. These fungi had conidia and ascospores similar in size and shape and were difficult to distinguish on the bark, but differed based on position relative to the bark, stromatic tissue types and internal morphology of the stroma. *Cryphonectria cubensis* occurred on bark specimens from Zanzibar, Malaysia and Indonesia. A fungus with orange (colour 15) to sienna (colour 15i) stromata was found on the material from Zanzibar. Furthermore, herbarium materials, originating from inoculation with isolate CMW 10781 from Indonesia, contained fruiting structures with different characteristics to *C. cubensis* or the other fungus with the orange to sienna coloured stromata. These different fungi are discussed in greater detail under the following sections and morphological features are summarised in Table 3.

Cryphonectria cubensis on clove

Structures typical of *C. cubensis* (Table 3) were found on the clove specimens from Zanzibar (IMI 45449, IMI 45450, IMI 45440), Malaysia (IMI 56425a, IMI 58569, IMI

58388, IMI 58567, IMI 58568) and Indonesia (PREM 57470). Conidiomata were either pyriform with attenuated necks (Figs. 1a, 1c), or pulvinate since necks were broken or the structures were not fully developed (Figs. 1b, 1d). The tissue type in these stromata was characteristic of *C. cubensis* (Table 3) with base tissue *textura globulosa* (Fig. 1e) and neck tissue *textura porrecta* (Fig. 1f). Structures with a tissue type resembling that of *C. cubensis* were found on the type specimen of *E. eugeniae* (IMI 44954), but these were too brittle for thorough examination. Conidia (Fig. 1g, Table 3) were similar to those on specimens of *C. cubensis* on *Eucalyptus* (IMI 279614, IMI 304273, PREM 57297, IMI 284438, PREM 57294) and those previously described for *C. cubensis* (Bruner 1917, Hodges 1980, Myburg et al. 2002a, b).

The internal structure of conidiomata of *C. cubensis* was variable on clove. Pulvinate, blackened multilocular structures with convoluted and multilocular conidial chambers below the bark (Fig. 1d) were observed on clove tissue from Zanzibar (IMI 45440). The tissue type of the erumpent parts, as well as the spore shape and size [3.5-4(-4.5) x 1-1.5 µm] were similar to those of *C. cubensis*. The same extent of differences was observed for the clove and *Eucalyptus* material inoculated with *C. cubensis* (PREM 57469) and studied by Hodges et al. (1986). A small number of conidiomata were observed on this material, with structures on clove semi-immersed and conidial locules strongly convoluted and occurring underneath the bark.

The teleomorph of *C. cubensis* (Table 3, Figs. 1 h, 1i) on specimens IMI 45450 and IMI 45440 was frequently observed developing underneath anamorph structures (Fig. 1i). Stromatal development (Table 3) was prosenchymatous, orange (15) to luteous (17) and

restricted to the area around the base of the perithecial necks (Fig. 1j). Ascospores (Fig. 1k) were similar to those of *C. cubensis* (Table 3) as previously observed (Bruner 1917, Hodges 1980).

Other fungi on clove specimens

A fungus with stromatal structure and colour different from that of *C. cubensis* (Table 3) was found on some specimens from Zanzibar (IMI 45452, IMI 44951), studied in the original descriptions of *E. eugeniae* (Nutman and Roberts 1952; Reid and Booth 1969). These structures were only conidiomatal and occurred between structures of *C. cubensis*. They were erumpent, pulvinate (Fig. 2a) with several convoluted locules beneath the bark (Fig. 2b). Stromatic tissue (Table 3) was densely prosenchymatous (Fig. 2c), different from that of *C. cubensis* that is *textura globulosa* (Fig. 1e). Conidia from the orange structures (Fig. 2d) were similar in size and shape to those of *C. cubensis* (Table 3). No isolates exist for these specimens and it is impossible to study them further.

Specimens from Indonesia (PREM 57470) that gave rise to isolates of *C. cubensis* (CMW 8649, CMW 8650, CMW 8651) also contained ascomata different from those of *C. cubensis*. These ascomata superficially resembled the teleomorph of *C. cubensis* and also had one-septate, fusoid ascospores. They differed from *C. cubensis* in that stromatic tissue was densely prosenchymatous and orange to sienna. The latter characteristics were similar to those of the orange to sienna fungus on specimens from Zanzibar, but thorough comparisons between the fungus from Zanzibar and the Indonesian structures were hindered by the fact that stromata of the Indonesian specimens were very few in number. Furthermore, no isolates exist for these structures and for the present, we are

unable to draw a definitive conclusion regarding the identity of the fungus associated with these structures on the Indonesian material.

Eucalyptus sticks (PREM 57473) that had been inoculated with isolate CMW 10781 from Indonesia, showed structures different to those arising from the *C. cubensis* isolates (CMW 8649, CMW 8650, CMW 8651). Conidiomata (Table 3) were blackened (Fig. 3a), sometimes with a luteous (19) apex, with pseudoparenchymatous tissue (Figs. 3b, 3c). These structures and the tissues associated with them were also different from those of the other fungus (IMI 45452, IMI 44951) with orange to sienna stromata from Zanzibar (Figs. 2b, 2c). Conidia were also distinct in being more cylindrical (Fig. 3d), but length measurements were in the same size range as *C. cubensis* (Table 3). The shape and internal structure of the fruiting bodies were too variable to draw any definite conclusions on the identity of this fungus. The *C. cubensis* isolates, however, produced ascomata and conidiomata that were similar to those of *C. cubensis* on the clove specimens from nature and showed little variation amongst each other.

Sequencing

Amplification products for the DNA regions considered in this study were approximately 600 bp (ITS) and 550 bp (β-tubulin) in size. A combined sequence data set, comprising of ITS ribosomal and β-tubulin gene sequences included 1505 aligned sequence (Appendix 3) characters, of which 879 were constant, 40 parsimony-uninformative and 586 parsimony-informative. A strict consensus tree (tree length = 1198 steps,

consistency index/CI = 0.8 and retention index/RI = 0.9) was computed (Fig. 4) from the seventeen trees generated in the heuristic search.

The phylogram generated for the combined sequence data set (Fig. 4) showed three groups of fungi, clustering separately from the outgroup taxon represented by the *D. ambigua* isolates. The first clade (bootstrap support 100%) represents *C. cubensis* isolated from *Eucalyptus* species and clove originating from Southeast Asia (bootstrap support 98%), South America (bootstrap support 80%) and South Africa (bootstrap support 62%). The second group represents isolates that also originated on clove in Indonesia (bootstrap support 100%). A third group (bootstrap support 100%) is characterised by *C. parasitica, C. radicalis*, *C. macrospora* and *C. nitschkei* and represents species that characterise the genus *Cryphonectria sensu stricto* (Myburg et al. 2002a).

Three sub-groups of fungi make up the *C. cubensis* clade. These groups, previously identified by Myburg et al. (2002a), represent three geographical areas, where *C. cubensis* is known to occur. In the present study, one group represents *C. cubensis* isolated from *Eucalyptus* and clove originating from countries in Southeast Asia and Australia (bootstrap support 98%). The clove isolates (CMW 8649, CMW 8650, CMW 8651) from Indonesia as well as the Indonesian clove isolate (CMW 3839) used in the study of Myburg et al. (1999) clustered within this Southeast Asian/Australian clade. A clove isolate from Zanzibar (CMW 10774) also grouped in the Southeast Asian clade.

The second group within the *C. cubensis* clade (bootstrap support 80%) included Brazilian isolates from clove (CMW 10775, CMW 10776, CMW 10777, CMW 10778) as well as Brazilian (CMW 1853) and Venezuelan (CMW 8757) *C. cubensis* isolates from *Eucalyptus*. This clade also contained *C. cubensis* isolates from the Congo (CMW 10667, CMW 10668) that have been reported previously to group within the South American sub-clade (Roux et al. 2003).

The third sub-clade in the larger *C. cubensis* group included isolates originating from South Africa (bootstrap support 62%). This clade grouped separately from the South American and Southeast Asian *C. cubensis* group (bootstrap support 100%) and appears to represent a distinct taxon as previously shown by Myburg et al. (2002a).

A group of isolates originating on clove in Indonesia (CMW 10779, CMW 10780, CMW 10781) formed a separate and discrete clade, separately from the other clove isolates in the *C. cubensis* clade (bootstrap support 100%). This group was also separate from *C. parasitica*, *C. radicalis*, *C. macrospora* and *C. nitschkei* (bootstrap support 100%). The isolates in this clade were those that had cultural and morphological characteristics different to those of *C. cubensis*.

DISCUSSION

In this study, we have been able to confirm unequivocally that *C. cubensis* occurs on clove. This was based on ribosomal ITS and β-tubulin gene sequence data for fungi isolated from clove originating from South America, Indonesia and central Africa. We have linked these results to morphological characteristics for relevant herbarium

specimens collected from clove in Indonesia, Malaysia and Zanzibar. However, morphological and phylogenetic data from this study also indicate the presence of other fungi related to *Cryphonectria* occurring on clove.

The presence of a fungus other than *C. cubensis* on the clove specimens used in the original description of *E. eugeniae* raises doubt as to which fungus was referred to in the original description of *E. eugeniae*. *Cryphonectria cubensis* and the second fungus with orange stromata are similar and their conidia are undistinguishable. It is, therefore, likely that previous workers could have unwittingly assumed that these fungi represented a single taxon. The teleomorph description of *E. eugeniae* clearly refers to *C. cubensis,* since it describes perithecia developing below the conidiomata (Nutman and Roberts 1952, Reid and Booth 1969). The description of the anamorph of *E. eugeniae*, however, could relate to either *C. cubensis*, or the fungus with the orange anamorph, which we have found on specimens. The identity of *E. eugeniae* is connected to the type specimen of this fungus (IMI 44954), which contains structures with the same tissue type as *C. cubensis*. The synonymy of *E. eugeniae* with *C. cubensis* is, therefore, valid and the other fungi occurring on clove will require independent names.

A fungus, represented by isolates CMW 10779, CMW 10780 and CMW 10781, different from both *C. cubensis* and the fungus with orange to sienna stromata from Zanzibar, was isolated from cankers on clove in northern Sumatra and Kalimantan, Indonesia. DNA sequence data clearly show that this fungus is different from *C. cubensis* and the other *Cryphonectria* sp., yet it is closely related. These isolates could not be connected to morphological structures on host tissue. Bark inoculations on *Eucalyptus* yielded

information on conidial and tissue morphology, but structural morphology was too variable to be used in descriptions. Additional specimens and isolates of this third fungus on clove will be necessary before a name can be provided for it.

Results of this study have shown that *C. cubensis* occurs on clove in South America, Southeast Asia and central Africa. Isolates from clove reside in two phylogenetic groups that were previously defined by Myburg et al. (1999, 2002a) for *C. cubensis* isolates from *Eucalyptus*. It was interesting to discover that *C. cubensis* isolates from central Africa included those from both the Southeast Asian and South American phylogenetic lineages. These phylogenetic data suggest that *C. cubensis* has been introduced into Africa on two separate occasions. South African *C. cubensis* isolates, however, clearly reside in a separate lineage with a distinct origin as recently shown by Myburg et al. (2002a).

The presence of *Cryphonectria* spp. on clove appears to be considerably more complex than previously known. Based on detailed comparisons of DNA sequence and morphological characteristics, we have found that at least two closely related and similar fungi can occur on a single clove specimen. The lack of cultures linked to herbarium specimens has made conclusive identifications of these fungi difficult. However, there is good evidence to show that at least three different species of *Cryphonectria* occur on clove and future collections should make it possible to provide names for the two unidentified species.

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Table 1. Isolates used in sequencing analyses.

Culture no.[a]	Species	Host	Origin	Collector	GenBank accession numbers for ITS and β-tubulin sequence data
CMW 10774	*Cryphonectria cubensis*	*Syzygium aromaticum.*	Tanzania, Zanzibar	n.a	AF 492130, AF 492131, AF 492132
CMW 10775	*Cryphonectria cubensis*	*Syzygium aromaticum*	Brazil	C.S. Hodges	AY 084003, AY 084015, AY 084027
CMW 10776	*Cryphonectria cubensis*	*Syzygium aromaticum*	Brazil	C.S. Hodges	AY 084004, AY 084016, AY 084028
CMW 10777	*Cryphonectria cubensis*	*Syzygium aromaticum*	Brazil	C.S. Hodges	AY 084005, AY 084017, AY 084029
CMW 10778	*Cryphonectria cubensis*	*Syzygium aromaticum*	Brazil	C.S. Hodges	AY 084006, AY 084018, AY 084030
CMW 3839	*Cryphonectria cubensis*	*Syzygium aromaticum*	Indonesia	M.J. Wingfield	AF 046904, AY 084011, AY 084023
CMW 8649	*Cryphonectria cubensis*	*Syzygium aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084000, AY 084012, AY 084025
CMW 8650	*Cryphonectria cubensis*	*Syzygium aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084001, AY 084013, AY 084024
CMW 8651	*Cryphonectria cubensis*	*Syzygium aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084002, AY 084014, AY 084026
CMW 8756	*Cryphonectria cubensis*	*Syzygium aromaticum*	Indonesia	M.J. Wingfield	AF 046896, AF 273077, AF 285165
CMW 9903	*Cryphonectria cubensis*	*Syzygium aromaticum*	Kalimantan, Indonesia	C.S. Hodges	AF 292044, AF 273066, AF 273461
CMW 9906	*Cryphonectria cubensis*	*Syzygium aromaticum*	Kalimantan, Indonesia	C.S. Hodges	AF 292045, AF 273065, AF 273460
CMW 1853	*Cryphonectria cubensis*	*Syzygium aromaticum*	Brazil	n.a	AF 046891, AF 273070, AF 273465
CMW 8757	*Cryphonectria cubensis*	*Eucalyptus* sp.	Venezuela	M.J. Wingfield	AF 046897, AF 273069, AF 273464
CMW 10667	*Cryphonectria cubensis*	*Eucalyptus* sp.	Republic of Congo	J. Roux	AY 063477, AY 063479, AY 063481
CMW 10668	*Cryphonectria cubensis*	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 535121, AF 535123, AF 535125
CMW 1856	*Cryphonectria cubensis*	*Eucalyptus* sp.	Hawaii	n.a	AY 083999, AY 084010, AY 084022
CMW 2631	*Cryphonectria cubensis*	*Eucalyptus marginata*	Australia	E. Davison	AF 543823, AF543824, AF523825
CMW 2632	*Cryphonectria cubensis*	*Eucalyptus marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
CMW 2113	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
CMW 62	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 292041, AF 273063, AF 273458
CMW 8755	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273458
CMW 10463	*Cryphonectria macrospora*	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350
CMW 10518	*Cryphonectria nitschkei*	*Quercus* sp.	Japan	T. Kobayashi	AF 452118, AF 525706, AF 525713
CMW 10455	*Cryphonectria radicalis*	*Quercus suber*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712
CMW 10477	*Cryphonectria radicalis*	*Quercus suber*	Italy	A. Biraghi	AF 368328, AF 368347, AF 368346
CMW 7047	*Cryphonectria parasitica*	*Quercus virginiana*	USA	R.J. Stipes	AF 368329, AF 273073, AF 273469
CMW 7048	*Cryphonectria parasitica*	*Quercus virginiana*	USA	R.J. Stipes	AF 368330, AF 273076, AF 273470
CMW 10779	*Cryphonectria* sp.	*Syzygium aromaticum*	Somosir, Indonesia	M.J. Wingfield	AY 084007, AY 084019, AY 084031
CMW 10780	*Cryphonectria* sp.	*Syzygium aromaticum*	Somosir, Indonesia	M.J. Wingfield	AY 084008, AY 084020, AY 084032
CMW 10781	*Cryphonectria* sp.	*Syzygium aromaticum*	Kalimantan, Indonesia	M.J. Wingfield	AY 084009, AY 084021, AY 084033
CMW 5288	*Diaporthe ambigua*	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821
CMW 5587	*Diaporthe ambigua*	*Malus domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF 543822

[a] Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.

Table 2. Specimens used in morphological comparisons.

Herbarium no.*	Identity	Host	Origin	Date	Collector
IMI 44954 (holotype)	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 44945	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 45440	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 45445	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 45443	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 45448a	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	n.a.
IMI 45446	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 44953	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 44951	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 45452	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	J. Nutman & F.M. Roberts
IMI 49266	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	n.a.	J. Nutman & F.M. Roberts
IMI 45449	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	n.a.
IMI 45450	*Endothia eugeniae*	*Syzygium aromaticum*	Zanzibar	1951	n.a.
IMI 279614	*Cryphonectria cubensis*	*Eucalyptus urophylla*	Cameroon	1983	I.A.S. Gibson
IMI 56425a	*Endothia eugeniae*	Isolate ex *Eugenia* sp. on elm twigs	Malaysia	1954	W.J. Cherewick
IMI 58569	*Endothia eugeniae*	*Syzygium aromaticum*	Malaysia	1954	A. Johnston
IMI 58388	*Endothia eugeniae*	*Syzygium aromaticum*	Malaysia	1954	A. Johnston
IMI 58567	*Endothia eugeniae*	*Eugenia* sp.	Malaysia	n.a.	A. Johnston
IMI 58568	*Endothia eugeniae*	*Eugenia* sp.	Malaysia	1954	A. Johnston
IMI 350626	*Cryphonectria cubensis*	*Syzygium aromaticum*	Singapore	1991	C.P. Yik
PREM 57469	*Cryphonectria cubensis*	Inoculations into *E. saligna* and *S. aromaticum*	n.a.	1986	C.S. Hodges
PREM 57470[$]	*Cryphonectria cubensis* and unknown fungus	*Syzygium aromaticum*	Sulawesi, Indonesia	2001	M.J. Wingfield
PREM 57471	*Cryphonectria cubensis*	Inoculation of CMW 8649 into *E. grandis*	n.a.	2002	M. Gryzenhout
PREM 57472	*Cryphonectria cubensis*	Inoculation of CMW 8650 into *E. grandis*	n.a.	2001	M. Gryzenhout
PREM 57473	unknown	Inoculation of isolate CMW 10781 into *E. grandis*	n.a.	2001	M. Gryzenhout
IMI 304273	*Cryphonectria cubensis*	*Eucalyptus aromatica*	Malaysia	1986	Low Chow Fong
PREM 57297	*Cryphonectria cubensis*	*Eucalyptus* sp.	Indonesia	2001	M.J. Wingfield
IMI 284438	*Cryphonectria cubensis*	*Eucalyptus grandis/Eugenia* sp.	Venezuela	1983	C.S. Hodges
PREM 57294	*Cryphonectria cubensis*	*Eucalyptus grandis*	Colombia	2000	M.J. Wingfield

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

* IMI, Herbarium, CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, UK.

[$] Vouchered specimens linked to isolates CMW 8649 (PREM 57471), CMW 8650 (PREM 57472) and CMW 8651.

Table 3. Key morphological characteristics of the different fungi found on herbarium material of clove.

Fungus	Origin	Conidioma				Ascoma			
		External colour	Structure	Stromatic tissue	Conidia	Stroma colour	Structure	Stromatic tissue	Ascopores
Cryphonectria cubensis	Zanzibar, Malaysia, Indonesia	Dark mouse grey (15'''''k)	Pyriform with attenuated neck, or pulvinate, unilocular occasionally multilocular, convoluted	Umber (15m), base *textura globulosa*, neck *textura porrecta*	Oval to ovoid, aseptate, 3-4 (-4.5) x 1-1.5 (-2) µm	Orange (15) to pale luteous (19d) stroma with blackened perithecial necks	Semi-immersed, slightly erumpent, frequently formed underneath conidioma	Limited, diffuse, prosenchy-matous	Fusoid, one-septate, (5-) 6-7.5 (-8) x 1.5-2.5 µm
Orange to sienna fungus	Zanzibar	Orange (15) to sienna (15i)	Erumpent, elongated pulvinate, convoluted multilocular	Orange (15) to sienna (15i), lower part often lighter, dense, prosenchy-matous	Oval to ovoid, aseptate, (3-) 3.5-4 (-4.5) x 1-1.5 (-2) µm	-	-	-	-
CMW 10781 artificial inoculation	Indonesia	Blackened with luteous (19) apex	Generally ovoid	Umber (15m), pseudoparen-chymatous	Cylindrical aseptate, (2.5-) 3-3.5 (-4) x 1 µm	n.a.	n.a.	n.a.	n.a.

Fig. 1. Light micrographs of *Cryphonectria cubensis* occurring on clove. **a.** Pyriform conidiomata. **b.** Pulvinate conidiomata without necks. **c.** Longitudinal section through conidioma with neck attached. **d.** Longitudinal section through multilocular, pulvinate conidioma. **e.** Base tissue. **f.** Neck tissue. **g.** Conidia. **h.** Ascomata. **i.** Longitudinal section through perithecia occurring underneath conidioma (arrow). **j.** Stromatic tissue of ascoma. **k.** Ascospores. (Scale bars: **a**, **h** 200μm; **b-d**, **i**; 100 μm; **e-g**, **j**, **k** 10 μm.).

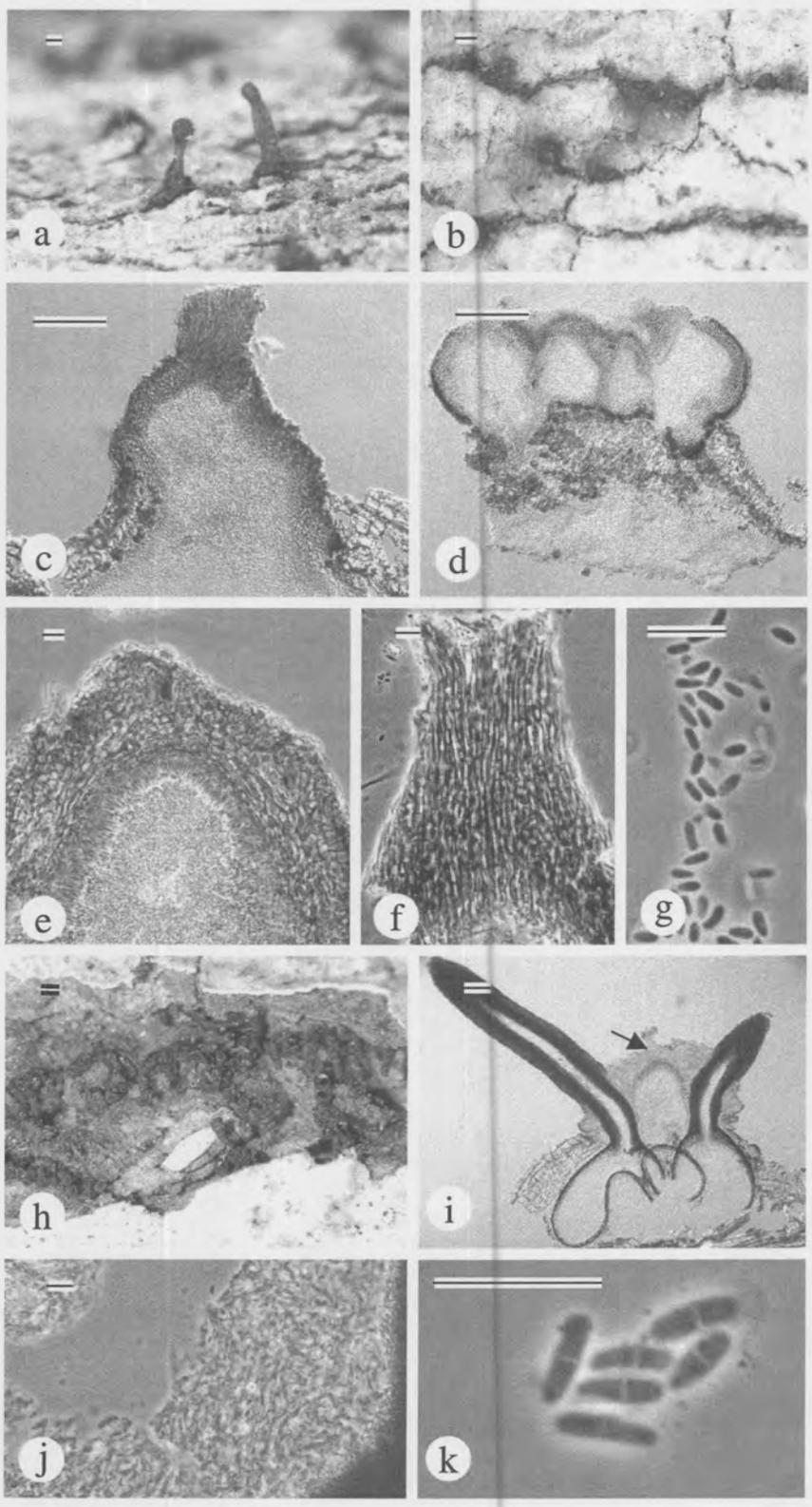


Fig. 2. Light micrographs of the unknown fungus with an orange to sienna anamorph occurring on clove in Zanzibar. **a**. Conidioma. **b**. Longitudinal section through conidioma. **c**. Stromatic tissue. **d**. Conidia. (Scale bars: **a**, **b**, 100 μm; **c**, **d** 10 μm.)

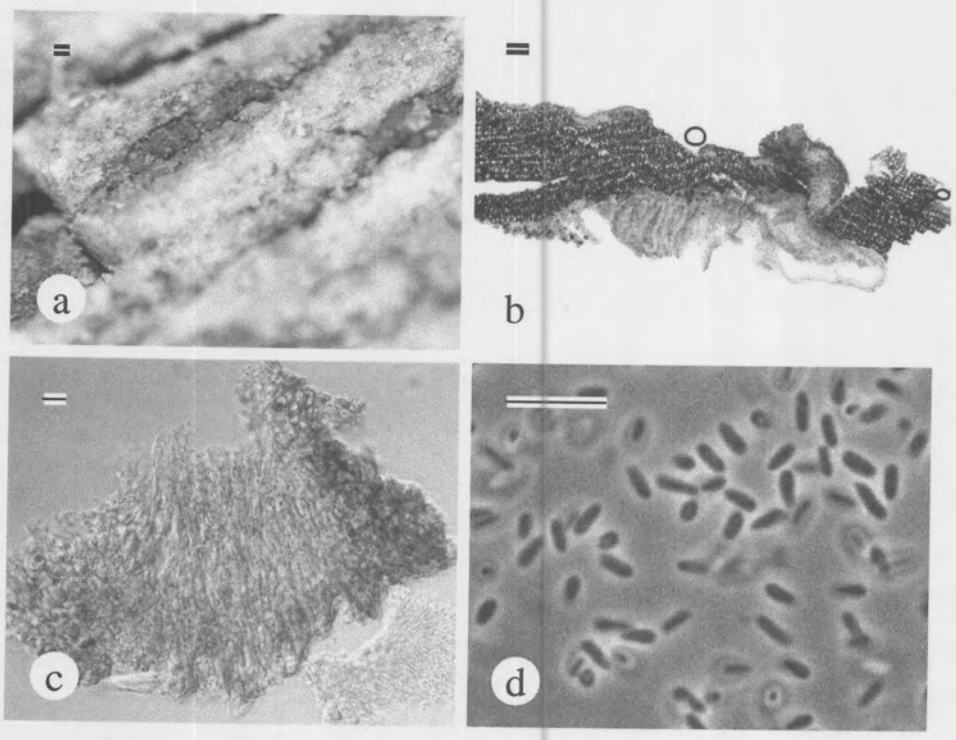


Fig. 3. Light micrographs of fruiting structures produced by isolate CMW 10781 in artificial inoculations on *Eucalyptus* sticks. **a**. Longitudinal section through superficial conidioma. **b-c**. Stromatic tissue. **d**. Conidia. (Scale bars: **a** 100 μm; **b**, **c**, **d** 10 μm).

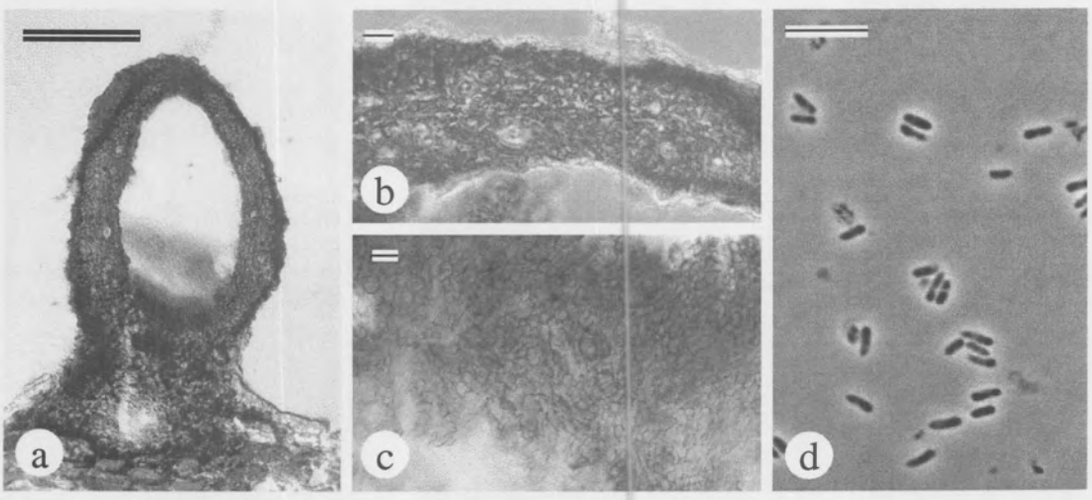
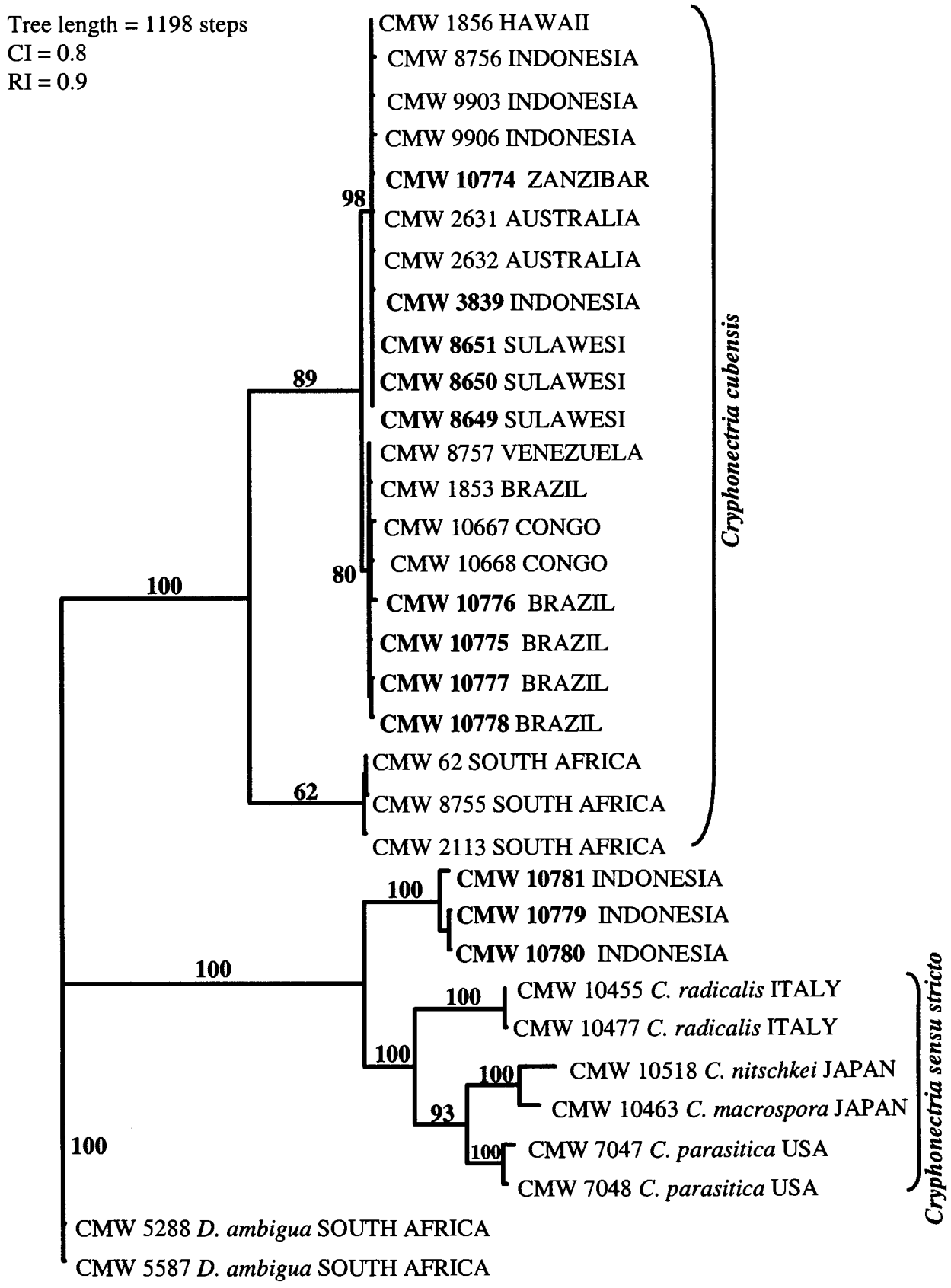


Fig. 4. Strict consensus tree (tree length = 1198 steps, CI = 0.8, RI = 0.9) computed from seventeen trees generated after heuristic search of a combined data set including ribosomal DNA and β-tubulin gene sequences. Bootstrap values (1000 replicates) are indicated above the inter nodes. Taxa in bold represent those sequenced in the present study. The *Diaporthe ambigua* isolates included in this study were used as outgroups to root the phylogenetic tree.



CHAPTER 5

Phylogenetic relationships of *Cryphonectria* and *Endothia* species, based on DNA sequence data and morphology.

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PHYLOGENETIC RELATIONSHIPS OF *CRYPHONECTRIA* AND *ENDOTHIA* SPECIES, BASED ON DNA SEQUENCE DATA AND MORPHOLOGY.

ABSTRACT

The fungal genera *Endothia* and *Cryphonectria* include some of the most important pathogens of forest trees. Despite available new technology, no comprehensive comparative study based on DNA sequence data and morphology has been done on all available isolates representing *Cryphonectria* and *Endothia* species. The main objectives of this study were to assess the phylogenetic relationships among species of *Cryphonectria* and *Endothia* for which cultures are available and to establish a taxonomic framework based on DNA sequence and morphological data that will aid future studies and identification of species residing in these and related genera. Comparisons were based on sequence variation found in the ITS region of the ribosomal RNA operon and two regions of the β-tubulin gene. Besides comparing sequence data, the morphology of these species was also examined. The phylogenetic data indicated that *Endothia* and *Cryphonectria* reside in two distinct phylogenetic clades. *Cryphonectria parasitica*, *C. macrospora*, *C. nitschkei*, *C. eucalypti* and *C. radicalis* represented the *Cryphonectria* clade. *Endothia* was characterised by *E. gyrosa* and *E. singul*aris isolates. An isolate representing *E. viridistroma* grouped outside the *Endothia* clade and separately from other groups. Other clades outside that encompassing

Cryphonectria, were those represented by the *C. cubensis* isolates and fungi isolated from *Elaeocarpus dentatus* originating from New Zealand. These clades could be distinguished from *Endothia* and *Cryphonectria*, based on anamorph morphology, stromatal structure and ascospore septation. *Cryphonectria* and *Endothia*, therefore, appear to be paraphyletic and taxonomic relationships for these fungi need to be revised.

INTRODUCTION

The fungal genera *Cryphonectria* and *Endothia* (order Diaporthales) have relatively few members, but include some of the most serious pathogens of forest trees in the world. These genera have been subjected to several classical taxonomic treatments (Barr 1978, Hodges 1980, Kobayashi 1970, Roane et al. 1986a, Shear et al. 1917), most of which were based on morphological comparisons. Most important of these, is Barr's (1978) monograph on the Diaporthales, which had a fundamental and important impact on the taxonomy of *Endothia*. Prior to the work of Barr, *Cryphonectria* was synonymous with the older *Endothia* (Kobayashi 1970, Shear et al. 1917, Von Höhnel 1909). Barr (1978), however, segregated *Cryphonectria* from *Endothia* based on the differences in ascospore septation and stromatal morphology. Of the thirteen species originally treated in *Endothia*, only three were retained, i.e. *E. gyrosa* (Schw.: Fr.) Fr. (type species), *E. viridistroma* Wehm. and *E. singularis* (H. & B. Syd.) Shear & Stevens. The remaining species were transferred to *Cryphonectria* and these included the type species *C. gyrosa* (Berk. & Br.) Sacc. (= *E. tropicalis* Shear & Stevens), *C. cubensis* (Bruner) Hodges, *C. havanensis* (Bruner) Barr, *C. macrospora* (Kobayashi & Ito) Barr, *C. nitschkei* (Otth.) Barr, *C. parasitica* (Murr.) Barr and *C. radicalis* (Schw.: Fr.) Barr. Other already described species, *C. longirostris* (Earle) Micales & Stipes and *C. coccolobii* (Vizioli)

Micales & Stipes, were not mentioned by Barr (1978), but were placed in *Cryphonectria* by Micales and Stipes (1987) based on similarities in morphology with other species in *Cryphonectria.*

Of the species in *Endothia* and *Cryphonectria* only *E. gyrosa*, *C. parasitica* and *C. cubensis* are known to be serious pathogens. The remaining members of these genera are considered saprophytic (Roane et al. 1986b). *Endothia gyrosa* causes cankers on various hardwood species in the USA and is known as the causal agent of pin oak (*Quercus palustris* Muench.) blight (Appel and Stipes 1986, Roane et al. 1974, Snow et al. 1974, Stipes and Phipps 1971). *Cryphonectria parasitica* is well-known for the devastation that it has caused to the American chestnut, *Castanea dentata* Borkh. (Anagnostakis 1987, Heiniger and Rigling 1994). This fungal pathogen has destroyed the American chestnut as a major forest tree and has resulted in significant change to the ecology of the eastern hardwood forests (Anagnostakis 1987). *Cryphonectria cubensis* is another important pathogen that causes a serious canker disease of plantation *Eucalyptus* species in tropical and subtropical areas of the world (Hodges et al. 1976, Hodges et al. 1979, Sharma et al. 1985a, b, Wingfield et al. 1989). This fungus is recognised as the causal agent of die-back on clove (*Syzygium aromaticum* (L.) Murr. & Perry) (Myrtaceae) (Hodges et al. 1986) and a serious canker disease of *Tibouchina* species (Melastomataceae) (Myburg et al. 2002b, Wingfield et al. 2001). *Cryphonectria eucalypti* is a canker pathogen of *Eucalyptus* trees and occurs in South Africa (Gryzenhout et al. 2003, Van der Westhuizen et al. 1993) and Australia (Old et al. 1986, Walker et al. 1985, Yuan and Mohammed 1997). This pathogen was previously known as *E. gyrosa*, but was found to represent a distinct and new species (Venter et al. 2001, 2002).

Morphologically, *Endothia* is characterised by strongly developed, widely erumpent stromata with predominantly pseudoparenchymatous tissue (Barr 1978, Micales and Stipes 1987). Perithecia are usually born in an upright, diatrypoid configuration (Barr 1978, Micales and Stipes 1987). In contrast, the stromata of *Cryphonectria* are semi-immersed in the bark and not as strongly developed as those of *Endothia* (Barr 1978, Micales and Stipes 1987). Stromatic tissue is predominantly prosenchymatous and the perithecia are often forced into a valsoid configuration by surrounding bark tissue (Barr 1978, Micales and Stipes 1987). Furthermore, *Cryphonectria* is distinguished by fusoid to ellipsoid, one-septate ascospores, while *Endothia* has cylindrical to allantoid, aseptate ascospores (Barr 1978, Micales and Stipes 1987). The phylogenetic studies of Venter et al. (2002), however, showed that ascospore septation is not a valid character at generic identification, and that stromatal morphology is more useful.

It is difficult to distinguish between species of *Endothia* and *Cryphonectria* based on morphology. Distinction is mainly restricted to size differences in fruiting structures (Roane 1986a, Kobayashi 1970). No method has been developed to distinguish unequivocally between all species of the two genera. Pigment production (Roane and Stipes 1978), disc electrophoresis of intramycelial enzymes (Stipes et al. 1982), tolerance to antibiotics (Micales and Stipes 1986) and optimal temperatures for growth (Stipes and Ratliff 1973) could only be used to distinguish between some species, especially *C. parasitica* and *E. gyrosa*.

Myburg et al. (1999) provided the first phylogenetic data on representatives of *Endothia* and *Cryphonectria*. The aim was to resolve taxonomic questions pertaining to *C. cubensis*. This study supported the conspecificity of *E. eugeniae* with *C. cubensis* and

showed clearly that *C. parasitica* is different from *E. gyrosa*. *Cryphonectria cubensis* isolates were also found to reside in two well-resolved sub-clades, reflecting a South American and a Southeast Asian group. The study of Myburg et al. (1999) was based on sequence variation within the ITS1 and ITS2 regions of the ribosomal RNA operon. A third sub-clade including isolates from South Africa, was recognised when β-tubulin and histone *H3* gene sequences were used in phylogenetic analyses (Myburg et al. 2002a).

In a phylogenetic study conducted by Venter et al. (2002), additional species of *Cryphonectria* and *Endothia* were included to examine the generic placement of the new species, *C. eucalypti*. In this study, isolates representing *C. parasitica*, *C. radicalis*, *C. macrospora*, *E. gyrosa* and *E. singularis* were included. *Endothia* and *Cryphonectria* grouped as two distinct phylogenetic clades. Only a subset of isolates currently available to us was used in the study of Venter et al. (2002). No comprehensive study including morphology and phylogenetic data has thus been undertaken on all available isolates representing the different species of these two genera. The objective of the present study was, therefore, to compare all available species of *Endothia* and *Cryphonectria* for which cultures and vouchered specimens exist. More specifically, our aim was to re-evaluate the generic distinctions between *Cryphonectria* and *Endothia* and thus to provide a taxonomic basis, based on DNA sequence data and morphological characteristics, for future studies of species assemblages residing in them.

MATERIALS AND METHODS

Isolates studied

Isolates included in this study (Table 1) represent most of the species retained in the genera *Cryphonectria* and *Endothia* (Barr 1978, Micales and Stipes 1987). A number of these isolates were obtained from the culture collection of R.J. Stipes. Authentic cultures are not available for *C. coccolobii*, *C. longirostris* or *C. havanensis* and these could not be included in this study. The isolate labelled as E40 (CMW 10453) in the collection of R.J. Stipes was previously found to be *C. cubensis* (Micales et al. 1987) and not representative of *C. havanensis* from Cuba. Isolates of *C. eucalypti*, the most recent addition to *Cryphonectria* (Venter et al. 2002), were also included in this study. All isolates (Table 1) are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and a duplicate set of sub-cultures have been deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

DNA extractions, ribosomal RNA (ITS1, 5.8S, ITS2) and β-tubulin gene amplification

DNA was extracted as described by Myburg et al. (1999). Amplification of the ITS 1, 5.8S and ITS 2 regions of the ribosomal RNA operon as well as two regions within the β-tubulin gene were as described by Myburg et al. (1999) and Myburg et al. (2002a) respectively. The primer pairs that were used to amplify the respective regions were the following: ITS1 and ITS 4 (White et al. 1990), Bt1a and Bt1b (Glass and Donaldson

1995) and Bt2a and Bt2b (Glass and Donaldson 1995). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany).

Ribosomal RNA (ITS1, 5.8S, ITS2) and β-tubulin gene sequencing

PCR products were sequenced in both directions using the same primer pairs that were used in the amplification reactions. Sequencing reactions were achieved using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, United Kingdom). The nucleotide sequences were determined with an ABI PRISM 3100™ automated DNA sequencer.

Sequence alignment and analyses

Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software was used to analyse the DNA sequences. All sequences generated were aligned in a data matrix using CLUSTAL X (Thompson et al. 1997) and the alignment was checked manually. Sections of the ITS and the β-tubulin introns were highly variable. This resulted in difficulty when aligning sequence data. An analysis (data not shown) of only the exon regions of the β-tubulin gene produced a phylogenetic tree with a similar topology to that obtained when full data set was considered collectively. However, there was no resolution at the intraspecific level using this more conservative data set and all the sequence data were thus retained in the analyses, where gaps were treated as missing data.

Subsequent phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford 1998). A 500 replicate partition-homogeneity test (PHT) was executed to determine if the ribosomal (ITS1, 5.8S, ITS2) and β-tubulin gene sequence data sets (1a/1b, 2a/2b) could be combined as one data set prior to phylogenetic analyses. Gene sequences were analysed using heuristic searches with tree-bisection-reconnection (TBR) and MULTREES options (saving all optimal trees) effective. The confidence levels of the tree branch nodes generated in the phylogenetic analysis were determined by a 1000 replicate bootstrap analysis. *Diaporthe ambigua* Nitschke, a known canker pathogen of stone and pome fruit trees (Smit et al. 1996, 1997) was included as outgroup taxon to root the phylogenetic tree. Sequences were deposited in Genbank and the accession numbers are listed in TABLE I. The sequence alignments and phylogenetic tree (FIG. 1) were deposited in TreeBase (submission ID number = SN 1205).

Morphological studies

General morphological features such as stromatal and spore morphology, were examined microscopically for relevant herbarium specimens of *Endothia* and *Cryphonectria* (Table 2). As far as possible, the type specimens of the different species in the phylogenetic tree were studied. Specimens from New Zealand, linked to some of the isolates used in the phylogenetic study (Table 2), were also included.

Fruiting structures were embedded in Leica mountant (Setpoint Premier, Johannesburg, South Africa) after rehydration in boiling water for 1 min. Sections were made with a Leica CM1100 cryostat (Setpoint Premier) at –20 C and were 12-16 μm thick. Sections

were dropped in water, transferred to a microscope slide, mounted in lacto-phenol and examined using phase contrast and differential interference contrast light microscopy.

RESULTS

Ribosomal RNA (ITS1, 5.8S, ITS2) and β-tubulin gene amplification and sequencing

Amplification products for the respective gene regions were between 550bp and 600bp in size (data not shown). Sequences for isolates generated in this study were aligned with sequence data from previous studies (Table 1). The PHT performed between the ribosomal and β-tubulin gene sequence data sets generated a P-value of 0.01. This indicated that there was no significant conflict between the data sets and that they could be combined in subsequent phylogenetic analyses. The ribosomal (ITS1, 5.8S, ITS2) and β-tubulin (1a/1b and 2a/2b) sequence data sets were thus analysed together in the parsimony analyses.

The resulting combined data set comprised of 28 sequences of which one, *D. ambigua*, was used as the outgroup taxon. Manual alignment of the combined β-tubulin gene and ribosomal DNA sequence data resulted in a total of 1510 characters (Appendix 4). Of these 932 characters were constant, 132 variable characters were parsimony uninformative and 446 variable characters were parsimony informative. No sequence characters were excluded. The heuristic search produced twenty-three trees, which were converted to a strict consensus tree (tree length = 1154 steps, consistency index/CI = 0.6888, retention index/RI = 0.8376).

The consensus tree (Fig. 1) showed a well-resolved clade labelled as "*Cryphonectria* spp." and representing the taxa *C. parasitica, C. nitschkei, C. macrospora, C. eucalypti* and *C. radicalis* from Europe. Isolates of *C. radicalis* formed two distinct groups. One is represented by isolates CMW 10477 and CMW 10455 while the other is represented by isolate CMW 10484 and an isolate from *Quercus* identified as *Endothiella gyrosa* Sacc. (CMW 10436) (bootstrap value = 100%).

Two groups of isolates originally identified as species of *Cryphonectria* did not group within the main *Cryphonectria* clade. The first of these included unidentified isolates from New Zealand, which were originally labelled *C. radicalis* (CMW 10469, CMW 10470) and *C. gyrosa* (CMW 10471) (bootstrap support = 100%). These isolates originated from *Elaeocarpus dentatus* Vahl.

The second group of isolates that clustered outside the *Cryphonectria* clade were those representing *C. cubensis* (bootstrap = 100%). Within this *C. cubensis sensu lato* clade, the three sub-clades as previously defined by Myburg et al. (2002a), were evident and represented *C. cubensis* originating from South America/Congo, Southeast Asia and South Africa, respectively.

Endothia was represented by *E. gyrosa* and *E. singularis* isolates from the USA. The remaining *Endothia* species, *E. viridistroma*, grouped separately from the *Endothia* and *Cryphonectria* clades. A BLAST search on the ITS sequence data generated for the *E. viridistroma* isolate revealed that it has a 97% similarity to *Cytospora eucalypticola* Van der Westh. (Genbank Accession number = AF192321, BLAST result = 835 bits), a weak

pathogen of *Eucalyptus* in Australia and South Africa (Old et al. 1986, Van der Westhuizen 1965).

Morphological studies

The different groups found in the phylogenetic tree could be distinguished based on morphology (Fig. 2). The most important distinguishing character was anamorph morphology. The conidiomata of *E. gyrosa* (Fig. 2a) and *E. singularis* (Fig. 2b) were tuberculate and locules were numerous and minute, while those of the *Cryphonectria* spp. (i.e. *C. parasitica*, *C. radicalis* from Europe, *C. nitschkei*, *C. macrospora*) (Fig. 2c) were pulvinate and locules were few in number and large. Those of *C. eucalypti* (Fig. 2d) were similar to those of the other *Cryphonectria* spp. (Fig. 2c). The conidiomata on the specimens of *C. gyrosa* and *C. radicalis* from New Zealand (Fig. 2e) were unique, since single conidiomata were ovoid, superficial and unilocular. More complex conidiomatal structures on the New Zealand specimens (containing more than one ovoid structure) were multilocular with irregular conidial locules (Fig. 2e). Conidiomata of *C. cubensis* (Fig. 2f) were also different and were convoluted, generally unilocular, superficial and pycnidia-like. The conidiomata of *C. cubensis* were blackened, unlike the orange conidiomata of the other species of *Cryphonectria* and *Endothia*.

The ascomata of *E. gyrosa* (Fig. 2a), *E. singularis* (Fig. 2b) and the New Zealand specimens (Fig. 2e) were erumpent, strongly developed and superficial. Perithecia were diatrypoid and the bases situated in fungal tissue above the level of the bark. The ascomata of the *Cryphonectria* spp. (Fig. 2c) and *C. eucalypti* (Fig. 2d) were also erumpent, but were semi-immersed and perithecia were valsoid, with the bases

surrounded by bark tissue beneath the level of the bark. Ascomata of *C. cubensis* specimens (Fig. 2f) had weak to no stromatal tissue development, and the protruding necks of the perithecia were covered with brown tissue, which was different to the orange necks of the other specimens of *Cryphonectria*.

Ascospores of *E. gyrosa* (Fig. 2a) and *E. singularis* (Fig. 2b) were aseptate, cylindrical to allantoid. Those of the *Cryphonectria* spp. (Fig. 2c) and *C. cubensis* (Fig. 2f) were one-septate, ellipsoid to fusoid. The specimens (K 109807, K 109809, BPI 614797, BPI 614526, BPI 797701) connected to the type species of *Cryphonectria*, *C. gyrosa* from Sri Lanka, also had one septate ascospores. *Cryphonectria eucalypti* (FIG. 2d) had aseptate, cylindrical to allantoid ascospores that were different to those of other *Cryphonectria* species, and more similar to those of *Endothia* species. For specimens representing the New Zealand clade (Fig. 2e), ascospores were different to those of *Cryphonectria* species and *C. cubensis* in having 1-3 septa. Conidia of *E. gyrosa* (Fig. 2a), *E. singularis* (Fig. 2b), the *Cryphonectria* species (Fig. 2c), *C. eucalypti* (Fig. 2d) and the specimens from New Zealand (Fig. 2e) were aseptate, minute and cylindrical. The conidia of *C. cubensis* (Fig. 2f) differed from those of the others in being more oval than cylindrical.

Features of *E. viridistroma* that led to its placement in *Endothia* are the large, erumpent, tuberculate, superficial stromata (Fig. 2g). Perithecia have a similar orientation to those of *E. gyrosa* (Fig. 2g) and conidial locules are numerous and irregular to ellipsoid (Fig. 2g) (Wehmeyer 1936). Ascospores are aseptate and allantoid to slightly ellipsoid, and conidia are aseptate, cylindrical to allantoid (Fig. 2g) (Wehmeyer 1936). *Endothia viridistroma* is, however, atypical of all the other species of *Endothia* and *Cryphonectria* since the stromata of this species has a dark green exterior and green interior.

DISCUSSION

This study presents a phylogenetic analysis of a large group of isolates that were identified in *Cryphonectria* and the closely related genus *Endothia*. The majority of these are linked to vouchered specimens representing different species of *Cryphonectria* and *Endothia*, e.g. *C. parasitica*, *C. radicalis* (Europe), *C. nitschkei*, *C. macrospora*, *C. eucalypti*, *E. gyrosa* and *E. singularis*. Sequence and morphological data provide evidence that *Endothia* and *Cryphonectria* represent separate genera. These data also show that *C. cubensis* should be excluded from *Cryphonectria*. Similarly, taxa labelled as *Cryphonectria* spp., occurring on *Elaeocarpus dentatus* from New Zealand, do not reside in *Cryphonectria* and the taxon representing *E. viridistroma* does not belong in *Endothia*. The sequence and morphological data that are now available should facilitate future segregation among species in these genera. Studies including more specimens and isolates for each phylogenetic group should now be undertaken to formally describe the new generic groups proposed in this study.

To the best of our knowledge, all species for which cultures are currently available were included in this study. It is unfortunate that isolates of *C. longirostris*, *C. coccolobii* and an authentic isolate of *C. havanensis* from Cuba, the described origin of *C. havanensis* (Bruner 1916), are unavailable. An isolate (CMW 10471) that was originally identified as *C. gyrosa*, isolated from specimen PDD 32619, was phylogenetically and morphologically linked to the New Zealand group studied. Specimens in this group had morphological characteristics atypical of the type species of *Cryphonectria* and other *Cryphonectria* spp. The latter two groups have one-septate ascospores (with the exception of *C. eucalypti*), while the specimens linked to the New Zealand clade are

characterised by one to three septate ascospores. We were, therefore, not able to use isolate CMW 10471 as a representative of *C. gyrosa*, which is the type species of *Cryphonectria*.

Numerous challenges exist regarding the type species of *Endothia* and *Cryphonectria*. *Endothia gyrosa* (Fries 1849) and *C. gyrosa* (Berkeley and Broome 1875) were described in the 1800's and no cultures exist that can be linked to these names. The type specimen of *E. gyrosa* has been separated and moved among a number of herbarium collections in the past (Shear et al. 1917). The only remaining fragment of the original type material of *E. gyrosa*, which is designated as a co-type, contains only conidiomata (Shear et al. 1917). Comparisons of teleomorph morphology using the current collections are thus impossible.

In the case of *C. gyrosa*, ambiguities include the existence of two sets of herbarium specimens that are linked to this fungus. The first (K 109807, K 109809) is connected to the original description of *C. gyrosa* (basionym *Diatrype gyrosa* Berk & Br.) from Sri Lanka and the host was specified only as "sticks". Shear et al. (1917) obtained an alternative set of specimens, presumably of the same fungus, from Sri Lanka (BPI 614526, BPI 614797) on *Elaeocarpus glandulifer* Mast., when they transferred *C. gyrosa* to *E. tropicalis*. This was after *Cryphonectria* had been reduced to synonymy with *Endothia* (Von Höhnel 1909). These BPI specimens (BPI 614526, BPI 614797) were thus designated as the type specimens of *E. tropicalis* (Shear et al. 1917), while one of the original specimens connected to the 1875 description of *C. gyrosa* (K 109809, designated originally as number 290) was mentioned only as an additional collection examined (Shear et al. 1917).

In subsequent reviews on the taxonomy of *C. gyrosa* hosts other than *Elaeocarpus glandulifer* have been mentioned. These include an *Elaeagnus* sp. (Barr 1978), as well as *Elaeocarpus dentatus*, *Myrsine salicina* Heward, several *Quercus* spp., *Quintinia serrata* A. Cunn. and *Shiia sieboldii* Makino (Roane 1986a). The review of *C. gyrosa* by Barr (1978) was possibly based on specimen BPI 797701 that is stated to have originated from the original host *Elaeagnus glandulifer*, and collected from the same locality (i.e. Hakgala, Sri Lanka) as BPI 614526 and BPI 614797. There has, however, never been a plant species with the name *Elaeagnus glandulifer* (International Plant Name Index Query, http://www.ipni.org/ipni/query_ipni.html), and the host for the abovementioned material given on the herbarium packet, should probably have been *Elaeocarpus glandulifer* Mast. Mention of *C. gyrosa* on *Quercus* spp. and *S. sieboldii* (Roane 1986a), possibly originates in reports of this fungus from Japan (Kobayashi and Ito 1956, Kobayashi 1970). *Quintinia serrata* and *M. salicina* are, however, not mentioned as hosts of *C. gyrosa* in Japan (Kobayashi and Ito 1956, Kobayashi 1970), and the source of these reports is unclear. These contradictions regarding the appropriate type specimen for *C. gyrosa* need to be addressed and will probably rely upon new collections from the original collection sites.

Isolates from New Zealand considered in this study and labelled as *C. radicalis* and *C. gyrosa*, were not related to other *C. radicalis* isolates within the *Cryphonectria* clade. Nor did they resemble *C. gyrosa* specimens from Sri Lanka. These isolates from New Zealand were also not similar to the group accommodating *C. cubensis* or the phyloclade representing *Endothia*. The New Zealand isolates originated from *Elaeocarpus dentatus* and it is probable that the isolate labelled *C. gyrosa* (CMW 10471) was misidentified as

this species, because it also has large, erumpent, pulvinate stromata and originated from the *Eleaocarpus* (Roane 1986a, Shear et al. 1917). Ascospores of the New Zealand specimens labelled as *C. gyrosa* and *C. radicalis* specimens were, however, unusual in being two or three septate (Fig. 2e). They are, therefore, different from the type specimens connected to *C. gyrosa* from Sri Lanka and other specimens of *C. radicalis* that have two-celled ascospores. This feature, the ovoid anamorph structures and the grouping of these isolates separately from other *Cryphonectria* and *Endothia* isolates in the phylogenetic analysis, suggests that they most likely represent a discrete genus.

Our phylogenetic and morphological results provide added evidence that *C. cubensis* represents a distinct genus closely related to *Cryphonectria* and *Endothia*. Isolates of *C. cubensis* formed a distinct group separate from other *Cryphonectria* spp. The blackened, superficial to slightly immersed, pyriform bases with attenuated neck of the anamorph of *C. cubensis* (Bruner 1917, Hodges 1980, Myburg et al. 2002a) and reduced stromatic development and extending perithecial necks with dark brown tissue (Hodges 1980, Myburg et al. 2003), furthermore distinguishes *C. cubensis* from the *Cryphonectria* spp., the *Endothia* spp., the unidentified species occurring on *Elaeocarpus dentatus* from New Zealand and the type specimens of *C. gyrosa* from Sri Lanka.

The distinct morphology of *C. cubensis,* when compared with that of other *Cryphonectria* species, has led to uncertainty as to where *C. cubensis*, previously known as *Diaporthe cubensis* Bruner, should be placed (Bruner 1917, Hodges 1980). It has been suggested that *C. cubensis* could belong in the genus *Cryptodiaporthe*, with a *Cystosporella* anamorph (Roane 1986a). Recent phylogenetic studies based on LSU rDNA, however, showed that *C. cubensis* does not group with other *Cryptodiaporthe*

species (Castlebury et al. 2002, Zhang and Blackwell 2001). One species of *Cryptodiaporthe*, *C. corni* (Wehm.) Petr., however, did group close to *C. cubensis*, but was not representative of the genus *Cryptodiaporthe* (Castlebury et al. 2002, Zhang and Blackwell 2001).

Isolates labelled as *C. radicalis* from Europe formed two sub-groups within the greater *Cryphonectria* clade. The one sub-clade (CMW 10477, CMW 10455) presumably represents *C. radicalis*, but the identity of isolates in the other sub-clade (CMW 10436, CMW 10484) is unknown. *Cryphonectria radicalis* has been reported to occur widely in Europe (Anagnostakis 1983, Hoegger et al. 2002, Shear et al. 1917), and also in the USA (Shear et al. 1917) and Japan (Kobayashi 1970). Despite this fact, few isolates exist and it is reportedly difficult to find the fungus in the United States (M. Milgroom, personal communication). This might be due to its displacement by the virulent *C. parasitica* that was previously not present in its natural habitat (Anagnostakis 1983, Hoegger et al. 2002). An alternative hypothesis is that *C. radicalis* is not easily noticed due to the presence of the more commonly found and pathogenic *C. parasitica* (Hoegger et al. 2002). The correct taxonomic placement of isolates in the two sub-clades representing *C. radicalis* from Europe is currently impossible, since there are no herbarium specimens linked to European isolates of *C. radicalis*.

An isolate labelled as *Endothiella gyrosa* from Portugal (CMW 10436), grouped within the European *C. radicalis* clade. *Endothiella* is currently the recognized anamorph genus for both *Endothia* and *Cryphonectria* (Hawksworth et al. 1996). This specimen was isolated from *Quercus suber* L., the same host as that of the Italian *C. radicalis* isolates. We believe that this isolate was misidentified and should have been designated as *C.*

radicalis. This, however, illustrates the difficulty of identifying species of *Endothia* and *Cryphonectria,* in the absence of teleomorph specimens. Conidia of these two genera are similar and stromatal morphology of *Cryphonectria* species can sometimes be superficial and strongly developed, similar to those of *E. gyrosa*. This could be due to host tissue characteristics and environmental conditions (Cannon 1988, Hodges et al. 1986, Shear et al. 1917).

The ascospores of *C. eucalypti* differ from those of other *Cryphonectria* spp. in being aseptate. In this study, isolates of this fungus grouped together with *Cryphonectria* species and not close to those in the *Endothia* clade. This finding supports a previous report (Venter et al. 2002) that stromatal structure is an important taxonomic feature for this group of fungi. The aseptate ascospores of *C. eucalypti,* in contrast to septate ascospores of other *Cryphonectria* species, however, raise the question as to whether this fungus represents a distinct genus. At present, phylogenetic data are insufficient to support transferring *C. eucalypti* to a discrete genus.

The *E. viridistroma* specimens included in this study have green stromata (Roane 1986a, Wehmeyer 1936), which is unlike other species of *Endothia,* which have orange stromata (Barr 1978, Shear et al. 1917, Roane 1986a). Results of a BLAST search on the ITS ribosomal sequence data generated for this *E. viridistroma* isolate, showed sequence similarities with *Cytospora eucalypticola*. *Endothia viridistroma*, however, has large, widely erumpent, pulvinate stromata with diatrypoid perithecia (Roane 1986a, Wehmeyer 1936). This is in contrast to the immersed, typically valsoid, blackened stromata of *Valsa* species and their multilocular *Cytospora* anamorphs (Spielman 1984). It is, therefore, unlikely that *E. viridistroma* can be accommodated in *Valsa*. We believe

that the *E. viridistroma* isolate in our collection was misidentified and does not represent the fungus originally described as *E. viridistroma*. The taxonomic relationships of *E. viridistroma* will be difficult to resolve, since no other isolates of this species exist and herbarium specimens contain insufficient stromata for meaningful taxonomic study.

The results of this study reflect the importance of linking isolates to vouchered herbarium material in order to identify the defined taxa. The genera *Cryphonectria* and *Endothia* include very important fungal pathogens and it is essential that these species are correctly identified. One such example relates to *C. cubensis sensu lato.* Results of the present study and that of Myburg et al. (2002a) indicate that *C. cubensis sensu lato* includes isolates reflecting three geographically distinctive groups. Of these, the South African *C. cubensis* isolates are different from *C. cubensis* in other parts of the world and they are also more pathogenic (Myburg et al. 2002a, Roux et al. 2003). This discovery has important implications for the global security of *Eucalyptus* species, both in their native range and in countries where these trees are propagated t commercially. Further studies and possibly the development of rapid techniques to identify these fungi should thus be undertaken.

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Table 1. Isolates used in this study.

Isolate no.[a]	Additional numbers[b]	Original label name of taxon[c]	Host	Origin	Collector	Genbank Accession no.
CMW 2113	-	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
CMW 8755	-	*C. cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273459
CMW 62	-	*C. cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 292041, AF 273063, AF 273458
CMW 1840	-	*C. cubensis*	*Eucalyptus camaldulensis*	China	unknown	AF 046890, AF 273071, AF 273466
CMW 1853	-	*C. cubensis*	*Syzygium aromaticum*	Brazil	unknown	AF 036891, AF 273070, AF 273465
CMW 8757	-	*C. cubensis*	*Eucalyptus*	Venezuela	M.J. Wingfield	AF 046897 AF 273069 AF 273464
CMW 8758	-	*C. cubensis*	*Eucalyptus*	Venezuela	M.J. Wingfield	AF 046898 AF 273068 AF 273463
CMW 8756	-	*C. cubensis*	*Eucalyptus*	Indonesia	M.J. Wingfield	AF 046896, AF 273077, AF 375606
CMW 2632	-	*C. cubensis*	*Eucalyptus marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
CMW 10453	E40, CBS 505.63	*C. havanensis*	*Eucalyptus saligna*	Congo	unknown	AY 063476, AY 063478, AY 063480
CMW 10463	E54	*C. macrospora*	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350

Table 1. (continued)

Isolate no.[a]	Additional numbers[b]	Original label name of taxon[c]	Host	Origin	Collector	Genbank Accession no.
CMW 10518	E53	*C. nitschkei*	*Quercus*	Japan	T. Kobayashi	AF 452118 AF 525706, AF 525713
CMW 1651	-	*C. parasitica*	*Castanea dentata*	USA	unknown	AF 046901, AF 273074, AF 273467
CMW 1652	-	*C. parasitica*	*Castanea dentata*	USA	unknown	AF 046902, AF 273075, AF 273468
CMW 10455	E42, CBS 238.54	*C. radicalis*	*Quercus suber*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712
CMW 10477	E76, CBS 240.54	*C. radicalis*	*Castanea sativa*	Italy	A. Biraghi	AF 368328, AF 368347, AF 368346
CMW 10484	E83, CBS 240.54	*C. radicalis*	*Castanea sativa*	Italy	A. Biraghi	AF 368327, AF 368349, AF 368349
CMW 10469	E67	*C. radicalis*	*Elaeocarpus dentatus*	New Zealand	G. Samuels	AF 452111, AF 525707, AF 525714
CMW 10470	E68	*C. radicalis*	*Elaeocarpus dentatus*	New Zealand	G. Samuels	AF 452112, AF 525708, AF 525715
CMW 10471	E70	*C. gyrosa*	*Elaeocarpus dentatus*	New Zealand	G. Samuels	AF 452116, AF 525709, AF 525716
CMW 7037	-	*C. eucalypti*	*Eucalyptus*	Australia	M.J. Wingfield	AF 232880, AF 368343, AF 368342
CMW 7036	-	*C. eucalypti*	*Eucalyptus*	South Africa	M.J. Wingfield	AF 232878, AF 368341, AF 368340

Table 1. (continued)

Isolate no.[a]	Additional numbers[b]	Original label name of taxon[c]	Host	Origin	Collector	Genbank Accession no.
CMW 10436	E14, CBS 165.30	*Endothiella gyrosa*	*Quercus suber*	Portugal	B. d'Oliveira	AF 452117, AF 525703, AF 525710
CMW 2091	E13	*Endothia gyrosa*	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336
CMW 10442	-	*E. gyrosa*	*Quercus palustris*	USA	R.J. Stipes	AF 368326, AF 368339, AF 368338
CMW 10465	E58	*E. singularis*	unknown	USA	unknown	AF 368323, AF 368333, AF 368332
CMW 10454	E41, CBS 202.36	*E. viridistroma*	*Cercis canadensis* Castigl.	USA	J.H. Miller	AF 452120, AF 525704, AF 525711
CMW 2498	-	*Diaporthe ambigua*	*Malus sylvestris*	Netherlands	S. Truter	AF 046906, AF 273072, AF 273471

[a] Taxa presented in bold represent those for which sequences were generated in this study. Sequences for the other taxa were obtained from the previous studies of (Myburg et al. 1999, Myburg et al. 2002a, Roux et al. 2003, Venter et al. 2002). Isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.

[b] Alternative numbers refer to those deposited in the Centraalbureau voor Schimmelcultures (**CBS**), P.O. Box 85167, 3508 AD Utrecht, The Netherlands and those from the culture collection (**E**) of Prof. R. J. Stipes now housed in the culture collection (CMW) of FABI (see **a**).

[c] Names of taxa are those on the original labels. As a result of this study "*C. havanensis*" (CMW 10453) now represents *C. cubensis* and "*C. radicalis*" (CMW 10469, CMW 10470), "*C. gyrosa*" (CMW 10471), "*Endothiella gyrosa*" (CMW 10436) and "*E. viridistroma*" (CMW 10454) unidentified taxa.

Table 2. Hebarium specimens examined in this study.

Herbarium number*	Current name of taxon	Original label name on specimen	Original host name on label	Origin	Collector	Date
BPI 631857	*Cryphonectria cubensis* (type)	*Diaporthe cubensis* Bruner	*Eucalyptus botryoides* Sm.	Cuba	S.C. Bruner	1916
PREM 57297	*C. cubensis*	*Cryphonectria cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	2001
PREM 57294	*C. cubensis*	*C. cubensis*	*E. grandis*	Colombia	M.J. Wingfield	2000
PREM 57293	*C. cubensis*	*C. cubensis*	*E. grandis*	South Africa	M. Venter	2001
K 109807	*C. gyrosa* (type)	*Nectria gyrosa* (#638)	Bark	Sri Lanka	n.a.	1868
K 109809	*C. gyrosa*	n.a. (#290)	Bark	Mount Eliya, Sri Lanka	G.H.K. Thwaites	n.a.
BPI 614797	*C. gyrosa*	*E. tropicalis* (type)	*Elaeocarpus glandulifer* Mast.	Hakgala, Sri Lanka	T. Petch	1913
BPI 614526	*C. gyrosa*	*E. tropicalis* (type)	*Elaeocarpus glandulifer*	Hakgala, Sri Lanka	T. Petch	1913
BPI 797701	*C. gyrosa*	*E. tropicalis*	*Elaeagnus glandulifer*	Hakgala, Sri Lanka	n.a.	n.a.
PDD 32619[1]	*C. gyrosa*	*E. tropicalis*	*Elaeocarpus dentatus*	Auckland, New Zealand	G.J. Samuels	1973

Table 2. (continued)

Herbarium number*	Current name of taxon	Original label name on specimen	Original host name on label	Origin	Collector	Date
PDD 20056	*C. gyrosa*	*E. tropicalis*	*Elaeocarpus hookerianus* Raoul	Southland, New Zealand	J.M. Dingley	1948
PDD 21944	*C. gyrosa*	*E. tropicalis*	*Elaeocarpus dentatus*	Auckland, New Zealand	J.M. Dingley	1963
NYBG 31874 [2]	*C. radicalis*	*E. radicalis* (Schw.: Fr.) Ces. & de Not.	Dead tree	Auckland, New Zealand	R.E. Beever	1973
TFM 1057	*C. macrospora* (type)	*E. macrospora* Kobayashi & Ito (type)	*Shiia siebordii* Makino	Japan	T. Kobayashi	1954
TFM 1045	*C. nitschkei* (type)	*E. nitschkei* Otth	*Quercus grosseserrata* Bl.	Japan	T. Kobayashi	1954
CUP 2926	*C. parasitica*	*Diaporthe parasitica* Murrill	*Castanea dentata*	New York, USA	W.A. Murrill	1907
CUP 47983	*C. parasitica*	*E. parasitica* (Murrill) P.J. & H. W. Anderson	*Castanea dentata*	Md., USA	D.S. Welch	1938
BPI 797697	*C. radicalis*	*E. radicalis* (Schw.: Fr.) Fr.	*Castanea vesca*	Locarno, Switzerland	n.a.	1862
BPI 613739	*C. radicalis*	*E. fluens* (Sow.) Shear & Stevens	*Castanea vesca*	Stresa, Italy	C.L. Shear	1913

Table 2. (continued)

Herbarium number*	Current name of taxon	Original label name on specimen	Original host name on label	Origin	Collector	Date
PREM 56211	*C. eucalypti* (type)	*C. eucalypti* (type)	*Eucalyptus grandis* X *camaldulensis*	Nyalazi, South Africa	M. Venter	1998
PREM 56218	*E. gyrosa*	*E. gyrosa*	*Q. phellos* L.	Raleigh, USA	L. Grand	1997
BPI 614515	*E. singularis* (type)	*Calopactis singularis* Syd.	*Q. gambelli*	Colorado, USA	E. Bethel	1911
DAR 11235	*E. singularis*	*E. singularis*	*Q. gunnisonii*	Colorado, USA	G.G. Hedgcock and E. Bethel	1917
DAOM 3634	*E. viridistroma* (type)	*E. viridistroma* (type)	*Cercis canadensis*	Georgia, USA	J.H. Miller	1934
BPI 797702	*E. viridistroma*	*E. viridistroma*	*C. canadensis*	Georgia, USA	J.H. Miller	1934

***BPI**, U. S. National Fungus Collections, Systematic Botany and Mycology, Rm. 304, Bldg. 011A, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA.

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

TFM, Forestry and Forest Products Research Institute, P. O. Box 16, Tsukuba Norin Kenkyu, Danchi-Nai, Ibaraki, 305 Japan.

CUP, Plant Pathology Herbarium, Cornell University, 334 Plant Science Building, Ithaca, New York 14853-4203 USA.

PDD, Landcare Research New Zealand Limited, Private Bag 92 170, 120 Mt. Albert Road, Mt. Albert, Auckland, New Zealand.

DAR, Plant Pathology Herbarium, Orange Agricultural Institute, Forest Road, Orange, N. S. W. 2800, Australia.

DAOM, National Mycological Herbarium, Eastern Cereal and Oilseed Center (ECORC), Agriculture and Agri-Food Canada, Edifice Wm. Saunders Building. #49, Ottawa, Ontario, Canada, KIA 0C6.

K, Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, England, U.K.

[1] Specimen linked to isolate CMW 10471 (Table 1).

[2] Specimen linked to isolates CMW 10469 and CMW 10470 (Table 1).

Fig. 1. A strict consensus tree (tree length = 1918 steps, CI = 0.7106, RI = 0.8703) generated from a combined data set comprising ribosomal and β-tubulin gene sequences. Confidence levels of the tree branch nodes (>50%) are indicated above the nodes and were determined by a 1000 replicate bootstrap analysis. Branch lengths are indicated below the nodes. *Diaporthe ambigua* was used as the outgroup taxon.

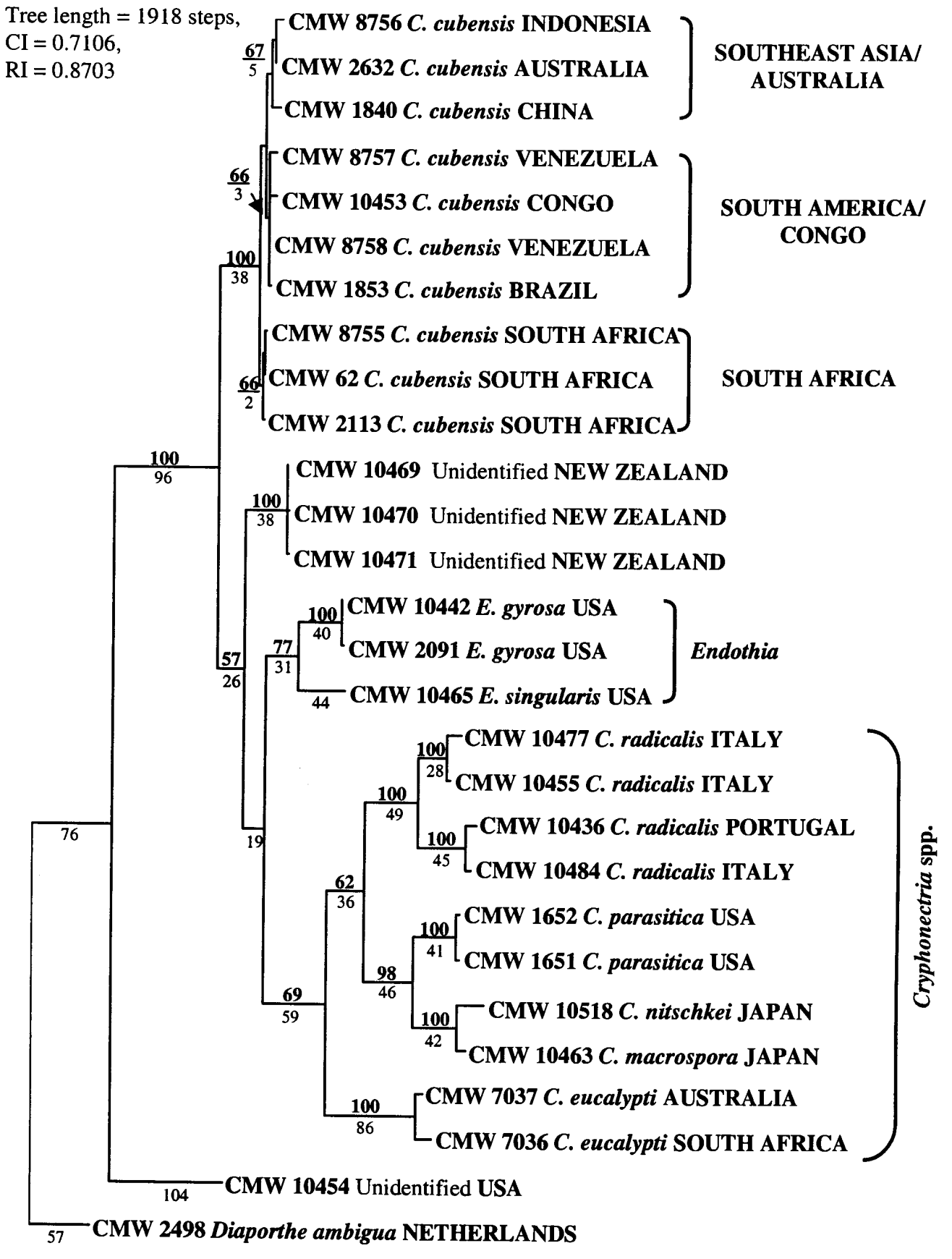
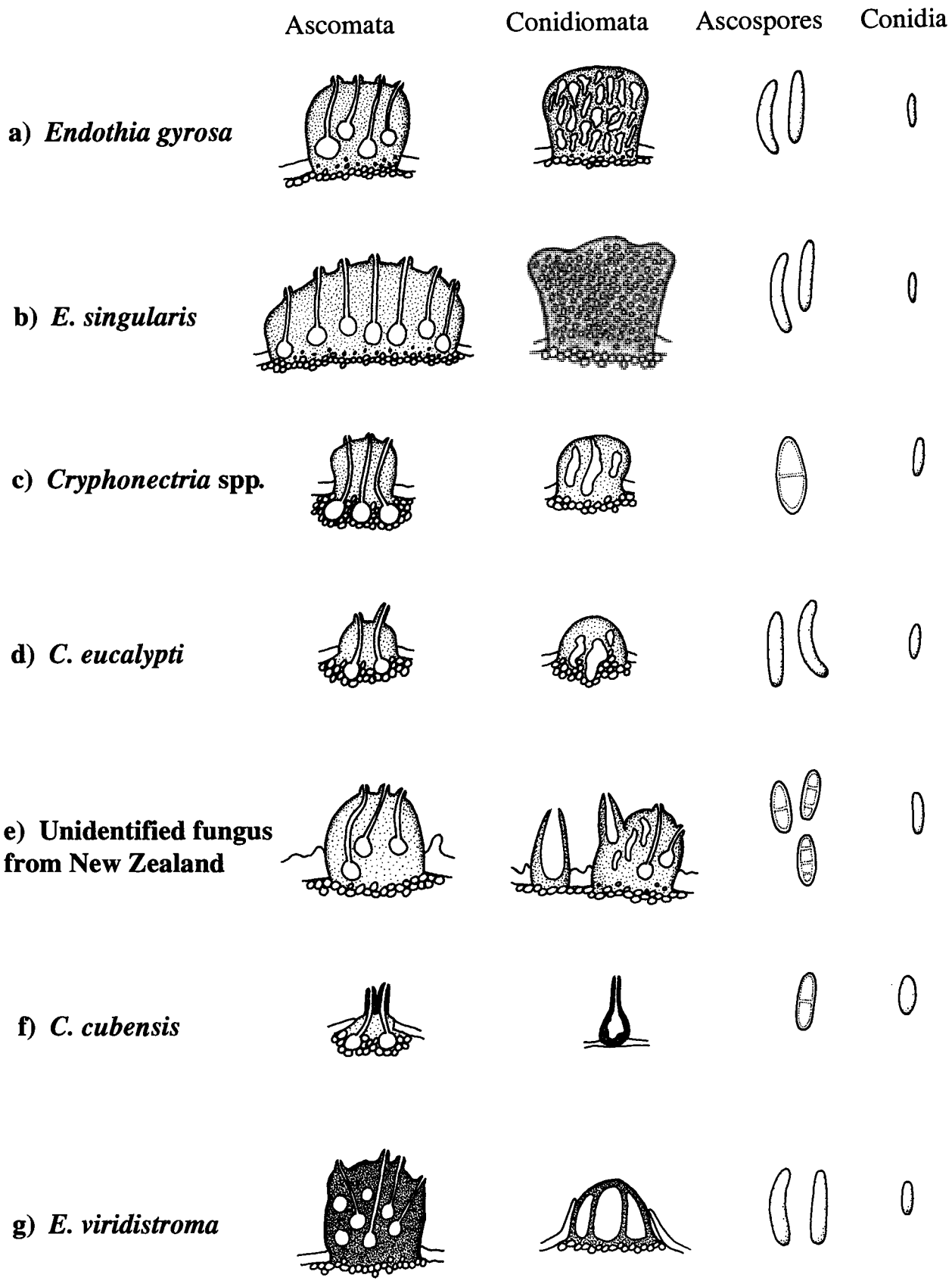

Tree length = 1918 steps,
CI = 0.7106,
RI = 0.8703
CMW 8756 C. cubensis INDONESIA
CMW 2632 C. cubensis AUSTRALIA
CMW 1840 C. cubensis CHINA
SOUTHEAST ASIA/ AUSTRALIA
CMW 8757 C. cubensis VENEZUELA
CMW 10453 C. cubensis CONGO
CMW 8758 C. cubensis VENEZUELA
CMW 1853 C. cubensis BRAZIL
SOUTH AMERICA/ CONGO
CMW 8755 C. cubensis SOUTH AFRICA
CMW 62 C. cubensis SOUTH AFRICA
CMW 2113 C. cubensis SOUTH AFRICA
SOUTH AFRICA
CMW 10469 Unidentified NEW ZEALAND
CMW 10470 Unidentified NEW ZEALAND
CMW 10471 Unidentified NEW ZEALAND
CMW 10442 E. gyrosa USA
CMW 2091 E. gyrosa USA
CMW 10465 E. singularis USA
Endothia
CMW 10477 C. radicalis ITALY
CMW 10455 C. radicalis ITALY
CMW 10436 C. radicalis PORTUGAL
CMW 10484 C. radicalis ITALY
CMW 1652 C. parasitica USA
CMW 1651 C. parasitica USA
CMW 10518 C. nitschkei JAPAN
CMW 10463 C. macrospora JAPAN
CMW 7037 C. eucalypti AUSTRALIA
CMW 7036 C. eucalypti SOUTH AFRICA
Cryphonectria spp.
CMW 10454 Unidentified USA
CMW 2498 Diaporthe ambigua NETHERLANDS

Fig. 2. Schematic drawings of the ascomata, conidiomata, ascopores and conidia of the fungi representing the different phylogenetic clades. **a.** *Endothia gyrosa*. **b.** *Endothia singularis*. **c.** *Cryphonectria* spp. representing *C. parasitica*, *C. radicalis* (Europe), *C. nitschkei*, *C. macrospora*. **d.** *Cryphonectria eucalypti*. **e.** Specimens labeled as *C. radicalis* and *C. gyrosa* from New Zealand. **f.** *Cryphonectria cubensis*. **g.** *Endothia viridistroma*.


Ascomata
Conidiomata
Ascospores
Conidia
a) Endothia gyrosa
b) E. singularis
c) Cryphonectria spp.
d) C. eucalypti
e) Unidentified fungus from New Zealand
f) C. cubensis
g) E. viridistroma

CHAPTER 6

DNA sequence data and morphology define *Cryphonectria* species on Fagaceae in Europe and Asia.

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DNA SEQUENCE DATA AND MORPHOLOGY DEFINE *CRYPHONECTRIA* SPECIES ON FAGACEAE IN EUROPE AND ASIA.

ABSTRACT

The genus *Cryphonectria* includes important tree pathogens as well as species believed to be saprophytes. While recent taxonomic studies have concentrated on North American and southern hemisphere *Cryphonectria* spp., little is known about Asian and European taxa found on *Castanea* and *Quercus*. A collection of isolates and specimens from woody hosts in Greece, Japan and China that could not be identified to the species level, has become available to us in recent years. In this study we identify these fungi and differentiate between the species occurring on *Quercus* and *Castanea* in Europe and Asia. Identification was achieved by morphological and phylogenetic comparisons between the unidentified fungi and established *Cryphonectria* and *Endothia* species. Phylogenetic comparisons were based on sequence data from the ribosomal ITS operon and two regions in the β-tubulin gene. Japanese and Chinese isolates showing different cultural features to those of *C. parasitica* from Japan and the USA, showed no sequence similarity with previously identified *C. parasitica* or *C. radicalis* isolates. They were related to *C. nitschkei* and *C. macrospora* from Japan, and specifically to an isolate assumed to be *C. havanensis* from

Japan. Specimens linked to these Japanese and Chinese isolates, had morphological features different from any of the Japanese herbarium specimens available to us, including specimens labelled as *C. havanensis* from Japan. A description and the name *C. clavata* are provided for this fungus. Furthermore, the fungal specimens in Japan that have been labelled as *C. havanensis* appeared to represent more than one undescribed species. Additional collections, more detailed morphological studies as well as phylogenetic analyses will be needed to fully resolve the identity of these fungi. Sequence and morphological data also suggested that there are two species currently representing *C. radicalis* in Europe. It is, however, not clear which of these represents the authentic *C. radicalis* and further investigations will be necessary to resolve this question.

INTRODUCTION

Cryphonectria and *Endothia* include fungal species that are both saprophytes and serious tree pathogens. A number of challenges exist when attempting to distinguish between these genera and species assigned to them. For example, the orange stromata of *Cryphonectria* and *Endothia* species are superficially similar. Additionally, differentiation of *Cryphonectria* and *Endothia* species is hindered by the fact that their ranges of spore size commonly overlap, and that ascospores for specimens are not always fully developed, resulting in variable measurements. Identification is further complicated when specimens or cultures do not produce spores. Taxonomic information is needed from both teleomorph and anamorph states because when only one morph is present, conclusive identification is difficult. Furthermore, fruiting structures, especially perithecia, are rarely produced in

culture, and cultural morphology is not sufficient to be used as the only means of identification.

Recent taxonomic studies, based on DNA sequence comparisons, have resolved a number of questions pertaining to the identification and differentiation of *Cryphonectria* and *Endothia* species (Myburg et al. 1999, Myburg et al. 2003, Venter et al. 2002). A comprehensive phylogenetic study on representative species of *Cryphonectria* and *Endothia*, for which cultures were available, indicated that these genera should be considered as separate taxonomic entities, even though they are closely related (Venter et al. 2002). However, studies such as those of Myburg et al. (1999), Myburg et al. (2003) and Venter et al. (2002) focussed primarily on species of *Cryphonectria* and *Endothia* that originated from the USA, Europe and countries in the Southern Hemisphere. Therefore, a similar study that focuses on the taxonomic and phylogenetic relationships of *Cryphonectria* and *Endothia* species with Asian origins is necessary.

The best-known species in *Cryphonectria* is *C. parasitica* (Murr.) Barr, the causal agent of chestnut blight, which practically eliminated the American chestnut (*Castanea dentata* Borkh.) during the last century (Anagnostakis 1987, Griffin 1986) after being introduced from eastern Asia, where it is native (Anagnostakis 1992, Milgroom et al. 1996, Shear and Stevens 1913, Shear and Stevens 1916). *Cryphonectria parasitica* also occurs on European chestnuts (*Castanea sativa* Mill.), although the disease has not been as severe as it has been in North America (Bazzigher and Miller 1991, Bissegger and Heiniger 1991, Heiniger and Rigling 1994). This is attributed to greater resistance in European chestnuts (Heiniger and

Rigling 1994, Metcalf 1908, Clapper 1952), differences in environmental conditions and the presence of naturally occurring hypovirulent *C. parasitica* strains in Europe (Grente 1965, Grente 1975, Heiniger and Rigling 1994).

Cryphonectria radicalis (Schw.: Fr.) Barr is a colonist of *Castanea* and *Quercus* species in the Northern Hemisphere (Anderson and Anderson 1912, Shear et al 1917). The fungus was first described in 1814 from England (Sowerby 1814) and later (1828) from the USA (Fries 1828, Shear et al. 1917). It was reported from southern Europe in 1863 (Shear et al. 1917) and from Japan in 1914 (Shear et al. 1917, Kobayashi 1970). *Cryphonectria radicalis* was, therefore, known in North America and Europe before *C. parasitica* was introduced. *Cryphonectria radicalis* has a special association with *C. parasitica* (Anagnostakis 1995, Hoegger et al. 2002) in that both species occur on the same host genera. Previous studies report species of *Castanea* and *Quercus* (Family: *Fagaceae*) as the most important hosts of *C. parasitica* and *C. radicalis* in Europe, North America (Shear et al. 1917, Roane 1986a) and eastern Asia (Kobayashi and Ito 1956, Kobayashi 1970). In a recent study aimed at isolating *C. parasitica* from dead chestnut stems in Switzerland, *C. radicalis* isolates were unintentionally collected (Hoegger et al. 2002). Identification of these isolates as *C. radicalis* was based on comparisons of morphology in culture, ascospore dimensions, mating behaviour and pathogenicity to chestnut plants.

In addition to *C. radicalis* and *C. parasitica*, *C. havanensis* (Bruner) Barr and *C. nitschkei* (Otth.) Barr have also been recorded on *Castanea* and *Quercus* spp. (Kobayashi and Ito 1956, Kobayashi 1970) making identification of the fungi on these hosts a challenge.

Furthermore, *Endothia singularis* (H. & B. Syd.) Shear and Stevens, a fungus with orange stromata typical of *Cryphonectria* species, but with aseptate ascospores, also occurs on *Castanea* and *Quercus* spp. in Japan (Kobayashi and Ito 1956, Kobayashi 1970) and a fungus reminiscent of *E. gyrosa* (Schw.: Fr.) Fr has been reported on *Quercus* from China (Teng 1934). Minor host species for this group of fungi in eastern Asia include *Castanopsis cuspidata* Schottky, a reported host for *Cryphonectria macrospora* (Kobayashi and Ito) Barr and *E. singularis* (Kobayashi 1970). Of the abovementioned species, only *E. gyrosa* is regarded as a pathogen, while *C. havanensis, C. nitschkei, C. macrospora* and *E. singularis* are considered opportunists or saprophytes (Roane et al. 1986, Kobayashi 1970 Shear et al. 1917).

A collection of isolates and specimens identified as *Cryphonectria* spp. and originating from *Quercus* and *Castanea* spp. in Greece, Japan and China form the basis of this study. Some of these isolates are those that Liu et al. (2003) treated in a recent study, which have not been described taxonomically. The objectives of this study were therefore to identify the isolates in the collection from Greece, Japan and China, to provide DNA sequence and morphological data to facilitate differentiation between the species occurring on *Quercus* and *Castanea* spp. in Europe and Asia, and to provide a full morphological description and name for the undescribed fungus mentioned in Lui et al. (2003).

MATERIALS AND METHODS

Collection of isolates and specimens

This study includes twenty-nine isolates (Table 1). Eight (CMW 10782 to CMW 10789) of these represented an unidentified fungus sampled from *Castanea* and *Quercus* spp. from Japan, China and Greece that produce less orange pigmentation than is characteristic of *C. parasitica*; three of these (CMW 10785 to CMW 10787) are from Liu et al. (2003). One Japanese isolate (CMW 10790) was morphologically similar to *C. parasitica*. Species of *Cryphonectria* and *Endothia* studied previously (Myburg et al. 2003, Venter et al. 2002) were included for comparative purposes. These are *C. parasitica* (CMW 1651, CMW 1652, CMW 10427, CMW 10431), *C. radicalis* (CMW, 10436, CMW 10455, CMW 10477), *C. macrospora* (CMW 10463), *E. gyrosa* (CMW 10442, CMW 2091) and *E. singularis* (CMW 10465). Four *C. radicalis* isolates (CMW 10791 to CMW 10794) from Europe recently studied by Hoegger et al. (2002) were incorporated as well as one isolate (CMW 11294) putatively identified as *C. havanensis* from Japan. Two *Diaporthe ambigua* Nitschke isolates (CMW 5288, CMW 5587) were included to serve as outgroup taxa in the phylogenetic analyses. Isolates of *C. radicalis* from Asia and North America are unfortunately not available and could not be included. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and duplicate cultures have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.

Herbarium specimens of the undescribed *Cryphonectria* species occurring in Japan, China and Europe (Table 2) were examined. Unfortunately only two of these specimens (FPH 7609, FPH 7610) are linked to isolates, i.e. CMW 10786 and CMW 10787. These specimens linked to the two isolates of unknown identity from *Castanea crenata* Sieb. & Succ. in Japan (Table 2) have been deposited in the herbarium of the Forestry and Forest Products Research Institute (FPH), P. O. Box 16, Tsukuba Norin Kenkyu, Danchi-Nai, Ibaraki, 305 Japan.

DNA isolations and amplification

DNA was isolated from cultures as described in Myburg et al. (1999). The ITS1 and ITS2 region of the ribosomal RNA operon, as well as the conserved 5.8S gene, were amplified using the primer set ITS1 and ITS4 (White et al. 1990). Two regions within the β-tubulin gene were amplified using primer pairs Bt1a with Bt1b and Bt2a with Bt2b (Glass and Donaldson 1995). The amplification reaction mixes, as well as the reaction conditions, were the same as those described in Myburg et al. (2002).

DNA sequencing and analyses

PCR products were sequenced in both directions using the same primer pairs that were used in the amplification reactions. Sequencing reactions were done using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS

(Perkin-Elmer, Warrington, United Kingdom). DNA sequences were determined using an ABI PRISM 3100™ automated DNA sequencer (Perkin Elmer, UK).

Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software was used to edit the DNA sequences. The sequences were manually aligned with sequence data sets from previous studies (Venter et al. 2002, Myburg et al. 2002). Phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) software version 4.0b (Swofford 1998). The ribosomal DNA (ITS1, 5.8S, ITS2) and the β-tubulin sequence data sets were subjected to a Templeton nonparametric Wilcoxon Signed Ranked (WSR) test to assess whether the two data sets could be combined in the subsequent phylogenetic analyses (Kellogg et al. 1996). Phylogenetic analyses were done using heuristic searches with tree-bisection-reconnection (TBR) and MULTREES (saving all optimal trees) options effective. Gaps were treated as fifth characters (NEWSTATE) in the heuristic searches. The confidence levels of the branching nodes were determined by a bootstrap analysis (1000 replications). Sequence data for two *D. ambigua* isolates were used to root the phylogenetic tree. Sequences generated in this study have been deposited in GenBank and accession numbers are listed in Table 1, together with accession numbers of previously deposited sequences (Venter et al. 2002, Myburg et al. 2002, Myburg et al. 2003).

Morphological comparisons

Fruiting structures on herbarium specimens were cut from the bark and rehydrated for one min. in boiling water. The structures were sectioned at –20 °C with a Leica CM1100 cryostat after embedding in Leica mountant (Setpoint Premier, Johannesburg, South Africa). Sections, 12-16 μm thick, were mounted on microscope slides in lactophenol. Spores from the various specimens were measured in 3% KOH and lactophenol. At least twenty spores for each specimen were measured, but fifty measurements of ascospores, asci, conidia and conidiophores were made for the holotype specimen of the new species described here. The distribution of measurements (µm) for each specimen is expressed as the range and the mean (± standard deviation) using the notation: (min-)(mean-std. dev.) – (mean+std. dev.)(-max). A range of measurements was taken from two structures for the conidiomata and ascomata and used in the species description. Standard colour notations of Rayner (1970) were used.

Growth in culture of isolates CMW 10786 and CMW 10787, which are linked to specimens FPH 7609 and FPH 7610, respectively, and representing the new species described in this study (Tables 1, 2), was studied on MEA [20 g/l malt extract agar (Biolab)]. These cultures were grown in the dark at temperatures ranging from 10 °C to 30 °C at 5 °C intervals and evaluated using the technique described by Venter et al. (2002).

RESULTS

DNA sequencing and analyses

Amplification of the ITS1/ITS2 ribosomal RNA operon and β-tubulin gene regions resulted in PCR products of between 550bp and 600bp in size (data not shown). The Templeton nonparametric Wilcoxon Signed Ranked (WSR) test performed on the combined ribosomal DNA and β-tubulin sequence data sets indicated that the respective gene regions could be analysed as a combined data set in the subsequent PAUP analyses. A total of twenty-nine isolates were included in the combined data set with the two *D. ambigua* isolates serving as outgroup taxa. The combined data set consisted of a total of 1541 characters (Appendix 5), of which 929 were constant, 27 were parsimony-uninformative and 585 parsimony-informative. The heuristic search produced one most parsimonious tree (tree length= 1203 steps, consistency index = 0.77 and retention index = 0.92) (Fig. 1).

Cryphonectria and *Endothia* were resolved into distinct groups in the phylogenetic tree (Fig. 1). The *Cryphonectria* group includes three clades (Fig. 1). The first of these (**Clade 1**) includes *C. parasitica* isolates originating from the USA and Japan. **Clade 2** incorporates the unidentified fungus represented by the isolates (CMW 10782 to CMW 10787) from *Quercus* and *Castanea* spp. in China and Japan. A Japanese isolate (CMW 11294) previously assigned the name *C. havanensis*, grouped with the abovementioned isolates from *Quercus* and *Castanea* spp. This isolate and those of unknown identity are most closesly related to isolates of *C. nitschkei* and *C. macrospora* from Japan.

The third clade (**Clade 3**) in the *Cryphonectria* group included isolates that have been referred to as *C. radicalis* from Europe. The *C. radicalis* isolates from Italy (CMW 10455, CMW 10477, CMW 10791), Greece (CMW 10788, CMW 10789) and Switzerland (CMW 10792, CMW 10793, CMW 10794) showed a high degree of sequence similarity. The isolates from Switzerland are the *C. radicalis* isolates obtained from Hoegger et al. (2002). However, two isolates, CMW 10436 from Portugal and CMW 10484 from Italy, which had been identified as *C. radicalis* (Myburg et al. 2003), grouped separately from the isolates representing *C. radicalis* from Italy, Greece and Switzerland.

Morphological comparisons

Identification of the unknown *Cryphonectria* sp.

The morphology of the fungus represented by isolate CMW 10786 (annotated KB1 in Liu et al. 2003) and residing in **Clade 2** of the phylogenetic tree could be described since specimen FPH 7609 is linked to this isolate. In this fungus, ascospores were (8.5-)10-11.5-(-12.5) µm long, (3.5-)4-4.5(-5) µm wide and conidia were 4-5.5(-6) µm long, (1-)1.5(-2) µm wide (Table 3). Specimen FPH 7610, linked to isolate CMW 10787 (annotated CD28 in Liu et al. 2003), had slightly longer conidia [(4.5-)5-6.5(-7) µm long, 1.5 µm wide] (Fig. 3), but this isolate also grouped in **Clade 2** of the phylogenetic tree.

Of all of the species previously reported on woody hosts in Japan (Kobayashi and Ito 1956, Kobayashi 1970), the ascospore and conidial dimensions of the unidentified specimens

(FPH 7609, FPH 7610) most closely resembled those of *C. nitschkei* and *C. havanensis*, determined in this study (Table 3). Furthermore, both isolates CMW 10786 and CMW 10787 grouped closely with an isolate annotated as *C. havanensis* (CMW 11294) in **Clade 2** of the phylogenetic tree, but distinct from the isolate of *C. nitschkei* (Fig. 1). Unfortunately the isolate labelled as *C. havanensis* is not linked to herbarium material. Herbarium specimens, labeled *C. havanensis* in our collection, that were studied are likewise not connected to isolates currently in our collection and could thus not be included in the phylogenetic tree.

Specimens labeled as *C. havanensis* originated from fagaceous and non-fagaceous hosts. Two of these specimens from *Quercus* spp. [FPH 1203 from *Q. variabilis* Blume (Fig. 2a) and FPH 1047 from *Q. glandulifera* Blume (Fig. 2b)], specimen FPH 2300 from a *Betula* sp. (*Betulaceae*) (Fig. 2c) and specimen FPH 1270 from *Pyrus sinensis* Lindl. (*Rosaceae*) (Fig. 2d) had ascospores comparable in size (Table 3). These measurements were similar to those given by Kobayashi (1970), which was given as an average size of 8-12.5 x 3-4 µm. Specimen FPH 633 from *Eucalyptus globulus* Labill. (*Myrtaceae*) also had structures with ascospores similar to the other specimens labelled *C. havanensis* (designated as "A. *C. havanensis*" in Table 3, Fig. 2e). Smaller fruiting structures were, however, also found on specimen FPH 633 (designated as "B. *C. havanensis*" in Table 3) with ascospores (Fig. 2f) different [(6-)6.5-9(-11) µm long, 3-2.5 µm wide] from those of the other fruiting structures on the same specimen.

Specimen FPH 1047 was the only specimen labeled as *C. havanensis* that contained conidiomata. The conidia (Table 3) were comparable to measurements (3.5-4.2 µm long, 0.5-1 µm wide, 4 x 0.8 µm in average) given by Kobayashi (1970), and it was also similar to conidia of the unknown fungus. Phialide morphology, however, differentiated between the specimens of the unidentified fungus and the *C. havanensis* specimen from *Q. glandulifera* (FPH 1047). It also distinguished the unidentified fungus from *C. nitschkei* (FPH 1045). Conidiogenous cells of *C. nitschkei* in general had inflated bases with the apices of the cells strongly attenuated (Fig. 3a). Conidiogenous cells of the specimen (FPH 1047) annotated as *C. havanensis* did not have inflated bases, but were evenly attenuated (Fig. 3b). Conidiogenous cells of specimens of the unidentified fungus (FPH 7609 and FPH 7610) could be distinguished from the other two specimens by the inflated apical cells of the branched conidiophores were often inflated (Figs. 4i, 4j, 5e).

Taxonomy of the unknown *Cryphonectria* sp.

The unknown fungus found on *Quercus* spp. and *Castanea crenata* in Japan and China grouped separately from other species in the phylogenetic tree (Fig. 1). The close grouping of the isolate (CMW 11294) that has been referred to as *C. havanensis*, to isolates of the unknown fungus, is, however, not supported by morphological characteristics of the conidiogenous cells. Since the *C. havanensis* isolate (CMW 11294) is not connected to herbarium material, we propose that this isolate is not truly representative of *C. havanensis*, but is similar to the unknown fungus.

The unknown fungus could be distinguished from specimens of putative *C. havanensis* and *C. nitschkei*, the species that it resembled most closely, based on its clavate conidiogenous cells. One specimen (FPH 7610) linked to the unknown fungus also had longer conidia than any of the other Japanese species (Table 3). We, therefore, propose that the unknown fungus from *Castanea crenata* in Japan and *Quercus* spp. in China, characterised by isolates CMW 10786 and CMW 10787 and the corresponding herbarium material (FPH 7609, FPH 7610), represents a distinct and new species of *Cryphonectria*, different from the fungus in Japan identified as *C. havanensis* (Kobayashi and Ito 1956, Kobayashi 1970). The following description is provided for the fungus:

PLEASE NOTE THAT THE FOLLOWING SPECIES DESCRIPTION IS PRESENTED HERE IN PRELIMINARY FORM AND SHOULD NOT BE CITED. THE DESCRIPTION WILL BE SUBMITTED FOR PUBLICATION IN SCIENTIFIC LITERATURE.

Cryphonectria clavata M. Gryzenhout & M. J. Wingfield, prov.nom. – Figs. 4, 5.

Etym.: Latin, clavata, refers to the inflated apices of the conidiogenous cells that give the impression of a club.

Stromata semi-immersa, erumpentia, pulvinata, sphaerica vel elongata, aurantiaca. Bases peritheciorum saepe a textura corticis circumcinctae, parietibus atratis. Colla peritheciorum atrata, basi perithecii centralia, in superficie stromatis pro ostiolis nigris in papillis brevibus, textura stromatali aurantiaca tectis emergentia. Asci

fusiformes, solum immaturi stipitati, octospori. Ascosporae fusiformes vel ovatae, interdum subfalcatae, semel septatae, in septo cum aut sine strictura parva, extremis obtusis. Stromata anamorpha multilocularia convoluta, loculis saepe cum peritheciis dispositis. Conidiophora cylindrica vel basin versus bulbosa, apicibus attenuatis vel cellula apicali inflata, saepe septata, infra septo cum aut sine ramis lateralibus. Cellulae conidiogenae enteroblasticae, phialidicae, collariculo incrassatioque periclinali inconspicuo. Conidia cylindrica, interdum subfalcata, aseptata. Coloniae in MEA lanuginosae, margine integro vel crenato, albae conidiomatibus aurantiacis, catillum 90 mm diametro octo diebus tegentes, incremento optimo ad 25°C.

HOLOTYPUS: JAPONIA, in ditione "Yamanashi", loco dicto Kobuchizawa: in cortice Castaneae crenatae, Aprilio 1998, M. G. Milgroom et S. Kaneko, FPH 7609; cultura viva CMW 10786.

EPITYPUS: JAPONIA, in ditione Kyoto", loco dicto Chudai: in cortice *Castaneae crenatae*, Aprilio 1998, M. G. Milgroom et S. Kaneko, FPH 7610, cultura viva CMW 10787.

Stromata erumpent, pulvinate, spherical to elongated (Figs. 4a, 4g, 5a, 5d), 230 – 330 μm high, 250 – 1630 μm long and 210 – 1010 μm wide above the level of the bark, orange (colour 15). Ascomata stromatic, semi-immersed in bark, region above level of bark ectostromatic, lower region entostromatic containing host cells and perithecial bases (Figs. 4b, 5a), pseudoparenchymatous tissue at edge of stromata (Fig. 4c), prosenchymatous tissue in center (Fig. 4d). Perithecial bases 162 – 286 μm long, 160 – 379 μm wide, globose to sub-globose, dark-walled, up to 16 per stroma (Fig. 4b, 5a), perithecial walls 15 – 20 μm diam. Perithecial necks 55 – 72 μm wide, length depending on depth of perithecium in stroma, slender, dark, periphysate, with a central position on base of perithecium (Fig. 4b, 5a), necks emerging at the stromatal surface as black ostioles in short papillae covered with orange stromatal tissue (Fig. 4a, 5a), papillae 104 – 168 μm in diam. Asci (42-)44.5-50.5(-

56) μm long, 7-8.5(-9.5) μm wide, fusiform, numerous, floating freely in perithecial cavity, stipitate only when immature, unitunicate with non-amyloid, refractive apical rings; asci with eight ascospores (Fig. 4e, 5b). Ascospores (8.5-)9.5-11.5(-12.5) μm long, (3-)3.5-4.5(-5) μm wide, fusiform to oval, sometimes slightly curved, with or without slight constriction at septum, ends obtuse, hyaline, one septate (Figs. 4f, 5c).

Anamorphic stromata multilocular and convoluted, locules often occurring in same stroma that contains perithecia, conidia expelled through opening at stromatal surface (Figs. 4g, 4h, 5d). Conidiophores (5.5-)7.5-17(-24) μm long, (1-)1.5-2 μm wide, cylindrical or bulbous base, apices attenuated or inflated, often septated with or without lateral branches beneath septum, hyaline (Figs. 4i, 4j, 5f). Conidiogenous cells enteroblastic, phialidic, determinate, apical or lateral on branches, hyaline, collarette and periclinal thickening inconspicuous (Figs. 4i, 4j, 5f). Conidia (3.5-)4-5.5(-6.5) μm long, (1-)1.5(-2) μm wide, hyaline, cylindrical, occasionally slightly curved, hyaline, aseptate (Figs. 4k, 5g).

CULTURAL CHARACTERISTICS: Cultures on MEA fluffy with a smooth to crenate margin, white with orange (colour 15) conidiomata, covering a 90 mm plate after minimum of eight days, optimum temperature for growth 25 °C.

HOLOTYPE: JAPAN, Yamanashi Prefecture, Kobuchizawa: bark of *Castanea crenata*, April 1998, M. G. Milgroom and S. Kaneko, FPH 7609; living culture CMW 10786.

EPITYPE: JAPAN, Kyoto Prefecture, Chudai: bark of *Castanea crenata*, April 1998, M. G. Milgroom and S. Kaneko, FPH 7610, living culture CMW 10787. This herbarium

specimen was chosen as epitype because the holotype specimen contains very few anamorph structures.

SUBSTRATE: Bark of *Quercus* and *Castanea* spp.

DISTRIBUTION: China, Japan,

Distinction between *C. radicalis* groups in Europe

Based on ascospore size, two groups could be distinguished for European specimens labelled as *C. radicalis* (Table 3). This is in agreement with the phylogenetic analysis (Fig. 1) showing two distinct groups for *C. radicalis* isolates from Europe. Although no specific connections between groups defined by sequence data and spore morphology are possible because none of the specimens are linked to isolates in the phylogenetic tree, the first group of specimens (BPI 797697, BPI 613739, BPI 612672 and BPI 797693) (Table 2), originating from *Castanea sativa* in Italy and Switzerland, had ascospores (6-)7-8.5 µm long, (2-)2.5-3 µm wide (Table 3). These dimensions are similar to those given for the European *C. radicalis* isolates (CMW 10792, CMW 10793 and CMW 10794) by Hoegger et al. (2002), and to the Japanese *C. radicalis* specimens (Table 3). Conidiomata in this first group of European specimens also had pale luteous (colour 19d) cells lining the conidial locules, similar to Japanese *C. radicalis* specimens.

Specimens of *C. radicalis* from North America were similar to the group of *C. radicalis* specimens with smaller ascospores from Europe. Ascospore sizes for a specimen from a *Quercus* sp. (NYBG 1963) and another specimen (CUP 6178) fell within the size range

given for *C. radicalis* specimens from Japan and the European group with smaller ascospores (Table 3). Furthermore, cells giving rise to conidiophores were pale luteous in colour. These features were similar to those in Japanese and the European specimens with smaller ascospores. Conidial sizes for the North American *C. radicalis* specimens (CUP 6178, NYBG 2018) were comparable with those from the rest of the world (Table 3).

The second group of specimens from Europe labelled *C. radicalis* (BPI 797696, BPI 797692, BPI, 1112743, BPI 797698, BPI 612660; Table 2), originating from Italy, Abkhazia and France on *Castanea sativa*, a *Quercus* sp. and *Carpinus betulus* Linn, respectively, had longer ascospores than those of the first group, but were similar in width (Table 3). The ascospore sizes of the fungus residing in the second group did not resemble those of any other *Cryphonectria* species examined in this study (Table 3). Conidia were similar in size to the first group of *C. radicalis* from Europe with smaller ascospores (Table 3).

DISCUSSION

The present study has provided a set of DNA sequences for the ribosomal ITS region and two regions of the β-tubulin gene representing the majority of *Cryphonectria* species known to occur on woody host species in Europe and Asia. Analysis of sequence data and morphological comparisons shows that various *Cryphonectria* species occur on *Quercus* and *Castanea* species in Europe and Asia, including *C. parasitica* and *C. radicalis*. We also describe a new species of *Cryphonectria*, *C. clavata*, from Japan and China, which is phylogenetically distinct from the other *Cryphonectria* species. *Cryphonectria clavata* is

phylogenetically most closely related to *C. nitschkei* and *C. macrospora* and can be morphologically distinguished from *C. macrospora* by its smaller ascospores, and from *C. nitschkei* by its inflated apical conidiogenous cells. The combination of morphological and DNA sequence data presented in this study should aid future researchers in making correct identifications of *Cryphonectria* species found in Eurasia. This is particularly so given the difficulty of making firm identifications of these fungi based on morphology alone.

The newly described species, *C. clavata*, was mentioned in a recent study of interspecies transmission of hypoviruses (Liu et al. 2003). While sampling *C. parasitica* isolates in Japan, the authors recognised that their collections included another *Cryphonectria* species that produced less pigment in culture than *C. parasitica*, which in general were more orange. This unknown sympatric species, along with *C. parasitica*, also contained *Cryphonectria hypovirus 1* (CHV-1), which could be transmitted between the two species in culture. Liu et al. (2003) showed through DNA sequencing and RFLP data of the ribosomal ITS DNA region sequence, that CMW 10785, CMW 10786, CMW 10787 (isolates 09494, KB1 and CD28, respectively, in Lui et al. [2003]) of this unknown species grouped separately from their *C. parasitica* isolates. In our study, we have been able to show conclusively, by additional sequence data and morphological comparisons, that this unknown *Cryphonectria* species is new, and provided the name *C. clavata* for it. This confirmed the discovery by Liu et al. (2003) that the virus transmission they observed both in the laboratory and in nature was between different fungal species.

Other than *C. parasitica*, *C. radicalis*, *C. nitschkei*, *C. macrospora* and *C. clavata*, a fungus identified as *C. havanensis* was previously reported from various hosts in Japan (Kobayashi and Ito 1965, Kobayashi 1970). These hosts, including *Quercus* spp, a *Betula* sp., *P. sinensis* and *E. globulus*, are members of different plant families. Morphological comparisons of the specimens from these host species showed that spore sizes overlap with each other, although additional morphological criteria, such as conidiophore morphology, should be included to determine whether these different specimens really represent a single taxon. For example, based on ascospore size, it seems likely that two different species occur on *E. globulus* in Japan.

Apart from the fact that specimens from Japan identified as *C. havanensis* possibly represent more than one species, the name for Japanese specimens of *C. havanensis* also needs to be revised. *Cryphonectria havanensis* from Japan was at first thought to be two species (Kobayashi and Ito 1965, Kobayashi 1970). The first of these was a fungus on dead bark of *Eucalyptus globulus* annotated as *E. havanensis* Bruner (Kobayashi and Ito 1956). The second fungus occurred on fagaceous hosts in Japan and was identified as *Endothia tropicalis* Shear & Stevens (Kobayashi and Ito 1956). *Endothia tropicalis* is originally known from Sri Lanka (Berkeley and Broome, 1875, Shear et al. 1917) and is currently the type species of *Cryphonectria*, *C. gyrosa* (Barr 1978). Prior to the work of Barr (1978), Kobayashi (1970) reduced *E. tropicalis* to synonymy with *C. havanensis*, which was originally described from *Eucalyptus* spp. in Cuba (Bruner 1916). Hence Japanese specimens of *E. tropicalis* and *E. havanensis* were amalgamated under the single name *E. havanensis* (Kobayashi 1970). Although not the aim of this study, collections of these fungi

from Japan should be compared with the type specimens from Sri Lanka and Cuba, to establish the true identity of the specimens annotated as *C. havanensis* in Japan. Additional collections of *C. havanensis* from Cuba and the fungus from Japan that include sequences for cultures linked to specimens, should also be sought to fully resolve this question.

Hoegger et al. (2002) showed that *C. radicalis* occurs sympatrically with *C. parasitica.* Our results support this finding. Both our data and those of Hoegger et al. (2002) show that *C. radicalis* continues to exist in Asia, Europe and the USA, even though it is apparently not common. This species can be distinguished from *C. parasitica* based on ascospore length and width, although in the absence of a teleomorph it will be difficult to distinguish between the two species because conidial dimensions of *C. parasitica* and *C. radicalis* overlap. *Cryphonectria parasitica* also produces mycelial fans in the wood and these are not present in the case of *C. radicalis* (Roane 1986a, Shear et al. 1917). Another important distinguishing characteristic between the two species is that *C. radicalis* colours growth medium purple due to the production of a pigment known as endothine red, while *C. parasitica* does not produce this pigment (Hoegger et al. 2002,Roane 1986b, Roane and Stipes 1978, Shear et al. 1917).

Results of the present study and those of Myburg et al. (2003) show the presence of two groups within the fungus known as *C. radicalis* in Europe. These groups were defined independently based on DNA sequence data and morphology. It is, however, difficult to resolve whether the two groups found based on DNA sequence data, corresponds with the two groups distinguished based on morphology. This is because isolates used in the

phylogenetic analyses were not linked to any specimens in the morphological comparisons. It is, furthermore, difficult to deduct from previous studies which of the morphological groups in Europe corresponded most closely with published data for *C. radicalis*. Shear et al. (1917) made comparisons of various key specimens that included European and North American material. They obtained ascospore dimensions of 6-10 µm long, 3-4.5 µm wide for *C. radicalis* that encompassed both morphological groups in Europe identified in the present study. Shear et al. (1917) also observed that *C. radicalis* had highly variable ascospores, and it is possible that they were treating the two different species that we are now able to distinguish based on DNA sequence data, as a single species. The study of Hoegger et al. (2002), however, may present a possible link because ascospore dimensions given for the isolates from Hoegger et al. (2002), correspond with measurements of the one group of *C. radicalis* specimens from Europe and Japan that had ascospores (6-)7-8.5 µm long. The isolates from Hoegger et al. (2002) were included in this study and resided in the group that includes *C. radicalis* isolates from Greece and Italy. Unfortunately, no morphological data are available for the isolates in the second phylogenetic clade, incorporating CMW 10436 from Portugal and CMW 10484 from Italy.

The presence of two groups labelled as *C. radicalis* in Europe, makes it unclear which of the groups represents the true *C. radicalis* in Europe. The type specimen of *C. radicalis* has a North American origin (Fries 1828). The specimens from the USA examined in this study, had ascospore ranges that corresponded most closely with those of the group of *C. radicalis* specimens from Europe with smaller ascospores. Furthermore, North American specimens also had pale luteous linings to the conidial locules, similar to these European specimens.

However, in order to verify the identity of the two taxa that apparently represent the fungus known as *C. radicalis* in Europe, more detailed morphological comparisons of herbarium collections from Europe with those of the type specimen from North America will be necessary. More thorough studies are also needed including collections of North American *C. radicalis* specimens since Shear et al. (1917) mentioned a second form of *C. radicalis*, named *E. fluens* var. *mississippiensis*, existing in North America. There is thus the possibility that *C. radicalis* in North America represents different fungi. Unfortunately the type specimen of *C. radicalis* and other specimens of *C. radicalis* from the USA, are not linked to living isolates that we have been able to obtain. Numerous enquiries lead us to believe that these isolates do not exist and new collections will be needed to resolve the identity of *C. radicalis* in the USA.

A number of questions relating to *C. radicalis* still remain. We have no knowledge regarding the relatedness of the fungi known as *C. radicalis* from Europe, Japan and from North America. It would be interesting to determine the relationships of the different continental groups of *C. radicalis*, in order to establish whether *C. radicalis sensu lato* has been moved around the world through human involvement, as was the case with *C. parasitica*; or whether it is the member of *Cryphonectria* with the widest geographical distribution. The data presented in this study should aid future researchers in answering these questions and making correct identifications of *Cryphonectria* species found in Eurasia. This is particularly so given the difficulty of making firm identifications of these fungi based on morphology. We recommend comparisons of DNA sequences in addition to analysis of morphological criteria in order to characterise new collections of these fungi.

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Table 1. List of isolates included in this study[a].

Isolate number[b]	Alternative isolate numbers[d]	Species	Host	Origin	Collector	GenBank Accession numbers
CMW 10782	-	*Cryphonectria clavata*	*Quercus mongolica*	Japan	M. Kusunoki	AF 140242, AF 140248, AF 140254
CMW 10783	-	*C. clavata*	*Quercus mongolica*	Japan	M. Kusunoki	AF 140244, AF 140250, AF 140256
CMW 10784	-	*C. clavata*	*Quercus mongolica*	Japan	M. Kusunoki	AF 140245, AF 140249, AF 140257
CMW 10785	09494	*C. clavata*	*Quercus* sp.	China	M. Milgroom and S. Kaneko	AF 140246, AF 140252, AF 140258
CMW 10786	KB1	*C. clavata*	*Castanea crenata*	Japan	M. Milgroom and S. Kaneko	AF 140247, AF 140251, AF 140259
CMW 10787	CD28	*C. clavata*	*Castanea crenata*	Japan	M. Milgroom and S. Kaneko	AF 214212, AF 214214, AF 214216
CMW 11294[c]	E57	*C. clavata*	*Quercus grosserata*	Japan	T. Kobayashi	AY 214211, AY 214213, AY 214215
CMW 10788	D15	*C. radicalis*	*Quercus*	Greece	P. Cortesi	AY 143075, AY 143077, AY 143079
CMW 10789	D31	*C. radicalis*	*Quercus*	Greece	P. Cortesi	AY 143076, AY 143078, AY 143080
CMW 10790	-	*C. parasitica*	*Quercus serrata*	Japan	M. Kusunoki	AF 140243, AF 140253, AF 140255
CMW 10791	M 285	*C. radicalis*	*Quercus suber*	Italy	M. Orsenigo	AF 548742, AF 548746, AF 548750
CMW 10792	M 2268	*C. radicalis*	*Castanea sativa* Mill.	Switzerland	U. Heiniger	AF 548743, AF 548747, AF 548751

Table 1. (continued)

Isolate number[b]	Alternative isolate numbers[d]	Species	Host	Origin	Collector	GenBank Accession numbers
CMW 10793	M 2269	*C. radicalis*	***Castanea sativa***	Switzerland	U. Heiniger	AF 548744, AF 548748, AF 548752
CMW 10794	M 2270	*C. radicalis*	*Castanea sativa*	Switzerland	U. Heiniger	AF 548745, AF 548749, AF 5487503
CMW 10427	ATCC 48197	*C. parasitica*	*Quercus virginiana* Mill.	USA	R.D. Wolfe	AF 368329, AF 273073, AF 273469
CMW 10431	ATCC 48198	*C. parasitica*	*Quercus virginiana*	USA	F.F. Lombard	AF 368330, AF 273076, AF273470
CMW 1651	-	*C. parasitica*	*Castanea dentata*	USA	P.J. Bedker	AF 046901, AF 273074, AF 273467
CMW 1652	-	*C. parasitica*	*Castanea dentata*	USA	P.J. Bedker	AF 046902, AF 273075, AF 273468
CMW 10436	CBS 165.30	*C. radicalis*	*Quercus suber*	Portugal	B. d'Oliviera	AF 452117, AF 525703, AF 525710
CMW 10455	CBS 238.54	*C. radicalis*	*Castanea dentata*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712
CMW 10477	CBS 240.54	*C. radicalis*	*Quercus suber*	Italy	M. Orsenigo	AF 368328, AF 368347, AF 368346
CMW 10484	-	*C. radicalis*	*Castanea sativa*	Italy	A. Biraghi	AF 368327, AF 368349, AF 368349
CMW 10518	-	*C. nitschkei*	*Quercus*	Japan	T. Kobayashi	AF 452118, AF 525706, AF 525713
CMW 10463	-	*C. macrospora*	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350

Table 1. (continued)

Isolate number[b]	Alternative isolate numbers[d]	Species	Host	Origin	Collector	GenBank Accession numbers
CMW 10465	-	*Endothia singularis*	unknown	USA	R.J. Stipes	AF 368323, AF 368333, AF 368332
CMW 10442	-	*E. gyrosa*	*Quercus palustris* L.	USA	R.J. Stipes	AF 368326, AF 368339, AF 368338
CMW 2091	ATCC 48192	*E. gyrosa*	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336
CMW 5288	-	*Diaporthe ambigua*	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821
CMW 5587	-	*D. ambigua*	*Malus domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF 543822

[a] All isolates are maintained in the culture collection (**CMW**) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

[b] Taxa presented in bold represent isolates sequenced in this study. Sequence data for the other taxa are from the studies of Venter et al. (2002), Myburg et al 2002), Myburg et al. (2003).

[c] This isolate was previously labelled as *Cryphonectria havanensis*.

[d] **ATCC** = American Type Culture Collection, P.O. Box 1549, Manassas, VA 20108, USA.

CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

FPH = Forestry and Forest Products Research Institute, P. O. Box 16, Tsukuba Norin Kenkyu, Danchi-Nai, Ibaraki, 305 Japan.

M = isolates used in Hoegger et al. (2002).

(**D15, D31**) = Isolates used in Cortesi et al. (2001).

(**09494, KB1, CD28**) = isolates used in Lui et al. (2003).

Table 2. Specimens of *Cryphonectria* species used in the morphological comparisons.

Identification	Herbarium allocation[a]	Host	Origin	Collector	Date
Cryphonectria parasitica	FPH 1326	*Castanea crenata*	Tsurukawa, Japan	T. Kobayashi	1953
C. parasitica	FPH 629	*Castanea crenata*	Koganei, Japan	T. Kobayashi	1953
C. parasitica	FPH 608	*Castanea crenata*	Matsudo, Japan	T. Kobayashi	1953
C. parasitica	FPH 600	*Castanea crenata*	Seki, Japan	T. Kobayashi	1953
C. radicalis	BPI 612660	n.a.	Como, Italy	C.L. Shear	1912
C. radicalis	BPI 612672	*Castanea sativa*	Etremblieres, Switzerland	C.L. Shear	1913
C. radicalis	BPI 613739	*Castanea sativa*	Stresa, Italy	C.L. Shear	1913
C. radicalis	BPI 1112743	*Quercus sp.*	Bois Bastard, France	F. Candoussau	1992
C. radicalis	BPI 797696	*Castanea* sp.	Rome, Italy	Prof. Liropoli	1877
C. radicalis	BPI 797697	*Castanea sativa*	Locarno, Switzerland	n.a.	1862
C. radicalis	BPI 797698	n.a.	Sciolze, Italy	n.a.	1873
C. radicalis	BPI 797692	*Carpinus betulus*	Abkehazia	Woronin	n.a.
C. radicalis	BPI 797693	*Castanea* sp.	Locarno, Switzerland	Denotaris	1862
C. radicalis	BPI 797694	*Castanea* sp.	Locarno, Switzerland	Daldini	1862
C. radicalis	BPI 797695	*Castanea* sp.	Como, Italy	n.a.	n.a.

Table 2. (continued)

Identification	Herbarium allocation[a]	Host	Origin	Collector	Date
C. radicalis	FPH 1200	*Quercus variabilis*	Meguro, Japan	T. Kobayashi	1953
C. radicalis	FPH 1072	*Quercus serrata* Thunb.	Machida, Japan	T. Kobayashi	1954
C. radicalis	FPH 2483	*Quercus salicina* Blume	Komayama, Japan	T. Kobayashi	1959
C. radicalis	FPH 601	*Alnus firma* Siebold & Zucc.	Nishina, Japan	T. Kobayashi	1955
C. radicalis	FPH 652	*Carpinus japonica* Blume	Asakawa, Japan	T. Kobayashi	1962
C. radicalis	NYBG 1963	*Quercus* sp.	Glatfelter, USA	C.L. Shear and N.E. Stevens	1913
C. radicalis	CUP 6178	Chestnut stump	Connellsville, USA	P.J. Anderson and H.W Anderson	1912
C. havanensis	FPH 633	*Eucalyptus globulus*	Meguro, Japan	T. Kobayashi	1954
C. havanensis	FPH 2300	*Betula* sp.	Yoshiwara, Japan	Zinno	1963
C. havanensis	FPH 1270	*Pyrus sinensis*	Inagi, Japan	T. Kobayashi	1960
C. havanensis	FPH 1203	*Quercus variabilis*	Seto, Japan	T. Kobayashi	1953
C. havanensis	FPH 1047	*Quercus glandulifera*	Japan	T. Kobayashi	1954

Table 2. (continued)

Identification	Herbarium allocation[a]	Host	Origin	Collector	Date
C. macrospora (type)	FPH 1057	*Castanopsis cuspidata*	Shinagawa, Japan	T. Kobayashi	1954
C. macrospora	FPH 1058	*Castanopsis cuspidata*	Shinagawa, Japan	T. Kobayashi	1954
C. nitschkei (type)	FPH 1045	*Quercus grosseserrata*	Meguro, Japan	T. Kobayashi	1954
C. clavata (holotype)	FPH 7609	*Castanea crenata*	Kobuchizawa Japan	M. Milgroom and S. Kaneko	1998
C. clavata (epitype)	FPH 7610	*Castanea crenata*	Chudai, Japan	M. Milgroom and S. Kaneko	1998

[a] **BPI** = U. S. National Fungus Collections, Systematic Botany and Mycology, Rm. 304, Bldg. 011A, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA.

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

FPH = Forestry and Forest Products Research Institute, P. O. Box 16, Tsukuba Norin Kenkyu, Danchi-Nai, Ibaraki, 305 Japan.

CUP = Plant Pathology Herbarium, Cornell University, 334 Plant Science Building, Ithaca, New York 14853-4203 USA,

.[b] Specimen FPH 7609 is linked to isolate CMW 10786, and specimen FPH 7610 is linked to isolate CMW 10787.

Table 3. Spores sizes for the different species studied. Species, and individual specimens that was considered separately, are mentioned in the order of decreasing ascospore length.

Label name	Specimens	Ascospore length	Ascospore width	Specimens	Conidial length	Conidial width
Cryphonectria macrospora	FPH 1057	14-17(-19)	(4.5-)5.5-7(-8)	FPH 1057 FPH 1058	3.5-4.5(-5)	1-1.5
C. nitschkei	FPH 1045	(9.5-)10-11.5(-12.5)	(3-)3.5-4.5(-5)	FPH 1045	3.5-5(-6)	(1-)1.5(-2)
C. havanensis (*Quercus*)	FPH 1203 FPH 1047	(8-)9.5-11.5(-13)	(3.5-)4-5(-5.5)	FPH 1047	(3-)3.5-4.5(-5)	1.5(-2)
C. clavata	FPH 7609	(8.5-)10-11.5(-12.5)	(3.5-)4-4.5(-5)	FPH 7609	4-5.5(-6)	(1-)1.5(-2)
C. clavata	FPH 7610	n.a.	n.a.	FPH 7610	(4.5-)5-6.5(-7)	1.5
C. havanensis (*Betula* sp.)	FPH 2300	(8-)9.5-11(-12.5)	(3-)3.5-4(-4.5)	n.a.	n.a.	n.a.
C. havanensis (*Pyrus sinensis*)	FPH 1270	10-12(-13.5)	(3-)3.5-4(-4.5)	n.a.	n.a.	n.a.

Table 3. (continued)

Label name	Specimens	Ascospore length	Ascospore width	Specimens	Conidial length	Conidial width
"A. *C. havanensis*" (*Eucalyptus globulus*)	FPH 633	9.5-12(-13.5)	3-3.5(-4.5)	n.a.	n.a.	n.a.
"B. *C. havanensis*" (*Eucalyptus globulus*)	FPH 633	(6-)6.5-9(-11)	3-2.5	n.a.	n.a.	n.a.
C. parasitica	FPH 629 FPH 1326	(7.5-)8-9(-9.5)	3.5-4(-4.5)	FPH 600 FPH 608 FPH 1326	(3-)3.5-4(-4.5)	1-1.5
C. radicalis, longer ascospores (Europe)	BPI797696 BPI 797692 BPI1112743 BPI 797698 BPI 612660	(7-)8-10(-12)	(2-)2.5-3.5(-4)	BPI1112743 BPI 797698 BPI 612660	(3-)3.5-4(-4.5)	1-1.5(-2)

Table 3. (continued)

Label name	Specimens	Ascospore length	Ascospore width	Specimens	Conidial length	Conidial width
C. radicalis Japan and China	FPH 652 FPH 2483	(5.5-)6.5-8(-9.5)	(2-)2.5-3.5	FPH601 FPH 652 FPH 1072 FPH 1200	3-4(-4.5)	1-1.5
C. radicalis smaller ascospores (Europe)	BPI 797697 BPI 613739 BPI 612672 BPI 797693	(6-)7-8.5	(2-)2.5-3	BPI 613739 BPI 612672 BPI 797693	(3-)3.5-4(-4.5)	1-1.5(-2)
C. radicalis NYBG 1963 (USA)	NYBG 1963	(5.5-)6.5-8.5(-10)	(2.5-)3-4	n.a.	n.a.	
C. radicalis CUP 6178 (USA)	CUP 6178	(5-)5.5-7(7.5)	2.5-3(-3.5)	CUP 6178	(2.5-)3-3.5(-4)	1-1.5

Fig. 1. Most parsimonious phylogenetic tree (tree length= 1203 steps, CI = 0.77 and RI = 0.92) generated from sequence variation within a combined ribosomal (ITS1, 5.8S, ITS2) and β-tubulin (1a/b, 2a/b) sequence data set. Bootstrap values >50% (1000 replicates) are indicated. The *Diaporthe ambigua* isolates were used as outgroup taxa to root the phylogenetic tree.

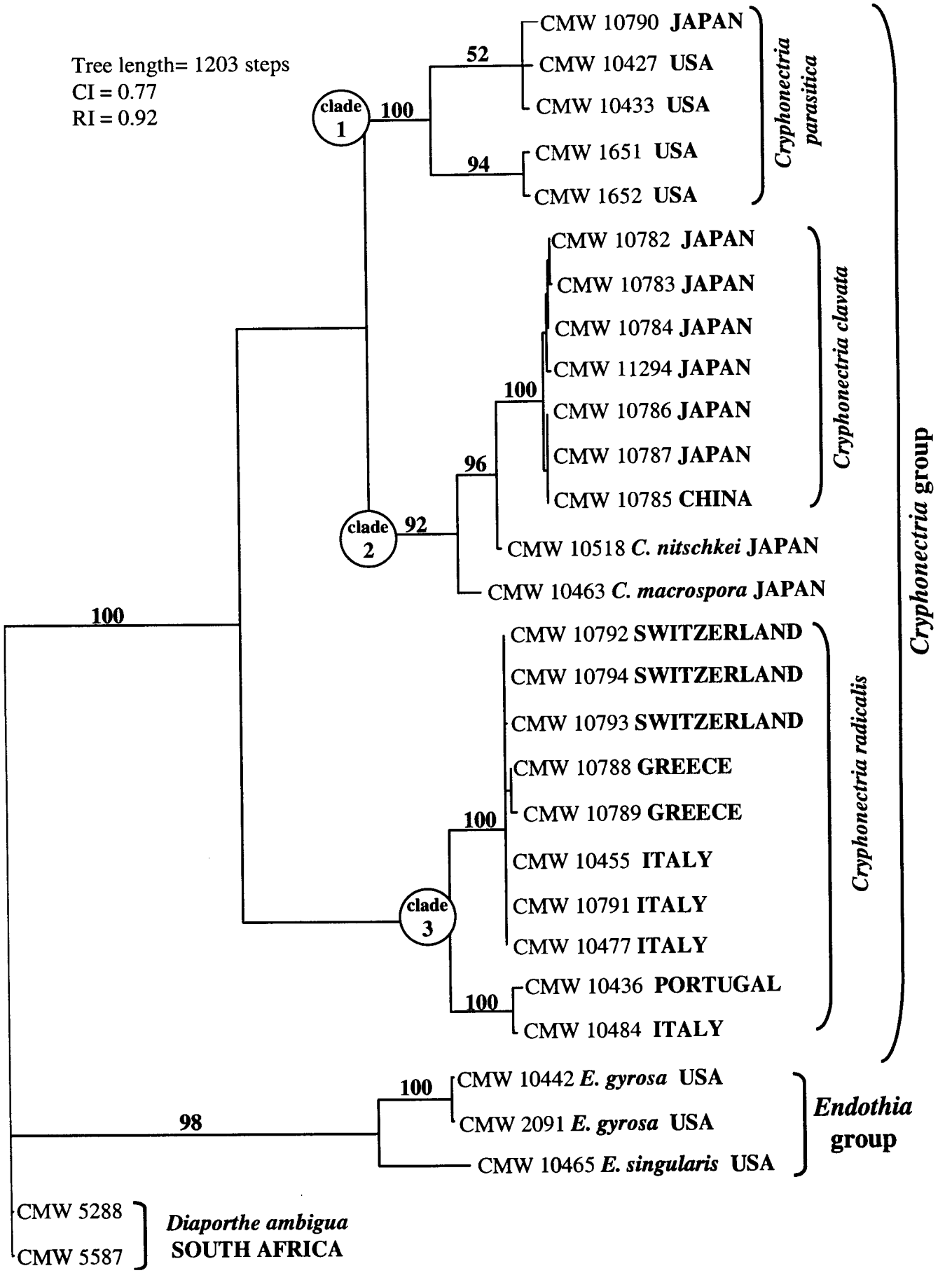

Tree length= 1203 steps
CI = 0.77
RI = 0.92
clade 1
clade 2
clade 3
100
52
94
100
96
92
100
100
100
98
CMW 10790 JAPAN
CMW 10427 USA
CMW 10433 USA
CMW 1651 USA
CMW 1652 USA
Cryphonectria parasitica
CMW 10782 JAPAN
CMW 10783 JAPAN
CMW 10784 JAPAN
CMW 11294 JAPAN
CMW 10786 JAPAN
CMW 10787 JAPAN
CMW 10785 CHINA
Cryphonectria clavata
CMW 10518 C. nitschkei JAPAN
CMW 10463 C. macrospora JAPAN
CMW 10792 SWITZERLAND
CMW 10794 SWITZERLAND
CMW 10793 SWITZERLAND
CMW 10788 GREECE
CMW 10789 GREECE
CMW 10455 ITALY
CMW 10791 ITALY
CMW 10477 ITALY
CMW 10436 PORTUGAL
CMW 10484 ITALY
Cryphonectria radicalis
Cryphonectria group
CMW 10442 E. gyrosa USA
CMW 2091 E. gyrosa USA
CMW 10465 E. singularis USA
Endothia group
CMW 5288
CMW 5587
Diaporthe ambigua
SOUTH AFRICA

Fig. 2. Micrographs of ascospores from fruiting structures on various specimens annotated as *Cryphonectria havanensis* from different hosts in Japan. **a**. FPH 1203 from *Quercus variabilis*. **b**. FPH 2300 from a *Betula* sp. **c**. FPH 1047 from *Q. glandulifera*. **d**. FPH 1270 from *Pyrus sinensis*. **e**. Larger ascospores on specimen FPH 633 ("A. *C. havanensis*") from *Eucalyptus globulus*. **f**. Smaller ascospores on specimen from FPH 633 ("B. *C. havanensis*") from *Eucalyptus globulus*. (Scale bars for **a-f** 10 μm).

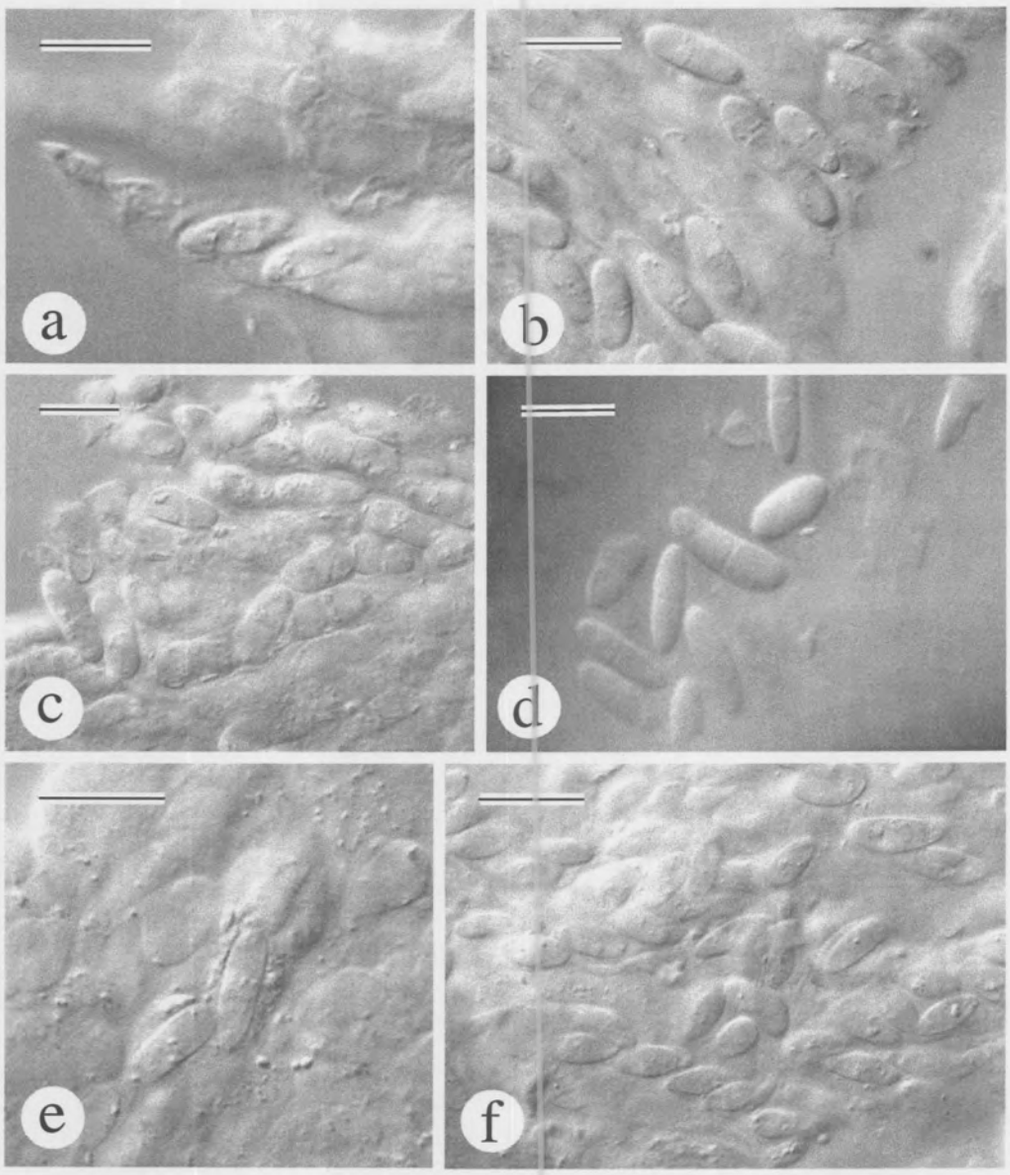


Fig. 3. Micrographs of phialides of two species of *Cryphonectria*. **a**. From type specimen (FPH 1045) of *C. nitschkei* from *Quercus grosseserrata*. **b**. From specimen (FPH 1047) of putative *C. havanensis* from *Q. glandulifera*. (Scale bars for **a**, **b** 10 μm).

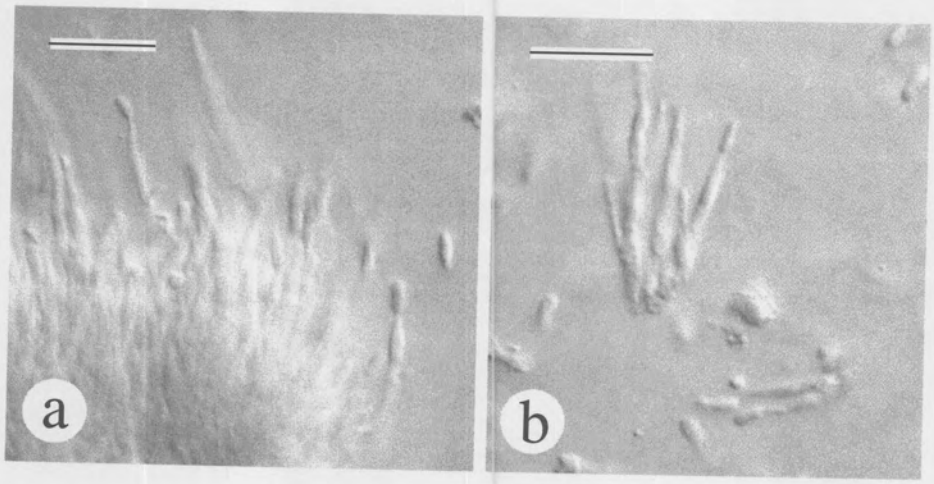


Fig. 4. Micrographs representing the fruiting structures of *Cryphonectria clavata*. **a**. Ascomata on bark. **b**. Longitudinal section through ascomata. **c**. Pseudoparenchymatous tissue. **d**. Prosenchymatous tissue. **e**. Ascus with ascospores. **f**. Ascospores. **g**. Conidioma on bark. **h**. Longitudinal section through conidioma. **i-j**. Conidiophores and conidiogenous cells. **k**. Conidia. (Scale bars for **a**, **b**, **g**, **h** 100 μm; **c**, **d** 20 μm; **e**, **f**, **i**, **j**, **k** 10 μm).

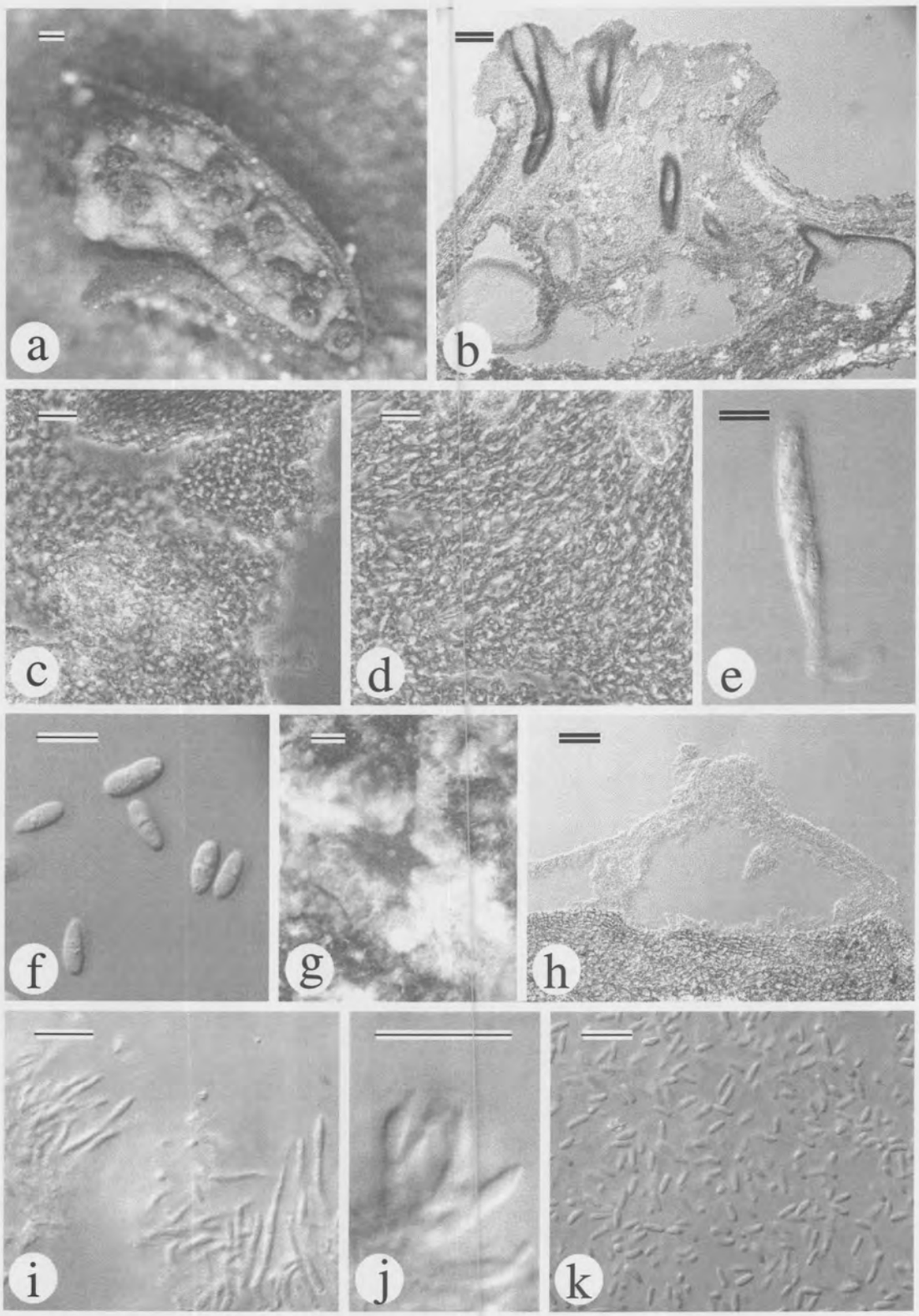
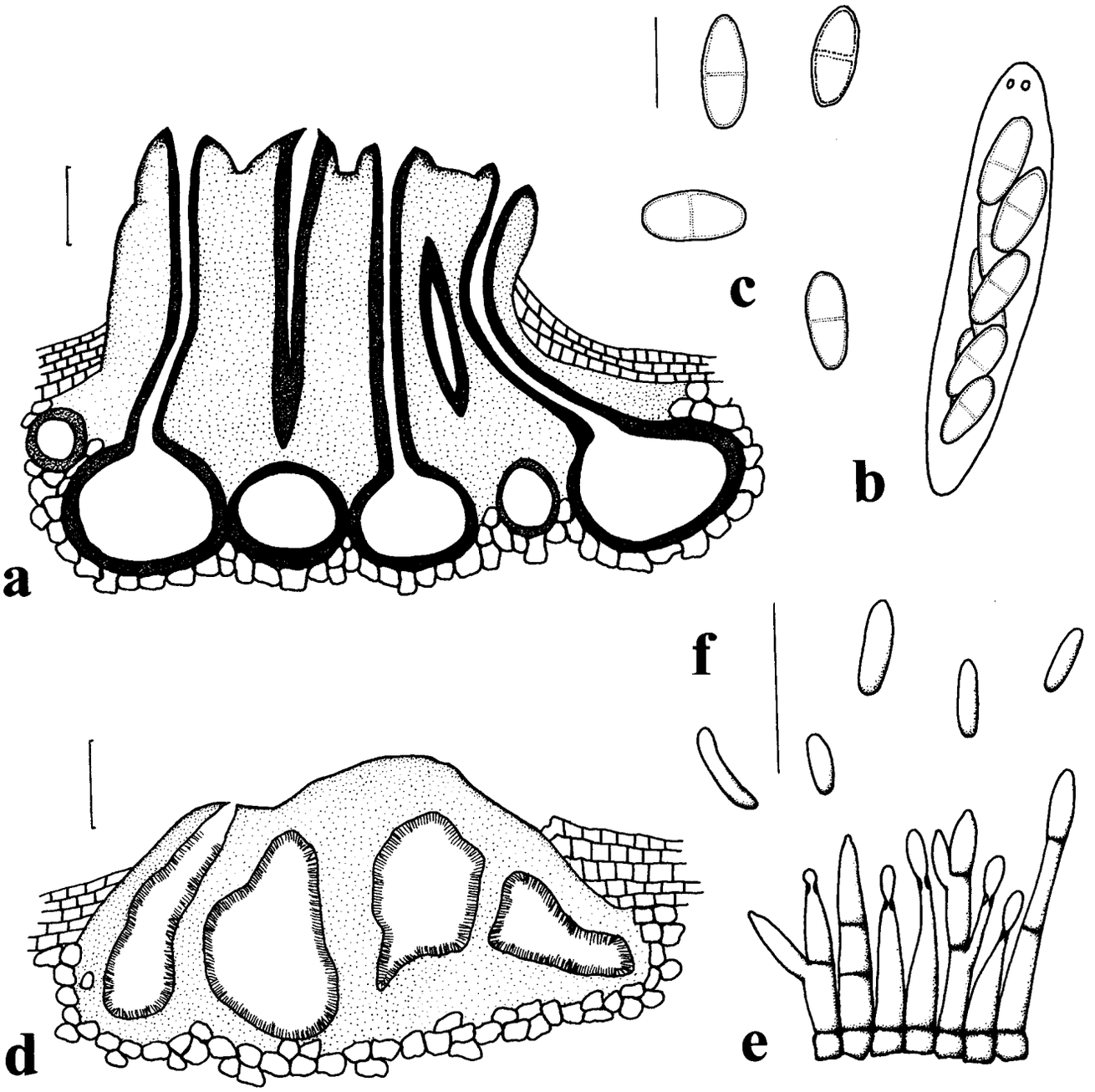


Fig. 5. Line drawings of the fruiting structures of *Cryphonectria clavata*. **a**. Ascoma. **b**. Ascus. **c**. Ascospores. **d**. Conidioma. **e**. Conidiophores and conidiogenous cells. **f**. Conidia. (Scale bars for **a**, **d** 100 μm; **b**, **c**, **e**, **f** 10 μm).



CHAPTER 7

Genera and species in the *Cryphonectria/Endothia* complex and their placement in the Diaporthales: A Molecular and Morphological synopsis.

GENERA AND SPECIES IN THE *CRYPHONECTRIA/ENDOTHIA* COMPLEX AND THEIR PLACEMENT IN THE DIAPORTHALES: A MOLECULAR AND MORPHOLOGICAL SYNOPSIS.

ABSTRACT

The order Diaporthales encompasses a number of important fungal plant pathogens, many of which reside in the genera *Cryphonectria* and *Endothia*. Collections of a number of newly described species, and as yet undescribed fungi, resembling species of *Cryphonectria* and *Endothia*, have recently been made from various hosts in different parts of the world. The aim of this study was to characterise these collections and to consider their relationships with all *Cryphonectria* and *Endothia* species available in culture. Identification and characterisation was based on morphological comparisons and sequence analyses of the ITS ribosomal DNA region and two regions in the β-tubulin gene. Sequence analyses showed that *Cryphonectria* and *Endothia* are characterised by a number of previously described as well as some presently undescribed species. The majority of the newly collected fungi resembling *Cryphonectria* spp., formed groups closely related to but distinct from *Cryphonectria*. This suggests that *Cryphonectria* is paraphyletic and includes a number of different genera. Family level relationships of *Cryphonectria* and *Endothia* were considered in terms of their relationships with recognised lineages in the Diaporthales, based on sequence data from the large subunit (LSU) nuclear rDNA. *Cryphonectria* and *Endothia*

species, including the related genera recognised in this study, formed a distinct group in the LSU rDNA phylogenetic tree. This supports the view that *Cryphonectria* and *Endothia*, as well as their closest relatives, should reside in a discrete family of the Diaporthales.

INTRODUCTION

The Diaporthales includes important plant pathogenic fungi. Members of this order are morphologically united by a *Diaporthe*-type centrum (Alexopoulos and Mims 1978, Barr 1978). Morphological criteria include perithecia with long necks, located in a pseudostroma with no paraphyses and thick-walled asci that are either evanescent with short stalks or intact (Alexopoulos and Mims 1978, Hawksworth et al. 1996). Features such as the presence or absence of stromatic tissue, stromatal tissue type, the position of the perithecia and perithecial beaks relative to the substrate, ascospore shape and ascospore septation have been used to differentiate between the families and genera in the Diaporthales (Barr 1978).

Cryphonectria and *Endothia* are amongst the better-studied genera in the Diaporthales. This is primarily because they include important tree pathogens. The best-known pathogens are *Cryphonectria parasitica* (Murr.) Barr, *C. cubensis* (Bruner) Hodges, *C. eucalypti* M. Venter and M.J. Wingf. and *Endothia gyrosa* (Schw.: Fr.) Fr. *Cryphonectria parasitica* and *E. gyrosa* are mainly found on members of the Fagaceae (Barr 1978, Kobayashi 1970, Roane 1986, Shear et al. 1917) while *C. cubensis* (Hodges 1980, Hodges et al. 1979, Hodges et al. 1986) and *C. eucalypti* mainly infect members of the Myrtaceae (Old et al. 1986, Venter et

Differentiation between species of *Cryphonectria* and *Endothia* has primarily been based on the morphological characteristics of their teleomorph states (Micales and Stipes 1987). *Cryphonectria* species have valsoid perithecia, semi-immersed stromata and one-septate ascospores, while *Endothia* species have diatrypoid perithecia, superficial stromata and aseptate ascospores (Barr 1978, Kobayashi 1970, Micales and Stipes 1987, Roane 1986, Shear et al. 1917). However, in a recent study that integrated DNA sequence data and morphology, Venter et al. (2002) showed that ascospore morphology is not a reliable characteristic to distinguish unequivocably between *Cryphonectria* and *Endothia*, but that stromatal morphology represents an important characteristic defining the two genera.

Cryphonectria was synonymised with *Endothia* by Von Höhnel (1909) and remained so until a monograph on the Diaporthales by Barr (1978). In this monograph, *Cryphonectria* was resurrected and various species were transferred from *Endothia* to *Cryphonectria.* These include *C. gyrosa* (Berk. & Br.) Sacc. (type species), *C. cubensis*, *C. havanensis* (Bruner) Barr, *C. macrospora* (Kobayashi & Ito) Barr, *C. nitschkei* (Otth.) Barr, *C. parasitica* and *C. radicalis* (Schw.: Fr.) Barr. Species currently treated in *Cryphonectria* but not included in Barr's (1978) monograph, are *C. longirostris* (Earle) Micales & Stipes and *C. coccolobii* (Vizioli) Micales & Stipes. These species were placed in *Cryphonectria* based on similar morphological characteristics (Micales and Stipes 1987). Only *Endothia gyrosa* (type species), *E. viridistroma* Wehmeyer and *E. singularis* (H. & B. Syd.) Shear and Stevens were retained in *Endothia.*

The newest member to the *Cryphonectria-Endothia* complex is *C. eucalypti* M. Venter & M. J. Wingf. (Venter et al. 2002). *Cryphonectria eucalypti*, previously considered conspecific with *E. gyrosa*, is the causal agent of a canker disease on *Eucalyptus* species in South Africa and Australia (Gryzenhout et al. 2003, Old et al. 1986, Venter et al. 2001, Venter et al. 2002, Walker et al. 1985). Venter et al. (2002) used both morphology and DNA sequence data to show that *E. gyrosa* from *Eucalyptus* in South Africa and Australia was different from *E. gyrosa* from the United States and more closely related to species of *Cryphonectria* even though the fungus had non-septate, allatoid ascospores typical of Endothia spp., and was subsequently described as *C. eucalypti*.

An additional *Cryphonectria* species will be formally described in near future. This species emerged from a study by Lui et al. (2003) and is characterised in Chapter 6 (this thesis) as *C. clavata* M. Gryzenhout & M. J. Wingf. nom. prov. This characterisation was based on ribosomal ITS and β-tubulin DNA sequence analyses as well as morphological comparisons of a large collection of *Cryphonectria* species on *Fagaceae* in Europe and Asia. Morphological and DNA sequence results indicated that *C. clavata* was different from all the *Cryphonectria* species for which cultures are currently available.

In a recent study of Myburg et al. (2003a) two new *Cryphonectria*-like fungi, other than *C. cubensis* (= *E. eugeniae* [Nutman & Roberts] Reid & Booth), were discovered on clove. This discovery was based on morphological comparisons and phylogenetic analyses. One of these newly discovered species was represented by isolates that grouped separately from the *C. cubensis* isolates in the phylogenetic tree and more closely to the clade representing

Cryphonectria. Morphological characterisation of this *Cryphonectria*-like clove fungus could not be resolved due to the lack of specimens linked to the isolates. The second species found on the clove specimens resembled the anamorph of *Cryphonectria* based on similar orange to sienna stroma. No isolates were available that could be linked to the specimens of this species. Description of these two fungi must await acquisition of isolates linked to specimens that can be connected to these fungi. The synonymy of *C. cubensis* and *E. eugeniae* (Hodges et al. 1986, Micales et al. 1987) was also confirmed in the study of Myburg et al. (2003a).

Two new genera, closely related to *Cryphonectria*, will be described in near future. The first will accommodate a fungus that is associated with a canker disease on *Terminalia ivorensis* A. Cheval. in Ecuador. Phylogenetic analyses showed that this fungus resides in a group separate from but closely related to *C. cubensis* and is distinguished by its orange, superficial, rostrate conidiomata (M. Gryzenhout, personal communication). Furthermore, morphological comparisons show that *C. longirostris* is similar to the fungus on *T. ivorensis* (M. Gryzenhout, personal communication). Nevertheless, *C. longirostris* could be differentiated from this fungus based on conidial size. *Cryphonectria longirostris* will, therefore, also be accommodated in this new genus (M. Gryzenhout, personal communication).

The second new genus related to *Cryphonectria* will include an undescribed fungal species occurring on *Miconia theaezans* Cogn. and *T. urvilleana*, trees native to Colombia, as well as on *Eucalyptus grandis* W.Hill ex Maiden in Colombia. This fungus is anamorphic, but

morphological and DNA sequence comparisons showed that it resides in the Diaporthales, close to *Cryphonectria*. The fungus is morphologically similar to *C. cubensis* based on its blackened, pyriform conidiomata, but can be distinguished from the latter species based on the distinct orange apices of the necks (M. Gryzenhout, personal communication).

Phylogenetic studies on *Cryphonectria* and *Endothia* (Myburg et al. 1999, Myburg et al. 2002a, Myburg et al. 2003b, Roux et al. 2003, Venter et al. 2001) have shown that taxonomic changes are needed for *C. cubensis*. Ribosomal ITS (Venter et al. 2001) and β-tubulin (Myburg et al. 2003b) sequence data have shown that *C. cubensis* should be considered in a genus separate but related to *Cryphonectria*. The sequence data were strongly supported by morphological characteristics such as dark brown tissue that covers the extending perithecial necks in the ascomata of *C. cubensis* as opposed to the orange tissue that covers the extending perithecial necks of typical *Cryphonectria* spp. In addition, *C. cubensis* has superficial, pyriform and blackened conidiomata different to the typical orange, pulvinate and semi-immersed conidiomata of *Cryphonectria* spp. (Gryzenhout et al. 2002, Myburg et al. 2003b). Furthermore, three phylogenetic sub-clades were observed within *C. cubensis* and these accommodated *C. cubensis* from South America/Congo, Southeast Asia/Australia and South Africa (Myburg et al. 2002a, Myburg et al. 2003b, Roux et al. 2003). Fungi residing in these sub-clades apparently represent distinct species (Myburg et al. 2002a).

Myburg et al. (2003b) studied isolates originating from *Elaeocarpus dentatus* Vahl. in New Zealand and previously thought to represent *C. gyrosa* and *C. radicalis*. DNA sequence data

comparisons revealed that this fungus should be described as a new species within a new genus, closely related to *Cryphonectria*, in the Diaporthales. Morphological features defining this group are ovoid, superficial conidiomata, and one to three septated ascospores (Myburg et al. 2003b).

In recent years, we have acquired a large number of new collections of *Cryphonectria* spp. as well as closely related species. In addition, new species or incorrectly identified species have been recognised that require further study. These fungi have been isolated from a variety of host species originating from different geographical areas of the world. The objective of this study was to provide a comprehensive synopsis for all previously described and new fungal collections awaiting description. These comparisons are based on ribosomal ITS and β-tubulin gene sequence data and morphological characteristics. Data have been extracted from a large number of previous studies (Heath et al. 2003, Myburg et al. 1999, Myburg et al. 2002a, Myburg et al. 2002b, Myburg et al. 2003a, Myburg et al. 2003b, Chapter 6 [this thesis], Roux et al. 2003, Venter et al. 2002, Wingfield et al. 2001). This study also includes fungi not previously studied as well as molecular and morphological data not published before. In order to understand the relationships among all these fungi it was necessary to determine their taxonomic position in relation to other members of the Diaporthales. This was achieved by determining LSU rDNA sequence data for these fungi and comparing it with the LSU rDNA sequence data used in publications treating the species in the Diaporthales (Castlebury et al. 2002, Zhang and Blackwell 2001).

MATERIALS AND METHODS

Isolates studied

Isolates included in this study (Table 1) represent all the species residing in *Cryphonectria* and *Endothia* for which isolates are currently available. These isolates also represent a broad range of geographical origins and hosts. As far as we are aware, no isolates exist for species such as *C. coccolobii*, authentic *C. havanensis* from Cuba, *C. gyrosa* and authentic *E. viridistroma*. These species could, therefore, not be included in the present study. The *E. viridistroma* isolate included in Myburg et al. (2003b) most likely represents a *Cytospora* sp. and has been excluded from this study. An isolate labelled as *C. gyrosa* (CMW 10471) and included in Myburg et al. (2003b) did not represent this fungus and could, therefore, not be included in the present study as *C. gyrosa*. Representatives of the newly described genera that will accommodate the fungi on *T. ivorensis* and *M. theaezans*, *T. urvilleana* and *Eucalyptus grandis* in Colombia were also included, as well as the unknown fungus found on clove in Indonesia (Myburg et al. 2003a) and waterberry in South Africa (M. Gryzenhout, personal communication) occurring together with *C. cubensis*. Furthermore, fungi thought to be species of *Cryphonectria*, but that we now believe represent new genera in the Diaporthales, have been incorporated to reflect their relationships with one another as well as with other *Cryphonectria* and *Endothia* spp. *Diaporthe ambigua* Nitschkei isolates were included as outgroup taxa to root the ITS/β-tubulin phylogenetic tree.

Representative isolates from the above mentioned groups of fungi were included in LSU sequence data analyses. These sequences were compared to the LSU sequence data sets generated in Zhang and Blackwell (2001) (Table 3) and Castlebury et al. (2002) (Table 4). The LSU sequence data for *Magnaporthe grisea* (T.T. Herbert) Yaegashi & Udugawa (AB 026819), *Pyricularia grisea* (Cooke) Sacc. (AF 362554), *Gaeumannomyces graminis* (Sacc.) Arx & D. Oliver (AF 362556) and *Gaeumannomyces graminis* (AF 362557) generated in the study of Castlebury et al. (2002), were used as outgroup taxa to root the LSU phylogenetic tree.

The isolates listed in Table 1 are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Many of the isolates are also available in other internationally recognised collections and the information is included in Tables 1 and 2.

DNA extractions

DNA was isolated from mycelium using the DNA extraction buffer of Raeder and Broda (1985). The DNA extraction buffer consisted of 200 mM Tris-HCl (pH 8.5), 250 mM NaCl, 25mM EDTA and 0.5% SDS. DNA quality was assessed by gel electrophoresis in a 1% agarose gel containing ethidium bromide. The DNA was visualised by exposing the agarose gel to a UV light source.

ITS rDNA and β-tubulin amplification

Reaction conditions to amplify the ITS1, 5.8S and ITS2 regions of the rRNA operon as well as two regions within the β-tubulin gene are described in the studies of Myburg et al (1999) and Myburg et al (2002a,b). The primer pairs used to amplify the ribosomal DNA (ITS1, 5.8S and ITS2) and β-tubulin gene regions are those designed and used by White et al. (1990) and Glass and Donaldson (1995), respectively. Amplified products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and directly used as templates in sequencing reactions.

LSU rDNA amplification

The partial LSU rDNA gene was amplified with primers pairs ITS3 (White et al. 1990) and LR3 (Rehner and Samuels 1994, Vilgalys and Hester 1990). PCR conditions were: 95°C for 3 min (denature), 30 cycles of 95°C for 30 s (denature), 56°C for 45s (anneal), 72°C for 1 min (elongation) and a final elongation step of 72°C for 4 min. LSU amplification products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and directly used as templates in subsequent sequencing reactions.

Sequencing

Sequencing reactions were as specified by the manufacturers of the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, United

Kingdom). Nucleotide sequence data were generated with an ABI PRISM 3100™ automated DNA sequencer (Perkin-Elmer, Warrington, United Kingdom). The primer pairs used in the respective sequencing reactions are as follows: ITS1 and ITS4 (amplifying the rDNA operon), Bt1a and Bt1b (amplifying β-tubulin region 1a/1b), Bt2a and Bt2b (amplifying β-tubulin region 2a/2b), LS1 and LR3 (amplifying LSU rDNA).

Sequence data analyses

The raw sequence data generated for the respective gene regions were edited using the Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software package. Gaps were inserted during sequence alignment and were treated as fifth characters (NEWSTATE) in the sequence analyses. Phylogenetic analyses were executed using the software package PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b8 (Swofford 1998).

Sequence data obtained from other studies

Sequence data from other studies were included in this study for comparative purposes. Ribosomal ITS and β-tubulin DNA sequence data were obtained from the studies of Heath et al. (2003), Myburg et al. (1999), Myburg et al. (2002a,b), Myburg et al. (2003a,b), Chapter 6 (this thesis), Venter et al. (2001), Venter et al. (2002), Roux et al. (2003) and Wingfield et al. (2001). Large subunit ribosomal RNA sequence data were obtained from Zhang and Blackwell (2001) and Castlebury et al. (2002). Sequence alignments used by

Zhang and Blackwell (2001) and Castlebury et al. (2002) were obtained from TreeBASE. TreeBASE study accession numbers are S 665 (Zhang and Blackwell 2001) and S 815 (Castlebury et al. 2002).

Analysis of ITS and β-tubulin sequence data

The ribosomal DNA (ITS1, 5.8S, ITS2) and β-tubulin sequence data sets were subjected to the Templeton nonparametric Wilcoxon Signed Ranked (WSR) (Kellogg et al. 1996) test to assess whether they could be combined as one sequence data set in the phylogenetic analyses. Heuristic searches, with tree-bisection-reconnection (TBR) and MULTREES options (saving all optimal trees) effective, were used to analyse the DNA sequences. The confidence levels of the tree branch nodes were determined by a 1000 replicate bootstrap analysis (Felsenstein 1985). GenBank accession numbers of sequences generated in this study as well as those from previous phylogenetic studies are listed in Table 1.

Analyses of LSU sequence data

The LSU rDNA region of twenty-seven taxa was amplified (Table 1) in the present study. These taxa represent *Cryphonectria* and *Endothia*, related taxa and new species (representatives from **clades 1-10** in the ITS/β-tubulin phylogenetic tree). Phylogenetic trees were generated by maximum parsimony (MP) using the heuristic search option, with random sequence addition (1000 replications) and tree-bisection-reconnection (TBR) branch swapping options of PAUP* 4.0b8 (Swofford 1998) effective. All the sequence characters

were unordered and given equal weight. Gaps were treated as missing data in the parsimony analyses. Branch support was determined with 1000 bootstrap replications (Felsenstein 1985), MULTREES and TBR options rejected and random sequence additions for the MP bootstraps changed to 10. LSU sequence data generated in this study were deposited in GenBank and the accession numbers are listed in Table 1. Accession numbers for LSU sequences obtained from Zhang and Blackwell (2001) and Castlebury et al. (2002) are listed in Tables 3 and 4 respectively.

Morphological observations

Herbarium specimens (Table 2) linked to the different genera and ten phylogenetic groups indicated in the ITS/β-tubulin phylogenetic tree (Table 1, Fig. 1) were compared. Wherever possible, type specimens were included. Newly collected specimens linked to the undescribed taxa represented in the ITS/β-tubulin phylogenetic tree have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM). No herbarium specimens are available for the *Endothiella* spp. on *Eucalyptus* spp. from New Zealand (isolates CMW 10010, CMW 10011 and CMW 10797) or isolate CMW 11297 annotated as *C. havanensis* from Mexico. Fruiting structures for the group of isolates from clove in Indonesia (CMW 10780, CMW 10779, CMW 10781) were obtained through artificial inoculations on stems of *E. grandis* (Myburg et al. 2003a).

Longitudinal sections of the stromata were made by rehydrating the fruiting structures in boiling water for 1 min and then embedding them in Leica mountant (Setpoint Premier,

Johannesburg, South Africa). Sections, 12-16 μm thick, were made with a Leica CM1100 cryostat (Setpoint Premier) at –20 °C. The sections were then dropped in water, transferred to microscope slides and mounted in lacto-phenol. These slides were studied using standard light microscopy. Spores were also examined in 3% KOH.

RESULTS

ITS/β-tubulin sequence analyses and morphological data

Results from the Templeton nonparametric Wilcoxon Signed Ranked test showed no significant conflict between the two data sets and the ITS ribosomal DNA and β-tubulin gene sequence data sets could be combined in the parsimony analyses. The combined ITS and β-tubulin sequence data sets included 106 taxa of which two *D. ambigua* isolates were incorporated as the outgroup taxa. A total of 1602 characters were included in the phylogenetic analyses of which 746 were constant, 96 variable but parsimony-uninformative and 760 variable and parsimony-informative. (Sequence alignments are available on request from the author as the document was too large to include in this thesis). The 'MaxTrees' limit (100) was reached in the heuristic search and a strict consensus tree (tree length = 3267 steps, consistency index/CI of = 0.51, retention index/RI of = 0.9) was computed for the 100 trees.

The phylogram derived from the combined sequence data (Fig. 1) depicts ten phylogenetically distinct clades, which have been numbered accordingly. Some of these

groups have previously been recognised while others have not yet been identified. The ten clades are each supported by unique morphological features.

Clade 1 (bootstrap support = 100%) includes *Cryphonectria cubensis* and is represented by isolates of *C. cubensis* from different parts of the world and from different hosts. Morphological features that define this clade are superficial, blackened pyriform conidiomata and ascomata with weakly developed orange to cinnamon stromatal tissue and blackened perithecial necks (Fig. 2a). Ascospores are fusoid, one-septate, and conidia are minute, aseptate, oblong to oval (Fig. 2a).

The three sub-groups of *C. cubensis* isolates from different parts of the world, previously described in Myburg et al. (2002a) were supported in the present study (Fig. 1). The South American sub-clade (bootstrap support = 77%) includes *C. cubensis* isolates from *Eucalyptus* spp. in Brazil and Venezuela. *Cryphonectria cubensis* isolates from *Eucalyptus* spp. in the Congo (Roux et al. 2003) and the *C. cubensis* isolates occurring on clove (*S. aromaticum*) in Brazil (Myburg et al. 2003a) grouped in the South American sub-clade. Recently obtained isolates from *Miconia* species (Melastomataceae) native to Colombia, i.e. *M. rubiginosa* (Bonpl.) DC. and *M. theaezans* (C. Rodas, personal communication), also resided in this sub-clade.

The Southeast Asian and Australian sub-clade (bootstrap support = 87%) included *C. cubensis* isolates from *Eucalyptus* spp. originating from Indonesia, China, Vietnam and Australia. *Cryphonectria cubensis* isolated from clove in Indonesia and Zanzibar (Myburg

et al. 2003b) grouped with those isolated from *Eucalyptus* spp. A *C. cubensis* isolate from Hawaii (CMW 1856) also grouped in this clade.

Isolates of *C. cubensis* in South Africa resided in a distinct sub-clade separately (bootstrap support = 87%) from the Southeast Asian/Australian and South American/Congo sub-clades. Isolates in this sub-clade represent those collected from *Eucalyptus* spp., *Tibouchina* spp. (Myburg et al. 2002b) and native waterberry trees (*Syzygium cordatum*) in South Africa (Heath et al. 2003).

Two additional groups, not previously recognised, are evident in the phylogram (Fig. 1). *Cryphonectria cubensis* isolates from a *Eucalyptus* sp. in Ecuador grouped separately from the other South American *C. cubensis* isolates (bootstrap support = 98%). Similarly, the *C. cubensis* isolates previously described from *T. urvilleana* in Colombia (Wingfield et al. 2001) (bootstrap support = 99%), grouped basal to the other sub-clades defined by geographical origin. A *C. cubensis* isolate from *M. theaezans* (CMW 9979) grouped together with these isolates from *T. urvilleana* in Colombia.

Clade 2 includes a group of isolates that represent the fungus occurring on *T. ivorensis* in Ecuador. This group clearly represents a distinct genus based on sequence data and morphological characteristics (bootstrap support = 100%). This fungus is more closely related to the group representing *C. cubensis sensu lato* than to any of the other groups in the phylogenetic tree. Based on morphology, this group is characterised by superficial, pyriform, orange conidiomata with long necks (Fig. 2b). Ascomata are seated in the bark,

surrounded by little stromatic tissue except for a sheath of tissue around the perithecial necks. Ascospores are fusoid to ellipsoid, one-septate, and conidia are minute, aseptate, cylindrical to oval (Fig. 2b).

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Clade 3 incorporates *C. eucalypti* isolates from South Africa and Australia (Venter et al. 2001, Venter et al. 2002) and fungi identified as *Endothiella* spp. occurring on *Eucalyptus* in New Zealand (bootstrap stupport = 100%). One of the isolates identified as an *Endothiella* sp. (CMW 10797), grouped separately (bootstrap support = 100%) from the other New Zealand *Endothiella* spp. as well as the *C. eucalypti* isolates and most probably represents a distinct species. This group of fungi have small, semi-immersed stromata containing convoluted conidial locules, and perithecia with bases surrounded by host tissue (Fig. 2c). Ascospores are cylindrical, sometimes allantoid and aseptate while conidia are minute, cylindrical and aseptate (Fig. 2c).

Isolates in **clades 4** to **6** grouped closely together in the phylogenetic tree (Fig.1). Morphology of **clades 5** and **6** is not yet fully resolved due to a lack of herbarium material or the presence of only one morph.

Clade 4 includes the isolates representing a fungus originating from *M. theaezans* in Colombia. The members of this genus have blackened, pyriform conidiomata (Fig. 2d). The apices of the conidiomatal necks are characteristically orange and conidia are minute, aseptate and oval (Fig. 2d). No teleomorph is known for this fungus.

Clade 5 represents undescribed fungi, occurring alongside *C. cubensis* on clove (*S. aromaticum*) in Indonesia (Myburg et al. 2003a) and waterberry (*S. cordatum*), a native tree species in South Africa (M. Gryzenhout, personal communication). The undescribed fungus on clove from Indonesia, represented by isolates CMW 10779, CMW 10780 and CMW 10781, is not connected to original host material. Fruiting structures have been produced on artificially inoculated *E. grandis* bark (Myburg et al. 2003a). Although these fruiting structures were too variable to draw definitive conclusions regarding the morphology of the fungus (Myburg et al. 2003a), fruiting structures were in general blackened, superficial and conical without long necks (Fig. 2e). Conidia were minute, cylindrical and aseptate (Fig. 2e). The teleomorph of this fungus is unknown. Fruiting structures of the fungus from *S. cordatum* (CMW 9978) in South Africa were only sexual and consisted of semi-immersed perithecia in well-developed, orange stromata (Fig. 2e). Ascospores are fusoid and one-septate (Fig. 2e).

Clade 6 comprises the unnamed fungus (CMW 9945, CMW 9946) isolated from *T. urvilleana* in New Zealand. Ascomatal structures representing isolates in this clade contains orange-brown stromatal tissue with semi-immersed perithecia (Fig. 2f). Ascospores are fusoid and one septate (Fig. 2f). No anamorph was found on the current herbarium specimens.

Isolates and specimens labelled as *C. havanensis* and collected on *Eucalyptus* in Mexico (CMW 11297, CMW 11298), and a fungus occurring on *Myrica faya* in Madeira (CMW 11299, CMW 11300) and the Azores (CMW 11301, CMW 11302) make up **Clade 7**. The

fungus collected from the Azores and Madeira, was associated with cankers of *M. faya* (Gardner and Hodges 1990, Hodges and Gardner 1992). Fruiting structures related to this group appear similar to those characterising *Cryphonectria sensu stricto*, but the stromata are more superficial and less developed (Fig. 2g). Ascospores are fusoid to ellipsoid, one-septate, and conidia minute, cylindrical and aseptate (Fig. 2g).

Cryphonectria sensu stricto is represented by isolates of *C. parasitica*, *C. nitschkei*, *C. radicalis* and *C. macrospora* that reside in **Clade 8**. This group was also recognised previously (Myburg et al. 2003b, Myburg et al. Chapter 6, Venter et al. 2002) and includes the new species, *C. clavata*, on *Quercus* and *Castanea* spp. from Japan (Myburg et al. Chapter 6). Two isolates, CMW 10436 and CMW 10484, identified as *C. radicalis*, grouped closely, but separately (bootstrap support = 100%) from the other *C. radicalis* isolates (CMW 10455, CMW 10477, CMW 10788-10789, CMW 10791-10794). These isolates (CMW 10436, CMW 10484) probably represent a distinct species, but due to the lack of herbarium specimens with fungal structures on host tissue, it is not possible to conclude which of the two groups represent authentic European *C. radicalis* (Myburg et al. 2003b, Myburg et al. Chapter 6). Species in **clade 8** are unified by conidiomata that are orange, multilocular and semi-immersed (Fig. 2h). Ascomata are orange, well developed, erumpent, semi-immersed, with perithecia in a valsoid orientation (Fig. 2h). Ascospores are fusoid to ellipsoid and one-septate while the conidia are minute, cylindrical and aseptate (Fig. 2h).

Endothia is represented by the two species *E. gyrosa* and *E. singularis* that reside in **Clade 9**. This group is well supported within the phylogenetic tree (bootstrap support = 91%) and

is distinct from any of the other phylogenetic groups. Species of *Endothia* are characterised by orange, superficial, strongly developed stromata with perithecia in a diatrypoid orientation, and numerous conidial locules (Fig. 2i). Ascospores are cylindrical to allantoid and aseptate, while conidia are minute, cylindrical and aseptate (Fig. 2i).

Clade 10 incorporates a group of fungi (CMW 10469, CMW 10470, CMW 10471) isolated from *Elaeocarpus dentatus* in New Zealand (Myburg et al. 2003b). This clade grouped basal to the other clades in the phylogenetic tree. The stromata of these fungi are large, superficial, orange, and perithecia are borne similar to those of *Endothia* (Fig. 2j). Conidiomata, however, have a conical shape (Fig. 2j). Ascospores are also very distinct from other species currently described in *Cryphonectria sensu lato* and have one to three septa (Fig. 2j). Conidia are cylindrical and aseptate (Fig. 2j).

Analysis of LSU rDNA sequences

The LSU sequence data set included 125 taxa, of which 20 sequences were obtained from Zhang and Blackwell (2001) (Table 3) and 76 sequences from Castlebury et al. (2002) (Table 4) of which four represented the outgroup taxa, i.e. *Magnaporthe grisea*, *Pyricularia grisea* and *Gaeumannomyces grisea* (2 representatives). Fifteen additional taxa representing the genera *Cryphonectria* and *Endothia* and fourteen isolates representing the suggested new genera and/or species were included (Table 1). The LSU sequence data set consisted of 552 total bases of which 408 were constant, 18 were parsimony-uninformative and 126 were parsimony-informative. (Sequence alignments are available on request from the author as

the document was too large to include in this thesis). The heuristic search done for the MP analyses resulted in 100 trees and a strict consensus tree (Fig. 3) were computed (tree length = 487 steps, CI = 0.37, RI = 0.87).

The LSU phylogenetic tree (Fig. 3) is similar to a portion of the tree generated by Castlebury et al. (2002), although the present study includes a substantially greater number of taxa representing the *Cryphonectria-Endothia* complex. Other lineages in the phylogram represent the families Gnomoniaceae *sensu stricto*, Melanconidaceae *sensu stricto*, a *Schizoparme* complex including the anamorph genera *Coniella* and *Pilidiella*, the Valsaceae *sensu stricto* and the Diaporthaceae *sensu stricto* (Castlebury et al. 2002, Zhang and Blackwell, 2001).

The *Cryphonectria-Endothia* complex, as it is presented in Fig. 3, includes *C. parasitica* (AF 277132), *Cryptodiaporthe corni* (AF 277133) and *Endothia eugeniae* (AF 277142) included in the study of Zhang and Blackwell (2001). *Endothiella gyrosa* (AF 362555), *Cryptodiaporthe corni* (AF 408343), *Chromendothia citrina* (AF 408335), *Cryphonectria macrospora* from Russia (AF 408340), *Cryphonectria nitschkei* from Russia (AF 408341), *Cryphonectria cubensis* (AF 408338) and *Cryphonectria havanensis* (AF 408339) were included in the study of Castlebury et al. (2002). The taxa selected as representatives of the different clades in the ITS/β-tubulin phylogram (Fig. 1) in this study, all grouped within the *Cryphonectria-Endothia* complex. The phylogenetic groupings in the LSU phylogram (Fig. 3) mirrored those of the ITS/β-tubulin phylogram (Fig. 1) and similar species grouped together.

The isolates representing the species *Cryptodiaporthe corni* (Castlebury et al. 2002, Zhang and Blackwell 2001) and *Chromendothia citrina* (Castlebury et al. 2002) grouped within the *Cryphonectria-Endothia* complex. The grouping of the *C. corni* isolate with those in the *Cryphonectria-Endothia* complex was strange as *C. corni* grouped separately from the type species of *Cryptodiaporthe*, *C. aesculi*. Castlebury et al. (2002) suggested that C. corni might reside in either *Cryphonectria* or *Endothia*. The *E. eugeniae* isolate included in the study of Zhang and Blackwell (2001) grouped with the undescribed fungi on clove (CMW 10781) (Myburg et al. 2003a) and *S. cordatum* in South Africa (CMW 9978). The isolates identified as *C. radicalis* and *C. gyrosa* (CMW 10469 and CMW 10470) from New Zealand (Myburg et al. 2003b) fall within the *Cryphonectria-Endothia* complex, even though they have three-septate ascospores, and grouped basal to those in the other clades in the ITS/β-tubulin tree (Fig.1). Isolates of *C. cubensis* grouped closely with the isolates of *C. cubensis* and the *C. havanensis* (AF 408339) included in the study of Castlebury et al. (2002). Interestingly, the *C. havanensis* (AF 408339) isolate, deposited in CBS as *C. havanensis* (CBS 505.63), is actually *C. cubensis*. Studies of Micales et al. (1987), Hodges et al. (1986) and Myburg et al. (2003b), based on cultural, morphological comparisons, protein profiles and phylogenetic analyses based on DNA sequence data have also shown that isolate CBS 503.63 was misidentified as *C. havanensis* and represents *C. cubensis*.

DISCUSSION

Ribosomal ITS and β-tubulin DNA sequence analyses show that a number of genera exist in a group of fungi that has previously been represented by *Cryphonectria* and *Endothia*. In

this study we putatively recognise eight new generic groups within the so-called *Cryphonectria-Endothia* complex. These generic groups are based on distinct morphological and/or molecular differences. These generic groups are represented by the following: *C. cubensis* (**clade 1**), the fungus occurring on *T. ivorensis* in Ecuador (**clade 2**), *C. eucalypti* and the *Endothiella* spp. from New Zealand (**clade 3**), the fungus on Colombian *M. theaezans* and *Tibouchina* spp. (**clade 4**), the fungus on Indonesian clove and South African waterberry trees occurring alongside *C. cubensis* (**clade 5**), the fungus on *T. urvilleana* from New Zealand (**clade 6**), the isolates from Mexico identified as *C. havanensis* and the fungus originating from Madeira and the Azores (**clade 7**) and the fungus on *E. dentatus* from New Zealand that is characterised by three-septate ascospores (**clade 10**). All species in these clades can be differentiated based on morphological features that are linked to stromatal shape and colour of the anamorph, position of both morphs relative to the bark and ascospore septation.

The LSU rDNA analysis of this study strongly support the view of Castlebury et al. (2002) that the *Cryphonectria-Endothia* complex based on distinct, monophyletic group within the Diaporthales and that it should be considered as a separate family in this order. The study of Castlebury et al. (2002) showed the distinct grouping of *Cryphonectria* and *Endothia* spp., although their results were based on only a small collection of isolates representing these genera. The large number of undescribed genera and species included in the present study add further justification for describing a family for these fungi as well as species of *Cryphonectria* and *Endothia*. This selection of species and genera are unified by the production of orange pigments in their stromatic tissue and in culture, as well as a purple

discolouration of the fruiting structures in 3% KOH and a yellow discolouration in lactic acid (Castlebury et al. 2002).

This study includes all the isolates currently known to us that represent species of *Cryphonectria* and *Endothia* and related genera. There are, however, some species for which no living cultures or authentic isolates, linked to previous descriptions, exist. These include *C. longirostris*, *C. coccolobii*, *C. havanensis* from Cuba or *C. gyrosa*, the type species of *Cryphonectria*. Morphological characteristics linked to clades 1-10 identified in this study, can be used to predict appropriate placements for the abovementioned taxa for which isolates are not available. These predictions would, however, be without phylogenetic support. For example, *C. longirostris* will be placed in the new genus that accommodates the fungus on *T. ivorensis* in Ecuador. This placement will be based on the fact that *C. longirostris* and the fungus on *T. ivorensis* have similar superficial rostrate conidiomata (M. Gryzenhout, personal communication).

The genera *Cryphonectria* and *Endothia* retain a significant taxonomic position in the Diaporthales, despite the fact that new generic groups have been identified. *Cryphonectria* and *Endothia* species represented in this study include some of the species that have been treated by authors such as Kobayashi (1970), Barr (1978), Roane, (1986), Micales and Stipes (1987) and Shear et al. (1917). These species are *C. parasitica*, *C. radicalis*, *C. macrospora*, *C. nitschkei*, *E. gyrosa* and *E. singularis*. It is unfortunate that no isolates linked to collections of *C. gyrosa* from Sri Lanka exist that can be used to confirm the relationship between the type species of *Cryphonectria* and the *Cryphonectria* species

included in this study. Isolates of *E. gyrosa*, the type species of *Endothia*, has been incorporated in this study although these isolates are not linked to the original type collection.

The remaining described species of *Cryphonectria*, namely *C. longirostris*, *C. cubensis*, *C. havanensis* and *C. coccolobii*, await further study. *Cryphonectria longirostris* will be transferred as a member of the new genus (**clade 2**) including the fungus on *T. ivorensis* in Ecuador (M. Gryzenhout, personal communication). *Cryphonectria cubensis sensu lato* is the only long-established species for which isolates exists, and it did not group with the other *Cryphonectria* spp. and will be described in a genus of its own. The taxonomic position of *C. havanensis* from Cuba and *C. coccolobii* still need to be considered. All of these species exhibit characteristics different from the type species *C. gyrosa* (K 109807) from Sri Lanka.

Cryphonectria cubensis sensu lato forms a well-delineated group (**clade 1**). The morphological characteristics of *Cryphonectria cubensis sensu lato*, that distinguish it from *Cryphonectria sensu stricto* and the type specimen of *C. gyrosa* (K 109807), are long, black perithecial necks and superficial, pyriform, blackened conidiomata (Myburg et al. 2002a). These morphological differences as well as the separate phylogenetic grouping support the suggestion of Myburg et al. (2002a), Myburg et al. (2003b) and Venter et al. (2001) that *C. cubensis sensu lato* should be considered in a discrete genus.

Cryphonectria cubensis sensu lato accommodates five sub-groups. These include isolates from Ecuador, South America/Congo, Colombia, South Africa and Southeast Asia/Australia. Isolates residing in these sub-groups will most likely be described as distinct species (M. Gryzenhout, personal communication). The proposed species are morphologically similar, but are well delineated based on distinct differences in the ITS and β-tubulin gene sequences.

Isolates of *C. cubensis sensu lato* have been reported to occur on a variety of host species throughout the world. These include species of *Eucalyptus* (Hodges et al. 1976), *Syzygium* (Heath et al. 2003, Hodges et al. 1986, Myburg et al. 2003a), *Miconia* (C. Rodas, personal communication) and *Tibouchina* (Myburg et al. 2002b, Wingfield et al. 2001). These tree species reside in the Myrtaceae and Melastomataceae (Myrtales). Two of these tree hosts, i.e. *Miconia* and *Tibouchina*, are native in South America, which suggests that *C. cubensis* comprising the South American/Congo phylogenetic clade (Fig. 1) might have an origin in South America. An origin in Indonesia has also been suggested by Hodges et al. (1986). This hypothesis could be valid for *C. cubensis* representing the Southeast Asian phylogenetic clade (Fig. 1) and occuring on native *Syzygium* species in Indonesia. Furthermore, it seems that *C. cubensis* isolates from South Africa have an origin on South African *Syzygium* species. Further studies at population level are needed to resolve these hypotheses regarding the origin of *C. cubensis* and its occurrence on *Eucalyptus*.

Myburg et al. (2003a) described the presence of a fungus resembling Cryphonectria occurring with *C. cubensis* on clove in Indonesia. Sequence data showed that this fungus

was closely related yet distinct from *Cryphonectria sensu stricto*. This relatedness was confirmed in the present study, but a conclusive identification of this *Cryphonectria*-like fungus could not be made due to lack of specimens on natural substrate that could be linked to these isolates. Artificial inoculations of an isolate of this fungus into *Eucalyptus* yielded conidiomata that were superficial, ovoid, black, and without necks (Myburg et al. 2003b), and which easily could be distinguished from the conidiomata of *C. cubensis* on eucalyptus which have long attenuated necks (Myburg et al. 2002a, Myburg et al. 2003a). This distinction should aid future collection and identification of this fungus on clove.

A *Cryphonectria*-like fungus was discovered occurring with *C. cubensis* on native waterberry trees (*S. cordatum*) in South Africa (M. Gryzenhout, personal communication). Phylogenetic analyses show that this fungus grouped with the *Cryphonectria*-like fungus occurring alongside *C. cubensis* on clove in Indonesia. Herbarium specimens of this fungus from waterberry contained teleomorphic stromata that were orange, semi-immersed and had short orange necks, different from the black necks of *C. cubensis*. Based on the close phylogenetic grouping of the *Cryphonectria*-like fungi from Indonesia and South Africa, we believe that this group of fungi should reside in a new genus. The teleomorph state of the fungus from Indonesia is unknown, while the anamorph state of the fungus from South Africa has not been found. Yet, the fungi represented in **clade 5** are morphologically characterised by blackened ovoid conidiomata without the long necks that is typical of *C. cubensis* on eucalyptus, and by semi-immersed ascomata with orange, well-developed stromatic tissue and orange tissue that covers the perithecial as they extend from the stromatal surface.

A canker pathogen on *Eucalyptus* in Australia and South Africa, previously known as *E. gyrosa*, recently has been described as a new species, *Cryphonectria eucalypti* (Venter et al. 2002). The placement of this fungus was justified based on stromatal similarities with *Cryphonectria* species and the close phylogenetic grouping of *C. eucalypti* isolates with species of *Cryphonectria* (Venter et al. 2002). When fungal groups that were more closely related to *Cryphonectria* were included, Myburg et al. (2003b) found that the *C. eucalypti* isolates were still more closely related to *Cryphonectria* than to species of *Endothia*, even though *C. eucalypti* has similar aseptate, allantoid ascospores usually considered characteristic of species of *Endothia*. Phylogenetic results from this study support this close relationship but also suggest that *C. eucalypti* most probably represents a distinct generic lineage (**clade 3**). The distinct grouping of *C eucalypti* isolates results from the inclusion of isolates that possibly represents undescribed species belonging to this generic lineage, namely the isolates (CMW 10010, CMW 10011) labelled as *Endothiella* spp. from New Zealand. This generic distinction is supported by the fact that ascospores of *C. eucalypti* are unlike those of *Cryphonectria*.

Clade 6 accommodates the fungus on *T. urvilleana* in New Zealand. Despite the fact that this fungus is represented in this study by only two isolates, the orange-brown stromatic tissue of this fungus and sequence data justifies treating this fungus in a discrete genus. Unfortunately the anamorph of this fungus is absent on the available herbarium material. It is likely that since anamorph morphology has been one of the most important morphological characters to distinguish among the different clades in the phylogenetic tree, the anamorph

of this fungus is likely to yield better morphological criteria to separate this clade from the others.

The group of fungi residing in **Clade 7** and tentatively identified as *C. havanensis* from Mexico, and those isolated from *M. faya* in the Azores and Madeira, require further study prior to deciding on their identity. The occurrence of a fungus reminiscent of a *Cryphonectria* species but different from *C. cubensis*, has been reported before from Florida, USA on *Eucalyptus grandis* (Barnard et al. 1987, Barnard et al. 1993). It is possible that the fungus occurring on *Eucalyptus* spp. in Mexico (CMW 11297, CMW 11298) will be similar to the fungus reported from Florida. Further comparisons will also be needed to determine whether these fungi, annotated as *C. havanensis*, represents *C. havanensis* that was first described from Cuba (Bruner 1916). It is also possible that *C. coccolobii*, a fungus found on *Coccoloba uvifera* (L.) L. (seagrape) in Bermuda (Vizioli 1923, Waterston 1947) and Florida (Barnard et al. 1993) will reside in this group. These questions will, however, be difficult to pursue in the absence of additional isolates linked to specimens.

The overall results of this study reflect the importance of establishing a clear and well-defined delineation for species residing in the genera *Cryphonectria* and *Endothia*, and those identified in this study that need further characterisation. It is also clear that there are many more species and genera in this group than previously thought and we believe that there are others that have not yet been discovered. Results of this study have provided a framework for further collecting and characterising of genera and species in the family that is typified

by *Cryphonectria*. To aid future identifications the following key to the possible genera linked to the different phylogenetic clades is presented.

PLEASE NOTE THAT THE FOLLOWING KEY PRESENTED HERE IS IN PRELIMINARY FORM AND SHOULD NOT BE CITED. A DETAILED KEY WILL BE PRESENTED IN A FUTURE STUDY.

KEY:

1a. Conidiomata black.....2

1b. Conidiomata orange.....3

2a. Conidiomata pyriform, slender necks with orange apices; teleomorph unknown.....
.....Fungus on *M. theaezans* and *Tibouchina* spp., Colombia **(Clade 4)**

2b. Conidiomata pyriform with attenuated, uniformly black necks; ascomata have black necks.....*C. cubensis* **(Clade 1)**

2c. Conidiomata superficial, ovoid without a neck; ascomata with orange perithecial necks.....Fungi on *Syzygium* spp. Indonesia, South Africa **(Clade 5)**

3a. Stromata superficial.....4

3b. Stromata semi-immersed.....6

4a. Conidiomata rostrate, long slender necks; ascomata semi-immersed, no stromatic tissue except sheath around perithecial necks.....
.....Fungus on *Terminalia ivorensis*, Ecuador **(Clade 2)**

4b. Stromata rounded.....5

5a. Large, tubercular, multilocular conidiomata; aseptate ascospores.....

....*Endothia* **(Clade 9)**

5b. Large, conical conidiomata; one to three septated ascospores.....

.....Fungus on *Elaeocarpus* sp., New Zealand **(Clade 10)**

5c. Small, pulvinate stroma, one septated ascospores.....

.....*C. havanensis*-type fungi **(Clade 7)**

6a. Large, erumpent stromata, perithecial necks emerging from stromatal surface papillate, one septate ascospores..... .....*Cryphonectria* **(Clade 8)**

6b. Small stromata, aseptated ascospores.....*C. eucalypti* **(Clade 3)**

6c. Small, orange-brown stromata, perithecial necks emerging from stromatal surface long, one-septated ascospores.....

.....Fungus on *Tibouchina* spp., New Zealand **(Clade 6)**

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Table 1. Taxa included in the phylogenetic analyses. Taxa in bold represent fungal isolates sequenced in the present study. Taxa names indicated as "Undescribed" represent new fungal species awaiting description.

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 11286	CRY 1471	*C. cubensis*	*Eucalyptus* sp.	Ecuador	M.J. Wingfield	AY 214289, AY 214217, AY 214253	AY 194096
CMW 11287	CRY 1473	*C. cubensis*	*Eucalyptus* sp.	Ecuador	M.J. Wingfield	AY 214290, AY 214218, AY214254	AY 194095
CMW 8757	CRY 268	*C. cubensis*	*Eucalyptus* sp.	Venezuela	M.J Wingfield	AF 046897, AF 273069, AF 273464	-
CMW 8758	CRY 243	*C. cubensis*	*Eucalyptus* sp.	Venezuela	M.J Wingfield	AF 046898, AF 273068, AF 273463	AY 194098
CMW 1853	CRY 138	*C. cubensis*	*Syzygium aromaticum*	Brazil	unknown	AF 036891, AF 273070, AF 273465	-
CMW 10667	-	*C. cubensis*	*Eucalyptus* sp.	Republic of Congo	M.J. Wingfield	AY 063477, AY 063479, AY 063481	-
CMW 10668	-	*C. cubensis*	*Eucalyptus* sp.	Republic of Congo	M.J Wingfield	AF 535121, AF 535123, AF 535125	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 10669	-	*C. cubensis*	*Eucalyptus* sp.	Republic of Congo	M.J Wingfield	AF 535122, AF 535124, AF 535126	-
CMW 10453	CRY 1533, E40, CBS 505.63	*C. havanensis* [c]	*E. saligna*	Demographic Republic of Congo	R.J. Stipes	AY 063476, AY 063478, AY 063480	-
CMW 9970	CRY 2357	*C. cubensis*	*Miconia rubiginosa*	Colombia	C. Rodas M.J. Wingfield	AY 214291, AY 214219, AY 214255	-
CMW 9996	CRY 2404	*C. cubensis*	*M. rubiginosa*	Colombia	C. Rodas M.J. Wingfield	AY 214292, AY 214220, AY 214256	-
CMW 10025	-	*C. cubensis*	*M. rubiginosa*	Colombia	C. Rodas M.J. Wingfield	AY 214293, AY 214221, AY 214257	-
CMW 10026	-	*C. cubensis*	*M. rubiginosa*	Colombia	C. Rodas M.J. Wingfield	AY 214294, AY 214222, AY 214258	-
CMW 10028	-	*C. cubensis*	*M. rubiginosa*	Colombia	C. Rodas M.J. Wingfield	AY 214295, AY 214223, AY 214259	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 10775	CRY 498	*Endothia eugeniae*	*Syzygium aromaticum*	Brazil	C.S. Hodges	AY 084003, AY 084015, AY 084027	-
CMW 10776	CRY 499	*E. eugeniae*	*S. aromaticum*	Brazil	C.S. Hodges	AY 084004, AY 084016, AY 084028	-
CMW 10777	CRY 500	*E. eugeniae*	*S. aromaticum*	Brazil	C.S. Hodges	AY 084005, AY 084017, AY 084029	-
CMW 10778	CRY 501	*E. eugeniae*	*S. aromaticum*	Brazil	C.S. Hodges	AY 084006, AY 084018, AY 084030	-
CMW 9979	-	*C. cubensis*	*Miconia theaezans*	Colombia	M.J. Wingfield	AY 214296, AY 214224, AY 214260	-
CMW 9980	-	*C. cubensis*	*M. theaezans*	Colombia	M.J. Wingfield	AY 214297, AY 214225, AY 214261	-
CMW 9993	-	*C. cubensis*	*M. theaezans*	Colombia	M.J. Wingfield	AY 214298, AY 214226, AY 214262	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 9927	CRY 368	*C. cubensis*	*Tibouchina urvilleana*	Colombia	C. Rodas, M.J. Wingfield	AF 265653, AF 292034, AF 292037	-
CMW 9928	CRY 371	*C. cubensis*	*T. urvilleana*	Colombia	C. Rodas, M.J. Wingfield	AF 265654, AF 292035, AF 292038	-
CMW 9929	CRY 378	*C. cubensis*	*T. urvilleana*	Colombia	C. Rodas M.J. Wingfield	AF 265656, AF 292036, AF 292039	-
CMW 9932	CRY 675	*C. cubensis*	*T. granulosa*	South Africa	M.J. Wingfield	AF 273472, AF 273062, AF 273457	-
CMW 9327	CRY 782	*C. cubensis*	*T. granulosa*	South Africa	M.J. Wingfield	AF 273473, AF 273060, AF 273455	-
CMW 9328	CRY 783	*C. cubensis*	*T. granulosa*	South Africa	M.J. Wingfield	AF 273474, AF 273061, AF 273456	-
CMW 62	CRY 98	*C. cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 292041, AF 273063, AF 273458	AY 194097

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 2113	CRY 0140	*C. cubensis*	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462	-
CMW 8755	CRY 144	*C. cubensis*	*E. grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273458	-
CMW 10192	-	*C. cubensis*	*Syzygium cordatum*	South Africa	M. Gryzenhout	AY 214299, AY 214227, AY 214263	-
CMW 8317	CRY 2089	*C. cubensis*	*E. camaldulensis*	Vietnam	M.J. Wingfield	AY 214300, AY 214228, AY 214264	-
CMW 8318	CRY 2090	*C. cubensis*	*E. camaldulensis*	Vietnam	M.J. Wingfield	AY 214301, AY 214229, AY 214265	-
CMW 1856	-	*C. cubensis*	*Eucalyptus* sp.	Hawaii	unknown	AY 083999, AY 084010, AY 084022	-
CMW 8756	CRY 289	*C. cubensis*	*E. grandis*	Indonesia	M.J. Wingfield	AF 046896, AF 273077, AF 375606	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 9903	CRY 555	*C. cubensis*	*Eucalyptus* sp.	Kalimantan	M.J. Wingfield	AF 292044, AF 273066, AF 273461	-
CMW 2632	-	*C. cubensis*	*E. marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607	-
CMW 3839	-	*E. eugeniae*	*Syzygium aromaticum*	Indonesia	M.J. Wingfield	AF 046904, AY 084011, AY 084023	-
CMW 8649		*E. eugeniae*	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084000, AY 084012, AY 084025	-
CMW 8650	-	*E. eugeniae*	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084001, AY 084013, AY 084024	-
CMW 8651	-	*E. eugeniae*	S. aromaticum	Sulawesi, Indonesia	M.J. Wingfield	AY 084002, AY 084014, AY 084026	
CMW 10774	CRY 497	*E. eugeniae*	S. aromaticum	Zanzibar, Tanzania	n.a	AF 492130, AF 492131, AF 492132	-
CMW 11288		*C. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214302, AY 214230, AY 214266	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 11289	-	*C. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214303, AY 214231, AY 214267	-
CMW 11290	-	*C. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214304, AY 214232, AY 2143268	-
CMW 11291	-	*C. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214305, AY 214233, AY 2143269	-
CMW 11292	-	*C. cubensis*	*Eucalyptus* sp.	Vietnam	M.J. Wingfield	AY 214306, AY 214234, AY2143270	-
CMW 11293	-	*C. cubensis*	*Eucalyptus* sp.	Vietnam	M.J. Wingfield	AY 214307, AY 214235, AY 2143271	-
CMW 1840	CRY 0127	*C. cubensis*	*Eucalyptus camaldulensis*	China	unknown	AF 046890, AF 273071, AF 273466	-
CMW 9971	CRY 2345	Undescribed	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167425, AY 167430, AY 167435	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 9972	CRY 2346	Undescribed	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167426, AY 167431, AY 167436	AY 194092
CMW 9973	CRY 2348	Undescribed	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167427, AY 167432, AY 167437	-
CMW 10796	CRY 2353	Undescribed	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438	-
CMW 9975	CRY 2355	Undescribed	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167429, AY 167434, AY 167439	-
CMW 10782	CRY 778	*Cryphonectria clavata*	*Quercus mongolica*	Japan	M. Kusunoki	AF 140242, AF 140248, AF 140254	-
CMW 10783	CRY 780	*C. clavata*	*Q. mongolica*	Japan	M. Kusunoki	AF 140244, AF 140250, AF 140256	-
CMW 10784	CRY 781	*C. clavata*	*Q. mongolica*	Japan	M. Kusunoki	AF 140245, AF 140249, AF 140257	-

Table 1. (continued)

Isolate numbers[a]	Additional numbers[b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 10785	CRY 1444	*C. clavata*	*Quercus* sp.	China	M. Milgroom and S. Kaneko	AF 140246, AF 140252, AF 140258	-
CMW 10786	CRY 1447	*C. clavata*	*Quercus* sp.	Japan	M. Milgroom and S. Kaneko	AF 140247, AF 140251, AF 140259	AY 194099
CMW 11294	E57	*C. clavata*	*Q. mongolica*	Japan	T. Kobayashi and S. Kaneko	AY 214211, AY 214213, AY 214215	-
CMW 10791	CRY 2789, E83	*C. radicalis*	*Q. suber*	Italy	M. Orsenigo	AF 548750, AF 548746, AF 548742	-
CMW 10455	CRY 1535, E42	*C. radicalis*	*Q. suber*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712	AY 194101
CMW 10477	CRY 1557, E76	*C. radicalis*	*Q. suber*	Italy	A. Biraghi	AF 368328, AF 368347, AF 368347	AY 194102
CMW 10788	CRY 809	*C. radicalis*	*Quercus* sp.	Greece	P. Cortesi	AY 143075, AY 143077, AY 143079	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 10789	CRY 810	*C. radicalis*	*Quercus* sp.	Greece	P. Cortesi	AY 143076, AY 143078, AY 143080	-
CMW 10436	CRY 1516, E14	*Endothiella gyrosa* [d]	*Q. suber*	Portugal	B. d'Oliveira	AF 452117, AF 525703, AF 525710	-
CMW 10484	CRY 1564, E83	*C. radicalis*	*Q. suber*	Italy	A. Biraghi	AF 368327, AF 368349, AF 368349	-
CMW 10792	CRY 2790	*C. radicalis*	*C. sativa*	Switzerland	U. Heiniger	AF 548751, AF 548747, AF 548743	-
CMW 10793	CRY 2791	*C. radicalis*	*C. sativa*	Switzerland	U. Heiniger	AF 548752, AF 548748, AF 548744	-
CMW 10794	CRY 2792	*C. radicalis*	*C. sativa*	Switzerland	U. Heiniger	AF 548753, AF 548749, AF 548745	-
CMW 10790	CRY 779	*C. parasitica*	*Q. serrata*	Japan	M. Kusunoki	AF 140243, AF 140253, AF 140255	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 7047	CRY 1507, E5	*C. parasitica*	*Q. virginiana*	USA	R.J. Stipes	AF 292042, AF 273073, AF 273469	-
CMW 7048	CRY 1511, E9	*C. parasitica*	*Q. virginiana*	USA	R.J. Stipes	AF 292043, AF 273076, AF 273470	AY 194100
CMW 1651	CRY 66	*C. parasitica*	*Q. virginiana*	USA	-	AF 046902, AF 273075, AF 273468	-
CMW 1652	CRY 44	*C. parasitica*	*Castanea dentata*	USA	-	AF 046901, AF 273074, AF 273467	-
CMW 10518	CRY 1669, E53	*C. nitschkei*	*Quercus* sp.	Japan	T. Kobayashi	AF 452118, AF 525706, AF 525713	-
CMW 10463	CRY 1543, E54	*C. macrospora*	*Castanopsis cupsidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350	-
CMW 7036	CRY 62	*C. eucalypti*	*E. delegatensis*	Australia	M.J. Wingfield	AF 232878, AF 368341, AF 368340	AY 194105

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 7037	CRY 45	*C. eucalypti*	*Eucalyptus* sp.	South Africa	M.J. Wingfield	AF 232880, AF 368343, AF 368342	AY 194106
CMW 10010	CRY 2401	*Endothiella* sp.	*E. fastigata*	New Zealand		AY 214308, AY 214236, AY 214272	AY 194112
CMW 10011	CRY 2402	*Endothiella* sp.	*Eucalyptus* sp.	New Zealand		AY 214309, AY 214237, AY 214273	AY 194113
CMW 10797	CRY 2399	*Endothiella* sp.	*E. regnans*	New Zealand		AY 214310. AY 214238. AY 214274	AY 1941011
CMW 10030	-	Undescribed	*Miconia theaezans*	Colombia	C. Rodas M.J. Wingfield	AY 214311, AY 214239, AY 214275	AY 194103
CMW 10032	-	Undescribed	*M. theaezans*	Colombia	C. Rodas M.J. Wingfield	AY 214312, AY 214240, AY 214276	AY 194104
CMW 10035	-	Undescribed	*M. theaezans*	Colombia	C. Rodas M.J. Wingfield	AY 214313, AY 214241, AY 214277	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 11295	-	Undescribed	*M. theaezans*	Colombia	C. Rodas and M.J. Wingfield	AY 214314, AY 214242, AY 214278	AY 194089
CMW 11296	-	Undescribed	*M. theaezans*	Colombia	C. Rodas and M.J. Wingfield	AY 214315, AY 214243, AY 214279	AY 194090
CMW 10779	CRY 543	*Cryphonectria* sp.	*Eugenia aromatica*	Indonesia	M.J. Wingfield	AY 084007, AY 084019, AY 084031	-
CMW 10780	CRY 544	*Cryphonectria* sp.	*E. aromatica*	Indonesia	M.J. Wingfield	AY 084008, AY 084020, AY 084032	-
CMW 10781	CRY 554	*Cryphonectria* sp.	*E. aromatica*	Indonesia	M.J. Wingfield	AY 084009, AY 084021, AY 084033	AY 194093
CMW 9978	-	*Cryphonectria* sp.	*Syzygium cordatum*	South Africa	M. Gryzenhout	AY 214316, AY 214244, AY 214280	AY 194094
CMW 9945	-	Undescribed	*Tibouchina urvilleana*	New Zealand	M.J. Wingfield	AY 214317, AY 214245, AY 214281	AY 194109

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 9946	-	Undescribed	*T. urvilleana*	New Zealand	M.J. Wingfield	AY 214318, AY 214246, AY 214282	AY 194110
CMW 11297	CRY 303	*Cryphonectria havanensis*	*Eucalyptus sp.*	Mexico	n.a	AY 214319, AY 214247, AY 214283	-
CMW 11298	CRY 514	*C. havanensis*	*Eucalyptus sp.*	Mexico	C.S. Hodges	AY 214320, AY 214248, AY 214284	AY 194091
CMW 11299	-	Undescribed	*Myrica faya*	Madeira	C.S. Hodges	AY 214321, AY 214249, AY 214285	AY 194087
CMW 11300	-	Undescribed	*M. faya*	Madeira	C.S. Hodges	AY 214322, AY 214250, AY 214286	AY 194088
CMW 11301	CRY 490	Undescribed	*M. faya*	Açores	C.S. Hodges	AY 214323, AY 214251, AY 214287	-
CMW 11302	CRY 491	Undescribed	*M. faya*	Açores	C.S. Hodges	AY 214324, AY 214252, AY 214288	-

Table 1. (continued)

Isolate numbers [a]	Additional numbers [b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 2091	CRY 1515, E13	*E. gyrosa*	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336	AY 194114
CMW 10442	CRY 1522, E27	*E. gyrosa*	*Q. palustris*	USA	G.J. Samuels	AF 368326, AF 368339, AF 368338	AY 194115
CMW 10465	CRY 1545, E58	*E. singularis*	unknown	USA	R.J. Stipes	AF 368323, AF 368333, AF 368332	-
CMW 10469	CRY 1549, E67	*C. radicalis*	Spragg's bush	New Zealand	G.J. Samuels	AF 452111, AF 525707, AF 525714	AY 194107
CMW 10470	CRY 1550, E68	*C. radicalis*	Spragg's bush	New Zealand	G.J. Samuels	AF 452112, AF 525708, AF 525715	AY 194108
CMW 10471	CRY 1551, E70	*C. gyrosa*	*Elaeocarpus dentatus*	New Zealand	G.J. Samuels	AF 452116, AF 525709, AF 525716	-
CMW 5288	-	*Diaporthe ambigua*	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821	-

Table 1. (continued)

Isolate numbers[a]	Additional numbers[b]	Original label name of taxon	Host	Origin	Collector	GenBank Accession numbers	
						ITS, β-tubulin	LSU
CMW 5587	-	*D. ambigua*	*M. domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF543822	-

[a] Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

[b] Additional numbers that is linked to the CMW isolates. These isolates are also maintained in the culture collection of FABI.

CRY = *Cryphonectria* culture collection.

E = Culture numbers of isolates previously maintained in the culture collection of Prof. R.J. Stipes.

CBS = Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

[c] "*C. havanensis*" = *C. cubensis*

[d] "*Endothiella gyrosa*" = *C. radicalis*

Table 2. Herbarium specimens studied.

Linked to phylogenetic clade (Fig. 1)	Herbarium number [a]	Linked culture number	Current name of taxon	Original host name on label	Origin	Collector	Date
1	BPI 631857 (type)	n.a.	*Cryphonectria cubensis*	*Eucalyptus botryoides* Sm.	Cuba	S.C. Bruner	1916
1	PREM 57297	n.a.	*C. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	2001
1	PREM 57294	n.a.	*C. cubensis*	*E. grandis*	Colombia	M.J. Wingfield	2000
1	PREM 57293	n.a.	*C. cubensis*	*E. grandis*	South Africa	M. Venter	2001
1	PREM 57518	CMW 11286 CMW 11287	*C. cubensis*	*Eucalyptus* sp.	Ecuador	M.J. Wingfield	2001
1	PREM 56914	CMW 9927 CMW 9928	*C. cubensis*	*Tibouchina urvilleana*	Colombia	M.J. Wingfield	1999
2	PREM 57519	CMW 9972 CMW 10796	Undescribed	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	2001
2	NYBG 4340 (type)		*C. longirostris*	Fallen tree	Puerto Rico	A. Heller	1900
3	PREM 56211	n.a.	*C. eucalypti* (type)	*Eucalyptus grandis* X *camaldulensis*	South Africa	M. Venter	1998

Table 2. (continued)

Linked to phylogenetic clade (Fig. 1)	Herbarium number [a]	Linked culture number	Current name of taxon	Original host name on label	Origin	Collector	Date
4	PREM 57520	CMW 10030 CMW 10032	Undescribed	*Miconia theaezans*	Colombia	C.A. Rodas	1998
5	PREM 57473	CMW 10781	Undescribed	Inoculation of isolate CMW 10781 into *E. grandis*	n.a.	M. Gryzenhout	2001
5	PREM 57521	CMW 9978	Undescribed	*Syzygium cordatum*	South Africa	M. Gryzenhout & R. Heath	2002
6	PREM 57522	CMW 9946	Undescribed	*T. urvilleana*	New Zealand	M.J. Wingfield	2002
7	PREM 57523	CMW 11298	*C. havanensis*	*E. saligna*	Mexico	C.S. Hodges	1998
7	PREM 57524	CMW 11299 CMW 11300	Undescribed	*Myrica faya*	Madeira	C.S. Hodges	2000
7	PREM 57525	CMW 11301 CMW 11302	Undescribed	*M. faya*	Azores	C.S. Hodges	unknown
8	K 109807	n.a.	*C. gyrosa* (type)	Bark	Sri Lanka	n.a.	1868
8	TFM 1057	n.a.	*C. macrospora* (type)	*Shiia sieboldii* Makino	Japan	T. Kobayashi	1954

Table 2. (continued)

Linked to phylogenetic clade (Fig. 1)	Herbarium number [a]	Linked culture number	Current name of taxon	Original host name on label	Origin	Collector	Date
8	TFM 1045	n.a.	*C. nitschkei* (type)	*Quercus grosseserrata* Bl.	Japan	T. Kobayashi	1954
8	CUP 2926	n.a.	*C. parasitica*	*Castanea dentata*	New York, USA	W.A. Murrill	1907
8	CUP 47983	n.a.	*C. parasitica*	*Castanea dentata*	Md., USA	D.S. Welch	1938
8	TFM 652	n.a.	*C. radicalis*	*Carpinus carpinoides*	Japan	T. Kobayashi	1962
8	BPI 797693	n.a.	*C. radicalis*	*Castanea* sp	Italy	Denotaris	1862
8	FPH 7609	n.a.	*C. clavata*	*Castanea crenata*	Japan	M. Milgroom	1998
9	PREM 56218	n.a.	*E. gyrosa*	*Q. phellos* L.	Raleigh, USA	L. Grand	1997
9	BPI 614515	n.a.	*E. singularis* (type)	*Q. gambelli*	Colorado, USA	E. Bethel	1911
10	PDD 32619	CMW 10471	Undescribed	*Elaeocarpus dentatus*	Auckland, New Zealand	G.J. Samuels	1973

Table 2. (continued)

Linked to phylogenetic clade (Fig. 1)	Herbarium number [a]	Linked culture number	Current name of taxon	Original host name on label	Origin	Collector	Date
10	PDD 20056	n.a.	Undescribed	*Elaeocarpus hookerianus* Raoul	Southland, New Zealand	J.M. Dingley	1948
10	PDD 21944	n.a.	Undescribed	*Elaeocarpus dentatus*	Auckland, New Zealand	J.M. Dingley	1963
10	NYBG 31874	CMW 10469 CMW 10470	Undescribed	Dead tree	Auckland, New Zealand	R.E. Beever	1973

[a] **BPI** = U.S. National Fungus Collections, Systematic Botany and Mycology, Rm. 304, Bldg. 011A, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA;

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

TFM = Forestry and Forest Products Research Institute, P. O. Box 16, Tsukuba Norin Kenkyu, Danchi-Nai, Ibaraki, 305 Japan;

CUP = Plant Pathology Herbarium, Cornell University, 334 Plant Science Building, Ithaca, New York 14853-4203 USA; PDD, Landcare Research New Zealand Limited, Private Bag 92 170, 120 Mt. Albert Road, Mt. Albert, Auckland, New Zealand;

DAR = Plant Pathology Herbarium, Orange Agricultural Institute, Forest Road, Orange, N. S. W. 2800, Australia;

DAOM = National Mycological Herbarium, Eastern Cereal and Oilseed Center (ECORC), Agriculture and Agri-Food Canada, Edifice Wm. Saunders Building. #49, Ottawa, Ontario, Canada, KlA 0C6.

CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

Table 3. List of taxa included in Zhang and Blackwell (2001).

Taxon	Host	Collector	Culture no.	GenBank
Ampiporthe castanea (Tulasne) Barr	*Castanea sativa*		CBS 392.93	AF 277128
Apiognomonia supraseptata Kaneko et Kobayashi	*Quercus glauca*		ATCC 58737	AF 277127
Apioplagiostoma aceriferum (Cooke) Petr.	*Acer campestre*		CBS 781.79	AF 277129
Apiosporopsis carpinea (Fr.) Sacc.	*Carpinus hetulus*		CBS 771.79	AF 277130
Cryphonectria parasitica (Murr.) Barr	*Castanea sp.*		S. Anagnostakis 713	AF 277132
Cryptodiaporthe corni (Wehmeyer) Perr.	*Cornus alternifolia*		ATCC 66834	AF 277133
Discula campestris (Pass.) Arx	*Acer sp.*	S. Anagnostakis	S. Anagnostakis	AF 277140
Diaporthe phaseolorum	n.a.	n.a.	n.a.	U47830
Discula destructiva Redlin 254 (type)	*Cornus florida*	S. Redlin	S. Redlin	AF 277137
Discula fraxinae Peck	*Fraxinus sp.*	S. Anagnostakis	S. Anagnostakis	AF 277138
Discula quercina (Cooke) Sacc.	*Fraxinus sp.*	A. Rossman	A. Rossman	--
Discula sp.326	*Quercus sp.*	S. Anagnostakis	S. Anagnostakis	AF 277139
Endothia eugeniae (Nutman and Roberts) J. Reid and C. Booth	*Syzygium aromaticum*		CBS 534.82	AF 277142
Gnomonia padicola (Libert) Klebahn	*Prunus padus*		CBS 845.79	AF 277134
Gnomonia setaceae (Pers. Ex Fr.) Ces and de Not.	*Castanea sativa*		CBS 863.79	AF 277135
Linospora caprae (DC.) Fuckel	*Salix caprea*		CBS 372.69	AF 277143
Melanconis marginalis (Peck) Wehmeyer	*Alnus tenuifolia*		ATCC 56907	AF 277144
Plagiostoma euphorbiae Fuckel	*Euphorbia palustris*		CBS 340.78	AF 277131
Pleuroceras pleurostylum (Auerswald) Barr	*Salix Helvetica*		CBS 906.79	AF 277145
Valsa ambiens ssp. *Leucostomoides* (Peck) Spielman	*Acer rubrum*		ATCC 52280	AF 277146

ATCC = American Type Culture Collection, P.O. Box 1549, Manassas, VA 20108, USA.

CBS = Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Table 4. List of taxa included in Castlebury et al. (2002).

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	GenBank LSU
Apiognomnia errabunda (Roberge) Höhn [anamorph *Discula umbrinella* (Berk. and Broome) M. Morelet]	Switzerland	*Fagus sylvatica*	M. Monod	--	AR 2813 (= CBS 109747)	AF 408334
Chromendothia citrina Lar. N. Vassiljeva	Russia	*Quercus mongolica* Fisch. ex Ledeb.	L. Vasilyeva	BPI 747935	AR 3446 (= CBS 109758)	AF 408335
Coniella australiensis Petr.	South Africa	Leaf litter	K.T. van Warmelo	BPI 748425	IMI 261318	AF 408336
***Coniella fragariae* (Oudem.) B. Sutton**	India	Soil	V.V. Bhatt	BPI 841767	IMI 081599	AF 408391
Coniella fragariae	USA: Minnesota	*Lythrum salicaria*	n.a.	BPI 747949	AR 3382 (= ATCC PTA-275)	AF 362553
Coniella musaiensis B. Sutton var. hibisci B. Sutton	?Africa	*Hibiscus* sp.	R.R. Cervantes	BPI 748426	AR 3534 (= CBS 109757)	AF 408337
Cryphonectria cubensis (Bruner) Hodges	Cameroon	*Eucalyptus urophylla* S.T. Blake	I.A.S Gibson	BPI 841768	CBS 101281	AF 408338
Cryphonectria havanensis (Bruner) M.E. Barr	Zaire	*Eucalyptus saligna* Sm.	Unknown	BPI 748427	CBS 505.63	AF 408339
Cryphonectria macrospora (Tak. Kobay. & Kaz. Itô) M.E. Barr	Russia	*Quercus mongolica*	L. Vasilyeva	BPI 748428	AR 3444 (= CBS 109764)	AF 408340

Table 4. (continued)

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	GenBank LSU
Cryphonectria nitschkei (G.H. Otto) M.E. Barr	Russia	*Quercus mongolica*	L. Vasilyeva	BPI 748429	AR 3433 (= CBS 109776)	AF 408341
Cryptodiaporthe aesculi (Fuckel) Petr.	Austria	*Aesculus hippocastanum*	W. Jaklitsch	BPI 748430	AR 3580 ex WJ 1695 (= CBS 109765)	AF 408342
Cryptodiaporthe corni (Wehm.) Petr.	USA: Maine	*Cornus alternifolia* L.f.	S. Redlin	BPI 747916	AR 2814 (= CBS 245.90)	AF 408343
Cryptodiaporthe hystrix (Tode) Petr.	Austria	*Acer pseudoplantanus*	W. Jaklitsch	BPI 748431	AR 3565 ex WJ 1491 (= CBS 109759)	AF 408344
Cryptodiaporthe salicella (Fr.) Petr.	Austria	*Salix.* sp	W. Jaklitsch	BPI 747938	AR 3455 ex WJ 1463 (= CBS 109775)	AF 408345
***Cryptosporella hypodermia* (Fr.) Sacc.**	Austria	*Ulmus minor* Mill.	W. Jaklitsch	BPI 748432	AR 3552 ex WJ 1694	AF 408346
Cryptosporella hypodermia	Austria	*Ulmus minor/laevis* Pall.	W. Jaklitsch	BPI 748433	AR 3566 ex WJ 1497 (= CBS 109753)	AF 408347
Diaporthe arctii	USA: New Jersey	*Ambrosia trifida*	n.a.	BPI 747273	AR 3450	AF 362562
Diaporthe decendens (Fr.) Fuckel	Austria	*Corylus avellana* L.	W. Jaklitsch	BPI 747942	AR 3459 ex WJ 1473 (= CBS 109772)	AF 408348
Diaporthe detrusa (Fr.) Fuckel	Austria	*Berberis vulgaris* L.	W. Jaklitsch	BPI 748434	AR 3424 ex WJ 1445 (= CBS 109770)	AF 408349

Table 4. (continued)

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	GenBank LSU
***Diaporthe eres* Nitschke**	Austria	*Acer campestre* L.	W. Jaklitsch	BPI 748435	AR 3538 ex WJ 1643 (= CBS 109767)	AF 408350
Diaporthe eres	Austria	*Corylus avellana*	n.a.	BPI 747936	AR 3519	AF 362565
Diaporthe fibrosa (Pers.:Fr) Nitschke	Austria	*Rhamus catharticus* L.	W. Jaklitsch	BPI 747929	AR 3425 ex WJ 1417 (= CBS 109751)	AF 408351
Diaporthe medusae Nitschke	Austria	*Laburnum anapyroides*	W. Jaklitsch	BPI 748231	AR 3422 ex WJ 1443 (= CBS 109492)	AF 3408352
Diaporthe oncostoma (Duby) Fuckel	Russia	*Robinia pseudoacacia* L.	L. Vasilyeva	BPI 747934	AR 3445 (= CBS 109741)	AF 408353
Diaporthe padi G. H. Otto	Austria	*Prunus padus* L.	W. Jaklitsch	BPI 748436	AR 3419 ex WJ 1458 (= CBS 109784)	AF 408354
Diaporthe pardalota (Mont.) Fuckel	Canada: British Columbia	*Epilobium augustifolium* L.	M. Barr	BPI 747946	AR 3478 ex MBB 10220 (= CBS 109768)	AF 408355
Diaporthe perjuncta Niessl	Austria	*Ulmus glabra* Huds.	W. Jaklitsch	BPI 748437	AR 3461 ex WJ 1480 (= CBS 109745)	AF 408356
Diaporthe pustulata (Desm,) Sacc.	Austria	*Acer pseudoplatanus*	W. Jaklitsch	BPI 747928	AR 3430 ex WJ 1428 (= CBS 109742)	AF 408357
Diaporthe pustulata	Austria	*Acer pseudoplatanus*	W. Jaklitsch	BPI 748438	AR 3535 ex WJ 1628 (= CBS 109760)	AF 408358
Discula destructiva	USA: Maryland	*Cornus florida*	n.a.	n.a.	ATCC 76230	AF 362568

Table 4. (continued)

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	GenBank LSU
Discula destructiva Redlin	USA: Washington	*Cornus nuttallii* Audubon	M. Daughtrey	BPI 1107757	AR 2596 (= CBS 109771)	AF 408359
***Ditopella ditopa* (Fr.:Fr.) J. Schröt.**	Austria	*Acer glutinosa* (L.) Gaertn.	W. Jaklitsch	BPI 748439	AR 3423 ex WJ 1443 (= CBS 109748)	AF 408360
Endothiella gyrosa	USA: Maryland	*Quercus sp.*	n.a.	n.a.	AR 3396	AF 362555
Gnomoniella fraxinae (anamorph: *Discula fraxinea*)	USA: Maryland	*Fraxinus pennsylvanica*	n.a.	n.a.	AR 2789	AF 362552
***Gnomonia gnomon* (Tode : Fr.) J. Schröt.**	Italy	*Carylus avellana*	M. Ribaldi	--	CBS 199.53	AF 408361
Gnomonia leptostyla (Fr.:Fr.) Ces. and de Not. [anamorph *Marssonina juglandis* (Lib.) Magnus)	USA: Illinois	*Juglans nigra* L.	D. Neely	BPI 747976	FAU 543	AF 408362
Gnomonia setacea	USA: New Jersey	*Quercus prinus*	n.a.	BPI 747274	AR 3451	AF 362563
Greeneria uvicola	USA: Ohio	*Vitis sp.*	n.a.	n.a.	n.a.	AF 362670
***Harkenessia eucalypti* Cooke**	Australia	*Eucalyptus regnans* F. Muell.	Z-q. Yuan	--	CBS 342.97	AF 408363
Harkenessia lythri D.F. Farr & Rossman	USA: Minnesota	*Lythrum salicaria* L.	E. Katovich	BPI 747560	AR 3383 (=ATCC PTA-2756)	AF 408363

Table 4. (continued)

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	GenBank LSU
***Hercospora tiliae* (Pers.:Fr.) Fr.**	Austria	*Tilia tomentosa* Moench	W. Jaklitsch	BPI 748440	AR 3526 ex WJ 1600 (= CBS 109746)	AF 408365
Leucostoma auerswaldi Nitschke	Austria	*Frangula alnus* Mill.	W. Jaklitsch	BPI 748456	AR 3428 ex WJ 1424 (= CBS 109774	AF 408384
Leucostoma cincta (Fr.:Fr.) Höhn	Russia	*Padus maackii* Rupr.	L. Vasilyeva	BPI 748441	AR 3415 (= CBS 109766))	AF 408366
Leucostoma nivea (Hoffm.:Fr.) Höhn	Austria	*Salix pupurea* L.	W. Jaklitsch	BPI 748442	AR3512 ex WJ 1555 (= CBS 109743)	AF 408367
Leucostoma nivea	Russia:	*Populus* sp.	n.a.	BPI 748232	AR 3413	AF 362558
Mazzantia napelli (Ces.) Sacc.	Austria	*Aconitum vulparia* Rchb.	W. Jaklitsch	BPI 748443	AR 3498 ex WJ 1531 (= CBS 109769)	AF 408368
Melanconis alni Tul.	Austria	*Alnus viridis* (Vill.) Lam. & DC.	W. Jaklitsch	BPI 748444	AR 3500 ex WJ 1542 (= CBS 109773)	AF 408371
Melanconis alni	Russia: Sakhalin Island	*Duschekia maximowiczii*	n.a.	BPI 748233	AR 3529	AF 362566
Melanconis desmazierii Petr.	Austria	*Tilia* sp.	W. Jaklitsch	BPI 748445	AR 3525 ex WJ 1588 (= CBS 109780)	AF 408372
Melanconis marginalis (Peck) Wehm.	Canada: British Columbia	*Alnus rubra* Bong.	M. Barr	BPI 748446	AR 3442 ex MBB 1021A (= CBS 109744)	AF 408373

Table 4. (continued)

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	GenBank LSU
***Melanconis stilbostoma* (Fr.) Tul.**	Austria	*Betula pendula* Roth	W. Jaklitsch	BPI 748447	AR 3501 ex WJ 1543 (= CBS 109778)	AF 408374
Melanconis stilbostoma	Russia: Sakhalin Island	*Betula sp.*	n.a.	BPI 748234	AR 3548	AF 362567
Ophiovalsa betulae (Tul. and C. Tul.) Petr. anamorph *Discula betulina* (Sacc.) Höhn.)	Austria	*Betula pendula*	W. Jaklitsch	BPI 748448	AR 3524 ex WJ 1610 (= CBS 109763)	AF 408375
***Ophiovalsa suffusa* (Fr.) Petr.** [anamorph Disculina vulgaris (Fr.) B. Sutton]	Austria	*Alnus incana* (L.) Moench	W. Jaklitsch	BPI 748449	AR 3496 ex WJ 1556 (= CBS 109750)	AF 408376
***Phragmaporthe conformis* (Berk. and Broome) Petr.**	Canada: British Columbia	*Alnus rubra*	M. Barr	BPI 748450	AR 3632 ex MBB 10338 (= CBS 109783)	AF 408377
***Pilidiella castaneicola* (Ellis and Everh.) Arx**	Korea	unknown	K.S. Bae	BPI 748451	CBS 143.97	AF 408378
Pilidiella granati (Sacc.) Aa	Cyprus	*Punica granatum* L.	R.M. Natrass	BPI 748452	CBS 152.33	AF 408379
Pilidiella granati	Turkey	*Punica granatum*	N. Kaskalöglu	BPI 748453	CBS 814.71	AF 408380
Plagiostoma conradii (Ellis) M.E. Barr	USA: New Jersey	*Hudsonia tomentosa* Nutt.	G. Bills	BPI 746482	AR 3488 (= CBS 109761)	AF 408381
***Plagiostoma euphorbiae* (Fuckel) Fuckel**	Netherlands	*Euphorbia palustris* L.	Unknown	--	CBS 340.78	AF 408382

Table 4. (continued)

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	Genbank LSU
Shizoparme botrytidis Samuals	Puerto Rico	Dead wood	S. Huhndorf	BPI 748454	SMH 1354 (= AR 3504)	AF 408383
Shizoparme straminea	USA: Virginia	*Rosa rugosa*	n.a.	BPI 797000	CBS 149.22	AF 362569
Valsa ambiens	Austria	*Fagus sylvatica*	n.a.	BPI 748237	AR 3516	AF 362564
Valsa cenisia De Not.	Austria	*Juniperus communis* L.	W. Jaklitsch	BPI 748457	AR 3522 ex WJ 1583 (= CBS 109752)	AF 408385
Valsa ceratosperma (Tode: Fr.) Maire	Russia	*Quercus mongolica*	L. Vasilyeva	BPI 748458	AR 3416 (= CBS 109756)	AF 408386
Valsa ceratosperma	Austria	*Quercus robur* L.	W. Jaklitsch	BPI 748459	AR 3426 ex WJ 1425 (= CBS 109756)	AF 408387
Valsa germanica	Austria	*Salix alba*	n.a.	BPI 748236	AR 3427	AF 362561
Valsa mali	Russia: Primorsky Territory	*Malus sp.*	n.a.	BPI 748235	AR 3417	AF 362559
Valsella adherens Fuckel	Russia	*Betula* sp.	L. Vasilyeva	BPI 748460	AR 3549 (= CBS 109782)	AF 408388
***Valsella salicis* Fuckel**	Italy	*Salix fragilis* L.	W. Jaklitsch	BPI 748461	AR 3514 ex WJ 1580 (= CBS 109754)	AF 408389
Wuestneia molokaiensis Crous & J.D. Rogers	USA: Hawaii	*Eucalyptus robusta* Sm.	J. Rogers	BPI 748462	AR 3578 (= CBS 109779)	AF 408390

Table 4. (continued)

Taxon [a]	Country	Host	Collector	Specimen no.[b]	Culture no. [c]	Genbank LSU
[d]*Magnaporthe grisea* (T.T. Herbert) Yaegashi & Udugawa	n.a.	n.a.	n.a.	n.a.	n.a.	AB 026819
[d]*Pyricularia grisea*	USA: Pennsylvania	*Lolium perenne*	n.a.	n.a.	AR 3390	AF 362554
[d]*Gaeumannomyces graminis* (Sacc.) Arx & D. Oliver	United Kingdom	*Avena* sp.	n.a.	n.a.	AR 3400	AF 362556
[d]*Gaeumannomyces graminis*	USA: Georgia	*Glycine* sp.	n.a.	n.a.	AR 3401	AF 362557

[a] Type species of genus in bold

[b] **BPI** = U.S. National Fungus Collection.

[c] **AR** = Amy Rossman, Systematic Botany and Mycology Laboratory, USDA-ARS, 10300 Baltimore Ave., Beltsville, Maryland. USA 20705.

ATCC = American Type Culture Collection, P.O. Box 1549, Manassas, VA 20108, USA.

CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

FAU = Maintained by Amy Rossman (see address above).

IMI = International Mycological Institute, now CABI, Inc.

MBB = Margeret Barr Bigelow, Sidney, British Colombia.

SMH = Sabine M. Huhndorf, Field Museum, Chicago, IL.

WJ = Walter Jaklitsch, Van den langen Lüssen 31/2. A-1190, Vienna.

[d] Isolates included as outgroup taxa.

Fig. 1. A strict consensus tree (tree length = 3267 steps, CI = 0.51, RI = 0.9) generated from a combined data set comprising ribosomal (ITS 1, 5.8S, ITS 2) and β-tubulin gene sequences. Confidence levels of the tree branch nodes are indicated and were determined by a 1000 replicate bootstrap analysis. Taxa in bold represent the isolates that were sequenced in the present study. Species names in capital letters represent host species. *Diaporthe ambigua* isolates were used as the outgroup taxa to root the phylogenetic tree.

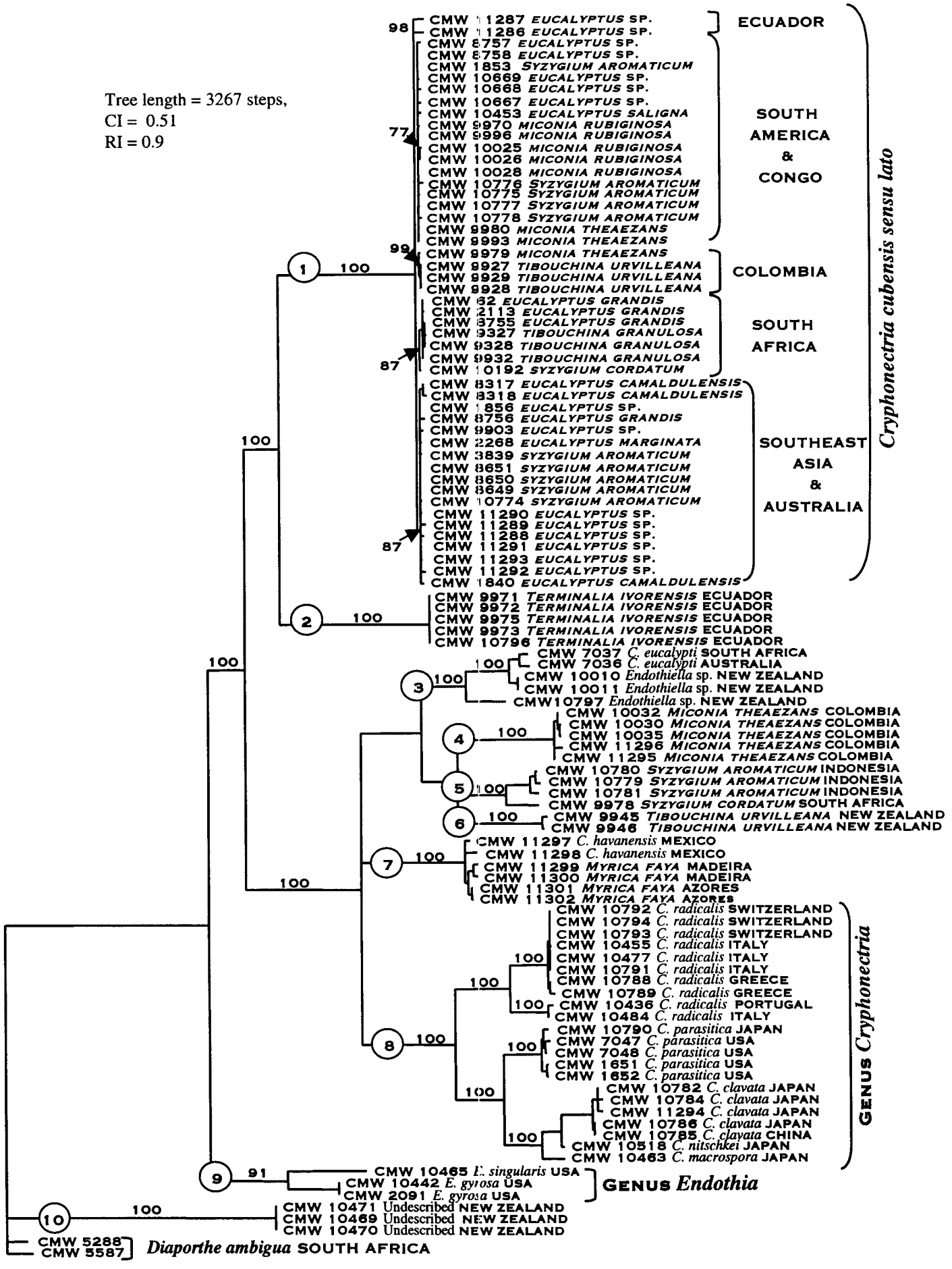
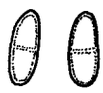
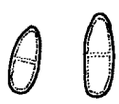

Tree length = 3267 steps,
CI = 0.51
RI = 0.9
CMW 11287 EUCALYPTUS SP.
CMW 11286 EUCALYPTUS SP.
CMW 8757 EUCALYPTUS SP.
CMW 8758 EUCALYPTUS SP.
CMW 1853 SYZYGIUM AROMATICUM
CMW 10669 EUCALYPTUS SP.
CMW 10668 EUCALYPTUS SP.
CMW 10667 EUCALYPTUS SP.
CMW 10453 EUCALYPTUS SALIGNA
CMW 9970 MICONIA RUBIGINOSA
CMW 9996 MICONIA RUBIGINOSA
CMW 10025 MICONIA RUBIGINOSA
CMW 10026 MICONIA RUBIGINOSA
CMW 10028 MICONIA RUBIGINOSA
CMW 10776 SYZYGIUM AROMATICUM
CMW 10775 SYZYGIUM AROMATICUM
CMW 10777 SYZYGIUM AROMATICUM
CMW 10778 SYZYGIUM AROMATICUM
CMW 9980 MICONIA THEAEZANS
CMW 9993 MICONIA THEAEZANS
CMW 9979 MICONIA THEAEZANS
CMW 9927 TIBOUCHINA URVILLEANA
CMW 9929 TIBOUCHINA URVILLEANA
CMW 9928 TIBOUCHINA URVILLEANA
CMW 62 EUCALYPTUS GRANDIS
CMW 2113 EUCALYPTUS GRANDIS
CMW 3755 EUCALYPTUS GRANDIS
CMW 9327 TIBOUCHINA GRANULOSA
CMW 9328 TIBOUCHINA GRANULOSA
CMW 9932 TIBOUCHINA GRANULOSA
CMW 10192 SYZYGIUM CORDATUM
CMW 8317 EUCALYPTUS CAMALDULENSIS
CMW 8318 EUCALYPTUS CAMALDULENSIS
CMW 1856 EUCALYPTUS SP.
CMW 8756 EUCALYPTUS GRANDIS
CMW 9903 EUCALYPTUS SP.
CMW 2268 EUCALYPTUS MARGINATA
CMW 3839 SYZYGIUM AROMATICUM
CMW 8651 SYZYGIUM AROMATICUM
CMW 8650 SYZYGIUM AROMATICUM
CMW 8649 SYZYGIUM AROMATICUM
CMW 10774 SYZYGIUM AROMATICUM
CMW 11290 EUCALYPTUS SP.
CMW 11289 EUCALYPTUS SP.
CMW 11288 EUCALYPTUS SP.
CMW 11291 EUCALYPTUS SP.
CMW 11293 EUCALYPTUS SP.
CMW 11292 EUCALYPTUS SP.
CMW 1840 EUCALYPTUS CAMALDULENSIS
ECUADOR
SOUTH AMERICA & CONGO
COLOMBIA
SOUTH AFRICA
SOUTHEAST ASIA & AUSTRALIA
Cryphonectria cubensis sensu lato
CMW 9971 TERMINALIA IVORENSIS ECUADOR
CMW 9972 TERMINALIA IVORENSIS ECUADOR
CMW 9975 TERMINALIA IVORENSIS ECUADOR
CMW 9973 TERMINALIA IVORENSIS ECUADOR
CMW 10796 TERMINALIA IVORENSIS ECUADOR
CMW 7037 C. eucalypti SOUTH AFRICA
CMW 7036 C. eucalypti AUSTRALIA
CMW 10010 Endothiella sp. NEW ZEALAND
CMW 10011 Endothiella sp. NEW ZEALAND
CMW10797 Endothiella sp. NEW ZEALAND
CMW 10032 MICONIA THEAEZANS COLOMBIA
CMW 10030 MICONIA THEAEZANS COLOMBIA
CMW 10035 MICONIA THEAEZANS COLOMBIA
CMW 11296 MICONIA THEAEZANS COLOMBIA
CMW 11295 MICONIA THEAEZANS COLOMBIA
CMW 10780 SYZYGIUM AROMATICUM INDONESIA
CMW 10779 SYZYGIUM AROMATICUM INDONESIA
CMW 10781 SYZYGIUM AROMATICUM INDONESIA
CMW 9978 SYZYGIUM CORDATUM SOUTH AFRICA
CMW 9945 TIBOUCHINA URVILLEANA NEW ZEALAND
CMW 9946 TIBOUCHINA URVILLEANA NEW ZEALAND
CMW 11297 C. havanensis MEXICO
CMW 11298 C. havanensis MEXICO
CMW 11299 MYRICA FAYA MADEIRA
CMW 11300 MYRICA FAYA MADEIRA
CMW 11301 MYRICA FAYA AZORES
CMW 11302 MYRICA FAYA AZORES
CMW 10792 C. radicalis SWITZERLAND
CMW 10794 C. radicalis SWITZERLAND
CMW 10793 C. radicalis SWITZERLAND
CMW 10455 C. radicalis ITALY
CMW 10477 C. radicalis ITALY
CMW 10791 C. radicalis ITALY
CMW 10788 C. radicalis GREECE
CMW 10789 C. radicalis GREECE
CMW 10436 C. radicalis PORTUGAL
CMW 10484 C. radicalis ITALY
CMW 10790 C. parasitica JAPAN
CMW 7047 C. parasitica USA
CMW 7048 C. parasitica USA
CMW 1651 C. parasitica USA
CMW 1652 C. parasitica USA
CMW 10782 C. clavata JAPAN
CMW 10784 C. clavata JAPAN
CMW 11294 C. clavata JAPAN
CMW 10786 C. clavata JAPAN
CMW 10785 C. clavata CHINA
CMW 10518 C. nitschkei JAPAN
CMW 10463 C. macrospora JAPAN
GENUS Cryphonectria
CMW 10465 E. singularis USA
CMW 10442 E. gyrosa USA
CMW 2091 E. gyrosa USA
GENUS Endothia
CMW 10471 Undescribed NEW ZEALAND
CMW 10469 Undescribed NEW ZEALAND
CMW 10470 Undescribed NEW ZEALAND
CMW 5288
CMW 5587
Diaporthe ambigua SOUTH AFRICA

Fig. 2. Schematic drawings of the conidiomata, ascomata, ascopores and conidia for the fungal groups represented by the different phylogenetic clades. **a**. *Cryphonectria cubensis sensu lato* (**clade 1**). **b.** The fungus on *Terminalia ivorensis* from Ecuador (**clade 2**). **c.** *Cryphonectria eucalypti* and other species (**clade 3**). **d.** The fungus from Colombia (**clade 4**). **e**. Fungal species from *Syzygium* spp. in Indonesia and South Africa (**clade 5**). **f.** Fungus from *Tibouchina urvilleana* in New Zealand (**clade 6**). **g.** Fungi from Mexico, Azores and Madeira (**clade 7**). **h.** *Cryphonectria* (**clade 8,** ascospores not representing those of *C. eucalypti*). **i.** *Endothia* (**clade 9**). **j.** Fungus from *Elaeocarpus* spp. in New Zealand (**clade 10**).

Clade	Conidioma	Ascoma	Ascospores	Conidia
a) *Cryphonectria cubensis* **Clade 1**				
b) Fungus on *Terminalia ivorensis* **Clade 2**				
c) *C. eucalypti* **Clade 3**				
d) Fungus from Colombia **Clade 4**		—	—	
e) Fungus on *Syzygium* spp. **Clade 5**				
f) Fungus on *Tibouchina* from New Zealand **Clade 6**	—			—
g) Fungi from Mexico, Açores and Madeira **Clade 7**				
h) *Cryphonectria* **Clade 8**				
i) ***Endothia*** **Clade 9**				
) Fungus on *Elaeocarpus* rom New Zealand **Clade 10**				

APPENDIXES

Appendix 1. Raw sequence data of the two regions within the β-tubulin gene (designated as β-tub 1a/1b and β-tub 2a/2b) and the ITS1, conserved 5.8S and ITS2 regions of the rDNA operon. The start of each region is indicated above the alignment. The exon regions of the β-tubulin gene as well as the conserved 5.8S region of the rDNA operon are indicated in red. Unknown sequence characters are indicated with a "**N**", while gaps inserted to achieve sequences alignment are indicated with "**-**". Bases matching those of **CMW 2113** are indicated with a "**.**".

```
[                 10        20        30        40        50        60        70        80        90]
[          β-tub 1a/1b →    .         .         .         .         .         .         .         .]
CMW 2113   TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACGGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG ATGGCTGCCT
CMW 8755   .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 8758   GA........ .......... ..A....... .......C.. .......... .......... .......... .......... ..........
CMW 8757   .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 1853   .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 1840   .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 8756   .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 2632   .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 1652   .......... .......... .......... .CC.A..C.. C......... .......... .......... .......... ..........
CMW 7047   .......... .......... .......... .CC....C.. C......... .......... .......... .......... ..........
CMW 7048   .......... .......... ..T....... .CC....C.. C......... .......... .......... .......... ..........
CMW 2498   .......... .......... .......... .C.....C.. T..T...C.C ........A. .......... .......... ..........

[                100        110        120        130        140        150        160        170        180]
[                 .         .         .         .         .         .         .         .         .]
CMW 2113   CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC CCCC--GCC- ---CCTCGC- -GCCTCGGGG CGCCTC---G
CMW 8755   .......... .......... .......... .......... .......... .......... .......... .......... A..A......
CMW 62     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758   .......... .......... .......... .......... .......... T......... .......... ........A. A..A......
CMW 8757   .......... .......... .......... .......... .......... T......... .......... ........A. A..A......
CMW 1853   .......... .......... .......... .......... .......... T......... .......... .......AA. A..A......
CMW 1840   .......... .......... .......... .......... .......... .......... .......... .......... A..A......
CMW 8756   .......... .......... .......... .......... .......... .......... .......... .......... A..A......
CMW 2632   .......... .......... .......... .......... .......... .......... .......... .......... N..A......
CMW 1652   .......... .......... ........A. ....T..... .........T TT.TT..T.T TTT......A A.T....AC. AA.G..TTG.
CMW 7047   .......... .......... ........A. ....T..... .........T TT.TT..T.T TTT......A G.T..A.ACA AA.G.TTCG.
CMW 7048   .......... .......... ........A. ....T..... .........T TT.TT..T.T TTT......A G.T....ACA AA.G..TTG.
CMW 2498   .......... .......... .......... ....T..... .......... ...TAA.T.C .........A .-.AAATAAA ATGG..GCGC

[                190        200        210        220        230        240        250        260        270]
[                 .         .         .         .         .         .         .         .         .]
CMW 2113   GCCG------ -AAGCTCGTC TGCTAACCCT CATCGTCC-- -AGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 8755   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758   .......... ......T... ..T....T.. T......... .......... .......... .......... T......... ........T.
CMW 8757   .......... ......T... .......T.. T......... .......... .......... .......... T......... ........T.
CMW 1853   .......... ......T... .......T.. T......... ....-..... .......... .......... T......... ........T.
CMW 1840   .......... ......T... .......T.. T......... .......... .......... .......... T......... ..........
CMW 8756   .......... ......T... .......T.. T......... .......... .......... .......... T......... ..........
CMW 2632   .......... ......T... .......T.. T......... .......... .......... .......... T......... ..........
CMW 1652   ..T.TTTGGC T..C.CT... .-T.CT.T.. TCC.C.T.TC T........T .......... .......A.. .......... ..........
CMW 7047   ..T.TTGGGC T..C.CT... .-T.CT.T.. TCC.C.T.TC T.....G..T .......... .......A.. .......... ..........
CMW 7048   ..T.TTTGGC T..C.CT... .-T.CT.T.. TCC.C.T.TT T........T .......... .......A.. .......... ..........
CMW 2498   ..T....... .--------- -----..A.. ----..G.TC T........A .......... .......... .......... ..........
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```
[                 280        290        300        310        320        330        340        350        360]
[                  .          .          .          .          .          .          .          .          .]
CMW 2113    TCCAGAGCAA GAACTCGTCC TACTTCGTCG AGTGGATCCC CAACAACGTC CAGACCGCCC TCTGCTCCAT CCCCCCCAAG GGTCTCAAGA
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .........A. .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1652    .......... .......... .......... .......T.. .......... .......... .......... T......... ..C.......
CMW 7047    .......... .......... .......... .......T.. .......... .......... .T........ T......G.. ..C..A....
CMW 7048    .......... .......... .......... .......T.. .......... .......... .......... T......G.. ..C.......
CMW 2498    .......... ......A... .......... .......... .......... .......... .......G.. ...T...... ..........

[                 370        380        390        400        410        420        430        440        450]
[                  .          .          .          .          .          .          .          .          .]
CMW 2113    TGTCCTCCAC CTTTGTTGGC AACTCCACTG CCATCCAGGA GCTCTTCAAG CGTATCGGCG AGCAGTTCAC TGCTATGTTC CGTCGCAAGG
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1652    .......G.. ......C... ........C. .......... .......... ...G...... .......T.. C..C...... ..G.......
CMW 7047    .......G.. ......C... ........C. .......... ......A... ...G...... .......... C..C...... ..G.......
CMW 7048    .......G.. ......C... ........C. .......... .......... ...G...... .......T.. C..C...... ..G.......
CMW 2498    .......T.. ...C..G..T .....G.... .T........ ...G...... ...G.G.... .......... ...C...... ..G.......

[                 460        470        480        490        500        510        520        530        540]
[                  .          .   β-tub 2a/2b          .          .          .          .          .          .]
CMW 2113    CTTTCTTGCA TTGGTACACT GGGACGCGAC ACGGCGGTCT CGAGACCGCG AT-GGTAGTG GTGGCTTCAG TACTGACCGC GACCGC-AGG
CMW 8755    .......... .......... .......... .......... ........T. .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... ........T. ......G... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... ........T. ......G... .......... .......... ..........
CMW 1853    .......... .......... .......... .......... ........T. ......G... .......... .......... ..........
CMW 1840    .......... .......... .......... .......... ........T. ......G... .......... .......... ..........
CMW 8756    .......... .......... .......... .......... ........T. ......G... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... ........T. ......G... .......... .......... ..........
CMW 1652    .......... .......... .....A...T ..A..TACA. ...------C ...C..GCA. CA.A...GGA .G......T. ...AAT....
CMW 7047    .......... .......... .....A...T ..A..TACA. ...------C ...C..GCA. CA.A...GGA .G......T. ...AAT....
CMW 7048    .......... .......... .....A...T ..A..TACA. ...------C ...C..GCA. CA.A...GGA .G......T. ...AAT....
CMW 2498    .......... .......... ..C..-..C. G..A..C..G AC.CG.GA.A ..AC.ACC.C .AA..A..GT .G......T. ...ATTT...
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```
[                 550       560       570       580       590       600       610       620       630]
[                  .         .         .         .         .         .         .         .         .]
CMW 2113    CAAACCATCT CTGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTACGTACC CT---CCTGT TGCACCAGGC GG-----CGC GCCTCGAGCT
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .........C .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .........C .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......... .........C .......... .......... ..........
CMW 1840    .......... .......... .......... .......... .......... .........C .......... .......... ..........
CMW 8756    .......... .......... .......... .......... .......... .....T...C .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .........C .......... .......... ..........
CMW 1652    .......... .C........ .......... .......... .......... ..ATCT.G.C .T.C.A..CA A.ACAGA... .A..T.....
CMW 7047    .......... .C........ .......... .......... .......... ..ATCT.G.C .T.C.A..CA A.ACAGA... .A..T.....
CMW 7048    .......... .C........ .......... .......... .......... ..ATCT.G.C .T.C.A..CA A.ACAGA... .A..T.....
CMW 2498    .......... .......... .......... .......... ....T.C... TCACATTCCC ...C.ACT.A TCTTGGC.T. T.T..CG...

[                 640       650       660       670       680       690       700       710       720]
[                  .         .         .         .         .         .         .         .         .]
CMW 2113    TCCC-GCTGA CCACTGCACA GCTACAACGG CACCTCCGAG CTCCAGCTCG AGCGCATGAA CGTCTACTTC AACGAGGTAT GTCTG-TCGG
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840    .......... ....C..... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... ....C..... .......... .......... ..T....... .......... .......... .......... ..........
CMW 2632    .......... ....C..... .......... .......... .......... .......... .......... .......... ..........
CMW 1652    .T..T..... ....CA..T. .......... .......... .......... .......... .........T .......... ....TA....
CMW 7047    .T..T..... ....CA..T. .......... .......... .......... .......... .........T .......... ....TA....
CMW 7048    .T..T..... ....CA..T. .......... .......... .......... .......... .........T .......... ....TA....
CMW 2498    .GG..A.... .A.TC...T. .T........ ...T..T... .......... .......... .......... .........A ...AA.----

[                 730       740       750       760       770       780       790       800       810]
[                  .         .         .         .         .         .         .         .         .]
CMW 2113    G--ACCAGGC T-GGCGCGTC -ATCCCGCC- ----CGCGAA CCCCCTGTGC GTGACC---- ---------- ------GAGC TCCCGCT---
CMW 8755    .......... ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    .......... ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 1840    ........T. ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    ........T. ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... ....G..... .......... .......... .......... .......... .......... .......... ..........
CMW 1652    .TG.T..A.. ..CAA..T.. ..C.T..G.. AA.C.C.CCC ......T.C. .G.G..TT.. GACTTCTGGT ATAGGC.... .T..T..TCT
CMW 7047    .TG.T..A.. ..CAA..T.. C.C.T..G.C A.CC.C.CCC ......T.C. .G.G..CTC. GACTTCTGGT ATAGGC.... .T..T..TCT
CMW 7048    .TG.T..A.. ..CAA..T.. C.C.T..G.C AACC.C.CCC ......T.C. .G.G..CTC. GACTTCTGGT ATAGGC.... .T..T..TCT
CMW 2498    -..--..ACT GCACAT.A.. C....GA... ..AT.T.C.. .A.GG.T.A. T--G..GTCG CCCG...... ........C. .--.......
```

```
[                   820       830       840       850       860       870       880       890       900]
[                    .         .         .         .         .         .         .         .         .]
CMW 2113    GACGCGCTCC TGTCACAGGC CTCCGGCAAC AAGTATGTCC CCCGCGCCGT CCTCGTCGAT CTCGAGCCCG GCACCATGGA CGCCGTCCGT
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .......... ........T. .......... .......... ........T. .......... ..........
CMW 2632    .......... .......... .......... ........T. .......... .......... ........T. .......... ..........
CMW 1652    ........T. .TGTC..... .......... ........T. .......... .......... .......... .T........ T........C
CMW 7047    ........T. .TGTC..... .......... ........T. .......... .......... .......... .T........ T........C
CMW 7048    ........T. .TGTC..... .......... ........T. .......... .......... .......... .T........ T........C
CMW 2498    A...T.-.TA .CG.C..... .......... ........T. .T........ .......... .......... .T........ ..........

[                   910       920       930       940       950       960       970       980       990]
[                    .         .         .         .         .   Histone H3 →    .         .         .]
CMW 2113    GCCGGCCCCT TCGGCCAGCT GTTCCGCCCC GACAACTTCG TCTTCGGCCA GTCCACCGGT GGCAAGGCCC CCCGTAAGCA GCTCGCCTCC
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .......-.. .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1652    ..T....... .T..T..... ......T... .......... ....T..... ......T... ........T. .......... ......G...
CMW 7047    ..T....... .T..T..... ......T... .......... ....T..... ......T... ........T. .......... ......G...
CMW 7048    ..T....... .T..T..... ......T... .......... ....T..... ......T... ........T. .......... ......G...
CMW 2498    .....T.... .T........ C......... .......... .......... .......... .......... .......... ..........

[                  1000      1010      1020      1030      1040      1050      1060      1070      1080]
[                    .         .         .         .         .         .         .         .         .]
CMW 2113    AAGGCTGCTC GCAAGTCCGC CCCCTCCACC GGTGGTGTCA AGAAGCCTCA CCGCTACAAG CCCGGTACTG TCGCTCTGCG TGAGATTCGT
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .........T .......... .......... .......... ........C. .......... ..........
CMW 8757    .......... .......... .........T .......... .......... .......... ........C. .......... ..........
CMW 1853    .......... .......... .........T .......... .......... .......... ........C. .......... ..........
CMW 1840    .......... .......... .........T .......... .......... .......... ........C. .......... ..........
CMW 8756    .......... .......... .........T .......... .......... .......... ........C. .......... ..........
CMW 2632    .......... .......... .........T .......... .......... .......... ........C. .......... ..........
CMW 1652    ........C. .......... .......... ..A....... .......... .......... ..T.....C. .......... ......C...
CMW 7047    ........C. .......... .......... ..A....... .......... .......... ..T.....C. .......... ......C...
CMW 7048    ........C. .......... .......... ..A....... .......... .......... ..T.....C. .......... ......C...
CMW 2498    .......... .......... G..A..T... ..A....... .......... .......... ..T.....C. .G........ ......C...
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```
[                  1090       1100       1110       1120       1130       1140       1150       1160     1170]
[                  .          .          .          .          .          .          .          .          .]
CMW 2113   CGCTACCAGA AGTCCACCGA GTTGCTTATC CGCAAGCTCC CCTTCCAGCG CCTGGTGAGC AACCAACCCC TTTGACCGCC GTCGTGTCAT
CMW 8755   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758   .....T.... .......... .C........ ........T. ....T..A.. ...A...... .......... ....C..... ..........
CMW 8757   .....T.... .......... .C........ .......... .......... ...A...... .......... ....C..... ..........
CMW 1853   .....T.... .......... .C........ .......... .......... .......... .......... ....C..... ..........
CMW 1840   .......... .......... .C........ .......... .......... .......... .......... ....C..... ..........
CMW 8756   .......... .......... .C........ .......... .......... .......... .......... ....C..... ..........
CMW 2632   .......... .......... .C........ .......... .......... .......... .......... ....C..... ..........
CMW 1652   .......... .......... .C....C... .......... .......... T........T CTT---.... ..CTCGTT.. T..C..GG.C
CMW 7047   .......... .......... .C....C... .......... .......... T........T CTT---.... ..CTCGTT.. T..C..GG.C
CMW 7048   .......... .......... .C....C... .......... .......... T........T CTT---.... ..CTCGTT.. T..C..GG.C
CMW 2498   .......... ..AG...... .C....G... .......... .......... T.....AT.T TG.--..... .CGC.AT... CA.CACG.--

[                  1180       1190       1200       1210       1220       1230       1240       1250     1260]
[                  .          .          .          .          .          .          .          .          ]
CMW 2113   G--------- -TGCCTTTGC TGACTCGTTC TGTTTCCCTA C--GACAGGT CCGTGAGATC GCCCAGGACT TCAAGTCCGA CCTCCGCTTC
CMW 8755   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758   .......... .......... .......... .......... .....T.... .......... .......... .T........ .....T..T
CMW 8757   .......... .......... .......... .......... .....T.... .......... .......... .......... ..........
CMW 1853   .......... .......... .......... .......... .....T.... .......... .......... .......... ..........
CMW 1840   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1652   .ATTTGTGTC GC.A..C... ATCT.AC.GA C.CC.T..AC .CCA...... ...C.....T .......... .......... T.....T...
CMW 7047   .ATTTGTGTC GC.A..C... ATCT.AC.GA C.CC.T..AC .CCA...... ...C.....T .......... .......... T.....T...
CMW 7048   .ATTTGTGTC GC.A..C... ATCT.AC.GA C.CC.T..AC .CCA...... ...C.....T .......... .......... T.....T...
CMW 2498   -......... .C.A.CC... ....-.A.CA .CGCCT...T ...A.T.... .......... .......... .......... .........T

[                  1270       1280       1290       1300       1310       1320       1330       1340     1350]
[                  .          .          .          .          .          .          .          .          .]
CMW 2113   CAGTCCTCCG CCATCGGTGC CCTGCAGGAG TCCGTCGAGT CCTACCTCGT CTCTCTCTTC GAGGACACCA ACCTGTGCGC CATCCACGCC
CMW 8755   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758   .....T..G. .A..T.... .......... .......... .T........ .......... .......... ..T....... ...T......
CMW 8757   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840   .......... .......... .......... .......... .......... .......... .......... .T........ ..........
CMW 8756   .......... .......... .......... .......... .......... .......... .......... .T........ ..........
CMW 2632   .......... .......... .......... .......... .......... .......... .......... .T........ ..........
CMW 1652   .....G.... .......... G......... .....T.... .......T.. ...C...... .......... .......... ..........
CMW 7047   .....G.... .......... G......... .....T.... .......T.. ...C...... .......... .......... ..........
CMW 7048   .....G.... .......... G......... .....T.... .......T.. ...C...... .......... .......... ..........
CMW 2498   .....T.... .......... .......... ..G....... .T.....A.. .......... .......... .......... ..........
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```
[                    1360    ]
[                    .       ]
CMW 2113     AAGCGTGTCA CCATC
CMW 8755     .......... .....
CMW 62       .......... .....
CMW 8758     .......... ....T
CMW 8757     .......... .....
CMW 1853     .......... .....
CMW 1840     .......... .....
CMW 8756     .......... .....
CMW 2632     .......... .....
CMW 1652     .......... .....
CMW 7047     .......... .....
CMW 7048     .......... .....
CMW 2498     .......... .....
```

Appendix 2. Raw sequence data of the two regions sequenced within the β-tubulin gene (designated as β-tub 1a/1b and β-tub 2a/2b) and the ITS1, conserved 5.8S and ITS2 regions of the rDNA operon. The start of each region is indicated above the alignment. The exon regions of the β-tubulin gene as well as the conserved 5.8S region of the rDNA operon are in bold and boxed. Unknown sequence characters are indicated with a "**N**", while gaps inserted to achieve sequences alignment are indicated with "**-**". Bases matching those of **CMW 8757** are indicated with a "**.**".

```
[                    10         20         30         40         50]
[          β-tub 1a/1b →        .          .          .          .]
CMW 8757   TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG
CMW 1853   .......... .......... .......... .......... ..........
CMW 9927   .......... .......... .......... .......... ..........
CMW 9929   .......... .......... .......... .......... ..........
CMW 9928   .......... .......... .......... .......... ..........
CMW 9327   .......... .......... .......... .......... ..........
CMW 9328   .......... .......... .......... .......... ..........
CMW 9932   .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... ..........
CMW 2113   .......... .......... .......... .......G.. ..........
CMW 8755   .......... .......... .......... .......... ..........
CMW 8756   .......... .......... .......... .......... ..........
CMW 9906   .......... .......... .......... .......... ..........
CMW 9903   .......... .......... ........G. .......... ..........
CMW 1651   .......... .......... .......... .CC....... C.........
CMW 1654   .......... .......... .......... .CC.A..... C.........
CMW 2498   .......... .......... .......... .C........ T..T...C.C

[                    60         70         80         90        100]
[                     .          .          .          .          .]
CMW 8757   ACCCAGCAGA TGTTCGACCC CAAGAACATG ATGGCTGCCT CTGACTTCCG
CMW 1853   .......... .......... .......... .......... ..........
CMW 9927   .......... .......... .......... .......... ..........
CMW 9929   .......... .......... .......... .......... ..........
CMW 9928   .......... .......... .......... .......... ..........
CMW 9327   .......... .......... .......... .......... ..........
CMW 9328   .......... .......... .......... .......... ..........
CMW 9932   .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... ..........
CMW 2113   .......... .......... .......... .......... ..........
CMW 8755   .......... .......... .......... .......... ..........
CMW 8756   .......... .......... .......... .......... ..........
CMW 9906   .......... .......... .......... .......... ..........
CMW 9903   .......... .......... .......... .......... ..........
CMW 1651   .......... .......... .......... .......... ..........
CMW 1654   .......... .......... .......... .......... ..........
CMW 2498   ........A. .......... .......... .......... ..........

[                   110        120        130        140        150]
[                     .          .          .          .          .]
CMW 8757   CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC TCCCGCCCCT
CMW 1853   .......... .......... .......... .......... ..........
CMW 9927   .......... .......... .......... .......... C.........
CMW 9929   .......... .......... .......... .......... C.........
CMW 9928   .......... .......... .......... .......... C.........
CMW 9327   .......... .......... .......... .......... C.........
CMW 9328   .......... .......... .......... .......... C.........
CMW 9932   .......... .......... .......... .......... C.........
CMW 62     .......... .......... .......... .......... C.........
CMW 2113   .......... .......... .......... .......... C.........
CMW 8755   .......... .......... .......... .......... C.........
CMW 8756   .......... .......... .......... .......... C.........
CMW 9906   .......... .......... .......... .......... C.........
CMW 9903   .......... .......... .......... .......... C.........
CMW 1651   .......... ........A. ....T..... .........T .T.TTG..T.
CMW 1654   .......... ........A. ....T..... .........T .T.TTGT.T.
CMW 2498   .......... .......... ....T..... .......... C..TAAGT.C
```

```
[                    160        170        180        190        200]
[                     .          .          .          .          .]
CMW 8757     CGCGCCTCGG AGAGCATCGG CCGAA-G-CT T-----GTCT G-CTAACTCT
CMW 1853     .........A .......... .......... .......... ..........
CMW 9927     .......... G......... ....G..... .......... ..........
CMW 9929     .......... G......... ....G..... .......... ..........
CMW 9928     .......... G......... ....G..... .......... ..........
CMW 9327     .......... G.C..C.... .......... C......... .......C..
CMW 9328     .......... G.C..C.... .......... C......... .......C..
CMW 9932     .......... G.C..C.... .......... C......... .......C..
CMW 62       .......... G.C..C.... .......... C......... .......C..
CMW 2113     .......... G.C..C.... .......... C......... .......C..
CMW 8755     .......... G......... .......... C......... .......C..
CMW 8756     .......... G......... .......... .......... ..........
CMW 9906     .......... G......... .......... .......... ..........
CMW 9903     .......... G......... ..C....... .......... ..........
CMW 1651     TT.CT.G.AA GT--.-...A -....C.T.. .GGGCT..T. .G.....C..
CMW 1654     TT.CT.G.AA GT--.-...A -....C.T.. .GGGCT..T. .G.....C..
CMW 2498     .CTCG.A.AA .T.AA..-.. T..CGC.... GACACT..-- -G..------

[                    210        220        230        240        250]
[                     .          .          .          .          .]
CMW 8757     TATCG---TC ---------- --C-AGCCGT GGCAAGGTCT CCATGAAGGA
CMW 1853     .......... .......... .......-.. .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     C......... .......... .......... .......... ..........
CMW 9328     C......... .......... .......... .......... ..........
CMW 9932     C......... .......... .......... .......... ..........
CMW 62       C......... .......... .......... .......... ..........
CMW 2113     C......... .......... .......... .......... ..........
CMW 8755     C......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     G-..TTTC.. TCTTCCCCTT CT.A...... ..T....... ..........
CMW 1654     G-..TTTC.. TCTTCCCCTT CT.T...... ..T....... ..........
CMW 2498     -----...-- .......... ...T...... ..A....... ..........

[                    260        270        280        290        300]
[                     .          .          .          .          .]
CMW 8757     GGTTGAGGAC CAGATGCGCA ATGTCCAGAG CAAGAACTCG TCCTACTTCG
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     ...C...... .......... .......... .......... ..........
CMW 9929     ...C...... .......... .......... .......... ..........
CMW 9928     ...C...... .......... .......... .......... ..........
CMW 9327     ...C...... .......... .C........ .......... ..........
CMW 9328     ...C...... .......... .C........ .......... ..........
CMW 9932     ...C...... .......... .C........ .......... ..........
CMW 62       ...C...... .......... .C........ .......... ..........
CMW 2113     ...C...... .......... .C........ .......... ..........
CMW 8755     ...C...... .......... .C........ .......... ..........
CMW 8756     .......... .......... .C........ .......... ..........
CMW 9906     .......... .......... .C........ .......... ..........
CMW 9903     .......... .......... .C........ .......... ..........
CMW 1651     A..C...... .......... .C........ .......... ..........
CMW 1654     A..C...... .......... .C........ .......... ..........
CMW 2498     ...C...... .......... .C........ .........A ..........
```

```
[                    310        320        330        340        350]
[                    .          .          .          .          .]
CMW 8757     TCGAGTGGAT CCCCAACAAC GTCCAGACCG CCCTCTGCTC CATCCCCCCC
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... ....T..... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... ....T..... ..........
CMW 1651     .......... T......... .......... .......... ...T......
CMW 1654     .......... T......... .......... .......... ...T......
CMW 2498     .......... .......... .......... .......... G.....T...

[                    360        370        380        390        400]
[                    .          .          .          .          .]
CMW 8757     AAGGGTCTCA AGATGTCCTC CACCTTTGTT GGCAACTCCA CTGCCATCCA
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .C........
CMW 1651     .....C.... .......... G........C .......... .C........
CMW 1654     .....C.... .......... G........C .......... .C........
CMW 2498     .......... .......... T.....C..G ..T.....G. ....T.....

[                    410        420        430        440        450]
[                    .          .          .          .          .]
CMW 8757     GGAGCTCTTC AAGCGTATCG GCGAGCAGTT CACTGCTATG TTCCGTCGCA
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .......... ......G... .......... T..C..C... .....G....
CMW 1654     .......... ......G... .......... T..C..C... .....G....
CMW 2498     ......G... ......G.G. .......... ......C... .....G....
```

```
[                    460        470        480        490       500]
[                     .          .     β-tub 2a/2b → .          .]
CMW 8757     AGGCTTTCTT GCATTGGTAC ACTGGGACGC GAC--ACGGC GGTCT-CGAG
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... .....G....
CMW 9328     .......... .......... .......... .......... .....G....
CMW 9932     .......... .......... .......... .......... .....G....
CMW 62      .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .......... .......... ........A. ..T....A.. TACA....--
CMW 1654     .......... .......... ........A. ..T....A.. TACA....--
CMW 2498     .......... .......... .....C..CG CCGCG..-.. T----....C

[                    510        520        530        540       550]
[                     .          .          .          .          .]
CMW 8757     ACCGTGATGG TGGTGGTGGC TTCAGT---- ---ACTGACC GCG-ACCGC-
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... ..T....... .......... .......C..
CMW 9929     .......... .......... ..T....... .......... .......C..
CMW 9928     .......... .......... ..T....... .......... .......C..
CMW 9327     ....A..... .A........ .......... .......... ...G......
CMW 9328     ....A..... .A........ .......... .......... ...G......
CMW 9932     ....A..... .A........ .......... .......... ...G......
CMW 62      ....C..... .A........ .......... .......... ..........
CMW 2113     ....C..... .A........ .......... .......... ..........
CMW 8755     .......... .A........ .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     ....C..... .A.......G .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     ..-----..C. ..CA.CA.A. ..GGA..... ...G...... T.....AAT.
CMW 1654     ..-----..C. ..CA.CA.A. ..GGA..... ...G...... T.....AAT.
CMW 2498     ..GCGACA-- -ATAC.ACCT C-G.AGCATC GTTG...... T.....ATTT

[                    560        570        580        590       600]
[                     .          .          .          .          .]
CMW 8757     AGGCAAACCA TCTCTGGCGA GCACGGCCTC GACAGCAATG GCGTGTACGT
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .......... ....C..... .......... .......... ..........
CMW 1654     .......... ....C..... .......... .......... ..........
CMW 2498     .......... .......... .......... .......... .......T.C
```

```
[                    610        620        630        640       650]
[                     .          .          .          .          .]
CMW 8757     ACCCTCCTGC TGCACCAGGC GGCGCGCCTC GAG------- ----------
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... ........A. .......... .......... ..........
CMW 9929     .......... ........A. .......... .......... ..........
CMW 9928     .......... ........A. .......... .......... ..........
CMW 9327     .........T .......... .......... .......... ..........
CMW 9328     .........T .......... .......... .......... ..........
CMW 9932     .........T .......... .......... .......... ..........
CMW 62       .........T .......... .......... .......... ..........
CMW 2113     .........T .......... .......... .......... ..........
CMW 8755     .........T .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .....AT--. .---.--... TT.C.AAGCA -..ACAGACG CGACTTGAGC
CMW 1654     .....AT--. .---.--... TT.C.AAGCA -..ACAGACG CGACTTGAGC
CMW 2498     ...-..ACAT .C.CTGCCCA CTGATCTTGG CCTCTCTTCC GG.CTTGGCA

[                    660        670        680        690       700]
[                     .          .          .          .          .]
CMW 8757     CTTCCCGCTG ACCACTGCAC AGCTACAACG G-CACCTCCG AGCTCCAGCT
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .....C.... .......... .......... ..........
CMW 9906     .......... .....C.... .......... .......... ..........
CMW 9903     .......... .....C.... .......... .......... ..........
CMW 1651     T....T.... .....CA..T .......... .......... ..........
CMW 1654     T....T.... .....CA..T .......... .......... ..........
CMW 2498     -------... ..A.TC...T ..T....... .....T..T. ..........

[                    710        720        730        740       750]
[                     .          .          .          .          .]
CMW 8757     CGAGCGCATG AACGTCTACT TCAACGAGGT ATGTCTGTCG GGACCA----
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .......... .......... .T........ ......TATC ..GTG.TACA
CMW 1654     .......... .......... .T........ ......TATC ..GTG.T.CA
CMW 2498     .......... .......... .......... .A...AACAA CTG.ACATCA
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[                    760        770        780        790       800]
[                     .          .          .          .          .]
CMW 8757     GGCTGGGGCG TCATCCCGCC CGCGA----- ACCCCCTGTG CGT-------
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     ......C... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .T........ .......... .......... .......... ..........
CMW 9906     .T........ ........T. .......... .......... ..........
CMW 9903     .T........ ........T. .......... .......... ..........
CMW 1651     A...ACAAGC .TCCA..TGG GC.A-..... ......CCCC .CCTTTCCGG
CMW 1654     A...-CAAGC .TC-A..T.G GC-A-..... ......CCCC .CCTTTCCGG
CMW 2498     TC.ATCC.AC -....T.CAA .A..GTTTAC TG..GTC.CC ..-.......

[                    810        820        830        840       850]
[                     .          .          .          .          .]
CMW 8757     -GACCGAGCT CCCGCTGACG C-GC-----T CCT-GTCA-- ----------
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......-.. ..-.T..... .......... T..T...... ..........
CMW 1651     G.C.TTCTGA .TTCTG.TAT AG..GAGCA. ....----CT TCTGACGCG.
CMW 1654     G.C.TT--GA .TTCTG.TAT AG..GAGCT. ....----CT TCTGACGCG.
CMW 2498     .....TC... AA..TGTTAT .GC......- ---.----.. ..........

[                    860        870        880        890       900]
[                     .          .          .          .          .]
CMW 8757     ---------C A-GGCCTCCG GCAACAAGTA TGTCCCCCGC GCCGTCCTCG
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... ...T...... ..........
CMW 9906     .......... .......... .......... ...T...... ..........
CMW 9903     .......... .A...T..G. .......... ...T...... ........T.
CMW 1651     CTTCTTGTC. .......... .......... ...T...... ..........
CMW 1654     CTTCTTGTC. .......... .......... ...T...... ..........
CMW 2498     .......... .......... .......... ...T..T... ..........
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[                    910        920        930        940       950]
[                    .          .          .          .          .]
CMW 8757     TCGATCTCGA GCCCGGCACC ATGGACGCCG TCCGTGCCGG CCCCTTCGGC
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     ....C..... .......... .......... .......... ..........
CMW 9929     ....C..... .......... .......... .......... ..........
CMW 9928     ....C..... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... ...T...... .......... .......... ..........
CMW 9906     .......... ...T...... .......... .......... ..........
CMW 9903     .T........ A..T...... .......... .T........ ..........
CMW 1651     .......... ......T... .....T.... ....C..T.. ......T..T
CMW 1654     .......... ......T... .....T.... ....C..T.. ......T..T
CMW 2498     .......... ......T... .......... .......... T.....T...

[                    960        970        980        990      1000]
[                    .          .          .          ITS1 →     .]
CMW 8757     CAGCTGTTCC GCCCCGACAA CTTCGTCTTC GGCCAGTCCC CCAGATACCC
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     ..A....... .......... .......... .......... ..........
CMW 9929     ..A....... .......... .......... .......... ..........
CMW 9928     ..A....... .......... .......... .......... ..........
CMW 9327     .......... .......... ...T...... .......... ..........
CMW 9328     .......... .......... ...T...... .......... ..........
CMW 9932     .......... .......... ...T...... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .G........ .......... .......... ..........
CMW 1651     .......... .T........ .........T .......... ..........
CMW 1654     .......... .T........ .........T .......... ..........
CMW 2498     .....C.... .......... .......... .......... .....A....

[                   1010       1020       1030       1040      1050]
[                   .          .          .          .          .]
CMW 8757     TTTGTGAACT TATA-CCTTT TTATC-GTTG CCTCGGCGCC GAGCC----G
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... ........G. .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .......... ....A..A.. .......... .........T .....TCTG.
CMW 1654     .......... ....A..A.. .......... .........T .....TCTG.
CMW 2498     .......... .......--- .....T.... .........T -...T....-
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```
[                    1060       1070       1080       1090     1100]
[                    .          .          .          .          .]
CMW 8757     GGAGTGCT-- ---------- ---------C TTCTGTGCTC CC--------
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     ..G.G.G.TG GCGAAGGCAG ATTTTCTTC. ....CCC... ..TCCCCCCC
CMW 1654     ..G.G.G.TG GCGAAGGCAG ATTTTCTTC. ....CCC... ..TCCCCCCC
CMW 2498     -----.G... .......... ........C. C..G.G..-. ..T.......

[                    1110       1120       1130       1140     1150]
[                    .          .          .          .          .]
CMW 8757     -----CCACC GCGCAAGCAG T------GGA GCAGGCCCGC CGGCGGCCCA
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... ........G. .......... .......... ..........
CMW 9929     .......... ........G. .......... .......... ..........
CMW 9928     .......... ........G. .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     CTCTT..... .T....A.G. .TGTTGG... .......... ..........
CMW 1654     CTCTT..... .T....A.G. .TGTTGG... .......... ..........
CMW 2498     .....-.... -.T.GG.TGT .......-.. .ACA.....T ........A.

[                    1160       1170       1180       1190     1200]
[                    .          .          .          .          .]
CMW 8757     CCAAA-CTCT TTGTTTTTAG AACGTATCTC TTCTGAGTGT TTATA-ACAA
CMW 1853     .T........ .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .T........ .........T ...C...... ........AC A..A.C.A..
CMW 1654     .T........ .........T ...C...... ........AC A..A.C.A..
CMW 2498     ..T....... .-.......C .CT.A.A..- --.....CAC A--A..--.C
```

```
[                    1210       1220       1230       1240      1250]
[                    .    5.8S →          .          .          .]
CMW 8757     A-CAAATGAA TCAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .A-....... .......... .......... .......... ..........
CMW 1654     .A-....... .......... .......... .......... ..........
CMW 2498     .T-....... .......... .......... .......... ..........

[                    1260       1270       1280       1290      1300]
[                    .          .          .          .          .]
CMW 8757     TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .......... .......... .......... .......... ..........
CMW 1654     .......... .......... .......... .......... ..........
CMW 2498     .......... .......... .......... .......... ..........

[                    1310       1320       1330       1340      1350]
[                    .          .          .          .          .]
CMW 8757     AATCATCGAA TCTTTGAACG CACATTGCGC CCGCTGGAAT TCCAGCGGGC
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... ...T...... ..........
CMW 9328     .......... .......... .......... ...T...... ..........
CMW 9932     .......... .......... .......... ...T...... ..........
CMW 62       .......... .......... .......... ...T...... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... ..CG...... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .....G.... .......... .......... .......... ..........
CMW 1654     .....G.... .......... .......... .......... ..........
CMW 2498     .......... .......... .......... ..T....T.. ...G.A....
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```
[                    1360       1370       1380       1390    1400]
[                    .          . ITS2 →   .          .          .]
CMW 8757     ATGCCTGTTC GAGCGTCATT TCAACCCTCA AGCCTGGCTT GGTGTTGGGG
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     ...-...... .......... .......... ...T...... ..........
CMW 1654     ...-...... .......... .......... ...T...... ..........
CMW 2498     .......... .......... .......... .......... ....A.....

[                    1410       1420       1430       1440    1450]
[                    .          .          .          .          .]
CMW 8757     CACTACCTGT TC-ACAGCGG GTAGGCCCTG AAATTTAATG GCGGGCTCGC
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......G.. ..........
CMW 9929     .......... .......... .......... .......G.. ..........
CMW 9928     .......... .......... .......... .......G.. ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     ....G..... .TT....... .......... .......G.. ..........
CMW 9906     ....G..... .TT....... .......... .......G.. ..........
CMW 9903     ....G..... .TT....... .......... .......G.. ..........
CMW 1651     T......C.. AAA..----. .......... .......G.. ..........
CMW 1654     T......C.. AAA..----. .......... .......G.. ..........
CMW 2498     ....G.T.C. .ACC..AGAA .C........ .....C.G.. ...A......

[                    1460       1470       1480       1490    1500]
[                    .          .          .          .          .]
CMW 8757     TAAGACTCTG AGCGTAGTAG TTTTTATC-- ----ACCTCG CTTTGGAAGG
CMW 1853     .......... .......... .......... .......... ..........
CMW 9927     .......... .......... .......... .......... ..........
CMW 9929     .......... .......... .......... .......... ..........
CMW 9928     .......... .......... .......... .......... ..........
CMW 9327     .......... .......... .......... .......... ..........
CMW 9328     .......... .......... .......... .......... ..........
CMW 9932     .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..........
CMW 8756     .......... .A........ .......... .......... ..........
CMW 9906     .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... ..........
CMW 1651     .......... .......... .....T.TTC TTCA...... ..........
CMW 1654     .......... ...C...... .....T.TTC TTCA...... ..........
CMW 2498     C.G...C.C. ....C..... ..--A.A... ....-..... ..C.......
```

```
[                    1510        1520    1530]
[                    .           .          .]
CMW 8757     AT-TAGCGGT -GCTCTTGCC G-TAAAACC
CMW 1853     .......... .......... .........
CMW 9927     .......... .......... .........
CMW 9929     .......... .......... .........
CMW 9928     .......... .......... .........
CMW 9327     .......... .......... .........
CMW 9328     .......... .......... .........
CMW 9932     .......... .......... .........
CMW 62       .......... .......... .........
CMW 2113     .......... .......... .........
CMW 8755     .......... .......... .........
CMW 8756     .....CGA.. ...C...... .........
CMW 9906     .......... .......... .........
CMW 9903     .......... .......... .........
CMW 1651     .......... T......... .........
CMW 1654     .......... T......... .........
CMW 2498     CCC.G..... ...C..-... .T....-..
```

Appendix 3. Raw sequence data of the two regions within the β-tubulin gene (designated as β-tub 1a/1b and β-tub 2a/2b) and the ITS1, conserved 5.8S and ITS2 regions of the rDNA operon. The start of each region is indicated above the alignment. The exon regions of the β-tubulin gene as well as the conserved 5.8S region of the rDNA operon are indicated in bold and boxed. Unknown sequence characters are indicated with a "**N**", while gaps inserted to achieve sequences alignment are indicated with "**-**". Bases matching those of **CMW 1856** are indicated with a "**.**"

```
[                   10         20         30         40         50         60         70         80]
[          β-tub 1a/1b →     .          .          .          .          .          .          .]
CMW 1856   TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 8756   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906   .......... .......... ........G. .......... .......... .......... .......... ..........
CMW 2631   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113   .......... .......... .......... .......G.. .......... .......... .......... ..........
CMW 8755   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667  .......... .......... .......... .......... ...A...... .......G.. .......... ..........
CMW 10668  .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774  .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776  .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775  .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777  .......... .......... .......... .......... .......... ........A. .......... ..........
CMW 10778  .......... .......... .......... .......... .......... ........A. .......... ..........
CMW 10781  .......... .......... .......... .CC....... T......... .......... .......... ..........
CMW 10779  .......... .......... .......... .CC....... T......... .......... .......... ..........
CMW 10780  .......... .......... .......... .CC....... T......... .......... .......... ..........
CMW 10455  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10477  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10518  .......... .......T.. .......... .CC.A..... T......... .......... .......... ..........
CMW 10463  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 7047   .......... .......... .......... .CC....... C......... .......... .......... ..........
CMW 7048   .......... .......... ..T....... .CC....... C......... .......... .......... ..........
CMW 5288   .......... C......... ........T. .C........ .......C.C .......... .......... ..........
CMW 5587   .......... C......... ........T. .C........ .......C.C .......... .......... ..........
```

```
[                    90         100        110        120        130        140        150       160]
[                     .          .          .          .          .          .          .         .]
CMW 1856     ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC CCCCGCCCCT CGCGCCTCGG
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... T......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... T......... .........A
CMW 10667    .......... .......... .......... .......... .......... .......... T......... ..........
CMW 10668    .....C.... .C........ .......... .......... .......... .......... T......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .........T .....A.... .......... .......... T.......T. .........A
CMW 10775    .......... .......... .......... .......... .......... .......... T......... ..........
CMW 10777    .......... .......... .........T .......... .......... .......... T......... .......G..
CMW 10778    .......... .......... .......... .......... .......... .......... T......... G......G..
CMW 10781    .......... .......... .........G .....A.... ....T..... .......... .A..T..T.A .AGA....C.
CMW 10779    .......... .......... .......... .......... ....T..... .......... .A..T..T.A .AGA....C.
CMW 10780    .......... .......... .......... .......... ....T..... .......... .A..T..T.A .AGA....C.
CMW 10455    .......... .......... .......... .......... ....T..... .........T GTTTTTTTT. TT.TT...TT
CMW 10477    .......... .......... .......... .......... ....T..... .........T GTTTTTTTT. TT.TT...TT
CMW 10518    .......... .......... .........T ........T. ....T..... .........T TT.T.TGT.. TCT-------
CMW 10463    .......... .......... T......... ........A. ....T..... .........T TT.TATA... T---------
CMW 7047     .......... .......... .......... ........A. ....T..... .........T TT.TTGT.T. TT--------
CMW 7048     .......... .......... .......... ........A. ....T..... .........T TT.TTGT.T. TT--------
CMW 5288     .....C.... .......... T..T...... .......... ....T..... .......... ...T.AG.A. .T--------
CMW 5587     .....C.... .......... T..T...... .......... ....T..... .......... ...T.AG.A. .T--------
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[                    170        180        190        200        210        220        230      240]
[                     .          .          .          .          .          .          .         .]
CMW 1856    GGAGCA---- ---------- -TCGGCCGAA GCTT------ ---GTCTGCT AACTCTTATC GTC------- ----------
CMW 8756    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906    .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 2631    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      ..C..C.... .......... .......... ...C...... .......... ...C..C... .......... ..........
CMW 2113    ..C..C.... .......... .......... ...C...... .......... ...C..C... .......... ..........
CMW 8755    .......... .......... .......... ...C...... .......... ...C..C... .......... ..........
CMW 8757    A......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667   A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668   A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775   A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778   A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781   A.GT.-.... .......... ....--..-- -..C...... .....G.... ...C.CCC.. CC.CTCCCCC CCAAAA....
CMW 10779   A.GT.-.... .......... ....--..-- -..C...... .....G.... ...C.CCC.. CC.C.CC... ..AAAA....
CMW 10780   A.GT.-.... .......... ....--..-- -..C...... .....G.... ...C.CCC.. CC.C.CC... ..AAAA....
CMW 10455   CCCCTTGCCT CCTCGCAAGT C...ATAA.G T.G.CTCTGG CTT..T.... ...C-.GT.T C..TCCCCCC CCCCCCAAC.
CMW 10477   CCCCTTGCCT CCTCGCAAGT C...ATGA.G T.G.CTCTGG CTT..T.... ...C-.GT.T C..TCCCCCC CCCCCCAAC.
CMW 10518   -------... CCTCGCAGGC C..CATGA.C ATC.TG..GG CTTT.TG... ...C.CATGT T..TCTCTTT CCCCCTTC..
CMW 10463   -------... .CTCGCAAGC C...ATGA.C ATC.CG..GG CTTC.TG... ...C.CACGT T..TCTCTTT C......CTC
CMW 7047    -------... CCTCGCAGGT C.A.A.AA.C .T..CG..GG CTGT.GG... ...C..GTCT T..TCTCTT. CCCC.TTCTC
CMW 7048    -------... CCTCGCAGGT C...A.AA.C .TC.TG..GG CTGT.TG... ...C..GTCT T..TCTCTT. CCCC.TTCTT
CMW 5288    -----CCACA CGACCCAAGT .--------- ----...... ..GT.TGCGC GCTGACAC.G T--...CTTC ..........
CMW 5587    -----CCACA CGACCCAAGT .--------- ----...... ..GT.TGCGC GCTGACAC.G T--...CTTC ..........
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[                    250        260        270        280        290        300        310        320]
[                     .          .          .          .          .          .          .          .]
CMW 1856     CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT TGAGGACCAG ATGCGCAACG TCCAGAGCAA GAACTCGTCC TACTTCGTCG
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... C......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... C......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... C......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .........T. .......... .......... ..........
CMW 1853     ....-..... .......... .......... .......... .........T. .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .........T. .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .........T. .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .........T. .........G .......... ..........
CMW 10775    .......... .......... .......... .......... .........T. .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .........T. .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .........T. .......... .......... ..........
CMW 10781    .......... ........T. .......... C......... .......... .......... .........G .....T....
CMW 10779    .......... ........T. .......... C......... .......... .......... .........G .....T....
CMW 10780    .......... ........T. .......... C......... .......... .......... .........G .....T....
CMW 10455    .......... .......... .......... A......... .......... .......... .......... ..........
CMW 10477    .......... .......... .......... A......... .......... .......... .......... ..........
CMW 10518    T......... .G........ .......... C......... .......... .T........ .......... ..........
CMW 10463    T......... .......... .......... C......... .......... .......... .......... ..........
CMW 7047     T.....G..T .......... .......A.. C......... .......... .......... .......... ..........
CMW 7048     T........T .......... .......A.. C......... .......... .......... .......... ..........
CMW 5288     T........A .......... .......... C......... .......... .......... .......... ..........
CMW 5587     T........A .......... .......... C......... .......... .......... .......... ..........
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[                    330        340        350        360        370        380        390        400]
[                     .          .          .          .          .          .          .          .]
CMW 1856     AGTGGATCCC CAACAACGTC CAGACCGCCC TCTGCTCCAT CCCCCCCAAG GGTCTCAAGA TGTCCTCCAC CTTTGTTGGC
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .T........ .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    ..G....... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781    .......... ......T... .......... .......T.. .......... ..C....... .......... ......C...
CMW 10779    .......... ......T... .......... .......T.. .......... ..C....... .......... ......C...
CMW 10780    .......... ......T... .......... .......T.. .......... ..C....... .......... ......C...
CMW 10455    .......... .......... .......... .......... .......... ..C....... .......... ......C...
CMW 10477    .......... .......... .......... .......... .......... ..C....... .......... ......C...
CMW 10518    .A........ .......... ..A....... .......... ......T... ..C....... .......T.. ......C...
CMW 10463    .A........ .......... .......... .......... .......... ..C....... .......T.. ......C...
CMW 7047     .......T.. .......... .......... .T........ T......G. ..C..A.... .......G.. ......C...
CMW 7048     .......T.. .......... .......... .......... T......G. ..C....... .......G.. ......C...
CMW 5288     .A.....T.. .......... .......... .G.....G.. ...T...... .......... .......T.. ...C.....T
CMW 5587     .A.....T.. .......... .......... .G.....G.. ...T...... .......... .......T.. ...C.....T
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[                    410        420        430        440        450        460        470        480]
[                    .          .          .          .          .          .          .          .]
CMW 1856     AACTCCACTG CCATCCAGGA GCTCTTCAAG CGTATCGGCG AGCAGTTCAC TGCTATGTTC CGTCGCAAGG CTTTCTTGCA
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     ........C. .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781    ........C. .......... .......... ...G.T.... .A........ C..C...... .......... ..........
CMW 10779    ........C. .......... .......... ...G.T.... .......... C..C...... .......... ..........
CMW 10780    ........C. .......... .......... ...G.T.... .......... C..C...... .......... ..........
CMW 10455    ........C. .......... .......... ...G...... .......... C..C...... .......... ..........
CMW 10477    ........C. .......... .......... ...G...... .......... C..C...... .......... ..........
CMW 10518    ........C. .......... .......... ...G...... .......... C..C...... .......... ..........
CMW 10463    ........C. .......... .......... ...G...... .......... C..C...... .......... ..........
CMW 7047     ........C. .......... ......A... ...G...... .......... C..C...... ..G....... ..........
CMW 7048     ........C. .......... .......... ...G...... .......T.. C..C...... ..G....... ..........
CMW 5288     .....G.... .T........ ...G...... ...G...... .......... ...C...... A.G....... ..........
CMW 5587     .....G.... .T........ ...G...... ...G...... .......... ...C...... A.G....... ..........
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[                    490        500        510        520        530        540        550       560]
[                     .  β-tub 2a/2b →      .          .          .          .          .         .]
CMW 1856     TTGGTACACT GGCAAACCAT CTCTGGCGAG CACGGCCTCG ACAGCAATGG CGTGTACGT- ----ACCCTC C-----TGCT
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ........T.
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ........T.
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ........T.
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    C......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    C......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781    .......... .......... ...C...... .......... .......... ......T..A CAGT.....A .ACGGC....
CMW 10779    .......... .......... ...C...... .......... .......... ......T..A CAGT.....A .ACGGC....
CMW 10780    .......... .......... ...C...... .......... .......... ......T..A CAGT.....A .ACGGC....
CMW 10455    ......T... .......... ...C...... .......... ......G... ......T..A CCGTG..T.A .ACCGGCTT.
CMW 10477    ......T... .......... ...C...... .......... ......G... ......T..A CCGTG..T.A .ACCGGCTT.
CMW 10518    .......... .......... ...C...... ..T....... .......... ......T..A CTCT..-..- -..CGGCTT-
CMW 10463    .......... .......... ...C...... ..T..T.... .......... ......T..A CCCT..-..- -..CGGCTT-
CMW 7047     .......... .......... ...C...... .......... .......... .........A CCCT.T-..- -..CGGCTT-
CMW 7048     .......... .......... ...C...... .......... .......... .........A CCCT.T-..- -..CGGCTT-
CMW 5288     .......... .......... .......... .....T.... .......... .........A CCTCGTA-.. .CCTGCC-.A
CMW 5587     .......... .......... .......... .....T.... .......... ......T..A CCTCGTA-.. .CCTGCC-.A
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[                    570        580        590        600        610        620        630        640]
[                    .          .          .          .          .          .          .          .]
CMW 1856     GCACCAGG-- ----CGG--- CGCGCCTCGA GCTT-CCC-G CTGACCA-CC GCACAGCTAC AACGGCACCT CCGAGCTCCA
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .........T .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .........T .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .........T .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .........T .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .........T .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .........T .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .........T .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .........T .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .........T .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .........T .......... .......... ..........
CMW 10781    ----....CC GAGA.A.ACG ...C..--.G ....T..T.. .......... .......... .......... ..........
CMW 10779    ----....CC GAGA.A.ACG ...C..--.G ....T..T.. .......... .......... .......... ..........
CMW 10780    ----....CC GAGA.A.ACG ...C..--.G ....T..T.. .......... .......... .......... ..........
CMW 10455    .-C....ACA A.GA.A.ACG ...CTT--.. ...CT..TT. .......A.. A......... ..T....... ..........
CMW 10477    .-C....ACA A.GA.A.ACG ...CTT--.. ...CT..TT. .......A.. A......... ..T....... ..........
CMW 10518    -.C....-CA A.GATA.ACG ..ACTTGT.. .AC.T..T.. ....T..... A..T...... .......... ..........
CMW 10463    -.C....-CA A.GATA.ACG ..ACTTGT.. ....T..T.. .....G.... A..T...... .......... ..........
CMW 7047     -.C.A..-CA A.GA.A.ACG ..ACTT--.. ....T..T.. .......... A..T...... .......... ..........
CMW 7048     -.C.A..-CA A.GA.A.ACG ..ACTT--.. ....T..T.. .......... A..T...... .......... ..........
CMW 5288     CTGGTCTCGT CCTCTCCCTC ---------G ....GG.-.A .....A...T ......T... ........T. ..........
CMW 5587     CTGGTCTCGT CCTCTCCCTC ---------G ....GG.-.A .....A..TT ......T... ........T. ..........
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[                    650       660       670       680       690       700       710       720]
[                     .         .         .         .         .         .         .         .]
CMW 1856     GCTCGAGCGC ATGAACGTCT ACTTCAACGA GGTATGTCTG T--------- CGG--GAC-C A-GGCTGGGG CGTCATCCC-
CMW 8756     .......... .......... .......... .......... .......... .......... ...T...... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... ...T...... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... ...T...... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... ...T...... ..........
CMW 10776    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781    ...T...... .......... .......... .........-T .AT....... ...GC..... ......C.A. .AC....TT.
CMW 10779    ...T...... .......... .......... .........-T .AT....... ...GC..... ......C.A. .AC....TT.
CMW 10780    ...T...... .......... .......... .........-T .AT....... ...GC..... ......C.A. .AC....TTT
CMW 10455    .......... .......... .......... ........CT .ATCATCCAT ...GT..... .A....CAA. .A..CAT.TC
CMW 10477    .......... .......... .......... ........CT .ATCATCCAT ...GT..... .A....CAA. .A..CAT.TC
CMW 10518    .......... .......... .......... .........-T .AT....... ...GT..... ......C.A. .A..CAT.TC
CMW 10463    .......... .......... .......... .........-T .AT....... ...GT..... ......C.A. .A..CAT.T.
CMW 7047     .......... .......... ....T..... .........-T .AT....... ...GT..T.. .A-...CAA. .T..CA..TC
CMW 7048     .......... .......... ....T..... .........-T .AT....... ...GT..T.. .A-...CAA. .T..CA..TC
CMW 5288     .......... .......... .......... ....A...-- AACAGCCACG TC.TCA.TT. .AATT..ACA ACCT.CGG.A
CMW 5587     .......... .......... .......... ....A...-- AACAGCCACG TC.TCA.TC. .AATT..ACA ACCT.CGG.A
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[                   730        740        750        760        770        780        790       800]
[                    .          .          .          .          .          .          .          .]
CMW 1856    GCCC-GC-GA --ACCCCC-- ---------- --TGTGCGT- -----GACC- --------GA GC-----TCC CG----CTGA
CMW 8756    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903    .T........ .......... .......... .......... .......... .......... .......... ..........
CMW 9906    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778   .......... .......... .......... .......... .......... .......... .......... .C........
CMW 10781   ...T...T.. .C......CC CCCCCTT... ..-------. ....C....T CT.GGCGT.. C.GGGCG... .-CTT.....
CMW 10779   ...T...T.. AC......CC CCCCCTT.CT TT-------. ....C....T CT.GGCGT.. C.GGGCG... .-CTT.....
CMW 10780   ...TT..T.. .C......CC CCCCCTT.CT TT-------. ....C....T CTTGGCGT.. C.GGGCG... .-CTT.....
CMW 10455   .A..TTGG.C CCC..AA.CC CCTCTCCC.. ..-------. ........TT CT.GGCATAG ..GAAGT... .TCTTT....
CMW 10477   .A..TTGG.C CCC..AA.CC CCTCTCCC.. ..-------. ........TT CT.GGCATAG ..GAAGT... .TCTTT....
CMW 10518   AG--.--.-C CC----A.CC CTGTTCC... ..-------. ..CTCC..TT CT.GGTACAG ..GAGCT... -TCTT.....
CMW 10463   ----.--.-- .C.A....CC CCCCCTCCCA AA.CCCG.GC CCCTC...TT CT.GGCATAG ..GAGCT... -TCTT.....
CMW 7047    .G.-.--.-- .C......CC CCCCC...CT TTC.G.GCCC TC......TT CT.GGTATAG ..GAGCT... -TCTT.....
CMW 7048    .G.-.--.-- .C.A....CC CCCCCC..CT TTCCG.G.CC CTC.....TT CT.GGTATAG ..GAGCT... -TCTT.....
CMW 5288    ----.--.-- ..-------.. ......TGGT TTCCC..CG. ....TCG..A AGGCCTTGCT AACGCAT.-- --.....-.AT
CMW 5587    ----.--.-- ..-------.. ......TGGT TTC.C..CG. ....TCG..A AGGCCTTGCT AACGCAT.-- --.....-.AT
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[                    810        820        830        840        850        860        870       880]
[                    .          .          .          .          .          .          .          .]
CMW 1856     CGCG-CTCCT GTCACAGGCC TCCGGCAACA AGTATGTTCC CCGCGCCGTC CTCGTCGATC TCGAGCCTGG CACCATGGAC
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 2113     .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 8755     .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 8757     .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 1853     .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 10667    .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 10668    .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 10775    .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 10777    .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 10778    .......... .......... .......... .......C.. .......... .......... .......C.. ..........
CMW 10781    .......G.. CGTT...... .......... .......C.. .........T .......... .......C.. T........T
CMW 10779    ....G..... CGTT...... .......... .......C.. .........T .......... .......C.. T........T
CMW 10780    .......... CGTT...... .......... .......C.. .........T .......... .......C.. T........T
CMW 10455    ....CT.TT. ...-.....T ....C..... .......... .......... .......... .......G.. T........T
CMW 10477    ....CT.TT. ...-.....T ....C..... .......... .......... .......... .......G.. T........T
CMW 10518    .......T.. TATC...... ...A...... .......... ......T... .......... .......C.. T.........
CMW 10463    .......T.. TATC...... ...A...... .......... ......A... .......... .......C.. T........T
CMW 7047     .......T.. TGTC...... .......... .......... .......... .......... .......C.. T........T
CMW 7048     .......T.. TGTC...... .......... .......... .......... .......... .......C.. T........T
CMW 5288     ...C----- ----...... .......... .......G.. T......... .......... .......C.. T.........
CMW 5587     ...C----- ----...... .......... .......G.. T......... .......... .......C.. T.........
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[                    890       900       910       920       930       940       950       960]
[                     .         .         .         .         .         .   ITS 1 →          .]
CMW 1856     GCCGTCCGTG CCGGCCCCTT CGGCCAGCTG TTCCGCCCCG ACAACTTCGT CTTCGGCCAG TCCCCCAGAT ACCC-TTTGT
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......A.. ..........
CMW 2632     .......... .......... .......... ........G. .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .......... .......-.. ..G....... ..........
CMW 10668    .......... .......... .......... ...-...... .......... .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781    ........C. .......... T..T...... .....T.... .......... ...T...... .......... ....C.A...
CMW 10779    ........C. .......... T..T...... .....T.... .......... ...T...... .......... ....C.A...
CMW 10780    ........C. .......... T..T...... .....T.... .......... ...T...... .......... ....C.A...
CMW 10455    ........C. .......... T..T...... .....T.... .......... ...T...... .......... ..........
CMW 10477    ........C. .......... T..T...... .....T.... .......... ...T...... .......... ..........
CMW 10518    ........C. .......... T......... .....T.... .......... ...T...... .......... ..........
CMW 10463    ........C. .......... T..T...... .....T.... .......... ...T...... .......... ..........
CMW 7047     ........C. .T........ T..T...... .....T.... .......... ...T...... .......... ..........
CMW 7048     ........C. .T........ T..T...... .....T.... .......... ...T...... .......... ..........
CMW 5288     .......... ....T..... .......... .......... .......... .......... .........A ..........
CMW 5587     .......... ....T..... .......... .......... .......... .......... .........A ..........
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[                    970        980        990        1000       1010       1020       1030    1040]
[                     .          .          .          .          .          .          .       .]
CMW 1856     GAACTTATA- CCTTTTT-AT C--GTTGCCT CGGCGCCGAG CC----GGGA GTGCTCTTCT GTGC------ ----------
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .T........ .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .T........ .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .T........ .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781    ...A...... .......A.A ATT....... T......... ..C.GG.... .ACGGGA.A. AGAAAAATAT TCTTTTCTTT
CMW 10779    .......... .......A.A ATT....... .......... ..C.GG.... .ACGGGA.A. AGAAAAATAT .CTTTTCTTT
CMW 10780    .......... .......A.A ATT....... .......... ..C.GG.... .ACGGGA.A. AGAAAAATAT .CTTTTCTTT
CMW 10455    .......... ..A....... .......... ......T... ..C.GG...G AG.GAAAAAA AAAAAAAAAA GGGGGGAAAT
CMW 10477    .......... ..A....... ...-...... ......T... ..C.GG...G AG.GAAAAAA AAAAAAAAAA GGGGGGAAAT
CMW 10518    .....C.... ..A....... .......... ......T... ..CCGG...G .ATT.T.--G AGAGAGTC.. TCTCTCTCCT
CMW 10463    .....C.... ..A....... .......... ......T... ..CCGG...G .ATT.T.--G AGAGAGTC.. TCTCTCTCCT
CMW 7047     .........A ..A....... .......... ......T... ..C.GG...G .G.T.GGC-G AA.GCAGA.. TTTTCTTCCT
CMW 7048     .........A ..A....... .......... ......T... ..C.GG...G .G.T.GGC-G AA.GCAGA.. TTTTCTTCCT
CMW 5288     .......... ...----... .......... ....---..A GGCCGGCC-- ---------- ----...... ..........
CMW 5587     .......... ...----... .......... ....---..A GGCCGGCC-- ---------- ----...... ..........
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[                    1050       1060       1070       1080       1090       1100       1110     1120]
[                    .          .          .          .          .          .          .          .]
CMW 1856     ------TCCC C--------- ---------- -CACC---GC GCAAGCAGT- ---------- --GGAGCAGG CCCGCCGGCG
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781    TCCCGN.... .TCCCTT... .......... .....ACC.T ....A.G..G TGTTG....G TG........ ..........
CMW 10779    TCCCGC.... .TCCCT.... .......... .....ACC.T ....A.G..G TGTTG....G TG........ ..........
CMW 10780    TCCCGC.... .TCCCTT... .......... .....ACC.T ....A.G..G TGTTG....G TG........ ..........
CMW 10455    TCTGTT.... .TTTTTCTTT TTCCCCCCCT .--T.CCCTT CATCCGT..A AAATCGGGTG CT........ ..........
CMW 10477    TCTGTT.... .TTTTTCTTT TTCCCCCCCT .--T.CCCTT CATCCGT..A AAATCGGGTG CT........ ..........
CMW 10518    TCCTTC---- -..TCGC... .......... CTT.TC...T ....A.G... TGTTG..... ..-....... ..........
CMW 10463    TCCTTC---- -..TCGC... .......... CTT.TACC.T ....A.G... TGTTG..... ..-....... ..........
CMW 7047     TC........ .TCCC..... TCCCCCCCCT CTT..ACC.T ....A.G... TGTTG..... .G........ ..........
CMW 7048     TC........ .TCCC..... TCCCCCCCCT CTT..ACC.T ....A.G... TGTTG..... .G........ ..........
CMW 5288     .....---- -......... TCCCCACCGA GG--.CCCTT .GG.A..A-. .......... .........C ..GC.G.CG.
CMW 5587     .....---- -......... TCCCCACCGA GG--.CCCTT .GG.A..A-. .......... .........C ..GC.G.CG.
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[                    1130       1140       1150       1160       1170       1180       1190    1200]
[                     .          .          .          .          .          .       5.8S →     .]
CMW 1856    GCCCACCAAA CTCTTTGTTT TTAGAA-CGT ATCTCTTCTG AGTGTTTATA ACAAACAAA- -TGAATCAAA ACTTTCAACA
CMW 8756    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853    ......T... .......... .......... .......... .......... .......... .......... ..........
CMW 10667   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775   ......T... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   ......T... .......... .......... .......... .......... .......... .......... ..........
CMW 10778   .......... .......... -......... .......... .......... .......... .......... ..........
CMW 10781   ....GTT... A.T..-.... ...A..AA.. .......... ...AA...A. CA...A...A A......... ..........
CMW 10779   ....GTT... .....-.... ...A..A... .......... ...AA...A. CA...A...A A......... ..........
CMW 10780   ....GTT... .....-.... ...A..A... .......... ...AA...A. CA...A...A A......... ..........
CMW 10455   .....TT... .......... ...A....C. .......... ...T.--.A. .-...A...A A......... ..........
CMW 10477   .....TT... .......... ...A....C. .......... ...T.--.A. .-...A...A A......... ..........
CMW 10518   .......... .......... ...T....C. .......... ...ACA.T.. .-...A...A A......... ..........
CMW 10463   .......... .......... ...T....C. .......... ...ACA.T.. .-...A...A A......... ..........
CMW 7047    ......T... .......... ...T....C. .......... ...ACA..A. CA...A...A .......... ..........
CMW 7048    ......T... .......... ...T....C. .......... ...ACA..A. CA...A...A .......... ..........
CMW 5288    C.-A...... .....-.... C.TAG-.T.A ....---... ...----.A. .A.....T.A A......... ..........
CMW 5587    C.-A...... .....-.... C.TAG-.T.A ....---... ...----.A. .A.....T.A A......... ..........
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[                    1210       1220       1230       1240       1250       1260       1270     1280]
[                    .          .          .          .          .          .          .          .]
CMW 1856             ACGGATCTCT TGGTTCTGGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA
CMW 8756             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8651             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62               .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10667            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781            .......... .......... .......... .......... .......... .........T .......... ..........
CMW 10779            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10780            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10455            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10477            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10518            .......... .......... .........A .......... .......... .......... .......... ..........
CMW 10463            .......... .......... .........A .......... .......... .......... .......... ..........
CMW 7047             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 7048             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 5288             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 5587             .......... .......... .......... .......... .......... .......... .......... ..........
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[                    1290       1300       1310       1320       1330       1340       1350     1360]
[                     .          .          .          .          .          .     ITS 2 →       .]
CMW 1856    TCGAATCTTT GAACGCACAT TGCGCCCGCT GGAATTCCAG CGGGCAT-GC CTGTTCGAGC GTCATTTCAA CCCTCAAGCC
CMW 8756    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9903    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631    .......... .......... .......... .......... .......C.. .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......C.. .......... .......... ..........
CMW 3839    .......... .......... .......... .......... .......C.. .......... .......... ..........
CMW 8651    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62      .......... .......... .......CG. .......... .......... .......... .......... ..........
CMW 2113    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......CG. .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......C.. .......... .......... ..........
CMW 1853    .......... .......... .......... .......... .......C.. .......... .......... ..........
CMW 10667   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10668   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10774   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10778   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10781   .......... .......... .......... .......... .......... .......... .......... .........T
CMW 10779   .......... .......... .......... .......... .......... .......... .......... .........T
CMW 10780   .......... .......... .......... .......... .......... .......... .......... .........T
CMW 10455   .......... .......... .......... .......... .......... .......... .......... .........T
CMW 10477   .......... .......... .......... .......... .......... .......... .......... .........T
CMW 10518   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10463   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 7047    .......... .......... .......... .......... .......... .......... .......... .........T
CMW 7048    .......... .......... .......... .......... .......... .......... .......... .........T
CMW 5288    .......... .......... .......T.. ..T.....G. A......... .......... .......... ..........
CMW 5587    .......... .......... .......T.. ..T.....G. A......... .......... .......... ..........
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[                    1370       1380       1390       1400       1410       1420       1430     1440]
[                    .          .          .          .          .          .          .          .]
CMW 1856     TGGCTTGGTG TTGGGGCACT GCCTGTTTTA CAGCGGGTAG GCCCTGAAAT TTAGTGGCGG GCTCGCTAAG ACTCTGAGCG
CMW 8756     .......... .......... .......... .......... .......... .......... .......... .......A..
CMW 9903     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9906     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2631     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .....N.... .......... .......... .......... .......... .......... ..........
CMW 8651     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8650     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8649     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 2113     .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 8755     .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 8757     .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 1853     .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 10667    .......... .......... A.......-. .......... .......... ...A...... .......... ..........
CMW 10668    .......... .......... A.......-. .......... .......... ...A...... .......... ..........
CMW 10774    .......... .......... .......... .......... ......G... .......... .......... ..........
CMW 10776    .......... .......... A.......-. ........G. .......... ...A...... .......... ..........
CMW 10775    .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 10777    .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 10778    .......... .......... A......C-. .......... .......... ...A...... .......... ..........
CMW 10781    .......... ......T... A..C..---. A.A...-... .......... .......... .......... ..........
CMW 10779    .......... ......T... A..CC.---. A.A...-.G. .......... .......... .......... ..........
CMW 10780    .......... ......T... A..CC.---. A.A...-... .......... .......... .......... ..........
CMW 10455    .A........ .......... A.TC..---. A.A...-... .......... C......... .......... ..........
CMW 10477    .A........ .......... A.TC..---. A.A...-... .......... C......... .......... ..........
CMW 10518    .......... ......T... A..C..---C A.A...-... .......... .......... .......... ..........
CMW 10463    .......... ......T... A..C..---C A.A...-... .......... .......... .......... ..........
CMW 7047     .......... ......T... A..C..---. A.A...-... .......... .......... .......... ..........
CMW 7048     .......... ......T... A..C..---. A.A...-... .......... .......... .......... ..........
CMW 5288     .......... A......... ..T.CCGAG. GG.A.--C.. .......... C........A ......C.G. ..C.C.....
CMW 5587     .......... A......... ..T.CCGAG. GG.A.--C.. .......... C........A ......C.G. ..C.C.....
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[                    1450       1460       1470       1480       1490       1500   ]
[                    .          .          .          .          .          .      ]
CMW 1856     TAGTA--GTT TTTAT---CA -CCTCGCTTT GGAA-GGATT A-GCGG-TGC TCTTGCCGTA AAACC
CMW 8756     .......... .......... .......... .......... ..CGA..... C......... .....
CMW 9903     .......... .......... .......... .......... .......... .......... .....
CMW 9906     .......... .......... .......... .......... .......... .......... .....
CMW 2631     .......... .......... .......... .......... ..-....... .......... .....
CMW 2632     .......... .......... .......... .......... .A........ .......... .....
CMW 3839     .......... .......... .......... .......... ....A..... .......... .....
CMW 8651     .......... .......... .......... .......... .......... .......... .....
CMW 8650     .......... .......... .......... .......... .......... .......... .....
CMW 8649     .......... .......... .......... .......... .......... .......... .....
CMW 62       .......... .......... .......... .......... .......... .......... .....
CMW 2113     .......... .......... .......... .......... .......... .......... .....
CMW 8755     .......... .......... .......... .......... .......... .......... .....
CMW 8757     .......... .......... .......... .......... .......... .......... .....
CMW 1853     .......... .......... .......... .......... .......... .......... .....
CMW 10667    .......... .......... .......... .......... .......... .......... ..-..
CMW 10668    .......... .......... .......... .......... .......... .......... ..-..
CMW 10774    .......... .......... .......... .......... ....A..... .......... .....
CMW 10776    .......... .......... .......... .......... .......... .......... .....
CMW 10775    .......... .......... .......... .......... .......... .......... .....
CMW 10777    .......... .......... .......... .......... .......... ......G... .....
CMW 10778    .......... .......... .......... .......... .......... .......... .....
CMW 10781    .....GT... ...T-CT... A......... .......... ......T... .......... .....
CMW 10779    .....GT... ...T-CT... A......... .......... ......T... .......... .....
CMW 10780    .....GT... ...T-CT... A......... .......... ......T... .......... .....
CMW 10455    .....GTT.. ...T.CTT.C A......... ....A..... ......T... .T........ .....
CMW 10477    .....GTT.. ...T.CTT.C A......... ....A..... ......T... .T........ .....
CMW 10518    .....GTT.. ..---..... A......... .......... ......T... .......... .....
CMW 10463    .....GTT.. ..---..... A......... .......... ......T... .......... .....
CMW 7047     .....GTT.. ...T.CTT.. A......... .......... ......T... .......... .....
CMW 7048     .....GTT.. ...T.CTT.. A......... .......... ......T... .......... .....
CMW 5288     .....GTTA- .A.--...-- .-......CC .......CCC TG........ C..-.....T .....
CMW 5587     .....GTTA- .A.--...-- .-......CC .......CCC TG........ C..-.....T .....
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Appendix 4. Raw sequence data of the two regions within the β-tubulin gene (designated as β-tub 1a/1b and β-tub 2a/2b) and the ITS1, conserved 5.8S and ITS2 regions of the rDNA operon. The start of each region is indicated above the alignment. The exon regions of the β-tubulin gene as well as the conserved 5.8S region of the rDNA operon are in bold and boxed. Unknown sequence characters are indicated with a "**N**", while gaps inserted to achieve sequences alignment are indicated with "**-**". Bases matching those of **CMW 8756** are indicated with a "**.**

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[                   10         20         30         40         50         60         70        80]
[          β-tub 1a/1b →      .          .          .          .          .          .          .]
CMW 8756   TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 1840   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757   GA........ .......... ..A....... .......... .......... .......... .......... ..........
CMW 10453  .......... .......... .......... .......... .......... ........A. .......... ..........
CMW 8758   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113   .......... .......... .......... .......G.. .......... .......... .......... ..........
CMW 10469  .......... ......T... .......... .......... C..G...... .......... .......... ..........
CMW 10470  .......... ......T... .......... .......... C..G...... .......... .......... ..........
CMW 10471  .......... ......T... .......... .......... C..G...... .......... .......... ..........
CMW 10442  .......... ......TT.. .......... .C........ C......... .......... .......... ..........
CMW 2091   .......... ......TT.. .......... .C........ C......... .......... .......... ..........
CMW 10465  .......... .......T.. .......... .C........ C......... .......... .......... ..........
CMW 10477  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10455  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10436  .......... ....CA.T.. ..G.....T. .CC.G..... T......... .......... .......... A.........
CMW 10484  .......... .......T.. ........T. .CC....... T......... .....C.... .......... ..........
CMW 1652   .......... .......... .......... .CC.A..... C......... .......... .......... ..........
CMW 1651   .......... .......... .......... .CC....... C......... .......... .......... ..........
CMW 7037   .......... .......... ..T....... .CC....... .......... .......... .......... ..........
CMW 7036   .......... .......... ..T....... .CC....... .......... ........A. .......... ..........
CMW 10518  .......... .......T.. .......... .CC.A..... T......... .......... .......... ..........
CMW 10463  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10454  .......... .......T.. .......... .......... T..T...C.C .......... .......... ..........
CMW 2498   .......... .......... .......... .C........ T..T...C.C ........A. .......... ..........
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[                    90         100        110        120        130        140        150       160]
[                     .          .          .          .          .          .          .         .]
CMW 8756     ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC CCCCGCCCCT CGCGCCT---
CMW 1840     .......... .......... .......... .......... .......... .......... .......... .......---
CMW 2632     .......... .......... .......... .......... .......... .......... .......... .......---
CMW 8757     .......... .......... .......... .......... .......... .......... T......... .......---
CMW 10453    .......... .......... .......... .......... .......... .......... T......... .......---
CMW 8758     .......... .......... .......... .......... .......... .......... T......... .......---
CMW 1853     .......... .......... .......... .......... .......... .......... T......... .......---
CMW 8755     .......... .......... .......... .......... .......... .......... .......... .......---
CMW 62       .......... .......... .......... .......... .......... .......... .......... .......---
CMW 2113     .......... .......... .......... .......... .......... .......... .......... .......---
CMW 10469    .......... .......... .......... .......... ....T..... .........- ----------- ..TAT..---
CMW 10470    .......... .......... .......... .......... ....T..... .........- ----------- ..TAT..---
CMW 10471    .......... .......... .......... .......... ....T..... .........- ----------- ..TAT..---
CMW 10442    .....C.... .......... ......C..T .......... ....T..... .........G GT-TC....C ACACA.CC--
CMW 2091     .....C.... .......... ......C..T .......... ....T..... .........G GT-TC....C ACACA.CC--
CMW 10465    .....C.... .......... .......... .......... ....T..... .........G GT.TC....C .AACA.CCCA
CMW 10477    .......... .......... .......... .......... ....T..... .........G GTTTTTTTT. TT.TT..TTC
CMW 10455    .......... .......... .......... .......... ....T..... .........G GTTTTTTTT. TT.TT..TTC
CMW 10436    .......... .......... .......... .......... ....T..... .........- ----TATTT. TT.TT.----
CMW 10484    .......... T......... .......... .......... ....T..... .........- ----TATTT. TT.TT.----
CMW 1652     .......... .......... .......... ........A. ....T..... .........- ----TTT.T. GT.TTT----
CMW 1651     .......... .......... .......... ........A. ....T..... .........- ----TTT.T. GC.TTT----
CMW 7037     .......... .G........ ......C..T .......... ....T..... ......C..- ----TTT.TG .TTCTT.---
CMW 7036     .......... .G........ ......C... .......... ....T..... ......C..- ----TTTT-G T--CTT----
CMW 10518    .......... .......... .........T ........T. ....T..... .........- ----TTT.TG T.TCTT----
CMW 10463    .......... .......... T......... ........A. ....T..... .........- ----TTT.TA TA.CTT----
CMW 10454    ........T. .......... .........T .......... ....T..... .........- ----TTAT.. .TAT.-----
CMW 2498     .......... .......... .......... .......... ....T..... .........- ----C...TA A.TC.-----
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[                    170        180        190        200        210        220        230      240]
[                     .          .          .          .          .          .          .         .]
CMW 8756     ---------- CGGGG-AGCA TC-------- ------GGCC GAAGCTTGTC T---GCTAAC TCT-----TA TCG--TC---
CMW 1840     ---------- .....-.... ..-------- ------.... .......... .---...... ...-----.. ...--..---
CMW 2632     ---------- .....-.... ..-------- ------.... .......... .---...... ...-----.. ...--..---
CMW 8757     ---------- ...A.-.... ..-------- ------.... .......... .---.T.... ...-----.. ...--..---
CMW 10453    ---------- ...A.-.... ..-------- ------.... .......... .---...... ...-----.. ...--..---
CMW 8758     ---------- ...A.-.... ..-------- ------.... .......... .---...... ...-----.. ...--..---
CMW 1853     ---------- ..AA.-.... ..-------- ------.... .......... .---...... ...-----.. ...--..---
CMW 8755     ---------- .....-.... ..-------- ------.... ......C... .---...... C..-----C. ...--..---
CMW 62       ---------- .....-C..C ..-------- ------.... ......C... .---...... C..-----C. ...--..---
CMW 2113     ---------- .....-C..C ..-------- ------.... ......C... .---...... C..-----C. ...--..---
CMW 10469    ---------- ..A..-CA.G .--------- ---------T T.G.T..... .---...... C.------.. ..C--..---
CMW 10470    ---------- ..A..-CA.G .--------- ---------T T.G.T..... .---...... C.------.. ..C--..---
CMW 10471    ---------- ..A..-CA.G .--------- ---------T T.G.T..... .---...... C.------.. ..C--..---
CMW 10442    ---------- GTT..-C..C .TTGGGGGGC ------T.T. AGG......T .TTT...G.. C.C-----.. ..CC-..---
CMW 2091     ---------- GCT..-C..C .TTGGGGGGC ------T.T. AGG......T .TTT...G.. C.C-----.. ..CC-..---
CMW 10465    GACC----CC .T...-C..C .TTGGCGGGA GAGGGCT.T. AGG......T .CT-...... C.C-----.. ..CCC..---
CMW 10477    CCCCTTGCCT .CTC.-CAAG .TTCGATAAA GTCGTCTCT- --G......T .--------- C-.GTTTC.C ..CCCC.CCC
CMW 10455    CCCCTTGCCT .CTC.-CAAG .TTCGATAAA GTCGTCTCT- --G......T .--------- C-.GTTTC.C ..CCCC.CCC
CMW 10436    ---CTTGCCT .CTC.-CAGG ..TGGATGAA CTCGTCTTT- --G......T .--------- C-.GCCT-.C C.CCCC.CCC
CMW 10484    ---CTTGCCT .CTC.-CAAG ..TGGATGAA CTCGTCTCT- --G......T .--------- C-.GCCT-.C C.CCCC.CCC
CMW 1652     ---------T .CTC.-CAAG ..TCGACGAA --CGTCTTG- --G...GT.T G--------- C..GTCTT.C ..TCT..CCC
CMW 1651     ---------T .CTC.-CAAG ..TCGACGAA --CGTCTTG- --G...GT.T G--------- C..GTCTT.C ..TCT..CCC
CMW 7037     ------CTCT ATCTCACAA. ..TCGGATCC ACC-TCTCG- --G......T .TT-...... C..GCTTTCC ..TC-..CCC
CMW 7036     ------CTCT GTCTCACA.. ..TCGGATCC ACC-TCTCG- --G......T .TT-...... C..GCTTTCC ..TC-..CCC
CMW 10518    -------CTC .TC.--CAGG C.TCCATGAA --CATCTTG- --G....T.T G--------- C.CATGTT.C ..TCT.TCCC
CMW 10463    ---------- .TC.--CAAG C.TCGATGAA --CATCTCG- --G....C.T G--------- C.CACGTT.C ..TCT.TCCT
CMW 10454    ---------- .CA..-CTTC G.TG------ -----CTTG- --GCGCGTCG .---...G.. .A.--GTT.- ----------
CMW 2498     ---------- .CTC.-CA.. AATA------ ---------- --.AA.G... GCGC...G.. A..GTGCT-- ----------
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[                    250       260       270       280       290       300       310       320]
[                    .         .         .         .         .         .         .         .]
CMW 8756     ------CAGC CGTGGCAAGG TCTCCATGAA GGAGGTTGAG GACCAGATGC GCAACGTCCA GAGCAAGAAC TCGTCCTACT
CMW 1840     ------.... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     ------.... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     ------.... .......... .......... .......... .......... ....T..... .......... ..........
CMW 10453    ------.... .......... .......... .......... .......... ....T..... .......... ..........
CMW 8758     ------.... .......... .......... .......... .......... ....T..... .......... ..........
CMW 1853     ------.... .......... .......... .......... .......... ....T..... .......... ..........
CMW 8755     ------.... .......... .......... ......C... .......... .......... .......... ..........
CMW 62       ------.... .......... .......... ......C... .......... .......... .......... ..........
CMW 2113     ------.... .......... .......... ......C... .......... .......... .......... ..........
CMW 10469    ------.... .......... ....T..... ......C... .......... .......... .......... ..........
CMW 10470    ------.... .......... ....T..... ......C... .......... .......... .......... ..........
CMW 10471    ------.... .......G.. ....T..... ......C... .......... .......... .......... ..........
CMW 10442    ------.... .......... .......... ......C... .......... .......... .......... ..........
CMW 2091     ------.... .......... .......... ......C... .....A.... .......... .......... ..........
CMW 10465    ------.... .......... .......... ......C... .......... .......... .......... ..........
CMW 10477    CCCAAC.... .......... .......... ......A... .......... .......... .......... ..........
CMW 10455    CCCAAC.... .......... .......... ......A... .......... .......... .......... ..........
CMW 10436    CCCAACT... .......... .......... ......C... .......... .......... .......... ..........
CMW 10484    CCCAACT... .......... .......... ......C... .......... .......... .......... ..........
CMW 1652     TTCT-CT... .....T.... .......... ...A..C... .......... .......... .......... ..........
CMW 1651     TTCT-CA... .....T.... .......... ...A..C... .......... .......... .......... ..........
CMW 7037     TA----.... .......... .......... ......C... .......... .......... .......... ..........
CMW 7036     TA----.... .......... .......... ......C... .......... .......... .......... ..........
CMW 10518    CCTT-CT... .......G.. .......... ......C... .......... .......T.. .......... ..........
CMW 10463    -----CT... .......... .......... ......C... .......... .......... .......... ..........
CMW 10454    ------T... .......... .......... ......C... .......... .......... .......... ..T.......
CMW 2498     -----CT... .....A.... .......... ......C... .......... .......... .......... ..A.......
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[                    330        340        350        360        370        380        390        400]
[                     .          .          .          .          .          .          .          .]
CMW 8756             TCGTCGAGTG GATCCCCAAC AACGTCCAGA CCGCCCTCTG CTCCATCCCC CCCAAGGGTC TCAAGATGTC CTCCACCTTT
CMW 1840             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757             ....A..... .......... .......... .......... .......... .......... .......... ..........
CMW 10453            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62               .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10469            .......... .......... .......... .......... .........T .......... .......... .........C
CMW 10470            .......... .......... .......... .......... .........T ....G..... .......... .........C
CMW 10471            .......... .......... .......... .......... .........T .......... .A........ .........C
CMW 10442            .......... .......... .......... .......... .......... ....G..... .......... ..........
CMW 2091             .......... .......... .......... .......... .......... ....G..... .......... ..........
CMW 10465            .......... .......... .......... .......T.. .......... .......... .......... ..........
CMW 10477            .......... .......... .......... .......... .......... ........C. .......... ..........
CMW 10455            .......... .......... .......... .......... .......... ........C. .......... ..........
CMW 10436            .......... .......... .......... .......... .......... ........C. .......... ..........
CMW 10484            .......... .......... .......... .......... .......... ........C. .......... ..........
CMW 1652             .......... ...T...... .......... .......... ......T... ........C. .......... ...G......
CMW 1651             .......... ...T...... .......... .......... ......T... ........C. .......... ...G......
CMW 7037             .......... .......... .......... .......T.. .......... ....G...C. .......... ..........
CMW 7036             .......... .......... .......... .......T.. .......... ....G...C. .......... ..........
CMW 10518            .......A.. .......... ........A. .......... .......... ..T.....C. .......... ...T......
CMW 10463            .......A.. .......... .......... .......... .......... ........C. .......... ...T......
CMW 10454            .......... .......... .......... .......T.. ...G.....T ....G...C. .......... ...T.....C
CMW 2498             .......... .......... .......... .......... ...G.....T .......... .......... ...T.....C
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[                    410        420        430        440        450        460        470        480]
[                     .          .   β-tub 2a/2b       .          .          .          .          .]
CMW 8756     GTTGGCAACT CCACTGCCAT CCAGGAGCTC TTCAAGCGTA TCGGCGAGCA GTTCACTGCT ATGTTCCGTC GCAAGGCTTT
CMW 1840     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10453    .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10469    .......... .T........ .......... .........G .......... .......... .......... ..........
CMW 10470    .......... .T........ .......... .........G .....A.... .......... .......... ..........
CMW 10471    .......... .T........ .......... .........G .......... .......... .......... ..........
CMW 10442    .......... .T..C..... .........T ........CG .T........ ......C..C .......... .......C..
CMW 2091     .......... .T..C..... .........T ........CG .T........ ......C..C .......... .......C..
CMW 10465    .......... ....C..T.. .......... ........CG .T........ ......C..C .......... .......C..
CMW 10477    ..C....... ....C..... .......... .........G .......... ......C..C .......... ..........
CMW 10455    ..C....... ....C..... .......... .........G .......... ......C..C .......... ..........
CMW 10436    ..C....... ....C..... .......... .........G .......... .........C .......... ..........
CMW 10484    ..C....... ....C..... .......... .........G .......... .........C .......... ..........
CMW 1652     ..C....... ....C..... .......... .........G .......... ...T..C..C ........G. ..........
CMW 1651     ..C....... ....C..... .......... .........G .......... ...T..C..C ........G. ..........
CMW 7037     ..C....... ....C..... .......... .........G .T........ ......C..C ........G. ..........
CMW 7036     ..C....... ....C..... .......... .........G .T........ ......C..C ........G. ..........
CMW 10518    ..C....... ....C..... .......... .........G .......... ......C..C .......... ..........
CMW 10463    ..C....... ....C..... .......... .........G .......... ......C..C .......... ..........
CMW 10454    ..G....... .G.....T.. .........T .........G .......... .......... .......... ..........
CMW 2498     ..G..T.... .G.....T.. .........G .........G .G........ .........C ........G. ..........
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[                    490        500        510        520        530        540          550       560]
[                    .          .          .          .          .          .            .          .]
CMW 8756     CTTGCATTGG TACACTGGCA AACCATCTCT GGCGAGCACG GCCTCGACAG CAATGGCGTG TACGT---AC CCT-------
CMW 1840     .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 2632     .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 8757     .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 10453    .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 8758     .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 1853     .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 8755     .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 62       .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 2113     .......... .......... .......... .......... .......... .......... .....---.. ...-------
CMW 10469    .......... .......... .........C .......... .T........ .......... ..T..---.. ...------C
CMW 10470    .......... .......... .........C .......... .T........ .......... ..T..---.. ...------C
CMW 10471    .......... .......... .........C .......... .T........ .......... ..T..---.. ...------C
CMW 10442    .......... .....C.... .........C .......... .......... .G........ ..T..TGT.. ...-------
CMW 2091     .......... .....C.... .........C .......... .......... .G........ ..T..TGT.. ...-------
CMW 10465    .......... .....C.... .........C ........T. .......... .......... ..T..---.. T..-------
CMW 10477    .......... ..T....... .........C .......... .......... ..G....... ..T..---.. .G.GCCTTAC
CMW 10455    .......... ..T....... .........C .......... .......... ..G....... ..T..---.. .G.GCCTTAC
CMW 10436    .......... ..T....... .........C ........T. .......... ..G....... ..T..---.. .G.ACCTTAC
CMW 10484    .......... ..T....... .........C ........T. .......... ..G....... ..T..---.. .G.ACCTTAC
CMW 1652     .......... .......... .........C .......... .......... .......... .....---.. ...ATCT---
CMW 1651     .......... .......... .........C .......... .......... .......... .....---.. ...ATCT---
CMW 7037     .......... .......... .........G .......... .......... .......... ..T..---.. .ACACCATAC
CMW 7036     .......... .......... .........G .......... .......... .......... ..T..---.. .ACACCATAC
CMW 10518    .......... .......... .........C ........T. .......... .......... ..T..---.. T..ACCT---
CMW 10463    .......... .......... .........C ........T. .T........ .......... ..T..---.. ...ACCT---
CMW 10454    .......... .......... ...T...... .......... .T........ .G........ .....----- --AACCT---
CMW 2498     .......... .......... .......... .......... .......... .......... ..T.C----- ---ACCT---
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[                    570        580        590        600        610        620        630        640]
[                     .          .          .          .          .          .          .          .]
CMW 8756     -CCTGCTG-C ACCAGGC--- ---GGCGCGC CTC--GAG-- CTTCCC-GCT GACCA-CCGC ACAGCTACAA CGGCACCTCC
CMW 1840     -.......-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 2632     -.......-. .......--- ---....... ...--...-- ......-... .....-.... .......... ..........
CMW 8757     -.......-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 10453    -.......-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 8758     -.......-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 1853     -.......-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 8755     -....T..-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 62       -....T..-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 2113     -....T..-. .......--- ---....... ...--...-- ......-... .....-.T.. .......... ..........
CMW 10469    A.TGCTC--- C..G...--- ---.A..... ...--...-- ......-... .....-.A.. .......... ..........
CMW 10470    A.TGCTC--- C..G...--- ---.A..... ...--...-- ......-... .....-.A.. .......... ..........
CMW 10471    A.TGCTC--- C..G...--- ---.A..... ...--...-- ......-... .....-.A.. .......... ..........
CMW 10442    A.TGCTG--- C..G.C.--- ---.A..... ...--.G.CC ......C... .....-.... .......... ..........
CMW 2091     A.TGCTG--- C..G.C.--- ---.A..... ...--.G.CC ......C... .....-.... .......... ..........
CMW 10465    A...CGGCTT C..CA..AAG ATA.A....A ..TGT...A- .....T-... ..T..-..A. .T........ ..........
CMW 10477    A..G...TTG C....A.AAG ACA.A..... ..T--...CT ..C.TT-... .....A..A. .......... T.........
CMW 10455    A..G...TTG C....A.AAG ACA.A..... ..T--...CT ..C.TT-... .....A..A. .......... T.........
CMW 10436    A..G...TTG C....A.AAG ACA.A..... ..T--...CT ..C.TT-... .....A..A. .T........ T.........
CMW 10484    A..G...TTG C....A.AAG ACA.A..... ..T--...CT ..C.TT-... .....A..A. .T........ T.........
CMW 1652     --.G...T-- C...A..AAG ACA.A....A ..T--...CT T.C.T--... .....-..A. .T........ ..........
CMW 1651     --.G...T-- C...A..AAG ACA.A....A ..T--...CT T.C.T--... .....-..A. .T........ ..........
CMW 7037     C.TACAC.G. GG.CCA.GCA AGAT.GA... GG.TC.G.CT ..C.T--... .....C.... .T........ ..........
CMW 7036     C.TACAC.G. GG.CCA.GCA AGAT.GA... GG.TC.G.CT T.C.T--... A....C.... GT........ ..........
CMW 10518    --.G...T-- C..CA..AAG ATA.A....A ..TGT...AC T.C.T--... ..T..-..A. .T........ ..........
CMW 10463    --.G...T-- C..CA..AAG ATA.A....A ..TGT...CT T.C.T--... ...G.-..A. .T........ ..........
CMW 10454    --..C.AT-- C-.CAA.TTG CGC.TTTGC. .A--GAGCCT .GCAGTGA.. .....-T.AA ....T..... ......T..T
CMW 2498     --.ACA.T-- C..T.C.CAC TGATCTTG.. ...TCTTCCG GC.TGGCA.. ...A.-T... .T..T..... ......T..T
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[                    650       660       670       680       690       700       710       720]
[                    .         .         .         .         .         .         .         .]
CMW 8756             GAGCTTCAGC TCGAGCGCAT GAACGTCTAC TTCAACGAGG TATGTC--TG TC-------G GG--AC-CAG T-CTGGGGCG
CMW 1840             .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... .-........
CMW 2632             .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-........
CMW 8757             .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-........
CMW 10453            .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-........
CMW 8758             .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-........
CMW 1853             .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-........
CMW 8755             .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-........
CMW 62               .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-........
CMW 2113             .....C.... .......... .......... .......... ......--.. ..-------. ..--..-... G-....C...
CMW 10469            .....C.... .......G.. .......... .......... .....A--.A .T-------. ..-C..-..A G-..---TGA
CMW 10470            .....C.... .......G.. .......... .......... .....A--.A .T-------. ..-C..-..A G-..---TGA
CMW 10471            .....C.... .......G.. .......... .......... .....A--.A .T-------. ..-C..-..A G-..---TGA
CMW 10442            .....C.... .......... .......... .......... .......--.A .G-------. ..-G..-... GC.C---TG-
CMW 2091             .....C.... .......... .......... .......... .......--.A .G-------. ..-G..-... GC.C---TG-
CMW 10465            .....C.... .......... .......... .......... .......-T.A ..-------. ..TG..-... G-..---CGA
CMW 10477            .....C.... .......... .......... .......... ......CT.A ..ATCCATC. ..TG..-..A GG..---CAA
CMW 10455            .....C.... .......... .......... .......... ......CT.A ..ATCCATC. ..TG..-..A GG..---CAA
CMW 10436            .....C.... .......... .......... .......... ......CT.A ..ATC----. ..TG..-... G-..---CGA
CMW 10484            .....C.... .......... .......... .......... ......CT.A ..ATC----. ..TG..-... G-..---CGA
CMW 1652             .....C.... .......... .......... ..T....... .......-T.A ..-------. ..TG.T-..A G-..---CAA
CMW 1651             .....C.... .......... .......... ..T....... .......-T.A ..-------. ..TG.TA..A G-..A--CAA
CMW 7037             .....C.... .......... .......... .......... .......-T.A ..-------. ..TG..-... GC..---CGA
CMW 7036             .....C.... .......... .......... .......... .......-T.. ..-------. .CTG..-... GC..---C.A
CMW 10518            .....C.... .......... .......... .......... .......-T.A ..-------. ..TG..-... G-..---CGA
CMW 10463            .....C.... .......... .......... .......... .......-T.A ..-------. ..TG..-... G-..---CGA
CMW 10454            .....C.... .......... .......... .......... .......--AA .A-------. -.AT..-ATT GC...ATC.C
CMW 2498             .....C.... .......... .......... .......... ..A...--AA CA-------A C-TGCACATC AT.CATCCGA
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[                   730       740       750       760       770       780       790      800]
[                    .         .         .         .         .         .         .         .]
CMW 8756      T-CATCCCGC CCG------- --------CG AACCCCC--T GTG------- --CGTGACCG AG-CTCCCG- --------CT
CMW 1840      .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 2632      .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 8757      .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 10453     .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 8758      .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 1853      .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 8755      .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 62        .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 2113      .-........ ...------- --------.. .......--. ...------- --........ ..-......- --------..
CMW 10469     --.CGT.AT. T.-------- GCCCG----- CGG.TT.--. .G-------- --.A...... ..-.....T- --------..
CMW 10470     --.CGT.AT. T.-------- GCCCG----- CGG.TT.--. .G-------- --.A...... ..-.....T- --------..
CMW 10471     --.CGT.AT. T.-------- GCCCG----- CGG.TT.--. .G-------- --.A...... ..-.....T- --------..
CMW 10442     --GCGTG--- ---------- GCCCCGCC.. CGG....--. .G-------- --........ ..-.....A- --------..
CMW 2091      --GCGTG--- ---------- GCCCCGCC.. CGG....--. .G-------- --........ ..-.....A- --------..
CMW 10465     G-.....AT. T.AGCCCA-- CCCCTGTT.C CT..A.TTC. .G-------- --TACAGG.. ..CT...-TC TT------..
CMW 10477     G-.....AT. T..ACCTTGG GCCCCCCAAC CC..T.T-CC CG--ACTTCT GG.A.AGG.. .AGT....TC TTT-----..
CMW 10455     G-.....AT. T..ACCTTGG GCCCCCCAAC CC..T.T-CC CG--ACTTCT GG.A.AGG.. .AGT....TC TTT-----..
CMW 10436     A-.....AT. T..ACAT--- -CCCCCCC.C CCTTTTT-CC CG--ATTTTG GGAAAAGG.A .ACT.T..TT TTTTTAACGC
CMW 10484     A-.....AT. T..ACAT--- -CCCCCCC.C CC.TTTT-CC .A--AATTTG GGAAAAGGGA .-CT.T..TT TTTTTAAAGG
CMW 1652      GCTTC-A.CT .G.C-A---- ACCCCCCC.C CC.TTT.CGG .GCCTT--GA CTTC..GTAT ..G.GA-GCT TCCTCTT-..
CMW 1651      GCTTC.A.CT GG.CCA---- ACCCCCCC.C CC.TTT.CGG .GCCTTCTGA CTTC..GTAT ..G.GA-GCA TCCTCTT-..
CMW 7037      GC....-AT. .T.CCTCCTC CCTCCTCAT- ---....TCG .G.CTTTTGT GG-CCTGA.C GAG..TG.C- ----CTT-..
CMW 7036      GC....-AT. .T.CCTCCTG CCTCCTCCT- ---T..ATCG .GACTTCTGT GG-CCTGA.C GAG..TG.C- ----CTT-..
CMW 10518     GCATC.-AT. T.AGCCCA-- CCCCTGTT-- ---------- --CCCTC-CA CTTC..GTAC ..G.GA-GCT TCCTCTT-..
CMW 10463     GCATC.-AT. T.AACCCC-- CCCCCCCT.C C.AAT..CGG .CCCCTC-GA CTTC..G.AT ..G.GA-GCT TCCTCTT-..
CMW 10454     CC-C....CA A.TCCACCCC ACCCCCCAGC T.TG------ -----GCTGC CTTCGCT.TC TCGAG.T.TA --------T.
CMW 2498      CCATCT..AA .AC------- ---------- ------GGT. TA-------- ---C..C.GT C.C.C---.G ACCTCGC-TA
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[                    810        820        830        840        850        860        870       880]
[                    .          .          .          .          .          .          .          .]
CMW 8756     GACGCGC--- -------T-C CTGTCACAGG CCTCCGGCAA CAAGTATGTT CCCCGCGCCG TCCTCGTCGA TCTCGAGCCT
CMW 1840     .......--- -------.-. .......... .......... .......... .......... .......... .........C
CMW 2632     .......--- -------.-. .......... .......... .......... .......... .......... ..........
CMW 8757     .......--- -------.-. .......... .......... .........C .......... .......... .........C
CMW 10453    .......--- -------.-. .......... .......... .........C .......... .......... .........C
CMW 8758     .......--- -------.-. .......... .......... .........C .......... .......... .........C
CMW 1853     .......--- -------.-. .......... .......... .........C .......... .......... .........C
CMW 8755     .......--- -------.-. .......... .......... .........C .......... .......... .........C
CMW 62       .......--- -------.-. .......... .......... .........C .......... .......... .........C
CMW 2113     .......--- -------.-. .......... .......... .........C .......... .......... .........C
CMW 10469    .......--- -------.-. T....-.... .......... ...A...... .......... .......T.. .........C
CMW 10470    .......--- -------.-. T....-.... .......... ...A...... .......... .......T.. .........C
CMW 10471    .......--- -------.-. T....-.... .......... ...A...... .......... .......T.. .........C
CMW 10442    .......--- -------.-. .....-.... .......... .........C .......... .......... .........C
CMW 2091     .......--- -------.-. .....-.... .......... .........C .......... .......... .........C
CMW 10465    .......--- -------.T. T.A..-.... .....A.... .......... ........T. .......... .........C
CMW 10477    .......--- -------.TT T....-.... .T....C... .......... .......... .......... .........G
CMW 10455    .......--- -------.TT T....-.... .T....C... .......... .......... .......... .........G
CMW 10436    -C.TTTTTTT TTTTTTT.TT T.----.... .T....C... .......... .......... .......... .........C
CMW 10484    .C.TTTTTTT TTTTTTT.TT T.----.... .T....C... .......... .......... .......... .........C
CMW 1652     .......--- -------.T. T....-.... .......... .......... .......... .......... .........C
CMW 1651     .......--- -------.T. T....-.... .......... .......... .......... .......... .........C
CMW 7037     ......T--- -------.T. TC...-.... .......... .......... .......... .......... .........C
CMW 7036     ......T--- -------.T. TC...-.... .......... .......... .......... .......... .........C
CMW 10518    .......--- -------.T. T.A..-.... .....A.... .......... ........T. .......... .........C
CMW 10463    .......--- -------.T. T.A..-.... .....A.... .......... ........A. .......... .........C
CMW 10454    ......T--- -------.-. TC...-.... .T........ .......... ..T....... .......... .........C
CMW 2498     -...T.T--- -------.-A TC.C.-.... .......... .......... ..T....... .......... .........C
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[                    890        900        910        920        930        940        950       960]
[                     .          .          .          .          .          .          .     ITS1 →]
CMW 8756     GGCACCATGG ACGCCGTCCG TGCCGGCCCC TTCGGCCAGC TGTTCCGCCC CGACAACTTC GTCTTCGGCC AGTCCCCCAG
CMW 1840     .......... .......... .......... .......... .......... .......... .......... .........A
CMW 2632     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10453    .......... .......... .......... .......... .......... .......... .......... ...T......
CMW 8758     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10469    ..T....... .......... C.....T... ..T..G.... .......T.. .......... .......... ..........
CMW 10470    ..T....... .......... C.....T... ..T..G.... .......T.. .......... .......... ..........
CMW 10471    ..T....... .......... C.....T... ..T..G.... .......T.. .......... .......... ..........
CMW 10442    ..T....... .......... C......... .......... .......... .......... .......... ..........
CMW 2091     ..T....... .......... C......... .......... .......... .......... .......... ..........
CMW 10465    ..T....... .......... C......... ..T....... .......T.. .......... .....T.... ..........
CMW 10477    ..T....... .T........ C......... ..T..T.... .......T.. .......... .....T.... ..........
CMW 10455    ..T....... .T........ C......... ..T..T.... .......T.. .......... .....T.... ..........
CMW 10436    ..T....... .T........ C......... ..T..T.... .......T.. .......... .....T.... ..........
CMW 10484    ..T....... .T........ C......... ..T..T.... .......T.. .......... .....T.... ..........
CMW 1652     ..T....... .T........ C..T...... ..T..T.... .......T.. .......... .....T.... ..........
CMW 1651     ..T....... .T........ C..T...... ..T..T.... .......T.. .......... .....T.... ..........
CMW 7037     ..T....... .T........ C......... .......... .......T.. .......... .......... ..........
CMW 7036     ..T....... .T........ C......... .......... .......T.. .......... .......... ..........
CMW 10518    ..T....... .......... C......... ..T....... .......T.. .......... .....T.... ..........
CMW 10463    ..T....... .T........ C......... ..T..T.... .......T.. .......... .....T.... ..........
CMW 10454    ..T....... ....T..... ......T... .....A.... .......... .......... ..T....... ..........
CMW 2498     ..T....... .......... ......T... ..T....... .C........ .......... .......... ..........
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[                    970        980        990        1000       1010       1020       1030    1040]
[                    .          .          .          .          .          .          .          .]
CMW 8756     ATACCCT-TT GTGAACTTAT ACCTTTTT-- -ATCGTTGCC TCGGCGCCGA GC-CGGGAGT ---------- ----------
CMW 1840     .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 2632     .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 8757     .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 10453    .......-.. .......... ........-- -......... .......... ..-T...... ---------- ----------
CMW 8758     .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 1853     .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 8755     .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 62       .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 2113     .......-.. .......... ........-- -......... .......... ..-....... ---------- ----------
CMW 10469    .......-.. .......... ....A...-- -......... .......T.. ..-....G.C ---------- ----------
CMW 10470    .......-.. .......... ....A...-- -......... .......T.. ..-....G.C ---------- ----------
CMW 10471    .......-.. .......... ....A...-- -......... .......T.. ..-....G.C ---------- ----------
CMW 10442    .......-.. .......... ...A....-- -......... .......T.. ..-T...G.C ---------- ----------
CMW 2091     .......-.. .......... ...A....-- -......... .......T.. ..-T...G.C ---------- ----------
CMW 10465    .......-.. .......... ...A....T- -......... .......T.. ..-T...G.C ---------- ----------
CMW 10477    .......-.. .......... ...A....-- -......... .......T.. ..C....G.G AGGGAAAAAA AAAAAAAAAA
CMW 10455    .......-.. .......... ...A....-- -......... .......T.. ..C....G.- AGGGAAAAAA AAAAAAAAAA
CMW 10436    .......-.. .......... ...AC...-- -G........ .......T.. ..C....G.G GAGGAAACAA GTAAAAGAGG
CMW 10484    .......-.. .......... ...AC...-- -G........ .......T.. ..C....G.G GAGGAAACAA GTAAAAGAGG
CMW 1652     .......-.. .......... .A.CA...T- -......... .......T.. ..CTCT.G.G G--------- -GGGGTTGGC
CMW 1651     .......-.. .......... .A.CA...T- -......... .......T.. ..CTCT.G.G G--------- -GGGGTTGGC
CMW 7037     .......-A. .......... ........TT A......... .......T.. ..C....G.G ---------- --AA------
CMW 7036     .......-A. .......... ........TT -......... .......T.. ..C....G.G G--------- --AA------
CMW 10518    .......-.. .......C.. .-.CA...T- -......... .......T.. ..C.C..G.G G--------- --GA-TTTTT
CMW 10463    .......-.. .......C.. .-.CA...T- -......... .......T.. ..C.C..G.G G--------- --GA-TTTTT
CMW 10454    .A.....T.. .......... ....A..--- -......... .......T.- ---------- ---------- ----------
CMW 2498     .A.....-.. .......... .....A.--- -C.-...... .......TA- ---------- ---------- ----------
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[                    1050       1060       1070       1080       1090       1100       1110     1120]
[                     .          .          .          .          .          .          .          .]
CMW 8756     ---------- ---------G CTCTT--CTG TGCTCCCCC- ---------- ACCGCGCAAG CA---GTG-- -GAGCAGGCC
CMW 1840     ---------- ---------. .....--... .........- ---------- .........C G.---...-- -.........
CMW 2632     ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 8757     ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 10453    ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 8758     ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 1853     ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 8755     ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 62       ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 2113     ---------- ---------. .....--... .........- ---------- .......... ..---...-- -.........
CMW 10469    ---------- ---------A ....CTT... ..TG.....C ---------C ....T..... .G---...-- -.........
CMW 10470    ---------- ---------A ....CTT... ..TG.....C ---------C ....T..... .G---...-- -.........
CMW 10471    ---------- ---------A ....CTT... ..TG.....C ---------C ....T..... .G---...-- -.........
CMW 10442    ---------- ---------A ....C--... ...C.....- ---------- ....T..... .G---...-- -.........
CMW 2091     ---------- ---------A ....C--... ...C.....- ---------- ....T..... .G---...-- -.........
CMW 10465    ---------- ---------A ....C--... .ATG.....C CCT------T ....TA.TC. .G---...G- -.........
CMW 10477    GGGGGGAAAT TTGTTTCCCC ..T..T-T.T .TTC.....C TTCCCCTTTA T...G.A..A ATCGG.G.CT G..A......
CMW 10455    GGGGGGAAAT TCTGTTTCCC ..T..C--.T .TTC.....C TTCCCCTTCA T...T.T..A ATCGG...CT G.......G.
CMW 10436    AGAATCTTTT TC---TCCTT ..T..C-T.T .TTC.....C TTCCCCTCCA T...T.T... A.CGGT..CT G.........
CMW 10484    AGAATCTTTT TC---TCCTT ..T..C-T.T .TTC.....C TTCCCCTCCA T...T.T... A.CGGT..CT G.........
CMW 1652     GAAGGCAGAT TTTCTTCCTT ...CCC-TCC CT.C.....C T----CTTCC ....T....A .GGTT..TGG G.........
CMW 1651     GAAGGCAGAT TTTCTTCCTT ...CCC-TCC CT.C.....C T----CTTCC ....T....A .GGTT..TGG G.........
CMW 7037     GAG---AAAG CTTG------ ..T.CCCTCC CT.C...-TT C-----ACGG GTGTAAA..C .CAGT..TG- -.........
CMW 7036     GAG---AAAG CTTG------ ..T.CCCTCC CT.C....TT C-----ACGG GTGTAAA..C .-AGT..TG- -.........
CMW 10518    GAG---AGAG TCTC-----T ....CC-T.C CTTCT.G..T TCT------- --..T....A .GGTT..TG- -.........
CMW 10463    GAG---AGAG TCTC-----T ....CC-T.C CTTCT.G..T TCT------- ....T....A .GGTT..TG- -.........
CMW 10454    ---------- ---------. ..GCC----C ..TC..T..G GG--GATAGG GG.C.C.TCT .CGGA.G.GA CA-.......
CMW 2498     ---------- ---------. ..G------- -.TC..T.GG G--------G C..T.A.CCT .GGGT..T-- -...ACA...
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[                    1130       1140       1150       1160       1170       1180       1190    1200]
[                    .          .          .          .          .          .          .       5.8S]
CMW 8756     CGCCGGCGGC CCACCAAACT CTTTGTTTTT AGAA-CGTAT CTCTTCTGAG TG--TTTATA ACAAACAAAT GAATCAAAAC
CMW 1840     .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 2632     .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 8757     .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 10453    .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 8758     .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 1853     .......... ....T..... .......... ....-..... .......... ..--...... .......... ..........
CMW 8755     .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 62       .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 2113     .......... .......... .......... ....-..... .......... ..--...... .......... ..........
CMW 10469    .......... ...T...... ..C....... ....-..... .......... .T--....-. .......... ..........
CMW 10470    .......... ...T...... ..C....... ....-..... .......... .T--....-. .......... ..........
CMW 10471    .......... ...T...... ..C....... ....-..... .......... .T--....-. .......... ..........
CMW 10442    .......... .......... .......... ...C-..... ...C...... ..--....C. .-........ ..........
CMW 2091     .......... .......... .......... ...C-..... ...C...... ..--....C. .-........ ..........
CMW 10465    .......... .T........ ...-.G.... ....-..... .......... .---....C. .-..CA...A T.........
CMW 10477    .C........ ...TT..... T......... .A..-.C... ..T..T.... .T--.A-.A. .A...A.... ..........
CMW 10455    .......... ...TT..... .....G.... .A..A.C... .....T.... .T--.A-.A. .A...A.... ..........
CMW 10436    .......... ...T...... .......... .A..-.C... .......... .A--AA..A. CA...A.... ..........
CMW 10484    .......... ...T...... .......... .A..-.C... .......... .A--AA..A. CA...A.... ..........
CMW 1652     .......... ....T..... .......... .T..-.C... .......... .ACA.AA.C. .-...A.... ..........
CMW 1651     .......... ....T..... .......... .T..-.C... .......... .ACA.AA.C. .-...A.... ..........
CMW 7037     .......... ...TT..... ...GT..... .T..-.C... .......... .---..C... .-.C.A.... ..........
CMW 7036     .......... ...TT..... ...GT..... .T..-.C... .......... .---..C... .-.C.A.... ..........
CMW 10518    .......... .......... .......... .T..-.C... .......... .ACA....A. .-...A.... ..........
CMW 10463    .......... .......... .......... .T..-.C... .......... .ACA....A. .-...A.... ..........
CMW 10454    .......... .T..TT.... ...-...... .CTG---AG. A......... .------.A. G.TTCT.... ..........
CMW 2498     ..T....... .A...T..A. ...-...... .C.C---.GA AA..-..... C------.C. .A.-CAT... ..........
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[                    1200       1210       1220       1230       1240       1250       1260     1270]
[                    .          .          .          .          .          .          .          .]
CMW 8756             TTTCAACAAC GGATCTCTTG GTTCTGGCAT CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
CMW 1840             .......... .......... .......... .......... ....C.C... ...C....C. .......... ..........
CMW 2632             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10453            .......... .......... .......... .......... .C..A..... .......... .......... ..........
CMW 8758             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62               .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10469            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10470            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10471            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10442            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2091             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10465            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10477            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10455            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10436            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10484            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1652             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 7037             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 7036             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10518            .......... .......... .......... .......A.. .......... .......... .......... ..........
CMW 10463            .......... .......... .......... .......A.. .......... .......... .......... ..........
CMW 10454            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2498             .......... .......... .......... .......... .......... .......... .......... ..........
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[                    1280       1290       1300       1310       1320       1330       1340     1350]
[                     .          .          .          .          .          .          .   ITS 2 →]
CMW 8756             GTGAATCATC GAATCTTTGA ACGCACATTG CGCCCCGTGG AATTCCAGCG GGCATGCCTG TTCGAGCGTC ATTTCAACCC
CMW 1840             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 2632             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 8757             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10453            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 8758             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 1853             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 8755             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62               .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10469            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10470            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10471            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10442            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 2091             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10465            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10477            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10455            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10436            .......... .......... .......... .....GC... C......... .......... .......... ..........
CMW 10484            .......... .......... .......... .....GC... C......... .......... .......... ..........
CMW 1652             ........G. .......... .......... .....GC... .......... ......-... .......... ..........
CMW 1651             ........G. .......... .......... .....GC... .......... ......-... .......... ..........
CMW 7037             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 7036             .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10518            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10463            .......... .......... .......... .....GC... .......... .......... .......... ..........
CMW 10454            .......... .......... .......... .....TC... T......A.. .......... .......... ..........
CMW 2498             .......... .......... .......... .....TC... T.....G.A. .......... .......... ..........
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[                    1360       1370       1380       1390       1400       1410       1420    1430]
[                    .          .          .          .          .          .          .          .]
CMW 8756     TCAAGCC--- TGGCTTGGTG TTGGGGCACT GCCTG----- TTTTACAGCG GGTAGGCCCT GAAATTTAGT GGCGGGCTCG
CMW 1840     .......--- .......... .......... ....----- .......... .......... .......... ..........
CMW 2632     .......--- .......... .......... ....----- .......... .......... .......... ..........
CMW 8757     .......--- .......... .......... A...----- ..C-...... .......... ........A. ..........
CMW 10453    .......--- .......... .......... A...----- ...-...... .......... ........A. ..........
CMW 8758     .......--- .......... .......... A...----- ..C-...... .......... ........A. ..........
CMW 1853     .......--- .......... .......... A...----- ..C-...... .......... ........A. ..........
CMW 8755     .......--- .......... .......... A...----- ..C-...... .......... ........A. ..........
CMW 62       .......--- .......... .......... A...----- ..C-...... .......... ........A. ..........
CMW 2113     .......--- .......... .......... A...----- ..C-...... .......... ........A. ..........
CMW 10469    .......--- .......... .......... A...----- .AAA..---- .......... .......... ..........
CMW 10470    .......--- .......... .......... A...----- .AAA..---- .......... .......... ..........
CMW 10471    .......--- .......... .......... A...----- .AAA..---- .......... .......... ..........
CMW 10442    .......--- .......... .......... A...----- .ACA..---- .......... .......... ..........
CMW 2091     .......--- .......... .......... A...----- .ACA..---- .......... .......... ..........
CMW 10465    .......--- .A........ .-........ A...----- .ACA..---- .......... .....A.... ..........
CMW 10477    ......T--- .A........ .......... A.TC----- .AAA..---- .......... .....C.... ..........
CMW 10455    ......T--- .A........ .......... A.TC----- .AAA..---- .......... .....C.... ..........
CMW 10436    ......T--- .......... .......... A..C----- .AAA..---- .......... .......... ..........
CMW 10484    ......T--- .......... .......... A..C----- .AAA..---- .......... .......... ..........
CMW 1652     ......T--- .......... ......T... A..C----- .AAA..---- .......... .......... ..........
CMW 1651     ......T--- .......... ......T... A..C----- .AAA..---- .......... .......... ..........
CMW 7037     .......CCT .......... .......... A..C----- .AAA..---- .......... .......... ..........
CMW 7036     .......CCT .......... .......... A..CC----- .AAC..---- .......... ......G... ..........
CMW 10518    .......--- .......... ......T... A..C----- .CAA..---- .......... .......... ..........
CMW 10463    .......--- .......... ......T... A..C----- .CAA..---- .......... .......... ..........
CMW 10454    .......--- .A........ ........T. A....ACTGT ..AC.GGAG- ....A..... .....C.... ....A.....
CMW 2498     .......--- .......... A.......-- --...-CTTC ..ACC..AGA A.C....... ......C... ....A.....
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[                    1440       1450       1460       1470       1480       1490       1500     1510]
[                     .          .          .          .          .          .          .         .]
CMW 8756     CTAAGACTCT GAACGTAGTA GTTTTTAT-- ----CACCTC GCTTTGGAA- GGATTA-CGA GTGCCCTT-G CCGTAAAACC
CMW 1840     .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. ..........
CMW 2632     .......... ..G....... ........-- ----...... .........- ......AGCG ....T...-. ..........
CMW 8757     .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. ..........
CMW 10453    .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. .......C..
CMW 8758     .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. ..........
CMW 1853     .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. ..........
CMW 8755     .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. ..........
CMW 62       .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. ..........
CMW 2113     .......... ..G....... ........-- ----...... .........- ......-GCG ....T...-. ..........
CMW 10469    .......... ..G....... ...--..C-- ----...... .........- .A....-GCG ....T...-. ..........
CMW 10470    .......... ..G....... ...--..C-- ----...... .........- .A....-GCG ....T...-. ..........
CMW 10471    .......... ..G....... ...--..C-- ----...... .........- .A....-GCG ....T...-. ..........
CMW 10442    .......... ..G....... ...--...-- ----...... .........- ......-GCG ....T...-. ..........
CMW 2091     .......... ..G....... ...--...-- ----...... .........- ......-GCG ....T...-. ..........
CMW 10465    .......... AGG....... ...--...-- ----...... .........- ......-GCG ....T...-. ..........
CMW 10477    .......... ..G....... ......T.TT CTTC...... .........A ......-GCG ..TG.T..T. ..........
CMW 10455    .......... ..G....... ......T.TT CTTC...... .........A ......-GCG ..TG.T..T. ..........
CMW 10436    .......... ..G....... ......T.-- ---C...... .........- ......-GCG ..TG.T..T. ..........
CMW 10484    .......... ..G....... ......T.-- ---C...... .........- ......-GCG ..TG.T..T. ..........
CMW 1652     .......... ..G.C..... ......T.TT CTTCA..... .........- ......-GCG ..TG.TC.T. ..........
CMW 1651     .......... ..G....... ......T.TT CTTCA..... .........- ......-GCG ..TG.TC.T. ..........
CMW 7037     .......... ..G....... ........-- ---CA..... .........- ......-GCG ..TG.TC.T. ..........
CMW 7036     .......... ..G....... ........-- ---CA..... .........- ......-GCG ..TG.TC.T. ..........
CMW 10518    .......... ..G....... ......T--- ---CA..... .........- ......-GCG ..TG.TC.T. ..........
CMW 10463    .......... ..G....... ......T--- ---CA..... .........- ......-GCG ..TG.TC.T. ..........
CMW 10454    .C.G.....C ..G..C.... ..AA------ ---AAC.... ........TC .T.C.G-GCG CG.....--. ..........
CMW 2498     .C.G...C.C ..G..C.... ...A------ ---AAC.... ...C.....G .CCC.G-GCG ........--. ....T.....
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Appendix 5. Raw sequence data of the two regions (designated as β-tub 1a/1b and β-tub 2a/2b) within the β-tubulin gene and the ITS1, 5.8S and ITS2 regions of the rDNA operon. The start of each region is indicated above the alignment. The exon regions of the β-tubulin gene as well as the conserved 5.8S region of the rDNA operon are in bold and boxed. Unknown sequence characters are indicated with a "**N**", while gaps inserted to achieve sequences alignment are indicated with "**-**". Bases matching those of **CMW 8757** are indicated with a "**.**"

```
[                   10         20         30         40         50         60         70         80]
[          β-tub 1a/b →         .          .          .          .          .          .          .]
CMW 8757   TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 8758   GA........ .......... ..A....... .......... .......... .......... .......... ..........
CMW 1853   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113   .......... .......... .......... .......G.. .......... .......... .......... ..........
CMW 8755   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2628   .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10782  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10784  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10783  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10786  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10787  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 11294  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10785  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10518  .......... .......T.. .......... .CC.A..... T......... .......... .......... ..........
CMW 10463  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10484  .......... .......T.. ........T. .CC....... T......... .....C.... .......... ..........
CMW 10436  .......... ....CA.T.. ..G.....T. .CC.G..... T......... .......... .......... A.........
CMW 10790  .......... .......... .......... .CC....... C......... .......... .......... ..........
CMW 7047   .......... .......... .......... .CC....... C......... .......... .......... ..........
CMW 7048   .......... .......... ..T....... .CC....... C......... .......... .......... ..........
CMW 1651   .......... .......... .......... .CC....... C......... .......... .......... ..........
CMW 1652   .......... .......... .......... .CC.A..... C......... .......... .......... ..........
CMW 10477  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10455  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10788  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10789  .......... .......T.. .......... .CC....... T......... .......... .......... ..........
CMW 10442  .......... ......TT.. .......... .C........ C......... .......... .......... ..........
CMW 2091   .......... ......TT.. .......... .C........ C......... .......... .......... ..........
CMW 10465  .......... .......T.. .......... .C........ C......... .......... .......... ..........
CMW 5288   .......... C......... ........T. .C........ .......C.C .......... .......... ..........
CMW 5587   .......... C......... ........T. .C........ .......C.C .......... .......... ..........
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[                    90         100        110        120        130        140        150      160]
[                     .          .          .          .          .          .          .         .]
CMW 8757     ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC TCCCGCCCC- ----------
CMW 8758     .......... .......... .......... .......... .......... .......... .........- ----------
CMW 1853     .......... .......... .......... .......... .......... .......... .........- ----------
CMW 62       .......... .......... .......... .......... .......... .......... C........- ----------
CMW 2113     .......... .......... .......... .......... .......... .......... C........- ----------
CMW 8755     .......... .......... .......... .......... .......... .......... C........- ----------
CMW 1840     .......... .......... .......... .......... .......... .......... C........- ----------
CMW 8756     .......... .......... .......... .......... .......... .......... C........- ----------
CMW 2628     .......... .......... .......... .......... .......... .......... C........- ----------
CMW 10782    .......... .......... .........T ........T. ....T..... .........T .T.T.TGT.T TCTCC-----
CMW 10784    .......... .......... .........T ........T. ....T....A .........T .T.T.TGT.T TCTCC-----
CMW 10783    .......... .......... .........T ........T. ....T..... .........T .T.T.TGT.T TCTCC-----
CMW 10786    .......... .......... .........T ........T. ....T..... .........T .T.T.TGT.T TCTCC-----
CMW 10787    .......... .......... .........T ........T. ....T..... .........T .T.T.TGT.T TCTCC-----
CMW 11294    .......... .......... .........T ........T. ....T..... .........T .T.T.TGT.T TCTCC-----
CMW 10785    .......... .......... .........T ........T. ....T..... .........T .T.T.TGT.T TCTCC-----
CMW 10518    .......... .......... .........T ........T. ....T..... .........T .T.T.TGT.T TCTCC-----
CMW 10463    .......... .......... T......... ........A. ....T..... .........T .T.TATA..T TC--------
CMW 10484    .......... T......... .......... .......... ....T..... .........T ATTTTTT.TT CCTT------
CMW 10436    .......... .......... .......... .......... ....T..... .........T ATTTTTT.TT CCTT------
CMW 10790    .......... .......... .......... ........A. ....T..... .........T .T.TT-GT.T TTTCC-----
CMW 7047     .......... .......... .......... ........A. ....T..... .........T .T.TT-GT.T TTTCC-----
CMW 7048     .......... .......... .......... ........A. ....T..... .........T .T.TT-GT.T TTTCC-----
CMW 1651     .......... .......... .......... ........A. ....T..... .........T .T.TT-G..T TTTCC-----
CMW 1652     .......... .......... .......... ........A. ....T..... .........T .T.TT-GT.T TTTCC-----
CMW 10477    .......... .......... .......... .......... ....T..... .........G GTTTTTTTTT TTCTTCTTTC
CMW 10455    .......... .......... .......... .......... ....T..... .........G GTTTTTTTTT TTCTTCTTTC
CMW 10788    .......... .......... .......... .......... ....T..... .........G GTTTTTTTTT TTCTTCTTTC
CMW 10789    .......... .......... .......... .......... ....T..... .........G GTTTTTTTTT TTCTTCTTTC
CMW 10442    .....C.... .......... ......C..T .......... ....T..... .........G GT-------- --------TC
CMW 2091     .....C.... .......... ......C..T .......... ....T..... .........G GT-------- --------TC
CMW 10465    .....C.... .......... .......... .......... ....T..... .........G GT.------- --------TC
CMW 5288     .....C.... .......... T..T...... .......... ....T..... .......... C..T.AG.AT CT--------
CMW 5587     .....C.... .......... T..T...... .......... ....T..... .......... C..T.AG.AT CT--------
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[                    170        180        190        200        210        220        230      240]
[                    .          .          .          .          .          .          .          .]
CMW 8757     ---------- --TCGC--GC CTCGGAGAGC ATCGGCCGAA ---------- ---GCTTGTC T---GCTAAC TCTTATC---
CMW 8758     ---------- --....--.. .......... .......... ---------- --......... .---.T.... .......---
CMW 1853     ---------- --....--.. ....A..... .......... ---------- --......... .---...... .......---
CMW 62       ---------- --....--.. .....G.C.. C......... ---------- ---...C... .---...... C..C...---
CMW 2113     ---------- --....--.. .....G.C.. C......... ---------- ---...C... .---...... C..C...---
CMW 8755     ---------- --....--.. .....G.... .......... ---------- ---...C... .---...... C..C...---
CMW 1840     ---------- --....--.. .....G.... .......... ---------- --......... .---...... .......---
CMW 8756     ---------- --....--.. .....G.... .......... ---------- --......... .---...... .......---
CMW 2628     ---------- --....--.. .....G.... .......... ---------- --......... .---...... .......---
CMW 10782    ---------- --....AG.. ...CAT..A. ...TTG---- ---------- --G....T.T G---...... C.C-..GTTT
CMW 10784    ---------- --....AG.. ...CAT..A. ...TTG---- ---------- --G....T.T G---...... C.C-..GTTT
CMW 10783    ---------- --....AG.. ...CAT..A. ...TTG---- ---------- --G....T.T G---...... C.C-..GTTT
CMW 10786    ---------- --....AG.. ...CAT..A. ...TTG---- ---------- --G....T.T G---...... C.C-..GTTT
CMW 10787    ---------- --....AA.. ...CAT..A. ...TTG---- ---------- --G....T.T G---...... C.C-..GTTT
CMW 11294    ---------- --....AA.. ...CAT..A. ...TTG---- ---------- --G....T.T G---...... C.C-..GTTT
CMW 10785    ---------- --....AA.. ...CAT..A. ...TTG---- ---------- --G....T.T G   ...... C.C ..GTTT
CMW 10518    ---------- --....AG.. ...CAT..A. ...TTG---- ---------- --G....T.T G---...... C.C-..GTTT
CMW 10463    ---------- --....AA.. ....AT..A. ...TCG---- ---------- --G....C.T G---...... C.C-.CGTTT
CMW 10484    ------GCCT CC....AA.T ..G.AT..A. ---TCGTCTC T--------- --G......T .---...... C-.GCC-TTC
CMW 10436    ------GCCT CC....AG.T ..G.AT..A. ---TCGTCTT T--------- --G......T .---...... C-.GCC-TTC
CMW 10790    ---------- --....AG.T ....AC..A. G..T.G---- ---------- --G...GT.G G---...... C..GTCTTTC
CMW 7047     ---------- --....AG.T ..A.ACA.A. G.TTCG---- ---------- --G...GT.G G---...... C..GTCTTTC
CMW 7048     ---------- --....AG.T ....ACA.A. G..TTG---- ---------- --G...GT.T G---...... C..GTCTTTC
CMW 1651     ---------- --....AA.T ....AC..A. G..TTG---- ---------- --G...GT.T G---...... C..GTCTTTC
CMW 1652     ---------- --....AA.T ....AC..A. G..TTG---- ---------- --G...GT.T G---...... C..GTCTTTC
CMW 10477    CCCCTTGCCT CC....AA.T T...ATA.AG ---TCGTCTC T--------- --G......T .---...... C-.GT.TCTC
CMW 10455    CCCCTTGCCT CC....AA.T T...ATA.AG ---TCGTCTC T--------- --G......T .---...... C-.GT.TCTC
CMW 10788    CCCCTTGCCT CC....AA.T T...AT..AG ---TCGTCTC T--------- --G......T .---...... C-.GT.TTTT
CMW 10789    CCCCTTGCCT CC....AA.T T...AT..AG ---TCGTCTC T--------- --G......T .---...... C-.GT.TTTT
CMW 10442    CCCCCACACA CCCGTTGG-- ------CGC. T.T..-G.G- ----GCTGTC AGG......T .TTT...G.. C.C-----TA
CMW 2091     CCCCCACACA CCCGCTGG-- ------CGC. T.T..-G.G- ----GCTGTC AGG......T .TTT...G.. C.C-----TA
CMW 10465    CCCCCCAACA CCC.AGACC. .CT..GCGC. T.T...G.G. GAGGGCTGTC AGG......T .CT-...... C.C-----TA
CMW 5288     -----CCACA CGA.C.AA.T ---------- ---------- ---------- -------..T .GC-..GCTG A.ACTGT---
CMW 5587     -----CCACA CGA.C.AA.T ---------- ---------- ---------- -------..T .GC-..GCTG A.ACTGT---
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[                    250        260        270        280        290        300        310        320]
[                     .          .          .          .          .          .          .          .]
CMW 8757     ---------- ---GTCCAGC CGTGGCAAGG TCTCCATGAA GGAGGTTGAG GACCAGATGC GCAATGTCCA GAGCAAGAAC
CMW 8758     ---------- ---....... .......... .......... .......... .......... .......... ..........
CMW 1853     ---------- ---....... .......... .......... .......... .......... .......... ..........
CMW 62       ---------- ---....... .......... .......... ......C... .......... ....C..... ..........
CMW 2113     ---------- ---....... .......... .......... ......C... .......... ....C..... ..........
CMW 8755     ---------- ---....... .......... .......... ......C... .......... ....C..... ..........
CMW 1840     ---------- ---....... .......... .......... .......... .......... ....C..... ..........
CMW 8756     ---------- ---....... .......... .......... .......... .......... ....C..... ..........
CMW 2628     ---------- ---....... .......... .......... .......... .......... ....C..... ..........
CMW 10782    CTCTCTTTCC CCCT..T... .......... .......... ......C... .......... ....C..T.. ..........
CMW 10784    CTCTCTTTCC CCCT..T... .......... .......... ......C... .......... ....C..T.. ..........
CMW 10783    CTCTCTTTCC CCCT..T... .......... .......... ......C... .......... ....C..T.. ..........
CMW 10786    CTCTCTTTCC CCCT..T... .......... .......... ......C... .....A.... ....C..T.. ..........
CMW 10787    CTCTCTTTCC CCCT..T... .......... .......... ......C... .......... ....C..T.. ..........
CMW 11294    CTCTCTTTCC CCCT..T... .......... .......... ......C... .......... ....C..T.. ..........
CMW 10785    CTCTCTTTCC CCCT..T... .......... .......... ......C... .......... ....C..T.. ..........
CMW 10518    CTCTCTTTCC CCCT..T... .......G.. .......... ......C... .......... ....C..T.. ..........
CMW 10463    CTCTCTTTCC ----..T... .......... .......... ......C... .......... ....C..... ..........
CMW 10484    CCCCCCCCCC CCCAA.T... .......... .......... ......C... .......... ....C..... ..........
CMW 10436    CCCCCCCCCC CCCAA.T... .......... .......... ......C... .......... ....C..... ..........
CMW 10790    TCTCTTCCCC TT-C..T... .....T.... .......... ...A..C... .......... ....C..... ..........
CMW 7047     TCTCTTCCCC TT-C..T... ..G..T.... .......... ...A..C... .......... ....C..... ..........
CMW 7048     TCTCTTCCCC TT-C.TT... .....T.... .......... ...A..C... .......... ....C..... ..........
CMW 1651     TCTCTTCCCC TT-C..A... .....T.... .......... ...A..C... .......... ....C..... ..........
CMW 1652     TCTCTTCCCC TT-C..T... .....T.... .......... ...A..C... .......... ....C..... ..........
CMW 10477    TCCCCCCCCC CCCAA..... .......... .......... ......A... .......... ....C..... ..........
CMW 10455    TCCCCCCCCC CCCAA..... .......... .......... ......A... .......... ....C..... ..........
CMW 10788    TCCCCCCCCC C--AA...AG ..G..G.... .......... ......A... .....A.... ....C..... ..........
CMW 10789    TCCCCCCCCC C--AA...AG ..G..G.... .......... ......A... .....A.... ....C..... ..........
CMW 10442    TCC------- ---C...... .......... .......... ......C... .......... ....C..... ..........
CMW 2091     TCC------- ---C...... .......... .......... ......C... .....A.... ....C..... ..........
CMW 10465    TCC------- --CC...... .......... .......... ......C... .......... ....C..... ..........
CMW 5288     ---------- --CT..T... .....A.... .......... ......C... .......... ....C..... ..........
CMW 5587     ---------- --CT..T... .....A.... .......... ......C... .......... ....C..... ..........
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[                    330        340        350        360        370        380        390        400]
[                     .          .          .          .          .          .          .          .]
CMW 8757     TCGTCCTACT TCGTCGAGTG GATCCCCAAC AACGTCCAGA CCGCCCTCTG CTCCATCCCC CCCAAGGGTC TCAAGATGTC
CMW 8758     .......... ....A..... .......... .......... .......... .......... .......... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62       .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2628     .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10782    .......... .......A.. .......... .......... .......... .......... ..T.G...C. ..........
CMW 10784    .......... .......A.. .......... .......... .......... .......... ..T.G...C. ..........
CMW 10783    .......... .......A.. .......... .......... .......... .......... ..T.G...C. ..........
CMW 10786    .......... .......A.. .......... .......... .......... .......... ..T.G...C. ..........
CMW 10787    .......... .......A.. .......... .......... .......... .......... ..T.....C. ..........
CMW 11294    .......... .......A.. .......... .......... .......... .......... ..T.G...C. ..........
CMW 10785    .......... .......A.. .......... .......... .......... .......... ..T.G...C. ..........
CMW 10518    .......... .......A.. .......... ........A. .......... .......... ..T.....C. ..........
CMW 10463    .......... .......A.. .......... .......... .......... .......... ........C. ..........
CMW 10484    .......... .......... .......... .......... .......... .......... ........C. ..........
CMW 10436    .......... .......... .......... .......... .......... .......... ........C. ..........
CMW 10790    .......... .......... ...T...... ........A. .......... ......T... ........C. ..........
CMW 7047     .......... .......... ...T...... .......... .......T.. ......T... ....G...C. .A........
CMW 7048     .......... .......... ...T...... .......... .......... ......T... ....G...C. ..........
CMW 1651     .......... .......... ...T...... .......... .......... ......T... ........C. ..........
CMW 1652     .......... .......... ...T...... .......... .......... ......T... ........C. ..........
CMW 10477    .......... .......... .......... .......... .......... .......... ........C. ..........
CMW 10455    .......... .......... .......... .......... .......... .......... ........C. ..........
CMW 10788    .......... .......... .......... ........A. .......T.. T..A...... ..A.G...C. .A..A.....
CMW 10789    .......... .......... .......... ........A. .......T.. T..A...... ..A.G...C. .A..A.....
CMW 10442    .......... .......... .......... .......... .......... .......... ....G..... ..........
CMW 2091     .......... .......... .......... .......... .......... .......... ....G..... ..........
CMW 10465    .......... .......... .......... .......... .......T.. .......... .......... ..........
CMW 5288     .......... .......A.. ...T...... .......... .......G.. ...G.....T .......... ..........
CMW 5587     .......... .......A.. ...T...... .......... .......G.. ...G.....T .......... ..........
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[                    410        420        430        440        450        460        470        480]
[                     .          .          .          .          .          .          .          .]
CMW 8757             CTCCACCTTT GTTGGCAACT CCACTGCCAT CCAGGAGCTC TTCAAGCGTA TCGGCGAGCA GTTCACTGCT ATGTTCCGTC
CMW 8758             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62               .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2628             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10782            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10784            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10783            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10786            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10787            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 11294            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10785            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10518            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10463            ...T...... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10484            .......... ..C....... ....C..... .......... .........G .......... .........C ..........
CMW 10436            .......... ..C....... ....C..... .......... .........G .......... .........C ..........
CMW 10790            ...G...... ..C....... ....C..... .......... .........G .......... ...T..C..C ........G.
CMW 7047             ...G...... ..C....... ....C..... .......... ..A......G .......... ......C..C ........G.
CMW 7048             ...G...... ..C....... ....C..... .......... .........G .......... ...T..C..C ........G.
CMW 1651             ...G...... ..C....... ....C..... .......... .........G .......... ...T..C..C ........G.
CMW 1652             ...G...... ..C....... ....C..... .......... .........G .......... ...T..C..C ........G.
CMW 10477            .......... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10455            .......... ..C....... ....C..... .......... .........G .......... ......C..C ..........
CMW 10788            ...T...... ..G..G.... ....C..... T..A..A..T ..T.....GG .G..G..A.. ......C..C .........A
CMW 10789            ...T...... ..G..G.... ....C..... T..A..A..T ..T.....GG .G..G..A.. ......C..C .........A
CMW 10442            .......... .......... .T..C..... .........T ........CG .T........ ......C..C ..........
CMW 2091             .......... .......... .T..C..... .........T ........CG .T........ ......C..C ..........
CMW 10465            .......... .......... ....C..T.. .......... ........CG .T........ ......C..C ..........
CMW 5288             ...T.....C .....T.... .G.....T.. .........G .........G .......... .........C ......A.G.
CMW 5587             ...T.....C .....T.... .G.....T.. .........G .........G .......... .........C ......A.G.
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[                    490       500       510       520       530       540       550       560]
[                     .         .     β-tub 2a/b →          .         .         .         .]
CMW 8757             GCAAGGCTTT CTTGCATTGG TACACTGGCA AACCATCTCT GGCGAGCACG GCCTCGACAG CAATGGCGTG TACGT---AC
CMW 8758             .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 1853             .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 62               .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 2113             .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 8755             .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 1840             .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 8756             .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 2628             .......... .......... .......... .......... .......... .......... .......... .....---..
CMW 10782            .......... .......... .......... .........C ........T. .......... .......... ..T..---..
CMW 10784            .......... .......... .......... ......T..C ..A.....T. .......... .......... ..T.G---..
CMW 10783            .......... .......... .......... .........C ........T. .......... .......... ..T..---..
CMW 10786            .......... .......... .......... .........C ........T. .......... .......... ..T..---..
CMW 10787            .......... .......... .......... .........C ........T. .......... .......... ..T..---..
CMW 11294            .......... .......... .......... .........C ........T. .......... .......... ..T..---..
CMW 10785            .......... .......... .......... .........C ........T. .......... .......... ..T..---..
CMW 10518            .......... .......... .......... .........C ........T. .......... .......... ..T..---..
CMW 10463            .......... .......... .......... .........C ........T. .T........ .......... ..T..---..
CMW 10484            .......... .......... ..T....... .........C ........T. .......... ..G....... ..T..---..
CMW 10436            .......... .......... ..T....... .........C ........T. .......... ..G....... ..T..---..
CMW 10790            .......... .......... .......... .........C .......... .......... .......... .....---..
CMW 7047             .......... .......... .......... .........C .......... .......... .......... .....---..
CMW 7048             .......... .......... .......... .........C .......... .......... .......... .....---..
CMW 1651             .......... .......... .......... .........C .......... .......... .......... .....---..
CMW 1652             .......... .......... .......... .........C .......... .......... .......... .....---..
CMW 10477            .......... .......... ..T....... .........C .......... .......... ..G....... ..T..---..
CMW 10455            .......... .......... ..T....... .........C .......... .......... ..G....... ..T..---..
CMW 10788            .A........ T......... ..T....... .........C .......... .......... ..G....... ..T..---..
CMW 10789            .A........ T......... ..T....... .........C .......... .......... ..G....... ..T..---..
CMW 10442            .......C.. .......... .....C.... .........C .......... .......... .G........ ..T..TGT..
CMW 2091             .......C.. .......... .....C.... .........C .......... .......... .G........ ..T..TGT..
CMW 10465            .......C.. .......... .....C.... .........C ........T. .......... .......... ..T..---..
CMW 5288             .......... .......... .......... .......... .......... .T........ .......... .....---..
CMW 5587             .......... .......... .......... .......... .......... .T........ .......... ..T..---..
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[                    570        580        590        600        610        620        630      640]
[                     .          .          .          .          .          .          .        .]
CMW 8757     CCT-CCT--- ----GCTG-C ACCAG-GCGG -----CGCGC CTC--GAG-C TTCCC--GCT GACCA-CTGC ACAGCTACAA
CMW 8758     ...-...--- ----....-. .....-.... -----..... ...--...-. .....--... .....-.... ..........
CMW 1853     ...-...--- ----....-. .....-.... -----..... ...--...-. .....--... .....-.... ..........
CMW 62       ...-...--- ----.T..-. .....-.... -----..... ...--...-. .....--... .....-.... ..........
CMW 2113     ...-...--- ----.T..-. .....-.... -----..... ...--...-. .....--... .....-.... ..........
CMW 8755     ...-...--- ----.T..-. .....-.... -----..... ...--...-. .....--... .....-.... ..........
CMW 1840     ...-...--- ----....-. .....-.... -----..... ...--...-. .....--... .....-.... ..........
CMW 8756     ...-...--- ----....-. .....-.... -----..... ...--...-. .....--... .....-.C.. ..........
CMW 2628     ...-...--- ----....-. .....-.... -----..... ...--...-. .....--... .....-.C.. ..........
CMW 10782    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 10784    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 10783    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 10786    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 10787    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 11294    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 10785    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 10518    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A. ....T--... ..T..-.CA. .T........
CMW 10463    ...A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...CT ....T--... ...G.-.CA. .T........
CMW 10484    .G.A...TAC ACCG...TTG C....ACAA. ACAGA..... ..T--...CT C...TT-... .....A.CA. .T........
CMW 10436    .G.A...TAC ACCG...TTG C....ACAA. ACAGA..... ..T--...CT C...TT-... .....A.CA. .T........
CMW 10790    ...AT..--- --CG...T-. C.A..-CAA. ACAGA....A ..T--...CT ....T--... .....-.CA. .T........
CMW 7047     ...AT..--- --CG...T-. C.A..-CAA. ACAGA....A ..T--...CT ....T--... .....-.CA. .T........
CMW 7048     ...AT..--- --CG...T-. C.A..-CAA. ACAGA....A ..T--...CT ....T--... .....-.CA. .T........
CMW 1651     ...AT..--- --CG...T-. C.A..-CAA. ACAGA....A ..T--...CT ....T--... .....-.CA. .T........
CMW 1652     ...AT..--- --CG...T-. C.A..-CAA. ACAGA....A ..T--...CT ....T--... .....-.CA. .T........
CMW 10477    .G.G...TAC ACCG...TTG C....ACAA. ACAGA..... ..T--...CT C...TT-... .....A.CA. ..........
CMW 10455    .G.G...TAC ACCG...TTG C....ACAA. ACAGA..... ..T--...CT C...TT-... .....A.CA. ..........
CMW 10788    .G.G...TAC ACCG...TTG C....ACAA. ACAGA..... ..T--...CT C...TT-... .....A.CA. ..........
CMW 10789    .G.G...TAC ACCG...TTG C....ACAA. ACAGA..... ..T--...CT C...TT-... .....A.CA. ..........
CMW 10442    ...------- ---ACTGCTG C..G.CC--- ---GA..... ...--.G.C. C.T..CC... .....-.C.. ..........
CMW 2091     ...------- ---ACTGCTG C..G.CC--- ---GA..... ...--.G.C. C.T..CC... .....-.C.. ..........
CMW 10465    T..A...--- --CG...T-. C....-CAA. ATAGA....A ..TGT...A- C.T..T-... ..T..-.CA. .T........
CMW 5288     .TCGTA.CC- ---CCTGCC. ..TG.TCTC. TCCTCTC--. ...-----GG C.TGGC-A.. ..-..A.... ....T.....
CMW 5587     .TCGTA.CC- ---CCTGCC. ..TG.TCTC. TCCTCTC--. ...-----GG C.TGGC-A.. ..-..AT... ....T.....
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[                    650       660       670       680       690       700       710       720]
[                     .         .         .         .         .         .         .         .]
CMW 8757     CGGCACCTCC GAGCTCCAGC TCGAGCGCAT GAACGTCTAC TTCAACGAGG TATGTC-TGT --------CG G--GACCA-G
CMW 8758     .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 1853     .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 62       .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 2113     .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 8755     .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 1840     .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 8756     .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 2628     .......... .......... .......... .......... .......... ......-... -------.. .--.....-.
CMW 10782    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 10784    .......... .......... .......... .......... .......... .....C.TA T-------.. .GT.....-.
CMW 10783    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 10786    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 10787    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 11294    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 10785    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 10518    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 10463    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 10484    T......... .......... .......... .......... .......... .....C.TA T----CAT.. .GT.....-.
CMW 10436    T......... .......... .......... .......... .......... .....C.TA T----CAT.. .GT.....-.
CMW 10790    .......... .......... .......... .......... ..T....... .....-.TA T-------.. .GT..T..-A
CMW 7047     .......... .......... .......... .......... ..T....... .....-.TA T-------.. .GT..T..-A
CMW 7048     .......... .......... .......... .......... ..T....... .....-.TA T-------.. .GT..T..-A
CMW 1651     .......... .......... .......... .......... ..T....... .....-.TA T-------.. .GT..T..-A
CMW 1652     .......... .......... .......... .......... ..T....... .....-.TA T-------.. .GT..T..-A
CMW 10477    T......... .......... .......... .......... .......... .....C.TA TCATCCAT.. .GT.....A.
CMW 10455    T......... .......... .......... .......... .......... .....C.TA TCATCCAT.. .GT.....A.
CMW 10788    T......... .......... .......... .......... .......... .....C.TA TCATCCAT.. .GT.....A.
CMW 10789    T......... .......... .......... .......... .......... .....C.TA TCATCCAT.. .GT.....A.
CMW 10442    .......... .......... .......... .......... .......... .....--TA T-------G. .G-.....-.
CMW 2091     .......... .......... .......... .......... .......... .....--TA T-------G. .G-.....-.
CMW 10465    .......... .......... .......... .......... .......... .....-.TA T-------.. .GT.....-.
CMW 5288     ......T... .......... .......... .......... .......... ..A...-AAC AGCCACGT.. TCAATT..AA
CMW 5587     ......T... .......... .......... .......... .......... ..A...-AAC AGCCACGT.. TCAAT...AA
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[                    730        740        750        760        770        780        790      800]
[                     .          .          .          .          .          .          .         .]
CMW 8757     GC--TGGGGC GTC-ATCCCG CCCGCGAACC CCCTGTGCGT ---------- ---------- ---------- -----GACCG
CMW 8758     ..--...... ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 1853     ..--...... ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 62       ..--...... ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 2113     ..--...C.. ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 8755     ..--...... ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 1840     T.--...... ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 8756     T.--...... ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 2628     ..--...... ...-...... .......... .......... ---------- ---------- ---------- -----.....
CMW 10782    ..--.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGTACAGG..
CMW 10784    ..--.C.A.. A.AC...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGTACAGG..
CMW 10783    ..--.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGTACAGG..
CMW 10786    ..--.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGTACAGG..
CMW 10787    ..--.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGACA.GG..
CMW 11294    ..--.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGTACAGG..
CMW 10785    ..--.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC                  ------T GGTACAGG..
CMW 10518    ..--.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGTACAGG..
CMW 10463    ..--.C.A.. A..C...T.A A..C---C.. ..------.CC TCCCAAATCC CGGGCCCCTC ---GACTTCT GGCATAGG..
CMW 10484    ..--.C.AA. A..C...T.. A.AT---C.. ...CCCC.C. -TTTCCGAAT TTTGGGAAAA GGGAA-CTTT --------.C
CMW 10436    ..--.C.AA. A..C...T.. A.AT---C.. ...CCCC.T. -TTTCCCGAT TTTGGGAAAA GGCAAACTTT --------.C
CMW 10790    ..--.CAA.. T..C.C.T.. G..A---... ...CCCC.CC -TTTCCGGGG CCC-TC---- ---GACTTCT GGTATAGG..
CMW 7047     ..--.CAA.. T..C.C.T.. G..A---C.. ...CCCC.CC -TTTCCGGGG CCC-TC---- ---GACTTCT GGTATAGG..
CMW 7048     ..--.CAA.. T..C.C.T.. G..A---... ...CCCC.CC CTTTCCGGGG CCC-TC---- ---GACTTCT GGTATAGG..
CMW 1651     ..--.CAA.. T..-.C.T.. G..A---... ...CCCC.CC -TTTCCGGGG CCT-TCT--- ---GACTTCT GGTATAGG..
CMW 1652     ..--.CAA.. T..-.C.T.. G.-A---... ...CCCC.CC -TTTCCGGGG CCTT------ ---GACTTCT GGTATAGG..
CMW 10477    ..--.CAA.. A..C...T.. A..TT.GG.. ...CAAC.CC CTCTCCC--- ---------- ---GACTTCT GGCATAGG..
CMW 10455    ..--.CAA.. A..C...T.. A..TT.GG.. ...CAAC.CC CTCTCCC--- ---------- ---GACTTCT GGCATAGG..
CMW 10788    ..--.CAA.. A..C...T.. A..TT.GG.. ...CAAC.CC CTCTCCC--- ---------- ---GACTTCT GGCATAGG..
CMW 10789    ..--.CAA.. A..C...T.. A..TT.GG.. ...CAAC.CC CTCTCCC--- ---------- ---GACTTCT GGCATAGG..
CMW 10442    ..CC...C.T ---------- --G..CCCG. ..GC.----- ---------- ---------- ---GCCCC-T GGCGT.....
CMW 2091     ..CC...C.T ---------- --G..CCCG. ..GC.----- ---------- ---------- ---GCCCC-T GGCGT.....
CMW 10465    .--C.C.A.. A..C...T.A G..C---... ..-...T.CC TCCACTTC-- ---------- ---------T GGTACAGG..
CMW 5288     TTTGACAAC. TA.GGCA--- ---------- --------TG GTTTCCCGCC G---TCGC-C AAGGCCTTG- ----------
CMW 5587     TTTGACAAC. TA.GGCA--- ---------- --------TG GTTTCGCGCC G---TCGC-C AAGGCCTTG- ----------
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[                    810        820        830        840        850        860        870        880]
[                     .          .          .          .          .          .          .          .]
CMW 8757     AGC-TCCC-- --GCTGACGC GCT-CCT--- -GTCA--CAG GCCTCCGGCA ACAAGTATGT CCCCCGCGCC GTCCTCGTCG
CMW 8758     ...-....-- --........ ...-...--- -....--... .......... .......... .......... ..........
CMW 1853     ...-....-- --........ ...-...--- -....--... .......... .......... .......... ..........
CMW 62       ...-....-- --........ ...-...--- -....--... .......... .......... .......... ..........
CMW 2113     ...-....-- --........ ...-...--- -....--... .......... .......... .......... ..........
CMW 8755     ...-....-- --........ ...-...--- -....--... .......... .......... .......... ..........
CMW 1840     ...-....-- --........ ...-...--- -....--... .......... .......... T......... ..........
CMW 8756     ...-....-- --........ ...-...--- -....--... .......... .......... T......... ..........
CMW 2628     ...-....-- --........ ...-...--- -....--... .......... .......... T......... ..........
CMW 10782    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 10784    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 10783    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 10786    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 10787    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 11294    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 10785    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 10518    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 10463    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........A ..........
CMW 10484    TTTT.TTAAC GC..CTTTTT TT.TTT.TTT TT.T---... ..T....C.. .......... T......... ..........
CMW 10436    TTTT.TTAAC GC-.CTTTTT TT.TTT.TTT TT.T---... ..T....C.. .......... T......... ..........
CMW 10790    ...T...-TC TT-....... ...T.T.--- -...---... .......... .......... T......... ..........
CMW 7047     ...T...-TC TT-....... ...T.T.--- -...---... .......... .......... T......... ..........
CMW 7048     ...T...-TC TT-....... ...T.T.--- -...---... .......... .......... T......... ..........
CMW 1651     ...A...-TC TT-....... ...T.T.--- -...---... .......... .......... T......... ..........
CMW 1652     ...T...-TC TT-....... ...T.T.--- -...---... .......... .......... T......... ..........
CMW 10477    .AGT....TC TTT....... ...TTT.--- -...---... ..T....C.. .......... T......... ..........
CMW 10455    .AGT....TC TTT....... ...TTT.--- -...---... ..T....C.. .......... T......... ..........
CMW 10788    .AGT....TC TTT....... ...TTT.--- -...---... ..T....C.. .......... T......... ..........
CMW 10789    .AGT....TC TTT....... ...TTT.--- -...---... ..T....C.. .......... T......... ..........
CMW 10442    ...-....A- ---....... ...-...--- -...---... .......... .......... .......... ..........
CMW 2091     ...-....A- ---....... ...-...--- -...---... .......... .......... .......... ..........
CMW 10465    ...T...-TC TT-....... ...T.T.--- -A..---... ......A... .......... T........T ..........
CMW 5288     ---------- ---..A...- --CATT.--- -A..GCC... .......... .......... G..T...... ..........
CMW 5587     ---------- ---..A...- --CATT.--- -A..GCC... .......... .......... G..T...... ..........
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[                    890        900        910        920        930        940        950        960]
[                    .          .          .          .          .          .          .          .]
CMW 8757             ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG CTGTTCCGCC CCGACAACTT CGTCTTCGGC
CMW 8758             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1853             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62               .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2113             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1840             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756             .......... T......... .......... .......... .......... .......... .......... ..........
CMW 2628             .......... T......... .......... .......... .......... .......... .......... ..........
CMW 10782            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 10784            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 10783            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 10786            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 10787            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 11294            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 10785            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 10518            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 10463            .......... ...T...... ..T....... .C........ ...T..T... ........T. .......... ......T...
CMW 10484            .......... ...T...... ..T....... .C........ ...T..T... ........T. .......... ......T...
CMW 10436            .......... ...T...... ..T....... .C........ ...T..T... ........T. .......... ......T...
CMW 10790            .......... ...T...... ..T....... .C..T..... ...T..T... ........T. .......... ......T...
CMW 7047             .......... ...T...... ..T....... .C..T..... ...T..T... ........T. .......... ......T...
CMW 7048             .......... ...T...... ..T....... .C..T..... ...T..T... ........T. .......... ......T...
CMW 1651             .......... ...T...... ..T....... .C..T..... ...T..T... ........T. .......... ......T...
CMW 1652             .......... ...T...... ..T....... .C..T..... ...T..T... ........T. .......... ......T...
CMW 10477            .......... G..T...... ..T....... .C........ ...T..T... ........T. .......... ......T...
CMW 10455            .......... G..T...... ..T....... .C........ ...T..T... ........T. .......... ......T...
CMW 10788            .......... G..T...... ..T....... .C........ ...T..T... ........T. .......... ......T...
CMW 10789            .......... G..T...... ..T....... .C........ ...T..T... ........T. .......... ......T...
CMW 10442            .......... ...T...... .......... .C........ .......... .......... .......... ..........
CMW 2091             .......... ...T...... .......... .C........ .......... .......... .......... ..........
CMW 10465            .......... ...T...... .......... .C........ ...T...... ........T. .......... ......T...
CMW 5288             .......... ...T...... .......... .......T.. .......... .......... .......... ..........
CMW 5587             .......... ...T...... .......... .......T.. .......... .......... .......... ..........
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[                    970        980        990        1000       1010       1020       1030     1040]
[                ITS 1 →      .          .          .          .          .          .          .]
CMW 8757         CAGTCCCCCA GATACCCTTT GTGAACTTAT A-CCTTTTT- ATCGTTGCCT CGGCGCCGAG CC--GGGAGT GCTCTTCTGT
CMW 8758         .......... .......... .......... .-......-. .......... .......... ..--...... ..........
CMW 1853         .......... .......... .......... .-......-. .......... .......... ..--...... ..........
CMW 62           .......... .......... .......... .-......-. .......... .......... ..--...... ..........
CMW 2113         .......... .......... .......... .-......-. .......... .......... ..--...... ..........
CMW 8755         .......... .......... .......... .-......-. .......... .......... ..--...... ..........
CMW 1840         .......... A......... .......... .-......-. .......... .......... ..--...... ..........
CMW 8756         .......... .......... .......... .-......-. .......... .......... ..--...... ..........
CMW 2628         .......... .......... .......... .-......-. .......... .......... ..--...... ..........
CMW 10782        .......... .......... .......... .-..A...T. .......... ......T... ..CC...G.G .T.GG.A.TG
CMW 10784        .......... .......... .......... .-..A...-. .......... ......T... ..CCC..G.G .T.GG.A.TG
CMW 10783        .......... .......... .......... .-..A...T. .......... ......T... ..CC...G.G .T.GG.A.TG
CMW 10786        .......... .......... .......... .-..A...-. .......... .....-T... ..CC...G.G .T.GG.A.TG
CMW 10787        .......... .......... .......... .-..A...-. .......... .....-T... ..CC...G.G .T.GG.A.TG
CMW 11294        .......... .......... .......... .-..A...T. .......... ......T... ..CC...G.G .T.GG.A.TG
CMW 10785        .......... .......... .......... .-..A...-. .......... ......T... ..CC...G.G .T.GG.A.TG
CMW 10518        .......... .......... .......C.. .-..A...-. .......... ......T... ..CC...G.G .A.T..T---
CMW 10463        .......... .......... .......C.. .-..A...-. .......... ......T... ..CC...G.G .A.T..T---
CMW 10484        .......... .......... .......... .-..AC...- G......... ......T... ..C-...G.G .AGGAAACAA
CMW 10436        .......... .......... .......... .-..AC...- G......... ......T... ..C-...G.G -AGGAAACAA
CMW 10790        .......... .......... .......... .A..A...- .......... ......T... ..C-...G.G .---------
CMW 7047         .......... .......... .......... .A..A...- .......... ......T... ..C-...G.G .GG-------
CMW 7048         .......... .......... .......... .A..A...- .......... ......T... ..C-...G.G .GG-------
CMW 1651         .......... .......... .......... .A..A...- .......... ......T... ..TCT..G.G .GGG------
CMW 1652         .......... .......... .......... .A..A...- .......... ......T... ..TCT..G.G .GGG------
CMW 10477        .......... .......... .......... .-..A...- .......... ......T... ..C-...G.- -GAGGGAAAA
CMW 10455        .......... .......... .......... .-..A...- .......... ......T... ..C-...G-- -GAGGGAAAA
CMW 10788        .......... .......... .......... .-..A...- .......... ......T... ..C-...G.- -GAGGGAAAA
CMW 10789        .......... .......... .......... .-..A...- .......... ......T... ..C-...G.- -GAGGGAAAA
CMW 10442        .......... .......... .......... .-..A...- .......... ......T... .T--...G.C A....C....
CMW 2091         .......... .......... .......... .-..A...- .......... ......T... .T--...G.C A....C....
CMW 10465        .......... .......... .......... .-..A...T. .......... ......T... .T--...G.C A....C....
CMW 5288         .......... ..A....... .......... .-...----- .......... ....---..A GGCC..CC-- ----------
CMW 5587         .......... ..A....... .......... .-...----- .......... ....---..A GGCC..CC-- ----------
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[                    1050       1060       1070       1080       1090       1100       1110     1120]
[                    .          .          .          .          .          .          .          .]
CMW 8757     GC-------- ---------- ---------- ---------- ---------- ---------- ------TCCC C-CACCG--C
CMW 8758     ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 1853     ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 62       ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 2113     ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 8755     ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 1840     ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 8756     ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 2628     ..-------- ---------- ---------- ---------- ---------- ---------- ------.... .-.....--.
CMW 10782    AAAGAGAATA AAAAAATACT T-TTTTCTCT CTCTCTCCC- -----CTCT- ---CCCCCC- GC-----.TT .-T....--T
CMW 10784    AAAGAGAATA AAAAAATACT T-TTTTCTCT CTCTCTCCC- -----CTCT- ---CCCCCC- GC-----.TT .-T....--T
CMW 10783    AAAGAGAATA AAAAAATACT T-TTTTCTCT CTCTCTCCC- -----CTCT- ---CCCCCC- GC-----.TT .-T....--T
CMW 10786    AAAGAGAAGA AAAAAATACT T-TTTTCTCT CTCTCTTCC- -----CTCC- ---CCCCCCC GC-----.TT .-T....--T
CMW 10787    AAAGAGAAGA AAAAAATACT T-TTTTCTCT CTCTCTTCC- -----CTCC- ---CCCCCCC GC-----.TT .-T....--T
CMW 11294    AAAGAGAATA AAAAAATACT T-TTTTCTCT CTCTCTCTC- ---CCCTCT- ---CCCCCC- GC-----.TT .-T....--T
CMW 10785    AAAGAGAAGA AAAAAATACT T-TTTTCTCT CTCTCTTCC- -----CTCC- ---CCCCCCC GC-----.TT .-T....--T
CMW 10518    ---GAGA-GA G--------- -TCTCTCTCT CCTTCCTTC- -----TCGC- ---------- -------.TT .-T--..--T
CMW 10463    ---GAGA-GA G--------- -TCTCTCTCT CCTTCCTTC- -----TCGC- ---------- -------.TT .-T....--T
CMW 10484    .TAAAAGAGG AGAATCTTTT T-------CT CCTTCTTTTC TTTTT----- ----TCCCCC CCT---.... .T.CATCCGT
CMW 10436    .TAAAAGAGG AGAATCTTTT T-------CT CCTTCTTTTC TTTTT----- ----TCCCCC CCT---.... .T.CATCCGT
CMW 10790    --------TT GGCGAAGGCA G-ATTTTCTT CCTTCTCCC- -----CTCC- ---CTTCCCC CCCCCC..TT .-.....--T
CMW 7047     --------TT GGCGAAGGCA G-ATTTTCTT CCTTCTCCC- -----CTCC- ---CTCCCCC CCC---..TT .-.....--T
CMW 7048     --------TT GGCGAAGGCA G-ATTTTCTT CCTTCTCCC- -----CTCC- ---CTCCCCC CCC---..TT .-.....--T
CMW 1651     --------TT GGCGAAGGCA G-ATTTTCTT CCTTCTCCC- -----CTCC- ---CTCCCCC CCC---..TT .-.....--T
CMW 1652     --------TT GGCGAAGGCA G-ATTTTCTT CCTTCTCCC- -----CTCC- ---CTCCCCC CCC---..TT .-.....--T
CMW 10477    AAAAAAAAAA AA--GGGGGG AAATTT-GTT TCCCCCTTTT TTTTTT---- ----TCCCCC CCT---.... .TTTATCCGG
CMW 10455    AAAAAAAAAA AA--GGGGGG AAATTCTGTT TCCCCTTTT- CTTTT----- ----TCCCCC CCT---.... .TTCATCCGT
CMW 10788    AAAAAAAAAA AAAAGGGGGG AA-TTCTGTT TCCCCTTTTT CTTTT----- ----TCCCCC CCT---.... .TTCATCCGT
CMW 10789    AAAAAAAAAA AAAAGGGGGG AA--TCTGTT TCCCCTTTTT CTTTT----- ----TCCCCC CCT---.... .TTCATCCGT
CMW 10442    --GCC----- ---------- ---------- ---------- ---------- ---------- -------... .---.ACCGT
CMW 2091     --GCC----- ---------- ---------- ---------- ---------- ---------- -------... .---.ACCGT
CMW 10465    ATGCC----- ---------- ---------- ---------- ---------- ---------- -----CC... .T--TACCGT
CMW 5288     ---------- ---------- ---------- ---------- ------TCCC CACCGAGGCC CCTT------ ----------
CMW 5587     ---------- ---------- ---------- ---------- ------TCCC CACCGAGGCC CCTT------ ----------
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[                    1130       1140       1150       1160       1170       1180       1190     1200]
[                     .          .          .          .          .          .          .         .]
CMW 8757     GCAAG--CAG T------GGA G--CAGGCCC GCCGGCGGCC CACCAAACTC TTTGTTTTTA GAA-CGTATC TCTTCTGAGT
CMW 8758     .....--... .------... .--....... .......... .......... .......... ...-...... ..........
CMW 1853     .....--... .------... .--....... .......... ...T...... .......... ...-...... ..........
CMW 62       .....--... .------... .--....... .......... .......... .......... ...-...... ..........
CMW 2113     .....--... .------... .--....... .......... .......... .......... ...-...... ..........
CMW 8755     .....--... .------... .--....... .......... .......... .......... ...-...... ..........
CMW 1840     ....C--G.. .------... .--....... .......... .......... .......... ...-...... ..........
CMW 8756     .....--... .------... .--....... .......... .......... .......... ...-...... ..........
CMW 2628     .....--... .------... .--....... .......... .......... .......... ...-...... ..........
CMW 10782    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 10784    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 10783    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 10786    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 10787    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 11294    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..C.TATCT CT.CTGAGTA
CMW 10785    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 10518    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 10463    ....A--.G. .TGTT--... .--....... .......... .......... .......... T..-.C.... ..........
CMW 10484    .T...AA.G. .TGCT--... .--....... .......... ..T....... .......... A..-.C.... ..........
CMW 10436    .T...AA.G. .TGCT--... .--....... .......... ..T....... .......... A..-.C.... ..........
CMW 10790    ....A--.G. .TGTTGG... .--....... .......... ...T...... .......... T..-.C.... ..........
CMW 7047     ....A--.G. .TGTTGG... .--....... .......... ...T...... .......... T..-.C.... ..........
CMW 7048     ....A--.G. .TGTTGG... .--....... .......... ...T...... .......... T..-.C.... ..........
CMW 1651     ....A--.G. .TGTTGG... .--....... .......... ...T...... .......... T..-.C.... ..........
CMW 1652     ....A--.G. .TGTTGG... .--....... .......... ...T...... .......... T..-.C.... ..........
CMW 10477    .A..AAT.G. GGGCT--... A--....... C......... ..TT.....T .......... A..-.C.... .T..T.....
CMW 10455    .T..AAT.G. GTGCT--... .--....G.. .......... ..TT...... ....G..... A..A.C.... ....T.....
CMW 10788    .T..AAT.G. GTGGT--... .--....... .......... ..TT...... .......... A..-.CC... ....T.....
CMW 10789    .T..AAT.G. GTGCT--... .--....... C......... ..TT...... .......... A..-.CC... ....T.....
CMW 10442    ..-------A AGCGGT---G .AG....... .......... .......... .......... ..C-...... ..C.......
CMW 2091     ..-------A AGCGGT---G .AG....... .......... .......... .......... ..C-...... ..C.......
CMW 10465    A.----T.GC G---GT--.G .AG....... .......... T......... ..-.G..... ...-...... ..........
CMW 5288     --------G. GAACAA---G .AG...C..G C.G.CG.C.- A......... ..-....C.T AG----.GAA ...-......
CMW 5587     --------G. GAACAA---G .AG...C..G C.G.CG.C.- A......... ..-....C.T AG----.GAA ...-......
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[                    1210       1220       1230       1240       1250       1260       1270     1280]
[                     .          .      5.8S →        .          .          .          .          .]
CMW 8757     GTTTATAACA AA-CAAA-TG AATCAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT
CMW 8758     .......... ..-....-.. .......... .......... .......... .......... .......... ..........
CMW 1853     .......... ..-....-.. .......... .......... .......... .......... .......... ..........
CMW 62       .......... ..-....-.. .......... .......... .......... .......... .......... ..........
CMW 2113     .......... ..-....-.. .......... .......... .......... .......... .......... ..........
CMW 8755     .......... ..-....-.. .......... .......... .......... .......... .......... ..........
CMW 1840     .......... ..-....-.. .......... .......... .......... .......... .......... ...C.C....
CMW 8756     .......... ..-....-.. .......... .......... .......... .......... .......... ..........
CMW 2628     .......... ..-....-.. .......... .......... .......... .......... .......... ..........
CMW 10782    ACA.TA..-. ..AA----.. .......... .......... .......... .......... .......... ..........
CMW 10784    ACA.TA..-. ..AA----.. .......... .......... .......... .......... .......... ..........
CMW 10783    ACA.TA..-. ..AA..--.. .......... .......... .......... .......... .......... ..........
CMW 10786    ACA.TA..-. ..AA.---.. .......... .......... .......... .......... .......... ..........
CMW 10787    ACA.TA..-. ..AA.---.. .......... .......... .......... .......... .......... ..........
CMW 11294    CA...A..-. ..AA.---.. .......... .......... .......... .......... .......... ..........
CMW 10785    ACA.TA..-. ..AA.---.. .......... .......... .......... .......... .......... ..........
CMW 10518    ACA.T...-. ..AA...A.. .......... .......... .......... .......... ......A... ..........
CMW 10463    ACA.T...-. ..AA...A.. .......... .......... .......... .......... ......A... ..........
CMW 10484    AAA..A.-.. ..AA...-.. .......... .......... .......... .......... .......... ..........
CMW 10436    AAA..A.-.. ..AA...-.. .......... .......... .......... .......... .......... ..........
CMW 10790    ACA..A.-.. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 7047     ACA..A.-.. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 7048     ACA..A.-.. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 1651     ACA..A.-.. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 1652     ACA..A.-.. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 10477    T.-A.A.--. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 10455    T.-A.A.--. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 10788    T.-A.A.--. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 10789    T.-A.A.--. ..AA...A.. .......... .......... .......... .......... .......... ..........
CMW 10442    .....C..A. .--....-.. .......... .......... .......... .......... .......... ..........
CMW 2091     .....C..A. .--....-.. .......... .......... .......... .......... .......... ..........
CMW 10465    -....C..A. --CA...A.- .......... .......... .......... .......... .......... ..........
CMW 5288     ---A.A..A. .-CAT..A.. .......... .......... .......... .......... .......... ..........
CMW 5587     ---A.A..A. .-CAT..A.. .......... .......... .......... .......... .......... ..........
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[                    1290       1300       1310       1320       1330       1340       1350     1360]
[                    .          .          .          .          .          .          .          .]
CMW 8757             GCGATAAGTA ATGTGAATTG CAGAATTCAG TGAATCATCG AATCTTTGAA CGCACATTGC GCCCGCTGGA ATTCCAGCGG
CMW 8758             .......... .......... .......... .......... .......... .......... ....CG.... ..........
CMW 1853             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 62               .......... .......... .......... .......... .......... .......... ....CG.... ..........
CMW 2113             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755             .......... .......... .......... .......... .......... .......... ....CG.... ..........
CMW 1840             ..C....C.. .......... .......... .......... .......... .......... .......... ..........
CMW 8756             .......... .......... .......... .......... .......... .......... ....CG.... ..........
CMW 2628             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10782            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10784            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10783            .......... .........C AGC..AA... .......... .......... .......... .......... ..........
CMW 10786            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10787            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 11294            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10785            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10518            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10463            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10484            .......... .......... .......... .......... .......... .......... .........C ..........
CMW 10436            .......... .......... .......... .......... .......... .......... .........C ..........
CMW 10790            .......... .......... .......... G......... .......... .......... .......... ..........
CMW 7047             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 7048             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651             .......... .......... .......... .......G.. .......... .......... .......... ..........
CMW 1652             .......... .......... .......... .......G.. .......... .......... .......... ..........
CMW 10477            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10455            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10788            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10789            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10442            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2091             .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10465            .......... .......... .......... .......... .......... .......... .......... ..........
CMW 5288             .......... .......... .......... .......... .......... .......... ....T....T .....G.A..
CMW 5587             .......... .......... .......... .......... .......... .......... ....T....T .....G.A..
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[                    1370       1380       1390       1400       1410       1420       1430     1440]
[                     .          .   ITS 2 →          .          .          .          .         .]
CMW 8757     GCATGCCTGT TCGAGCGTCA TTTCAACCCT CAAGCCTGGC TTGGTGTTGG GGCACTACCT GTTC-ACAGC GGGTAGGCCC
CMW 8758     .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW 1853     .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW 62       .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW 2113     .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW 1840     .......... .......... .......... .......... .......... ......G... ...TT..... ..........
CMW 8756     .......... .......... .......... .......... .......... ......G... ...TT..... ..........
CMW 2628     .......... .......... .......... .......... .......... ......G... ...TT..... ..........
CMW 10782    .......... .......G.. .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 10784    .......... .......... .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 10783    .......... .......... .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 10786    .......... .......... .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 10787    .......... .......... .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 11294    .......... .......... .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 10785    .......... .......... .......... .......... .......... ..T......C ..---CA.A. .. .......
CMW 10518    .......... .......... .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 10463    .......... .......... .......... .......... .......... ..T......C ..---CA.A. ..-.......
CMW 10484    .......... .......... .......... .....T.... .......... .........C ..---.A.A. ..-.......
CMW 10436    .......... .......... .......... .....T.... .......... .........C ..---.A.A. ..-.......
CMW 10790    .......... .......... .......... .....T.... .......... ..T......C ..---.A.A. ..-.......
CMW 7047     .......... .......... .......... .....T.... .......... ..T......C ..---.A.A. ..-.......
CMW 7048     .......... .......... .......... .....T.... .......... ..T......C ..---.A.A. ..-.......
CMW 1651     .....-.... .......... .......... .....T.... .......... ..T......C ..---.A.A. ..-.......
CMW 1652     .....-.... .......... .......... .....T.... .......... ..T......C ..---.A.A. ..-.......
CMW 10477    .......... .......... .......... .....T.A.. .......... ........TC ..---.A.A. ..-.......
CMW 10455    .......... .......... .......... .....T.A.. .......... ........TC ..---.A.A. ..-.......
CMW 10788    .......... .......... .......... .....T.A.. .......... ........TC ..---.A.A. ..-.......
CMW 10789    .......... .......... .......... .....T.A.. .......... ........TC ..---.A.A. ..-.......
CMW 10442    .......... .......... .......... .......... .......... .......... ..---...A. ..-.......
CMW 2091     .......... .......... .......... .......... .......... .......... ..---...A. ..-.......
CMW 10465    .......... .......... .......... .......A.. .......-.. .......... ..---...A. ..-.......
CMW 5288     .......... .......... .......... .......... ......A... ......G.T. CC--G.G..G .A.C......
CMW 5587     .......... .......... .......... .......... ......A... ......G.T. CC--G.G..G .A.C......
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[                    1450       1460       1470       1480       1490       1500       1510    1520]
[                    .          .          .          .          .          .          .          .]
CMW 8757     TGAAATTTAA TGGCGGGCTC GCTAAGACTC TGAGCGTAGT AGTTTTTAT- -----CACCT CGCTTTGGAA -GGATTA-GC
CMW 8758     .......... .......... .......... .......... .........- -----..... .......... -......-..
CMW 1853     .......... .......... .......... .......... .........- -----..... .......... -......-..
CMW 62       .......... .......... .......... .......... .........- -----..... .......... -......-..
CMW 2113     .......... .......... .......... .......... .........- -----..... .......... -......-..
CMW 8755     .......... .......... .......... .......... .........- -----..... .......... -......-..
CMW 1840     .........G .......... .......... .......... .........- -----..... .......... -......-..
CMW 8756     .........G .......... .......... ...A...... .........- -----..... .......... -......-CG
CMW 2628     .........G .......... .......... .......... .........- -----..... .......... -......A..
CMW 10782    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-..
CMW 10784    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-..
CMW 10783    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-..
CMW 10786    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-..
CMW 10787    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-..
CMW 11294    .........G .......... .....N.... .......... .......T-- ----CA.... .......... -......-..
CMW 10785    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-
CMW 10518    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-..
CMW 10463    .........G .......... .......... .......... .......T-- ----CA.... .......... -......-..
CMW 10484    .........G .......... .......... .......... .......T.- ----C..... .......... -......-..
CMW 10436    .........G .......... .......... .......... .......T.- ----C..... .......... -......-..
CMW 10790    .........G .......... .......... .......... .......T.T TCTTCA.... .......... -......-..
CMW 7047     .........G .......... .......... .......... .......T.T TCTTCA.... .......... -......-..
CMW 7048     .........G .......... .......... .......... .......T.T TCTTCA.... .......... -......-..
CMW 1651     .........G .......... .......... .......... .......T.T TCTTCA.... .......... -......-..
CMW 1652     .........G .......... .......... .....C.... .......T.T TCTTCA.... .......... -......-..
CMW 10477    ......C..G .......... .......... .......... .......T.T TCTTC..... .......... A......-..
CMW 10455    ......C..G .......... .......... .......... .......T.T TCTTC..... .......... A......-..
CMW 10788    ......C..G .......... .......... .......... .......T.T TCTTC..... .......... A......-..
CMW 10789    ......C..G .......... .......... .......... .......T.T TCTTC..... .......... A......-..
CMW 10442    .........G .......... .......... .......... .....--..- -----..... .......... G.-....-..
CMW 2091     .........G .......... .......... .......... .....--..- -----..... .......... G.-....-..
CMW 10465    ......A..G .......... .......... .AG....... .....--..- -----..... .......... G.-....-..
CMW 5288     ......C..G .....A.... ..C.G...C. C......... ....A.-..- ---------C TCGC.CC.G. A..CCCTG..
CMW 5587     ......C..G .....A.... ..C.G...C. C......... ....A.-..- ---------C TCGC.CC.G. A..CCCTG..
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[                    1530       1540]
[                    .          .   ]
CMW 8757     GG-TGCTCTT GCCGTAAAAC C
CMW 8758     ..-....... .......... .
CMW 1853     ..-....... .......... .
CMW 62       ..-....... .......... .
CMW 2113     ..-....... .......... .
CMW 8755     ..-....... .......... .
CMW 1840     ..-....... .......... .
CMW 8756     A.-...C... .......... .
CMW 2628     ..-....... .......... .
CMW 10782    ..T....... .........A .
CMW 10784    ..T....... .........A .
CMW 10783    ..T....... .........A .
CMW 10786    ..T....... .......... .
CMW 10787    ..T....... .......... .
CMW 11294    ..T....... .........G .
CMW 10785    ..T....... .......... .
CMW 10518    ..T....... .......... .
CMW 10463    ..T....... .......... .
CMW 10484    ..T....T.. .......... .
CMW 10436    ..T....T.. .......... .
CMW 10790    ..T....... .......... .
CMW 7047     ..T....... .......... .
CMW 7048     ..T....... .......... .
CMW 1651     ..T....... .......... .
CMW 1652     ..T....... .......... .
CMW 10477    ..T....T.. .......... .
CMW 10455    ..T....T.. .......... .
CMW 10788    ..T....T.. .......... .
CMW 10789    ..T....T.. .......... .
CMW 10442    ..-....... .......... .
CMW 2091     ..-....... .......... .
CMW 10465    ..-....... .......... .
CMW 5288     ..-...C..- .....T.... .
CMW 5587     ..-...C..- .....T.... .
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