

**Taxonomy and biology of ophiostomatoid fungi  
associated with conifer-infesting bark beetles**

by

**XuDong Zhou**

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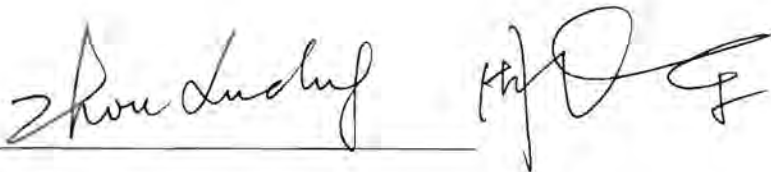
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**Supervisor: Prof. M. J. Wingfield**

**Co-supervisor: Prof. B. D. Wingfield**

## 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 faculty.



XuDong Zhou

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## PREFACE

Bark beetles (Coleoptera: Scolytidae) are generally regarded as forest pests and act as vectors of ophiostomatoid fungi. Many *Ophiostoma* spp. can cause sapstain in lumber and logs, reducing the value of the wood. Several species are pathogenic to trees and other crops. In South Africa, sapstain fungi degrade high quality pine logs and lead to significant financial losses to the forestry industry. Three exotic pine bark beetle species have been reported from South Africa, and considerable research has been done on these insects. Little is known, however, regarding the fungi associated with them, or the interactions between fungus, bark beetle and host tree. The primary aim of the study was, therefore, to identify *Ophiostoma* spp. associated with pine bark beetles in South Africa, evaluate the pathogenicity of the most common fungal associates to pines, and gain an understanding of the population biology of the most pathogenic fungus. The study also provided the opportunity to consider the taxonomy of selected fungal isolates from pine bark beetles from other countries, obtained during the course of the study. In order to assist the reader, the thesis is divided into four sections: literature review, taxonomy, pathology, and population biology.

### LITERATURE REVIEW

In Chapter 1, current knowledge regarding ophiostomatoid fungi and pine bark beetles in South Africa, as well as the association between fungi and bark beetles, is reviewed. The review also includes a brief summary of the ophiostomatoid fungi associated with pine bark beetles in other Southern Hemisphere countries such as Australia, Chile, and New Zealand.

### TAXONOMY

Three species of exotic pine bark beetles, *Hylastes angustatus*, *Hylurgus ligniperda* and *Orthotomicus erosus*, have been reported to occur on *Pinus* spp. in South Africa. All these bark beetles were introduced into South Africa from Europe. In Chapter 2, I present the results of a survey of ophiostomatoid fungi associated with these three bark beetle species. During the course of the two-year survey, 1558 samples (beetles and galleries) were collected from *P. patula* and *P. elliottii* plantations in Mpumalanga and KwaZulu-Natal provinces. In total, 1254 fungal isolates were recovered and 500 of these are identified in the Chapter. Three species are recorded for the first time from South Africa.

Similar to the situation in South Africa, *Hylurgus ligniperda* and *Hylastes ater*, which are native

to Europe, commonly occur on exotic *Pinus radiata* in Chile. Specimens of these bark beetles and their galleries were obtained and the five ophiostomatoid species isolated from these specimens are illustrated and described in Chapter 3. All of these species are recorded from Chile for the first time.

In contrast to the situation in South Africa and Chile, *Ips calligraphus* and *Dendroctonus mexicanus* are native to Mexico and occur on many species of pine in that country. Chapter 4 presents the results of isolations from these two bark beetle species obtained from their natural environment. The results of this study showed that six *Ophiostoma* spp. were associated with the two beetle species. One of these is recorded for the first time from Mexico. The collection also included a previously undescribed species isolated from both beetle species, which is described in the Chapter.

In South-western China, a native bark beetle, *Tomicus piniperda*, has destroyed more than 0.5 million ha of *Pinus yunnanensis* in the past 15 years. Isolates of a blue stain fungus were obtained, but not identified, in a previous study. The fungus is morphologically similar to the anamorph of *Ophiostoma crassivaginatium* and to *Leptographium pyrinum*. Based on comparisons using light and scanning electron microscopy, this fungus is described as a new species in Chapter 5.

*Ophiostoma galeiformis* was isolated from the three studies on ophiostomatoid fungi associated with bark beetles from South Africa, Chile, and Mexico, respectively. The species concept of this fungus, however, has been confused over the years due to a lack of sexual structures on the type specimen and contamination of the ex-type culture. The availability of fresh *O. galeiformis* cultures provided the opportunity to study the fungus further and results are presented in Chapter 6.

## PATHOLOGY

In Chapter 1 it was shown that *Ophiostoma ips*, *Leptographium serpens*, and *L. lundbergii*, occur most frequently with the three exotic pine bark beetle species in South Africa. In Chapter 7, the pathogenicity of these three fungi was tested on *Pinus elliottii* and *P. radiata* in South African plantations. Results indicated that the inoculated fungi caused resin exudation and sapwood discoloration around inoculation points. Of the three species, *O. ips* was the most pathogenic.



## POPULATION BIOLOGY

A single ascospore isolate of *O. ips* was selected for the development of twelve pairs of simple sequence repeat markers (SSR). *Ophiostoma ips* was selected based on its prevalence on all three bark beetle species occurring in South Africa, and its economic importance as a sapstain agent and possible pathogen. All markers were found to be polymorphic when tested on 7 isolates of *O. ips* collected from different parts of the world. Results of this study are reported in Chapter 8.

The twelve pairs of polymorphic markers developed in the study presented in Chapter 8 were applied to examine the population structure of five populations of *O. ips*, one each from Chile, Europe, and the USA, and two from South Africa. The genetic variation, genetic distance, and mode of reproduction within and between the different populations were considered.



**Summary**

**Opsomming**

## SUMMARY

The ophiostomatoid fungi include genera such as *Ophiostoma*, *Ceratocystis*, *Ceratocystiopsis*, and *Leptographium*. Several species in these genera are severe pathogens of trees and other crops. The majority of these species, however, are the causal agents of sapstain in lumber and logs, reducing the value of the wood. In South Africa, sapstain fungi degrade high quality pine logs causing significant financial losses to the forestry industry. Sapstain fungi, especially *Ophiostoma* spp., are often associated with bark beetles (Coleoptera: Scolytidae), many of which are regarded as forest pests. Three species of exotic pine bark beetles, *Hylastes angustatus*, *Hylurgus ligniperda*, and *Orthotomicus erosus*, occur on mature *Pinus* spp. in South Africa. The primary aim of this study was to identify ophiostomatoid fungi associated with pine bark beetles in South Africa, evaluate the pathogenicity of the most common of these fungal species, and gain an understanding of the population biology of the most pathogenic fungus.

During the course of the study, the opportunity arose to include introduced ophiostomatoid fungi associated with exotic pine bark beetles from Chile, and native species from Mexico and China, where little research in this field has been conducted. From Chile, five ophiostomatoid species were reported for the first time. From Mexico, six species were reported, including a new species, *Ophiostoma pulvinisporum* nom. prov. From China, another new species, *Leptographium yunnanense* sp. nov., was described.

The availability of fresh *O. galeiformis* cultures from the studies from South Africa, Chile and Mexico, as well as isolates obtained from Scotland, provided the opportunity to address the confusion that has existed in the taxonomy of this species. An epitype based on a collection from the area where *O. galeiformis* was first collected in Scotland, was designated. This study will provide a foundation for further work on the phylogeny of this species complex.

The survey on ophiostomatoid fungi associated with pine bark beetles in South Africa showed that *Ophiostoma ips*, *Leptographium serpens*, and *L. lundbergii* occur most frequently. In pathogenicity tests of these three fungi to pines, *O. ips* gave the longest lesions, and was the most pathogenic. *Ophiostoma ips* was, for this reason, selected for population studies. Twelve pairs of polymorphic simple sequence repeat markers (SSR) were then developed from *O. ips*. These markers were designed and used to examine the population structure of five populations of *O. ips* from Chile, Europe, South Africa, and the USA. Results support the hypothesis that *O. ips* was introduced into exotic pine-growing countries such as Chile and South Africa, together with the bark beetles native to Europe.

This study treats a number of questions pertaining to the ophiostomatoid fungi associated with pine-infesting beetles. It also represents the first comprehensive study of the topic in South Africa. Thus, new species have been discovered and some insight is presented into the population biology of one species of *Ophiostoma*. To the best of our knowledge this is the first population biology study of a sapstain fungus on conifers. While some important questions have been answered relating to the ophiostomatoid fungi, many remain. It is my hope that this study will provide a firm foundation for further work on the biology and taxonomy of *Ophiostoma* spp. associated with conifers-infesting bark beetles.

## OPSOMMING

Die ophiostomatoïede swamme sluit genera in soos *Ophiostoma*, *Ceratocystis*, *Ceratocystiopsis*, en *Leptographium*. Verskeie van hierdie spesies is ernstige patogene van bome en ander gewasse. Die meerderheid van die spesies verkleur egter die saphout van planke en stompe, wat lei tot 'n daling in die waarde van hout. In Suid-Afrika val sapverkleuringswamme hoë kwaliteit dennestompe aan, wat beduidende finansiële verliese vir die bosbou-industrie tot gevolg het. Sapverkleuringswamme, veral *Ophiostoma* spesies, word dikwels met baskewers (Coleoptera:Scolytidae) geassosieer, waarvan baie beskou word as peste in woude en plantasies. Drie spesies van uitheemse denne-baskewers, *Hylastes angustatus*, *Hylurgus ligniperda*, en *Orthotomicus erosus*, kom voor op volwasse *Pinus* spesies in Suid-Afrika. Die primêre doel van hierdie studie was om ophiostomatoïede swamme wat met denne-baskewers in Suid-Afrika geassosieer is, te identifiseer, die patogenisiteit van die algemeenste spesies te evalueer, en om die populasiebiologie van die mees patogeniese een te bestudeer.

Tydens die verloop van die studie het die geleentheid ontstaan om ophiostomatoïede swamme wat met uitheemse denne-baskewers in Chile geassosieer is, asook inheemses van Mexiko en China, waar weinig navorsing in hierdie veld gedoen is, in die studie in te sluit. Van Chile is vyf ophiostomatoïede spesies vir die eerste keer gerapporteer. Van Mexiko is ses spesies gerapporteer, waaronder 'n nuwe spesie, *Ophiostoma pulvinisporum* nom. prov. Van China, is nog 'n nuwe spesie, *Leptographium yunnanense* sp. nov., beskryf.

Die beskikbaarheid van vars *O. galeiformis* kulture van die studies in Suid-Afrika, Chile en Mexiko, asook isolate wat onlangs in Skotland versamel is, het die geleentheid gebied om die verwarring wat in die taksonomie van hierdie spesie bestaan, aan te spreek. 'n Epitipe, gebaseer op 'n versameling isolate van die area waar *O. galeiformis* die eerste keer in Skotland gevind is, is aangewys. Hierdie studie sal voorts dien as 'n fondasie vir verdere werk op die filogenie van die spesiekompleks.

Die opname van ophiostomatoïede swamme wat met baskewers in Suid-Afrika geassosieer word, het gewys dat *Ophiostoma ips*, *Leptographium serpens*, en *L. lundbergii*, die meeste voorkom. In patogenisiteitstoetse van hierdie drie fungi op denne, het *O. ips* die langste letsels veroorsaak, en was dus die mees patogeniese. *Ophiostoma ips* is vir hierdie rede gekies vir populasiestudies. Twaalf pare van polimorfiese eenvoudige basispaaropeenvolgingsherhalingsmerkers (SSR) is toe

vanaf *O. ips* ontwikkel. Hierdie merkers is ontwerp en gebruik om die populasiestruktuur van vyf populasies van *O. ips* van onderskeidelik Chile, Europa, Suid-Afrika, en die VSA, te bestudeer. Die resultate ondersteun die hipotese dat *O. ips* ingevoer is saam met Europese baskewers na lande soos Chile en Suid-Afrika waar uitheemse dennebome op groot skaal aangeplant word.

Hierdie studie behandel verskeie vraagstukke met betrekking tot die ophiostomatoïede swamme wat met baskewers geassosieer word. Dit verteenwoordig ook die eerste omvattende studie van die onderwerp in Suid-Afrika. Nuwe spesies is ontdek en beskryf en insig in die populasiestruktuur van een van die *Ophiostoma* spesies is verky. Volgens ons kennis, is dit die eerste populasiestudie van 'n sapverkleuringswam op naaldhoutsoorte. Terwyl verskeie belangrike vraagstukke in verband met die ophiostomatoïede swamme beantwoord is, is daar ander vrae wat onbeantwoord bly. Ek glo dat hierdie studie 'n sterk fondasie sal vorm vir verdere werk op die biologie en taksonomie van *Ophiostoma* spesies geassosieerd met naaldhout-infesterende baskewers.