Botryosphaeriaceae associated with *Acacia* species in southern Africa with special reference to *A. mellifera*

by

F.J.J van der Walt

Submitted in partial fulfillment of the requirements for the degree

MAGISTER SCIENTAE

in the Faculty of Natural and Agricultural Sciences, Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa

(October 2008)

Supervisor: Dr. Gert J. Marais
Co-supervisors: Prof. Jolanda Roux
Prof. Michael J. Wingfield
Dr. Bernard Slippers

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I would like to thank the following organizations and people:

My supervisors Dr. Bernard Slippers, Prof. Jolanda Roux, Prof. Michael Wingfield and Dr. Gert Marais for their guidance during these studies and assistance with the editing of the dissertation.

Prof. Michael Wingfield for the opportunity to complete my study at FABI.

Dr. Gert Marais for his guidance and understanding through my study. Also a special thanks for everyone that is part of the Centre for Applied Mycological Studies (CAMS) for their help and encouragement.

Dr. Percy M. Chimwamurombe and Mr. Jean Damascene Uzabakiriho from the University of Namibia who invited us to survey diseased *Acacia* spp. in that country and who provided field assistance during our visit. I would also like to thank the farmers of Namibia.

Flight Sergeant Mauritz of the Ditholo Training area in Pretoria, Mr. PJ Fourie and Mr. Nico Grobler for their permission to collect plant material.

I also want to acknowledge Mr. Ronald Heath, Miss. Marelize van Wyk, Dr. Gert Marais, Dr. Martin Coetzee and Prof. Jolanda Roux who assisted with my field work.

The Department of Science and Technology (DST)/National Research Foundation (NRF), Centre of Excellence in Tree Health Biotechnology (CTHB), the National Research Foundation (NRF), Centre for Applied Mycological Studies (CAMS), University of Pretoria and the THRIP initiative of the Department of Trade and Industry, South Africa for financial assistance.

PREFACE

Tree species belonging to the genus *Acacia* have a significant impact ecologically and economically in southern Africa. Together with the African baobab, these trees are recognized as icons of the African landscape. They are widely distributed in this area and extensively used by local communities as sources of energy, stock feed, medical remedies and building material. There is still a substantial lack of knowledge regarding the ecological association between these plants and other living organisms such as fungi. This is, however, not new to the African continent where fungi are generally poorly studied and collected, and it is envisaged that many new fungal species will be discovered as scientists focus their efforts more on this geographical niche.

An example of the lack of knowledge on the fungal biodiversity in Africa is reflected in the limited reports of members of the Botryosphaeriaceae, described to date from *Acacia* spp.. A review on phytopathogens in South Africa by Crous *et al*. (2000) indicated no records of the Botryosphaeriaceae associated with native *Acacia* spp. Despite the importance of many species within the Botryosphaeriaceae as pathogens, knowledge about the true diversity and taxonomy of species in this family is limited, especially where native plant communities are concerned.

This dissertation attempts to contribute to knowledge on the associations between members of the Botryosphaeriaceae and indigenous *Acacia* trees in southern Africa, and the possible role they may play in diseases of these trees. Chapter 1 represents a literature review that focuses on fungi previously associated with *Acacia* spp. on the African continent. Information provided in this chapter refers to available reports on

pathogens and saprophytes occurring on *Acacia* spp. that are both native and non-native to Africa. Special reference is made to those occurring in southern Africa. Due to the concern of the introduction of new pathogens in areas where native and non-native plants are co-existing, emphises is also placed on the possibility of pathogen-host jumps between native and non-native *Acacia* spp. The potential threat they might pose to the future biosecurity of these important trees is discussed.

Acacia mellifera, also known as the blackthorn, is one of the native African *Acacia* spp. that has been extensively studied. This tree is threatened by a serious die-back disease with symptoms similar to the die-back typically caused by members of the Botryosphaeriaceae. In an effort to understand the association of the Botryosphaeriaceae with native *Acacia* spp. in southern Africa, a study was undertaken to search for the presence of these fungi on especially *A. mellifera* in Namibia and the Pretoria area in South Africa. Other *Acacia* spp. were also sampled in cases where they were present in the same areas as *A. mellifera*. These results are presented in chapter 2.

In a previous study, the fungal diversity of native trees and plant species in the Northern Cape Province of South Africa was studied. This resulted in the isolation of a number of fungi that resembled the morphological characteristics of the Botryosphaeriaceae. In chapter 3, these fungi were further identified to species level based on morphological and phylogenetic characteristics.

In chapter 4 an attempt was made to compare the Botryosphaeriaceae that are associated with important native trees with those occurring on non-native trees. To

accomplish this, a pilot study was done to investigate the presence of the Botryosphaeriaceae on *A. mearnsii* in the Gauteng Province of South Africa. Results from chapters 2 and 3 on native *Acacia* spp. from Namibia and South Africa served as the bases of comparison for this chapter. Results of previous studies that investigated diseases of plantation grown *A. mearnsii* were also included for comparison.

Lastly, a summary is included to review the results of this study and also the significance and impact these results made, not only on the taxonomy of the Botryosphaeriaceae, but also understanding the fungal biodiversity of indigenous tree species in southern Africa. To date, this is the most extensive study of the Botryosphaeriaceae associated with native African *Acacia* spp. and it is also the first study that resulted in the describtion of so many new species in this group of fungi from a single host. Results from this study indicated that there is a significantly greater diversity in the Botryosphaeriaceae associated with native *Acacia* spp. in southern Africa than was previously thought. This dissertation attempts to form the basis for future studies to finally understand the interactions between the Botryosphaeriaceae and their native hosts as well as their role and threat as pathogens to indigenous and economically important plants.

Chapter 1

Fungi associated with *Acacia* spp. in Africa – A review

ABSTRACT

Acacia spp. occur throughout tropical, temperate and semi-arid to arid regions of the world, and they have a significant impact ecologically and economically. This cosmopolitan genus belongs to the family Leguminosae, subfamily Mimosoideae, and includes almost 1400 species. Although diseases of plantation *Acacia* spp., grown as non-natives, have been extensively documented, diseases of endemic *Acacia* spp. have been poorly studied. The importance of the genus *Acacia* is clearly identifiable and attention needs to be given to diseases affecting these trees. This is especially true with the planting of non-native *Acacia* spp. in close proximity to native species in many countries. This review focuses on reported pathogens and other fungi associated with *Acacia* spp. occurring in Africa, with special reference given to species in southern Africa.

1.0 INTRODUCTION

Acacia Miller is the second largest genus in the family Leguminosae, subfamily Mimosoideae (Maslin *et al*., 2003a, b; Rico-Arce, 2001), including 1350 described species (Maslin *et al*., 2003a, b; Orchard and Maslin, 2005). This cosmopolitan genus, treated here in the broad sense, occurs from tropical to arid regions in Africa (150 spp.), Asia and the Pacific region (95 spp.), Americas (185 spp.) and Australia (~1000 spp.) (Jawad *et al*., 2000; Luckow *et al*., 2005; Miller and Bayer, 2003; Maslin *et al*., 2003a, b; Orchard and Maslin, 2003, 2005).

Recent molecular and morphological data have shown that the genus *Acacia* is polyphyletic and that it does not represent the monophyletic entity formerly described (Clarke *et al*., 2000; Luckow *et al*., 2005; Miller and Bayer, 2001, 2003; Murphy *et al*., 2003; Orchard and Maslin, 2003, 2005; Rico-Arce, 2001). This has led to recent suggestions that the genus should be redefined. In 2005, the recommendation was accepted to conserve the name *Acacia*, against a new Australian type, *A. penninervis* (found in the temperate regions of eastern Australia) and no longer *A. scorpioides* (L.) W. F. Wight (=*A. nilotica* (L.) Del.) (originating from tropical Africa & western Asia) (Maslin, 2005; Maslin and Orchard, 2005). Clarification on the new sub-genera and name changes has yet to be reached. However, expected changes are explained by Jawad *et al*. (2000) and Luckow *et al*. (2005). For the purpose of this review, the name *Acacia* is used in the context as originally described based on an African holotype. This simplifies the discussion of diseases in this review that have mainly been described under the single generic name.

Acacia spp. play an important role ecologically and they contribute to the economies of many countries of the world. They are important sources of fuel (wood or charcoal), medicines, gums, tannins, building material, fibre, rope, honey and are also used in agro-forestry and for aesthetic purposes (Midgley and Bond, 2001; Mahdi *et al*., 2006; Van Rooyen *et al*., 2001; Van Wyk and Van Wyk, 1997; Venter and Venter, 2002). In addition, they play a significant role in nitrogen fixation (Brockwell *et al*., 2005; Midgley and Bond, 2001).

A number of Australian *Acacia* spp. form the basis for multi-million dollar commercial forestry operations, providing pulp for paper and viscose production. In this regard, plantations have been established in Africa, Indonesia, Malaysia, South America and the Indian continent (Brown, 2000; Chamshama and Nwonwu, 2004; Midgley and Turnbull, 2003). In Africa, the majority of plantations can be found in South Africa and the Mediterranean countries of North Africa (Brown, 2000). They are mainly planted for the production of paper and rayon, fuel wood, tannins, human and animal food, for land rehabilitation, conservation and as windbreaks (Bakshi, 1976; Gibson, 1980; Midgley and Turnbull, 2003). Large-scale plantations of Australian *Acacia* spp. in Africa were established during the 1800's up until the early/mid 1900's (Sherry, 1971; Chamshama and Nwonwu, 2004). Many *Acacia* spp. were introduced into southern Africa over the years. This was usually for commercial purposes (Palgrave, 2002). Australian *Acacia* spp. are particularly popular for planting, due to their adaptability, fast growth, high quality timber and tannins (Bakshi, 1976; Gibson, 1980; Midgley and Turnbull, 2003).

Fungal diseases of *Acacia* spp. have largely been neglected, except for those occurring on the more economically important plantation *Acacia* spp., which are non-native to Africa, South East Asia and South America. Lists of pathogens and associated diseases have been produced including those of Gibson (1975, 1980), Old *et al*. (2000, 2002) and Roux *et al*. (1995). There were also studies that specifically focused on diseases of plantation *Acacia* spp. in southern Africa (Roux and Wingfield, 1997; Roux, 2002).

In contrast, very little attention has been given to diseases and fungi associated with *Acacia* spp. growing as natives in natural ecosystems. In this regard, the importance of endemic *Acacia* spp. and the protection of associated native ecosystems from diseases, particularly those caused by alien invasive pathogens, are of crucial importance, particularly in Africa. This is especially true with the planting of non-native *Acacia* spp. in close proximity to native species in many countries, allowing for the possibility of fungal pathogen-host jumps (Blaney and Kotanen, 2001; Slippers *et al*., 2005).

According to Wingfield and Day (2001), one of the biggest threats to non-native trees used in plantations in Africa is that of introduced pathogens and pests. These authors indicated that there is an increase in the frequency of new records of pathogens attacking these hosts. This clearly has significant cost implications that negatively affect trade and the development of plantations.

This review mainly aims to provide a summary of fungi associated with *Acacia* spp. growing on the African continent. This includes information available on reported

pathogenic and non-pathogenic fungi, as well as their association with native and non-native *Acacia* spp. in southern Africa. Attention is also given to recorded and possible pathogen-host jumps occurring between native and non-native *Acacia* spp. and the potential threat they might pose to the future biosecurity of these important trees.

It should be noted that significant taxonomic changes have taken place in some species and associated genera since the first reports by Doidge in the 1920's. The purpose of this review is not to validate the correctness of names, but rather to provide an overview of the reports and findings over the past century. In cases where we are aware of name changes the information is accordingly indicated in the review.

2.0 FUNGI ASSOCIATED WITH AFRICAN *ACACIA* SPP.

The majority of reports of fungi occuring on native *Acacia* spp. in Africa were published by Doidge (Doidge, 1927, 1939, 1941, 1942, 1948, 1950; Doidge *et al*., 1953) during the early 1920's to 1950's and extensive reviews of the topic were produced by Gibson (1975, 1980). The pathogens most frequently listed by these authors were of rusts belonging to the genus *Ravenelia* Berk. (TABLE I). *Ravenelia evansii* Syd. is the most frequently observed, and an unknown *Ravenelia* sp. has been reported on seventeen different *Acacia* spp. No further information is provided whether this represent a single fungal species or whether the isolates include different species within the same genus. The distribution range of these species is from central to eastern Africa, although most reports refer to southern Africa. Symptoms caused by these fungi include leaf and phyllode rust, characterized by leaf pustules, leaf cast

and malformation of leaves, phyllodes and also flowers (Gibson, 1975, 1980). The ecological impact of these rust pathogens on the *Acacia* spp. has not been investigated.

Numerous species of *Fomes, Ganoderma* and *Phellinus* have been reported to cause either heart, root or trunk rot on African *Acacia* spp. *Fomes* spp. have been most frequently reported, however, many reports of these fungi include little or no information on the specific geographic region of occurrence of these fungi. Reports are mainly from southern Africa but also include Ethiopia, Kenya and Zambia (Browne, 1968; Doidge, 1950; James, 1983; Spaulding, 1961; Van der Byl, 1922) (TABLE I). It should be noted here that significant taxonomic revisions of the Aphylophoraceae has been done since the publication of these reports (Kim and Jung, 2000).

Due to the lack of knowledge on the association of fungi with native *Acacia* spp. in southern Africa, a preliminary survey of fungi associated with native plant and tree species in the Northern Cape area was done (Van der Walt *et al*., 2007). Amongst the species surveyed were *A. erioloba* E. May., *A. haematoxylon* Willd., *A. karoo* Hayne, *A. mellifera* (M. Vahl.) Benth. and *A. tortilis* (Forks.) Hayne. Some of the fungi that emerged from this study, and that might include significant pathogens, included species of the Botryosphaeriaceae (reported as *Diplodia*- and *Sphaeropsis*-like), *Fusarium* spp. and *Phoma* spp. (TABLE I). While pathogenicity tests and more detailed studies are required, the study emphasizes the lack of knowledge regarding diseases of these important trees. It should also be noted that significant changes have

occurred in the taxonomy of the Botryosphaeriaceae (Crous *et al*., 2006; Phillips *et al*., 2008) and these were not reflected in the study of Van der Walt *et al*. (2007).

Diseases of *A. mellifera* (blackthorn) have recieved some attention in recent years. This plant is one of the most valuable native trees in Africa (Venter and Venter, 2002; Van Wyk and Van Wyk, 1997; Van Rooyen *et al*., 2001) and it is thought to be threatened by a die-back disease, which has killed thousands of hectares of trees (Holz and Schreuder, 1989). *Phoma glomerata* (Cda) Wollenw. & Hochapf., *P. eupyrena* Sacc., *P. cava* Schulz. and *Cytospora chrysosperma* Pers.: Fr. have been suggested as the causal agents of the disease (Holz and Schreuder, 1989) (TABLE I). The disease is characterized by leaf chlorosis, defoliation, twig and branch die-back, and canker formation at the bases of the trunks. This however, differs from other die-back symptoms that include internal decay of the sapwood and heartwood at the base of the trunks and upper tap root regions (Holz and Schreuder, 1989). This die-back is also typical of die-back caused by fungi in the Botryosphaeriaceae.

3.0 FUNGI ASSOCIATED WITH NON-NATIVE *ACACIA* SPP. IN AFRICA

The most important Australian *Acacia* sp. planted in Africa, and more specifically, southern Africa, is *A. mearnsii* de Wild., also commonly known as the black wattle (Sherry, 1971). This tree was introduced into South Africa in 1864 (Sherry, 1971; Midgley and Turnbull, 2003) and is now planted in Kenya, South Africa, Swaziland, Tanzania, Uganda and Zimbabwe (Brown, 2000). Two other Australian *Acacia* spp., tested for commercial deployment in Africa, include *A. dealbata* Link. (silver wattle) and *A. decurrens* (green wattle), which have been planted in Kenya, South Africa,

Tanzania and Uganda. However, these trees have been replaced by *A. mearnsii* in these countries due to its higher tannin yield (Bakshi, 1976; Gibson, 1980; Sherry, 1971). Because of the economic importance of *A. mearnsii*, a number of studies have been conducted to identify and manage fungal diseases associated with this tree species in Africa. The diseases of *A. dealbata* and *A. decurrens* have also been studied in cases where these plants were grown commercially.

3.1 Fungi associated with *Acacia dealbata* and *A. decurrens* in Africa

There are only six reports of diseases on *A. dealbata* from Africa, and they do not include detailed information on the extent and impact caused by the diseases (TABLE II). The reports are mainly from South Africa with one report from Tanzania and Uganda respectively. There are no reports of rust fungi on these hosts from Africa.

Only two stem diseases have been recorded on *A. dealbata* in Africa. *Ceratocystis albifundus* De Beer, Wingfield and Morris has been found causing gummosis and wilting of this tree in South Africa (Morris *et al*., 1993; Roux *et al*., 2001a). *Cylindrocladium scoparium* Morgan, has been recorded as the causal agent of diseases such as leaf drop, stem disease and post emergence damping off in South Africa (Bakshi, 1976; Gibson, 1980) (TABLE II).

A larger number of diseases have been reported on *A. decurrens* in Africa than those on *A. dealbata* (TABLE II). However, most reports date back more than fifty years and detailed information on symptoms and specific geographic regions of occurrence of

the diseases is very limited. Most include rot fungi that are not considered as primary pathogens.

Species of *Polystictus* and *Trametes* are the most commonly described fungi recorded on *A. decurrens* and species inlcude *P. hirsutus* (Schw.) Cooke, *P. occidentalis* (Klotzsh) Fr., *P. polyzonus* (Pers.) Cooke, *P. sanguineus* (Linn. Ex Fr.) Fr., *P. versicolor* (Linn. Ex Fr.) Fr., *T. cingulata* Berk., *T. meyenii* (Klotzsch) Lloyd, *T. trabea* (Pers. Ex Fr.) Bres. and *T. violacea* Lloyd. (Doidge, 1950) (TABLE II). It should be noted that significant taxonomic revisions has been done in these genera and the Aphylophoraceae (Kim and Jung, 2000).

3.2 Fungi associated with *Acacia mearnsii* in Africa

Due to its economic importance, especially in South Africa, various studies on the diseases affecting *A. mearnsii* have been conducted. The last review treating diseases of *A. mearnsii* was that of Roux *et al*. (1995). The following provides an update after considerable new research has emerged on the topic since the review by these authors.

Most reports of new diseases have emerged from two surveys. The first was a survey during 1994 to 1995, covering two important commercial wattle-growing areas in South Africa (Roux and Wingfield, 1997). The second survey formed part of a study of diseases on plantation tree species in eastern and southern Africa that was undertaken by Roux *et al*. (2005).

A wide range of fungi were reported from the first survey, which included the Bloemendal Experimental Farm at Pieterrnaritzburg (KwaZulu/Natal Midlands),

various farms surrounding the town of Piet Retief (South Eastern Mpumalanga Province) and a small number of trees sampled from bush wattle stands in Alexandria (Eastern Cape Province). One of these was *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. that was previously reported from South Africa, but the specific localities were not indicated (Stephens and Goldschmidt, 1938; Gibson, 1975). A more recent study has now shown that this fungus occurs throughout the plantations of Kwa-Zulu Natal, South Africa (Roux and Wingfield, 1997). Another fungus includes *Botryosphaeria dothidea* (Roux and Wingfield, 1997; Roux *et al*., 1997) (TABLE II), however, due to recent taxonomic changes in the Botryosphaeriaceae (Crous *et al*., 2006; Phillips *et al*., 2008) this name may be inaccurate. The taxonomic placement of this fungus has yet to be confirmed.

Cylindrocladium candelabrum Viégas has been reported form *A. mearnsii* (Doidge, 1950; Gibson, 1975; Sherry, 1971). This fungus was later shown, based on molecular comparisons, to represent *C. pauciramosum* C.L.N. Schoch & Crous (Lombard *et al*., 2004) and not *C. candelabrum* as previously described. These authors also reported this fungus on plants in nurseries from South Africa (TABLE II).

Three reports on *A. mearnsii* emerged from the second survey (Roux *et al*., 2005). These included Armillaria root rot in Kenya, Phytophthora butt-rot reported from Kenya and Tanzania and *Ceratocystis albifundus* in Kenya and Tanzania causing rapid wilting and death of trees of all ages (TABLE II).

One of the most recent reports on *A. mearnsii* is *Fusarium acaciae-mearnsii* O'Donnell, T. Aoki, Kistler et Geiser (O'Donnell *et al*., 2004), first reported as *F.*

graminearum (Roux *et al*., 2001b). This fungus was isolated during a routine survey of diseases of *A. mearnsii* and *Eucalyptus grandis* Hill: Maid. in South Africa causing branch cankers (Roux *et al*., 2001b). Pathogenicity tests were conducted on *A. mearnsii*, which produced lesions that were characterized by black discoloration of the outer bark surface and the formation of sunken cankers. *Fusarium graminearum* sensu lato is a well-known pathogen of grain crops and this was the first report of the fungus as a pathogen of woody hosts (Roux *et al*., 2001b). The isolates occurring on *A. mearnsii* have subsequently been provided with a new name, *F. acaciae-mearnsii* (O'Donnell *et al*., 2004) (TABLE II).

Pink disease caused by *Erythricium salmonicolor* (Berk. & Broome) Burdsall.) (reported as *Corticium salmonicolor* B. & Br.) is a well-known pathogen on *A. mearnsii* in Indonesia, Malaysia and Mauritius (Roberts, 1957; Brown, 1968; Bakshi, 1976; Lenné, 1992). This pathogen (also reported as *Co. salmonicolor*) was first recorded on this host from South Africa by Spaulding (1961), Brown (1968) and Bakshi (1976). The most recent report was from the Kwazulu-Natal Midlands, near Howick, South Africa (Roux and Coetzee, 2005). These authors identified this pathogen as *E. salmonicolor*, and also showed a clear separation between this species and fungi in the genera *Corticium* and *Phanerochaete* (TABLE II). The occurrence of this pathogen on trees in South Africa could be a cause for concern.

3.3 Fungi associated with non-native *Acacia* spp. outside of Africa

This review mainly focuses on *Acacia* spp. and their associated fungi on the African continent. Therefore, reports outside this scope are not discussed, except some recent studies that contain information that could be relevant to the African situation.

Various Australian *Acacia* spp. other than *A. mearnsii* are planted successfully in over 70 countries worldwide (Gibson, 1980; Midgley and Turnbull, 2003; Old *et al*., 2000, 2002). Many diseases have been reported on these hosts, including foliage and stem diseases, root-, butt-, stem and heart rots (Bakshi, 1976; Gibson, 1980; Midgley and Turnbull, 2003; Old *et al*., 2000, 2002; Spaulding, 1961, 1964).

During 1995 to 1996, a series of disease surveys was done of native stands, trials and plantation-grown *Acacia* spp. in Australia, India, Indonesia, Malaysia and Thailand (Barry, 2002). Trees included *A. auriculiformis* A. Cunn., *A. aulacocarpa* A. Cunn ex Benth., *A. crassicarpa* Benth. and *A. mangium* Willd. in tropical areas of southeast Asia, India and Australia. From this survey, a manual was published of the diseases associated with these trees (Old *et al*., 2000). Results indicated five significant diseases as well as the possible pathogens that could be linked to these diseases. These included root rot (caused by a *Ganoderma* complex), pink disease (caused by *E. salmonicolor*), stem canker (caused by e.g. *L. theobromae* and other *Botryosphaeriaceae* spp.), heartrot (caused by wood decay fungi) and phyllode rust (caused by *Atelocauda digitata* (G. Wint.) Cummins and Y. Hiratsuka).

Erythricium salmonicolor, the cause of pink disease, has a wide host range, ranging from forest- to fruit trees and a world wide distribution (Brown, 1968; Old *et al*., 2000, 2002). This fungus was reported (as *Co. salmonicolor*) on *A. decurrens* in

South Africa, Malaysia and Mauritius (Bakshi, 1976; Brown, 1968; Gibson, 1980) and *A. mearnsii* from Indonesia, Malaysia and Mauritius (Bakshi, 1976; Brown, 1968; Gibson, 1980; Lenné, 1992; Spaulding, 1961). Recently, it was also recorded from native South African tree species (Roux and Coetzee, 2005), representing a possible host jump between unrelated tree species. There is thus a risk that this fungus could also spread to native African *Acacia* spp.

There are various reports of *Ganoderma* spp. on both native and non-native *Acacia* spp. in Africa (TABLE I, II), as well as various reports from countries outside of Africa (Bakshi, 1976; Browne, 1968; Gibson, 1980; Lenné, 1992; Old *et al*., 2000, 2002). *Ganoderma philippii* (Bres. & Henn. ex Sacc.) Bres., for example is identified as one of the most serious threats to *A. mangium* plantations in Indonesia and Malaysia (Lee, 2004; Old *et al*., 2000, 2002).

Passalora perplexa Beilharz, Pascoe, M.J. Wingf. & Crous, an important leaf blight pathogen was recently reported from lesions on blighted phyllodes of *A. crassicarpa* in Australia and Indonesia (Beilharz *et al*., 2004). However, this fungus is highly host specific, thus it is very unlikely that it would affect native African *Acacia* spp.

4.0 HOST JUMPS: PATHOGEN MOVEMENT FROM NATIVE TO NON-NATIVE *ACACIA* SPP.

Movement of fungal pathogens between different countries and continents is one of the most serious concerns to tree health worldwide (Slippers *et al*., 2005; Wingfield *et al*., 2001a, b; Wingfield, 2003). With increased trade between countries, the

possibility of this happening has increased dramatically in the last few decades (Blaney and Kotanen, 2001; Dwinell, 2001; Wingfield and Day, 2001). Although plantations are expanding in Africa (Brown, 2000; Palmberg-Lerche *et al*., 2002), many countries do not have effective quarantine systems in place to constrain the movement of pests and pathogens (Wingfield and Day, 2001). These factors, together with the adaptability of fungal pathogens, greatly increases the potential and risk of host jumps of pathogens between different plant genera and even families (Balney and Kotanen, 2001; Slippers *et al*., 2005; Wingfield *et al*., 2001a; Wingfield and Day, 2001; Wingfield, 2003).

Plantation forestry, based on non-native tree species such as Australian *Acacia* spp., in Africa has grown remarkably (Brown, 2000; Palmberg-Lerche *et al*., 2002). One of the factors that has accounted for this is unquestionably the separation of these plants from the pests and diseases that restrict their growth where they are native (Wingfield and Day, 2001; Wingfield, 2003). Logically, these plantations are seriously threatened by pathogens that might be introduced from the countries where they originate. In this regard, where such pathogens have already become established in new environments, the chance of them being introduced into additional countries is substantially enhanced. A second and less-well recognized threat is where pathogens of native plants become adapted to infect non-natives. This adaptation or the so-called "host-jumps" present a threat not only to the trees in their new environments, but also to the trees in their country of origin. Available data from research indicates an increase in frequency of such host jumps occurring and thus the emergence of so-called new pathogens is likely (Wingfield *et al*., 2001b; Wingfield and Day, 2001; Wingfield, 2003).

There are some important contemporary examples of pathogen host jumps associated with non-native plantation forestry that have emerged. Perhaps the best known is that of Eucalyptus rust caused by *Puccinia psidii* Winter. This fungus jumped from native Myrtaceae to non-native Eucalyptus trees in South America (Coutinho *et al*., 1998; Glen *et al*., 2007). Another intriguing example is of various *Chrysoporthe* spp. (previously *Cryphonectria cubensis* (Bruner) Hodges) reported on native Myrtaceae and Melostomataceae, including non-native *Eucalyptus* spp. in Southeast Asia, South America and Africa (Gryzenhout *et al*., 2004; Wingfield, 2003). Another example is species in the Botryosphaeriaceae that occurs on native *Syzygium cordatum* Hochst.ex C.Krauss. and introduced *Eucalyptus* spp. (Pavlic *et al*., 2007) in South Africa.

4.1 Potential host jump threats for *Acacia* spp. in Africa

Ceratocystis albifundus, considered to be the most important pathogen of *A. mearnsii* in South Africa, is hypothesized to be native to southern Africa (Barnes *et al*., 2003; Roux *et al*., 2001a). This view is based on the fact that it is known only from the African continent. It was also first isolated from three native *Protea* spp. from South Africa (Gorter, 1977) and it has a wide host range on native African tree species (Roux *et al*., 2007). This fungus is also highly virulent on non-native plantation grown *A. mearnsii* trees (Roux *et al*., 1999) and population biology studies have shown that it has a high level of genetic diversity in Uganda and South Africa(Roux *et al*., 2001a; Barnes *et al*., 2005).

Ceratocystis albifundus was first recorded in South Africa on *A. mearnsii* in the late 1980's as *C. fimbriata* (Morris *et al*., 1993). It was, however, later shown, using

morphological and DNA sequence-based comparisons, that the pathogen represents a novel taxon that was re-named *C. albifundus* (Wingfield *et al*., 1996). Infection normally results in rapid wilting, often accompanied by gummosis, and eventual death of susceptible trees (Morris *et al*., 1993; Roux *et al*., 1999). The pathogen has been recorded from *A. mearnsii* in South Africa, Uganda, Tanzania and Kenya (Morris *et al*., 1993; Roux and Wingfield, 1997, 2001; Roux *et al*., 2005, 2007). *Ceratocystis albifundus* has also been recorded on *A. dealbata* and *A. decurrens* from South Africa, causing gummosis and wilting (Roux *et al*., 2001a) (TABLE II).

The host range of *C. albifundus* on native South African tree species spans eight different genera, in seven different families (Roux *et al*., 2007). No associated disease has to date been reported on these native hosts, further supporting the view that *C. albifundus* is native to Africa. *Ceratocystis albifundus* could represent a great ecological threat if it were to be introduced into Australia, where *A. mearnsii* and *A. decurrens* are native. The potential threat is emphasized by the fact that many tree genera related to the native South African hosts, such as those in the Proteaceae, also occur in Australia. Effectively, planting Australian *Acacia* spp. in Africa has provided an African fungus with the opportunity to jump to a new, susceptible host, and thus to establish a bridge for the potential introduction and establishment in a country such as Australia.

The Botryosphaeriaceae have a wide geographic distribution and host range and some species can cause disease on stems, branches, twigs and leaves. Symptoms include shoot blight, leaf spot, fruit and seed rot, “witches broom”, die-back and cankers and in severe cases tree death (Denman *et al*., 2000; Smith *et al*., 1996; Slippers and

Wingfield, 2007). A few species have been associated with *Acacia* spp. For example, *L. theobromae*, reported as *Botryodiplodia theobromae* Pat. [(syn. of *Botryosphaeria rhodina* (Cooke) von Arx.] was recorded as the causal agent of root disease of *A. nilotica* in Kenya and India (Lenné, 1992). This pathogen has also been recorded on *A. auriculiformis* in India (Lenné, 1992), *A. mangium* in Malaysia (Lenné, 1992) and *A. mearnsii* where it causes a root disease and stem cankers (Stephens and Goldsschmidt, 1938; Gibson, 1975; Roux and Wingfield, 1997). Another species, *B. dothidea,* causes stem and branch cankers, tip die-back and pith discolouration of *A. mearnsii* in the Eastern Cape, Kwa-Zulu Natal, Mapumalanga and Western Cape provinces of South Africa (Roux and Wingfield, 1997; Roux *et al*., 1997) (TABLE II). However, it should be noted that due to the extensive recent revision of the Botryosphaeriaceae, the fungus name used in these reports probably needs revision (Crous *et al*., 2006; Phillips *et al*., 2008).

Because of their wide distribution and host range the Botryosphaeriaceae pose considerable risks as pathogens that can jump between native and non-native tree hosts (Slippers and Wingfield, 2007). According to these authors they appear to be able to infect different hosts and contribute to the genetic diversity and fitness of an already existing population once they have been introduced into a new environment. Because of this threat, the identification and knowledge of the biology of these pathogens are crucial, especially when they occur on infected plants that are transported world wide (Wingfield *et al*., 2001a, b).

From the above examples it should be clear that the potential for, and impact of, host jumps between different *Acacia* spp. is a growing and relatively poorly understood

threat. It is important that this problem and the implications of the devastating effect that it could have, are realised and addressed.

5.0 CONCLUSIONS

Acacia spp. native to Africa cover vast areas of the continent and play an important role economically and ecologically and are vital to people living on the continent. Considerable attention has been paid to diseases of plantation *Acacia* spp. (non-native in Africa) but the diseases of the less economically important and native *Acacia* spp. have been virtually ignored.

Since the establishment of non-native plant species through agricultural and forestry practices, the emergence of new plant pathogens has been dramatically altered. Emerging threats of disease are constantly observed in the forestry industry and it is becoming more relevant to understand diseases caused by indigenous mycoflora and the ability of pathogenic organisms to colonize and infect existing and new hosts.

Although *Acacia* spp. comprise a significant part of the indigenous flora of southern Africa, there is still a considerable lack of knowledge regarding their associated fungi. Understanding these relationships will contribute to improving our approach to the protection of indigenous flora on the African content. This will also increase our knowledge of the biodiversity of African fungi and their role in ecosystems. The aim of this thesis is, therefore, to contribute to and gain knowledge of fungal pathogens associated with native African *Acacia* spp. This is achieved by focusing on a few

selected *Acacia* spp. and will hopefully lay the foundation for a clearer understanding of these trees and their associated disease problems.

6.0 REFERENCES

Bakshi BK. 1976. Wattles – *Acacia* spp. Forest pathology principles and practice in forestry. F.K.I Press, Forest research institute and colleges, Dehra Dien, India, pp. 190–194.

Barnes I, Roux J, Wingfield BD, Dudzinski MJ, Old KM, Wingfield MJ. 2003. *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. Mycologia 95:865–871.

—, Nakabonge G, Roux J, Wingfield BD, Wingfield MJ. 2005. Comparison of populations of the wilt pathogen *Ceratocystis albifundus* in South Africa and Uganda. Plant Pathology 54:189–195.

Barry K. 2002. Heartrots in Plantation Hardwoods in Indonesia and Australia. ACIAR Technical Reports No. 51e, pp. 5.

Beilharz VC, Pascoe IG, Wingfield MJ, Tjahjoni B, Crous PW. 2004. *Passalora perplexa*, an important pleoanamorphic leaf blight pathogen of *Acacia crassicarpa* in Australia and Indonesia. Studies in Mycology 50:471–479.

Blaney CS, Kotanen PM. 2001. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. Journal of Applied Ecology 38:1104–1113.

Brockwell J, Searle SD, Jeavons AC, Waayers M. 2005. Nitrogen fixation in acacias: an untapped resource for sustainable plantations, farm forestry and land reclamation. ACIAR Monograph No. 115, pp. 132.

Browne FG. 1968. Pests and Diseases of Forest Plantation Trees: An annotated list of the principal species occurring in the British Commonwealth. Oxford University Press, Glasglow, London, pp. 1015−1017.

Brown C. 2002. The global outlook for future wood supply from forest plantations. Global Forest Products Outlook Study, Working Paper Series: FAO, Working Paper No: GFPOS/WP/03.

Chamshama SAO, Nwonwu FOC. 2004. Forest plantations in Sub-Saharan Africa. A report prepared for the project: Lessons learnt on Sustainable forest management in Africa. Sokoine University of Agriculture, Faculty of Forestry & Nature Conservation, Tanzania, pp. 1–55.

Clarke HD, Downic SR, Seigler DS. 2000. Implications of chloroplast DNA restriction site variation for systematics for *Acacia* (Fabacea: Mimosoideae). Systematic Botany 25:618–632.

Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW. 1998. Eucalyptus rust: a disease with the potential for serious international implications. Plant Disease 82:819–825.

Crous PW, Phillips AJL, Wingfield MJ. 1991. The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. South African Forestry Journal 157:69–85.

—. 1993. New and interesting records of South African fungi. XIII. Foliicolous microfungi. South African Journal of Botany, 59:602–610.

—, Phillips AJL, Baxter AP. 2000. Phytopathogenic Fungi from South Africa. Department of Plant Pathology Press, University of Stellenbosh, pp. 73–75.

—, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology 55:235–253.

Denman S, Crous PW, Taylor JE, Kang J-C, Pascoe I, Wingfield MJ. 2000. An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Studies in Mycology 45:129−140.

Doidge EM. 1927. A preliminary study of the South African Rust fungi. Bothalia 2:154−159.

—. 1939. South African Rust Fungi, Part III. Bothalia 3:487−512.

—. 1941. Some South African Valsaceae. Bothalia 4:47−74.

—. 1942. Revised describtions of *Phyllachora* and related fungi. Bothalia 4:421−463.

—. 1948. South African Rust Fungi, Part V. Bothalia 4:895−918.

—. 1950. The South African fungi and lichens to the end of 1945. Bothalia 5:844−848.

—, Bottomley AM, van der Plank JE, Pauer GD. 1953. A revised list of plant diseases in South Africa. Department of Agriculture, South Africa. Science Bulletin no. 346.

Dwinell LD. 2001. Exotic Forest Pests – A Global Issue. International online workshop to reduce the movement of forest pests with a minimal impact on trade held, April, 16–29, 2001. http://www.apsnet.org/online/proceedings/exoticpest/Papers

Gibson IAS. 1964. The impact of disease on forest production in Africa. FAO/IUFRO Symposium on internationally dangerous forest diseases and insects: Oxford.

—. 1975. The Leguminosae. In Diseases of Forest Trees Widely Planted as Exotics in the Tropics and Southern Hemisphere. Part I. Important members of the Myrtaceae, Leguminosae, Verbenaceae and Meliaceae. Commonwealth Forestry Institute, University of Oxford: Oxford. (ed. IAS Gibson), pp. 21–34.

—. 1980. Diseases of Forest Trees Widely Planted as Exotics in the Tropics and Southern Hemisphere, Part I. Important members of the Myrtaceae, Leguminosae, Verbenaceae and Meliaceae. Commonwealth Mycological Institute, Kew, pp. 21−27.

Glen M, Alfenas AC, Zauza EAV, Wingfield MJ, Mohammed C. 2007. *Puccinia psidii*: a threat to the Australian environment and economy – A review. Australasian Plant Pathology 36:1–16.

Gorter GJMA. 1977. Index of Plant Pathogens and the Diseases they Cause in Cultivated Plants in South Africa. Science Bulletin no. 392. Pretoria, South Africa: Plant Protection Research Institute, Department of Agricultural Technical Services, pp. 13, 90.

—. 1993. A revised list of South African Erysiphaceae (powdery mildews) and their host plants. South African Journal of Botany 59:566–568.

Gryzenhout M, Myburg H, Van der Merwe NA, Wingfield BD, Wingfield MJ. 2004. *Chrysoporthe*, a new genus to accommodate *Cryphonectria cubensis.* Studies in Mycology 50:119–142.

Holz G, Schreuder W. 1989. Dieback of blackthorn (*Acacia mellifera* subsp. *detinens*) in South West Africa. Agricola 7.

Hughes SJ. 1952. Studies on micro fungi. XIV. *Stigmella, Stigmina, Camptomeris, Polythrincium* and *Fusicladiella*. Mycological Papers 49:1–25.

James AD. 1983. Handbook of Energy Crops.

http://www.hort.purdue.edu/newcrop/Indices/index_ab.html

Jawad JT, Seigler DS, Ebinger JE. 2000. A systematic treatment of *Acacia coulteri* (Fabaceae, Mimosoideae) and similar species in the world. Annals of the Missouri Botanical Garden 87:528–548.

Kim SY, Jung HS. 2000. Phylogenetic Relationships of the Aphyllophorales Inferred from Sequence Analysis of Nuclear Small Subunit Ribosomal DNA. The Journal of Microbiology 38:122–131.

Ledeboer MSJ. 1946. Developments in pathological research on wattles. Journal of South African Forestry Association 4:28–45.

Lee SS. 2004. Diseases and potential threats to *Acacia mangium* plantations in Malaysia. Forest Research Institute Malaysia, Kepong, Kuala Lumpur, Malaysia. Unasylva 217, 55:31–35.

Lenné JM. 1992. Diseases on multi purpose woody legumes in the tropics: A Review. Nitrogen Fixing Tree Research Reports 10:13−29.

Lombard L, Coutinho TA, Janse BJ, Winfield MJ. 2004. *Cylindrocladium pauciramosum*, the dominant fungal pathogen in *Eucalyptus* clonal propagation nurseries in South Africa. In MSc thesis: Fungal diseases in *Eucalyptus* and *Acacia* nurseries in South Africa. University of Pretoria, South Africa.

Lückhoff HA. 1964. Diseases of exotic plantation trees in the Republic of South Africa. FAO/IUFRO Symposium on internationally dangerous forest diseases and insects: Oxford, pp. 28−45.

Luckow M, Hughes C, Schrire B, Winter P, Fagg C, Fortunato R, Hurter J, Rico L, Breteler FJ, Bruneau A, Caccavari M, Craven L, Crisp M, Delgado A, Demissew S, Doyle JJ, Grether R, Harris S, Herendeen PS, Hernández HM, Hirsch AM, Jobson R, Klitgaard BB, Labat J-N, Lock M, MacKinder B, Pfeil B, Simpson BB, Smith GF, Sousa M, Timberlake J, van der Maesen JG, Van Wyk AE, Vorster P, Willis CK, Wieringa JJ, Wojciechowski MF. 2005. *Acacia*: the case against moving the type to Australia. Taxon 54:513–519.

Mahdi H, Palmina K, Glavtch I. 2006. Characterization of *Acacia nilotica* as an indigenous tanning material of Sudan. Journal of Tropical Forest Science 18:181–187.

Maslin BR, Orchard AE, West JG. 2003a. Nomenclatural and classification history of *Acacia* (Leguminosae: Mimosoideae), and the implications of generic subdivision. Unpublished paper was submitted to the Committee for Spermatophyta. http://www.worwidewattle.com/infogallery/nameissue/

—, Miller JT, Seigler DS. 2003b. Overview of the generic status of *Acacia* (Leguminosae: Mimosoideae). Australian Systematic Botany 16:1−18.

—. 2005. Generic and infrageneric names in *Acacia* following retypification of the genus. http://www.worwidewattle.com/infogallery/nameissue/

—, Orchard AE. 2005. *Acacia* – The final decision. http://www.worwidewattle.com/infogallery/nameissue/

Midgley JJ, Bond WJ. 2001. A synthesis of the demography of African acacias. Journal of Tropical Ecology 17:871−886.

Midgley SJ, Turnbull JW. 2003. Domestication and use of Australian Acacias: case studies of five important species. Australian Systematic Botany 16:89–102.

Miller JT, Bayer RJ. 2001. Molecular phylogentics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast *MATK* coding sequence and flanking *TRNK* intron spacer regions. American Journal of Botany 88:697–705.

—, —. 2003. Molecular phylogenetics of *Acacia* subgenera *Acacia* and *Aculifeirum* (Fabaceae: Mimosoideae), based on the chloroplast *matK* coding sequence and flanking *trnK* intron spacer regions. Australian Systematic Botany 16:27–33.

Morris MJ, Wingfield MJ, Walker J. 1988. First record of a rust on *Acacia mearnsii* in Southern Africa. Transactions of the British Mycological Society 90:324–327.

—, —, De Beer C. 1993. Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. Plant Pathology 42:814–817.

Murphy DJ, Miller JT, Bayer RJ, Ladiges PY. 2003. Molecular phylogeny of *Acacia* subgenus *Phyllodineae* (Mimosoideae: Leguminosae) based on DNA sequences of the internal transcribed spacer region. Australian Systematic Botany 16: 9–26.

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aokid T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genetics and Biology 41:600–623.

Old KM, See LS, Sharma JK, Yuan ZQ. 2000. A Manual of Diseases of Tropical Acacias in Australia, South-East Asia and India. Centre for International Forestry Research: Jakarta, Indonesia.

—, Vercoe TK, Floyd RB, Wingfield MJ, Roux J, Neser S. 2002. FAO/IPGRI: Technical guidelines for the save movement of germplasm. No. 20. *Acacia* spp. Food and Agriculture Organizaton of the United Nations, Rome/International Plant Genetic Resources Institute, Rome.

Olembo TW, Muguga EA. 1972. *Phoma herbarium* Westend: A pathogen on *Acacia mearnsii* De Wild. in Kenya. East African Agricultural and Forestry Journal, 201–206.

Orchard AE, Maslin BR. 2003. (1584) Proposal to conserve the name *Acacia* (Leguminosae: Mimosoideae) with a conserved type. Taxon 52:362–363.

—, —. 2005. The case for conserving *Acacia* with a new type. Taxon 54:509−512.

Palgrave MC. 2002. Trees of Southern Africa. New Edition in association with RB Drummond, Edited by Dr. EJ Moll. pp. 264–304.

Palmberg-Lerche C, Iversen PA, Sigaud P. 2002. Global data on forest plantation resources. Forest Genetic Resources Bulletin: FAO 29.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ. 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. Plant Pathology 50:1–13.

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW. 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21: 29–55.

Rico-Arce L. 2001. Geographical patterns in neotropical *Acacia* (Mimosoideae: Leguminosae). Australian Systematic Botany 16:41–48.

Roberts K. 1957. A list of fungi collected in wattle plantations. Report of the Wattle Research Institute, pp. 26–28.

Roux J, Kemp GHJ, Wingfield MJ. 1995. Diseases of Black Wattle in South Africa – A Review. South African Journal of Forestry 174:35−40.

—, Wingfield MJ. 1997. Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. Forest Ecology and Management 99:327−336.

—, —, Morris MJ. 1997. *Botryosphaeria dothidea* as a pathogen of *Acacia mearnsii* in South Africa. South African Journal of Science 93:xii.

—, Dunlop R, Wingfield MJ. 1999. Susceptibility of elite *Acacia mearnsii* families to Ceratocystis wilt in South Africa. Journal of Forestry Research 4:187–190.

—, Wingfield MJ. 2001. First report of Ceratocystis wilt of *Acacia mearnsii* in Uganda. Plant Disease 85:1029.

—, Harrington TC, Steimel JP, Wingfield MJ. 2001a. Genetic variation in the wattle wilt pathogen *Ceratocystis albifundus*. Mycoscience 42:327–32.

—, Steenkamp ET, Marasas WFO, Wingfield MJ, Wingfield BD. 2001b. Characterization of *Fusarium graminearum* from *Acacia* and *Eucalyptus* using ß-tubulin and histone gene sequences. Mycologia 93:704–711.

—. 2002. Threats to Wattle plantations III: Diseases of Black Wattle: The South African Research Experience. Institute for Commercial Forestry Research, Pietermaritzburg, South Africa, pp. 121−126.

—, Coetzee MPA. 2005. First report of pink disease on native trees in South Africa and phylogenetic placement of *Erythricium salmonicolor* in the Homobasidiomycetes. Plant Disease 89:1158–1163.

—, Meke G, Kanyi B, Mwangi L, Mbaga A, Hunter GC, Nakabonge G, Heath RN, Wingfield MJ. 2005. Diseases of plantation forestry trees in eastern and southern Africa. South African Journal of Science 101:1–5.

—, Heath RN, Labuschagne L, Nkuekam GK, Wingfield MJ. 2007. Occurrence of the wattle wilt pathogen, *Ceratocystis albifundus* on native South African trees. Forest Pathology 37:1–11.

Sherry SP. 1971. 'The black wattle (*Acacia mearnsii* De Wild.)'. University of Natal Press: Pietermaritzburg, South Africa, pp. 1−382.

Slippers B, Stenlid L, Wingfield MJ. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends in Ecology and Evolution 20:8.

—, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biodiversity Review 21:75−89.

Smith H, Wingfield MJ, Crous PW, Coutinho TA. 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. South African Journal of Botany 62:86−88.

Spaulding P. 1961. Foreign diseases of forest trees of the word, Agricultural Handbook 197, U.S. Department of Agriculture 86:281–282.

—. 1964. Foreign diseases of forest trees of the word, Agricultural Handbook 197, U.S. Department of Agriculture 86:22−25, 80−81, 118−121, 202−203, 237, 256−257, 281−283.

Stephens RP, Goldschmidt WB. 1938. A prelimanary report on aspects of wattle pathology. South Africa Forestry Journal 2:32–42.

Sutton BC, Swart HJ. 1986. Australian leaf-inhabiting fungi XXII. Transactions of the British Mycological Society 87:93–102.

Van der Byl PA. 1922. Fungi of the Stellenbosch district and immediative vicinity. Transactions of the Royal Society of South Africa 10:281–288.

Van der Walt FJJ, Marais GJ, Lübben A. 2007. Fungal biodiversity of indigenous trees and plants in the Northern Cape. South African Journal of Science 103:xiii–xiv.

Van Rooyen N, Bezuidenhout H, de Kock E. 2001. Flowering plants of the Kalahari dunes. First edition. Ekotrust cc. Pretoria, South Africa, pp. 26−31.

Van Wyk B, Van Wyk P. 1997. Field guide to trees of southern Africa. Featuring more than 1000 species. Struik Publishers. Cape Town, South Africa, pp. 490−498.

Venter F, Venter J-A. 2002. Making the most of indigenous trees. Second edition. Briza Publications. Pretoria, South Africa, pp. 14−34.

Victor D, Crous PW, Janse BJH, Van Zyl WH, Wingfield MJ, Alfenas AC. 1998. Systematic appraisel of species complexes within *Cylindrocladiella*. Mycological Research 102:273–279.

Wingfield MJ, De Beer C, Visser C, Wingfield BD. 1996. A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. Systematics and Applied Microbiology 19:191–202.

—, Day RK. 2001. Risk of Exotic Forest Pests-Continental Overview for Sub-Saharan Africa. International online workshop to reduce the movement of forest pests with a minimal impact on trade held, April, 16-29, 2001.
http://www.apsnet.org/online/proceedings/exoticpest/Papers/wingfield.htm

—, Slippers B, Roux J, Wingfield BD. 2001a. Worldwide Movement of Exotic Forest Fungi, Especially in the Tropics and the Southern Hemisphere. BioScience 51:134–140.

—, Roux J, Coutino T, Govender P, Wingfield BD. 2001b. Plantation disease and pest management in the next century. South African Forestry Journal 190:67–71.

—. 2003. Increasing threat of diseases to exotic plantation forests in the Southern Hemisphere: lessons from Cryphonectria canker. Australian Plant Pathology 32:133–139.

Wood A. 2006. First report of *Ravenelia natalensis* attacking Fever Trees (*Acacia xanthophloea*). http://saspp.org/content/view/97/16/

Zeiljlemaker FCJ. 1968. The gummosis of black wattle, a complex of diseases. *Wattle Research Institute*, pp. 34–37.

TABLE I: Fungi associated with African *Acacia* spp.

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. abyssinica Benth.	*Aecidium immersum* P. Henn.	Reddish galls on twigs	Eritraea	Spaulding, 1961, 1964; Gibson, 1980
	Ravenelia volkensii P. Henn.	Leaf pustules, leaf cast and lesions on twigs	Kenya	Gibson, 1980
	Stereum hirsitum (Willd. Ex Fr.) Fr.	Wood rot	Not indicated	Spaulding, 1961, 1964
A. ataxacantha DC.	*Eutypella acaciae* Doidge	Bark fleck	Gauteng and North West Province, South Africa	Doidge, 1941; Crous *et al*., 2000
	R. halsei Doidge	Rust	Kwa-Zulu Natal, South Africa	Doidge, 1939; Crous *et al*., 2000
A. benthami F. Müll.	*Phyllachora acaciae* P. Henn.	Tar spot	Kwa-Zulu Natal, South Africa	Doidge, 1942; Gibson, 1980; Crous *et al*., 2000
		Small shiny black spots	South Africa	
A. burkei Benth.	*R. escharoides* Syd.	Leaf rust	Gauteng, Northern Province and North West Province, South Africa	Doidge, 1927; Crous *et al*., 2000
		Leaf cast and pustules	South Africa, Sudan and Rhodesia	Gibson, 1980
	Septobasidium protractum Syd.	Felt	Gauteng, South Africa	Doidge, 1950; Crous *et al*., 2000
A. caffra (Thunb.) Willd.	*Camptomeris albizziae*	Discoloured spots with pale yellow brown pustules	South Africa	Hughes, 1952; Gibson, 1980
	Ceratocystis albifundus	Bark wounds	South Africa	Roux *et al*., 2007
	R. peglerae Pole Evans	Rust	Eastern Cape and Kwa-Zulu Natal, South Africa	Doidge, 1927; Crous *et al*., 2000
		Amphigenous pustules on leaves	South Africa	Gibson, 1980

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. caffra (Thunb.) Willd.	*R. pienaarii* Doidge	Rust	Gautneg, Kwa-Zulu Natal and North West Province, South Africa	Doidge, 1927; Crous *et al*., 2000
		Leaf cast	South Africa	Gibson, 1980
A. erioloba	*Acremonium* sp. ex *Acacia eriolaba*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Alternaria chlamydospora Mouch	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	A. citri Ellis & N. Pierce in N. Pierce	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Aspergillus flavus Link: Fr.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Asp. ustus (Bain). Thom & Church.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Chaetomium indicum Corda	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Collectotrichum sp. ex *Acacia eriolaba*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Coniothyrium sp. ex *Acacia eriolaba*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Diplodia sp. ex *Acacia eriolaba*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Drechslera neergaardii Danquah	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Dre. papendorfii (Van der Aa) Ellis comb. nov.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. erioloba	*Dre. rostrata* (Drechsler) Richardson & Fraser	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Fairmaniella leprosa (Fairm.) Petr. & Syd.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Fusarium equiseti (Corda) Sacc.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. sambucinum Fuckel	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Gliocladium. catenulatum Gilman & Abbott	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Gliocladium sp. ex *Acacia eriolaba*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Humicola grisea var. grisea Traaen	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Penicillium waksmanii Zaleski	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Pithomyces chartarum (Berkeley & Curtis) Ellis	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Stachybotrys parvispora Hughes	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Ulocladium sp. ex *Acacia eriolaba*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. gerrardi Benth. var. *gerrardi*	*Ph. acaciae* P. Henn.	Tar spot	Kwa-Zulu Natal and Mpumalanga, South Africa	Doidge, 1942; Crous *et al*., 2000
		Small shiny black spots	South Africa	Gibson, 1980
	R. evansii Syd	Rust	Kwa-Zulu Natal, South Africa	Doidge, 1939; Crous *et al*., 2000
	Ravenelia sp.	Pustules on leaves and small twigs	Zambia and Ethiopia	Gibson, 1980
A. haematoxylon	*A. chlamydospora*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Asp. niger var. *niger* Van Tieghem	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Chaetomium sp. ex *Acacia haematoxylon*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Diplodia sp. ex *Acacia haematoxylon*	Not reported	Northern Cape Province, South Africa	Van der Walt *et. al.*, 2007
	Fa. leprosa	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. sambucinum	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Gli. catenulatum	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Pen. variabile Sopp	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Periconia sp. ex *Acacia haematoxylon*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Phoma eupyrena	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. herbarum Westend	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Phoma sp. ex *Acacia haematoxylon*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. haematoxylon	*R. modesta* Doidge	Rust	Gauteng, South Arica	Doidge, 1948
	R. pretoriensis Syd.	Rust	Gauteng, South Arica	Doidge, 1950
A. hebeclada subsp. *hebeclada*	*R. modesta*	Rust	Gauteng, South Africa	Doidge, 1948; Crous *et al*., 2000
	R. pretoriensis	Rust	Gauteng, South Africa	Doidge, 1950; Crous *et al*., 2000
A. karoo	*A. chlamydospora*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	A. citri	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	A. pluriseptata (P. Karst. & Har.) Jørst.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Daedalea eatoni Berk.	Not reported	Not indicated	Doidge, 1950
	Diplodia. mutila	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Fa. leprosa	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Fomes rimosus (Berk.) Cooke	Not reported	Not indicated	Doidge, 1950
	Ganoderma lucidum (Leyss.) Karst.	Trunk rot	Gauteng, South Africa	Doidge, 1950; Crous *et al*., 2000
	Hexagona crinigera Fr.	Rot	Not indicated	Doidge, 1950
	Lenzites palisoti Fr.	Not reported	Not indicated	Doidge, 1950
	Omphalia integrella (Pers. Ex Fr.) uel.	Not reported	Not indicated	Doidge, 1950
	Patellaria atrata (Hedw.) Fr.	Not reported	Not indicated	Doidge, 1950

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. karoo	*Phellinus rabiniae* (Murril) A. Ames	Not reported	Free State, South Africa	Van der Byl, 1922; Crous *et al*., 2000
	P. hedericola (Durieu & Montagne) Boerema	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Phyllactinia acaciae Syd.	Not reported	Gauteng, South Africa	Gorter, 1993; Crous *et al*., 2000
	R. inornata Diet.	Leaf rust	Eastern Cape, South Africa	Doidge, 1927; Crous *et al*., 2000
		Leaf pustules	South Africa	Gibson, 1980
	R. macowaniana Patzschke	Branch & pod Rust	Eastern Cape, Free State, Kwa-Zulu Natal, North West, Western Cape, South Africa	Doidge, 1927; Crous *et al*., 2000
		Cup shaped lesions on deformed pods and pustules	South Africa	Gibson, 1980
	R. natalensis H. & P. Syd. & Evans	Rust	Kwa-Zulu Natal, South Africa	Doidge, 1927; Crous *et al*., 2000
	Schizophyllum commune	Not reported	Not indicated	Doidge, 1950
	Scytalidium sp. ex *Acacia karoo*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Sep. protractum Syd.	Felt	Gauteng, Kwa-Zulu Natal and Northern Province in South Africa	Doidge, 1950; Crous *et al*., 2000
	St. hirsutum (Wild.: Fr.) Gray	Rot	Not indicated	Doidge, 1950
	Stilbum aurantio-cinnabarinum Speg.	Not reported	Not indicated	Doidge, 1950

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. karoo	*Trametes capensis* Lloyd	Not reported	Not indicated	Doidge, 1950
A. luederitzii Engl. var. *retinens* (Sim.) Ross & Brenan	*R. modesta*	Rust	Gauteng, South Africa	Doidge, 1948; Crous *et al*., 2000
	R. pretoriensis	Rust	Gauteng, South Africa	Doidge, 1950; Crous *et al*., 2000
A. mellifera subsp. *detinens*	*A. alternata* (Fr.) Keissler	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	A. citri	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	A. chlamydospora	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Chaetomium sp. ex *Acacia mellifera*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Cladosporium cladosporioides (Fresen.) de Vries	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Coniella sp. ex *Acacia mellifera*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Cochliobolus spicifer Nelson	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Cytospora chrysosperma	Die-back	Namibia	Holz and Schreuder, 1989
	Diplodia sp. ex *Acacia mellifera*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Dre. hawaiiensis (Bugnicourt) Subram. & Jain ex Ellis	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mellifera subsp. *detinens*	*F. chlamydosporum* Wollenw. & Reinking	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. nygamai Burgess & Trimboli	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. oxysporum	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. proliferatum (Matsushima) Nirenberg	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. scirpi Lambotte & Fautr.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. solani (Mart.)	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	F. subglutinans (Wollenweb. & Reinking) Nelson, Toussoun & Marases	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Gliocladium sp. ex *Acacia mellifera*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Melanopsamma pomiformis (Persoon:Fries) Saccardo	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Microdiscula sp. ex *Acacia mellifera*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Monodictys levis (Wiltshire) Hughes	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Neomelanconium deightanii Petrak	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mellifera subsp. *detinens*	*Nigrospora sphaerica* (Sacc.) Masson	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. cava	Die-back	Namibia	Holz and Schreuder, 1989
	P. denisii Boerema nom. nov.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. eupyrena	Die-back	Namibia	Holz and Schreuder, 1989
		Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. glomerata	Die-back	Namibia	Holz and Schreuder, 1989
	P. hedericola (Durieu & Montagne) Boerema	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. herbarum	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. nebulosa (Pers.:Fr.) Berkeley	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. pomorum Thüm.	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	P. tropica Schneider & Boerema	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Oedocephalum sp. ex *Acacia mellifera*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	R. acaciae-melliferae Sacc.	Pustules on leaves	Ethiopia	Spaulding, 1961; Gibson, 1980
	R. escharioides	Leaf cast and pustules	South Africa and Sudan	Gibson, 1980
	R. tranvaalensis Doidge	Rust and Amphigenous leaf pustules	Gauteng, South Africa	Doidge, 1950; Crous *et al*., 2000

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mellifera subsp. *detinens*	*Sep. protractum*	Felt	Gauteng, Kwa-Zulu Natal and Northern Province, South Africa	Doidge, 1950; Crous *et al*., 2000
	Sphaeropsis sapinae	Not reported	Northern Cape Province, South Africa	Van der Walt *et. al.*, 2007
	Sta. parvispora	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
A. nigrescens Oliv.	*Sep. protractum*	Felt	Gauteng, Kwa-Zulu Natal and Northern Province, South Africa	Doidge, 1950; Crous *et al*., 2000
	R. transvaalensis	Amphigenous leaf pustules	South Africa	Doidge, 1950
	Ravevelia sp.	Pustules on leaves, twigs	Zambia and Ethiopia	Gibson, 1980
A. nilotica (L.) Wild. Ex Del. subsp. *kraussiana* (Benth.) Brenan	*Ph. acaciae* subsp. *acacia* var. *acacia*	Tar spot	Kwa-Zulu Natal, South Africa	Doidge, 1942; Crous *et al*., 2000
A. nilotica	*Cyt. acaciae* Oud.	Not reported	Not indicated	James, 1983
	Diatryphe acaciae	Not reported	Not indicated	James, 1983
	Dip. acaciae Tilak & Rokde	Not reported	Not indicated	James, 1983
	Fo. badius (Berk.) Cke.	Not reported	Not indicated	Brown, 1968; James, 1983
	Fo. endotheius (Berk.) Cooke	Not reported	Not indicated	James, 1983
	Fo. fastuosus Cooke	Not reported	Not indicated	James, 1983
	Fo. rimosus	Not reported	Not indicated	James, 1983
	Fusicoccum indicum Tassi	Not reported	Not indicated	James, 1983

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. nilotica	*Lasiodiplodia theobromae*	Root disease	Kenya	Lenné, 1992
	Phyl. acaciae	Not reported	Not indicated	James, 1983
	R. acaciae-arabicae Mundk. & Thirum.	Not reported	Not indicated	James, 1983
	Septogloeum acaciae Syd.	Not reported	Not indicated	James, 1983
	Septoria mortalensis Penz. & Sacc.	Not reported	Not indicated	James, 1983
	Sphaerostilbe acaciae Tilak	Not reported	Not indicated	James, 1983
A. rehmanniana Schinz	*Leptostromella acaciae* H. & P. Sydow	Not reported	Gauteng, South Africa	Doidge, 1950; Sutton *et al*., 1986
	R. evansii	Brown, black pustules on leaves	Ethiopia, Kenya, Zambia and South Africa	Doidge, 1950
		Rust	Gauteng and Kwa-Zulu Natal, South Africa	
A. robusta Burch. subsp. *robusta*	*Ph. acaciae*	Tar spot	Kwa-Zulu Natal, South Africa	Doidge, 1950; Crous *et al*., 2000
	Phyl. acaciae	Powdery mildew	Gauteng	Gorter, 1993; Crous *et al*., 2000
		Small shiny black spots	South Africa	Gibson, 1980
	R. evansii	Rust	Gauteng, Kwa-Zulu Natal and Nort West Province, South Africa	Doidge, 1927; Crous *et al*., 2000
		Brown, black pustules on leaves	Ethiopia, Kenya, Zambia and South Africa	Doidge, 1950; Spaulding, 1961

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. senegal (L.) Willd.	*Clad. herbarum* (Pers.: Fr.) Link	Not reported	Not indicated	James, 1983
	Fusarium Link ex Gray sp.	Not reported	Not indicated	James, 1983
	Polyporus hispidus Bull.	Not reported	Not indicated	Spaulding, 1961
	R. acaciae-senegalae Mundk. & Thurim.	Not reported	Not indicated	James, 1983
	R. acaciicola Sanwal.	Not reported	Not indicated	James, 1983
A. seyal Del.	*Ae. schweinfurthii*	Not reported	Not indicated	Spaulding, 1961
	Fo. rimosus	Not reported	Not indicated	James, 1983
	Fo. robiniae (Murr.) Sacc. et D. Sacc.	Not reported	Not indicated	Spaulding, 1961
	Gan. lucidum	Not reported	Not indicated	James, 1983
	Leveillula taurica (Lev.) Arnauld	Not reported	Sudan	Spaulding, 1961; Gibson, 1980
	R. evansii	Brown, black pustules on leaves	Not indicated	James, 1983
	R. macowaniana	Cup shaped leasions on deformed pods and pustules on young branchlets and leaves	Congo and South Africa	Spaulding, 1961; Gibson, 1980
	R. volkensii	Not reported	Not indicated	James, 1983
	T. meyerii	Not reported	Ethiopia, Kenya, Zambia and South Africa	Gibson, 1980

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. seyal Del.	*Uromyces schweinfurthii* Henn.	Not reported	Not indicated	James, 1983
A.sieberiana DC. var. *woodii* (Burtt Davy) Keay & Brenan	*R. evansii*	Rust	Gauteng and Kwa-Zulu Natal, South Africa	Doidge, 1950; Crous *et al*., 2000
A. tortilis (Forssk.) Hayne	*A. chlamydospora*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	A. pluriseptata	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Chaetomium sp. #1 ex *Acacia tortilis*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Chaetomium sp. #2 ex *Acacia tortilis*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Dip. mutila	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Diplodia sp. ex *Acacia tortilis*	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Fa. leprosa	Not reported	Northern Cape Province, South Africa	Van der Walt *et al*., 2007
	Pen. griseofulvum Dierckx	Not reported	Northern Cape area, South Africa	Van der Walt *et al*., 2007
	P. glomerata	Not reported	Northern Cape area, South Africa	Van der Walt *et al*., 2007
	Sordaria fimicola (Roberge ex Desmaz.) Ces. & De Not.	Not reported	Northern Cape area, South Africa	Van der Walt *et al*., 2007
	Sphaeropsis sp. ex *Acacia tortilis*	Not reported	Northern Cape area, South Africa	Van der Walt *et al*., 2007

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. xanthophloea Benth	*R. natalensis*	Galls (nursery disease)	Nelspruit, Mpumalanga, South Africa	Wood, 2006

TABLE II: Fungi associated with non-native *Acacia* spp. in Africa

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. dealbata	*Armilaria mellea*	Root rot	Tanzania	Bakshi, 1976
	Ceratocystis albifundus	Gummosis and wilt	South Africa	Gorter, 1977; Roux *et al*., 2001a
	Cylindrocladium scoparium	Leaf drop, stem disease and post emergence damping off	South Africa	Bakshi, 1976
	Cy. floridanum Sobers & Seymour	Leaf spot and root rot	Not indicated	Crous *et al*., 1991
	Fusarium oxysporum	Post emergence damping off	South Africa	Gibson, 1975; Bakshi, 1976
	Ganoderma applanatum (Pers.) Pat.	Heart rot	South Africa	Bakshi, 1976
A. decurrens	*Amauroderma rugosum* (Blume & Ness)	Not reported	Not indicated	Doidge, 1950
	Ar. mellea	Root rot	Tanzania	Spaulding, 1961, 1964; Bakshi, 1976
	Camptomeris albizziae	Leaf spot and defoliation	South Africa	Hughes, 1952; Bakshi, 1976
	Cam. verruculosa (Syd) Bessey	Discoloured spots with pale yellow brown pustules & leaf spots	South Africa	Spaulding, 1961, 1964; Bakshi, 1976
	C. albifundus	Gummosis and wilt	South Africa	Gorter, 1977; Roux *et al*., 2001a
	Daldina concentrica (Bolt.ex Fr.) Ces. & De Not.	Not reported	Not indicated	Doidge, 1950
	Erythricium salmonicolor	Pink disease	South Africa	Spaulding, 1961, 1964; Bakshi, 1976
	Gan. lucidum	Root rot	South Africa	Doidge, 1950

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. decurrens	*Lenzites palisoti* Fr.	Not reported	Not indicated	Doidge, 1950
	Macrophomina phaseolina	Root rot	South Africa	Luckhoff, 1964; Gibson, 1975
	Nectria coccinea (Pesr.) Fr.	Not reported	Not indicated	Doidge, 1950
	Physalospora abdita (Bert. & Curt.) N. E. Stevens	Stem canker	South Africa	Spaulding, 1961, 1964; Bakshi, 1976
	Polyporus fruticum (Berk.) E Curt.	Not reported	Not indicated	Doidge, 1950
	Polystictus hirsutus	Not reported	Not indicated	Doidge, 1950
	Po. occidentalis	Not reported	Not indicated	Doidge, 1950
	Po. polyzonus	Not reported	Not indicated	Doidge, 1950
	Po. sanguineus	Not reported	Not indicated	Doidge, 1950
	Po. versicolor	Not reported	Not indicated	Doidge, 1950
	Schizopyllum commune	Not reported	Not indicated	Doidge, 1950; Spaulding, 1961, 1964
	T. cingulata	Not reported	Not indicated	Doidge, 1950
	T. meyenii	Not reported	Not indicated	Doidge, 1950
	T. trabea	Not reported	Not indicated	Doidge, 1950
	T. violacea	Not reported	Not indicated	Doidge, 1950
A. mearnsii	*Alternaria* sp.	Not indicated	Eastern Cape, South Africa	Roux and Wingfield, 1997
	Am. rude (Berk.) G. H. Cunn.	Root rot	Eastern Cape, Kwa-Zulu Natal and Mapumalanga, South Africa	Van der Westhuizen and Eicker, 1994
	Am. rugosum	Collar rot	Not indicated	Gibson, 1964
	Aplosporella sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mearnsii	*Ar. mellea*	Root rot	Malawi, Tanzania and Zimbabwe	Brown, 1968; Bakshi, 1976; Gibson, 1975; Lenné, 1992
	Armillaria sp.	Root rot	Kenya	Roux *et al*., 2005
	Bartalinia sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Botryosphaeria dothidea	Stem & branch canker, tip die-back and pith discolouration	Eastern Cape, Kwa-Zulu Natal, Mapumalanga and Western Cape, South Africa	Roux and Wingfield, 1997; Roux *et al*., 1997
	Calonectria pauciramosa (anamorph: *Cylindrocladium pauciramosum*)	Damping-off	Kwa-Zulu Natal, South Africa,	Doidge *et al*., 1953
	Camarosporium sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Cam. albizziae	Leaf spot and defoliation	South Africa	Hughes, 1952; Gibson, 1975; Bakshi, 1976
	Cam. verruculosa	Leaf spot	Kwa-Zulu Natal, South Africa, Kenya and Sudan	Gibson, 1975
	Cephalosporium sp.	Not indicated	Mpumalanga	Roux and Wingfield, 1997
	C. albifundus	Gummosis and wilt	Eastern Cape and Kwa-Zulu Natal, South Africa, Kenya, Tanzania and Uganda	Morris *et al*., 1993; Roux and Wingfield, 1997, 2001; Roux *et al*., 1999, 2005
	Chaetomium sp.	Not indicated	Mpumalanga	Roux and Wingfield, 1997
	Cladosporium sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Coleophoma sp.	Not indicated	Mpumalanga	Roux and Wingfield, 1997

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mearnsii	*Coniophora arida* Fr. Karst.	Not reported	Not indicated	Sherry, 1971
	Coriolus hirsitus (Wolf. Ex Fr.) Quél.	Wood rot	South Africa	Doidge, 1950; Sherry, 1971
	Curvularia sp.	Not indicated	Mpumalanga	Roux and Wingfield, 1997
	Cylindrocarpon sp.	Not indicated	Eastern Cape	Roux and Wingfield, 1997
	Cylindrocladiella camelliae	Root rot	Kwa-Zulu Natal, South Africa	Crous, 1993
	Cyl. peruviana	Root rot	Kwa-Zulu Natal, South Africa	Victor *et al*., 1998
	Cy. pauciramosum	Damping-off and stem cankers and damage to plants in nurseries	South Africa	Roux and Wingfield, 1997; Lombard *et al*., 2004; Roux *et al*., 2005
	Cy. scoparium	Root disease & post emergence damping off	South Africa	Doidge, 1950
	Cytospora sp.	Not indicated	Eastern Cape, Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Diheterspora sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Diplodia sp. A	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Diplodia sp. B	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Diplodia-like	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Drechlera sp.	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mearnsii	*E. salmonicolor*	Pink disease	South Africa	Spaulding, 1961; Brown, 1968; Bakshi, 1976
			Kwazulu-Natal, South Africa	Roux and Coetzee, 2005
	Epicoccum sp.	Not indicated	Eastern Cape, Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	F. acaciae-mearnsii O'Donnell, T. Aoki, Kistler et Geiser	Die-back	South Africa	O'Donnell *et al*., 2004; Roux *et al*., 2001b
	F. acuminatum	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
	F. graminearum	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
	F. oxysporum	Post emergence damping off	South Africa	Spaulding, 1961; Gibson, 1975; Bakshi, 1976
	F. proliferatum	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
	F. solani	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	F. subglutinans	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Gan. applanatum	Heart rot	South Africa	Doidge, 1950
	Gan. lucidum	Trunk rot	Gauteng, Kwa-Zulu Natal, Mapumalanga, Northern Province and North West Province, South Africa	Gorter, 1977
	Gan. rugosum (Blume & Nees) Torren	White mottled heart rot	South Africa	Gibson, 1964; Lückhoff, 1964
	Gliocladium roseum	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Gliomastix sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mearnsii	*Glomeralla* sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Harknesia sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Helminthosporium sp.	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
	Hydnum henningsii Bres.	Wood rot	Not indicated	Roberts, 1957
	L. theobromae	Collar rot and stem cankers	South Africa	Stephens and Goldschmidt, 1938; Gibson, 1975; Roux and Wingfield, 1997
	Leptosphaerulina sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Libertella sp.	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
	Mac. phaseolina	Root rot	South Africa	Gibson, 1975
	Microsphaeropsis spp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Nigrospora oryzae	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Oidium sp.	Powdery mildew	Not indicated	Sherry, 1971
	Pestalotiopsis sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Pestalotiopsis sp. 2	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Pestalotiopsis sp. 3	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Phacidium sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Phoma sp.	Not indicated	Eastern Cape, Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	P. herbarum	Twig die-back	Kenya	Olembo, 1972

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mearnsii	*Phomopsis* sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Phytophthora boehmeriae Sawada	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
	Phy. meadii McRae	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
			South Africa	Zeijlemaker, 1968; Sherry, 1971
	Phy. nicotianae	Black butt	Kenya, Tanzania	Roux *et al*., 2005
			Kwazulu-Natal, Sout Africa	Roux and Wingfield, 1997
	Phy. parasitica	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Physalospora abdita (Berk. & Curt.) N. E. Stevens	Stem canker	South Africa	Gibson, 1975
	Pithomyces sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Pleurocytospora sp.	Not indicated	Mpumalanga, South Africa	Roux and Wingfield, 1997
	Po. hirsitus	Not reported	Not indicated	Roberts, 1957
	Po. subicculoides Lloyd.	Heart rot	Not indicated	Doidge, 1950
	Pythium sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Rhinocladiella sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Rhizotonia lamellifera Small.	Wilt, die-back (Alberts Falls Disease) & root rot	South Africa	Gibson, 1964; Lückhoff, 1964
	Schizophyllum commune	Collar rot	Western Cape, South Africa	Ledeboer, 1946
	Seiridium sp.	Not indicated	Eastern Cape, Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997

Host	Fungus	Symptoms/Disease	Area isolated	Reference
A. mearnsii	*Sphaeropsis* sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Stereum ostrea (Fr.) Fr.	Heart rot	Tanzania	Bakshi, 1976
	Stigmina verruculosa Syd.	Leaf spot	Not indicated	Doidge *et al*., 1953
	Thanatephorus cucumeris	Root rot and Wilt	Kwa-Zulu Natal and Mapumalanga, South Africa	Doidge *et al*., 1953
	T. hirsuta	Root rot	Not indicated	Doidge *et al*., 1950
	T. meyerii	Heart rot	Not indicated	Doidge *et al*., 1953
	T. roseola	Heart rot	Not indicated	Doidge *et al*., 1953
	Trichoderma sp.	Not indicated	Kwa-Zulu Natal, Mpumalanga, South Africa	Roux and Wingfield, 1997
	Tryblidopycnis pinastri Höhn	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Verticillium sp.	Not indicated	Kwa-Zulu Natal, South Africa	Roux and Wingfield, 1997
	Uromycladium alpinum	Leaf rust	Kwa-Zulu Natal, Mapumalanga, Western Cape, South Africa	Morris *et al*., 1988

Chapter 2

Botryosphaeriaceae associated with *Acacia mellifera* and other native *Acacia* spp. in South Africa and Namibia.

ABSTRACT

Little is known regarding the fungi associated with native *Acacia* spp. in southern Africa. This study specifically focusses on the presence of Botryosphaeriaceae species on these hosts in Namibia and Pretoria, South Africa. Ten species of the Botryosphaeriaceae were identified based on PCR-RFLP groupings, characterization of anamorph characters and comparisons of sequence data for the ITS and EF1-α gene regions. Species that had been formerly described include *Lasiodiplodia pseudotheobromae*, *Spencermartinsia viticola* and *Botryosphaeria dothidea*, for which a previously unknown *Dichomera* synanamorph is described for the first time. Six previously undescribed species; *Diplodia variabilis* prov. nom., *Dothiorella oblonga* prov. nom., *Fusicoccum avasmontanum* prov. nom., *L. pyriformis* prov. nom., *S. rosulata* prov. nom. and a new genus, *Mucodiplodia* prov. nom., represented by the single species *M. africana* prov. nom., were discovered and are described. Results of this study contribute to the understanding of the overall diversity of the Botryosphaeriaceae and emphasize the limited knowledge that exists regarding these fungi on native trees in southern Africa.

INTRODUCTION

Acacia spp. are economically and ecologically important trees in the southern hemisphere. These tree species, together with the African baobab (*Adansania digitata* Linn.), are recognized as icons of the African landscape. Despite this, there is a considerable lack of knowledge regarding the fungal species associated with African *Acacia* spp., at least when compared to Australian *Acacia* spp. This could be explained because of the economic importance of the latter group that are widely used in commercial plantations in Africa, South East Asia and South America (Sherry, 1971).

Acacia mellifera, also known as the blackthorn, is one of the native African *Acacia* spp. that has been extensively studied. It is regarded as one of the most valuable native trees found on cattle and game farms in southern Africa (Venter and Venter, 2002), but the blackthorn in Namibia is threatened by a serious die-back disease (Holz and Schreuder, 1989). Symptoms of this disease are typical of the die-back that is caused by members of the Botryosphaeriaceae that were previously accommodated in the single genus, *Botryosphaeria*, or its anamorphs (Crous *et al*., 2006a).

Fungi belonging to the Botryosphaeriaceae are common endophytes, but some are also opportunistic pathogens, causing disease of many woody species (Denman *et al*., 2000; Slippers and Wingfield, 2007). They have a wide distribution and host range, causing symptoms such as shoot blight, leaf spot, fruit and seed rot, as well as "witches broom". Typical symptoms include die-back and cankers and, in severe cases, death of the trees (Denman *et al*., 2000; Smith *et al*., 1996; Slippers and Wingfield, 2007). Yet, the ecological role and presence of this group of fungi

is poorly understood, particularly on plants in native communities from Africa. Information regarding their ecology, pathology and possible roles as endophytes is especially important where infected plants are shipped between countries and even continents (Slippers and Wingfield, 2007).

The taxonomy of the Botryosphaeriaceae has been complex and confusing for many years and this has frustrated studies regarding these fungi. Recent studies based on DNA sequence comparisons and phylogenetic inference, together with morphological characteristics, has made it possible to classify the Botryosphaeriaceae more accurately (Crous *et al*., 2006a; Jacobs and Rehner, 1998; Slippers and Wingfield, 2007). In this regard, a recent phylogenetic study recognized 10 lineages within the Botryosphaeriaceae and also suggested important name changes (Crous *et al*., 2006a).

Reports of Botryosphaeriaceae from *Acacia* spp. in Africa are limited. A review on phytopathogens in South Africa by Crous *et al*. (2000) indicated no records of Botryosphaeriaceae on native *Acacia* spp. Besides this review, only one member of the Botryosphaeriaceae, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., was reported causing a root disease on *Acacia nilotica (L.*) Del., which is native to Kenya (Lenné, 1992). There are only two reports of Botrysophaeriaceae on non-native *A. mearnsii* de Wild. trees from South Africa, including *L. theobromae* (Stephens and Goldschmidt, 1938; Gibson, 1975; Roux and Wingfield, 1997) and *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. (Roux and Wingfield, 1997; Roux *et al*., 1997). Roux and Wingfield (1997) also identified two *Diplodia* spp. and a *Sphaeropsis* sp. during a survey (1994 to 1995) of two important commercial wattle-growing areas in South Africa, however, the taxonomic placement of these species is yet to be determined.

In an effort to understand the association of the Botryosphaeriaceae with native *Acacia* species in southern Africa, a study was undertaken to search for the presence of these fungi on especially *A. mellifera* in Namibia and Pretoria, South Africa. Other *Acacia* spp. were also included in cases where they were present in these areas. To identify the Botryosphaeriaceae associated with these hosts, morphological characterization of the anamorph states, PCR-RFLP groupings and sequence data for the internal transcribed spacer (ITS) and translation elongation factor 1-α (EF 1-α) gene regions were used.

MATERIALS AND METHODS

Isolates

Samples were collected from Windhoek, Dordabis, Grootfontein and Rundu in Namibia and Ditholo Air Force Base, near Pretoria in South Africa. A total of 89 *Acacia* trees were sampled of which 69 were from Namibia and 20 from Pretoria. Trees from Namibia included *A. hebeclade* DC. (5), *A. karoo* Hayne (19) and *A. mellifera* (45), while only *A. mellifera* (20) was sampled in Pretoria. Both healthy branch tips and diseased plant material were collected. Healthy branch tips were used for isolations of the Botryosphaeriaceae existing as endophytes as described by Pavlic *et al*. (2004). Diseased plant material included symptoms such as lesions on branches, black pith in the branches, cankers, tip die-back, streaked lesions and a brownish black discoloration in the upper tap roots of dying trees. The plant material was surface sterilized with 76 % ethanol (v/v) and small pieces (approximately 5 mm^2) of symptomatic tissue were plated onto 2 % malt extract agar (MEA).

Symptomatic tissue was also placed in moist chambers comprised of plastic ziplock bags containing paper towels wetted with sterilized distilled water. The plates and moist chambers were incubated at room temperature. Isolations were done from structures produced on tissue in moist chambers and cultures were purified from the plates. Cultures resembling the Botryosphaeriaceae were identified and initially grouped based on their morphological characteristics when grown in a pure culture.

DNA isolation and PCR

DNA was extracted from cultures using a phenol:chloroform DNA extraction method modified from Raeder and Broda (1985). Modifications were similar to those of Barnes *et al*. (2001) except that the mycelium of seven-day-old cultures was scraped from the medium and transferred to Eppendorf tubes (1.5 mL) and extraction buffer was added. The buffer and mycelium were manually ground with a glass rod and incubated at 60 °C for 60 min in a heating block. Centrifugation was done at 10 000 rpm. DNA pellets were resuspended in 50 µL sterile SABAX water. RNAse (5 mg/mL) was added to DNA samples and incubated overnight at 37 °C to degrade residual protein and RNA.

Primers ITS1 and ITS4 (White *et al*., 1990) were used to amplify the 3′ end of the 18S (small sub-unit) rRNA gene, internal transcribed spacer (ITS1), the complete 5.8S rRNA gene, ITS2 and the 5′ end of the 28S (large subunit) rRNA gene. The 3′ end of the second exon to the 5′ end of the last exon and two variable introns of the elongation factor 1-α (EF1-α) gene were also amplified with the primers EF1F and EF2R (Jacobs *et al*., 2004).

In addition, the primers ITS1 and LR5 (Vilgalys and Hester, 1990) were used to amplify part of the nuclear rRNA operon, 3′ end of the 18S rRNA gene, the internal spacers, the 5.8S rRNA gene (as described above) and a part of the 5′ end of the 28 rRNA gene. This was done for a selection of isolates that was compared to the dataset of Crous *et al*. (2006a).

PCR reaction mixtures contained 5 − 10 ng of genomic DNA, 0.2 mM dNTP, 0.2 mM of each primer, 1.5 mM buffer (10 mM Tris-HCL, 1.5 mM $MgCl_2$, 50 mM KCL), 2.5 U *Taq* DNA polymerase and was adjusted to a final reaction volume of 25 µL with sterile distilled water. Parameters used for the PCR reactions included a 2 min step at 95 °C, followed by 10 cycles of 20 s at 94 °C, 40 s at 55 °C and 45 s at 72 °C. The 10 cycles were then repeated for another 30 cycles with a 5 s increase per cycle for the annealing step at 55 °C, and then a final 10 min cycle at 72 °C. Amplification of the EF1-α was problematic for some isolates for which the annealing temperatures were adjusted up to 68 °C and the $MgCl_2$ concentrations up to 1.6 mM. PCR products were separated by electrophoresis using 2 % agarose gels, stained with ethidium bromide and visualized under UV illumination. Size estimates were made using a 100 bp size marker.

PCR-RFLP

A PCR-RFLP test was used on the ITS amplicons to confirm the initial groupings of isolates based on culture morphology of all the isolates from Namibia. Profiles were obtained using one enzyme, *Hha*I (Fermentas International Inc., Canada), which is the analog for *Cfo*I (Roche Diagnostics, Indianapolis, USA.) to digest the ITS amplicons (Slippers *et al*., 2004b, 2006). Each PCR-RFLP reaction consisted of 20 µl PCR product, 2 µl 10× Buffer Tango and 1 µl enzyme (10

U/µl) (Fermentas International Inc., Canada). The final reaction volume was adjusted to 40 µl with sterile distilled water. The reaction was incubated at 37 °C for 16 hr in a warm block and the enzyme was inactivated at 80 °C for 20 min. The products were subjected to electrophoresis using a 3 % agarose gel at 60 V for 90 min, stained with ethidium bromide and visualized under UV illumination. Size estimates were made using a 100 bp size marker.

Sequencing and Phylogenetic analyses

Three representative isolates of each PCR-RFLP group, where possible, were used in sequence comparisons. The PCR products were purified using 6 % Sephadex columns (1.33 g in 20 mL sterile water) (Sigma, Steinheim, Germany). The same primers were used as in the initial PCR reactions and both strands of the amplicons were sequenced. Reactions were performed using an ABI PRISM 3100 Autosequencer (Applied BioSystems, Foster City, California, USA) and sequences were analyzed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California, USA).

Sequences were compared to those of other Botryosphaeriaceae sequences in GenBank using BLAST and related sequences were downloaded. Sequences were aligned with MAFFT (Katoh *et al*., 2002) version 5.8 (http://timpani.genome.ad.jp/~mafft/server/) and manually adjusted, after which phylogenetic analysis was done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2002). Trees were rooted with *Cercospora apii* Fresen. and *C. beticola* Sacc. A partition homogeneity test (Swofford, 2002) was conducted to test the congruence between sequence results for the ITS and EF1-α gene regions. Gaps were treated as fifth characters and trees were obtained via stepwise addition of 1000 replicates with the Mulpar

option in effect. The heuristic search option, based on parsimony with stepwise addition, was used to obtain the phylograms. Confidence intervals using 1000 bootstrap replicates were calculated.

Bayesian analyses were used to test the credibility of the branch nodes using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Trees were obtained through 2 000 000 generations, with every 100th tree sampled. The burnin was obtained through the sumt command in MrBayes and the first 6000 trees were discarded as the burnin period. Evolutionary models for the datasets (ITS: GTR+I+G and EF1-α: HKY+G) were obtained through Modeltest (Posada and Crandall, 1998) and four MCMC chains were run simultaneously in the analysis, with three heated chains and one cold chain. The sampled trees were summarized in a consensus tree showing posterior probabilities of the branches and drawn up with TreeView (Page, 1996).

Morphological characterization

All the isolates that were used for sequencing (TABLE I) were induced to sporulate on sterilized pine needles that were placed on 2 % water agar (WA) at 25 °C under UV light. Fungal structures were mounted on glass slides in lactic acid (75 %) and examined under a Zeiss Axioskop microscope and images were captured using a HRc Axiocam digital camera and Axiovision 3.1 software (Carl Zeiss Ltd., Germany). At least fifty measurements were made for each diagnostic character per isolate in order to describe taxa. Although not all cultures sporulated, at least fifty measurements were available for each species description. The minimum, maximum, standard deviation (SD), mean values and the length/width (l/w) ratios

were calculated and are presented in this study as (min −) ave. ± std. dev. (− max). Colours were identified based on the colour charts of Rayner (1970).

Growth characterization

To determine the growth rate of species, two to three isolates (7-day-old) were selected to represent each taxon (TABLE I). Agar discs (4 mm diam.), overgrown with mycelium, were placed mycelial side down at the centres of 90 mm Petri dishes that contained 2 % MEA. Petri dishes were incubated in the dark at temperatures ranging from 5 °C to 35 °C with 5 °C intervals. The diameter of the colonies was measured after two days for fast growing cultures and four days for slower growing cultures. Average growth rates were calculated by using five replicate plates for each isolate and temperature, and the averages were computed.

RESULTS

Isolates

Eighty three Botryosphaeriaceae isolates were collected of which eight were from *A. mellifera* in Pretoria and 75 isolates from *Acacia* spp. in Namibia. Namibian isolates could initially be assigned to 12 groups based on culture morphology. Isolates from Pretoria were not included in these groups as they were obtained at a later stage.

PCR-RFLP

PCR produced amplicons of ~580 bp for the ITS gene region, ~800 bp for the EF1-α gene and ~900 bp for the LSU. PCR-RFLP profiles obtained from the *Hha*I digested ITS amplicons identified 11 PCR-RFLP groups from the Namibian isolates. These results corresponded well with the groups that were based on culture morphology, except in the case of two PCR-RFLP profiles with similar banding patterns that represented two different groups based on culture morphology.

Sequencing and Phylogenetic analyses

Based on PCR-RFLP groupings, a total of 20 isolates, representing the Namibian isolates and all eight isolates from Pretoria, were sequenced. BLAST searches and detailed DNA sequence comparisons were done on all 28 sequences (TABLE I). These were then aligned with published sequences that were obtained from GeneBank (TABLE I), using MAFFT.

The partition homogeneity test on the combined ITS and EF1-α dataset produced a P-value of 0.01. The data were thus combined, forming a dataset that contained 84 taxa with 972 characters after alignment. A total of 13 variable characters were parsimony-uninformative, 658 variable characters were parsimony-informative and 301 characters were constant. Heuristic searches in PAUP yielded two most parsimonious trees. The overall topology of the two trees was the same with only minor variation within the major clades and as a result, one most parsimonious tree (FIG. 1) was chosen for presentation. This tree had a Consistency Index (CI) = 0.62, a Homoplasy Index (HI) = 0.38, a Retention Index (RI) = 0.92 and a Rescaled Consistency (RC) = 0.57. Results of the posterior probabilities corresponded well to values of the bootstrap analyses.

Based on mycelial characteristics, Namibian cultures could be separated into 12 groups. These were reduced to 11 groups using the PCR-RFLP comparisons and Blast results indicated eight putative species. Blast searches done for the isolates from Pretoria indicated only two putative species. However, phylogenetic analyses of the combined sequence data for the ITS and EF1-α gene (FIG. 1) regions finally identified 10 distinct taxa amongst the 83 isolates collected in this study. Eight main Clades (A–H), corresponding to genera in the Botryopshaeriaceae (Crous *et al*., 2006a; Phillips *et al*., 2008), were identified and these comprised of 30 sub-clades corresponding to species (FIG. 1). Sequences obtained from isolates in this study grouped in 10 of the 30 sub-clades. Previously described species included *Spencermartinsia viticola* A.J.L Phillips & Luque (sub-clade 2), *Lasiodiplodia pseudotheobromae* A.J.L. Phillips, A. Alves & Crous (sub-clade 21) and *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi* Corda) (sub-clade 13) (FIG. 1).

Clade A included two sub-clades, of which one included sequences from this study (sub-clade 1). Sub-clade 1 was identified as a sister clade to the *S. viticola* clade (sub-clade 2). Fixed polymorphisms were used to distinguish between these sub-clades (1 and 2) and seven fixed alleles across two loci were found (TABLE II). Two isolates, ICMP16819 and ICMP16824, formed a sister clade in a recent study done by Phillips *et al*. (2008), however, no formal describtion was provided due to a lack of morphological data. Sequences from sub-clade 1 were compared to these isolates (data not shown here) and results clearly showed a distinction between these species. This is futher discussed in more detail in chapter 4.

Clade B included sub-clade 3 which represented a new taxon that was identified based on a strong bootstrap and posterior probability values (FIG. 1). Other sub-clades identified were *Dothiorella iberica* (sub-clade 4) and *Do. sarmentorum* (sub-clade 5).

Clade C contained only one sequence that represented a single isolate (sub-clade 10). This unknown *Neofusicoccum* sp. is closely related to *N. vitifusiforme* Niekerk & Crous and *Dichomera eucalypti* (G. Winter) B. Sutton. Because this taxon is represented by only one culture, and the fact that it is genetically close to the mentioned species, its taxonomic status remains uncertain.

Clade D included two sub-clades, one representing *Botryosphaeria dothidea* (sub-clade 13) and the other a new taxon (sub-clade 14). Although sub-clade 14 is represented by a single isolate, this had significant sequence divergence from known species in both loci (TABLE III). This isolate has also shown to be distinct based on the ITS gene region from *B.mamane* D. E. Gardner and *B. corticis* (Demaree & Wilcox) Arx & E. Müll. (FIG. 3). The latter two species were not included in figure 1 because EF1-α sequences are not available for them.

Clade E contained eight sub-clades, two of which included sequences for isolates collected in this study (FIG. 1). Sub-clade 15 was identified as a sister species of *Lasiodiplodia crassispora* Burgess & Barber. Fixed allele polymorphisms were used to confirm the distinction of the two sub-clades (TABLE IV). Sub-clade 21 was identified as the recently described *L. pseudotheobromae*.

Clade F included only one sub-clade that contained isolates from this study (FIG. 1). Sub-clade 23 was identified as a distinct taxon from the closely related *Diplodia porosum* Niekerk & Crous (sub-clade 24), based on significant sequence divergence and strong bootstrap and posterior probability values. Clade G (sub-clade 29) was strongly separated from other major clades and is, therefore, seen as a unique genus in the Botryosphaeriaceae. This genus is closely related to *Aplosporella* (Clade H). Isolates residing in this grouping were compared to those in the LSU dataset of Crous *et al*. (2006a) (FIG. 2). Both phylogenetic trees (FIGS. 1, 2) indicated a taxonomic entity that is distinct from all the other genera in the group.

Morphological characterization

All the isolates used in the phylogenetic analyses were included in the morphological comparisons. Anamorph structures were produced on pine needles after 2−3 weeks. No teleomorph structures were observed and, therefore, descriptions are based only on the anamorph states. The morphological characteristics of the isolates corresponded to groups emerging from the phylogenetic analyses and details are included in the descriptions of isolates. Furthermore, a previously undescribed *Dichomera* synanamorph was identified in one of the *B. dothidea* cultures (FIG. 10) and its morphological characteristics are also described.

TAXONOMY

DNA sequence comparisons for the isolates from *Acacia* spp. revealed the presence of seven distinct taxa in the Botryosphaeriaceae that have not previously been identified. Six of these are provided with names here. The new taxa also included one group that represents an undescribed

genus. In addition to phylogenetic differences, all of the species can be recognised based on distinct anamorph characteristics and they are described below.

Mucodiplodia F.J.J. van der Walt, Slippers & G.J. Marais gen. nov. FIG. 4

Etymology: The name refers to the mucous sheath surrounding the *Diplodia*-like conidia. Conidiomata pycnidialia, singula, parce mycelio brevi tecta, subglobosa vel pyriformia, plerumque superficialia, sub-immersa vel immersa. Paraphyses hyalinae, filiformes. Cellulae conidiogenae hyalinae, holoblasticae, cylindricae vel ampulliformes. Conidia primo hyalina, ellipsoidea vel late ellipsoidea, interdum reniformia, parietibus modice crassis, contentis granulariis, laevia, apicibus interdum sub-papillatis, vagina persistenti mucosa.

Mucodiplodia africana F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 4

Conidiomata pycnidialia, singula, parce mycelio brevi tecta, subglobosa vel pyriformia, 364.8 µm diametro, plerumque superficialia, sub-immersa vel immersa. Paraphyses hyalinae, filiformes, non septata, (15.5−)17.2−32.6(−38.3) × (1.2−)1.5−2.3(−2.4) µm. Cellulae conidiogenae hyalinae, holoblasticae, cylindricae vel ampulliformes (2.5−)3.3−6.8(−7.4) × (1.5−)1.8−3.2(−4) µm. Conidia primo hyalina, non septata, ellipsoidea vel late ellipsoidea, interdum reniformia, parietibus modice crassis, contentis granulariis, laevia, apicibus interdum sub-papillatis, vagina persistenti mucosa (10.3−)12.2−16(−19) × (5.8−)6.7−9.9(−13.1) µm.

Conidiomata pycnidial, separate, covered in a small number of short hyphae, subglobose, up to 364.8 µm in diameter or occasionally pyriform, up to 464.5 µm in height, mostly superficial, occasionally semi-immersed or immersed. *Paraphysis* hyaline, filiform, aseptate, (15.5−)

17.2−32.6(−38.3) × (1.2−)1.5−2.3(−2.4) µm (average: 24.9 × 1.9 µm). *Conidiogenous cells* hyaline, holoblastic, cylindrical to ampilliform, proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently to form one or two annelations, (2.5−) 3.3−6.8(−7.4) × (1.5−)1.8−3.2(−4) µm (average: 5 × 2.5 µm). *Conidia* initially hyaline becoming honey colored to brown, aseptate, ellipsoidal to broadly ellipsoidal, occasionally reniform, moderately thick walled, granular content, smooth, encased in a persistent mucous sheath, occasionally slightly papillate apices, (10.3−)12.2−16(−19) × (5.8−)6.7−9.9(−13.1) µm (average of 100 spores: 14.1 × 8.3 µm, l/w ratio: 1.7).

Culture characteristics: mycelium blackish green grey (35""), occasionally blackish brown (9""m), effuse, little mycelium. Thread-like growth on the edges of colonies. Reverse olivaceous black (25""m) thread-like growth at edges. Thickening of hyphae, chlamydospore-like, immersed in water agar. *Temperatures for growth*: growth at minimum 10 °C, growth rate of 18 mm/day at an optimal temperature of 25 °C, growth at maximum 35 °C.

Etymology: The name refers to the continent of Africa from which this genus and species was first described.

Host: *Acacia mellifera*.

Distribution: Windhoek, Dordabis, Grootfontein and Rundu (Namibia).

Notes: Ten isolates were identified and described as *M. africana*. Nine of these were obtained from apparantly healthy tissue and one was from wood with streaked discolouration. Distinct morphological differences were observed between *Mucodiplodia* and all other genera in the Botryosphaeriaceae, of which the most closely related are *Saccharata* Denman & Crous and *Aplosporella prunicola* Damm & Crous.

Saccharata, described by Crous *et al*. (2004), has a *Fusicoccum* anamorph and a *Diplodia*-like synanamorph. Furthermore, *Saccharata* spp. have cylindrical conidiogenous cells compared to the cylindrical to ampiliform cells in *M. africana*. In addition, *M. africana* has paraphyses that have not been described for *Saccharata* (Crous *et al*., 2004). Spores of *Saccharata* are finely verruculose, sub-cylindrical to narrowly ellipsoidal with rounded ends compared to spores of *M. africana* that are smooth, ellipsoidal to broadly ellipsoidal. Conidia of *M. africana* can also occasionally be reniform or ovoid and constricted at the center. Furthermore, unlike species in *Saccharata*, conidia of *M. africana* can occasionally have slightly papillate apices and all conidia are encased in a persistent mucous sheath.

There are substantial morphological differences between *Mucodiplodia africana* and *Aplosporella prunicola* Damm & Crous (Damm *et al*., 2007a). Pycnidia and paraphyses of *A. prunicola* are much larger (up to 800 µm in length and 35–95 µm × 4–8 µm) than those of *Mucodiplodia* (up to 465 µm and 15–38 µm × 1–2 µm). Conidia of *A. prunicola* are also hyaline at first, turning dark brown with age, which is different to those of *Mucodiplodia* that become honey to brown coloured. There are also no papillate apices on the conidia of *A. prunicola* as are found in *M. africana* and most importantly, conidia of *A. prunicola* have no persistent mucous sheath.

Specimens examined: NAMIBIA, DORDABIS, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, HOLOTYPE, herb. PREM59640, culture ex-type CMW25424, CBS121777, CAMS001479; NAMIBIA, GROOTFONTEIN, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, PARATYPE, herb. PREM59641, culture ex-paratype CMW25425, CBS121778, CAMS001480; NAMIBIA, DORDABIS, fruiting structures induced on needles of *Pinus* sp. on

WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, PARATYPE, herb. PREM59642, culture ex-paratype CMW25426, CBS121779, CAMS001481.

Diplodia variabilis F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 5

Conidiomata pycnidialia, globosa vel subglobosa, 392 µm diametro, mycelio brevi tecta, superficialia, subimmersa vel immersa. Cellulae conidiogenae holoblasticae, cylindricae vel ampulliformes, periclinale incrassatae vel rare percurrente proliferantes, annulationes singulas vel binas formantes (5.7−)6.2−10.8(−16.3) × 2.6−5.3(−7.7) µm. Conidia non vel 1–3-septa, plerumque base septata, laeves, forma modice variabilia: cylindrica, ovoidea, interdum reniformia vel allantoidea, rare guttulata; parietibus modice crassis (24−)26.5−33.3(−37.1) × (8−)10.7−13.9(−17.2) µm.

Conidiomata pycnidial, globose to subglobose, covered in short hyphae, superficial, immersed or semi-immersed, up to 392 µm in diameter. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, holoblastic, cylindrical to ampiliform, (5.7−)6.2−10.8(−16.3) × 2.6−5.3(−7.7) µm (average: 7.3 × 3.4 µm), proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently to form one or two annelations. *Conidia* honey colored to black brown, aseptate or 1−3 septa, mostly truncated at the base, smooth, moderately thick-walled, variable in shape ranging from cylindrical to ovoid or occasionally reniform or allantoid, occasionally guttalate, (24−)26.5−33.3(−37.1) × (8−)10.7−13.9(−17.2) µm (average of 140 spores: 30 × 12.3 µm, l/w ratio: 2.4).

Culture characteristics: mycelium olivaceous (21"m) to olive buff (21'"d) or white to smoke grey (21""d), effuse. Reverse dark olive (21'"m) to buffy olive (21"k). Occasional thickening of

the hyphae, chlamydospore-like, blackish brown, intercalary, terminal and found superficial and immersed in water agar. *Temperatures for growth*: growth at minimum 10 °C, growth rate of 19 mm/day at an optimal temperature 25 °C, growth at maximum 35 °C.

Etymology: Name refers to the variability in the shape of the conidia in this fungus.

Hosts: *Acacia hebeclade*, *A. karoo* and *A. mellifera*.

Distribution: Windhoek, Dordabis and Grootfontein (Namibia).

Notes: Twenty five isolates were identified as *D. variabilis* and these were from leading edges of lesions on branches, streaks in the wood and black pith from branches and stems.

Specimens examined: NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, HOLOTYPE, herb. PREM59637, culture ex-type CMW25419, CBS121774, CAMS001474; NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25420, CAMS001475; NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, PARATYPE, herb. PREM59638, culture ex-paratype CMW25421, CBS121775, CAMS001476.

Dothiorella oblonga F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 6

Conidiomata pycnidialia, singula, mycelio breve tecta, ovoidea vel globosa, 551.9 µm diametro, interdum in fasciculis botryosis aggregata, superficialia vel immersa. Cellulae conidiogenae holoblasticae, cylindricae, subcylindricae vel late lageniformes, periclinale incrassatae vel rare percurrente proliferantes, annulationes singulas vel binas formantes (5.9−)8.3−11.7(−12.6) × (2.6−)4−4.7(−5.3) µm. Conidia non vel 1-septata, plerumque basi truncata et in septis constricta

vel in basi septi incrassata, parietibus modice crassis, ovoideae, oblongae vel ellipsoideae (18.7−)23.8−27(−28) × (9.9−)11.3−13.2(−14.9) µm.

Conidiomata pycnidial, separate, covered with short hyphae, ovoid, up to 840.4 µm in height or globose, up to 551.9 µm in diameter, occasionally aggregated into botryose clusters, superficial or immersed. *Ostioles* single, central, papillate. *Conidiophores* reduced to conidigenous cells. *Conidiogenous cells* hyaline, holoblastic, cylindrical to sub-cylindrical or broadly lageniform, (5.9−)8.3−11.7(−12.6) × (2.6−)4−4.7(−5.3) µm (average: 10 × 4.3 µm), proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently to form one or two annelations. *Conidia* honey colored to brown becoming blackish brown, aseptate or 1-septate, mostly truncated at the base and constricted at the septum or with a thickening at the base of the septum, moderately thick-walled, ovoid or oblong to ellipsoidal, (18.7−)23.8−27(−28) × (9.9−)11.3−13.2(−14.9) µm (average of 100 spores: 25.5 × 12.3 µm, l/w ratio: 2.1).

Culture characteristics: mycelium greyish olive (21"") or dark greyish olive (21""k) concentric zone at the edge with an effuse black (*1) centre. Reverse dark olive (21'"m) to blackish brown (9""m), thread-like growth in the centre of the colony. Occasional thickening of the hyphae, chlamydospore-like, blackish brown: intercalary, terminal and dictyosporus and found superficial and immersed in water agar. *Temperatures for growth*: growth at minimum 10 °C, growth rate of 18.5 mm/day at an optimal temperature 25 °C, growth at maximum 35 °C.

Etymology: Name refers to the oblong shaped conidia in the fungus.

Host: *Acacia mellifera*.

Distribution: Rundu (Namibia), Ditholo, Pretoria (South Africa).

Notes: Only three isolates were identified as *Do. oblonga* and all the isolations of this fungus came from healthy tissue. Despite the fact that this fungus seems to be rare, it has a broad distribution.

Specimens examined: SOUTH AFRICA, PRETORIA: Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, HOLOTYPE, herb. PREM59628, culture ex-type CMW25407, CBS121765, CAMS001462; SOUTH AFRICA, PRETORIA: Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, PARATYPE, herb. PREM59629, culture ex-paratype CMW25408, CBS121766, CAMS001463; NAMIBIA, RUNDU, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, PARATYPE, herb. PREM59627, culture ex-paratype CMW25406, CBS121764, CAMS001461.

***Neofusicoccum* sp.** FIG. 7A–C

Conidiomata aggregated into botryose clusters, covered in mycelium, immersed or semi-immersed, occasionally superficial, up to 953.7 µm in diameter. *Conidiogenous cells* hyaline, holoblastic, cylindrical, (5.9–)7.4–10.9(–11.2) × 2–3.7 µm (average: 9.2 × 2.6 µm). *Conidia* ellipsoidal to fusiform, hyaline, smooth, aseptate, (14.6–)17–20.9(–22.5) × (4.5–)5.2–6(–6.8) µm (average of 100 spores: 19.3 × 5.6 µm, l/w ratio: 3.4).

Culture characteristics: white cottony mycelium in centre of colony with effuse dark olive (21'"m) at the edge of the colony. Reverse, concentric zones, olive buff (21'"d). *Temperatures for growth*: growth at minimum 10 °C, growth rate of 15 mm/day at an optimal temperature 25 °C, growth at maximum 35 °C.

Host: *Acacia mellifera*.

Distribution: Windhoek (Namibia).

Notes: Only one isolate of this fungus was obtained and this came from healthy tissue. There were no significant morphological differences compared to the closely related species, nl. *N. vitifusiforme* Niekerk & Crous (Van Niekerk *et al*., 2004) and *Dichomera eucalypti* (G. Winter) B. Sutton. (Barber *et al*., 2005).

Specimens examined: NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25409, CAMS001464.

Fusicoccum avasmontanum F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIGS. 7D−G

Conidiomata pycnidialia, ovoidea vel ellipsoidalia basi complanata, 236 µm diametro, interdum globosa vel subglobosa, singula, mycelio tecta, superficialia vel subimmersa. Cellulae conidiogenae hyalinae, holoblasticae, cylindricae 5.6−8.6(−10.5) × (1.7−)1.8−2.6(−3.1) µm. Conidia ellipsoidea, basi apiceque rotundata, non septata (8.1−)8.8−11.3(-13) × (2.5−)2.9−3.9 (−5) µm.

Conidiomata pycnidial, ovoid to ellipsoidal with a flat base, up to 408.14 µm in height, occasionally globose to subglobose, up to 236 µm in diameter, separate, covered in mycelium, superficial or semi-immersed. *Conidiogenous cells* hyaline, holoblastic, cylindrical, 5.6−8.6 (−10.5) × (1.7−)1.8−2.6(−3.1) µm. *Conidia* ellipsoidal, rounded at the base and apex, hyaline, aseptate, (8.1−)8.8−11.3(−13) × (2.5−)2.9−3.9 (−5) µm (mean of 28 spores: 10.1 × 3.4 µm, l/w ratio: 3).

Culture characteristics: mycelium greyish olive (21"") to brownish olive (19"m), effuse. Reverse black (*1) to olive brown (17"'k). *Temperatures for growth*: growth at minimum 10 °C, growth rate of 9 mm/day at an optimal temperature 25 °C, growth at maximum 35 °C.

Etymology: Name refers to the Auasberg Mountain surrounding Windhoek.

Host: *Acacia mellifera*.

Distribution: Windhoek (Namibia).

Notes: Description is based on a single isolate from healthy tissue. Even though only one isolate was collected, obvious differences in morphology, host and geographical occurrence compared to those of known species strongly support the description of a unique species. There are clear morphological differences between *Fusicoccum aesculi* (Slippers *et al*., 2004a), which is the species most closely related to *F. avasmontanum*. Conidiogenous cells as well as conidia are significantly smaller. Conidiogenous cells and conidia of *F. avasmontanum* range from (5−10) × (1−3) and (8−13) × (2−5) µm respectively, compared to conideogenous cells and conidia of *F. aesculi*, (6−20) × (2−5) and (17−22) × (4−5) µm respectively. In addition, there was also significant sequence divergence based on ITS sequencing data compared to *Botryosphaeria mamane* and *B. cortices* which are the other two species residing in the group together with *F. avasmontanum* (FIG. 3**)**.

Specimens examined: NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, HOLOTYPE, herb. PREM59632, culture ex-type CMW25413, CBS121769, CAMS001468.

Lasiodiplodia pyriformis F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 8

Conidiomata pycnidialia,superficialia, subimmersa vel immersa, papillata, interdum in fasciculis botryosis aggregata, mycelio tecta, pycnidia singula globosa, 694.1 µm diametro. Paraphyses cylindricae, non septatae (26.9−)28.5−33.6 × 1.5−2 µm. Cellulae conidiogenae holoblasticae, cylindricae (7.1−)8.9−15.8 × (2.4−)3.2−6.4 µm. Conidia ovoidea, pyriformia, ellipsoidea vel subglobosa, apice rotundata, interdum basi truncata, parietibus crassis, contenta granularia; post mensem atrobrunnea, tenue longitudinaliter striata, non septata (19.2−)21.5−25.1(−28.1) × (13.3−)15.8−19.3(−21.6) µm.

Conidiomata pycnidial, superficial, semi-immersed to immersed, papillate, occasionally aggregated into botryose clusters, covered in mycelium, individual superficial pycnidia, globose, up to 694.1 µm in diameter. *Paraphyses* cylindrical, aseptate, hyaline, (26.9−)28.5−33.6 × 1.5−2 µm (mean: 31.1 × 1.1 µm). *Conidiogenous cells* hyaline, holoblastic, cylindrical, (7.1−)8.9−15.8 × (2.4−)3.2−6.4 µm (mean: 12.3 × 4.7 µm). *Conidia* initially hyaline becoming sepia in color, ovoid or pyriform to ellipsoidal or subglobose, thick-walled with granular content, rounded at apex and occasionally truncated at base, after ~ 4 weeks, faint longitudinal striations, aseptate, (19.2−)21.5−25.1(−28.1) × (13.3−)15.8−19.3(−21.6) µm (mean of 100 spores: 23.3 × 17.6 µm, l/w ratio: 1.3).

Culture characteristics: columns of aerial mycelium reaching the lid, dark greyish olive (21""k) with white to smoke grey (21""d) tufts. Reverse dark olive (21""m) to black (*1), thread-like growth in the middle of the colony. *Temperatures for growth*: growth at minimum 15 °C, growth rate of 38.5 mm/day at an optimal temperature 30 °C, growth at maximum 35 °C.

Etymology: Name refers to the ovoid to pyriform shaped spores of this fungus.

Host: *Acacia mellifera*.

Distribution: Dordabis and Grootfontein (Namibia).

Notes: This description is based on three isolates from the leading edges of lesions on branches. Differences between *L. pyriformis* and those species of *Lasiodiplodia* described by Burgess *et al*. (2006) include those of the conidia and the paraphyses. Conidia in *L. pyriformis* are significantly smaller than those of all described species and they also have very faint longitudinal striations, which is unlike those of other species. The paraphyses in *L. pyriformis* were also significantly smaller and the growth rates of cultures were substantially more rapid than has been found for other species of *Lasiodiplodia* (Burgess *et al*., 2006).

Specimens examined: NAMIBIA, DORDABIS, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, HOLOTYPE, herb. PREM59633, culture ex-type CMW25414, CBS121770, CAMS001469; NAMIBIA, DORDABIS, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, PARATYPE, herb. PREM59634, culture ex-paratype CMW25415, CBS121771, CAMS001470; NAMIBIA, GROOTFONTEIN, structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25416, CAMS001471.

Spencermartinsia rosulata F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 9

Conidiomata pycnidialia, multa, superficialia, immersa vel semi-immersa, ovoidea vel globosa, singula vel in fasciculis botryosis aggregata, 577.4 µm diametro. Cellulae conidiogenae holoblasticae, cylindricae vel subcylindricae, periclinale incrassatae vel rarius percurrente proliferantes, annulationes singulas vel binas formantes (3.9−)5.2−12(−17) × (2.8−)3.4−5 µm. Conidia 1-septata, rarius non septata, plerumque guttulata, ovoidea, subcylindrica vel ellipsoidea, apice rotundata, basi rotundata vel truncata, aliquando in septis constricta, parietibus modice crassis, laevibus (19−)21−24.2(−26.7) × (8.1−)10.3−10.4(−13) µm.

Conidiomata pycnidial, abundant, superficial, immersed or semi-immersed, separate or aggregated into botryose clusters. Individual pycnidia pyriform to ovoid, up to 782.9 µm in height and covered with little mycelium, but also globose, up to 577.4 µm in diameter that is totally covered in mycelium. *Ostioles* single, central, papillate. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, holoblastic, cylindrical to sub-cylindrical, (3.9−)5.2−12(−17) × (2.8−)3.4−5 µm (average: 7.9 × 4.3 µm), proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently to form one or two annelations. *Conidia* honey colored to brown becoming blackish brown, 1-septate, to a lesser extent aseptate, mostly guttalate, ovoid to sub-cylindrical or ellipsoidal, either with a rounded apex and base or a rounded apex and truncated base, occasionally constricted at the septa, moderately thick-walled, smooth, (19−)21−24.2(−26.7) × (8.1−)10.3−10.4(−13) µm (average of 157 spores: 22.6 × 10.4 µm, l/w ratio: 2.2).

Culture characteristics: greyish olive (21"") aerial mycelium, cottony and in a rosette form with lobed areas at edges of colony, can also be effuse. Occasionally with vinaceous grey (69""d) cottony areas. Reverse olivaceous black (25""m) to olive buff (21"'d) or olivaceous (21"m), lobed with circular growth in the middle of the colony. Abundent thickenings of the hyphae, chlamydospore-like, blackish brown: intercalary, terminal and dictyosporus and found superficial and immersed in water agar. *Temperatures for growth*: growth at minimum 10 °C, growth rate of 19 mm/day at an optimal temperature of 25 °C, growth at maximum 35 °C.

Etymology: Name refers to the prominent rosette-like growth pattern of the fungus in culture.

Hosts: *Acacia karoo* and *A. mellifera*.

Distribution: Windhoek, Dordabis and Grootfontein (Namibia), Ditholo, Pretoria (South Africa).

Notes: Thirty-one isolates were identified as *S. rosulata* and isolates were obtained from healthy material as well as from the leading edges of lesions on branches, black pith, streaks in the wood of branches and stems.

Specimens examined: NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, HOLOTYPE, herb. PREM59622, culture ex-type CMW25389, CBS121760, CAMS001444; NAMIBIA, GROOTFONTEIN, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25390, CAMS001445; NAMIBIA, DORDABIS, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25391, CAMS001446; SOUTH AFRICA, PRETORIA, Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, PARATYPE, herb. PREM59623, culture ex- paratype CMW25392, CBS121761, CAMS001447; SOUTH AFRICA, PRETORIA, Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, CMW25393, CAMS001448.

Botryosphaeria dothidea (Moug. Fr.) Ces. & De Not., Comment Soc. Crittog. Ital. 1:212.1863.

Anamorph. ***Fusicoccum aesculi*** Corda in Sturm, Deutschl. Fl., Abth. 3, 2:111. 1829.

Synanamorph. ***Dichomera*** FIG. 10

Notes: *F. aesculi* was redescribed by Pennycook and Samuels (1985) and revised by Slippers *et al*. (2004a). There were no differences in conidial morphology for the isolates collected in this study, compared to those in previous descriptions. Two isolates of this fungus originated from healthy tissue and one was obtained from the leading edge of a lesion on a branch. Interestingly a *Dichomera*-like synanamorph such as those found in other Botryosphaeriaceae (Barber *et al*.,

2005) was observed in the culture that was collected form the discoloured tissue. Conidia of both *F. aesculi* and the *Dichomera* synanamorph were observed in the same culture and in the same pycnidia (FIG. 10). Buff and black masses of spores were visible on the pycnidia containing mostly both types of conidia.

Conidiomata containing both types of conidia were globose to subglobose or sphaerical, up to 418.2 µm diameter, separate or aggregated into botryose clusters, covered in mycelium, buff or black mass of oozing spores, superficial or semi-immersed. Paraphyses were filiform or hamate, hyaline, (19.1−)24.9−34.9 × (1.6−)1.7−2.9 µm (average: 32.2 × 2.2 µm). Conidiogenous cells were hyaline, cylindrical, (6.8−)7.6−12.8 × 2−3.2 µm (average: 10.1 × 2.7 µm). Conidia were variable in spore morphology ranging from: ovoid, hat-shaped, pyriform, cylindrical, limoniform to narrowly limoniform, mostly truncated at base with a rounded apex, hyaline, unicellular, aseptate, muriformly septate with 1−3 transverse septa and 1−2 angular or longitudinal septa, (8.5−)9.9−13.3(−17.1) × (3.9−)5.6−7.3 µm (average of 50 spores: 11.6 × 6.3 µm, l/w ratio: 1.8). Mycelium of the cultures were olive grey (23""b), white to smoke grey (21""d), cottony or effuse and the reverse black (*1) to olive brown (17"'k), lobed.

Specimens examined: NAMIBIA, DORDABIS, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25410, CAMS001465; NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25411, CAMS001466; NAMIBIA, WINDHOEK, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25412, CBS121768, CAMS001467.

Spencermartinsia viticola A.J.L Phillips & Luque

Notes: *Dothiorella viticola*, now known as *Spencermartinsia viticola* (Phillips *et al*., 2008), was described from *Vitis vinifera* by Luque *et al*. (2005). Four isolates were obtained in this study and these all originated from healthy tissue. The morphology of the isolates collected in this study was very similar to that described by Luque *et al*. (2005) with only small differences. Isolates from this study showed distinctive abundant thickenings of the hyphae, chlamydospore-like structures that were intercalary, terminal and dictyosporus and found superficial and immersed in water agar. *Conidiomata* limited, separate, mostly globose to ovoid, covered in mycelium, superficial, up to 459.8 µm in diameter (average: 325.8 µm), individual superficial pycnidia up to 371.3 µm in height. *Conidiogenous cells* (7−)9.6−16 × 2.1−4.7(−6.5) µm (average: 12.6 × 3.4 µm). *Conidia* aseptate or 1-septate, mostly truncated at the base and constricted at the septum, ovoid to sub-cylindrical, (16.7−)18.1−21.1(−23.3) × (8.4−) 8.6−10.8 (−12.3) µm (average of 63 spores: 19.6 × 9.9 µm, l/w ratio: 2).

Specimens examined: SOUTH AFRICA, PRETORIA: Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, CMW25399, CAMS001454; SOUTH AFRICA, PRETORIA: Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, CMW253400, CAMS001455; SOUTH AFRICA, PRETORIA: Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, CMW25401, CAMS001456; SOUTH AFRICA, PRETORIA: Ditholo, fruiting structures induced on needles of *Pinus* sp. on WA, May 2006, *F.J.J. van der Walt* and *R.N. Heath*, CMW25402, CAMS001457.

Lasiodiplodia pseudotheobromae A.J.L Phillips, A. Alves & Crous

Notes: This species was recently described in a study on cryptic speciation in *L. theobromae* by Alves *et al*. (2008). Two isolates were obtained from healthy tissue in this study. There were no substantial differences between these isolates and those previously described except that the paraphyses in pycnidia from the present study were never longer than 45 µm compared to those of 58 µm described by these authors. In addition, they described a pink pigment in cultures grown at 35 °C, which was not seen in cultures from the present study. Furthermore, cultures of the previous study were able to grow at 10 °C but those collected here were not able to grow at this temperature.

Specimens examined: NAMIBIA, RUNDU, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25417, CAMS001472; NAMIBIA, RUNDU, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2006, *F.J.J. van der Walt* and *J. Roux*, CMW25418, CAMS001473.

DISCUSSION

This study is the most detailed to date on fungi of the Botryosphaeriaceae associated with native *Acacia* spp. in southern Africa, or in any other part of the world. Results indicate that there is a significantly greater diversity of the Botryosphaeriaceae on native *Acacia* spp. in southern Africa than previously thought. This is particularly evident given that only one member of the Botryosphaeriaceae was known on a native *Acacia* sp. from Africa (Lenné, 1992) prior to this study.

In total, 10 Botryopshaeriaceae species, of which six are described as new, were identified in this study. Newly described species included *Diplodia variabilis*, *Dothiorella oblonga*, *Fusicoccum avasmontanum*, *Lasiodiplodia pyriformis*, *Spencermartinsia rosulata* and a new genus, *Mucodiplodia*, represented by the single species *M. africana*. Previously described species included *S. viticola*, *L. pseudotheobromae* and *Botryosphaeria dothidea* with a previously unknown *Dichomera* synanamorph.

These results illustrate the unexplored richness of fungal biodiversity that might be expected on native plant hosts in southern Africa. It has been indicated by Crous *et al*. (2006b) that fungal diversity is poorly studied, not only on the African continent, but world wide. These authors estimated that there could be as many as seven new species of fungi per indigenous plant host. The 10 species found in this study, were isolated from one host representing a single fungal family. This might indicate that estimates by Crous *et al*. (2006b) are conservative. Recent studies on other native trees of southern Africa have also indicated that a single host can harbour a very diverse community of the Botryosphaeriaceae (Damm *et al*., 2007b; Pavlic *et al*., 2007; Slippers and Wingfield, 2007). Studies of this kind are thus, emphasizing the need to study the the factors underlying patterns related to the richness of the Botryosphaeriaceae, which might include host, climate and geographic distribution.

Three previously described species identified in this study included *Botryopshaeria dothidea*, *Lasiodiplodia pseudotheobromae* and *Spencermartinsia viticola*, of which all have wide host ranges and broad geographical distributions (Alves *et al*., 2008; Luque *et al*., 2005; Van Niekerk *et al*., 2004; Slippers *et al*., 2004a). Results obtained in this study further expand our knowledge on the distribution of these fungi, but also emphasize the view of Slippers and Wingfield (2007)

that some of the most damaging pathogens in the Botryosphaeriaceae have wide host and geographical distributions. *Botryosphaeria dothidea*, *L. pseudotheobromae* and *S. viticola* were, however, all isolated from healthy material and in very low numbers. Nevertheless, these species should be considered in future pathology studies, due to their wide host and geographical distributions, and their apparent ability to move between hosts.

The most dominant fungi identified from diseased *A. mellifera* trees were *Spencermartinsia rosulata* (39 % of the total number of isolates) and *Diplodia variabilis* (33 % of the total number of isolates). All the *D. variabilis* isolates were obtained from diseased material while only 44 % of *S. rosulata* isolates were obtained from diseased material. Symptoms from which these fungi were isolated included lesions on branches, black pith in the branches, branch and stem cankers, tip die-back and a brownish black discoloration in the upper taproots of the trees. Although their pathogenicity was not considered it is very likely that these fungi could play a role in the decline of the trees investigated and they deserve further study.

Nine of the Botryosphaeriaceae species, with the exception of *Diplodia variabilis*, were obtained from healthy plant tissue. Of these, eight species were generally present in very low numbers, from one isolate to three isolates per fungal species. Slippers and Wingfield (2007) found that most of the genera in the Botryosphaeriaceae are described as endophytes, which in certain cases dominate the endophyte community (Smith *et al.*, 1996). Our results add credence to the notion that most species in this family are endophytic, although their ecological role as endophytes in nature has received little attention to date.

It is noteworthy that the sampling from Namibia yielded 10 species of the Botryosphaeriaceae compared to the three species from a similar sample size from Pretoria on *A. mellifera*. An explanation for this might be the larger area sampled in Namibia that includes four sites compared to only one sampling site in Pretoria. On the other hand, it was also noteworthy that the trees in Namibia was visually more diseased and apparently under stress. In contrast, *A. mellifera* plants that were sampled in the Pretoria area appeared to be healthy. The Botryosphaeriaceae are known to act as opportunistic pathogens on stressed plants (Denman *et al*., 2000; Slippers and Wingfield, 2007) and, therefore, could have dominated this niche in Namibia, making the isolation of these fungi easier.

Phylogenetic analyses of DNA sequence data provided an efficient means to identify members of the Botryosphaeriaceae collected in this study. This was more effective than morphological characterization and PCR-RFLP. Initial morphological groupings, based on culture characteristics, corresponded well with PCR-RFLP groupings. While the identifications based on these two methods was uncertain, they provided a useful means of selecting isolates for DNA sequencing. In some cases, sequence data were not sufficient to reliably distinguish cryptic species. Here, fixed alleles across multiple loci were needed to be considered to achieve this goal. This is because fixed alleles across multiple loci provide an indication of barriers to genetic exchange or recombination and thus phylogenetically distinct species (Steenkamp *et al*., 2002; Taylor *et al*., 2000). Results of this study support numerous recent investigations (Crous *et al*., 2006a; Denman *et al*., 2000), showing that identification of species in the Botryosphaeriaceae can no longer be achieved without DNA sequence-based analyses.

After applying phylogenetic inference to identify the isolates from *Acacia* spp. as separate species, morphological characterization was used for the species descriptions. Once taxa were defined and morphologically described, these morphological data became useful to identify remaining isolates from *Acacia*. It has been noted before that morphological characteristics can provide a useful basis to rapidly identify species from a particular host and area (Slippers *et al*., 2004b). However, this can only be done once these characteristics have been clearly defined and tested based on DNA sequence comparisons.

The shape of *Dichomera*-like conidia observed in one of the *B. dothidea* cultures contradicts conclusions of Crous *et al*. (2006a), who suggested that it is possible to distinguish between the genera *Neofusicoccum* and *Fusicoccum* based on the morphology of their associated *Dichomera*-like synanamorphs. Conidia of *Dichomera* synanamorphs of *Fusicoccum* were considered to be fusiform to ellipsoid, while those associated with *Neofusicoccum Dichomera* synanamorphs were described as globose to pyriform (Crous *et al*., 2006a). Results of this study, however, show that *Dichomera* synanamorphs of *Fusicoccum* can also be globose to pyriform in shape. The conidial morphology of the synanamorphs is thus a questionable characteristic to distinguish between *Fusicoccum* and *Neofusicoccum*. This is also the first report of pleoanamorphism in *B. dothidea* in Africa.

Considerable changes have occurred in the taxonomy of the Botryosphaeriaceae in the last three years. Not only have a number of new species (Alves *et al*., 2008; Burgess *et al*., 2006; Damn *et al*., 2007b; Phillips *et al*., 2007) and new genera (Crous *et al*., 2006a; Damn *et al*., 2007a) been described, but changes have also been made to the higher level taxonomy of the family (Crous *et al*., 2006a; Phillips *et al*., 2008; Schoch *et al*., 2006). From the current study alone, a number of

new species and a new genus in the Botryosphaeriaceae emerged. These studies clearly emphasize that a great deal has yet to be learned regarding the taxonomic circumscription of the Botryosphaeriaceae, especially as new areas and hosts are explored.

A new genus, *Mucodiplodia*, is described in this study based on DNA sequence comparisons and morphological characteristics. This genus is most closely related to the recently described genus, *Aplosporella* Damm & Crous (Damm *et al*., 2007a), based on phylogenetic analyses. There were, however, substantial morphological differences to distinguish between these two genera. Pycnidia and paraphyses of *Aplosporella* are much larger in length than those of *Mucodiplodia*. Conidia of *Aplosporella* are also hyaline at first, turning dark brown with age, compared to those of *Mucodiplodia* that become honey to brown coloured. However, the two main distinctive characteristics between the two genera is the fact that there are no papillate apices on the conidia of *Aplosporella* and most importantly, conidia of *Aplosporella* have no persistent mucous sheath.

The description of these two closely related genera illustrates the diversity and complexity of the taxonomy of this group of fungi. Recent studies have shown that it is possible to classify the Botryosphaeriaceae more accurately when phylogenetic inference are used together with morphological characteristics (Crous *et al*., 2006a; Jacobs and Rehner, 1998; Slippers and Wingfield, 2007). In this study, phylogenetic inference provided the necessary data to distinction between *M. africana* and *A. prunicola* and, based on significant differences in morphological characteristics, a new genus is described here.

While this study has contributed significantly to our understanding of the Botryosphaeriaceae on native *Acacia* spp. in southern Africa, little is known regarding the ecological relevance of this

group of fungi on these and other native African trees. Some species most likely pose a threat as pathogens. This is due to their ability to be associated with many hosts and the apparent ease with which they can switch hosts (Slippers and Wingfield, 2007). Climate change and drought could be additional stress factors that are likely to increase the frequency of diseases caused by this group of fungi (Slippers and Wingfield, 2007). Understanding the ecology and biology of these fungi will be important in order to understand the potential threats. This study should serve as a foundation for such studies on *Acacia* spp. in the future.

REFERENCES

Alves A, Crous PW, Correia A, Phillips AJL. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity 28:1–13.

Barber PA, Burgess TJ, Hardy GE St J, Slippers B, Keane PJ, Wingfield MJ. 2005. *Botryosphaeria* species from *Eucalyptus* in Australia are pleoanamorphic, producing *Dichomera* synanamorphs in culture. Mycological Research 109:1347−1363.

Barnes I, Roux J, Coetzee MPA, Wingfield MJ. 2001. Characterization of *Seiridium* spp. associated with cypress canker based on β-tubulin and histone sequences. Plant Disease 85:317–321.

Burgess T, Barber PA, Mohali S, Pegg G, de Beer W, Wingfield MJ. 2006. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. Mycologia 98:423−435.

Crous PW, Phillips AJL, Baxter AP. 2000. Phytopathogenic Fungi from South Africa. Department of Plant Pathology Press, University of Stellenbosh, pp. 73–75.

—, Denman S, Taylor JE, Swart L, Palm ME. 2004. Cultivation and diseases of *Proteaceae*: *Leucadendron*, *Leucospermum* and *Protea*. CBS Biodiversity Series 2:1−228.

—, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006a. Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology 55:235−253.

—, Rong IH, Wood A, Lee S, Glen HF, Botha W, Slippers B, De Beer ZW, Wingfield MJ, Hawksworth DL. 2006b. How many species of fungi are there at the tip of Africa? Studies in Mycology 55:13−33.

Damn U, Fourie PH, Crous PW. 2007a. *Aplosporella prunicola*, a novel species of anamorphic Botryosphaeriaceae. Fungal Diversity 27: 35–43.

—, Crous PW, Fourie PH. 2007b. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa with describtions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99:664–680.

Denman S, Crous PW, Taylor JE, Kang J-C, Pascoe I, Wingfield MJ. 2000. An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Studies in Mycology 45:129−140.

Gibson IAS. 1975. The Leguminosae. In Diseases of Forest Trees Widely Planted as Exotics in the Tropics and Southern Hemisphere. Part I. Important members of the Myrtaceae, Leguminosae, Verbenaceae and Meliaceae. Commonwealth Forestry Institute, University of Oxford: Oxford. (ed. IAS Gibson), pp. 21–34.

Holz G, Schreuder W. 1989. Dieback of blackthorn (*Acacia mellifera* subsp. *detinens*) in South West Africa. Agricola 7.

Jacobs KA, Rehner SA. 1998. Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. Mycologia 90:601−610.

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD. 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycological Research 108:411−418.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059−3066.

Lenné JM. 1992. Diseases on multi purpose woody legumes in the tropics: A Review. Nitrogen Fixing Tree Research Reports 10:13−29.

Luque J, Martos S, Phillips AJL. 2005. *Botryosphaeria viticola* sp. nov. on grapevines: a new species with a *Dothiorella* anamorph. Mycologia 97:1111−1121.

Page RDM. 1996. Treeview: an application to display phylogenetic trees on personal computers. Computer Applied Biosciences 12:357–358.

Pavlic D, Slippers B, Coutinho TA, Gryzenhout M, Wingfield MJ. 2004. *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. Studies in Mycology 50:313–22.

—, —, —, Wingfield MJ. 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. Plant Pathology 56:624–636.

Pennycook SR, Samuels GJ. 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. Mycotaxon 24:445−458.

Phillips AJL, Crous PW, Arthur A. 2007. *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*. Fungal Diversity 25:141–155.

—, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW. 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21: 29–55.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics Applications Notes 14:817−818.

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1:17−20.

Rayner RW. 1970. A mycological colour chart. CMI and British Mycological Society, Kew, Surrey, U.K.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Roux J, Wingfield MJ. 1997. Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. Forest Ecology and Management 99:327−336.

—, Wingfield MJ, Morris MJ. 1997. *Botryosphaeria dothidea* as a pathogen of *Acacia mearnsii* in South Africa. South African Journal of Science 93:xii.

Schoch CL, Schoemaker RA, Seifert KA, Spatafora JW, Crous PW. 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98:1041–1052.

Sherry SP. 1971. 'The black wattle (*Acacia mearnsii* De Wild.)'. University of Natal Press: Pietermaritzburg, South Africa, p. 1−382.

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ. 2004a. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96:83−101.

—, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Carnegie AJ, Wingfield MJ. 2004b. Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. Studies in Mycology 50:343–358.

—, Smit WA, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ. 2006. Taxonomy, phylogeny and identification of Botryosphaeriaceae causing diseases on pome and stone fruit trees in South Africa and other regions of the world. Plant Pathology: 1−12.

—, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biology Reviews 21:75−89.

Smith H, Wingfield MJ, Crous PW, Coutinho TA. 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. South African Journal of Botany 62:86−88.

Steenkamp ET, Wingfield BD, Desjardins AE, Marasas WFO, Wingfield MJ. 2002. Cryptic speciation in *Fusarium subglutinans*. Mycologia 94:1032−1043.

Stephens RP, Goldschmidt WB. 1938. A prelimanary report on aspects of wattle pathology. South Africa Forestry Journal 2:32–42.

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31:21−32.

Van Niekerk JM, Crous PW, Groenewald JZ, Fourie PH, Halleen F. 2004**.** DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. Mycologia 96:781−798.

Venter F, Venter J-A. 2002. Making the most of indigenous trees. Second edition. Briza Publications. Pretoria, South Africa, pp. 14−34.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172:4238−4246.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a sequencing guide to methods and applications'. Academic press: San Diego, CA, pp. 315−322.

TABLE I: Isolates of the Botryosphaeriaceae considered in this study (FIG. 1–3).

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
[1]**STE-U6326**	[2]CBS121167	*Aplosporella prunicola*	*Prunus persica* var. *nucipersica*	U. Damm	Modimolle Limpopo Province, South Africa	*EF564375*		*EF564377*
STE-U6327		*A. prunicola*	*Prunus persica* var. *nucipersica*	U. Damm	Modimolle Limpopo Province, South Africa	*EF564376*		*EF564378*
[3]**CMW8000**		*Botryosphaeria dothidea*	*Prunus* sp.	B. Slippers	Switzerland, Crocifisso	*AY236949*	*AY236898*	
CBS116742		*B. dothidea*	*Olea europea*	I. Rumbos	Greece, Thessalia	*AY640254*	*AY640257*	
CBS116743		*B. dothidea*	*Populus nigra*	A.J.L. Phillips	Portugal, Braga	*AY640253*	*AY640256*	
CMW25410	[4]CAMS001465	*B. dothidea**	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	EU101304	EU101349	
CMW25411	CAMS001466	*B. dothidea**	*A. karoo*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	EU101305	EU101350	
CBS121768	CMW25412 CAMS001467	*B. dothidea* (*Dichomera*-like synanamorph)*	*A. karoo*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	EU101306	EU101351	
GS-97-58		*B. mamane*	*Sophora chrysophylla*	D. Gardner	Hawaii	*AF246929*		
GS-97-59		*B. mamane*	*Sophora chrysophylla*	D. Gardner	Hawaii	*AF246930*		
CBS119047		*B. corticis*	*Vaccinium corymbosum*	P.V. Oudemans	New Jersey, USA	*DQ299245*		
CBS119048		*B. corticis*	*Vaccinium corymbosum*	P.V. Oudemans	New Jersey, USA	*DQ299246*		

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CMW15952		*Dichomera eucalypti*	*Eucalyptus diversicolor*	T. Burgess & K.-L. Goei	Western Australia	*DQ093194.1*	*DQ093215*	
CMW15953		*Dic. eucalypti*	*E. diversicolor*	T. Burgess & K.-L. Goei	Western Australia	*DQ093195.1*	*DQ093216*	
[5]WAC12398	CMW15198	*Dic. eucalypti*	*E. diversicolor*	T. Burgess & K.-L. Goei	Western Australia	*AY744371.1*	*DQ093214*	
STE-U5908	CBS120835	*Diplodia africana*	*P. persica*	U. Damm	Paarl, Western Cape, South Africa	*EF445343*	*EF445382*	
STE-U5946	CBS121104	*D. africana*	*P. persica*	U. Damm	Paarl, Western Cape, South Africa	*EF445344*	*EF445383*	
CBS112549		*D. corticola*	*Quercus suber*	A. Alves	Portugal, Aveiro	*AY259100*	*AY573227*	
CBS112545		*D. corticola*	*Q. suber*	M.E. Sánchez & A. Trapero	Spain, Cádiz	*AY259089*	*AY573226*	
CBS112553		*D. mutila*	*V. vinifera*	A.J.L. Phillips	Portugal, Montemor-o-Novo	*AY259093*	*AY573219*	
CBS431.82		*D. mutila*	*Fraxinus excelsior*	H.A. van der Aa	Netherlands, Maarseveen	*AY236955*	*AY236904*	
CBS110496		*D. porosum*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343379*	*AY343340*	
CBS110574		*D. porosum*	*V. vinifera*	J.M. van Niekerk	South Africa	*AY343378*	*AY343339*	
CBS112555		*D. seriata*	*V. vinifera*	A.J.L. Phillips	Portugal, Montemor-o-Novo	*AY259094*	*AY573220*	
CMW7775		*D. seriata*	*Ribes* sp.	B. Slippers & G. Hudler	USA, New York	*AY236954*	*AY236903*	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CBS121774	CMW25419	*D. variabilis**	*A. karoo*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	EU101312	EU101357	
	CAMS001474							
CMW25420	CAMS001475	*D. variabilis**	*A. hebeclade*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	EU101313	EU101358	
CBS121775	CMW25421	*D. variabilis**	*A. karoo*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	EU101314	EU101359	
	CAMS001476							
CBS115041		*Dothiorella iberica*	*Quercus ilex*	J. Luque	Spain, Aragon	*AY573202*	*AY573222*	
CBS115040		*Do. iberica*	*Q. ilex*	J. Luque	Spain, Catalonia	*AY573214*	*AY573232*	
CBS121764	CMW25406	*Do. oblonga**	*A. mellifera*	F.J.J. van der Walt & J. Roux	Rundu, Namibia	EU101299	EU101344	
	CAMS001461							
CBS121765	CMW25407	*Do. oblonga**	*A. mellifera*	F.J.J. van der Walt & R.N.	Pretoria, South Africa	EU101300	EU101345	
	CAMS001462			Heath				
CBS121766	CMW25408	*Do. oblonga**	*A. mellifera*	F.J.J. van der Walt & R.N.	Pretoria, South Africa	EU101301	EU101346	
	CAMS001463			Heath				
[6]IMI63581b		*Do. sarmentorum*	*Ulmus* sp.	E.A. Ellis	England, Warwickshire	*AY573212*	*AY573235*	
CBS115038		*Do. sarmentorum*	*Malus pumila*	A.J.L. Phillips	Netherlands, Delft	*AY573206*	*AY573223*	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CBS121769	CMW25413 CAMS001468	*Fusicoccum avasmontanum* *	*A. mellifera*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	EU101303	EU101348	
CMW13488		*Lasiodiplodia crassispora*	*Eucalyptus urophylla*	S. Mohali	Acarigua, Venezuela	*DQ103552.1*	*DQ103559*	
CMW14691	WAC12533	*L. crassispora*	*Syzygium album*	T.I. Burgess & B. Dell	Kununurra, Australia	*DQ103550.1*	*DQ103557*	
CMW14688	WAC12534	*L. crassispora*	*S. album*	T.I. Burgess & B. Dell	Kununurra, Australia	*DQ103551.1*	*DQ103558*	
CBS115812	CMW14077	*L. gonobiensis*	*Syzygium cordatum*	D. Pavlic	Eastern Cape Province, South Africa	*AY639595*	*DQ103566*	
CBS116355	CMW14078	*L. gonobiensis*	*S. cordatum*	D. Pavlic	Eastern Cape Province, South Africa	*AY639594*	*DQ103567*	
CBS456.78		*L. parva*	Cassava-field soil	O. Rangel	Colombia	EF622083	EF622063	
CBS494.78		*L. parva*	Cassava-field soil	O. Rangel	Colombia	EF622084	EF622064	
STE-U5803	CBS120832	*L. plurivora*	*P. salicina*	U. Damm	Stellenbosh, Western Cape, South Africa	EF445362	EF445395	
STE-U4583	CBS121103	*L. plurivora*	*V. vinifera*	F. Halleen	South Africa	AY343482	EF445396	
CBS116459		*L. pseudotheobromae*	*Gmelina arborea*	J. Carranza-Velásquez	Costa Rica	EF622077	EF622057	
CBS116460		*L. pseudotheobromae*	*A. mangium*	J. Carranza-Velásquez	Costa Rica	EF622078	EF622058	
CMW25417	CAMS001472	*L. pseudotheobromae**	*A. mellifera*	F.J.J van der Walt & J. Roux	Rundu, Namibia	EU101310	EU101355	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CMW25418	CAMS001473	*L. pseudotheobromae* *	*A. mellifera*	F.J.J van der Walt & J. Roux	Rundu, Namibia	EU101311	EU101356	
CBS121770	CMW25414	*L. pyriformis**	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	EU101307	EU101352	
	CAMS001469							
CBS121771	CMW25415	*L. pyriformis**	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	EU101308	EU101353	
	CAMS001470							
CMW25416	CAMS001471	*L. pyriformis**	*A. mellifera*	F.J.J van der Walt & J. Roux	Grootfontein, Namibia	EU101309	EU101354	
WAC12538		*L. rubropurpurea*	*Eucalyptus grandis*	T.I. Burgess/G. Pegg	Tully, Queensland	*DQ103555*	*DQ103573*	
WAC12539		*L. rubropurpurea*	*E. grandis*	T.I. Burgess/G. Pegg	Tully, Queensland	*DQ103556*	*DQ103574*	
CBS164.96		*L. theobromae*	Unknown fruit on coral reef coast	A. Aptroot	Papua New Guinea, Madang	*AY640255*	*AY640258*	
CMW9074		*L. theobromae*	*Pinus* sp.	T. Burgess	Mexico	*AY236952*	*AY236901*	
CMW13512	WAC12540	*L. venezuelensis*	*A. mangium*	S. Mohali	Acarigua, Venezuela	*DQ103548*	*DQ103569*	
CMW13513		*L. venezuelensis*	*A. mangium*	S. Mohali	Acarigua, Venezuela	*DQ103549*	*DQ103570*	
CBS121777	CMW25424	*Mucodiplodia africana**	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	EU101315	EU101360	EU101380
	CAMS001479							

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CBS121778	CMW25425 CAMS001480	*M. africana**	*A. mellifera*	F.J.J van der Walt & J. Roux	Grootfontein, Namibia	EU101316	EU 101361	EU101381
CBS121779	CMW25426 CAMS001481	*M. africana**	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	EU101317	EU101362	EU101382
CBS112872		*Neofusicoccum australe*	*V. vinifera*	F. Halleen	South Africa	*AY343388*	*AY343347*	
CBS112877		*N. australe*	*V. vinifera*	F. Halleen	South Africa	*AY343385*	*AY343346*	
CBS110299		*N. luteum*	*V. vinifera*	A.J.L. Phillips	Portugal, Oeiras	*AY259091*	*AY573217*	
CMW9076		*N. luteum*	*Malus x domestica*	S.R. Pennycook	New Zealand	*AY236946*	*AY236893*	
CMW9081	[7]ICMP8003	*N. parvum*	*Populus nigra*	G.J. Samuels	New Zealand, Te Puke	*AY236943*	*AY236888*	
CBS110301		*N. parvum*	*V. vinifera*	A.J.L. Phillips	Portugal, Palmela	*AY259098*	*AY573221*	
CBS115475		*N. ribis*	*Ribes* sp.	G. Hudler	USA, New York	*AY236935*	*AY236877*	
CMW7773		*N. ribis*	*Ribes* sp.	G. Hudler	USA, New York	*AY236936*	*AY236878*	
CBS112878		*N. viticlavatum*	*V. vinifera*	F. Halleen	South Africa, Stellenbosch	*AY343380*	*AY343342*	
CBS112977		*N. viticlavatum*	*V. vinifera*	F. Halleen	South Africa	*AY343381*	*AY343341*	
CBS110887		*N. vitifusiforme*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343383*	*AY343343*	
CBS110880		*N. vitifusiforme*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343382*	*AY343344*	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CBS121767	CMW25409 CAMS001464	*Neofusicoccum* sp.*	*A. mellifera*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	EU101302	EU101347	
CBS121760	CMW25389 CAMS 001444	*Spencermartinsia rosulata**	*Acacia karoo*	F.J.J. van der Walt & J. Roux	Windhoek, Namibia	EU101290	EU101335	
CMW25390	CAMS001445	*S. rosulata**	*A. mellifera*	F.J.J. van der Walt & J. Roux	Grootfontein, Namibia	EU101291	EU101336	
CMW25391	CAMS001446	*S. rosulata**	*A. mellifera*	F.J.J. van der Walt & J. Roux	Dordabis, Nambia	EU101292	EU101337	
CBS121761	CMW25392 CAMS001447	*S. rosulata**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101293	EU101338	
CMW25393	CAMS001448	*S. rosulata**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101294	EU101339	
CBS117009		*S. viticola*	*Vitis vinifera cv. Garnatxa Negra*	J. Luque & S. Martos	Spain, Vimbodí	AY905554	AY905559	
CBS117006		*S. viticola*	*V. vinifera cv. Garnatxa Negra*	J. Luque & R. Mateu	Spain, Vimbodí	AY905555	AY905562	
CBS117008		*S. viticola*	*V. vinifera cv. Xarel·lo*	J. Luque & J. Reyes	Spain, Sant Sadurní d'Anoia	AY905557	AY905560	
CMW25399	CAMS001454	*S. viticola**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101295	EU101340	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CMW25400	CAMS001455	*S. viticola**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101296	EU101341	
CMW25401	CAMS001456	*S. viticola**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101297	EU101342	
CMW25402	CAMS001457	*S. viticola **	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101298	EU101343	
CBS119.25		*Cercospora apii*	*Apium graveolens*	L.J. Klotz	Unknown	*AY179949*	*AY179915*	
[8]CPC12031		*C. beticola*	Unknown	Unknown	Unknown	*DQ233339*	*DQ233365*	

Culture collections: [1]STE-U: Department of Plant Pathology, University of Stellenbosh, South Africa; [2]CBS-Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; [3]CMW- FABI, University of Pretoria, South Africa; [4]CAMS-Center for Applied Mycological Studies, University of Pretoria, South Africa; [5]WAC-Department of Agriculture Western Australia Plant Pathogen Collection; [6]IMI-CABI Bioscience, Egham, UK; [7]ICMP-International Collection of Micro-organisms from Plants, New Zealnd; [8]CPC-Cultue collection of Pedro Crous.

BOLD = Isolate accession numbers in bold signify cultures ex-type, or from samples that have been linked morphologically to the type material

ITALICS = Sequence numbers in italics were retrieved from the GenBank public database. All others were obtained in this study.

* Isolates used in this study for sequencing and phylogenetic analysis is indicated with an asterisk

TABLE II: Polymorphic nucleotides from sequence data of the ITS and EF1-α to show the relationships between *Spencermartinsia viticola* and *S. rosulata*. Polymorphisms unique to *S. rosulata* are in bold type and shaded. Isolates from this study are indicated with an asterisk *.

Identity	**Culture no.**	**ITS**		**EF1-α**				
		27	**85**	**49**	**72**	**203**	**204**	**245**
*S. viticola**	CMW25399	C	T	C	C	C	A	G
*S. viticola**	CMW25401	C	T	C	C	C	A	G
S. viticola	CBS117006	C	T	C	C	C	A	G
S. viticola	CBS117009	C	T	C	C	C	A	G
S. viticola	CBS117008	C	T	C	C	C	A	G
*S. rosulata**	CBS121760	**T**	**C**	**T**	**–-**	**T**	**C**	**T**
*S. rosulata**	CBS121761	**T**	**C**	**T**	**–**	**T**	**C**	**T**

TABLE III: Sequence differences of the ITS and EF1-α gene regions to show the relationships between *Botryosphaeria dothidea* and *Fusicoccum avasmontanum*. Differences unique to the *F. avasmontanum* are in bold type and shaded. Isolates from this study are indicated by an asterisk*.

Identity	**Culture no.**	**ITS**				
		56	**57**	**91 - 106**	**110**	**133 - 135**
F. avasmontanum *	CBS121769	**T**	**G**	**----------**	**G**	**---**
B. dothidea	CMW8000	–	–	GCCGCGGTTCTCCGCG	C	GGG
B. dothidea	CBS116742	–	–	GCCGCGGTTCTCCGCG	C	GGG
B. dothidea	CBS116743	–	–	GCCGCGGTTCTCCGCG	C	GGG

Identity	**Culture no.**	**EF1-α**	
		214 - 230	**236 - 239**
F. avasmontanum *	CBS121769	**----------**	**----**
B. dothidea	CMW8000	CTCCGCATCTGGATTTT	TTGT
B. dothidea	CBS116742	CTCCGCATCTGGATTTT	TTGT
B. dothidea	CBS116743	CTCCGCATCTGGATTTT	TTGT

TABLE IV: Polymorphic nucleotides (or alleles) from sequence data of the ITS and EF1-α to show the relationship between *Lasiodiplodia crassipora* and the sibling species *L. pyriformis*. Polymorphisms unique to *L. pyriformis* are in bold type and shaded. Isolates from this study are indicated by an asterisk *.

Identity	**Culture no.**	**ITS**	**EF1-α**
		466	**555**
*L. pyriformis**	CBS121770	**C**	**C**
*L. pyriformis**	CBS121771	**C**	**C**
*L. pyriformis**	CMW25416	**C**	**C**
L. crassispora	CMW13488	T	G
L. crassispora	WAC12533	T	G
L. crassispora	WAC12534	T	G

FIGURE 1: Most-parsimonious tree obtained through heuristic searches of the combined dataset of sequences for the ITS and EF1-α gene region. Bootstrap values ≥ 70 % are indicated above the line with posterior probabilities ≥ 95 % indicated below the line. The main clades corresponding to genera are indicated by Clades A-G and the taxa are indicated by the sub-clades 1–30. Isolates sequenced in this study are indicated with an asterisk * and the ex-type cultures are in bold. New species emerging from this study are as follows: *Spencermartinsia rosulata* (Sub-clade 1), *Dothiorella oblonga* (Sub-clade 3), *Fusicoccum avasmontanum* (Sub-clade 14), *Lasiodiplodia pyriformis* (Sub-clade 15), *Diplodia variabilis* (Sub-clade 23) and *Mucodiplodia africana* (Sub-clade 29).

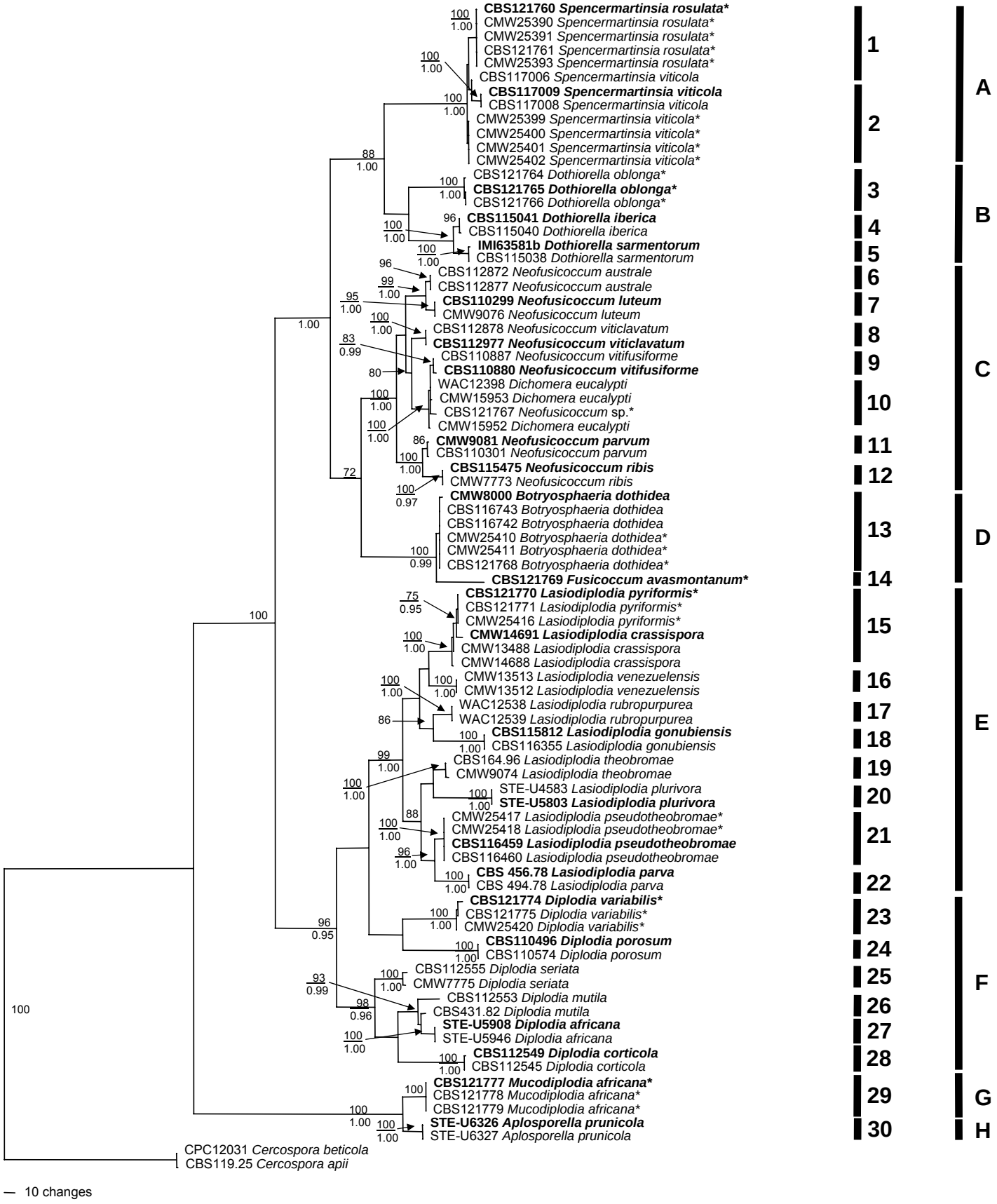

CBS121760 Spencermartinsia rosulata*
CMW25390 Spencermartinsia rosulata*
CMW25391 Spencermartinsia rosulata*
CBS121761 Spencermartinsia rosulata*
CMW25393 Spencermartinsia rosulata*
CBS117006 Spencermartinsia viticola
CBS117009 Spencermartinsia viticola
CBS117008 Spencermartinsia viticola
CMW25399 Spencermartinsia viticola*
CMW25400 Spencermartinsia viticola*
CMW25401 Spencermartinsia viticola*
CMW25402 Spencermartinsia viticola*
CBS121764 Dothiorella oblonga*
CBS121765 Dothiorella oblonga*
CBS121766 Dothiorella oblonga*
CBS115041 Dothiorella iberica
CBS115040 Dothiorella iberica
IMI63581b Dothiorella sarmentorum
CBS115038 Dothiorella sarmentorum
CBS112872 Neofusicoccum australe
CBS112877 Neofusicoccum australe
CBS110299 Neofusicoccum luteum
CMW9076 Neofusicoccum luteum
CBS112878 Neofusicoccum viticlavatum
CBS112977 Neofusicoccum viticlavatum
CBS110887 Neofusicoccum vitifusiforme
CBS110880 Neofusicoccum vitifusiforme
WAC12398 Dichomera eucalypti
CMW15953 Dichomera eucalypti
CBS121767 Neofusicoccum sp.*
CMW15952 Dichomera eucalypti
CMW9081 Neofusicoccum parvum
CBS110301 Neofusicoccum parvum
CBS115475 Neofusicoccum ribis
CMW7773 Neofusicoccum ribis
CMW8000 Botryosphaeria dothidea
CBS116743 Botryosphaeria dothidea
CBS116742 Botryosphaeria dothidea
CMW25410 Botryosphaeria dothidea*
CMW25411 Botryosphaeria dothidea*
CBS121768 Botryosphaeria dothidea*
CBS121769 Fusicoccum avasmontanum*
CBS121770 Lasiodiplodia pyriformis*
CBS121771 Lasiodiplodia pyriformis*
CMW25416 Lasiodiplodia pyriformis*
CMW14691 Lasiodiplodia crassispora
CMW13488 Lasiodiplodia crassispora
CMW14688 Lasiodiplodia crassispora
CMW13513 Lasiodiplodia venezuelensis
CMW13512 Lasiodiplodia venezuelensis
WAC12538 Lasiodiplodia rubropurpurea
WAC12539 Lasiodiplodia rubropurpurea
CBS115812 Lasiodiplodia gonubiensis
CBS116355 Lasiodiplodia gonubiensis
CBS164.96 Lasiodiplodia theobromae
CMW9074 Lasiodiplodia theobromae
STE-U4583 Lasiodiplodia plurivora
STE-U5803 Lasiodiplodia plurivora
CMW25417 Lasiodiplodia pseudotheobromae*
CMW25418 Lasiodiplodia pseudotheobromae*
CBS116459 Lasiodiplodia pseudotheobromae
CBS116460 Lasiodiplodia pseudotheobromae
CBS 456.78 Lasiodiplodia parva
CBS 494.78 Lasiodiplodia parva
CBS121774 Diplodia variabilis*
CBS121775 Diplodia variabilis*
CMW25420 Diplodia variabilis*
CBS110496 Diplodia porosum
CBS110574 Diplodia porosum
CBS112555 Diplodia seriata
CMW7775 Diplodia seriata
CBS112553 Diplodia mutila
CBS431.82 Diplodia mutila
STE-U5908 Diplodia africana
STE-U5946 Diplodia africana
CBS112549 Diplodia corticola
CBS112545 Diplodia corticola
CBS121777 Mucodiplodia africana*
CBS121778 Mucodiplodia africana*
CBS121779 Mucodiplodia africana*
STE-U6326 Aplosporella prunicola
STE-U6327 Aplosporella prunicola
CPC12031 Cercospora beticola
CBS119.25 Cercospora apii
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
A
B
C
D
E
F
G
H
10 changes

FIGURE 2: Most-parsimonious tree of the LSU representing the genera of the Botryosphaeriaceae (Crous *et al*., 2006). 100 parsimonious trees were found with no major changes in the topology amongst them. Isolates of the newly described genus, *Mucodiplodia*, that were sequenced in this study are indicated with an asterisk * and the ex-type culture is in bold. Bootstrap values ≥ 70 % are indicated.

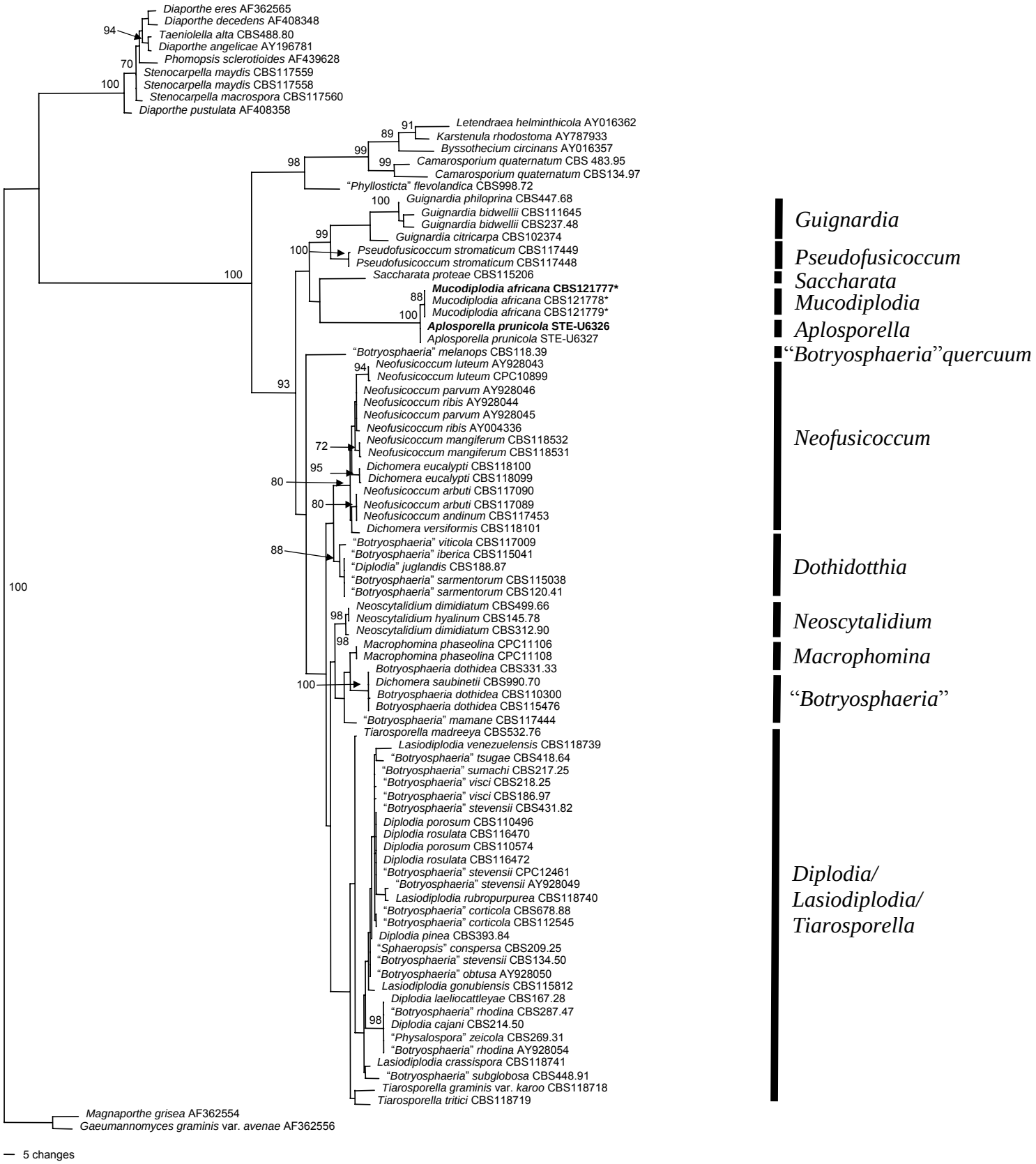

Diaporthe eres AF362565
Diaporthe decedens AF408348
Taeniolella alta CBS488.80
Diaporthe angelicae AY196781
Phomopsis sclerotioides AF439628
Stenocarpella maydis CBS117559
Stenocarpella maydis CBS117558
Stenocarpella macrospora CBS117560
Diaporthe pustulata AF408358
Letendraea helminthicola AY016362
Karstenula rhodostoma AY787933
Byssothecium circinans AY016357
Camarosporium quaternatum CBS 483.95
Camarosporium quaternatum CBS134.97
"Phyllosticta" flevolandica CBS998.72
Guignardia philoprina CBS447.68
Guignardia bidwellii CBS111645
Guignardia bidwellii CBS237.48
Guignardia citricarpa CBS102374
Pseudofusicoccum stromaticum CBS117449
Pseudofusicoccum stromaticum CBS117448
Saccharata proteae CBS115206
Mucodiplodia africana CBS121777*
Mucodiplodia africana CBS121778*
Mucodiplodia africana CBS121779*
Aplosporella prunicola STE-U6326
Aplosporella prunicola STE-U6327
"Botryosphaeria" melanops CBS118.39
Neofusicoccum luteum AY928043
Neofusicoccum luteum CPC10899
Neofusicoccum parvum AY928046
Neofusicoccum ribis AY928044
Neofusicoccum parvum AY928045
Neofusicoccum ribis AY004336
Neofusicoccum mangiferum CBS118532
Neofusicoccum mangiferum CBS118531
Dichomera eucalypti CBS118100
Dichomera eucalypti CBS118099
Neofusicoccum arbuti CBS117090
Neofusicoccum arbuti CBS117089
Neofusicoccum andinum CBS117453
Dichomera versiformis CBS118101
"Botryosphaeria" viticola CBS117009
"Botryosphaeria" iberica CBS115041
"Diplodia" juglandis CBS188.87
"Botryosphaeria" sarmentorum CBS115038
"Botryosphaeria" sarmentorum CBS120.41
Neoscytalidium dimidiatum CBS499.66
Neoscytalidium hyalinum CBS145.78
Neoscytalidium dimidiatum CBS312.90
Macrophomina phaseolina CPC11106
Macrophomina phaseolina CPC11108
Botryosphaeria dothidea CBS331.33
Dichomera saubinetii CBS990.70
Botryosphaeria dothidea CBS110300
Botryosphaeria dothidea CBS115476
"Botryosphaeria" mamane CBS117444
Tiarosporella madreeya CBS532.76
Lasiodiplodia venezuelensis CBS118739
"Botryosphaeria" tsugae CBS418.64
"Botryosphaeria" sumachi CBS217.25
"Botryosphaeria" visci CBS218.25
"Botryosphaeria" visci CBS186.97
"Botryosphaeria" stevensii CBS431.82
Diplodia porosum CBS110496
Diplodia rosulata CBS116470
Diplodia porosum CBS110574
Diplodia rosulata CBS116472
"Botryosphaeria" stevensii CPC12461
"Botryosphaeria" stevensii AY928049
Lasiodiplodia rubropurpurea CBS118740
"Botryosphaeria" corticola CBS678.88
"Botryosphaeria" corticola CBS112545
Diplodia pinea CBS393.84
"Sphaeropsis" conspersa CBS209.25
"Botryosphaeria" stevensii CBS134.50
"Botryosphaeria" obtusa AY928050
Lasiodiplodia gonubiensis CBS115812
Diplodia laeliocattleyae CBS167.28
"Botryosphaeria" rhodina CBS287.47
Diplodia cajani CBS214.50
"Physalospora" zeicola CBS269.31
"Botryosphaeria" rhodina AY928054
Lasiodiplodia crassispora CBS118741
"Botryosphaeria" subglobosa CBS448.91
Tiarosporella graminis var. karoo CBS118718
Tiarosporella tritici CBS118719
Magnaporthe grisea AF362554
Gaeumannomyces graminis var. avenae AF362556
5 changes
Guignardia
Pseudofusicoccum
Saccharata
Mucodiplodia
Aplosporella
"Botryosphaeria"quercuum
Neofusicoccum
Dothidotthia
Neoscytalidium
Macrophomina
"Botryosphaeria"
Diplodia/
Lasiodiplodia/
Tiarosporella

FIGURE 3: Most-parsimonious tree of the ITS gene region used to compare *Fusicoccum avasmontanum* with *Botryosphaeria mamane* and *B. corticis* that was not included in FIG. 1 because EF data are not available for it. The tree was rooted with *Dothiorella iberica* and *Do. sarmentorum*. Isolates used in this study are indicated by an asterisk * and the ex-type cultures are in bold. Bootstrap values ≥ 70 % are indicated.

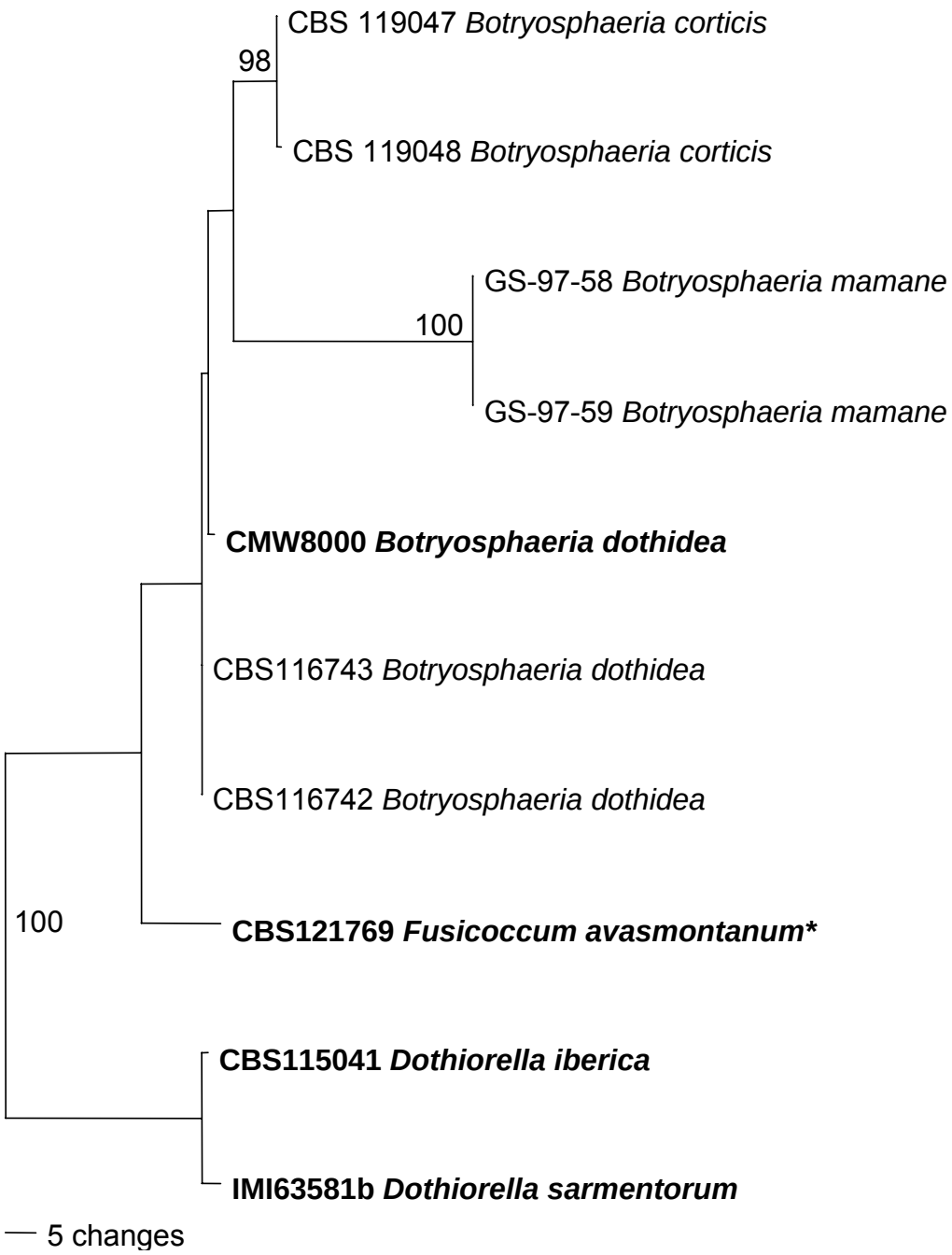

CBS 119047 Botryosphaeria corticis
98
CBS 119048 Botryosphaeria corticis
GS-97-58 Botryosphaeria mamane
100
GS-97-59 Botryosphaeria mamane
CMW8000 Botryosphaeria dothidea
CBS116743 Botryosphaeria dothidea
CBS116742 Botryosphaeria dothidea
100
CBS121769 Fusicoccum avasmontanum*
CBS115041 Dothiorella iberica
IMI63581b Dothiorella sarmentorum
5 changes

FIGURE 4: *Mucodiplodia africana*. A. Pycnidium on pine needles. B. Chlamydospore-like hyphae (arrows). C. Paraphysis. D. Conidiogenous cells with periclinal thickenings and annelations (arrow). E-F. Conidia encased in a persistent mucous sheath. E. Younger conidium (hyaline) and older conidium with slightly papillate apices (light brown). F. Older aseptate conidia. Bars: A = 100 µm; B = 50 µm; C-F = 10 µm.

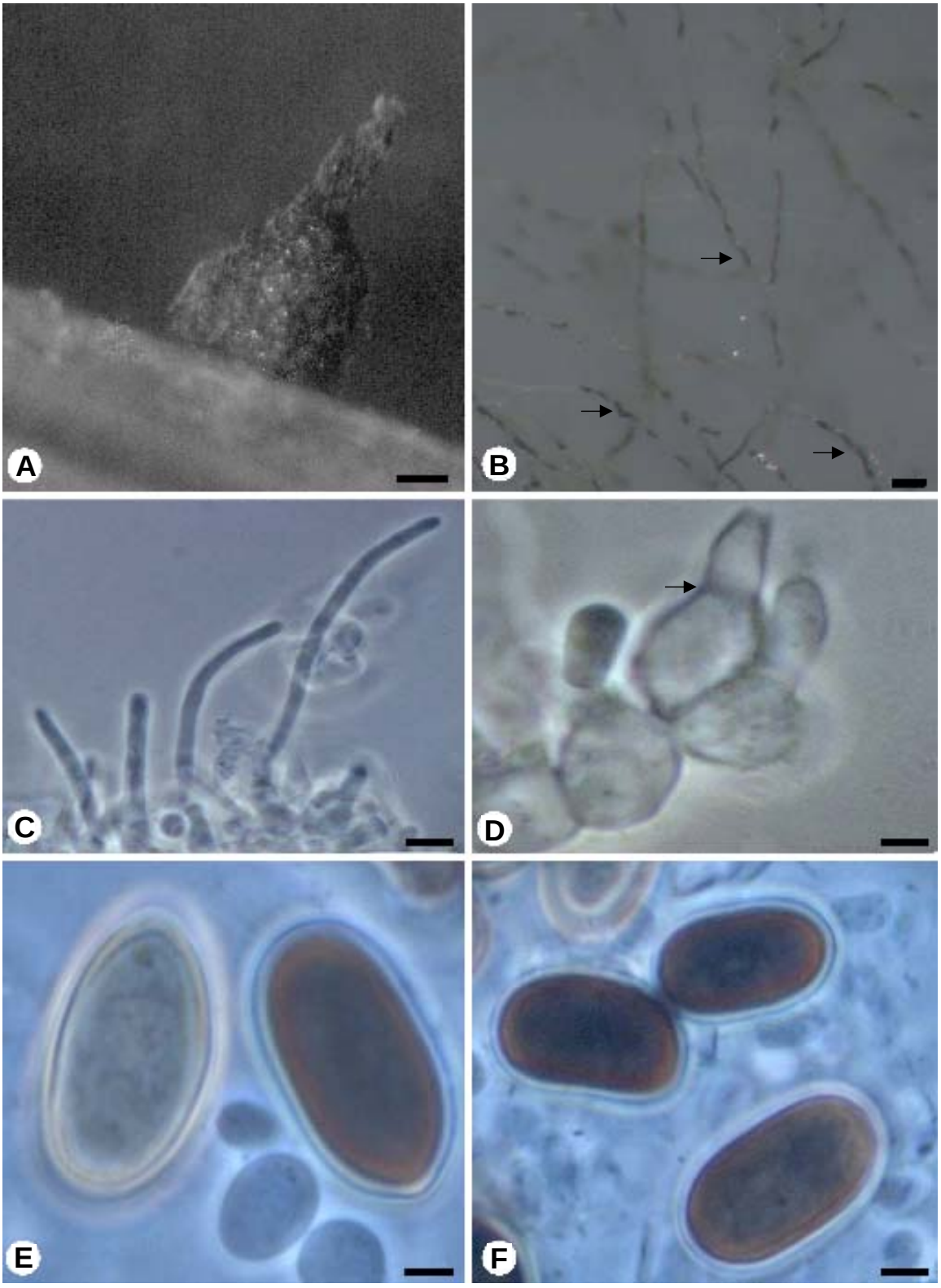

A
B
C
D
E
F

FIGURE 5: *Diplodia variabilis*. A. Pycnidium on pine needles. B. Chlamydospore-like thickenings of the hypae. C-D. Conidiogenous cells with periclinal thickenings (arrow). D. Conidiogenous cell with annelations. E-I. Conidia. Bars: A = 100 µm; B = 50 µm; C = 10 µm; D-I = 5 µm.

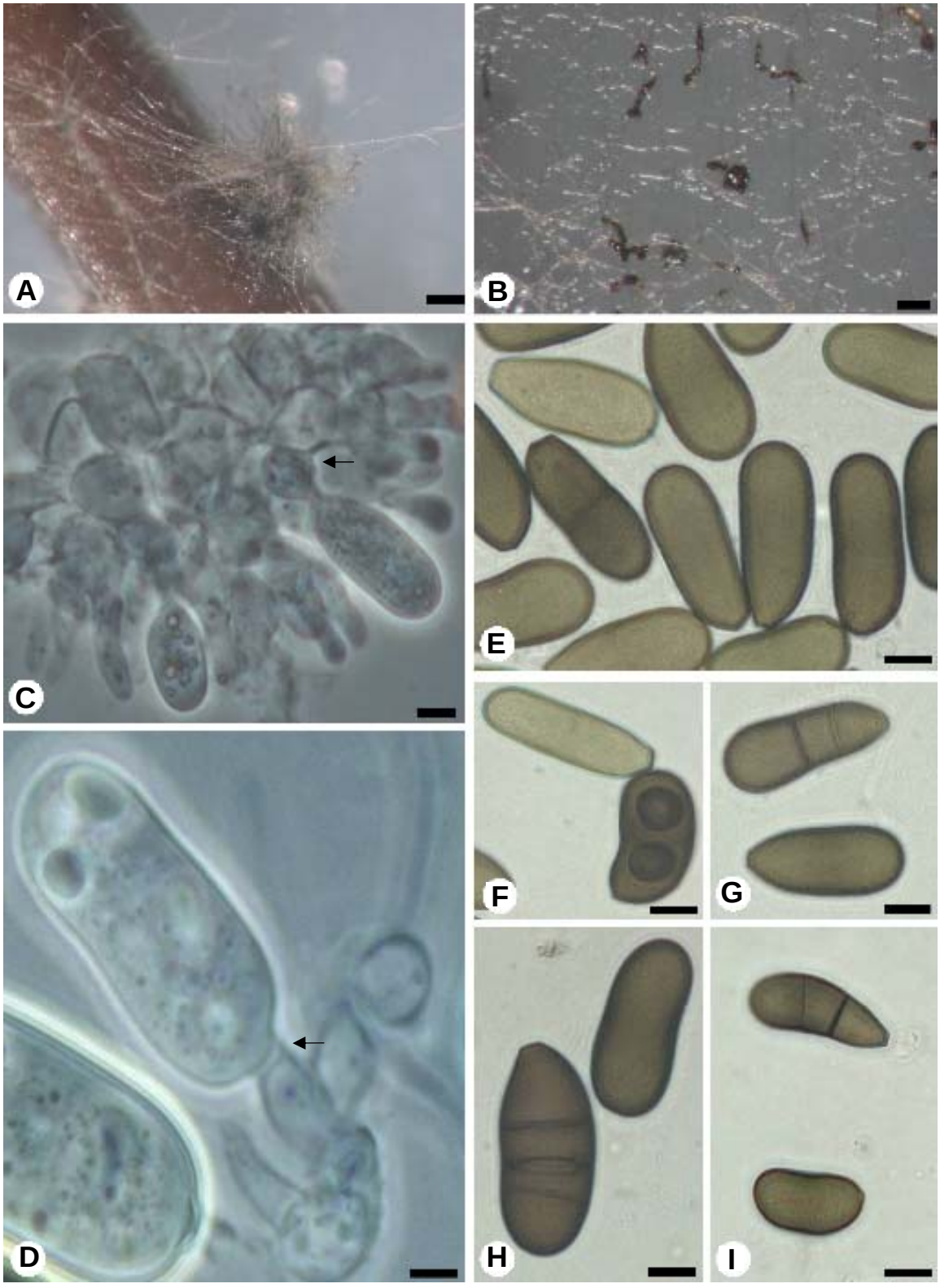

A
B
C
D
E
F
G
H
I

FIGURE 6: *Dothiorella oblonga*. A. Pycnidium on pine needles. B. Chlamydospore-like thickenings of the hypae (arrow). C-D. Conidiogenous cells with periclinal thickenings and annelations (arrows). E-F. Conidia. Bars: A = 100 µm; B = 50 µm; C, E = 10 µm; D, F = 5 µm.

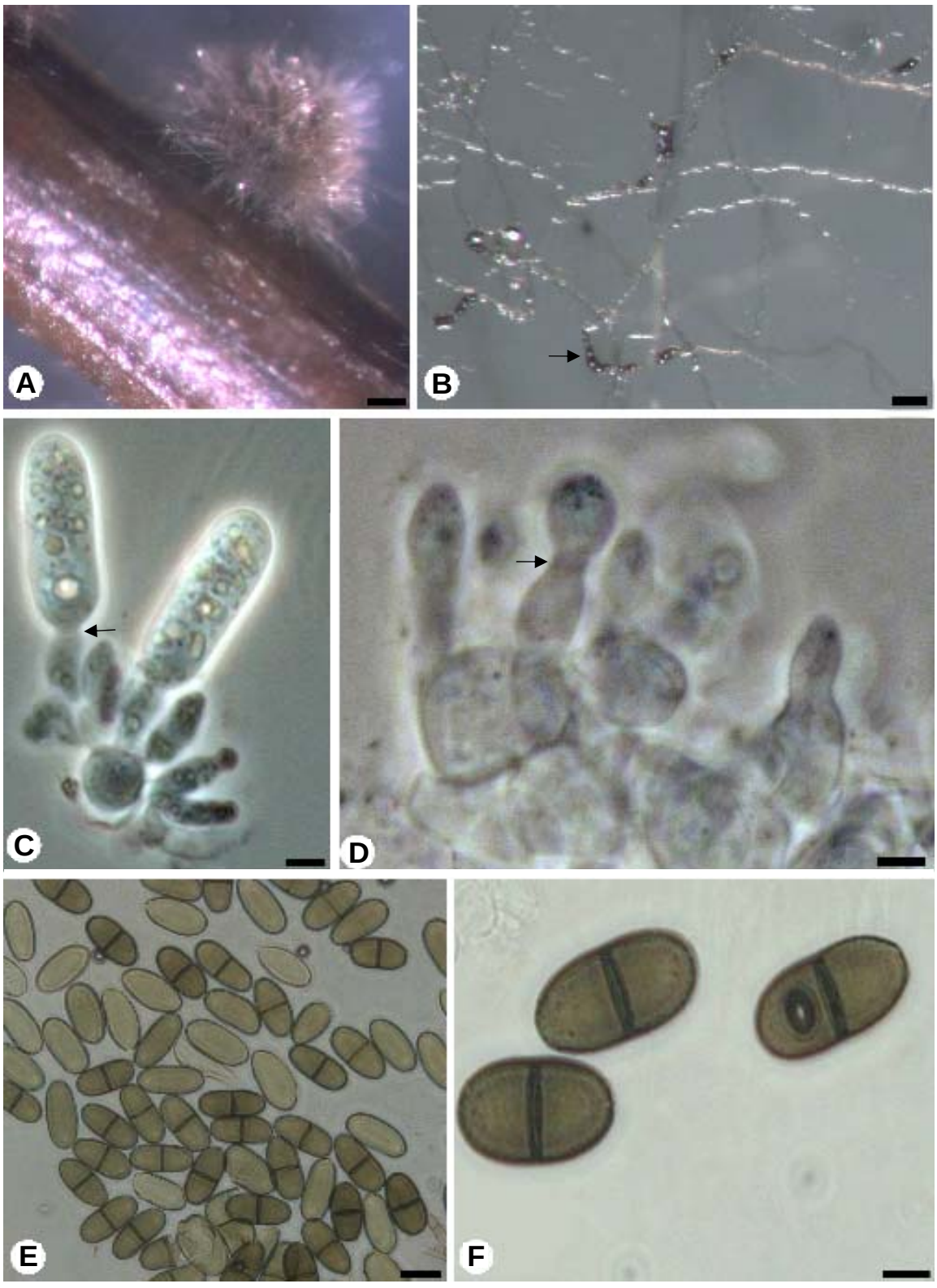

A
B
C
D
E
F

FIGURE 7: *Neofusicoccum* sp. A. Pycnidia in botryose clusters on pine needles. B. Conidiogenous cells (arrow). C. Conidia. Bars: A = 100 µm; B = 10 µm; C = 5 µm; D-G. *Fusicoccum avasmontanum*. D. Pynidium on wateragar. E-F. Conidiogenous cells (arrows). G. Conidia. Bars: D = 100 µm; E-G = 5 µm.

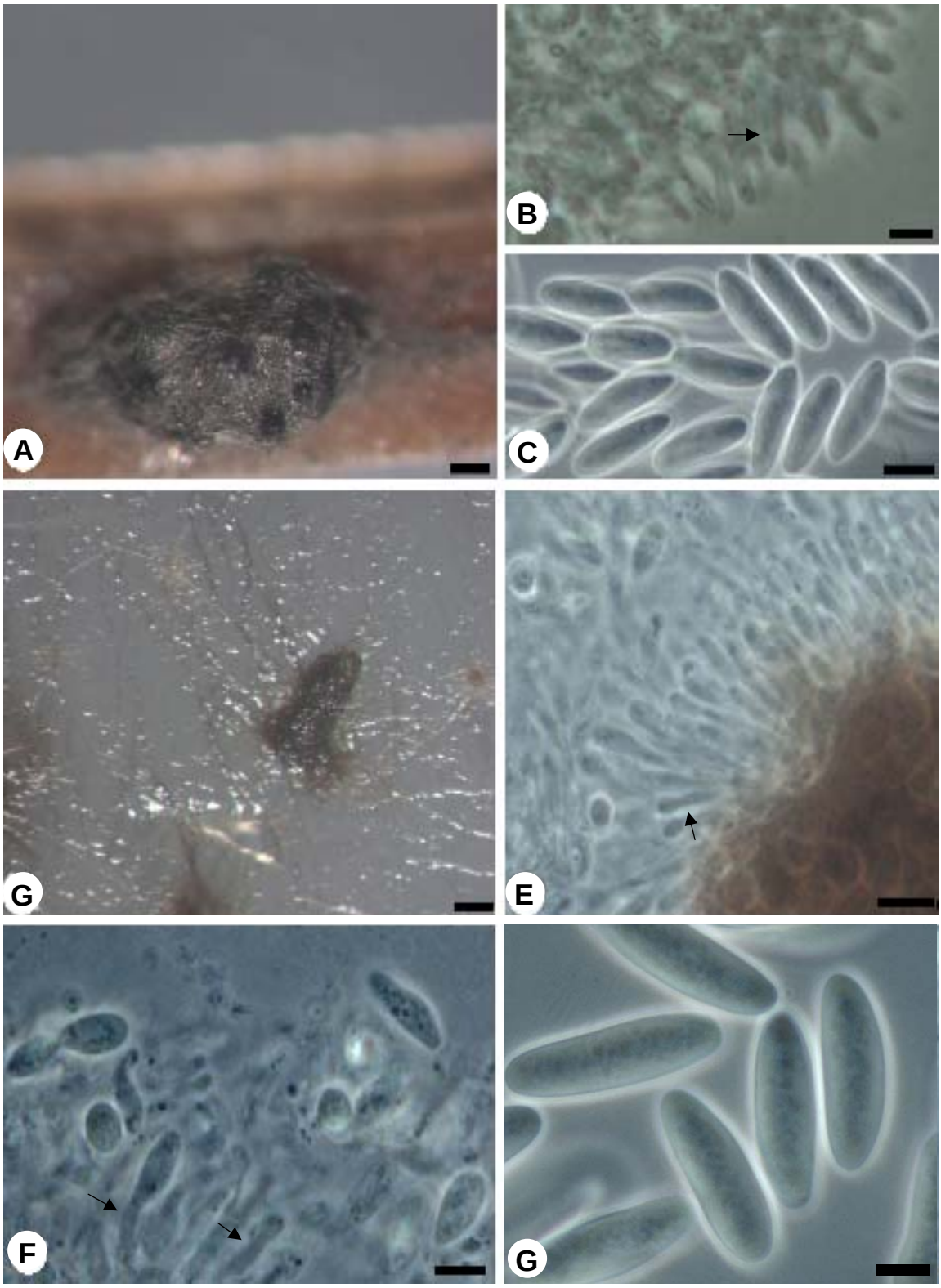

A
B
C
G
E
F
G

FIGURE 8: *Lasiodiplodia pyriformis*. A. Pycnidia on pine needles. B. Paraphysis (arrows). C. Conidium attached to a conidiogenous cell (arrow). D-F. Conidia. D. Younger conidia (hyaline) and older aseptate conidia (dark brown). F. Characteristic pyriform conidia. Bars: A = 100 µm; B, E, F = 10 µm; C, D = 5 µm.

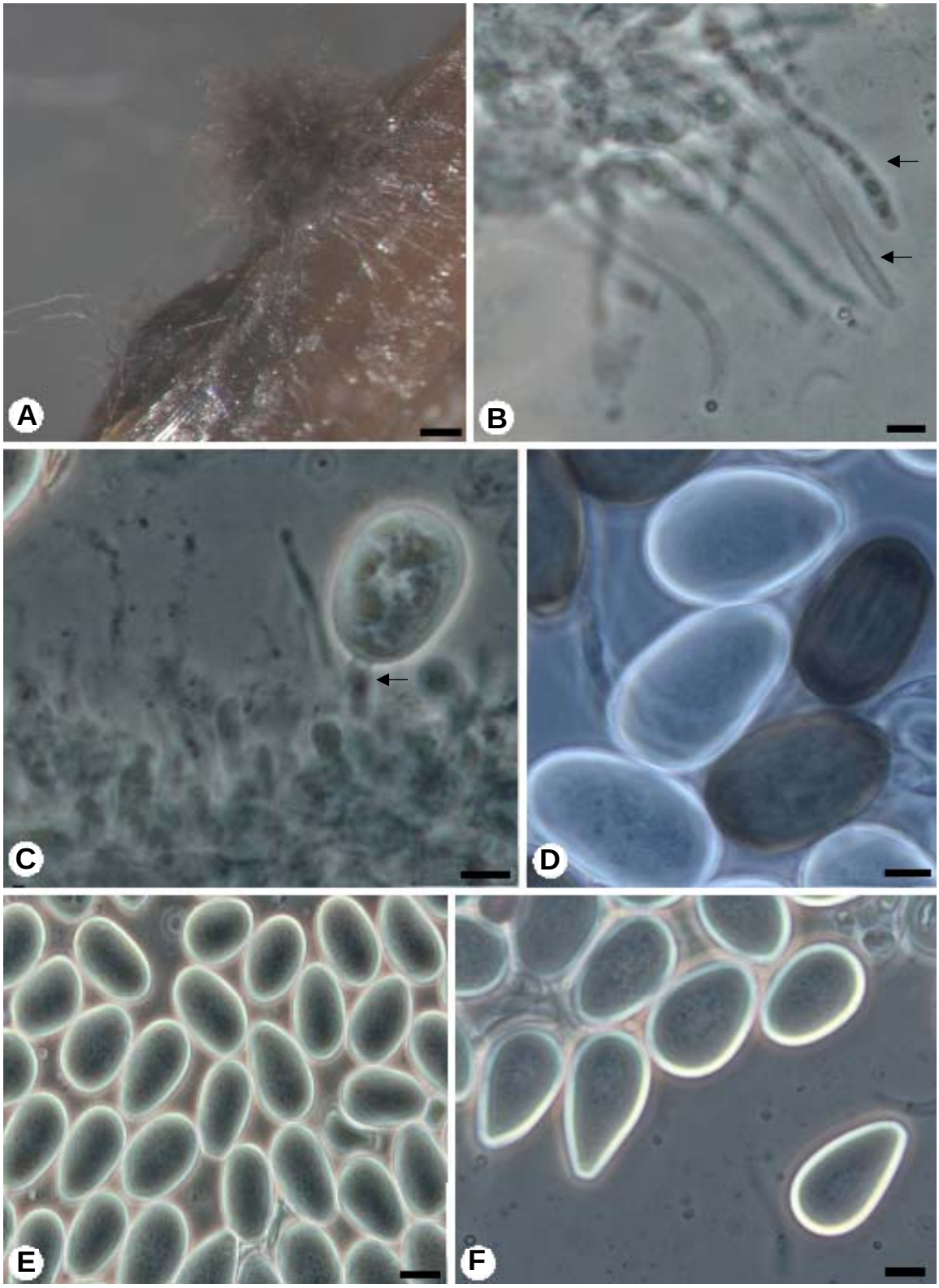

A
B
C
D
E
F

FIGURE 9: *Spencermartinsia rosulata*. A. Rosette-like culture morphology. B. Pycnidia on pine needles. C. Chlamydospore-like thickenings of the hypae (arrows). D. Conidia attached to conidiogenous cells (arrow). E. Conidiogenous cells with periclinal thickenings and annelations (arrow). F-G. Conidia. G. Guttalate conidia. Bars: B = 100 µm; C = 50 µm; D, G = 10 µm; E, F = 5 µm.

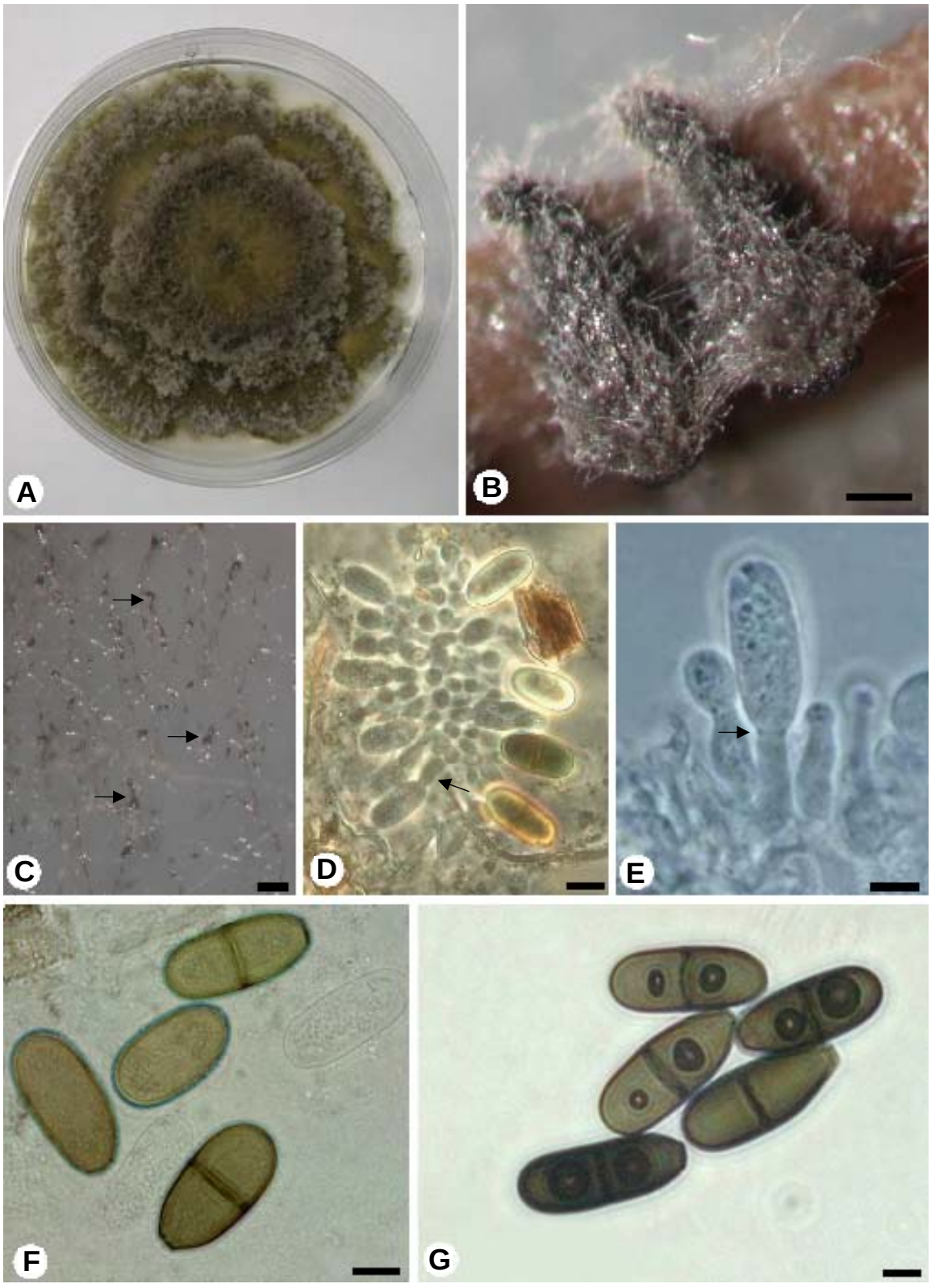
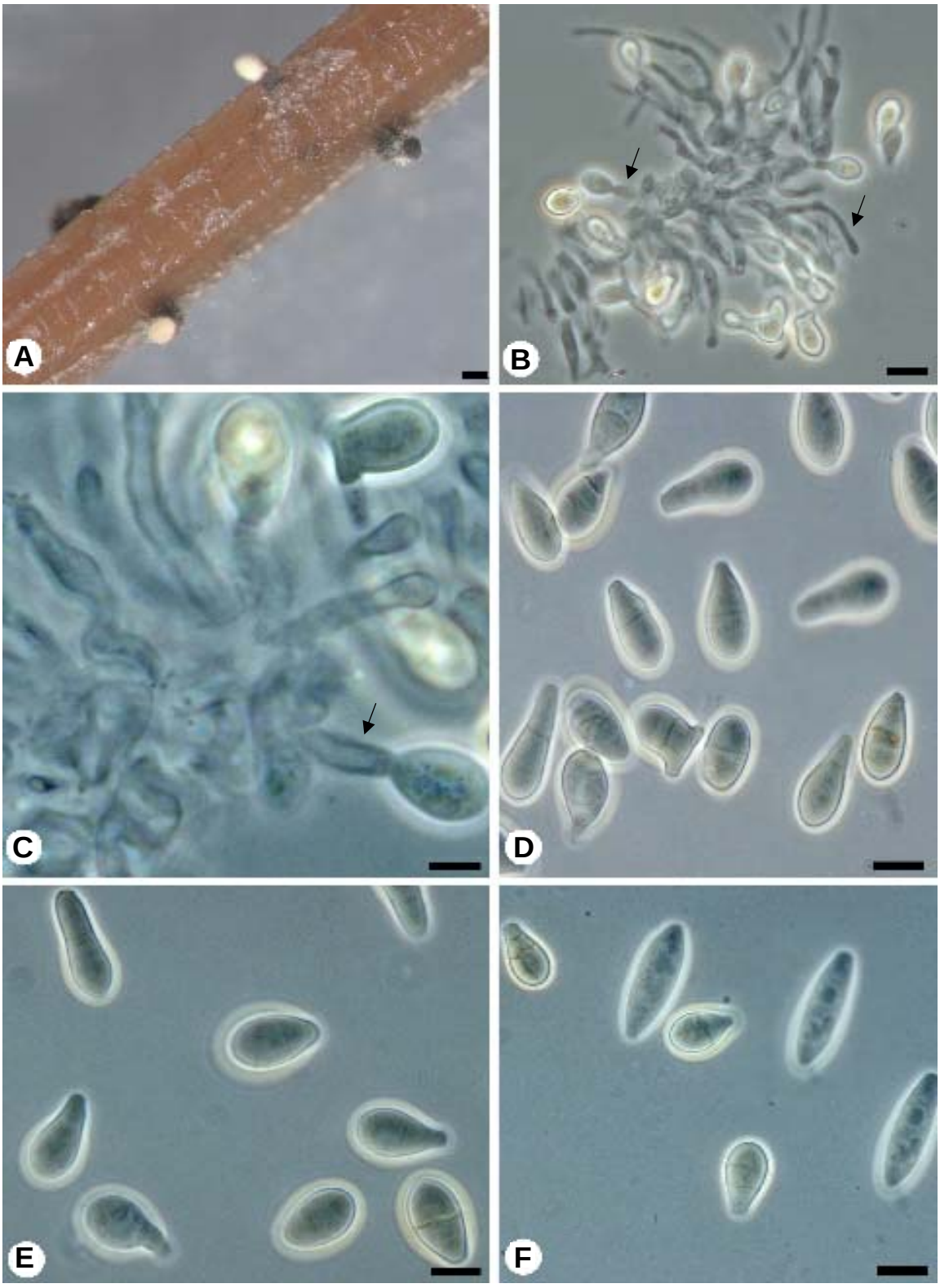

A
B
C
D
E
F
G

FIGURE 10. *Dichomera* synanamorph A. Pycnidia on pine needle oozing black and cream masses of spores. B. Paraphysis and conidiogenous cells (arrows). C. Conidiogenous cell (arrow). D-E. *Dichomera* spores. F. *Dichomera* and *Fusicoccum* spores. Bars: A = 100 µm; B = 10 µm; C-F = 5 µm.


A
B
C
D
E
F

Chapter 3

Botryosphaeriaceae associated with native *Acacia* spp. in the Northern Cape Province, South Africa

ABSTRACT

Acacia spp. are ecologically and economically important trees in southern Africa. A previous study (Chapter 2) has revealed a diverse suite of previously undescribed Botryosphaeriaceae from African *Acacia* spp. in Namibia and South Africa. In the current study, we consider the Botryosphaeriaceae on native *Acacia* spp. from the Northern Cape Province, South Africa and apply morphological and phylogenetic characteristics to identify them. Four species of the Botryosphaeriaceae were identified, including two recently described species, *Diplodia variabilis* and *Spencermartinsia rosulata*, and two previously undescribed species for which the names *Mucodiplodia papillata* prov. nom. and *S. capri-amissi* prov. nom. are provided.

INTRODUCTION

Acacia represents an important genus of native trees in Africa, where at least 144 species have been recorded (Maslin *et al*., 2003). Although these trees are widely distributed and used for many purposes, there remains a substantial lack of knowledge regarding the fungi associated with them in their natural habitats. This is perhaps not surprising given the fact that fungi in general have been poorly studied and collected on the African continent (Crous *et al*., 2006b) and it is envisaged that many new species will be described from this geographic region in future.

In a recent study, a number of fungi belonging to the Botryosphaeriaceae were described on African *Acacia* spp. from Namibia and South Africa (Chapter 2). This study included a previously undescribed genus and six new species. Together with other recent studies (Damn *et al*., 2007b; Pavlic *et al*., 2007), it is evident that the true diversity of this group of fungi has been underestimated in the past.

Members of the Botryosphaeriaceae are well known plant pathogens that sometimes have a wide host and geographical range (Denman *et al*., 2000; Slippers and Wingfield, 2007). They are also well known to have extended endophytic life cycles, existing in a dormant state within healthy plant tissues (Slippers and Wingfield, 2007). In many woody plant communities these fungi occur ubiquitously in the healthy tissue and sporulate on dead or dying tissues. Disease expression normally follows stress conditions (Slippers and Wingfield, 2007).

The threat of diseases caused by the Botryosphaeriaceae is most likely enhanced when these fungi are introduced into new areas on introduced plants and then jump to infect new hosts

(Slippers *et al*., 2005). Because of these concerns, and given predictions of widespread stress on plant communities in South Africa due to climate change (Slippers and Wingfield, 2007), enhanced knowledge of the Botryosphaeriaceae on important native tree species would be valuable.

Despite the importance of many species within the Botryosphaeriaceae as pathogens, knowledge about the true diversity and taxonomy of species in this family is limited in native plant communities. The Northern Cape Province of South Africa has a wide diversity of *Acacia* spp. with only one study done on the fungal biodiversity (Van der Walt *et al*., 2007). In that study, a number of fungi, resembling the morphological characteristics of the Botryosphaeriaceae, were isolated from native *Acacia* spp. in the Northern Cape Province. The aim of the current study was to identify the isolates from Van der Walt *et al*. (2007) based on morphology and comparisons of DNA sequence data.

MATERIALS AND METHODS

Isolates

Isolates were obtained from a preliminary survey conducted in 2005 in the Prieska area of South Africa. The area surveyed (Libertas farm) is situated on the south bank of the Orange River at the foot of the Doringberg, Northern Cape Province, South Africa. Samples were taken mainly from healthy tissue as the study formed part of a biodiversity study considering the mycoflora of the trees and other plants in the area (Van der Walt *et al*., 2007). Cultures were plated onto 2 % (w/v) malt extract agar (MEA) (Biolab, Midrand, South Africa), supplemented with Streptomycin

Sulphate (0.001 g vol^{-1}, SIGMA, Steinheim, Germany), and incubated at 25 °C. Cultures were initially grouped based on mycelial characteristics.

DNA isolation and PCR

A modification of the phenol:chloroform DNA extraction method of Raeder and Broda (1985) was used (also see chapter 2). Primers ITS1 and ITS4 (White *et al*., 1990) were used to amplify the internal transcribed spacer (ITS1 and ITS2) gene regions and the complete 5.8S rRNA gene of the ribosomal RNA (rRNA) operon. The elongation factor 1-α (EF1-α) gene region, from the last part of the second exon to the first part of the last exon, was amplified with the primers EF1F and EF2R (Jacobs *et al*., 2004). Primers ITS1 and LR5 (Vilgalys and Hester, 1990) were used to amplify part of the nuclear rRNA operon for a selection of isolates that were compared to the dataset of Crous *et al*. (2006a). PCR reaction mixtures, conditions and visualization of products were the same for all three sets of primers as were previously described (Chapter 2).

Sequencing and Phylogenetic analyses

PCR amplicons were cleaned, sequenced and analyzed using the same primers used to amplify the PCR products. Sequences from this study were compared to DNA sequences in GenBank using the BLAST search function. Based on the results of the BLAST searches, datasets were drawn up using sequences of published species most closely related to the isolates obtained from the Northern Cape. Sequences obtained from chapter 2 were also included to represent the diversity of Botryosphaeriaceae on native *Acacia* spp. in other areas.

Sequences for all gene regions were aligned using the online version of MAFFT (Katoh *et al*., 2002) version 5.8 (http://timpani.genome.ad.jp/~mafft/server/) and manually adjusted. Data for each gene region was analyzed separately and in a single dataset after testing the congruence of the ITS and EF1-α gene regions, using a partition homogeneity test in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2002). All trees were rooted to *Cercospora apii* Fresen. and *C. beticola* Sacc. A bootstrap analysis was done in PAUP, as well as Bayesian analyses using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), (see chapter 2), to calculate support values for the branch nodes. The first 6000 trees were discarded as the burnin period and evolutionary models for the datasets were the same as in chapter 2.

Morphological and Growth characterization

Sporulation of the isolates (TABLE I) collected from *Acacia* spp. in the Northern Cape was induced on sterilized pine needles on 2 % water agar (WA) at 25 °C under UV light. Fruiting bodies and conidia were examined by placing structures on glass slides in 75 % lactic acid. Analysis of the fungal structures and the capture of images were achieved as described in chapter 2. Not all isolates sporulated *in vitro*, but at least one isolate of each species produced fruiting structures. A minimum of fifty measurements was made per taxonomically informative character for each species. The minimum, maximum, standard deviation (SD), mean values and the length/width (l/w) ratios were calculated for each species and colours were applied using the color charts of Rayner (1970). Colony growth rates were determined as described previously (Chapter 2), except that colony diameters were measured after four days. Growth rates were calculated for all the isolates that were used for the phylogenetic analysis and morphological characterization (TABLE I).

RESULTS

Isolates

A total of 22 cultures resembling members of the Botryosphaeriaceae were obtained from *A. karoo* Hayne, *A. mellifera*, *A. tortilis* (Forks.) Hayne and *A. erioloba* E. May from the study by Van der Walt *et al*. (2007). Eight groups could be distinguished based on mycelial characteristics. Fifteen of the cultures resembled the rosette shaped cultures of *Spencermartinsia rosulata* F.J.J. van der Walt, Slippers & G.J. Marais, a fungus described from Namibia and Pretoria (see chapter 2). The other seven groups had mycelium that was more effuse on agar in Petri dishes, with no clear distinction based on colour or texture and they could not tentatively be assigned to any previously identified group.

Sequencing and Phylogenetic analyses

All 22 isolates were sequenced and Blast searches were done in GenBank that identified four possible species within the Botryosphaeriaceae (TABLE I). A partition homogeneity test was done on the ITS and EF1-α datasets and produced a P-value of 0.01. The combined datasets of the ITS and EF1-α gene regions contained 76 taxa and 966 characters. Six characters were parsimony-uninformative, 654 were parsimony-informative and 306 were constant. Heuristic searches in PAUP yielded 16 parsimonious trees. The overall topology of the trees generated from the individual ITS and EF1-α data sets were the same. As a result the most-parsimonious tree from the combined dataset was chosen for representation (FIG. 1) [Consistency Index (CI) = 0.66, Homoplasy Index (HI) = 0.34, Retention Index (RI) = 0.92 and Rescaled Consistency (RC) =

0.58]. The phylogenetic analysis resolved eight genera, and isolates from *Acacia* in southern Africa grouped into twelve distinct clades (1–12) (FIG. 1) for which the distribution was mapped (FIG. 2). Phylogenetic inference of the 22 isolates identified four taxa that resided in clades 1, 3, 10 and 12 (FIG. 1).

Spencermartinsia included three clades, corresponding to *S. rosulata* (clade 1), *S. viticola* (clade 2) and one unidentified taxon (clade 3) (FIG. 1). Two of these three clades included isolates obtained in this study. These are clade 1, representing *S. rosulata*, which was described from Namibia and Pretoria (Chapter 2), and clade 3, which appear to represent a new taxon based on its unique sequences, reflected in the very strong bootstrap and posterior probability values (100 % and 1.00 respectively). Sequences from clade 3 were compared to the dataset from Phillips *et al*. (2008) and it was clearly shown to be unrelated to an unidentified sister clade containing two sequences, ICMP16819 and ICMP16824. These comparisons are not reflected in this chapter but are further discussed in more detail in chapter 4.

Isolates from this study also grouped with those of *Diplodia variabilis* (clade 10) that was also recently described from Namibia (Chapter 2). The remaining isolates from this study were related to the newly described monophyletic genus in chapter 2 nl. *Mucodiplodia*. The isolates, however, resided in a distinct clade (11), separated from *M. africana* (clade 12) by a significant sequence divergence as well as strong bootstrap and posterior probability values (100 % and 0.95 respectively).

Morphological and Growth characterization

Most isolates included in this study sporulated in culture on pine needles. Descriptions were based on the anamorph states as no teleomorph structures were observed in culture and details of the morphological characteristics are provided in the descriptions of species below.

TAXONOMY

Based on phylogenetic inference, it can be concluded that there are two previously undescribed species of Botryosphaeriaceae amongst the isolates collected from *Acacia* spp. in the Northern Cape Province of South Africa. Descriptions of these species are given below, as well as additional notes on the two previously described species also encountered in this study.

Mucodiplodia papillata F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 3

Conidiomata pycnidialia, globosa, subglobosa vel sphaerica, 894 µm diametro, parce mycelio brevi tecta, subimmersa, immersa vel superficialia. Paraphyses hyalinae, filiformes (18.2−) 23.4−36.7 × (1.2−)1.8−2.8 µm. Cellulae conidiogenae hyalinae, holoblasticae, cylindricae vel ampulliformes (5.2−)5.3−9.4(−11.6) × (1.3−)1.4−2.5(−3.3) µm. Conidia primo hyalina, postea rubro-brunnescentes, ellipsoidea vel late ellipsoidea, interdum reniformia, ovoidea vel medio constricta, parietibus modice crassis, contentis granulariis, laevia, apicibus saepe manifeste papillatis, vagina persistenti mucosa (14.3−)15.8−18.2(−19.8) × (5.9−)6.5−8.8(−11.5) µm.

Conidiomata pycnidial, globose or spherical to subglobose, separate, covered in small amounts of short hyphae, up to 894 µm in diameter, mostly semi-immersed or immersed and occasionally superficial. *Paraphysis* hyaline, filiform, (18.2−)23.4−36.7 × (1.2−)1.8−2.8 µm (average: 30.1 × 2.2 µm). *Conidiogenous cells* hyaline, holoblastic, cylindrical to ampilliform, proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently to form one or two annelations, (5.2−)5.3−9.4(−11.6) × (1.3−)1.4−2.5(−3.3) µm (average: 7.4 × 2 µm). *Conidia* initially hyaline becoming blackish brown, ellipsoidal to broadly ellipsoidal, occasionally reniform, ovoid or constricted in the middle, moderately thick walled, granular content, smooth, frequently with prominent pappilate apices, encased in a persistent mucous sheath, (14.3−)15.8−18.2(−19.8) × (5.9−)6.5−8.8(−11.5) µm (average of 50 conidia: 17 × 7.6, l/w ratio: 2.2).

Culture characteristics: mycelium effuse, dark greenish olive (23"m) with an orange-rufous (11i) concentric zone near the edge of the colony, occasionally with a white mycelium. Reverse buffy brown (17'"i) with blackish brown (9""m) concentric zones and thread-like growth in the center. Occasionally a thickening of the hyphae, chlamydospore-like, immersed in the water agar, blackish brown. *Temperatures for growth*: growth at minimum 10 °C, growth rate of 69.7 mm after 4 days at an optimal temperature 25 °C, growth at maximum 35 °C.

Etymology: Name refers to the prominent papillation of the conidial apices.

Hosts: *Acacia erioloba*, *A. tortilis*.

Distribution: Prieska, Northern Cape (South Africa).

Notes: This is the second species to be described in *Mucodiplodia*. Conidial sizes of the two species overlap, but there are differences in conidial colour and shape that distinguish the two species morphologically. Conidia of *M. africana* are hyaline at first, turning honey to brown with age, while those of *M. papillata* turn blackish brown with age. Furthermore, conidia of *M.*

papillata may be constricted at the middle and can have very prominent papillate apices compared to those of *M. africana*.

Specimens examined: SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, HOLOTYPE, herb. PREM59643, culture ex-type CMW25427, CBS121780, CAMS001482; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, PARATYPE, herb. PREM59644, culture ex-paratype CMW25428, CBS121781, CAMS001483; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, PARATYPE, herb. PREM59645, culture ex-paratype CMW25429, CBS121782, CAMS001484.

Spencermartinsia capri-amissi F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 4

Conidiomata pycnidialia, singula vel in fasciculis botryosis aggregata, mycelio tecta, sphaerica vel globosa, 370 µm diametro, superficialia vel immersa. Cellulae conidiogenae hyalinae, cylindricae, subcylindricae vel late lageniformes, periclinale incrassatae vel rare percurrente proliferantes, annulationes singulas vel binas formantes (4.8−)5.1−8.8(−10.6) × (2.6−)3.1−4.4(−5.3) µm. Conidia 1-septata, rare non septata, basi plerumque truncata, in septis constricta, aut in basi septi incrassata et guttulata, interdum fusiformes vel reniformes (21.8−)23.1−26(−26.8) × (6.8−)8.7−10.7(−12.06) µm.

Conidiomata pycnidial, separate or occasionally aggregated into botryose clusters, covered with mycelium, spherical to globose, up to 370 µm in diameter, superficial or immersed. *Ostioles*

single, central, papillate. *Conidiophores* reduced to conidigenous cells. *Conidiogenous cells* hyaline, cylindrical to sub-cylindrical or broad lageniform, (4.8−)5.1−8.8(−10.6) × (2.6−)3.1−4.4(−5.3) µm (average: 7 × 3.8 µm), proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently to form one or two annelations. *Conidia* initially honey colored to brown becoming blackish brown, 1–septate and rarely aseptate, mostly truncated at the base and constricted at the septum or with a thickening at the base of the septum and guttalate, moderately thick-walled, cylindrical to ovoid or broadly ellipsoidal, occasionally fusiform or reniform, (21.8−)23.1−26(−26.8) × (6.8−)8.7−10.7(−12.06) µm (average of 50 conidia: 24.5 × 9.7 µm, l/w ratio: 2.5).

Culture characteristics: mycelium dark grayish brown (5""k) to dark grayish olive (21""k) with occasional smoke grey (21""d) tuft-like growth. Reverse dark olive (21"m) to black (*1) and irregular edges. Thickening of the vegetative hyphae, chlamydospore-like, blackish brown, intercalary, terminal and dictyosporus and found superficial and immersed in water agar. *Temperatures for growth*: growth at minimum 10 °C, growth rate of 41 mm after 4 days at an optimal temperature 25 °C, growth at maximum 30 °C.

Etymology: The name *capri-amissi* means “of the lost goat”, referring to the fact that Prieska, where this fungus was discovered, is a Khoisan word that means “place of the lost goat”.

Host: *Acacia erioloba*.

Distribution: Prieska, Northern Cape (South Africa).

Specimens examined: SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, HOLOTYPE, herb. PREM59626, culture ex-type CMW25404, CBS121878, CAMS001459; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, PARATYPE, herb. PREM59625,

culture ex-paratype CMW25403, CBS121763, CAMS001458; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, CMW25405, CAMS001460.

Diplodia variabilis F.J.J. van der Walt, Slippers & G.J. Marais.

Notes: Two isolates in this study were identified as *D. variabilis*, which was previously described from Namibia (Chapter 2). There were no significant differences in the morphology amongst isolates obtained here and those from the original description. Cultures were, however, slightly different where those from the original description (Chapter 2) were either olivaceous (21"m) to olive buff (21'"d) or white to smoke grey (21""d). Cultures from this study had what resembles a "clear zone" or effuse to very little growth at the centres of the colony with white tuft-like mycelial growth and dark olive-gray (23"""i) to brownish olive (19"m) mycelial growth surrounding the "clear zone", with small white tufts around the pycnidia.

Specimens examined: SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, herb. PREM59639, CMW25422, CBS121776, CAMS001477; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, CMW25423, CAMS001478.

Spencermartinsia rosulata F.J.J. van der Walt, Slippers & G.J. Marais

Notes: Fourteen isolates collected in this study were identified as *S. rosulata*, previously described from Namibia and Pretoria (Chapter 2). This fungus was the most dominant species

isolated in both studies and the results of the present investigation add evidence that it is a common species occurring on native *Acacia* spp. in southern Africa. There were no significant differences in the morphological characteristics of this species, except that isolates in this study had slightly smaller conidiogenous cells and conidial lengths. Conidiogenous cells were (7.6−) 8.6−10.3(−11.4) µm and conidia (16.5−)18.4−20.8(−22) µm in size compared with conidiogenous cells (3.9−)5.2−12(−17) µm and conidia (19−)21−24.2(−26.7) µm in size in the type specimen of this species as previously presented (Chapter 2).

Hosts: *Acacia karoo*, *A. mellifera*, *A. tortillis*.

Distribution: Northern Cape (South Africa).

Specimens examined: SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, herb. PREM59624, CMW25395, CBS121762, CAMS001450; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, CMW25394, CAMS001449; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, CMW25396, CAMS 001451; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, CMW25397, CAMS001452; SOUTH AFRICA, NORTHERN CAPE: Prieska, fruiting structures induced on needles of *Pinus* sp. on WA, Feb. 2005, *F.J.J. van der Walt* and *G.J. Marais*, CMW25398, CAMS001453.

DISCUSSION

Based on morphological characterization and phylogenetic inference, four species belonging to the Botryosphaeriaceae were found on native *Acacia* spp. in the Northern Cape Province of South Africa. These included two previously undescribed species for which the names *Mucodiplodia papillata* and *Spencermartinsia capri-amissi* have been provided. In addition, isolates of the previously described *Diplodia variabilis* and *S. rosulata* (Chapter 2) were encountered.

A lower diversity of the Botryosphaeriaceae was found in the current study compared to a recent study on native *Acacia* spp. in Namibia and Pretoria in South Africa (Chapter 2). Areas sampled from both these studies represent basically four major sites nl. northern Namibia (Grootfontein and Rundu), central Namibia (Windhoek and Dordabis), northern South Africa (Pretoria) and western South Africa (Northern Cape Province) (FIG. 2). By comparing these results, a total of six species of the Botryosphaeriaceae were found in northern Namibia and seven species in central Namibia, compared to the other two sites where only 3–4 species were found per site (FIG. 2). Sample sizes ranged from 10–35 trees per site (FIG. 2) which is relatively large but yet larger sample sizes may reveal additional new species.

Generally, a high diversity of the Botryosphaeriaceae was encountered at all four sites where native *Acacia* spp. have been sampled (FIG. 2). Some of the species were found at more than one site nl. *Spencermartinsia rosulata* (4 sites), *Dothiorella oblonga* (2 sites), *Lasiodiplodia pyriformis* (2 sites), *Diplodia variabilis* (2 sites) and *Mucodiplodia africana* (2 sites) (FIG. 2). However, each site yielded at least one or two species that were unique to that site. In total, there are six species nl. *Botryosphaeria dothidea*, *S. viticola*, *S. capri-amissi*, a *Neofusiscoccum* sp.,

Fusicoccum avasmontanum and *M. papillata*, that appears to occur only at specific sites. Whether the distribution of these species is dependant on fungal-host interactions or whether they are restricted based on geographical factors is not known. To realize the full extent of the distribution and host specificity of these fungi, further studies will be required that include more extensive sampling of hosts and geographical areas.

Members of the genus, *Spencermartinsia*, seem to be well represented on native *Acacia* spp. in southern Africa. These include *S. capri-amissi* described in this study, in addition to *S. rosulata* (Chapter 2) that were also recently described from this niche. *Spencermartinsia rosulata* appears to be the dominant species of the Botryosphaeriaceae on native *Acacia* spp. in southern Africa (FIG. 2). This fungus was obtained from both healthy and diseased material from Namibia and is the only species of the Botryosphaeriaceae that was found in all four sites (FIG. 2). This species might be specific to this host as no other reports exist that indicate its presence on any other native plant species in southern Africa (unpublished data, Slippers and Wingfield).

In a previous study (Chapter 2), *Spencermartinsia rosulata* and *Diplodia variabilis* were dominant in Namibia. The same trend was not observed in Pretoria or in the Northern Cape Province in this study. Here, only two isolates of *D. variabilis* were found in the Northern Cape compared to a total of 25 isolates in Namibia. As was discussed in more detail in chapter 2, trees in Namibia seemed to have been under greater stress compared to trees from the Pretoria area. No *D. variabilis* isolates were obtained from the apparently healthy trees in Pretoria and only two isolates were obtained in the Northern Cape Province that showed very little disease symptoms. Whether this fungus is mainly restricted geographically, or whether it contributes to disease symptoms on *Acacia* spp. is not clear and further work is needed to resolve these issues.

Three isolates from the current study, collected from *A. erioloba* and *A. tortilis*, were identified as a new species belonging to the recently described genus *Mucodiplodia* and the species was provided with the name *M. papillata*. The genus was recently described to accommodate a fungus, *M. africana*, which was isolated from *A. mellifera* in Namibia (Chapter 2). The current study contributes to the expansion of the host and geographic range of the genus *Mucodiplodia*. The identification of this new genus, together with the recently described genus, *Aplosporella* (Damn *et al*., 2007a), further illustrates that there is still much to learn about the true diversity of the Botryosphaeriaceae, especially on native trees in southern Africa. It is likely that similar studies will result in the discovery of more new species and even genera as native plants from southern Africa are sampled to study these interesting fungi.

Recent studies of fungi on native tree species in southern Africa indicated that there is a greater diversity of the Botryosphaeriaceae in this niche than previously anticipated. A number of new species have recently been described as molecular and morphological data were combined to characterize species (Alves *et al*., 2008; Burgess *et al*., 2006; Chapter 2; Damn *et al*., 2007b; Phillips *et al*., 2007). Similarly, a number of new genera had to be established to accommodate species that were atypical to any of the previously known genera (Chapter 2; Crous *et al*., 2006a; Damn *et al*., 2007a). At the higher taxonomic level, changes have also been made (Phillips *et al*., 2008) to accommodate these fungi in a new family (Botryosphaeriaceae) (Crous *et al*., 2006a) and order (Botryopshaeriales) (Schoch *et al*., 2006). Despite these substantial advances in understanding the taxonomy of the Botryosphaeriaceae, this study emphasizes that the taxonomy of this group of fungi is complex and much remains to be learned as new fungal-host interactions are discovered.

Members of the Botryosphaeriaceae are clearly prominent and diverse on *Acacia* spp. in southern Africa. The relevance of these fungi as pathogens and their potential risks to native hosts are still unclear and needs further consideration. Together with climate changes on a global scale, external environmental factors can affect the interactions between hosts and pathogens (Slippers and Wingfield, 2007). It is, therefore, important to understand these factors so that the role of pathogens to native and economically important plants can be realized.

REFERENCES

Alves A, Crous PW, Correia A, Phillips AJL. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity 28:1–13.

Burgess T, Barber PA, Mohali S, Pegg G, de Beer W, Wingfield MJ. 2006. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. Mycologia 98:423−435.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber PZ, Groenewald JZ. 2006a. Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology 55:235–253.

—, Rong IH, Wood A, Lee S, Glen HF, Botha W, Slippers B, De Beer ZW, Wingfield MJ, Hawksworth DL. 2006b. How many species of fungi are there at the tip of Africa? Studies in Mycology 55:13−33.

Damn U, Fourie PH, Crous PW. 2007a. *Aplosporella prunicola*, a novel species of anamorphic Botryosphaeriaceae. Fungal Diversity 27: 35–43.

—, Crous PW, Fourie PH. 2007b. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99:664–680.

Denman S, Crous PW, Taylor JE, Kang J-C, Pascoe I, Wingfield MJ. 2000. An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Studies in Mycology 45:129−140.

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD. 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycological Research 108:411–418.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066.

Maslin BR, Miller JT, Seigler DS. 2003. Overview of the generic status of *Acacia* (Leguminosae: Mimosoideae). Australian Systematic Botany 16:1–18.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ. 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. Plant Pathology 56:624–636.

Phillips AJL, Crous PW, Arthur A. 2007. *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*. Fungal Diversity 25:141–155.

—, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW. 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21: 29–55.

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1:17−20.

Rayner RW. 1970. A mycological colour chart. CMI and British Mycological Society, Kew, Surrey, U.K.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Schoch CL, Schoemaker RA, Seifert KA, Spatafora JW, Crous PW. 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98:1041–1052.

Slippers B, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biodiversity Review 21:75−89.

—, Stenlid L, Wingfield MJ. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends in Ecology and Evolution 20:8.

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

Van der Walt FJJ, Marais GJ, Lübben A. 2007. Fungal biodiversity of indigenous trees and plants in the Northern Cape. South African Journal of Science 103:xiii–xiv.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172:4238–4246.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a sequencing guide to methods and apllcations'. (Eds MA Innis, DH Gelfand, JJ Sninsky, TJ White), pp. 315–322.

TABLE I: Isolates that were included in the study to compare morphological and phylogenetic characteristics (FIG. 1).

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
[1]**STE-U6326**	[2]CBS121167	*Aplosporella prunicola*	*Prunus persica* var. *nucipersica*	U. Damm	Modimolle Limpopo Province, South Africa	*EF564375*		*EF564377*
STE-U6327		*A. prunicola*	*Prunus persica* var. *nucipersica*	U. Damm	Modimolle Limpopo Province, South Africa	*EF564376*		*EF564378*
[3]**CMW8000**		*Botryosphaeria dothidea*	*Prunus* sp.	B. Slippers	Switzerland, Crocifisso	*AY236949*	*AY236898*	
CMW25410	[4]CAMS001465	*B. dothidea*	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	*EU101304*	*EU101349*	
CMW25411	CAMS001466	*B. dothidea*	*A. karoo*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	*EU101305*	*EU101350*	
CBS112549		*Diplodia corticola*	*Quercus suber*	A. Alves	Portugal, Aveiro	*AY259100*	*AY573227*	
CBS112545		*D. corticola*	*Q. suber*	M.E. Sánchez & A. Trapero	Spain, Cádiz	*AY259089*	*AY573226*	
CBS112553		*D. mutila*	*V. vinifera*	A.J.L. Phillips	Portugal, Montemor-o-Novo	*AY259093*	*AY573219*	
CBS431.82		*D. mutila*	*Fraxinus excelsior*	H.A. van der Aa	Netherlands, Maarseveen	*AY236955*	*AY236904*	
CBS110496		*D. porosum*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343379*	*AY343340*	
CBS110574		*D. porosum*	*V. vinifera*	J.M. van Niekerk	South Africa	*AY343378*	*AY343339*	
CBS112555		*D. seriata*	*V. vinifera*	A.J.L. Phillips	Portugal, Montemor-o-Novo	*AY259094*	*AY573220*	
CMW7775		*D. seriata*	*Ribes* sp.	B. Slippers & G. Hudler	USA, New York	*AY236954*	*AY236903*	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CBS121774	CMW25419 CAMS001474	*D. variabilis*	*A. karoo*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	*EU101312*	*EU101357*	
CBS121775	CMW25421 CAMS001476	*D. variabilis*	*A. karoo*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	*EU101314*	*EU101359*	
CBS121776	CMW25422 CAMS001477	*D. variabilis**	*A. mellifera*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101326	EU101371	
CMW25423	CAMS001478	*D. variabilis**	*A. mellifera*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101327	EU101372	
CBS115041		*Dothiorella iberica*	*Quercus ilex*	J. Luque	Spain, Aragon	*AY573202*	*AY573222*	
CBS115040		*Do. iberica*	*Q. ilex*	J. Luque	Spain, Catalonia	*AY573214*	*AY573232*	
CBS121764	CMW25406 CAMS001461	*Do. oblonga*	*A. mellifera*	F.J.J. van der Walt & J. Roux	Rundu, Namibia	*EU101299*	*EU101344*	
CBS121765	CMW25407 CAMS001462	*Do. oblonga*	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	*EU101300*	*EU101345*	
CBS121766	CMW25408 CAMS001463	*Do. oblonga*	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	*EU101301*	*EU101346*	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
[5]**IMI63581b**		*Do. sarmentorum*	*Ulmus* sp.	E.A. Ellis	England, Warwickshire	*AY573212*	*AY573235*	
CBS115038		*Do. sarmentorum*	*Malus pumila*	A.J.L. Phillips	Netherlands, Delft	*AY573206*	*AY573223*	
CBS121769	CMW25413 CAMS001468	*Fusicoccum avasmontanum*	*A. mellifera*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	*EU101303*	*EU101348*	
CMW13488		*Lasiodiplodia crassispora*	*Eucalyptus urophylla*	S. Mohali	Acarigua, Venezuela	*DQ103552.1*	*DQ103559*	
CMW14691	[6]WAC12533	*L. crassispora*	*Syzygium album*	T.I. Burgess & B. Dell	Kununurra, Australia	*DQ103550.1*	*DQ103557*	
CMW14688	WAC12534	*L. crassispora*	*S. album*	T.I. Burgess & B. Dell	Kununurra, Australia	*DQ103551.1*	*DQ103558*	
CBS115812	CMW14077	*L. gonobiensis*	*Syzygium cordatum*	D. Pavlic	Eastern Cape Province, South Africa	*AY639595*	*DQ103566*	
CBS116355	CMW14078	*L. gonobiensis*	*S. cordatum*	D. Pavlic	Eastern Cape Province, South Africa	*AY639594*	*DQ103567*	
CBS121770	CMW25414 CAMS001469	*L. pyriformis*	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	*EU101307*	*EU101352*	
CBS121771	CMW25415 CAMS001470	*L. pyriformis*	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	*EU101308*	*EU101353*	
CBS116459		*L. pseudotheobromae*	*Gmelina arborea*	J. Carranza-Velásquez	Costa Rica	*EF622077*	*EF622057*	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CMW25417	CAMS001472	*L. pseudotheobromae*	*A. mellifera*	F.J.J van der Walt & J. Roux	Rundu, Namibia	*EU101310*	*EU101355*	
CMW25418	CAMS001473	*L. pseudotheobromae*	*A. mellifera*	F.J.J van der Walt & J. Roux	Rundu, Namibia	*EU101311*	*EU101356*	
WAC12538		*L. rubropurpurea*	*Eucalyptus grandis*	T.I. Burgess & G. Pegg	Tully, Queensland	*DQ103555*	*DQ103573*	
WAC12539		*L. rubropurpurea*	*E. grandis*	T.I. Burgess & G. Pegg	Tully, Queensland	*DQ103556*	*DQ103574*	
CBS164.96		*L. theobromae*	Unknown fruit on coral reef coast	A. Aptroot	Papua New Guinea, Madang	*AY640255*	*AY640258*	
CMW9074		*L. theobromae*	*Pinus* sp.	T. Burgess	Mexico	*AY236952*	*AY236901*	
CMW13512	WAC12540	*L. venezuelensis*	*A. mangium*	S. Mohali	Acarigua, Venezuela	*DQ103548*	*DQ103569*	
CMW13513		*L. venezuelensis*	*A. mangium*	S. Mohali	Acarigua, Venezuela	*DQ103549*	*DQ103570*	
CBS121777	CMW25424 CAMS001479	*Mucodiplodia africana*	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	*EU101315*	*EU101360*	*EU101380*
CBS121778	CMW25425 CAMS001480	*M. africana*	*A. mellifera*	F.J.J van der Walt & J. Roux	Grootfontein, Namibia	*EU101316*	*EU 101361*	*EU101381*
CBS121779	CMW25426 CAMS001481	*M. africana*	*A. mellifera*	F.J.J van der Walt & J. Roux	Dordabis, Namibia	*EU101317*	*EU101362*	*EU101382*

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CBS121780	CMW25427 CAMS001482	*M. papillata* *	*A. tortillas*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101328	EU101373	EU101383
CBS121781	CMW25428 CAMS001483	*M. papillata* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101329	EU101374	EU101384
CBS121782	CMW25429 CAMS001484	*M. papillata* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101330	EU101375	EU101385
CBS112872		*Neofusicoccum australe*	*V. vinifera*	F. Halleen	South Africa	*AY343388*	*AY343347*	
CBS112877		*N. australe*	*V. vinifera*	F. Halleen	South Africa	*AY343385*	*AY343346*	
CBS110299		*N. luteum*	*V. vinifera*	A.J.L. Phillips	Portugal, Oeiras	*AY259091*	*AY573217*	
CMW9076		*N. luteum*	*Malus x domestica*	S.R. Pennycook	New Zealand	*AY236946*	*AY236893*	
CMW9081	[7]ICMP8003	*N. parvum*	*Populus nigra*	G.J. Samuels	New Zealand, Te Puke	*AY236943*	*AY236888*	
CBS110301		*N. parvum*	*V. vinifera*	A.J.L. Phillips	Portugal, Palmela	*AY259098*	*AY573221*	
CBS115475		*N. ribis*	*Ribes* sp.	G. Hudler	USA, New York	*AY236935*	*AY236877*	
CMW7773		*N. ribis*	*Ribes* sp.	G. Hudler	USA, New York	*AY236936*	*AY236878*	
CBS112878		*N. viticlavatum*	*V. vinifera*	F. Halleen	South Africa, Stellenbosch	*AY343380*	*AY343342*	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CBS112977		*N. viticlavatum*	*V. vinifera*	F. Halleen	South Africa	*AY343381*	*AY343341*	
CBS110887		*N. vitifusiforme*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343383*	*AY343343*	
CBS110880		*N. vitifusiforme*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343382*	*AY343344*	
CBS121767	CMW25409 CAMS001464	*Neofusicoccum* sp.	*A. mellifera*	F.J.J van der Walt & J. Roux	Windhoek, Namibia	*EU101302*	*EU101347*	
CBS121763	CMW25403 CAMS001458	*Spencermartinsia capri-amissi* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101323	EU101368	
CBS121878	CMW25404 CAMS001459	*S. capri-amissi* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101324	EU101369	
CMW25405	CAMS001460	*S. capri-amissi* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101325	EU101370	
CBS121760	CMW25389 CAMS 001444	*S. rosulata*	*Acacia karoo*	F.J.J. van der Walt & J. Roux	Windhoek, Namibia	EU101290	EU101335	
CBS121761	CMW25392 CAMS001447	*S. rosulata*	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101293	EU101338	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CMW25394	CAMS001449	*S. rosulata* *	*A. karoo*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101318	EU101363	
CBS121762	CMW25395 CAMS001450	*S. rosulata* *	*A. mellifera*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101319	EU101364	
CMW25396	CAMS001451	*S. rosulata* *	*A. mellifera*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101320	EU101365	
CMW25397	CAMS001452	*S. rosulata* *	*A. tortillis*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101321	EU101366	
CMW25398	CAMS001453	*S. rosulata* *	*A. tortillis*	F.J.J. van der Walt & G.J. Marais	Nortern Cape, South Africa	EU101322	EU101367	
CBS117009		*S. viticola*	*Vitis vinifera cv. Garnatxa Negra*	J. Luque & S. Martos	Spain, Vimbodí	AY905554	AY905559	
CBS117008		*S. viticola*	*V. vinifera cv. Xarel·lo*	J. Luque & J. Reyes	Spain, Sant Sadurní d'Anoia	AY905557	AY905560	
CMW25399	CAMS001454	*S. viticola**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101295	EU101340	

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α	LSU
CMW25400	CAMS001455	*S. viticola**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101296	EU101341	
CBS119.25		*Cercospora apii*	*Apium graveolens*	L.J. Klotz	Unknown	*AY179949*	*AY179915*	
[8]CPC12031		*Cercospora beticola*	Unknown	Unknown	Unknown	*DQ233339*	*DQ233365*	

Culture collections: [1]STE-U: Department of Plant Pathology, University of Stellenbosh, South Africa; [2]CBS-Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; [3]CMW- FABI, University of Pretoria, South Africa; [4]CAMS-Center for Applied Mycological Studies, University of Pretoria, South Africa; [5]IMI-CABI Bioscience, Egham, UK; [6]WAC-Department of Agriculture Western Australia Plant Pathogen Collection; [7]ICMP-International Collection of Micro-organisms from Plants, New Zealnd; [8]CPC-Cultue collection of Pedro Crous.

BOLD = Isolate accession numbers in bold signify cultures ex-type, or from samples that have been linked morphologically to the type material

ITALICS = Sequence numbers in italics were retrieved from the GenBank public database or obtained from chapter 2. All others were obtained in this study.

* Isolates used in this study for sequencing and phylogenetic analysis is indicated with an asterisk

FIGURE 1: Most-parsimonious tree obtained through heuristic searches of the combined datasets of the ITS and EF1-α gene regions. Bootstrap values ≥ 70 % are indicated above the line and posterior probabilities ≥ 95 % under the line. The trees are rooted with *Cercospora apii* and *C. beticola*. The anamorphs are represented by the thick solid line. Clades 1–12 indicate all the species isolated from native *Acacia* spp. from southern Africa. Isolates used in this study are indicated by an asterisk (*) with the ex-type cultures indicated in bold.

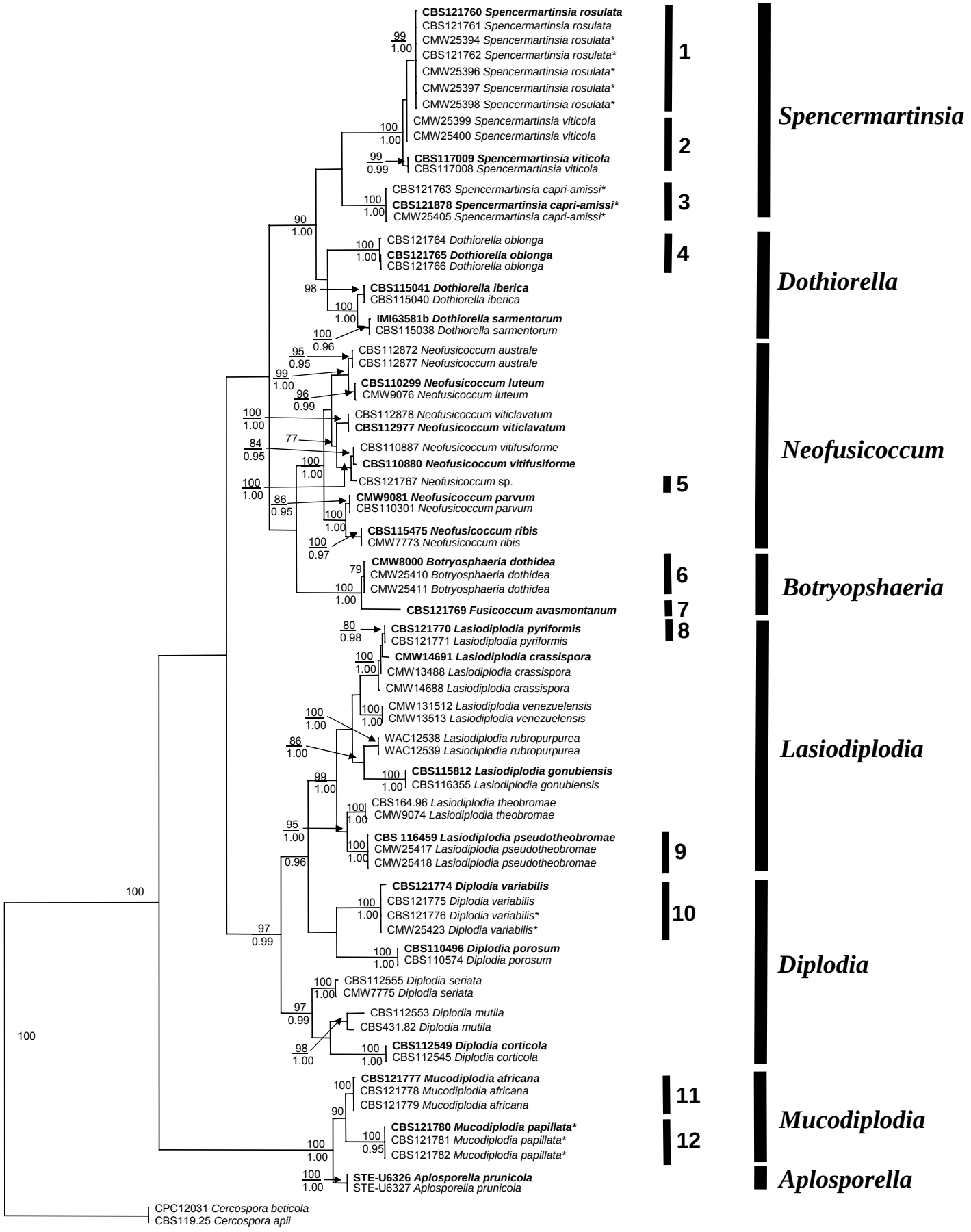
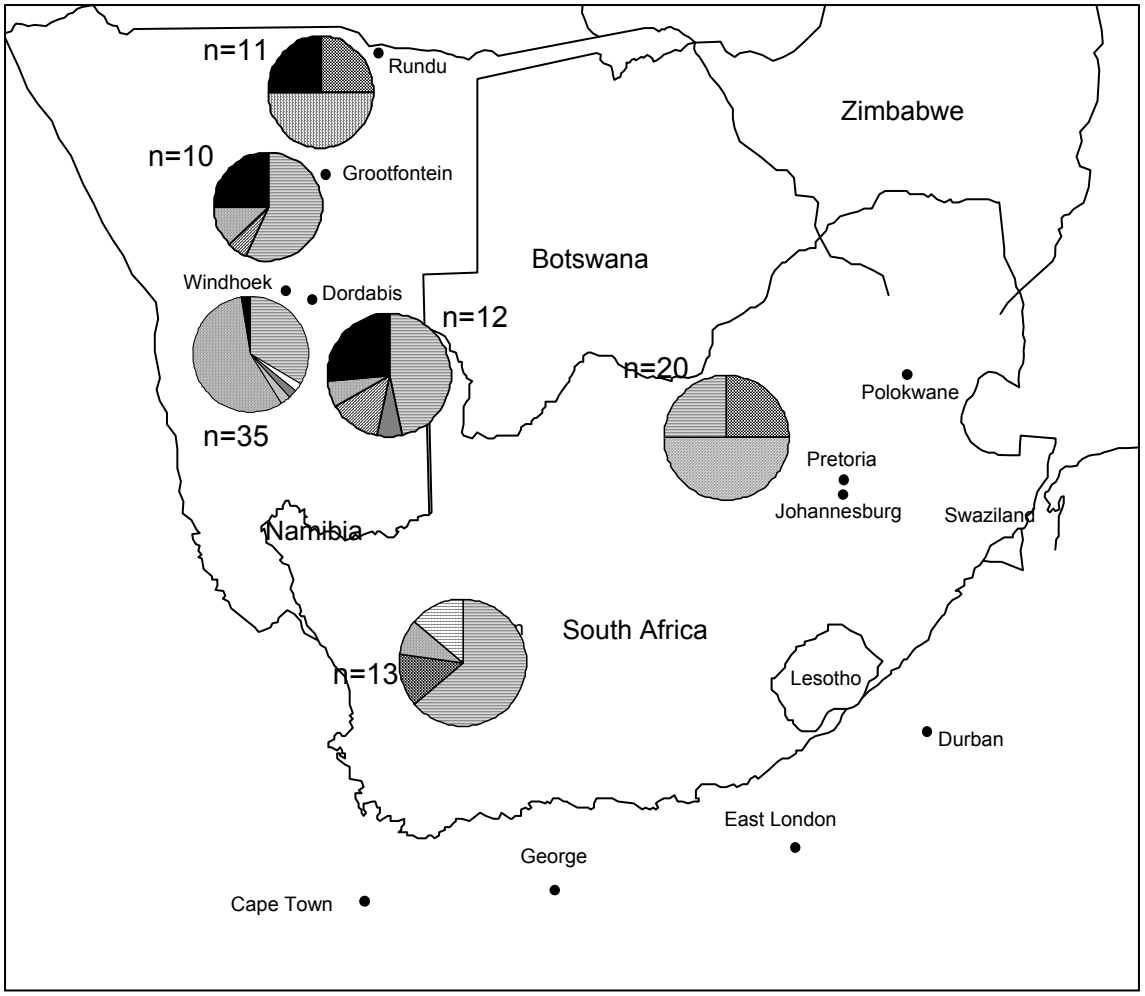

CBS121760 Spencermartinsia rosulata
CBS121761 Spencermartinsia rosulata
CMW25394 Spencermartinsia rosulata*
CBS121762 Spencermartinsia rosulata*
CMW25396 Spencermartinsia rosulata*
CMW25397 Spencermartinsia rosulata*
CMW25398 Spencermartinsia rosulata*
CMW25399 Spencermartinsia viticola
CMW25400 Spencermartinsia viticola
CBS117009 Spencermartinsia viticola
CBS117008 Spencermartinsia viticola
CBS121763 Spencermartinsia capri-amissi*
CBS121878 Spencermartinsia capri-amissi*
CMW25405 Spencermartinsia capri-amissi*
CBS121764 Dothiorella oblonga
CBS121765 Dothiorella oblonga
CBS121766 Dothiorella oblonga
CBS115041 Dothiorella iberica
CBS115040 Dothiorella iberica
IMI63581b Dothiorella sarmentorum
CBS115038 Dothiorella sarmentorum
CBS112872 Neofusicoccum australe
CBS112877 Neofusicoccum australe
CBS110299 Neofusicoccum luteum
CMW9076 Neofusicoccum luteum
CBS112878 Neofusicoccum viticlavatum
CBS112977 Neofusicoccum viticlavatum
CBS110887 Neofusicoccum vitifusiforme
CBS110880 Neofusicoccum vitifusiforme
CBS121767 Neofusicoccum sp.
CMW9081 Neofusicoccum parvum
CBS110301 Neofusicoccum parvum
CBS115475 Neofusicoccum ribis
CMW7773 Neofusicoccum ribis
CMW8000 Botryosphaeria dothidea
CMW25410 Botryosphaeria dothidea
CMW25411 Botryosphaeria dothidea
CBS121769 Fusicoccum avasmontanum
CBS121770 Lasiodiplodia pyriformis
CBS121771 Lasiodiplodia pyriformis
CMW14691 Lasiodiplodia crassispora
CMW13488 Lasiodiplodia crassispora
CMW14688 Lasiodiplodia crassispora
CMW131512 Lasiodiplodia venezuelensis
CMW13513 Lasiodiplodia venezuelensis
WAC12538 Lasiodiplodia rubropurpurea
WAC12539 Lasiodiplodia rubropurpurea
CBS115812 Lasiodiplodia gonubiensis
CBS116355 Lasiodiplodia gonubiensis
CBS164.96 Lasiodiplodia theobromae
CMW9074 Lasiodiplodia theobromae
CBS 116459 Lasiodiplodia pseudotheobromae
CMW25417 Lasiodiplodia pseudotheobromae
CMW25418 Lasiodiplodia pseudotheobromae
CBS121774 Diplodia variabilis
CBS121775 Diplodia variabilis
CBS121776 Diplodia variabilis*
CMW25423 Diplodia variabilis*
CBS110496 Diplodia porosum
CBS110574 Diplodia porosum
CBS112555 Diplodia seriata
CMW7775 Diplodia seriata
CBS112553 Diplodia mutila
CBS431.82 Diplodia mutila
CBS112549 Diplodia corticola
CBS112545 Diplodia corticola
CBS121777 Mucodiplodia africana
CBS121778 Mucodiplodia africana
CBS121779 Mucodiplodia africana
CBS121780 Mucodiplodia papillata*
CBS121781 Mucodiplodia papillata*
CBS121782 Mucodiplodia papillata*
STE-U6326 Aplosporella prunicola
STE-U6327 Aplosporella prunicola
CPC12031 Cercospora beticola
CBS119.25 Cercospora apii
1
2
3
4
5
6
7
8
9
10
11
12
Spencermartinsia
Dothiorella
Neofusicoccum
Botryopshaeria
Lasiodiplodia
Diplodia
Mucodiplodia
Aplosporella
10 changes

FIGURE 2: Distribution map of all the Botryosphaeriaceae species observed on native *Acacia* spp. previously (Chapter 2) and this study (TABLE I). Areas included represent four major sites nl. northern Namibia (Grootfontein and Rundu), central Namibia (Windhoek and Dordabis), northern South Africa (Pretoria) and western South Africa (Northern Cape Province). Sample sizes (n), fungal species, and their distribution are indicated.


n=11
Rundu
n=10
Grootfontein
Windhoek
Dordabis
n=35
n=12
Botswana
Zimbabwe
n=20
Polokwane
Pretoria
Johannesburg
Swaziland
Namibia
South Africa
n=13
Lesotho
Durban
East London
George
Cape Town

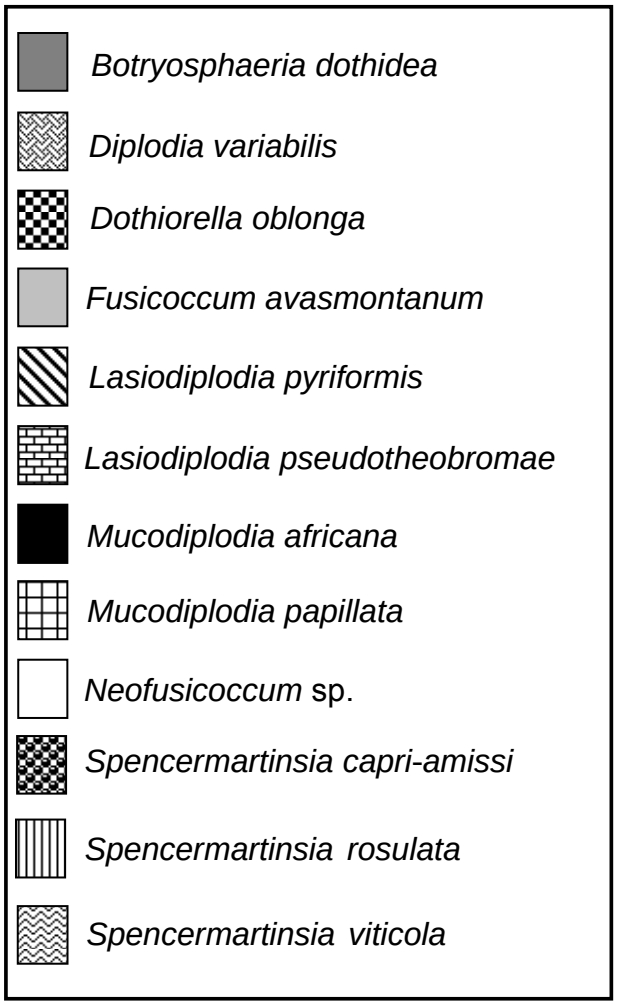

Botryosphaeria dothidea
Diplodia variabilis
Dothiorella oblonga
Fusicoccum avasmontanum
Lasiodiplodia pyriformis
Lasiodiplodia pseudotheobromae
Mucodiplodia africana
Mucodiplodia papillata
Neofusicoccum sp.
Spencermartinsia capri-amissi
Spencermartinsia rosulata
Spencermartinsia viticola

FIGURE 3: *Mucodiplodia papillata*. A. Pynidium on pine needles. B. Chlamydospore-like thickenings of the hypae (arrows). C. Paraphysis (arrow). D. Conidiogenous cells with periclinal thickening and annelations (arrow). E–G. Conidia encased in a persistent mucous sheath. E. Older conidia (reddish brown). F–G. Younger conium (hyaline) with prominent papillate apices and older conidium (reddish brown) (arrows). Bars: A = 100 µm; B =50 µm; C-F =5 µm; G =10 µm.

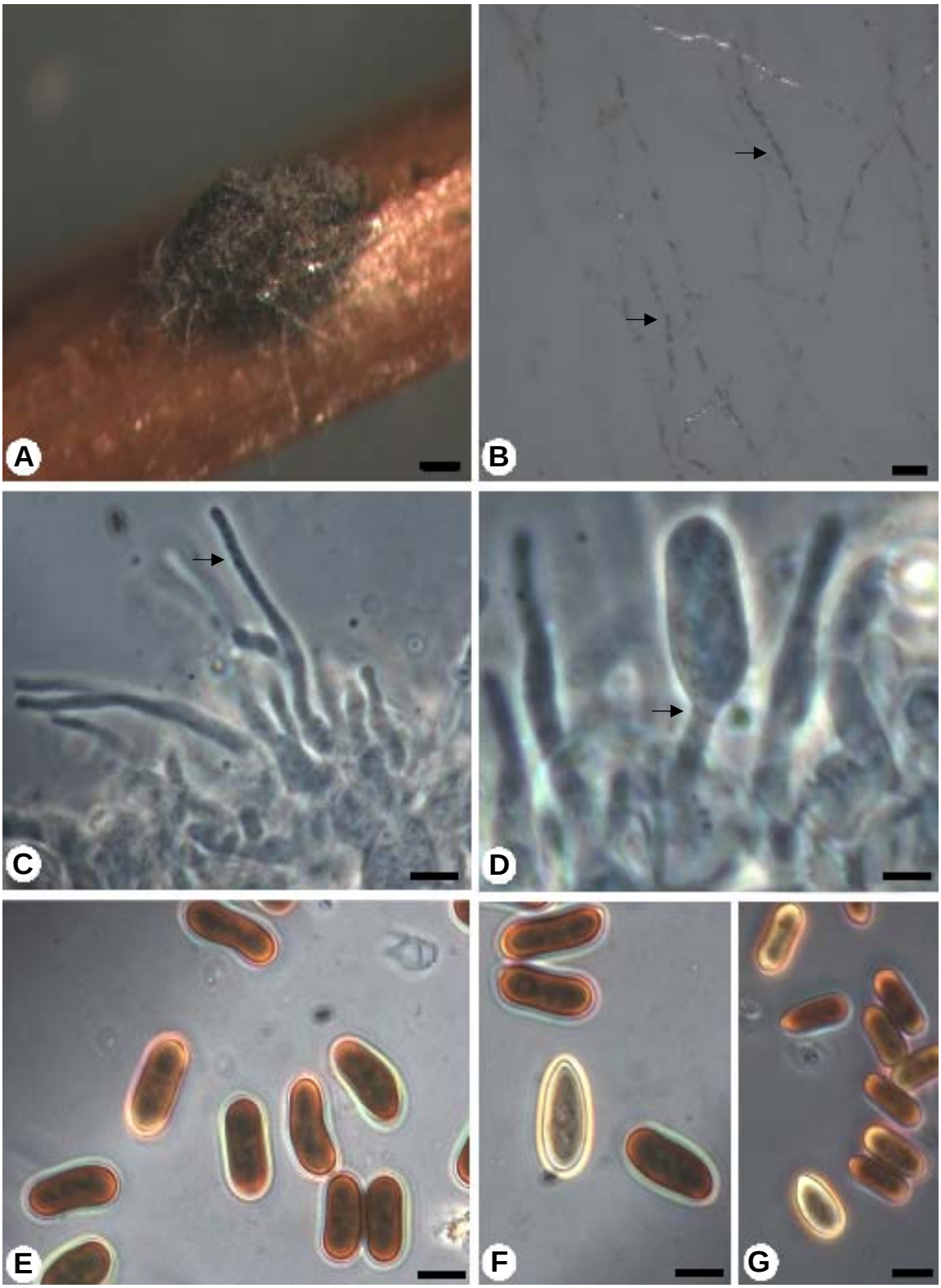
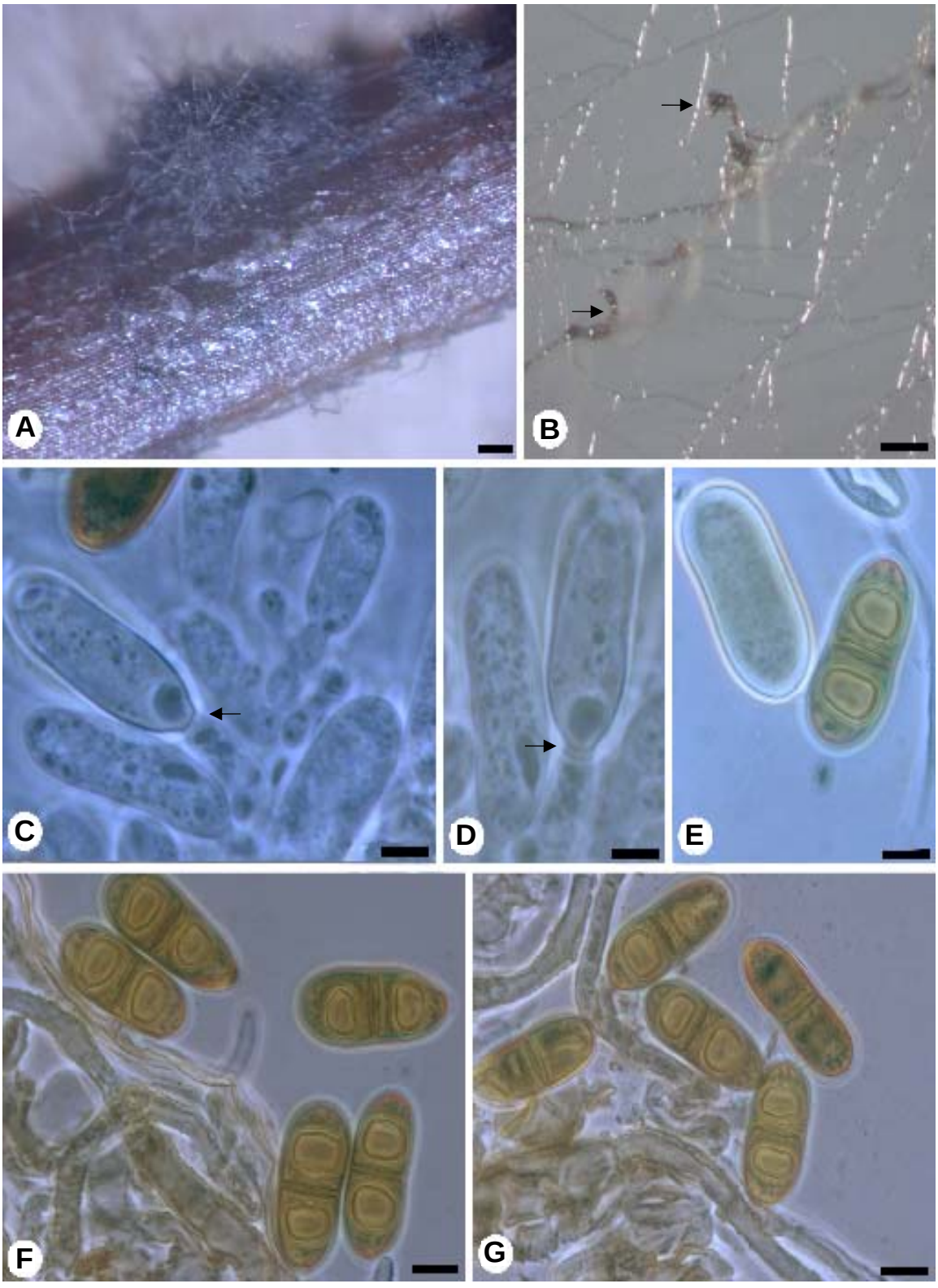

A
B
C
D
E
F
G

FIGURE 4: *Spencermartinsia capri-amissi*. A. Pycnidia on pine needles. B. Chlamydospore-like thickenings of the hypae (arrows). C–D. Conidia attached to conidiogenous cells with periclinal thickenings and annelations (arrows). E–G. Conidia. E. Younger aseptate spore with older darker brown, septate and guttalate conidia. Bars: A = 100 µm; B = 50 µm; C-G = 5 µm.


A
B
C
D
E
F
G

Chapter 4

Spencermartinsia nigra prov. nom., a new species associated with *Acacia mearnsii* De Wild. in the Gauteng Province, South Africa

ABSTRACT

Acacia mearnsii is regarded as one of the more important species planted in Africa, mainly for tannin production and high quality pulp. Recent studies on native *Acacia* spp. from Namibia and South Africa have revealed that there is a greater diversity in the Botryosphaeriaceae on a single tree species than might have been expected. In this study, we considered the presence of the Botryosphaeriaceae on *A. mearnsii* in the Gauteng Province in South Africa. A previously undescribed *Spencermartinsia* sp., emerged and it is described here as *S. nigra* prov. nom. based on morphology and DNA sequence data.

INTRODUCTION

The most important *Acacia* spp. planted in Africa, specifically southern Africa is *Acacia mearnsii* De Wild. (Sherry, 1971). It is mainly planted for tannin production and high quality pulp, but is also used for soil reclamation, wind breaks, mining timber and the wood is a source of fuel (Sherry, 1971). Due to the economic importance of this tree, a number of studies have considered the diseases affecting *A. mearnsii*, especially in eastern and southern Africa (Roux *et al*., 1995, 1997, 2005; Roux and Wingfield, 1997; Roux, 2002). Although there are many known diseases associated with *A. mearnsii*, there are only two known species of the Botryosphaeriaceae on this host from South Africa, namely *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Stephens and Goldschmidt, 1938; Gibson, 1975; Roux and Wingfield, 1997) and *Botryosphaeria dothidea* (Moug. Ex Fr.) Ces. & De Not. (Roux and Wingfield, 1997; Roux *et al*., 1997). This latter name may, however, be inaccurate due to its incorrect use in the past and recent taxonomic changes in the Botryosphaeriaceae (Crous *et al*., 2006; Slippers *et al*., 2004). It should, however, be noted that Roux and Wingfield (1997) also identified three fungal isolates as two *Diplodia* spp. and a *Sphaeropsis* sp. during a survey (1994 to 1995) of two important commercial wattle-growing areas in South Africa. The taxonomic placement of these species is yet to be determined.

Recent studies on native *Acacia* spp. in southern Africa (Chapter 2 and 3) have shown that these trees harbor an unusually high diversity of the Botryosphaeriaceae, with twelve species in eight genera. Nine of these species were new taxa and also included a new genus. The high level of diversity on native *Acacia* spp. has raised the question whether these species also occur on non-native *Acacia* spp., for example *A. mearnsii*.

In this study Botryosphaeriaceae collected from *A. mearnsii* trees in the Pretoria area, South Africa, were identified and compared with the Botryosphaeriaceae that are known to occur on native *A. mellifera* trees in the same area (see chapter 2). It was hoped that this would shed more light on the ability of the Botryosphaeriaceae to move between these important native and non-native trees.

MATERIALS AND METHODS

Isolates

Samples from 20 *A. mearnsii* trees, approximately four years old, were collected from Klipvlei farm (S 25° 29 33.0, E 028° 34 07.0), Pretoria, South Africa. Twenty healthy branch tips were collected from each tree and fungal endophytes were isolated as described in chapter 2. Samples were also collected from diseased plant tissue. Symptoms included lesions on branches, black piths in branches, cankers and tip die-back. Diseased plant material was surface disinfested with 76 % ethanol and small pieces of symptomatic tissue were plated onto 2 % (w/v) malt extract agar (MEA) (Biolab, Midrand, South Africa). Plates were incubated at 25 °C until fruiting structures were visible for further study.

DNA isolation and PCR

DNA was extracted using a modified phenol:chloroform DNA extraction method (Raeder and Broda, 1985) with modifications described previously (Chapter 2). The internal transcribed spacer (ITS1 and ITS2) gene regions were amplified using primers ITS1 and ITS4 (White *et al.*,

1990), as well as the elongation factor 1-α (EF1-α) gene using the primers EF1F and EF2R (Jacobs *et al*., 2004). Polymerase Chain Reaction (PCR) mixtures, PCR conditions and the visualization of products, with size estimates, were done as described previously (Chapter 2).

Sequencing and Phylogenetic analyses

The cleaning, sequencing and analysis of the PCR products were as described in Chapter 2. The BLAST search function on GenBank was used to compare sequences from this study to other Botryosphaeriaceae. GenBank sequences were aligned to sequences from this study using the online interface of MAFFT version 5.8 (http://timpani.genome.ad.jp/~mafft/server/) (Katoh *et al*., 2002) and a phylogenetic analyses were conducted using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2002). Sequences were manually adjusted and trees were rooted to *B. dothidea*. A partition homogeneity test (Swofford, 2002) was done to test the congruence between sequence results from the ITS and EF1-α gene regions. Phylogenetic analysis in PAUP and the Bayesian analysis with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) was done as described previously (Chapter 2). The first 600 trees were discarded as the burnin period. Modeltest (Posada and Crandall, 1998) identified the GTR+I+G model as the best fit for the ITS data set and the HKY+G model to best fit the EF1-α data set.

Morphological and Growth characterization

Cultures were induced to sporulate on sterilized pine needles on 2 % water agar (WA) at 25 °C under UV light. Morphological characteristics were analyzed by placing fungal structures onto glass slides in 75 % lactic acid. Fruiting bodies and conidia were examined and images were

captured as described previously (Chapter 2). Only one culture from the sample sporulated and this was used for morphological characterization, where fifty measurements were made for all diagnostic characters. Colours were assigned based on the colour charts of Rayner (1970) and the minimum, maximum, standard deviation (SD), mean value and the length/width (l/w) ratios were calculated.

The growth rate of the four isolates (TABLE I) were determined at seven different temperatures. Two seven-day-old cultures were selected and agar discs (4 mm diam.), overgrown with mycelium, were placed mycelial side down at the center of 90 mm Petri dishes containing 2 % MEA. Incubation temperatures ranged from 5 °C to 35 °C with 5 °C intervals and Petri dishes were incubated in the dark. Average growth rates were calculated by using five replicate plates for each isolate and temperature, and the averages were computed and colony diameters were measured after four days.

RESULTS

Sequencing and Phylogenetic analyses

Four isolates representing the Botryosphaeriaceae were obtained from the 20 *A. mearnsii* trees sampled in this study. BLAST searches on GenBank and preliminary phylogenetic analysis (data not shown) indicated that all four represented a single *Dothiorella* spp., although it is now placed in the genus, *Spencermartinsia*. These four sequences were aligned with selected sequences from the dataset of chapter 2 and 3 (TABLE I) in order to obtain a putative identity.

The partition homogeneity test on the combined ITS and EF1-α datasets produced a P-value of 0.01 showing that the data could be combined. The combined datasets for the ITS and EF1-α gene regions contained 32 taxa and 851 characters after alignment. Four characters were parsimony-uninformative, 356 were parsimony-informative and 491 constant. Heuristic searches in PAUP yielded only one parsimonious tree [Consistency Index (CI) = 0.72, Homoplasy Index (HI) = 0.28, Retention Index (RI) = 0.92 and Rescaled Consistency (RC) = 0.67] (FIG. 1). The overall topology of the ITS and EF1-α trees was the same. The four isolates from *A. mearnsii* grouped in a single unknown clade, which was supported with high bootstrap (100 %) and posterior probability (1.00) values (FIG. 1).

Morphological and Growth characterization

All the isolates used in the phylogenetic analyses were used for the morphological description but spore measurements could only be derived from the single culture that sporulated. Sporulation on pine needles was observed after 2 to 3 weeks and only anamorph structures were observed.

TAXONOMY

Spencermartinsia nigra F.J.J. van der Walt, Slippers & G.J. Marais prov. nom. FIG. 2

Conidiomata pycnidialia, multa, superficialia, immersa vel semi-immersa, singula vel in fasciculis botryosis aggregata, globosa vel ovoidea, 403 µm diametro, rarius pyriformia, mycelio brevi tecta. Cellulae conidiogenae hyalinae, holoblasticae, cylindricae vel late lageniformes vel ampulliformes, periclinale incrassatae vel rare percurrente proliferantes, annulationes singulas vel

binas formantes 7.5–13.3(–18) × (2.2–)2.2–5.4(–8.4) µm. Conidia 1-septata, rarius non vel 2-septata, ovoidea, subcylindrica vel ellipsoidea, apice basique rare rotundata, vel apice rotundata basi truncata, in septis constricta, parietibus modice crassis, laevibus (20–) 24.4–29.7(–36.2) × (8.6–)9.9–12.1(–13.4) µm.

Conidiomata pycnidial, abundant, superficial, immersed or semi-immersed, separate or aggregated into botryose clusters, globose or ovoid and to a lesser extent pyriform, up to 403 µm in diameter, covered in short mycelium. *Ostioles* single, central, papillate. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, holoblastic, cylindrical to broadly lageniform or ampilliform, 7.5–13.3(–18) × (2.2–)2.2–5.4(–8.4) µm (average: 10.4 × 3.8 µm), proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently to form one or two annelations. *Conidia* initially honey colored to brown becoming blackish brown, 1-septate, rarely aseptate or 2-septate, ovoid to sub-cylindrical or ellipsoidal, rarely with a rounded apex and base or a rounded apex and truncated base, constricted at the septa, moderately thick-walled, smooth, (20–) 24.4–29.7(–36.2) × (8.6–)9.9–12.1(–13.4) µm (average of 50 conidia: 27 × 11 µm, l/w ratio: 2.5).

Culture characteristics: mycelium effuse olivaceous black (25""m), with sparse olive grey (23""""b) mycelial growth, edges of colony irregular. Reverse black (*1). Occasional chlamydospore-like thickenings of the hyphae, blackish brown, intercalary, terminal and dictyosporus and found superficial and immersed in water agar. *Temperatures for growth*: growth at minimum 10 °C, growth rate of 54.7 mm after 4 days at an optimal temperature 25 °C, growth at maximum 25 °C.

Etymology: Name refers to the black colour of the colonies when viewed from the underside of plates.

Host: *Acacia mearnsii*.

Distribution: Pretoria (South Africa).

Specimens examined: SOUTH AFRICA, PRETORIA: Klipvlei farm, fruiting structures induced on needles of *Pinus* sp. on WA, Oct. 2006, *F.J.J. van der Walt* and *R.N. Heath*, HOLOTYPE, herb. PREM59647, culture ex-type CMW25432, CBS121783, CAMS001487; SOUTH AFRICA, PRETORIA: Klipvlei farm, fruiting structures induced on needles of *Pinus* sp. on WA, Oct. 2006, *F.J.J. van der Walt* and *R.N. Heath*, PARATYPE, herb. PREM59646, culture ex-paratype CMW25430, CBS121784, CAMS001485; SOUTH AFRICA, PRETORIA: Klipvlei farm, fruiting structures induced on needles of *Pinus* sp. on WA, Oct. 2006, *F.J.J. van der Walt* and *R.N. Heath*, PARATYPE, herb. PREM59648, culture ex-paratype CMW25433, CBS121785, CAMS001488; SOUTH AFRICA, PRETORIA: Klipvlei farm, fruiting structures induced on needles of *Pinus* sp. on WA, Oct. 2006, *F.J.J. van der Walt* and *R.N. Heath*, CMW25431, CAMS001486.

DISCUSSION

Recent studies have shown that native *Acacia* spp. in southern Africa harbour a wide variety of Botryosphaeriaceae (Chapters 2 and 3). It was, therefore, notable that only a single species in this group was isolated from the non-native *A. mearnsii* in this study. However, only 20 *A. mearnsii* trees were sampled and a larger sample size could have resulted in the isolation of other species within the Botryosphaeriaceae. This fungus was only isolated from apparently healthy tissue and was found to be unique among the Botryosphaeriaceae. It was consequently described here as *Spencermartinsia nigra*, and it is the third member of the Botryosphaeriaceae that has been reported on this host. The other two species include *Lasiodiplodia theobromae* (Stephens and

Goldschmidt, 1938; Gibson, 1975; Roux and Wingfield, 1997) and *Botryosphaeria dothidea* (Roux and Wingfield, 1997; Roux *et al*., 1997), including the two undescribed *Diplodia* spp. and *Sphaeropsis* sp. (Roux and Wingfield, 1997).

A recent study by Phillips *et al*. (2008) made a distinction between previously known *Dothiorella* spp. (Luque *et al*., 2005; Phillips *et al*., 2005) and described a new genus, *Spencermartinsia* AJL Phillips, A Alves & Crous, to accommodate *S. viticola*. These authors identified a sister clade to *Spencermartinsia* containing two isolates, ICMP16819 and ICMP16824, and due to the lack of morphological data no formal description was provided. Data obtained in chapters 2, 3 and 4 identified an additional three members of *Spencermartinsia* nl. *S. rosulata*, *S. capri-amissi* and *S. nigra*. This placed the two isolates in the study of Phillips *et al*. (2008) among the rest of the *Spencermartinsia* spp. (FIG. 1). Therefore, it is suggested that these two isolates will best reside in the latter genus.

Although we might have expected a different result, there was no evidence in this study that native and non-native *Acacia* spp. share species of the Botryosphaeriaceae. Based on previous studies, *S. rosulata* appears to be a dominant species on native *Acacia* spp. in Namibia, Pretoria and the Northern Cape Province (Chapters 2 and 3). This fungus was not isolated from the non-native *A. mearnsii* trees sampled in this study, even though the trees grow in close proximity to native *Acacia* spp. A more extensive sampling, including other geographic areas, is needed to present an unequivocal view of the observed pattern.

Previous studies on the Botryosphaeriaceae, associated with native (Chapter 2 and 3) and non-native *Acacia* spp.(Roux and Wingfield, 1997; Roux *et al*., 1997) in southern Africa, indicate that

there is little overlap of these fungi between hosts. *Botryosphaeria dothidea* is the only species previously reported from both native (Chapter 2) and non-native *Acacia* spp. in southern Africa (Roux and Wingfield, 1997; Roux *et al*., 1997). However, prior to 1997, the taxonomy of *B. dothidea* was confused and unclear, and consequently, the reported species could represent different taxa as defined in the contemporary taxonomic schemes of Slippers *et al*. (2004) and Crous *et al*. (2006).

Roux *et al*. (1997) reported *L. theobromae* on non-native *A. mearnsii* (Stephens and Goldschmidt, 1938; Gibson, 1975), which was not found in studies on native *Acacia* trees (Chapters 2 and 3), and neither was *S. nigra* that is described here. Recent studies that considered the Botryosphaeriaceae on native *Acacia* spp. (Chapters 2 and 3) identified 12 species that represent eight different genera, none of which are known from non-native *Acacia* spp., except *B. dothidea* (Chapter 2; Roux *et al*., 1997). The fact that native and non-native *Acacia* trees were not necessarily growing in the same areas makes any assumptions regarding their host preferences tenuous. However, it is known that strong geographical patterns can exist in species-host interactions where the Botryosphaeriaceae are of concern as pathogens (Slippers and Wingfield, 2007). There is consequently a clear and urgent need for more intensive studies to understand the distribution of the Botryosphaeriaceae among sympatrically occurring native and non-native *Acacia* spp. in southern Africa.

REFERENCES

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology 55:235–253.

Gibson IAS. 1975. The Leguminosae. In Diseases of Forest Trees Widely Planted as Exotics in the Tropics and Southern Hemisphere. Part I. Important members of the Myrtaceae, Leguminosae, Verbenaceae and Meliaceae. Commonwealth Forestry Institute, University of Oxford: Oxford. (ed. IAS Gibson), pp. 21–34.

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD. 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycological Research 108:411–418.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059–3066.

Luque J, Martos S, Phillips AJL. 2005. *Botryosphaeria viticola* sp. nov. on grapevines: a new species with a *Dothiorella* anamorph. Mycologia 97:1111−1121.

Phillips AJL, Alves A, Correia A, Luque J. 2005. Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. Mycologia 97:513–529.

—, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW. 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21: 29–55.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics Applications Note 14:817−818.

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1:17−20.

Rayner RW. 1970. A mycological colour chart. CMI and British Mycological Society, Kew, Surrey, U.K.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Roux J, Kemp GHJ, Wingfield MJ. 1995. Diseases of Black Wattle in South Africa - A Review. South African Journal of Forestry 174:35−40.

—, Wingfield MJ. 1997. Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. Forest Ecology and Management 99:327–336.

—, —, Morris MJ. 1997. *Botryosphaeria dothidea* as a pathogen of *Acacia mearnsii* in South Africa. South African Journal of Science 93:xii.

—. 2002. Threats to Wattle plantations III: Diseases of Black Wattle: The South African Research Experience. Institute for Commercial Forestry Research, Pietermaritzburg, South Africa, pp. 121–126.

—, Meke G, Kanyi B, Mwangi L, Mbaga A, Hunter GC, Nakabonge G, Heath RN, Wingfield MJ. 2005. Diseases of plantation forestry trees in eastern and southern Africa. South African Journal of Science 101:1–5.

Sherry SP. 1971. 'The black wattle (*Acacia mearnsii* De Wild.)'. University of Natal Press: Pietermaritzburg, South Africa, p. 1−382.

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ. 2004. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96:83−101.

—, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biology Reviews 21:75−89.

Stephens RP, Goldschmidt WB. 1938. A prelimanary report on aspects of wattle pathology. South Africa Forestry Journal 2:32–42.

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a sequencing guide to methods and apllcations'. (Eds MA Innis, DH Gelfand, JJ Sninsky, TJ White), pp. 315–322.

TABLE I: Isolates considered in the morphological and phylogenetic investigations (FIG. 1).

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α
[1]CBS121764	[2]CMW25406 [3]CAMS001461	*Dothiorella oblonga*	*A. mellifera*	F.J.J. van der Walt & J. Roux	Rundu, Namibia	EU101299	EU101344
CBS121765	CMW25407 CAMS001462	*Do. oblonga*	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101300	EU101345
CBS121766	CMW25408 CAMS001463	*Do. oblonga*	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101301	EU101346
CBS115041		*Do. iberica*	*Quercus ilex*	J. Luque	Spain, Aragon	*AY573202*	*AY573222*
CBS115040		*Do. iberica*	*Q. ilex*	J. Luque	Spain, Catalonia	*AY573214*	*AY573232*
[4]IMI63581b		*Do. sarmentorum*	*Ulmus* sp.	E.A. Ellis	England, Warwickshire	*AY573212*	*AY573235*
CBS115038		*Do. sarmentorum*	*Malus pumila*	A.J.L. Phillips	Netherlands, Delft	*AY573206*	*AY573223*
CBS112872		*Neofusicoccum australe*	*V. vinifera*	F. Halleen	South Africa	*AY343388*	*AY343347*
CBS112877		*N. australe*	*V. vinifera*	F. Halleen	South Africa	*AY343385*	*AY343346*
CBS110299		*N. luteum*	*V. vinifera*	A.J.L. Phillips	Portugal, Oeiras	*AY259091*	*AY573217*
CMW9076		*N. luteum*	*Malus x domestica*	S.R. Pennycook	New Zealand	*AY236946*	*AY236893*
CBS112878		*N. viticlavatum*	*V. vinifera*	F. Halleen	South Africa, Stellenbosch	*AY343380*	*AY343342*
CBS112977		*N. viticlavatum*	*V. vinifera*	F. Halleen	South Africa	*AY343381*	*AY343341*

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α
CBS110887		*N. vitifusiforme*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343383*	*AY343343*
CBS110880		*N. vitifusiforme*	*V. vinifera*	J.M. van Niekerk	South Africa, Stellenbosch	*AY343382*	*AY343344*
CBS121763	CMW25403 CAMS001458	*Spencermartinsia capri-amissi* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Northern Cape, South Africa	EU101323	EU101368
CBS121878	CMW25404 CAMS001459	*S. capri-amissi* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Northern Cape, South Africa	EU101324	EU101369
CMW25405	CAMS001460	*S. capri-amissi* *	*A. erioloba*	F.J.J. van der Walt & G.J. Marais	Northern Cape, South Africa	EU101325	EU101370
CBS121784	CMW25430 CAMS001485	*S. nigra* *	*A. mearnsii*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101331	EU101376
CMW25431	CAMS001486	*S. nigra* *	*A. mearnsii*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101332	EU101377
CBS121783	CMW25432 CAMS001487	*S. nigra* *	*A. mearnsii*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101333	EU101378
CBS121785	CMW25433 CAMS001488	*S. nigra* *	*A. mearnsii*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101334	EU101379
CBS117009		*S. viticola*	*Vitis vinifera* cv. Garnatxa Negra	J. Luque & S. Martos	Spain, Vimbodí	*AY 905554*	*AY 905559*
CMW25399	CAMS001454	*S. viticola*	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	*EU101295*	*EU101340*

Culture	Other nr.	Species	Host	Collectors	Locality	ITS	EF1-α
CMW25400	CAMS001455	*S. viticola**	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	*EU101296*	*EU101341*
CBS121760	CMW25389 CAMS001444	*S. rosulata*	*Acacia karoo*	F.J.J. van der Walt & J. Roux	Windhoek, Namibia	EU101290	EU101335
CBS121761	CMW25392 CAMS001447	*S. rosulata*	*A. mellifera*	F.J.J. van der Walt & R.N. Heath	Pretoria, South Africa	EU101293	EU101338
[5]ICMP16819		*Spencermartinsia* sp.	*Citrus sinensis*		New Zealand	*EU673320*	*EU673287*
ICMP16824		*Spencermartinsia* sp.	*Citrus sinensis*		New Zealand	*EU673321*	*EU673288*
CMW8000		*Botryosphaeria dothidea*	*Prunus* sp.	B. Slippers	Switzerland, Crocifisso	*AY236949*	*AY236898*
CBS116742		*B. dothidea*	*Olea europea*	I. Rumbos	Greece, Thessalia	*AY640254*	*AY640257*
CBS116743		*B. dothidea*	*Populus nigra*	A.J.L. Phillips	Portugal, Braga	*AY640253*	*AY640256*

Culture collections: [1]CBS-Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; [2]CMW- FABI, University of Pretoria, South Africa; [3]CAMS-Center for Applied Mycological Studies, University of Pretoria, South Africa; [4]IMI-CABI Bioscience, Egham, UK; [5]ICMP-International Collection of Micro-organisms from Plants.

BOLD = Isolate accession numbers in bold signify ex-type cultures, or from samples that have been linked morphologically and phylogeneticially to the type material.

ITALICS = Sequence numbers in italics were retrieved from the GenBank public database or obtained from chapter 2,3. All others were obtained in this study.

* Isolates used in this study for sequencing and phylogenetic inference are indicated by an asterisk

FIGURE 1: Parsimonious tree through heuristic searches of the combined datasets of the ITS and EF1-α gene regions. Bootstrap values ≥ 70 % are indicated above the line and posterior probabilities ≥ 95 % are indicated below the lines. Isolates used in this study are indicated by an asterisk * and the ex-type cultures are in bold.

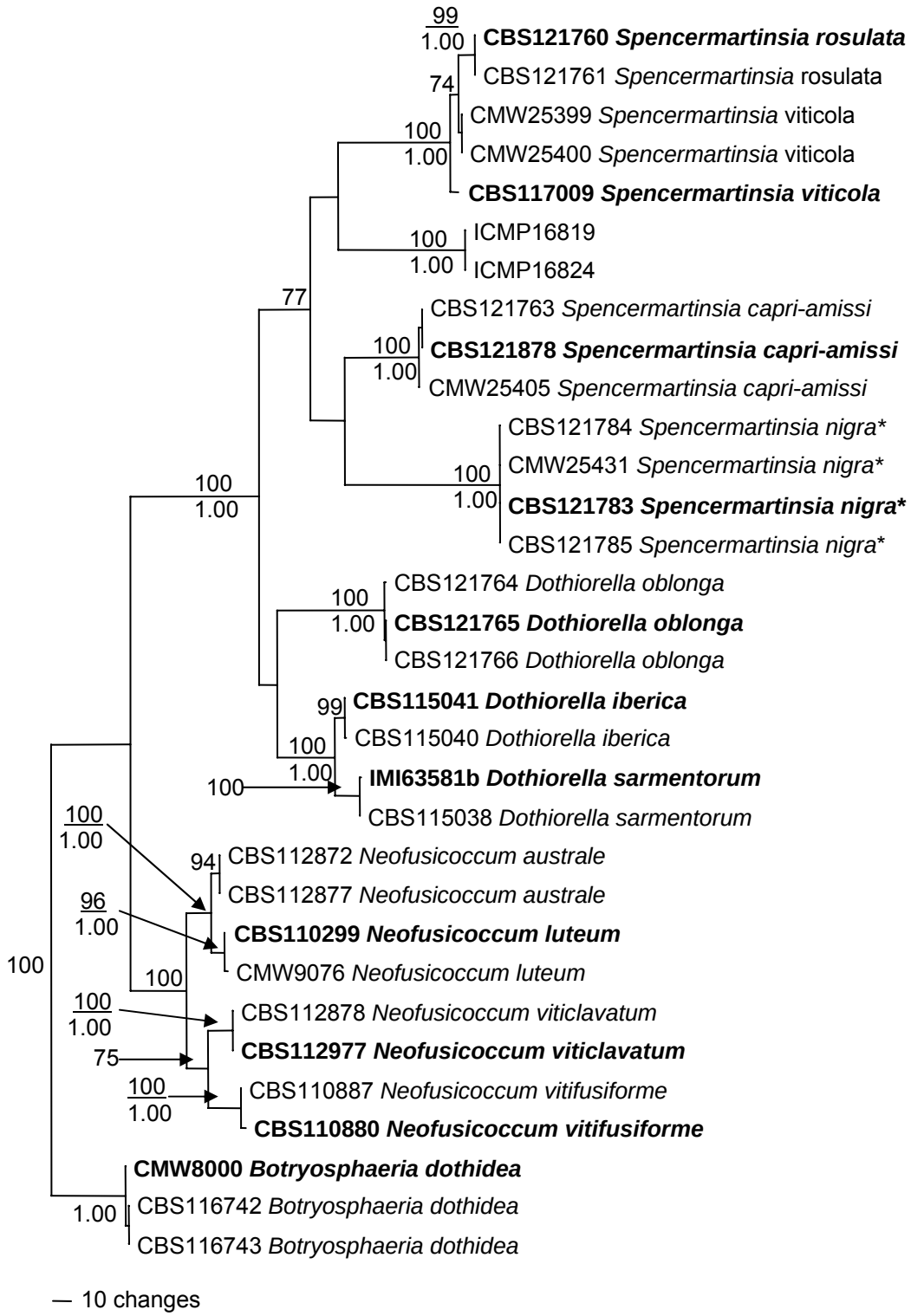
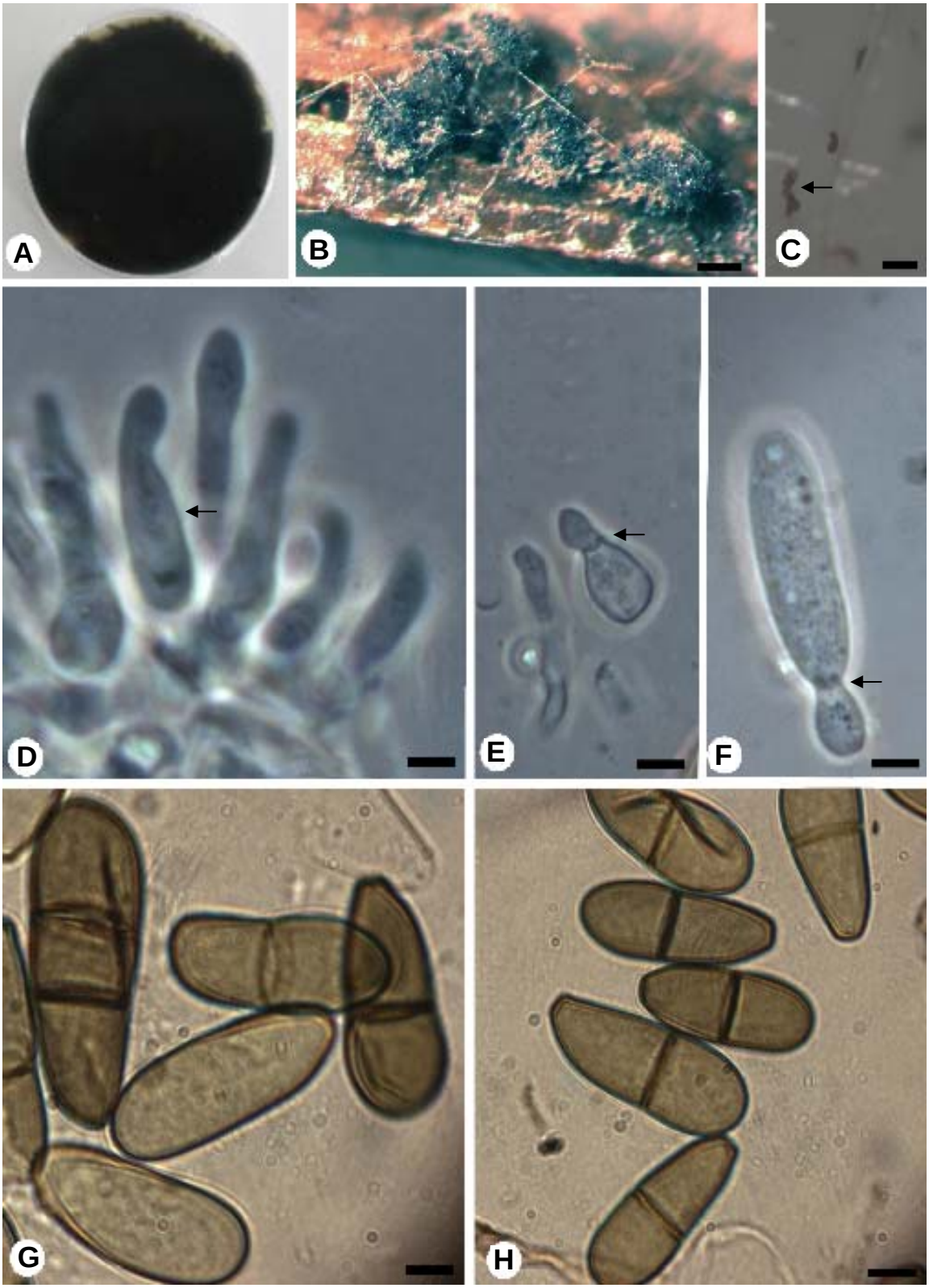

99
1.00
CBS121760 Spencermartinsia rosulata
CBS121761 Spencermartinsia rosulata
74
CMW25399 Spencermartinsia viticola
100
1.00
CMW25400 Spencermartinsia viticola
CBS117009 Spencermartinsia viticola
100
1.00
ICMP16819
ICMP16824
77
CBS121763 Spencermartinsia capri-amissi
100
1.00
CBS121878 Spencermartinsia capri-amissi
CMW25405 Spencermartinsia capri-amissi
CBS121784 Spencermartinsia nigra*
100
1.00
CMW25431 Spencermartinsia nigra*
CBS121783 Spencermartinsia nigra*
CBS121785 Spencermartinsia nigra*
100
1.00
CBS121764 Dothiorella oblonga
100
1.00
CBS121765 Dothiorella oblonga
CBS121766 Dothiorella oblonga
99
CBS115041 Dothiorella iberica
100
1.00
CBS115040 Dothiorella iberica
100
IMI63581b Dothiorella sarmentorum
CBS115038 Dothiorella sarmentorum
100
1.00
94
CBS112872 Neofusicoccum australe
CBS112877 Neofusicoccum australe
96
1.00
CBS110299 Neofusicoccum luteum
100
CMW9076 Neofusicoccum luteum
100
100
1.00
CBS112878 Neofusicoccum viticlavatum
75
CBS112977 Neofusicoccum viticlavatum
100
1.00
CBS110887 Neofusicoccum vitifusiforme
CBS110880 Neofusicoccum vitifusiforme
CMW8000 Botryosphaeria dothidea
1.00
CBS116742 Botryosphaeria dothidea
CBS116743 Botryosphaeria dothidea
— 10 changes

FIGURE 2: *Spencermartinsia nigra*. A. Black underside of a culture. B. Pynidia on pine needles. C. Chlamydospore-like hyphae (arrow). D. Conidiogenous cells (arrow). E, F. Conidiogenous cells with periclinal thickenings and annelations (arrows). G, H. Conidia. G. Guttalate conidia. Bars: B = 100 µm; C = 50 µm; D–H = 5 µm.


A
B
C
D
E
F
G
H

SUMMARY

Previously, only one member of the Botryosphaeriaceae, *Lasiodiplodia theobromae*, was known to occur on a native *Acacia* spp. in southern Africa. From this study alone, as indicated in chapters 2 and 3, a total of 12 members of the Botryosphaeriaceae were isolated from *Acacia* spp., of which nine was identified as new species. Formerly described species included *L. pseudotheobromae*, *Spencermartinsia viticola* and *Botryosphaeria dothidea*, for which a previously unknown *Dichomera* synanamorph is described here for the first time. Undescribed species included, *Diplodia variabilis*, *Dothiorella oblonga*, *Fusicoccum avasmontanum*, *L. pyriformis*, *S. rosulata*, *S. capri-amissi* and a new genus, *Mucodiplodia*, represented by two species, *M. africana* and *M. papillata*. An unknown *Neofusicoccum* sp. was also obtained but not described due to a lack of represented isolates.

While studying the diversity of the Botryosphaeriaceae on native *Acacia* spp., the question was raised whether these species also occur on non-native trees such as *A. mearnsii*. It was indicated in chapter 4 that only one Botryopshaeriaceae species was found to be assocaited with this host. The fungus was here described as *S. nigra*. Due to the limited species of the Botryosphaeriaceae found on *A. mearnsii*, no conclusive evidence could be found that native and non-native *Acacia* spp. can share the same species of the Botryosphaeriaceae. However, this study should only be seen as a pilot study as limited samples were available from only one area. More extensive sampling needs to be done to realize the full extent of the distribution of members of the Botryosphaeriaceae among native and non-native *Acacia* spp.

This is the most extensive study of the Botryosphaeriaceae associated with native *Acacia* spp. in southern Africa. To our knowledge this is also the first study that resulted in the description of so many new species of the Botryosphaeriaceae from a single host. Previous authors estimated that there could be as many as seven new species of fungi per indigenous plant host. However, results from this study may indicate this estimate to be conservative as all the new fungal species described here focused only on one fungal family. Nevertheless, this study has contributed significantly to our understanding of the Botryosphaeriaceae associated with indigenous *Acacia* spp. in southern Africa.

This study indicated that there is a significantly greater diversity of the Botryosphaeriaceae on native *Acacia* spp. in southern Africa than was previously thought. The relevance of these Botryosphaeriaceae as pathogens and their potential risks on native hosts is still unclear and needs further consideration. Together with climate changes on a global scale, plants and their pathogens are also affected. This can cause a shift in the relationships between host-pathogen interactions that could make previous assumptions inaccurate. It is, therefore, important to understand the interactions of the Botryosphaeriaceae and their native hosts so that their ability and threat as pathogens to native and economically important plants can be realized.