MOLECULAR PHYLOGENETIC STUDIES ON *ARMILLARIA* WITH SPECIFIC REFERENCE TO SOUTHERN HEMISPHERE SPECIES

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 as hitherto not been submitted for any degree at any university or faculty.

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PREFACE

Armillaria spp. are plant pathogenic fungi that cause the disease known as Armillaria root rot. Species in the genus are distributed throughout temperate and tropical regions of the world and have a broad range of plant hosts. *Armillaria* spp. from the Northern Hemisphere have received much attention in the past and their phylogenetic relationships are well resolved. There is, however, a dearth of information pertaining to phylogenetic relationships between *Armillaria* spp. from the Southern Hemisphere and their relationships with those from the Northern Hemisphere. This lack of knowledge has prompted the studies presented in this thesis.

The first chapter of this thesis presents a review of literature regarding *Armillaria*. The review is arranged in three sections. The first of these deals with the taxonomic history of *Armillaria*. This is followed by a broad discussion on species concepts and their application in *Armillaria* taxonomy. The last section is concerned mainly with the known phylogenetic relationships between species from the Northern Hemisphere and Africa; but also includes discussion on possible relationships between Australasian *Armillaria* spp.

Chapter Two of this thesis deals with the phylogenetic relationships between Australian and New Zealand *Armillaria* spp. Various species have previously been identified based on their basidiocarp morphology. A large volume of information about the distribution and the host ranges of these species is currently available. However, information pertaining to the phylogenetic relationships among these species has been conspicuously absent in systematic literature.

Chapter Three concerns the identity and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. Armillaria root rot is well known in South America and Indo-Malaysia but very little is known regarding the *Armillaria* spp. responsible for the disease. Studies presented in this chapter were, therefore, intended to add to the limited information regarding the species in these areas. Isolates were collected from infected trees with symptoms of Armillaria root rot in Chile, Indonesia and Malaysia. Basidiocarps were not present at the time of collection and field identification was, therefore, not possible. Isolates from basidiocarps with uncertain taxonomic status but resembling *A. novae-zelandiae* and *A. limonea* from Chile and Argentina, respectively, but with uncertain taxonomic status were also included in this study. The identities of all isolates

were determined by means of sequence comparisons, with those available for known *Armillaria* spp. The phylogenetic relationships among isolates of *Armillaria* spp. from the Southern Hemisphere were also determined.

Chapter Four of this thesis addresses the possible origin of *Armillaria* by investigating the phylogeny of *Armillaria* spp. from various floral kingdoms of the world. Earlier investigations revealed the phylogenetic relationships among *Armillaria* spp. from the Holarctic floral kingdom. Likewise, those from the Australian, Indo-Pacific and South American floral kingdoms were considered in chapters two and three of this thesis. The global phylogeny of the species in the genus, however, remained unresolved. The work presented in this chapter was, therefore, designed to determine the phylogenetic relationships between the *Armillaria* spp. from Africa, Australia, Europe, New-Zealand, South America and North America. In addition, the hypothesis that the genus originated in Gondwana was tested by estimating the date of divergence between non-Holarctic and Holarctic *Armillaria* spp.

Chapter Five deals with the phylogeny of African *Armillaria* isolates. One of my earlier studies showed that isolates thought to represent *A. fuscipes* reside in two monophyletic groups. The one group was suggested to represent *A. fuscipes* and the second group either *A. heimii* or an unknown species. Some isolates from the two monophyletic groups have, however, been found to represent the same biological species based on mating studies. The contradiction observed between phylogenetic studies and mating tests rendered the taxonomic position of the two African monophyletic groups refutable. The research presented in this chapter attempts to resolve the taxonomic status of isolates within two monophyletic groups.

Chapter Six deals with the identity of *Armillaria* isolates found on native *Protea* spp. and *Leucadendron* spp. in Kirstenbosh Botanical Gardens in the Western Cape Province of South Africa. Root rot disease on Proteaceae in this botanic garden is generally ascribed to *Phytophthora cinnamomi* infection. However, an investigation in 2000 revealed the presence of white mycelial fans between the bark and wood of dying plants, which are typical symptoms of Armillaria root rot. Basidiocarps were not found in the vicinity of the infected plants; consequently identification of the causal species based on morphology was not possible. The identity of the species was, therefore, determined based on DNA sequence data, their phylogenetic relationships with other *Armillaria* spp. and sexual compatibility tests.

Chapter Seven deals with identification of *Armillaria* isolates from Bhutan. Armillaria root rot is commonly encountered in fir and mixed forests in this mountain Kingdom. With the exception of one unsubstantiated record of *A. ostoyae,* virtually nothing is known about the species causing the disease in Bhutan. During a survey of tree diseases in 2001, isolates were collected from infected conifers showing typical symptoms of Armillaria root rot at four locations. Basidiocarps of the species causing the disease were, however, not found during the course of the survey. The identity of these isolates was therefore determined based on RFLP and DNA sequence data, their phylogenetic relationships with other Northern Hemisphere *Armillaria* spp. and sexual compatibility tests.

The last chapter of this thesis describes the development of an electronic RFLP identification tool for *Armillaria* spp. RFLP based methods provide a rapid and highly effective means for identification of *Armillaria* spp. The extensive use of this method has yielded a large number of PCR-RFLP profiles for various species. These profiles are currently available from a substantial and continuously growing set of publications. Identification using RFLP profiles, therefore, usually requires a cumbersome procedure of comparing profiles from unknown isolates with those that have been published. The software application described in this chapter circumvents this difficulty by providing all this information in a single database and employing an automated procedure for comparing RFLP profiles. The programme also allows for the addition of new information as it becomes available.

This thesis presents a collection of studies conducted over six year period that treat various aspects of *Armillaria* systematics. Three chapters deal with identification and phylogenetic relationships of unknown isolates from different locations in the world. Two chapters are specifically focussed on phylogenetic relationships among species from the Southern Hemisphere. One chapter deals with the global phylogeny of species from the Northern and the Southern Hemispheres. Each chapter is written in such a way that it can be read independently of the others; some repetition has, therefore, been unavoidable. It is my sincere hope that the work presented in thesis will advance our knowledge regarding *Armillaria* spp., their distribution and their relationships with one another.

Dedicated to my grandparents, Ouma Marietjie and Oupa Herman

"It is a strange fate that we should suffer so much fear and doubt over so small a thing."

BOROMIR

THE LORD OF THE RINGS,

THE FELLOWSHIP OF THE RING (THE MOVIE)

SUMMARY

Species of *Armillaria* are plant pathogens that cause the disease known as Armillaria root rot. Studies on the taxonomy and systematics of these fungi render a vital contribution to our ability to accurately identify them, as well as to our understanding of their distribution and ecology. This thesis represents an assemblage of studies that pertain to the taxonomy and phylogenetics of *Armillaria* species. The literature review presents an overview of the taxonomic history of the genus, the species concepts employed in fungal taxonomy, and the relevance of these concepts to *Armillaria*. It also discusses the phylogenetic relationships among *Armillaria* spp., to the extent that these were known prior to the studies constituting this thesis.

The major focus of the studies presented in this thesis was an investigation of the phylogenetic relationships between *Armillaria* spp. *Armillaria hinnulea* from Australia and New Zealand was shown to be closely related to the Northern Hemisphere *Armillaria* spp. The remainder of the *Armillaria* spp. from the two countries form a monophyletic group, thus confirming their separation based on morphology. A subsequent phylogenetic analysis for a global collection of *Armillaria* spp. suggests that species from the non-Holarctic floral kingdoms may be the ancestors of those from the Holarctic. Results also suggest that the genus probably originated in Gondwana. Phylogenetic and genetic analyses of isolates from Africa, which were previously considered to represent the same biological species, revealed two distinct phylogenetic species: *A. fuscipes* and an undescribed species.

Mating tests as well as RFLP and DNA sequence analyses were employed to determine the identity of vegetative isolates obtained during disease surveys from infected trees and shrubs. Isolates from South America and Indo-Malaysia were identified as *A. novae-zelandiae* and *A. luteobubalina* based on phylogenetic analyses. The Northern Hemisphere species, *A. gallica* and *A. mellea*, were shown to be the causal agents of Armillaria root rot on Proteaceae in Kirstenbosch Botanical Gardens, South Africa. Isolates from Bhutan were identified as *A. mellea* subsp. *nipponica* and an apparently undescribed species that we have referred to as Bhutanese Phylogenetic Species (BPS I).

Identification of *Armillaria* isolates is increasingly based on DNA sequence and RFLP data. There are currently a number of separate studies that present a confusing array of data for this

genus. To resolve this problem, a computer program was developed to provide an electronic database for managing RFLP profiles. It also includes an automated search algorithm for rapid identification of *Armillaria* isolates.

This thesis includes seven research chapters in addition to a comprehensive literature review. The collection of studies undoubtedly represents one of the most intensive efforts ever undertaken to identify *Armillaria* spp. This has been made possible through opportunities to collect isolates in many different countries and the availability of isolates from the collections of colleagues. New species have thus been recognised and intriguing patterns pertaining to the phylogeography of these fascinating fungi have begun to emerge. Additional evidence has emerged that, contrary to expectation, these soil-borne fungi have at least to some extent been dispersed across the globe by humans. This information and a considerably enhanced knowledge of the identity of *Armillaria* spp. should improve quarantine procedures to prevent their further spread.