



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

**MOLECULAR TAXONOMIC
STUDIES OF SELECTED SPECIES IN
THE *GIBBERELLA FUJIKUROI*
COMPLEX**

by

Emma Theodora Steenkamp

November 2000

Promoter: Prof. B.D. Wingfield

Co-promoter: Prof. M.J. Wingfield

Prof. T.A. Coutinho

Prof. W.F.O. Marasas

**Submitted in partial fulfillment of the requirements for the degree of Philosophiae Doctor
in the Faculty of Biological and Agricultural Science,
Department of Microbiology and Plant Pathology
at the University of Pretoria.**



TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
PREFACE	ii
CHAPTER 1 Literature review: The use of protein-coding genes in fungal taxonomy	1
CHAPTER 2 Differentiation of <i>Fusarium subglutinans</i> f. sp. <i>pini</i> by histone gene sequence data	52
CHAPTER 3 PCR-based identification of <i>MAT-1</i> and <i>MAT-2</i> in the <i>Gibberella fujikuroi</i> complex	70
CHAPTER 4 Molecular characterization of <i>Fusarium subglutinans</i> associated with mango malformation	86
CHAPTER 5 <i>Gibberella fujikuroi</i> mating population E associated with maize and teosinte species	103
CHAPTER 6 Cryptic speciation in <i>Gibberella fujikuroi</i> mating population E	120
CHAPTER 7 Molecular and morphological comparison of <i>Fusarium</i> species representing <i>F. subglutinans sensu lato</i>	141
SUMMARY	160
OPSOMMING	162
APPENDIX 1	A-1
APPENDIX 2	A-9
APPENDIX 3	A-14
APPENDIX 4	A-29
APPENDIX 5	A-42
APPENDIX 6	A-61

I wish to express my sincere thanks and appreciation to the following people and institutions:

Profs. B.D. Wingfield, M.J. Wingfield, W.F.O. Marasas and Dr. T.A. Coutinho, for their capable and enthusiastic guidance and advice;

Drs. J.F. Leslie, A.E. Desjardins and K.A. Zeller, as well as Ms. Henriette Britz and Ms. Riana Jacobs for their contribution to certain manuscripts, supply of cultures, useful discussions and advice on this project;

National Research Foundation (NRF) and the University of Pretoria for financial support;

Members of the Tree Pathology Co-operative Programme (TPCP) for opportunities to collect fungi and for financial support;

Department of Genetics at the University of Pretoria for use of facilities;

Department of Microbiology and Biochemistry at the University of the Orange Free State, for financial support and use of facilities during the first two years of this project;

My family and friends for their interest, advice and support throughout the course of this project.

PREFACE

Fusarium subglutinans forms part of the *Gibberella fujikuroi* complex. This species aggregate has been associated with different plant hosts, as well as at least three mating populations in the *G. fujikuroi* complex. However, *F. subglutinans* is a polyphyletic taxon (= *F. subglutinans sensu lato*), because each of these mating populations and lineages associated with the different hosts constitutes a discrete species. These different species are virtually identical when compared using morphological characteristics. The major goal of this study was to characterize some of the species representing *F. subglutinans sensu lato* using various protein-coding gene sequences. Methods to aid in the identification and differentiation of the fungi comprising *F. subglutinans sensu lato* were also developed. This thesis is, therefore, presented as a compilation of chapters that each deals with different approaches and techniques to study the taxonomy and biology of *F. subglutinans sensu lato*.

Most studies on the phylogeny and taxonomy of species in the *G. fujikuroi* complex have used protein-coding gene sequences. This is because the more widely used ribosomal DNA sequences do not provide sufficient resolution in these fungi. Their use is also complicated by the presence of paralogous internal transcribed spacer regions. Although several authors have presented extensive reviews on the use of ribosomal DNA sequences in taxonomy, no such reviews are available for the use of genes that encode proteins. A comprehensive literature review dealing with this issue is, therefore, presented in chapter one.

Pitch canker of mature pines and root rot of pine seedlings are important forestry diseases. The causal agent of these diseases is *F. subglutinans* f. sp. *pini*. This fungus is morphologically almost indistinguishable from other species of *F. subglutinans sensu lato*. The lack of a reliable identification system has hampered the implementation of disease management programs and quarantine measures. In chapter two, a PCR-based identification technique for *F. subglutinans* f. sp. *pini* is reported. This technique is based on the presence of unique restriction fragment length polymorphisms in the amplified portion of the histone *H3* gene of this fungus.

The biological species concept is frequently used for studying the fungi in the *G. fujikuroi* complex. Application of this concept for the classification of these fungi is relatively straightforward since they are heterothallic, having one of two possible mating types (*MAT-1* or *MAT-2*). To simplify the identification of isolates with opposite mating types, a PCR-based method was developed and is presented in chapter three. Partial DNA sequences for the two *MAT* loci (idiomorphs) were determined and used to construct mating type specific primers.

Chapter four reports on two distinct phylogenetic species associated with the disease known

as mango malformation. This study was based on the DNA sequence for the histone *H3* and β -tubulin genes. Both of the species associated with mango malformation display morphological characters typical of *F. subglutinans*. Since reliable identification procedures have not been available, the possible identity of these species was determined using a public domain nucleotide database and the internet-based programme BLAST.

Application of the biological species concept for classifying *Fusarium* isolates does not always yield definitive resolution of species. For example, sexually compatible isolates will be classified as the same species, whereas incompatible fungi do not necessarily represent separate species. Apart from "species barriers", factors such as female sterility and low fertility, greatly influence sexual compatibility tests. These factors may result in the genetic isolation of a population or species. Chapter five reports on such a genetically isolated population of isolates. Using the DNA sequence of two protein-coding genes, this population was shown to form part of the *G. fujikuroi* mating population E (*F. subglutinans sensu stricto*).

Chapter six reports on a recent speciation event within *G. fujikuroi* mating population E. The two cryptic species that emerged from the study are morphologically identical. They also have similar primary hosts, i.e. *Zea* spp. The speciation event was detected using a phylogenetic approach. For this purpose specific regions of three nuclear protein-coding genes, as well as three nuclear regions of unknown function were used.

Chapter seven represents a molecular and morphological comparison of the species comprising *F. subglutinans sensu lato*. All the available *Fusarium* isolates that were previously shown to display morphological characters that are typical of *F. subglutinans* were compared. From these comparisons, diagnostic morphological characters were identified. All these fungi were also compared using the gene sequences for three nuclear protein-coding genes, as well as the sequence for the mitochondrial small subunit ribosomal RNA gene. From the sequence of the most variable of these regions (elongation factor 1α), diagnostic restriction enzyme recognition sites were identified and used for species delimitation.

Each of the seven chapters of this thesis represents an independent unit, three of which have already appeared in print. Studies were undertaken over a five-year period and repetition between chapters has been unavoidable. All the available synonyms for each *Fusarium* species are also not always provided. This was done to avoid confusion, since the current taxonomic status of many *Fusarium* species in the *G. fujikuroi* complex remains uncertain. Many of the lineages in *F. subglutinans sensu lato* have been renamed, whereas others have not yet been formally described. The fact that many of these newly described species are invalid (Index of fungi, 1999, 6:979-980) further complicates their nomenclature.