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Phylogeny and Taxonomy of *Calonectria* and its *Cylindrocladium* anamorphs

By

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Declaration

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

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SUMMARY

Species in the genus *Calonectria* (anamorph: *Cylindrocladium*) are euascomycetes in the order *Hypocreales* and are important pathogens of a wide range of plant hosts globally. At the outset, this thesis considers the literature pertaining to species of *Calonectria* and especially the importance of the biological, morphological and phylogenetic species concepts on the taxonomy of this group. It is clear that DNA sequence comparisons have revolutionised the taxonomy of *Calonectria* and literature also highlights the importance of a polyphasic approach to species identification. Studies in this thesis treat a number of forest nursery disease problems caused by *Calonectria* spp. and new species are consequently described based on DNA sequence comparisons, morphological characteristics and sexual compatibility tests. As a consequence several cryptic species were also identified in the genus. Therefore, a multigene genealogy was constructed for all *Calonectria* spp. for which cultures were available and shown to group together in 13 subclades also supported by morphological similarities. As a consequence all *Cylindrocladium* spp. were circumscribed to the genus *Calonectria*, regardless whether the teleomorph state was present or not, based on new nomenclature regulations stated in Article 59.

TABLE OF CONTENTS

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PREFACE

Fungal species in the genus *Calonectria* (anamorph *Cylindrocladium*) are important plant pathogens of a wide variety of hosts particularly in tropical and sub-tropical regions of the world. For most of the taxonomic history of *Calonectria* spp., their identification has depended deeply on the morphology of the anamorph state, most frequently encountered in nature, and more recently the sexual compatibility of isolates. Advances in molecular technology and DNA sequencing have, however, resulted in the recognition of several species complexes that include numerous cryptic species.

Several studies focussing on the taxonomy of selected groups of *Calonectria* spp., have recently incorporated DNA sequence comparisons, morphology and sexual compatibility to identify cryptic species in these groups. Application of the Phylogenetic, Morphological and Biological Species Concepts has revolutionised the taxonomy of *Calonectria*. The first chapter of this thesis critically reviews and analyses published research on the application of these three species concepts as they pertain to the taxonomic history of *Calonectria* and places this research undertaken in this thesis in context.

The experimental part of this thesis is based on isolates collected over a period of five years from various geographical regions on plant hosts and in soil. In Chapter two, the mortality of rooted *Pinus* cuttings in a commercial forest nursery in Colombia was investigated. The *Calonectria* spp. responsible for plant deaths were identified using DNA sequence and morphological comparisons. Furthermore, pathogenicity of these *Calonectria* spp. was tested on *P. maximinoi* and *P. tecunumanii* in greenhouse studies.

Eucalyptus hybrid clones have emerged as the most widely planted forestry trees in China. The result has been large-scale uniform plantations to accommodate a high-demand for wood and wood-products. To support these *Eucalyptus* plantations, it is necessary to raise and maintain healthy plants in nurseries. Decline in *Eucalyptus* cutting production in a forest nursery in GuangDong Province, China, has been linked to cutting rot associated with several *Calonectria* spp. In Chapter three of this thesis, these *Calonectria* spp. were identified using DNA sequence and morphological

comparisons. Species in the *Ca. reteaudii* complex were re-considered and shown to accommodate two cryptic species.

Calonectria pauciramosa belongs to the *Ca. scoparia* complex and is a well-known plant pathogen of various hosts, worldwide. In some countries, it is regarded as the dominant pathogen in commercial forest nurseries. Several studies, in the past, have suggested that it accommodates cryptic species. These studies, however, included small numbers of isolates. The aim of chapter four was to identify these cryptic taxa in *Ca. pauciramosa*. This was accomplished using a large number of isolates subjected to DNA sequence comparisons, morphological observations and sexual compatibility tests.

Over a period of five years a large number of *Calonectria* isolates were collected from infected plant material or baited from soils. These isolates were obtained from many different geographical regions of the world. In Chapter 5, I identify these *Calonectria* isolates based on DNA sequence comparisons, morphological characteristics and sexual compatibility. This resulted in the identification of numerous new taxa in *Calonectria*. Subsequently, a multigene phylogeny was constructed for all *Calonectria* spp. for which cultures are available and particularly to determine the phylogenetic relationships within *Calonectria*.