Taxonomy and biology of the plant pathogenic fungus

Thielaviopsis basicola

by

Wilma Nel

Submitted in partial fulfilment of the requirements for the degree

Master of Science

In

The Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

05 December 2017

Supervisor: Prof Z. W. de Beer

Co-Supervisors: Dr. T. A. Duong, Dr. A. Hammerbacher, Prof. B.D. Wingfield and Prof. M. J. Wingfield

Declaration

I, the undersigned declare that the thesis/dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

_____

Miss Wilma Nel

(05 December 2017)

Dedication

I dedicate this dissertation to my grandfathers, Jan Nel and Wim Pel. In different ways you have inspired me to follow my dreams every day.

Acknowledgements

I would like to thank the following persons and/or institutes:

- Each of my supervisors, Prof. Wilhelm de Beer. Dr Tuan Duong, Dr Almuth Hammerbacher, Prof. Brenda Wingfield and Prof. Mike Wingfield. Thank you for all the time, effort and support that you have put in during the preparation of this dissertation. It has been a great privilege learning from each of you.
- My parents, Mike and Hanneke Nel. Thank you for standing by me through every decision I make.
- Dr Seonju Marincowitz, Ms. Valentina Nkosi and Ms. Lydia Twala for all your support. In many cases your contributions fall in the background of a project, but without your effort many projects will never happen.
- Dr. Markus Wilken. The help you gave me during my mating research was invaluable and it is a privilege to call you a friend.
- Ms. Miranda Procter, Mr. Seamus Morgan, Ms. Juanita Engelbrecht, and Mr. Francois Viljoen for all the support and interesting conversations. Because of you my days are always filled with laughter.
- All the remaining friends and collegues from the Forestry and Agricultural Biotechnology Institute (FABI).
- The members of the Tree Protection Co-operative Program, the DST-NRF Centre of Excellence in Tree Health Biotechnology, the National Research Foundation (NRF), the Genome Research Institute (GRI) and the University of Pretoria for funding my project.

Contents

Preface

The *Ceratocystidaceae* is a family of filamentous Ascomycete fungi that belongs to the order Microascales. At present, ten genera are accepted in the family, including a variety of plant pathogenic species. Among these species is *Thielaviopsis basicola*, an agriculturally important pathogen that causes root rot in many crop species resulting in severe losses to industry.

Thielaviopsis basicola was first described in the mid 1800's, from the roots of two plant species. Since its description, there has been much confusion surrounding the correct taxonomic placement of the species, which resulted in the fungus being treated in various genera. The most recent generic placement in *Thielaviopsis* for the species was done in 2002, around the time when DNA sequencing and phylogenetic analyses became a more standard taxonomic tool. However, as the techniques improved over the past decade, additional phylogenetic investigations involving the *Ceratocystidaceae* were done and in 2014 questions again arose regarding the placement of *T. basicola*.

Although the pathogen has now been known for more than 150 years, its sexual state and reproductive strategy remains unknown. As this species is well known and extensive studies have been conducted investigating various aspects of its biology, some researchers have started to conclude that the pathogen might reproduce exclusively asexually. With so many unresolved questions pertaining to this species, we were presented with the opportunity to do a comprehensive study investigating its taxonomic placement and reproductive strategy.

The **first chapter** of this dissertation consists of a comprehensive review of the literature pertaining to *T. basicola* specifically focusing on its taxonomic history and biology. Based on results from previous taxonomic investigations included in the literature review, we hypothesised that the species most likely constitutes a new genus in the *Ceratocystidaceae*. Thus, **Chapter two** focuses on the generic placement of the species in the family. Results from this chapter separated the isolates included into two well supported lineages that were described as separate species in a new genus, distinct from *Thielaviopsis* and all other genera in the *Ceratocystidaceae*. We investigated the possibility that an existing genus name might already be available to accommodate the species. Although *T. basicola* was treated in several genera over the years, only one of these names was available to accommodate our species. However, in **Chapter three** we formally propose that the candidate genus name should be rejected due to the unavailability of a type specimen for the type species, and dubious and contradicting descriptions and illustrations of the type species in various publications by the same author.

Aside from the taxonomy, many questions have been raised regarding the mating behaviour of *T. basicola*. In recent years, genome sequencing has been used to characterize mating loci and determine the mating strategies of multiple fungal species that were once suggested to be asexual. In **Chapter four** we discuss the processes involved in sequencing and assembly of the first whole genome sequences for an isolate representative of the new genus described in **Chapter two**. In **Chapter five** we interrogate

this genome to determine the mating strategy for the first of the two species of the new genus. Based on the data obtained for the first species, we were also able to design primers and determine the mating behaviour of its newly described sister species.

This page has intentionally been left blank

Chapter 1: A review of the taxonomy and biology of the black rot pathogen *Thielaviopsis basicola*

Abstract:

Thielaviopsis basicola (Ascomycota: *Ceratocystidaceae*) is an important pathogen of numerous agricultural and ornamental plant species. The fungus is a hemibiotroph, invading the living cells of its hosts, causing black necrotic lesions as it spreads to adjacent cells. The dead patches on the roots result in stunting of the plants and in some cases severe yield losses, especially in the cotton, tobacco, and groundnut industry. Since its first description in the mid 1800's substantial research has been conducted on *T. basicola*, particularly regarding its mode of infection, biology and control. A complex and controversial taxonomic history resulted in much confusion in the literature with regards to its taxonomic placement. The objective of this review is to clarify the taxonomic literature where possible and raise important questions regarding the phylogenetic placement and biology of this important plant pathogen.

Introduction

Thielaviopsis (*T.*) *basicola* (Berk. & Broome) Ferr. is an important plant pathogen affecting more than 170 plant species (**Table 1**), recorded on all continents except Antarctica (**Table 2**). It is considered to be a natural soil inhabitant because it has been found in virgin soils far from cultivated lands (Stover, 1950, Yarwood, 1974). Since its first description in the genus *Torula* Pers. in 1850 (Berkeley & Broome, 1850), *T. basicola* has had a tumultuous taxonomic history, being treated in several different genera and assigned numerous different species names.

Thielaviopsis basicola was originally thought to be a saprophyte (Massee, 1912, Gayed, 1972), but many investigations into its mode of infection on multiple hosts (**Table 3**), revealed that it is a hemibiotrophic plant pathogen (Bateman, 1963). This hemibiotroph (Hood & Shew, 1997a, Mims *et al.*, 2000, Punja, 2004) has a short biotrophic phase where penetration and colonization of living host root tissue occurs. Its life cycle then progresses to a necrotrophic phase during which cells are lysed and consumed by the fungus. As infection continues, dark necrotic lesions become visible on the plant roots, this causes decreased water and nutrient uptake resulting in stunting of the affected plants (Noshad *et al.*, 2006, Pereg, 2013).

During sporulation, which usually occurs during the necrotrophic phase, characteristic dark-coloured, thick walled chlamydospores (**Fig. 1**) are produced in abundance (King & Presley, 1942). These spores are so characteristic of *T. basicola* that they are generally considered sufficient to identify the pathogen. Apart from these chlamydospores, endoconidia are also produced by the fungus (**Fig. 2**). These spores are produced in chains from tubular conidiophores that are common in species of the *Ceratocystidaceae* that also accommodates *T. basicola* based on phylogenetic inference (De Beer *et al.*, 2014),

Chlamydospores of *T. basicola* are long-lived and act as the most important survival structures during dry or cold periods. (Tsao & Bricker, 1966, Meyer *et al.*, 1994). The endoconidia are produced abundantly and are thought to play a role in secondary infection (Mathre & Ravenscroft, 1966, Papavizas & Lewis, 1971a, Meyer *et al.*, 1994). They also contribute to survival outside the host, with a secondary state of the spore, known as the secondary chlamydospore, being able to survive for more than 15 months (Stover, 1950, Schipper, 1970) (**Fig. 3**).

Whether *T. basicola* has a broad host range or represents groups of differentially specialized races, is not well understood. In early investigations of pathogenicity Johnson (1916) showed that a single isolate of *T. basicola* could cause disease in more than 100 plant species. He hypothesised (Johnson, 1916, Johnson & Hartman, 1919) that the variation in symptom development observed in his experiments resulted from the natural susceptibility or resistance of hosts rather than the inherent ability of the fungus to cause disease on any specific plant. However, the difficulty or in some cases inability to induce

disease symptoms in some plant species known to be susceptible (e.g. *Lathyrus orodatus* and *Daucus carota*) raises questions as to whether there could be host specialized races of *T. basicola*.

Many published investigations provide evidence for host specificity in *T. basicola*. However, these studies also show that there is great variation in susceptibility between different genotypes, cultivars and varieties of a single host species (**Table 4**). More recent studies on the relatedness of different *T. basicola* isolates, both at the DNA and protein level, provide support for the existence of host specialized groups among isolates of this fungus (Geldenhuis *et al.*, 2006, Coumans *et al.*, 2011).

More than 500 research articles have been published with a focus on *T. basicola.* At least 110 articles have appeared in the past 15 years (**Fig. 4**), which illustrates that there is still current interest in this plant pathogen. There have been many studies regarding the biology, control and natural interactions of *T. basicola* with its environment and each could form the topic of an independent review. A recent phylogenetic study raised questions regarding the generic status of the fungus (De Beer *et al.*, 2014) that could substantially alter our general understanding of its biology. Consequently, this review is primarily focussed on the taxonomy of *T. basicola*, addressing its historical and current classification. This focus also provides an opportunity to provide a perspective on various elements of its biology.

The taxonomy of *Thielaviopsis basicola*

Ferraris (1912) was the first to treat *T. basicola* in *Thielaviopsis.* The genus was first described by Went (1893) to accommodate the species *T. ethacetica*, an asexual fungus found causing disease on sugarcane.

Nomenclature

Thielaviopsis basicola (Berk. & Broome) Ferr., Flora Italica Cryptogama Pars. I: Fungi, Hyphales, Tuberculariaceae – Stilbaceae. Fasc. 6: 113. (1912) [MB#119974]

≡ *Torula basicola* Berk. & Broome, XL-Notices of British Fungi, Ann. Mag. Nat. Hist. Ser. 2. 5(30): 461 (1850) (Basionym) [MB#162876]

= *Trichocladium basicola* (Berk. & Broome) Carmich., Genera of Hyphomycetes, p. 185. (1980) [MB#116231]

= *Helminthosporium fragile* Sorokïn, Hedw. 15: 113. (1876) [MB#229991]

= *Clasterosporium fragile* (Sorokïn) Sacc., Syll. Fung. 4: 386. (1886) [MB#198687]

?= *Milowia nivea* Massee, J. R. Microsc. Soc., IV 2: 841-845. (1884) [*nom. dub.*] [MB#209202]

= *Chalara elegans* Nag Raj & Kendr., A Monograph of *Chalara* and Allied Genera, p. 111. (1975) [MB#310994]

Morphology based descriptions

The first formal description of *T. basicola* and the causal agent of black root rot was by Berkeley and Broome in 1850 (**Fig. 5 a, b**). They described a fungus growing at the base of *Pisum sativum* (pea) and "*Nemophila auriculata"* stems. The fungus was named *Torula (Ta.) basicola* "distinguished from most other *Torulae* by its articulations not being constricted". At the time of its description the effect of *Ta. basicola* on its host was unknown and the authors speculated that it could either be a primary or secondary pathogen.

The identity of the host, *Nemophila auriculata,* from which *Ta. basicola* was described, remains uncertain as this species is not listed in The International Plant Names Index (2017) or any other works of reference. The closest possible species name to it is *Nemophila auriculiflora*. However, *N. auriculiflora* was described 30 years after the description of *Ta. basicola* in 1870 by Elisa de Vilmorin (in Fl. Pleine Terre, Ed. 3, 746. 1870, not seen), presumably from France. Furthermore, the species is currently treated as synonym of *Nemophila menziesii* (Hassler, 2017), which is native to western North America (Hooker & Arnott, 1841, Chandler 1902), and not listed among the flora of the British Isles (Fitter & Peat 1994). Unfortunately, reports of the fungus often still list *Nemophila auriculata* as host (Weber & Tribe, 2004, Abbas *et al.*, 2007), even though its identity has not been resolved.

Ferraris (1912) transferred the species described by Berkeley and Broome (1850) to *Thielaviopsis* (Went, 1893) establishing the new combination *Thielaviopsis basicola* (Berk. & Broome) Ferr. He considered the chlamydospores described for *Thielaviopsis* similar to those produced by the fungus described by Berkeley and Broome (1850).

The name *T. basicola* (Berk. & Broome) Ferr. remained undisputed until 1975. Nag Raj & Kendrick (1975) stated in their monograph on *Chalara* and other genera that the name *T. basicola* (Berk. & Broome) Ferr. was applicable only to the chlamydospore producing state of the fungus because it was the only state mentioned in the description of Berkeley & Broome (1850). They suggested that the commonly produced endoconidial state of the fungus should be described separately in the genus *Chalara* (Corda, 1838, Corda, 1842, Rabenhorst, 1844). However, their initial argument ruled the epithet *basicola* unusable for the endoconidial state and they consequently proposed the name *Chalara elegans* Nag Raj & Kendrick for the fungus. Under the dual nomenclature system still in place at the time, both asexual names were considered valid and were used interchangeably in literature for many years.

Carmichael *et al*. (1980) suggested the name *Trichocladium basicola* (Berk. & Broome) Carmich. for the chlamydospore-producing state of the fungus. They noted that the genus *Trichocladium* accommodated the thick-walled chlamydospores produced by the fungus more adequately than did *Thielaviopsis*. However, the name *Trichocladium basicola* has been used only occasionally in literature.

In the years following the description of *Ta. basicola* Berk. & Broome, the fungus was mistakenly described as the new species *Helminthosporium fragile* and *Milowia nivea*. The first of these descriptions was in 1876 by Sorokïn (**Fig. 5 e, f**) and the second description was in 1884 by Massee (**Fig. 5 c, d**).

Sorokïn (1876) described a fungus occurring on *Cochlearia armoracia* (horseradish) as *Helminthosporium fragile* Sorokïn. The species was transferred to the *Clasterosporium* by Saccardo (1886a) who realized this fungus was more closely related to *Torula* than *Helminthosporium*. However, Saccardo did not connect this species with *Torula basicola*, listing both species separately in his publications (Saccardo, 1886a, Saccardo, 1886b). The similarity between *Ta. basicola* Berk. & Broome and *C. fragile* (Sorok.) Sacc. was first proposed in 1912 by Ferraris when he transferred *Ta. basicola* Berk. & Broome to *Thielaviopsis*.

Massee (1884) described a fungus occurring on the decaying leaves of *Blysmus compressus* (flat-sedge) and named it *Milowia nivea*, and erecting a new genus to accommodate the species. In his original description, Massee (1884) seemingly mistook the chlamydospore chains as ascospores developing within an ascus and described globose, moniliform conidia not mentioned in any of the other descriptions of the fungus. The nature of the supposed "asci" was questioned by Cooke (1885), and Massee (1893) amended his original description with the statement "The conidia present the appearance of spores in an ascus" acknowledging his earlier error.

Massee (1912) himself synonymized *M. nivea* Massee with *Ta. basicola* Berk. & Broome and presented figures resembling the fungus much more closely than those in his original description (**Fig. 6 a-c)**. However, this synonymy has not gone without scrutiny from other authors (Johnson, 1916, Nag Raj & Kendrick, 1975). Nag Raj & Kendrick (1975) for example questioned the interpretation of the globose, moniliform conidia. Because the original specimen used in the Massee (1884) description appears to have been lost Nag Raj & Kendrick (1975), their questions remain unanswered. In the final section of this review, I discuss what I believe may be some of the structures that Massee (1884) described.

Zopf (1876) described what he believed was the sexual state of *Ta. basicola* Berk. & Broome. He found perithecial structures intermingled with the chlamydospores of the fungus described by Berkeley & Broome (1850) on the roots of the plant *Senecio elegans* (redpurple ragwort) (Zopf, 1876). He proposed a teleomorph-anamorph connection between the two species and provided the perithecia-producing state with the name *Thielavia (Tl.) basicola* Zopf. No images were presented by Zopf (1876) and figures (**Fig. 7**) of the two states were only published 15 years later (Zopf, 1891). Under dual nomenclature both names were accepted as valid, but priority was given to the sexual name. Many subsequent studies thus referred to this fungus as *Tl. basicola* (Berk. & Broome) Zopf even when only the asexual state of the fungus was observed.

The connection between *Tl. basicola* Zopf and *T. basicola* (Berk. & Broome) Ferr. was supported in several publications (Ferraris, 1912, Massee, 1912, Brierley, 1915, Johnson, 1916, Johnson & Hartman, 1919), but the teleomorph-anamorph connection was disproved by McCormick (1925). She performed multiple experiments and the results showed conclusively that *Tl. basicola* Zopf had no sexual connection to *T. basicola* (Berk. & Broome) Ferr. and that the two species were unrelated. Yet, even after McCormick (1925) had published her findings, some authors continued to refer to the fungus as *Tl. basicola* Zopf (Gilbert, 1926, Conant, 1927, Sattler, 1936).

DNA-based taxonomic investigations

The names *T. basicola* (Berk. & Broome) Ferr. and *C. elegans* Nag Raj & Kendrick remained unchallenged until the advent of molecular and phylogenetic techniques. Paulin & Harrington (2000) grouped six species of *Chalara* (*C. australis, C. elegans, C. neocaledoniae, C. ovoidea, C. populi* and *C. thielavioides*) in the *Ceratocystidaceae*, the most important of which for the case of this review *C. elegans.* Although no taxonomic changes were provided, Paulin & Harrington (2000) proposed that the name *T. basicola* (Berk. & Broome) Ferr. should be considered preferentially in literature. This was because cultures of *C. elegans* Nag Raj & Kendrick, as well as the five other *Chalara* spp. with *Ceratocystis* affinities, grow very differently to the generic type, *C. fusidioides* (Corda) Rabenh. In a subsequent paper, Paulin-Mahady *et al.* (2002) amended the generic description of *Thielaviopsis* (Went, 1893) to incorporate all six *Chalara* spp., as well as others, with *Ceratocystis* affinities in *Thielaviopsis*. From this point onwards, the name *T. basicola* (Berk. & Broome) Ferr. has consistently been used for the black root rot pathogen.

Neither of the studies by Paulin and Harrington (2000; 2002) included the type species of the genus *Chalara*. Because the phylogenetic placement of *C. fusidioides* was only resolved much later, their proposal to include all the *Chalara* species with *Ceratocystis* affinities in *Thielaviopsis* was premature and not strictly appropriate. However, when the phylogenetic placement had been resolved for *C. fusidioides*, it was shown not to reside in the *Ceratocystidaceae* (Réblová *et al.*, 2011) substantiating the earlier taxonomic revision by Paulin-Mahady *et al.* (2002) and rendering *T. basicola* as the valid name for the fungus.

Early phylogenetic investigations involving *T. basicola* were based on LSU, SSU and ITS sequence data, but the results from Naidoo *et al.* (2013) have shown that ITS based investigations can be unreliable for some species in the *Ceratocystidaceae* as multiple copies of the gene can be present within the genome of a single species. LSU and SSU are highly conserved (White *et al.*, 1990, Stielow *et al.*, 2015) regions and sequence and although data for these regions can distinguish between most genera, they fail to provide sufficient resolution to distinguish between all species in a family. Consequently, De Beer *et al.* (2014) applied the recently introduced one fungus one name principle (Hawksworth *et al.*, 2011) to redefine *Ceratocystidaceae* using a multigene phylogenetic approach.

Their results, involving three gene regions (60S, LSU & MCM7), did not accommodate *T. basicola* (Berk. & Broome) Ferr. in the redefined *Thielaviopsis* or any of the other six genera defined in the family at the time. They refrained from defining a new genus for the monophyletic lineage containing *T. basicola,* as an ex-type isolate representing the species was not available for their study. Consequently, the generic placement of *T. basicola* remained unresolved.

The unknown sexual state of T*hielaviopsis basicola*

Thielavia basicola *is not the sexual state of* Thielaviopsis basicola

As mentioned earlier in this review, the first study to show that *T. basicola* and *Tl. basicola* were not different morphs of the same fungus was performed by McCormick (1925). She performed multiple experiments, each of which provided evidence that the two states belonged to different species of fungi.

During the first part of her research she noticed differences in single spore cultures originating from the ascospores of *Tl. basicola* and endoconidia of *T. basicola*. The ascospore colonies of *Tl. basicola* did not form the characteristic chlamydospores commonly used in identifications of *T. basicola.* Although the *Tl. basicola* culture formed perithecia sporadically, the single endoconidia cultures of *T. basicola* never produced this morph. When performing co-culture experiments using the single spore cultures, she noticed that *Tl. basicola* colonies produced perithecia much more rapidly and abundantly in the presence of *T. basicola,* than when maintained in culture on its own.

Based on her early observations, McCormick (1925) performed co-inoculation experiments with the single spore isolate of *Tl. basicola* and those of multiple other fungal species. In mixed culture, *Tl. basicola* was rapidly overgrown, but in some cases where it could co-colonize the plate, it often developed perithecia in a similar manner when cultured together with *T. basicola*. Because the *Tl. basicola* colonies were so often overgrown in co-culture, McCormick (1925) incorporated extracts from different fungi into her growth media. When cultured on these extract-enriched medium plates, *Tl. basicola* was found to produce perithecia much more abundantly than when cultured on standard media. This led McCormick (1925) to conclude that *Tl. basicola* could not be the sexual state of *T. basicola* and that there must be some form of relationship between the two fungi explaining their prevalent co-occurrence.

More evidence to disprove the sexual-asexual relationship between the two species was presented by Lucas (1948, 1949). He found that single spore isolates of *Tl. basicola* and *T. basicola* reacted differently to different nutrient and incubation conditions. He also found that black root rot of tobacco could only be induced using cultures of *T. basicola* and not *Tl. basicola*. Because of this, Lucas (1948, 1949) concluded that physiological changes induced in the roots during infection by *T. basicola* allows establishment of *Tl. basicola* and is likely one of the reasons the two species often occur together.

Stover (1950) also investigated the relationship between *Tl. basicola* and *T. basicola* by using experiments that were similar to those of McCormick (1925) and Lucas (1948, 1949). During co-inoculation experiments of the two fungi he noted that *Tl. basicola* quickly overgrew the *T. basicola* isolates, but that the two species did not appear to have any overall antagonistic effects towards each other. Like Lucas (1948, 1949), he found that *Tl. basicola* was unable to induce root disease when inoculated on its own and that perithecial development only occurred when *Tl. basicola* and *T. basicola* was inoculated onto plant roots together. These finding supported the conclusions made by Lucas (1948, 1949) and Stover (1950) and further suggested that the relationship between the two species was most likely commensal, resulting in the increased perithecia formation by *Tl. basicola* when the two species occur together.

Questions regarding sexuality

Subsequent to the work of McCormick (1925), there have been limited attempts to induce a sexual state for *T. basicola*. The only clear attempt to induce sexual reproduction in the fungus, was made by Johnson & Valleau (1935). These authors made crosses with multiple single-spore isolates of *T. basicola* and observed them for periods of up to one year, but no sexual state was ever observed. Paulin & Harrington (2000) and Paulin-Mahady *et al.* (2002) concluded that this species represented an asexual fungus. However, because the species has been shown to reside in the *Ceratocystidaceae* where sexual structures are common (De Beer *et al*. 2014), asexuality seems unlikely.

Both heterothallism and homothallism can be found in species of the *Ceratocystidaceae*. Heterothallism requires outcrossing between two isolates of opposite mating types. In ascomycetes, these strains can have either the *MAT1-1* or the *MAT1-2* idiomorph (Yoder *et al.*, 1986, Turgeon & Yoder, 2000, Wilken *et al.*, 2017). Contemporary studies using whole genome sequences revealed that various asexual members of the *Ceratocystidaceae*, such as *Chalaropsis thielavioides* (previously *Chalara thielavioides*), a phylogenetically closely related species to *T. basicola* (Paulin-Mahady *et al.*, 2002, De Beer *et al.*, 2014) are heterothallic. Consequently, this could also be the case for *T. basicola*, because populations of the species appear to be clonal in countries where population genetic studies have been carried out (Geldenhuis *et al.*, 2006). The absence of one of the mating types could explain the absence of sexual reproduction.

In homothallic fungi, a single isolate of a species can undergo sexual reproduction and, in the case of the *Ceratocystidaceae*, perithecial development can readily be seen in culture. However, four types of homothallism have been identified in members of the *Ceratocystidaceae*: primary homothallism, pseudohomothallism, mating type switching, and unisexuality (Wilson *et al.*, 2015). Although primary homothallism, pseudohomothallism and unisexuality seems unlikely to be the mating strategy utilised by *T. basicola*, some characteristics of mating type switching individuals have been seen in isolates of *T. basicola* (M. Wilken, personal communication).

Although sexual reproduction has not been observed in *T. basicola*, there does appear to be some genetic variability in the global population of the species. Phylogenetic analyses of both microsatellite (Geldenhuis *et al.*, 2006) and ITS data (Coumans *et al.*, 2011) split isolates of the species into two distinct lineages, referred to by Geldenhuis *et al.* (2006) as clade A and clade B. In both reports, isolates in the two clades separate into many smaller groups often reflecting geographic and host origin. These groupings were reflected by RAPD analyses performed in the early 2000's (Punja & Sun, 2000, Nehl *et al.*, 2002, Nehl *et al.*, 2004). In these analyses, two unique banding patterns were usually seen, likely representing Clade A and B (Geldenhuis *et al.*, 2006), and further variation could be seen among isolates residing in the same clade, likely reflecting the smaller groupings based on host and geographic origin. This genetic variability and adaptation suggests that some level of genetic exchange is taking place. However, whether this is due to sexual recombination or some other factor, remains to be determined.

Two natural morphotypes and their possible significance in sexual reproduction

In nature two different morphotypes (in previous work referred to as wild-types (Stover, 1950)) of *T. basicola* have been identified, a brown-type and a grey-type (**Fig. 8**). The distinction between morphotypes appears to be mostly based on their colour morphology in culture. Brown-type isolates are colonies that contain brown or olive hues, whereas grey-type colonies are those containing shades of grey to black with no brown or olive tones (Stover, 1950, Stover, 1956). These two types have been further classified based on other morphological and even some geographical features (Allison, 1938, Stover, 1950, Huang & Patrick, 1971). Both types undergo morphological changes (**Fig. 9)** over time in culture, but brown-type cultures more often undergo changes in pigmentation and growth rate whereas grey-type cultures more often undergo changes in mycelial growth (Stover, 1950). It is possible for a brown-type to mutate into a grey-type and *vice versa* in culture, but this happens very rarely (Huang & Patrick, 1971). A grey-type mutating to a brown-type occurs so rarely that it was once thought impossible and arises more readily when an isolate is passed through a host than in culture.

An interesting characteristic of the type species of the genus *Ceratocystis, Ct. fimbriata*, is the fact that it can undergo uni-directional mating switching. In this type of mating behaviour a typically self-fertile, homothallic isolate becomes self-sterile (Wilken *et al.*, 2014). However, when a self-fertile isolate mutates to become self-sterile it also undergoes a change in colour, changing from dark coloured colonies to light coloured colonies (Webster, 1967) (Wilken, Personal communication). Because nothing is known about the mating strategy of *T. basicola* it raises the question if these two natural types might represent isolates with differences in their mating capabilities like those of *Ct. fimbriata?* However, as no perithecia are seen in either the brown or grey type cultures, which would be expected if they are undergoing uni-directional mating type switching, this might not be the cause of the colour variation in these species.

The true reason behind these differences in colony colour is currently unknown (Stover, 1950), but besides being influenced by mating, the differences could be due to variation in expression of genes that form part of the melanin biosynthesis cluster. Fungi have been found to utilize various melanin biosynthesis pathways. The most widely utilized is the dihydroxynaphthalene (DHN) pathway (Jacobson, 2000, Langfelder *et al.*, 2003, Eisenman & Casadevall, 2012) and experiments by Wheeler & Stipanovic (1979) showed that this is likely the pathway utilized by *T. basicola* as well. However, it has also been found that disruption of chitin metabolism or the enzymes that catalyse the various steps in this biosynthesis pathway can influence or eliminate colour production in melanotic fungal species (Zimmerman *et al.*, 1995, Butler & Day, 1998, Cordero & Casadevall, 2017). This in turn can also influence the ability of a fungal species to sexually reproduce (Zimmerman *et al.*, 1995). Although Wheeler & Stipanovic (1979) stated that the difference in colour between the grey and brown type was down to differences in the abundance of their chlamydospores, hyphal and endoconidial production, this has not been my experience. Instead, the alteration in the expression in one of the late enzymes of the DHN pathway may be responsible for this difference in appearance.

Isolate variability

Macroscopic variation

Thielaviopsis basicola is very variable and unstable in culture. Variations in growth rate, mycelial type, colony colour and spore formation can often be seen, not only between different isolates of the fungus but also for different cultures of the same isolate. For this reason, some publications in the mid 1900's focussed specifically on investigating the macroscopic and microscopic variation among isolates of the species (Johnson & Valleau, 1935, Rawlings, 1940, Huang & Patrick, 1971, Maduewesi *et al.*, 1976). These variations in culture could arise from many different factors, but in the case of *T. basicola*, studies suggest that it likely has to do with either genetic change or culture condition (Huang & Patrick, 1971, Hood & Shew, 1997b).

Aside from the variability in morphology found in naturally isolated cultures of *T. basicola*, differences in morphology often also arise in sub-cultured, single colonies in regions referred to as "sectors". These "sectors" are wedge-shaped regions in a colony that have a morphology different to the rest of the colony or the parental colony (**Fig. 10**). More than ten sectors have been reported for some isolates (Rawlings, 1940), although the occurrence of so many in a single culture is rare. These sectors likely arise because adjacent chlamydospores in a chain of these structures can give rise to different cultural variants. Some authors have suggested that the individual spores in a chain might not be genetically identical (Huang & Patrick, 1971, Punja & Sun, 2000). However, minor epigenetic differences between the chlamydospores within a chain of spores seems a more likely reason for the morphological variations observed in culture.

Albino mutations give rise to very obvious sectors in culture. These sectors that arise in cultures of melanotic fungal species are typically due to mutation or disruption early in the melanin biosynthesis gene cluster (Zimmerman *et al.,* 1995, Langfelder *et al.,* 2003). As mentioned in the previous section, *T. basicola* appears to utilize the DHN-melanin pathway and the disruption leading to the development of an albino mutant is likely at the polyketide synthase (PKS) encoding region, which codes for the enzyme that catalyses the first step in DHN-melanin production (Langfelder *et al.*, 2003, Eisenman & Casadevall, 2012). Albino mutants have never been found to revert back to the original wild-type morphology naturally, but introduction of any of intermediate compounds (scylatone, vermalone, and 1,8-DHN) produced after the PKS step in the pathway into the growth media can cause a reversion of an albino to its melanised form (Wheeler & Stipanovic, 1979). This implies that the pathway remains active, giving more evidence that disruption of PKS is responsible for the change.

Albino mutants of phytopathogenic fungal species commonly have lower levels of pathogenicity, fitness and they can show differences in their structural morphology compared to their melanised counterparts (Jacobson, 2000, Langfelder *et al.*, 2003, Eisenman & Casadevall, 2012, Cordero & Casadevall, 2017). However, single spore cultures of *T. basicola* made from albino sectors result in cultures, which other than their loss in pigmentation, show very little difference to coloured colonies and continue to produce chlamydospores (Stover, 1950, Wheeler & Stipanovic, 1979, Punja & Sun, 2000). An albino mutant has in one study been observed to be slightly more pathogenic when compared to brown or grey isolates (Johnson & Valleau, 1935). However, as this was found only in a single experiment, using sterilized soil, this ability should be confirmed in non-sterilized soil environments.

Microscopic variation

Isolates of *T. basicola* can differ in their microscopic morphology. There are some microscopic differences that are present between the two morphotypes discussed earlier (**Table 5**), but many differences are also seen for isolates of the same morphotype. A very apparent difference between cultures is in their spore production. When Nag Raj & Kendrick (1975) described *C. elegans*, they argued that the endoconidial state should have preference when naming the fungus as this state is never lost in culture. However, based on early reports, this argument is not entirely correct as cultures of *T. basicola* containing only chlamydospores, only endoconidia or variations in the production of both spore types have been reported in cultures grown on PDA (Johnson & Valleau, 1935).

Although variation can be found in isolates grown on the same media, one of the most important factors in this variation appears to be nutrient availability and especially nutrient deficiency. For example, many of the structures depicted in the description of *Milowia nivea* Massee are not commonly seen when working with cultures of *T. basicola*, but some of them have been described in cultures growing under high levels of nutrient stress (**Fig. 11**).

In their monograph, Nag Raj & Kendrick (1975) explicitly question the nature of the globose, moniliform conidia (**Fig. 11a**) described by Massee (1884). However, when studying the effects of available nutrients on culture variability, Hood & Shew (1997b) described the development of "hyphae consisting of irregularly swollen and bulbous cells" and they published an image of these hyphae (**Fig. 11c**) that resemble those depicted by Massee (1884). These same structures were also described by Huang & Patrick (1971) 26 years earlier, while investigating cultural variation due to nutrient availability.

Studies on the histopathology of infection by *T. basicola* have included illustrations of hyphae that could be similar to those described by Massee (1884). Stover (1950) described the morphology of the fungus invading tobacco cells and noted that "the hyphae colonizing the cells were swollen and constricted at intervals" and globose and moniliform hyphae were obvious in a micrograph in this study. Other authors (Christou, 1962, Mathre *et al.,* 1966, Mims *et al.,* 2000) have also described similar beaded hyphae colonizing the roots of various plant species. For this reason, it is reasonable to conclude that the structures described by Massee (1884) were likely the same swollen and constricted hyphae described by many subsequent authors and that some of the other structures he described could have arisen due to stress factors in culture. This might also have been because the isolate with which he was working was from a species of grass. This is in contrast to the fact that the Poales are considered non-hosts of *T. basicola*, but some can support germination and development of the fungus (Rothrock & Nehl, 2000).

Conclusion

The black root rot fungus now known as *Thielaviopsis basicola* has had a complex taxonomic history since it was first described more than 150 years ago. However, results of recent investigations suggest that the taxonomy of the species has yet to be fully resolved. Although amplification of the LSU, SSU, and MCM7 regions is sufficient to resolve the generic level placement of species in the *Ceratocystidaceae* (De Beer *et al.,* 2014), the limited number of *T. basicola* isolates for which sequence data are available has prevented its accurate placement. Using these gene regions and newly developed primers for additional gene regions on a larger number of *T. basicola* isolates, should make it possible to answer remaining genus and species level questions relating to this important fungus.

A sexual state for *Tl. basicola* has yet to be discovered. This has led many authors to speculate that this fungus is a strictly asexual pathogen. However, new tools including for example whole genome sequencing will make it possible to determine the thallism of *T. basicola*. This in turn could contribute to a better understanding of the reproductive biology of the pathogen.

References

Abbas SQ, Niaz M & Ghaffar A (2007) *Thielaviopsis basicola*: a potential threat to agriculture and forestry in Pakistan. *Pakistan Journal of Botany* **39**: 985-990.

AbdAllah EF, Hashem A, Bahkali AH & Al-Huqail A (2011) First report of black root rot disease (*Thielaviopsis basicola*) of carrot in Saudi Arabia. *African Journal of Microbiology Research* **5**: 2867-2869.

Aderhold, R (1905) Impfversuche mit *Thielavia basicola* Zopf. *Arbeiten aus der Biologischen Abteilung für Land- und Forstwirtschaft am Kaiserlichen Gesundheitsamte* **4**: 463-465.

Allen SJ (1990) *Thielaviopsis basicola*, a new record on cotton in Australia. *Australasian Plant Pathology* **19**: 24-25.

Allison C (1938) Physiologic specialization of *Thielaviopsis basicola* on tobacco. *Phytopathology* **28**: 1.

Anderson TR & Patrick ZA (1978) Mycophagous amoeboid organisms from soil that perforate spores of *Thielaviopsis basicola* and *Cochliobolus sativus*. *Phytopathology* **68**: 1618-1626.

Arnold GWR (1986) *Lista de Hongos Fitopatógenos de Cuba*. Editorial Científico-Técnica, Cuba, La Habana.

Baard SW & Laubscher C (1985) Histopathology of blackhull incited by *Thielaviopsis basicola* in groundnuts. *Phytophylactica* **17**: 85-88.

Baker KF, Davis LH & Thomas HE (1953) Grafting failure of ornamentals induced by *Thielaviopsis basicola*. *Plant Disease Reporter* **37**: 526.

Bateman DF (1963) Influence of host & nonhost plants upon populations of *Thielaviopsis basicola* in soil. *Phytopathology* **53**: 1174-1177.

Berkeley MJ & Broome CE (1850) Notices of British fungi XL. *Annals and Magazine of Natural History Series 2*. **5**: 455-466.

Blume MC & Harman GE (1979) *Thielaviopsis basicola*: A component of the pea root rot complex in New York State. *Phytopathology* **69**: 785-788.

Bogdan C & Tihomir M (2000) Tobacco black root rot: *Thielaviopsis basicola* (Berk. & Broome) Ferraris; *Chala elegans* Nag Raj & Kendr. *Glasnik Zaštite Bilja* **23**: 203-206.

Bodker L, Leroul N & Smedegaard-Petersen V (1993) The occurrence in Denmark of black root rot of pea caused by *Thielaviopsis basicola*. *Plant Pathology* **42**: 820-823.

Borges RCF, Santos MDM, Macedo MA, Martins I, Nascimento AG, Boiteux LS, Fonseca MEN & Mello SCM (2014) First report of a wilt disease of *Tectona grandis* caused by *Thielaviopsis basicola* in Brazil. *New Disease Reports* **30**: 17.

Bosshard E, Heller W, Ladner J, Rüegg J, Schwizer T & Widmer A (2007) Decline of stone fruit trees in Switzerland. *Acta Horticulturae* **734**: 363-365.

Bowden RL, Wiese MV, Crock JE & Auld DL (1985) Root rot of chickpeas and lentils caused by *Thielaviopsis basicola*. *Plant Disease* **69**: 1089-1091.

Brierley WB (1915) The 'Endoconidia' of *Thielavia basicola* Zopf. *Annals of Botany* **29**: 483-493.

Burkholder WH (1916) Some root diseases of the bean. *Phytopathology* **6**: 104.

Butler MJ & Day AW (1998) Fungal melanins: A review. *Canadian Journal of Microbiology* **44**: 1115-1136.

Canter-Visscher TW & Over De Linden AJ (1972) Root and basal stem rot of grapevine caused by *Thielaviopsis basicola* (Berk. and Br.) Ferraris. *New Zealand Journal of Agricultural Research* **15**: 184-185.

Carmichael JW, Kendrick WB, Conners IL & Sigler L (1980) *Genera of Hyphomycetes*. The University of Alberta Press, Edmonton, Alberta, Canada.

Chandler, HP (1902). A revision of the genus *Nemophila*. *Botanical Gazette* **34**: 194-215.

Chittenden FJ (1911) Report [on investigations of sweet pea diseases]. *The Sweet Pea Annual* 35-39.

Christias C & Baker KF (1967) Chitinase as a factor in germination of chlamydospores of *Thielaviopsis basicola*. *Phytopathology* **57**: 1363-1367.

Christias C & Baker KF (1970) Ultrastructure and cleavage of chlamydospore chains of *Thielaviopsis basicola*. *Canadian Journal of Botany* **48**: 2305-2308.

Christou T (1962) Penetration and host-parasite relationships of *Thielavlopsis basicola* in the Bean plant. *Phytopathology* **52**: 194-198.

Cilliers AJ (2001) Resistance in new groundnut breeding lines to black pod rot caused by *Chalara elegans*. *South African Journal of Plant and Soil* **18**: 174-175.

Conant GH (1927) Histological studies of resistance in tobacco to *Thielavia basicola*. *American Journal of Botany* **14:** 457-480.

Cooke MC (1885) New British fungi. *Grevillea* **14**: 1-7.

Copes WE & Hendrix FF (1996) Chemical disinfestation of greenhouse growing surface materials contaminated with *Thielaviopsis basicola*. *Plant Disease* **80**: 885-886.

Corda ACJ (1838) *Icones Fungorum hucusque Cognitorum: Abbildungen der Pilze und Schwaemme*. J.G. Calve, Czechoslovakia, Prague.

Corda ACJ (1842) *Icones Fungorum hucusque Cognitorum: Abbildungen der Pilze und Schwaemme*. J.G. Calve Praque.

Cordero RJB & Casadevall A (2017) Functions of fungal melanin beyond virulence. *Fungal Biology Reviews* **31**: 99-112.

Coumans JVF, Harvey J, Backhouse D, Poljak A, Raftery MJ, Nehl D, Katz ME & Pereg L (2011) Proteomic assessment of host-associated microevolution in the fungus *Thielaviopsis basicola*. *Environmental Microbiology* **13**: 576-588.

Da Silva GS & Costa DLM (2013) Occurrence of black rot of carrots in the state of Maranhão. *Summa Phytopathologica* **39:**294**.**

Dalbosco M, El Tassa SOM & Duarte V (2004) Ocorrência de podridão negra, causada por *Chalara elegans*, em raízes de cenoura no Rio Grande do Sul. *Fitopatologia Brasileira* **29**: 336-336.

De Beer ZW, Duong TA, Barnes I, Wingfield BD & Wingfield MJ (2014) Redefining *Ceratocystis* and allied genera. *Studies in Mycology* **79**: 187-219.

Delgado A, Franco GM, Páez JI, Vega JM, Carmona E & Avilés M (2005) Incidence of cotton seedling diseases caused by *Rhizoctonia solani* and *Thielaviopsis basicola* in relation to previous crop, residue management and nutrients availability in soils in SW Spain. *Journal of Phytopathology* **153**: 710-714.

Delvecchio VG, Corbaz R & Turian G (1969) An ultrastructural study of the hyphae, endoconidia and chlamydospores of *Thielaviopsis basicola*. *Microbiology* **58**: 23-27.

Doidge EM (1950) The South African fungi and lichens to the end of 1945. *Bothalia* **5:**1-1094.

Doroszewska T & Przybys M (2007) Charakterystyka odpornosci gatunkow Nicotiana na czarna zgnilizne korzeni *Thielaviopsis basicola* [Berk. and Broome] Ferr. *Zeszyty Problemowe Postępów Nauk Rolniczych* **517**: 253-266.

Eisenman HC & Casadevall A (2012) Synthesis and assembly of fungal melanin. *Applied Microbiological Biotechnology* **93**: 931-940.

Farrow WM (1954) Tropical soil fungi. *Mycologia* **46**: 632-646.

Feder WA & Ford HW (1963) *Thielaviopsis basicola* associated with declining sweet orange seedlings in Florida. *Plant Disease Reporter* **47**: 666-668.

Ferraris TA (1912) *Pars 1: Fungi, Hyphales, Dematiaceae*. *Flora Italica Cryptogamica Vol. 8.* Stabilimento tipografico L. Cappelli, Rocca San Casciano, Italy.

Fitter, AH, Peat, HJ (1994). The ecological flora aatabase. *Journal of Ecology* **82**: 415-425. http://www.ecoflora.co.uk

Garibaldi A, Gilardi G & Gullino ML (2005) First Report of Root Rot Incited by *Thielaviopsis basicola* on Lamb's Lettuce (*Valerianella olitoria*) in Europe. *Plant Disease* **89**: 205-205.

Gayed SK (1969) Relation between tobacco leaf and root necrosis induced by *Thielaviopsis basicola* and its bearing on the nature of tobacco resistance to black root rot. *Phytopathology* **59**: 1596-1600.

Gayed SK (1972) Host range and persistence of *Thielaviopsis basicola* in tobacco soil. *Canadian Journal of Plant Science* **52**: 869-873.

Gayed SK (1970) The effect of benomyl on tobacco leaf necrosis induced by *Thielaviopsis basicola*. *Netherlands Journal of Plant Pathology* **76**: 125-128.

Geldenhuis MM, Roux J, Cilliers AJ, Wingfield BD & Wingfield MJ (2006) Clonality in South African isolates and evidence for a European origin of the root pathogen *Thielaviopsis basicola*. *Mycological Research* **110**: 306-311.

Gilbert WW (1909) The root-rot of tobacco caused by *Thielavia basicola*. *United States Department of Agriculture Bureau of Plant Industry Bulletin* **158.**

Gilbert WW (1926) An improved method of isolation of *Thielavia basicola*. *Phytopathology* **16**: 579.

Hassler M (2017) World Plants: Synonymic Checklists of the Vascular Plants of the World (version Feb 2017). (Roskov Y, Abucay L, Orrell T, *et al.*, eds.), Species 2000: Naturalis, Leiden, the Netherlands, Digital resource at www.catalogueoflife.org/col.

Hawes CR & Beckett A (1977) Conidium ontogeny in *Thielaviopsis basicola*. *Transactions of the British Mycological Society* **68**: 304-307.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, Abaci Ö & Aime C (2011) The Amsterdam declaration on fungal nomenclature. *IMA Fungus.* **2**: 105-112.

Hoestra H (1965) *Thielaviopsis basicola*, a factor in the cherry replant problem in the Netherlands. *Netherlands Journal of Plant Pathology* **71**: 180-182.

Hood ME & Shew HD (1996) Pathogenesis of *Thielaviopsis basicola* on a susceptible and a resistant cultivar of burley tobacco. *Phylopathology* **86**: 38-44

Hood ME & Shew HD (1997a) Reassessment of the role of saprophytic activity in the ecology of *Thielaviopsis basicola*. *Phytopathology* **87**: 1214-1219.

Hood ME & Shew HD (1997b) The influence of nutrients on development, resting hyphae and aleuriospore induction of *Thielaviopsis basicola*. *Mycologia*. **89:** 793-800.

Hood ME & Shew HD (1997c) Initial cellular interactions between *Thielaviopsis basicola* and tobacco root hairs. *Phytopathology* **87**: 228-235.

Hooker, WJ, Arnott, GAW (1841). *The Botany of Captain Beechey's Voyage*. HG Bohn, London.

Huang HC & Patrick ZA (1971) Variability of *Thielaviopsis basicola* in culture. *Canadian Journal of Botany* **49**: 1041-1047.

Huang J & Kang Z (2010) Detection of *Thielaviopsis basicola* in soil with real-time quantitative PCR assays. *Microbiological Research* **165**: 411-417.

Huang YT & Chen CY (2010) Two *Thielaviopsis* species on carrots in Taiwan. *Fungal Science* **25**: 69-74.

Jacobson ES (2000) Pathogenic roles for fungal melanins. *Clinical Microbiology Reviews* **13**: 708-717.

Johnson EM & Valleau WD (1935) Cultural variations of *Thielaviopsis basicola*. *Phytopathology* **25**: 1011-1018.

Johnson J (1916) Host plants of *Thielavia basicola*. *Journal of Agricultural Research* **7**: 289-300.

Johnson J & Hartman RE (1919) Influence of soil environment on the root-rot of tobacco. *Journal of Agricultural Research* **17**: 41-86.

Kamyshko OP (1968) Soil mycoflora of Gyjuvan district of Bukhara oblast. *Mycology & Phytopatholog.* **2**: 367-378 [In Russian].

Kasuyama S & Tanimei K (2008) Black root rot of sweet pea (*Lathyrus odoratus* L.), Japanese hornwort (*Cryptotaenia japonica* Hassk.) and root rot of garden pansy (*viola* × *wittrockiana* Gams) caused

by *Thielaviopsis basicola* in Okayama Prefecture. *Bulletin of the Agricultural Experiment Station, Okayama Prefectural General Agriculture Center (Japan).* **26**: 61-64.

Keller JR & Shanks JB (1955) Poinsettia root rot. *Phytopathology* **45**: 552-558.

Keller JR & Potter HS (1954) *Thielaviopsis* associated with root rot of some ornamental plants. *Plant Disease Reporter* **38**: 354-355.

King CJ & Presley JT (1942) A root rot of cotton caused by *Thielaviopsis basicola*. *Phytopathology* **32**: 752-761.

Kirchner O (1906) *Die Krankheiten und Beschädigungen unserer landwirtschaftlichen Kulturpflanzen: eine Anleitung zu ihrer Erkennung und Bekämpfung fur Landwirte, Gärtner, u.a.* Ulmer, E, Stuttgart, Germany.

Koike ST & Henderson DM (1998) Black root rot, caused by *Thielaviopsis basicola*, on tomato transplants in California. *Plant Disease* **82**: 447-447.

Lambe RC & Wills WH (1978) Pathogenicity of *Thielaviopsis basicola* to Japanese holly. *Plant Disease Reporter* **62**: 859-863.

Langfelder K, Streibel M, Jahn B, Haase G & Brakhage AA (2003) Biosynthesis of fungal melanins and their importace for human pathogenic fungi. *Fungal Genetics and Biology* **38**: 143-158.

Larter LNH & Martyn EB (1943) *A Preliminary List of Plant Diseases in Jamaica*. Imperial Mycological Institute, Kew, London, England.

Lim LL & Cole A (1984) Infection and colonisation of white clover seedling roots by fungi. *New Zealand Journal of Agricultural Research* **27**: 459-465.

Linderman RG & Toussoun TA (1967) Behavior of chlamydospores and endoconidia of *Thielaviopsis basicola* in nonsterilized soil. *Phytopathology* **57**: 729-731.

Linderman RG & Toussoun TA (1968) Pathogenesis of *Thielaviopsis basicola* in nonsterile soil. *Phytopathology* **58**: 1578-1583.

Lloyd AB & Lockwood JL (1963) Effect of soil temperature, host variety & fungus strain on *Thielaviopsis* root rot of peas. *Phytopathology* **53**: 329-331.

Lockwood JL, Yoder DL & Smith NA (1970) *Thielaviopsis basicola* root rot of soybeans in Michigan. *Plant Disease Reporter* **54**: 849-850.

Lucas GB (1948) Comparative cultural studies with *Thielavia basicola* and *Thielaviopsis basicola. Phytopathology* **38**: 16. [Abstract].

Lucas GB (1949) Studies on the morphology and cytology of *Thielavia basicola* Zopf. *Mycologia* **41**: 553-560.

Maduewesi JNC, Sneh B & Lockwood JL (1976) Improved selective media for estimating populations of *Thielaviopsis basicola* in soil [from soybean fields] on dilution plates [Fungus diseases]. *Phytopathology* **66:** 526-530

Maier CR & Staffeldt EE (1960) Cultural variability of selected isolates of *Rhizoctonia solani* and *Thielaviopsis basicola*, and the variability in their pathogenicity to Acala and Pima cotton, respectively. *Plant Disease Reporter* **44**: 956-961.

Massee GE (1884) Description and life-history of a new fungus, *Milowia nivea*. *Journal of the Royal Microscopical Society* **4**: 842-845.

Massee GE (1893) *British fungus-flora. A classified text-book of mycology*. George Bell & Sons, New York, London.

Massee GE (1912) A Disease of sweet peas, asters, and other plants. (*Thielavia basicola*, Zopf.). *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)*. **1**: 44-52.

Mathre DE & Ravenscroft AV (1966) Physiology of germination of chlamydospores and endoconidia of *Thielaviopsis basicola*. *Phytopathology* **56**: 337-342.

Mathre DE, Ravenscroft AV & Garber RH (1966) The role of *Thielaviopsis basicola* as a primary cause of yield reduction in cotton in California. *Phytopathology* **56**: 1213-1216.

Mauk PA & Hine RB (1988) Infection, colonization of *Gossypium hirsutum* and *G. barbadense*, and development of black root rot caused by *Thielaviopsis basicola*. *Phytopathology* **78**: 1662-1667.

McCormick FA (1925) Perithecia of *Thielavia basicola* Zopf in culture and the stimulation of their production by extracts from other fungi. *Connecticut Agricultural Experiment Station* **269**: 539-554.

McGovern RJ & Seijo TE (1999) Outbreak of black root rot in *Catharanthus roseus* caused by *Thielaviopsis basicola*. *Plant Disease* **83**: 396-396.

Meyer JR, Shew HD & Harrison UJ (1994) Inhibition of germination and growth of *Thielaviopsis basicola* by aluminum. *Phytopathology* **84**: 598-602.

Michel VV (2009) First report of *Chalara elegans* on roots of Black Elderberry. *Plant Disease* **93**: 963-963.

Michel VV (2015) First report of *Chalara elegans* causing wilt on *Lisianthus* in Switzerland. *Plant Disease* **99**: 1187.

Miczyńska Z & Jeziorska Z (1970) Virulence of four *Thielaviopsis basicola* (Berk. et Br.) Ferr. strains isolated in Pulawy. *Acta Microbiologica Polonica B* **2**: 121-126.

Mims CW, Copes WE & Richardson EA (2000) Ultrastructure of the penetration and infection of pansy roots by *Thielaviopsis basicola*. *Phytopathology* **90**: 843-850.

Mosma S & Struck C (2013) Studies on penetration, infection and colonization of lupin roots infected by *Thielaviopsis basicola*. *Science Research Reporter* **3**: 97-101.

Nag Raj TR & Kendrick WB (1975) *A monograph of Chalara and allied genera*. Wilfrid Laurier University Press, Waterloo, Ontario, Canada.

Naidoo K, Steenkamp ET, Coetzee MPA, Wingfield MJ & Wingfield BD (2013) Concerted evolution in the Ribosomal RNA Cistron. *PLOS ONE* **8**: e59355.

Nan ZB, Long PG, Skipp RA & Hopcroft DH (1992) Microscopy of invasion of red clover roots by *Trichocladium basicola*, and effects of benomyl and prochloraz. *Plant Pathology* **41**: 449-461.

Nehl DB, Aitken EAB & Harvey JA (2002) Genetic diversity of *Thielaviopsis basicola*. In *Field to Fashion 11th Australian Cotton Conference* (Vol. 1, p. 685-687). Australian Cotton Growers.

Nehl D, Harvey JA & Aitken EAB (2004) Occurrence of the black root rot fungus in soils surrounding Australian cotton properties. *Crop and Pasture Science*, **64**: 1112-1126.

Noshad DB, Punja ZK & Riseman A (2006) First report of *Thielaviopsis basicola* on *Daphne cneorum*. *Canadian Journal of Plant Pathology* **28**: 310-312.

O'Gara PJ (1915) Occurrence of *Thielavia basicola* as a root parasite of watermelons in the Salt Lake Valley, Utah. *Science* **42**: 314-314.

O'Brien RG & Davis RD (1994) Lettuce black root rot - a disease caused by *Chalara elegans*. *Australasian Plant Pathology* **23**: 106-111.

Papavizas GC & Lewis JA (1971) Survival of endoconidia and chlamydospores of *Thielaviopsis basicola* as affected by soil environmental factors. *Phytopathology* **61**: 108-113.

Patrick ZA, Toussoun TA & Thorpe HJ (1965) Germination of chlamydospores of *Thielaviopsis basicola*. *Phytopathology* **55**: 466-467.

Paulin-Mahady AE, Harrington TC & McNew D (2002) Phylogenetic and taxonomic evaluation of *Chalara, Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. *Mycologia* **94**: 62-72.

Paulin AE & Harrington TC (2000) Phylogenetic placement of anamorphic species of *Chalara* among *Ceratocystis* and other ascomycetes. *Studies in Mycology* **45**: 169-186.

Peglion V (1891) Marciume radicale delle piantine di tabacco causato dalla *Thielavia basicola* Zopf. *Centralblatt für Bakteriologie und Parasitenkunde, Abt. II* **3**: 580-584.

Pereg LL (2013) Black root rot of cotton in Australia: The host, the pathogen and disease management. *Crop and Pasture Science* **64**: 1112-1126.

Peterson JL (1967) Root and crown rot of Perennial *Phlox* caused by *Thielaviopsis basicola*. *Plant Disease Reporter* **51**: 436-437.

Pierre RE & Wilkinson RE (1970) Histopathological relationship of *Fusarium* and *Thielaviopsis* with beans. *Phytopathology* **60**: l-824.

Popov VI & Zdrojevskaya SD (1969) Regulation of soil fungistasis - a method to combat soil pathogens. *Mycology & Phytopathology*. **3**: 48-52. [In Russian].

Prinsloo GC (1986) Black root rot of chicory in South Africa. *Phytophylactica* **18**: 225-226

Prinsloo GC, Baard SW & Ferreira JF (1992) A scanning electron microscope study of the infection and colonization of chicory roots by *Thielaviopsis basicola*. *Phytophylactica* **24**: 293-296.

Punja ZK (2004) Virulence of *Chalara elegans* on bean leaves, and host-tissue responses to infection. *Canadian Journal of Plant Pathology* **26**: 52-62.

Punja ZK & Sun LJ (2000) Morphological and molecular characterization of *Chalara elegans* (*Thielaviopsis basicola*), cause of black root rot on diverse plant species. *Canadian Journal of Botany* **77**: 1801-1812.

Punja ZK, Chittaranjan S & Gaye MM (1992) Development of black root rot caused by *Chalara elegans* on fresh market carrots. *Canadian Journal of Plant Pathology* **14**: 299-309.

Rabenhorst L (1844) *Deutschlands Kryptogamen-Flora*. Eduard Kummer, Germany, Leipzig.

Ramette A, Moënne-Loccoz Y & Défago G (2003) Prevalence of fluorescent pseudomonads producing antifungal phloroglucinols and/or hydrogen cyanide in soils naturally suppressive or conducive to tobacco black root rot. *FEMS Microbiology Ecology* **44**: 35-43.

Rawlings RE (1940) Observations on the cultural and pathogenic habits of *Thielaviopsis basicola* (Berk. & Br.) Ferraris. *Annals of the Missouri Botanical Garden* **27**: 561-598.

Réblová M, Gams W & Štěpánek V (2011) The new hyphomycete genera *Brachyalara* and *Infundichalara*, the similar *Exochalara* and species of *'Phialophora* sect. *Catenulatae'* (*Leotiomycetes*). *Fungal Diversity* **46**: 67-86.

Rieuf P (1970) Parasites et saprophytes des plantes au Maroc. *Les cahiers de recherche agronomique* **28**: 179-357.

Riggs W & Mims CW (2000) Ultrastructure of chlamydospore development in the plant pathogenic fungus *Thielaviopsis basicola*. *Mycologia* **92**: 123-129.

Rosenbaun J (1912) Infection experiments with *Thielavia basicola* on ginseng. *Phytopathology* **2**: 191-199.

Rothrock CS & Nehl DB (2000) Reproductive potential of *Thielaviopsis basicola* on plant species and response of chlamydospores to host and nonhost exudates. *Phytopathology* **90**: S67.

Saccardo PA (1886a) *Clasterosporium fragile* (Sorok.) Sacc. *Sylloge Fungorum* **4**: 386.

Saccardo PA (1886b) *Torula basicola*. *Sylloge Fungorum* **4**: 257.

Sattler F (1936) Zur biologie von *Thielavia basicola* (B. et Br.) Zopf. *Phytopathologische Zeitschrift*. **9**: 1-51.

Schippers B (1970) Survival of endoconidia of *Thielaviopsis basicola* in soil. *Netherlands Journal of Plant Pathology* **76**: 206-211.

Selby AD (1896) Investigations of plant diseases in forcing house and garden. *Ohio Agricultural Experiment Station Bulletin* **73**: 221-246.

Selby AD (1904) Tobacco diseases and tobacco breeding. *Ohio Agricultural Experiment Station Bulletin* **156**: 87-114.

Simmonds JH (1966) *Host Index of Plant Diseases in Queensland*. Queensland Department of Primary Industries, Queensland, Australia.

Smith EF (1899) Wilt disease of cotton, watermelon and cowpea (*Neocosmospora* nov. gen.). *US Department of Agriculture Division of Vegetable Physiology and Pathology Bulletin* **17**:1-54.

Sorauer P (1893) Uber die Wurzelbraune der Cyclamen. *Zeitschrift für Pflanzenkrankheiten* **5**: 18-20.

Sorokïn NV (1876) Ueber *Helminthosporium fragile* sp. n. *Hedwigia* **15**: 113.

Staffeldt EE (1959) *Thielaviopsis basicola*, a part of the cotton (*Gossypum hirsutum*) seedling disease complex in New Mexico. *Plant Disease Reporter* **43**: 506-508.

Stanghellini ME & Barta DJ (1990) *Thielaviopsis* root rot of corn-salad. *Plant Disease* **74**: 81.

Stielow JB, Lévesque CA, Seifert KA, *et al.* (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia.* **35**: 242-263.

Stover RH (1950a) The black root rot disease of tobacco: I. Studies on the causal organism *Thielaviopsis basicola*. *Canadian Journal of Research* **28**: 445-470.

Stover RH (1950b) The black root rot disease of tobacco: II. Physiologic specialization of *Thielaviopsis basicola* on *Nicotiana tabacum*. *Canadian Journal of Research* **28**: 726-738.

Stover RH (1956) Effect of nutrition on growth and chlamydospore formation in brown and gray cultures of *Thielaviopsis basicola*. *Canadian Journal of Botany* **34**: 459-472.

Taubenhaus JJ (1914) *The diseases of the sweet pea*. Delaware College Agricultural Experiment Station, Delaware, USA.

Thaxter R (1892) Fungus in violet roots. *Connecticut Agricultural Experiment Station Annual Report*. New Haven, Connecticut, USA. p. 166-167.

The International Plant Names Index. (2012) Published on the Internet http://www.ipni.org [accessed 25 November 2017].

Thomas CA & Papavizas GC (1965) Susceptibility of sesame and castorbean to *Thielaviopsis basicola*. *Plant Disease Reporter* **49**: 256-257.

Tiddens BA (1934) Ueber die wurzelfaule der *Primula obconica*, veruscht durch *Thielaviopsis basicola* Ferraris. *Phytopathologische Zeitschrift.* **7**: 223-230.

Tosi L, Giovannetti M, Zazzerini A & Torre G (1988) Influence of mycorrhizal tobacco roots, incorporated into the soil, on the development of *Thielaviopsis basicola*. *Journal of Phytopathology* **122**: 186-189.

Tsao PH & Bricker JL (1966) Chlamydospores of *Thielaviopsis basicola* as surviving propagules in natural soils. *Phytopathology* **56**: 1012-1014.

Tsao PH (1962) Prevalence of *Thielaviopsis basicola* in California citrus soils. *Plant Disease Reporter* **46**: 357-359.

Tsao PH (1963) The relative susceptibility of certain varieties and hybrids of citrus species and relatives of *Thielaviopsis basicola*. *Plant Disease Reporter* **47**: 437-439.

Tsao PH & Van Gundy SD (1962) *Thielaviopsis basicola* as a citrus root pathogen. *Phytopathology* **52**: 781-786.

Tsao PW & Tsao PH (1970) Electron microscopic observations on the spore wall and operculum formation in chlamydospores of *Thielaviopsis basicola*. *Phytopathology* **60**: 613-616.

Turgeon BG & Yoder OC (2000) Proposed nomenclature for mating type genes of filamentous ascomycetes. *Fungal Genetics and Biology* **31**: 1-5.

Van der Ven R & van der Want JPH (1948) With a summary: *Thielaviopsis basicola* (Berk. et Br.) Ferraris the cause of a tobacco disease, new for the Netherlands. *Tijdschrift over Plantenziekten* **54**: 142-146.

Van Wyk M, Wingfield BD, Clegg PA & Wingfield MJ (2009) *Ceratocystis larium* sp. nov., a new species from *Styrax benzoin* wounds associated with incense harvesting in Indonesia. *Persoonia.* **22**: 75-82.

Vörös J (1958) Fungi imperfecti aus Ungarn. *Sydowia* **12**: 251.

Walker NR, Rothrock CS & Kirkpatrick TL (1999b) An important new pest interaction on cotton, *Meloidogyne incognita* and *Thielaviopsis basicola*. *Special Reports - University of Arkansas Agricultural Experiment Station* **193**: 91-96.

Weber RWS & Tribe HT (2004) Moulds that should be better known: *Thielaviopsis basicola* and *T. thielavioides*, two ubiquitous moulds on carrots sold in shops. *Mycologist* **18**: 6-10.

Webster RK (1967) The origin of self-sterile, cross-fertile strains and culture sterility in *Ceratocystis fimbriata*. *Mycologia* **59**: 212-221.

Went, FAFC (1893). De ananasziekte van het suikerriet. *Mededeelingen van het Proefstation West-Java* **V**: 1-8.

Wheeler MH & Stipanovic RD (1979) Melanin biosynthesis in *Thielaviopsis basicola*. *Experimental Mycology* **3**: 340-350.

Wheeler TA, Hake KD & Dever JK (2000) Survey of *Meloidogyne incognita* and *Thielaviopsis basicola*: their impact on cotton fruiting and producers' management choices in infested fields. *Journal of Nematology* **32**: 576-583.

White TJ, Bruns T, Lee S & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ & White TJ, eds., *PCR Protocols: A sequencing guide to methods and applications.* Academic Press, San Diego p. 315–322.

Wick RL & Moore LD (1983) Histopathology of root disease incited by *Thielaviopsis basicola* in *Ilex crenata*. *Phytopathology*. **73**: 561-564.

Wilken PM, Steenkamp ET, Wingfield MJ, De Beer ZW & Wingfield BD (2014) DNA Loss at the *Ceratocystis fimbriata* mating locus results in self-sterility. *PLOS ONE* **9**: e92180.

Wilken PM, Steenkamp ET, Wingfield MJ, De Beer ZW & Wingfield BD (2017) Which MAT gene? *Pezizomycotina* (Ascomycota) mating-type gene nomenclature reconsidered. *Fungal Biology Review* **35:** 242-263.

Wills WH & Lambe RC (1978) Pathogenicity of *Thielaviopsis basicola* from Japanese holly (*Ilex crenata)* to some other host plants. *Plant Disease Reporter* **61**: 1102-1106.

Wilson AM, Wilken PM, Van der Nest MA, Steenkamp ET, Wingfield MJ & Wingfield BD (2015) Homothallism: an umbrella term for describing diverse sexual behaviours. *IMA Fungus* **6**: 207-214.

Yarwood CE (1981) The occurrence of *Chalara elegans*. *Mycologia* 524-530.

Yarwood CE (1946) Isolation of *Thielaviopsis basicola* from soil by means of carrot disks. *Mycologia* **38**: 346-348.

Yarwood CE (1974) Habitats of *Thielaviopsis* in California. *Plant Disease Reporter* **58**: 54-56.

Yoder OC, Valent B & Chumley F (1986) Genetic nomenclature and practice for plant pathogenic fungi. *Phytopathology* **76**: 383-385.

Yoshinari S, Tamami K & Mayama M (2010) Postharvest development of black root rot caused by *Chalara elegans* on Tokushima-grown carrots and the possibility of disease control. *Annual Bulletin of Research Institute of Human Life Science.* **4**: 29-37.

Young BR (1970) Root rot of passionfruit vine (*Passiflora edulis* Sims.) in the Auckland area. *New Zealand Journal of Agricultural Research* **13**: 119-125.

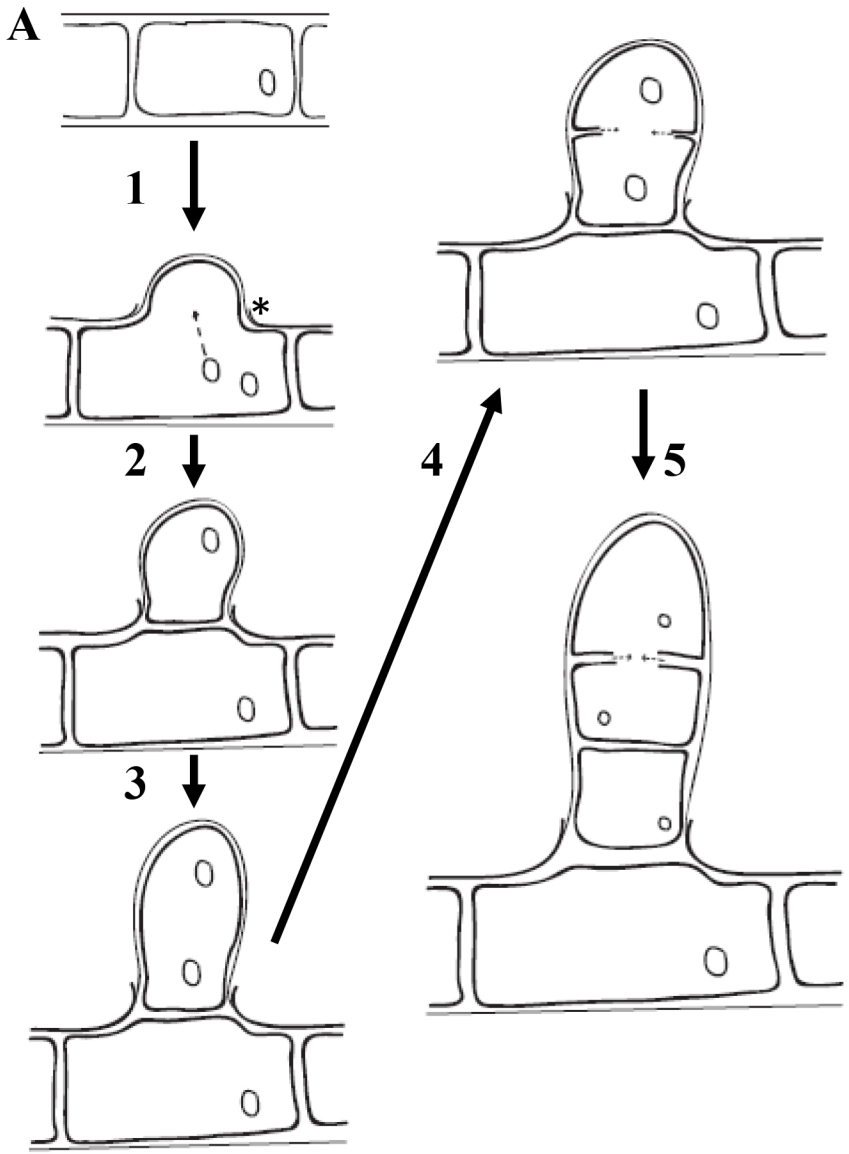
Zimmerman WC, Blanchette RA, Burnes TA & Farrell RL (1995) Melanin and perithecial development in *Ophiostoma piliferum*. *Mycologia* **87**: 857-863.

Zopf W (1876) *Thielavia basicola* Zopf. Genus Novum Perisporiacearum. *Verhandlungen des Botanischen Vereins für die Provinz Brandenburg* **18**: 101-105.

Zopf W (1891) Über die Wurzelbräune der Lupinen, eine neue Pilzkrankheit. *Zeitschrift für Pflanzenkrankheiten* **1**: 72-76.

Figures

Figure 1: Development and morphology of the chlamydospores of *Thielaviopsis basicola*. [A] Development of the chlamydospore chain [images produced from descriptions given in Delvecchio et al. (1969), Christias & Baker (1970), Tsao & Tsao (1970), and Rigs & Mims (2000).]: (A1) The development of the chlamydospore chain begins with the enteroblastic development of a small protrusion in a mycelial cell which ruptures the outer cell wall of the parental cell (*). The nucleus of the parental cell divides and a daughter nucleus migrates into this protrusion. (A2) As the protrusion continues to elongate a septum develops in a centripetal fashion separating the developing chlamydospore chain from the parental cell. (A3) After the septum has completely formed, the nucleus divides again and one of the daughter nuclei migrate into the apical region of the elongating protrusion. (A4) A second septum develops inward in a centripetal fashion dividing the growing protrusion. (A5) The process of nuclear division and septum formation continues until the chain contains about six segments, but his number can vary depending on growth conditions and medium. After the chain has completed its development, the chlamydospores within it develop their characteristic dark walls simultaneously, and the chain envelope forms. [B] The internal morphology of the chlamydospore chain and the separation of spores [images from Riggs & Mims (2000)]: (B1, B2) The contents of each chlamydospore is surrounded by a two-layered cell wall (W). The first is an outer electron-dense layer and the second is a thin inner electron-transparent layer. The entire chain of chlamydospores is also surrounded by a wall composed of two layers known as the chain envelope (CE) that is likely part of the cell wall of the parental cell. The CE also consists of an outer electron-dense layer and an inner electron-transparent layer. However, the CE is only about a third of the thickness of the chlamydospore walls. The thickest part of the chlamydospore walls are at the four corners, where the inner electron-transparent layer extends into the corners, creating a weak spot (arrow heads) where the operculum develops during spore germination. An electron-transparent layer (arrows) separates the chlamydospores in the chain from each other. (B3) However, the spores are not separated at the septal pore regions which are usually blocked by a septal pore plug (arrow) that extends into each spore after maturity. (B4) In the presence of chitin, the electron-transparent inner layer of the CE is digested inwardly, allowing movement of the chlamydospores within the still intact, outer electron-dense layer. As the chlamydospores start moving apart, the envelope eventually breaks. Chlamydospore separation completes when the septal pore plug pulls from one of the joined spores or breaks into two under the stress of the moving segments.


A
1
2
3
4
5
*

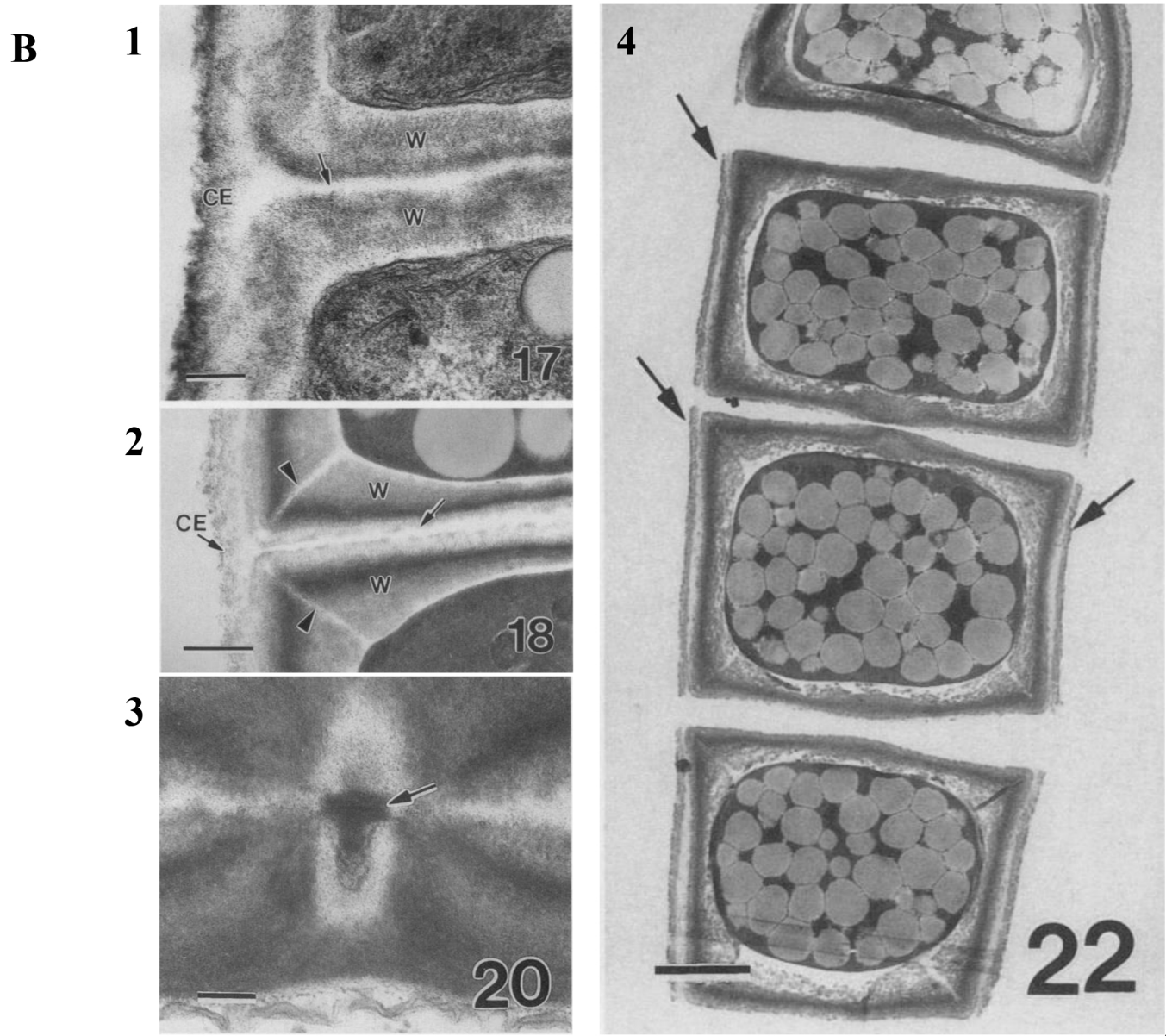
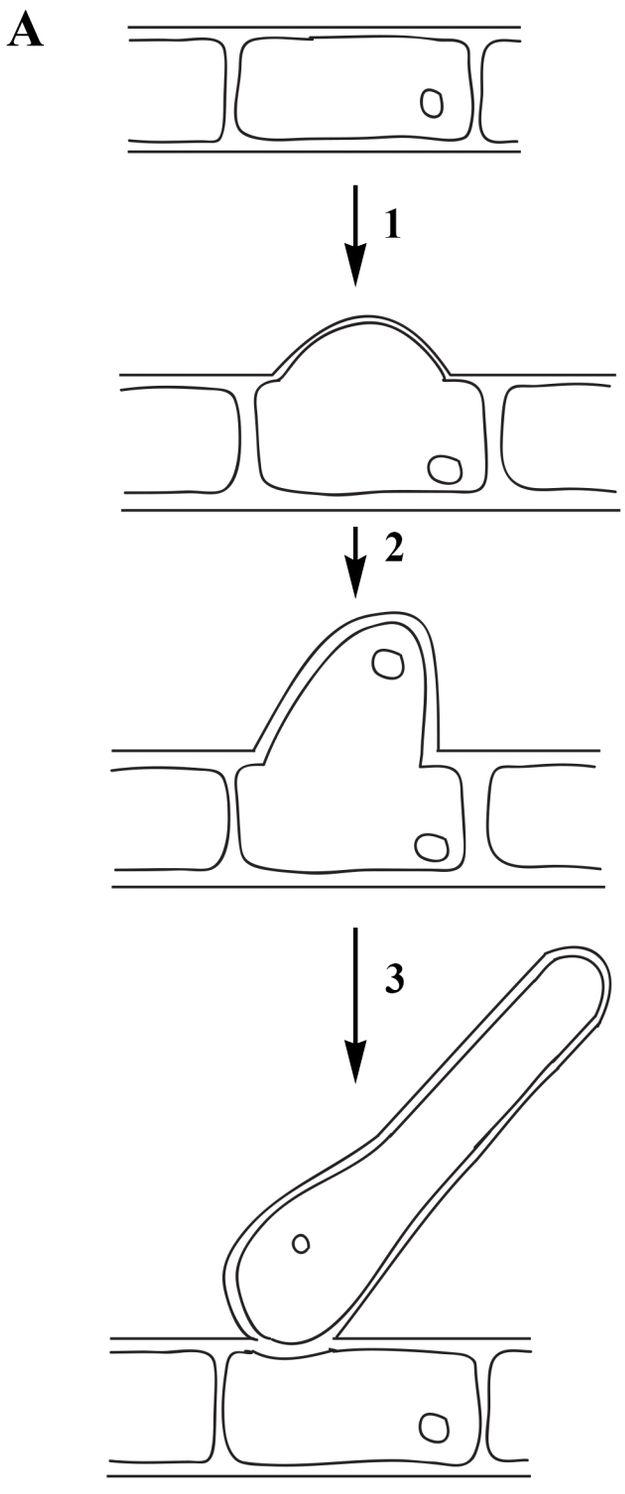

B
1
W
CE
W
17
2
W
CE
W
18
3
20
4
22

Figure 2: Development of the conidiogenous cell and endoconidia of *Thielaviopsis basicola* [adapted from Brierly (1915)]. [A] Development of the conidiogenous cell: (A1) The development of the conidiogenous cell begins with the formation of a thin walled protrusion in a mycelial cell containing a single nucleus. (A2) After some time, the nucleus of the parent cell divides and one of the daughter nuclei migrates into the developing protrusion. (A3) The protrusion then starts to take on the characteristic vial shape of the phialide and becomes cut off from the parent cell by the formation of a transverse wall at its base. [B] Development of the endoconidia: (B1) After the conidiogenous cell has completed its development, its nucleus divides and one of the daughter nuclei migrate into the apical region of the cell. (B2) The nucleus becomes cut off from the rest of the conidiogenous cell by the development of a septum that grows inwards from a transverse plain, finally closing off at its centre. (B3) The walls of the endoconidium split into an outer closed sheath and an internal cell enclosed in a thin, hyaline membrane that is only about half as thick as the original cell wall. (B4) The outer sheath splits revealing the endoconidium. (B5) The nucleus of the conidiogenous cell divides again and one of the daughter nuclei migrate to the apical region of the cell. (B6) A wall develops right below the transverse plain where the original septum developed giving rise to the second endoconidium immediately below the first. (B7) Continuous development of subsequent endoconidia in this manner, forces the preceding one out of the conidiogenous cell.


A
1
2
3

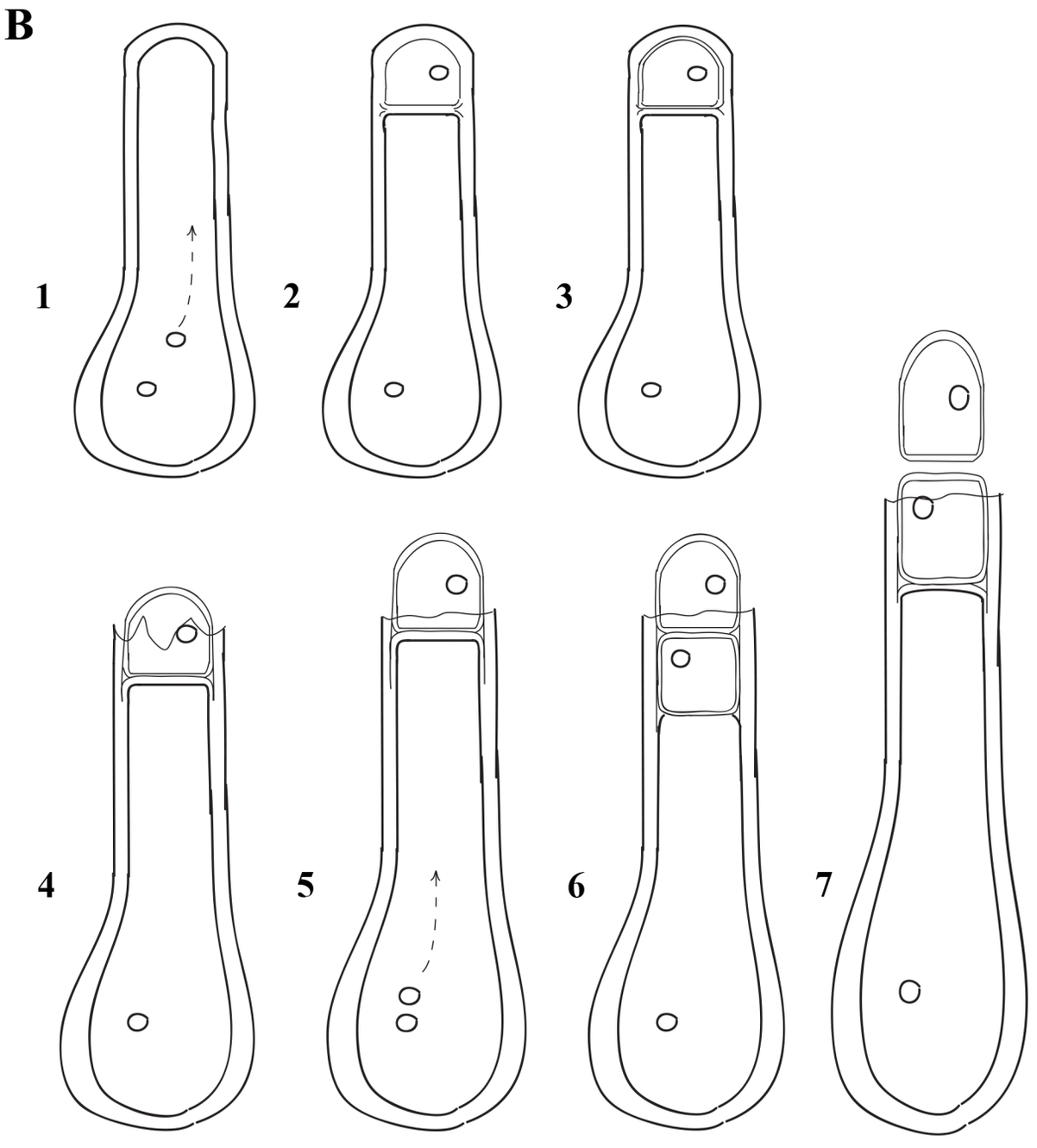

B
1
2
3
4
5
6
7

Figure 3: Secondary chlamydospores as illustrated and photographed by (a) Stover (1950a), (b) Schippers (1975), and (c, d) myself during the present study (Chapter 2). The exact mode of conidiogenises and origin of the veil-like structure remains unknown.

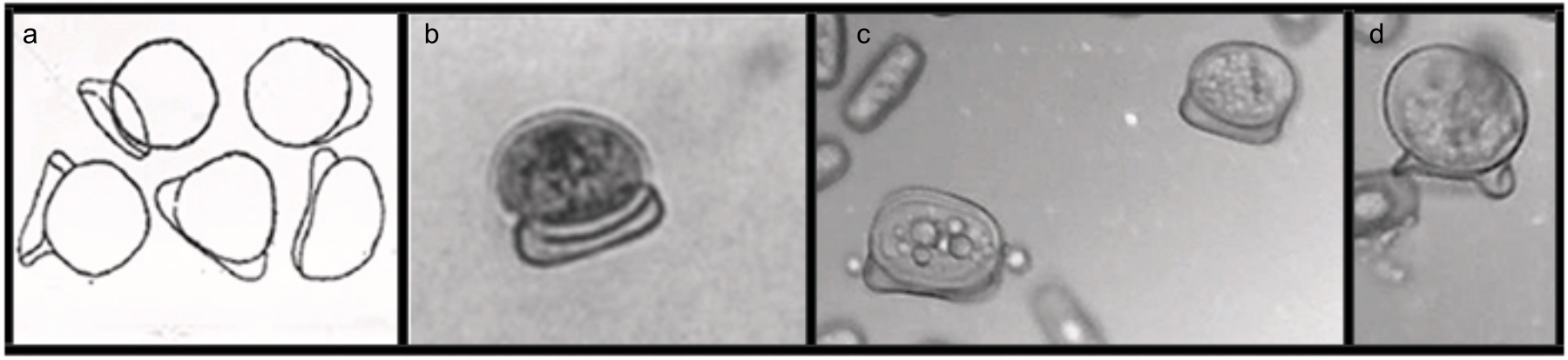

a
b
c
d

Figure 4: Number of research papers published in the period from 1992 to 2016 containing the names "*Thielaviopsis basicola*" or "*Chalara elegans*" in their titles. (Databases used: Google Scholar and Scopus)

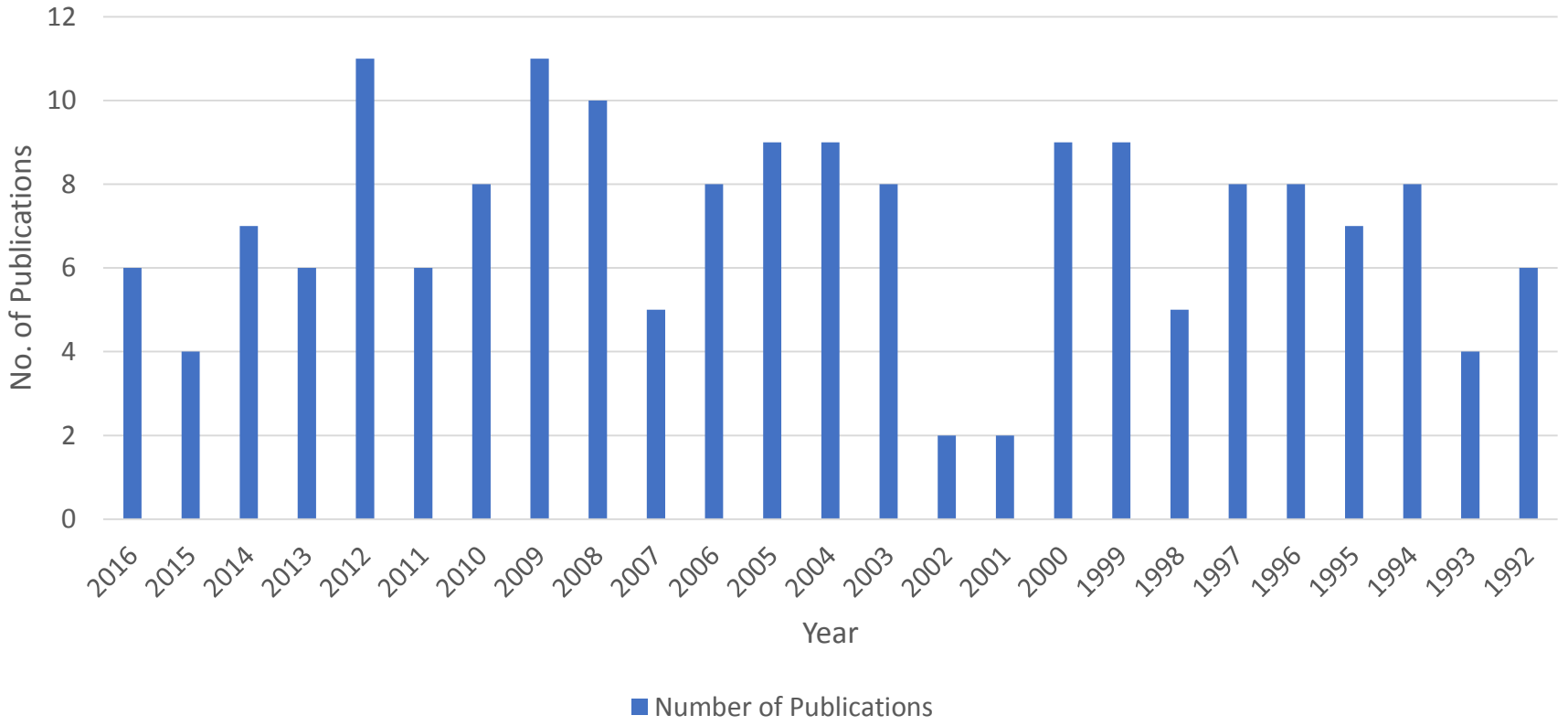

12
10
8
6
4
2
0
No. of Publications
2016
2015
2014
2013
2012
2011
2010
2009
2008
2007
2006
2005
2004
2003
2002
2001
2000
1999
1998
1997
1996
1995
1994
1993
1992
Year
Number of Publications

Figure 5: Illustrations of chlamydospores in the descriptions of *Thielaviopsis basicola* and its synonyms. (a, b) Figures of *Torula basicola* described by Berkeley and Broome (1850). (c, d) Figures of *Milowia nivea* described by Massee (1884). (e, f) Figures of *Helminthosporium fragile* described by Sorokin (1876).

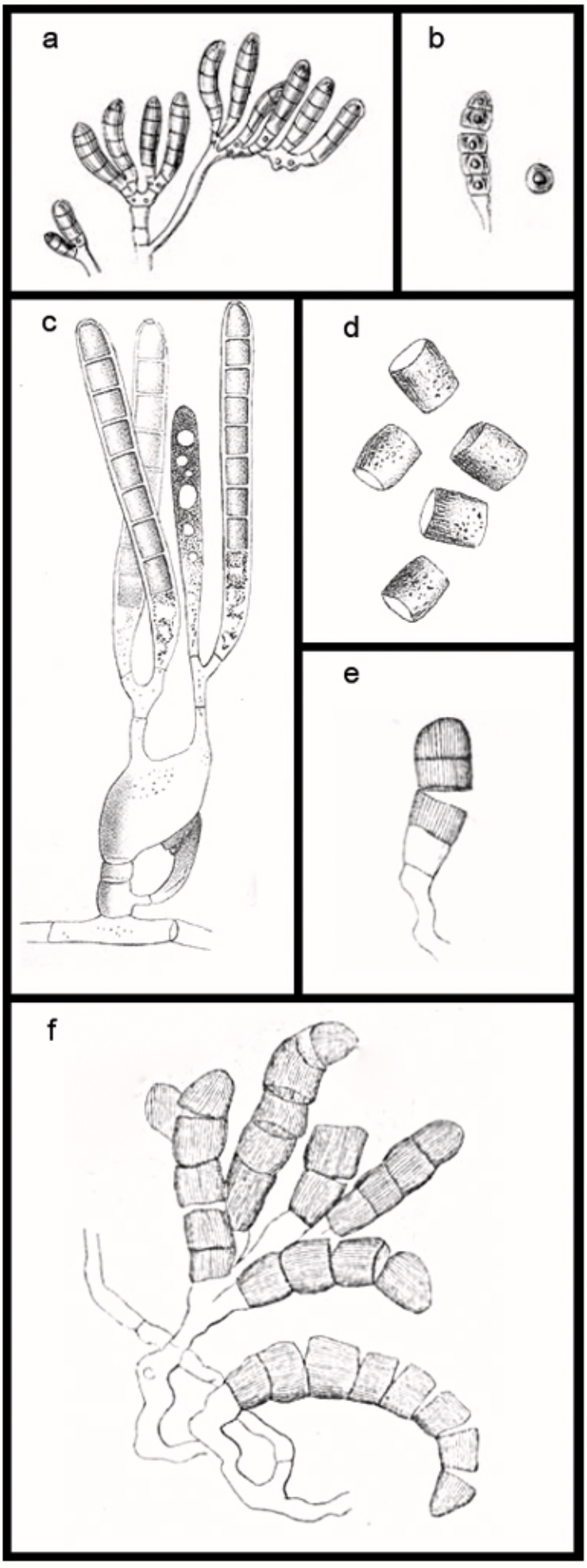

a
b
c
d
e
f

Figure 6: Drawings made by Massee (1912) in the paper where he synonymized *Milowia nivea* with *Thielaviopsis basicola.*

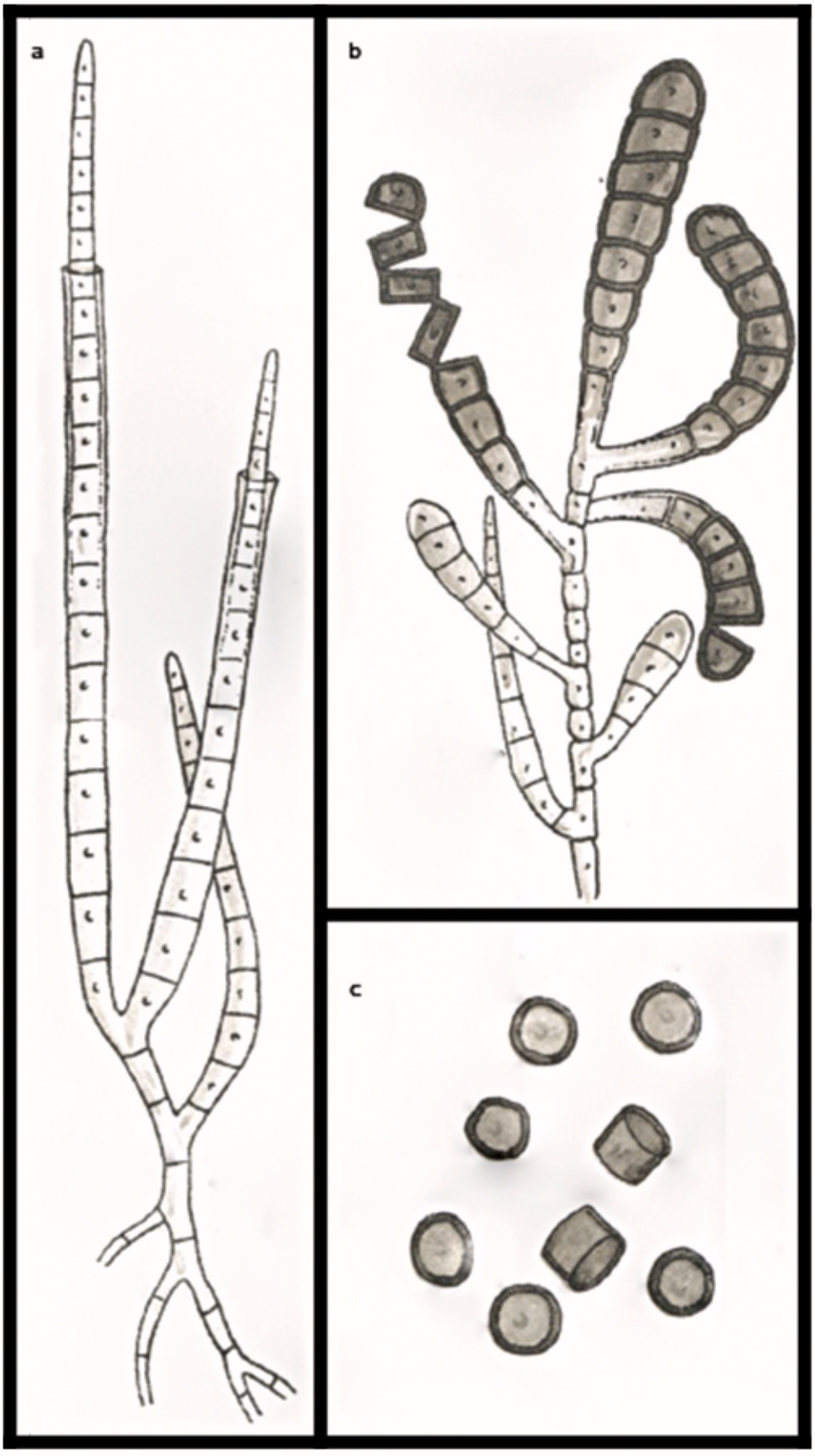

a
b
c

Figure 7: Drawings by Zopf (1891) of what he considered the (a-c) asexual state (*Thielaviopsis basicola*) and (d-h) sexual state (*Thielavia basicola*) of the same fungus.

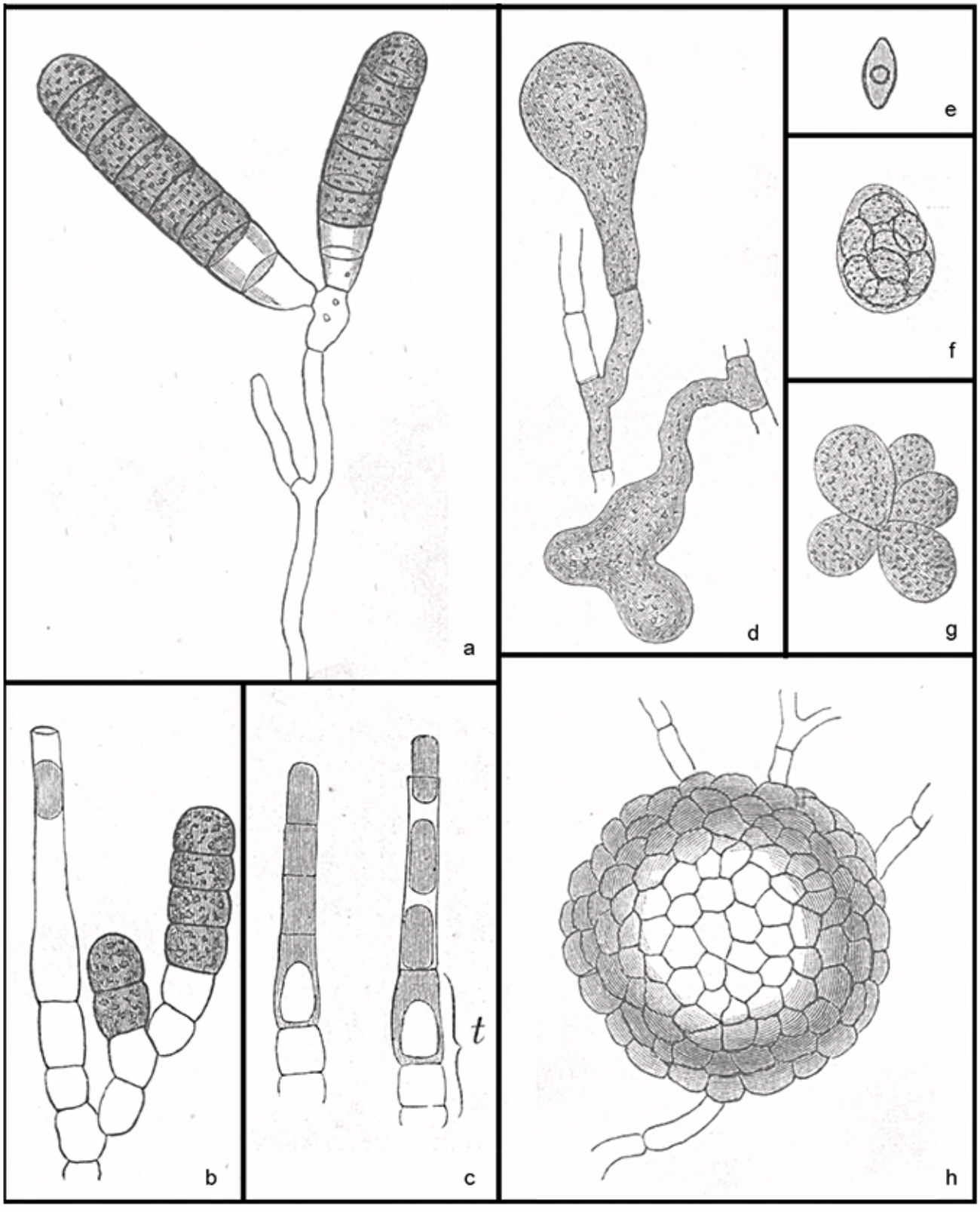

a
b
c
t
d
e
f
g
h

Figure 8: A brown type isolate (a) and a grey type isolate (b) of *T. basicola* photographed during the present study (Chapter 2).

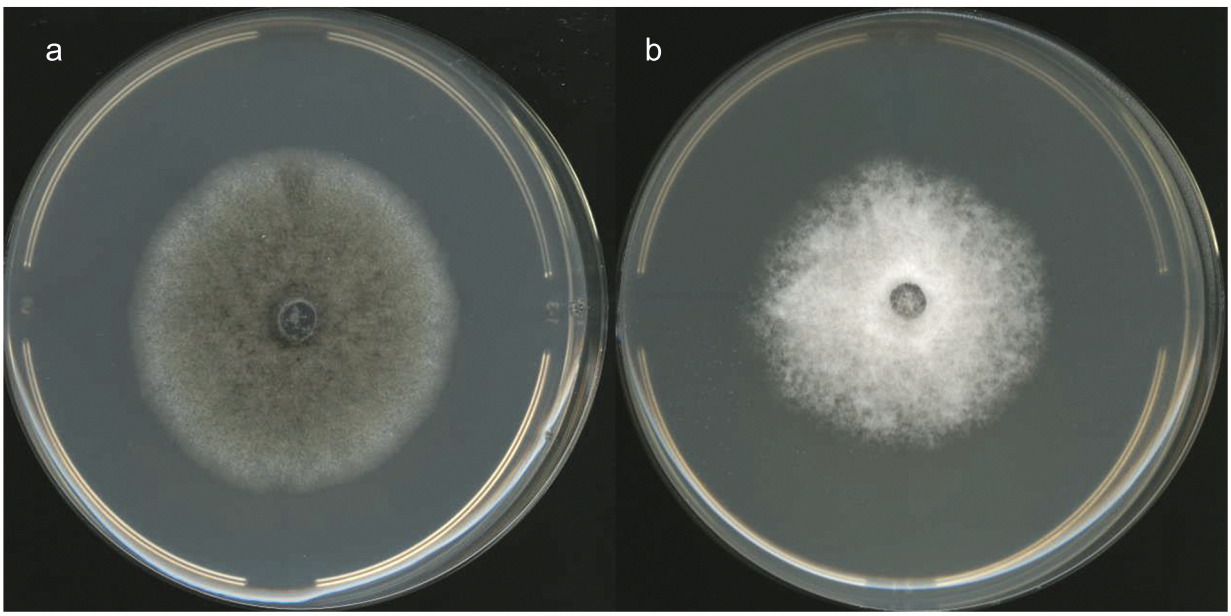
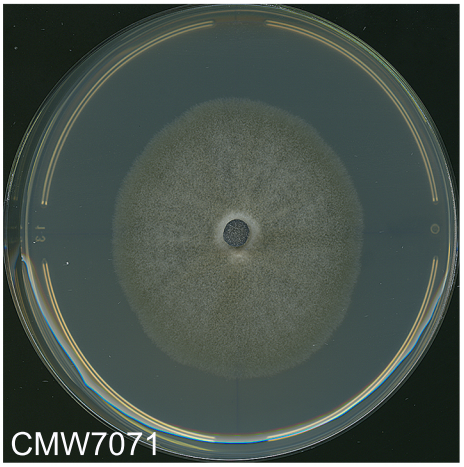
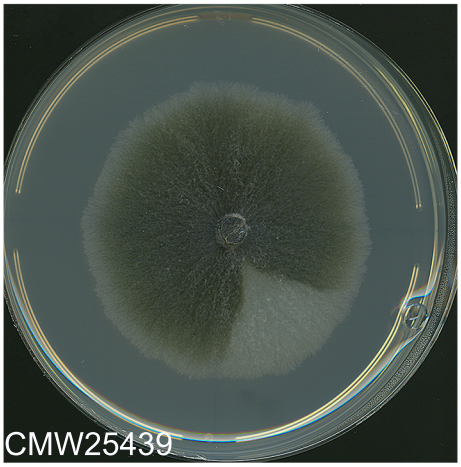
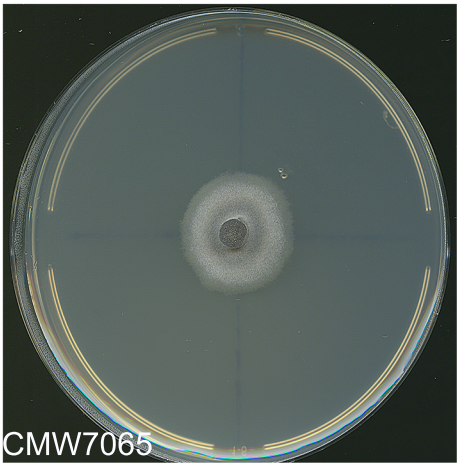

a
b

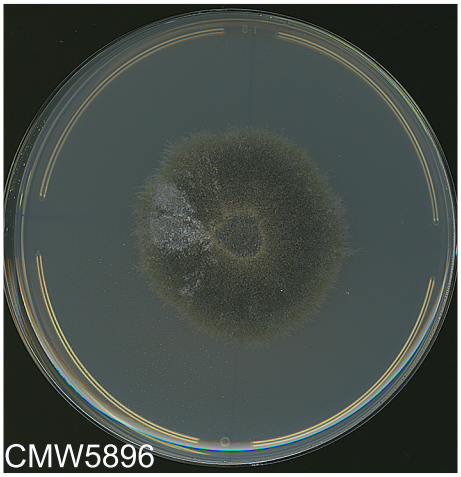
Figure 9: Some culture morphologies of a number of *Thielaviopsis basicola* isolates. All cultures shown are 10 days old and clear differences can be observed in colony colour, mycelial growth and overall culture growth rate.

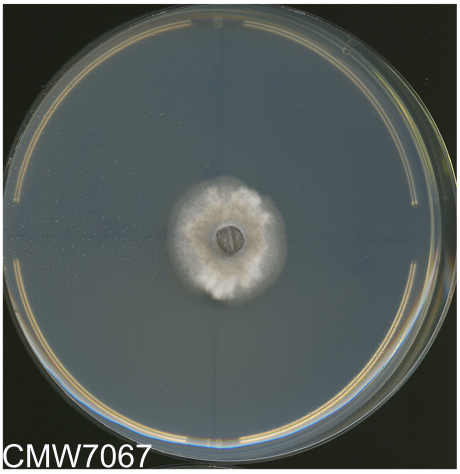

CMW7071

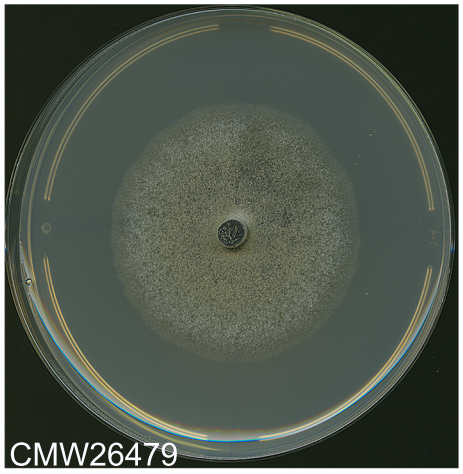

CMW25439

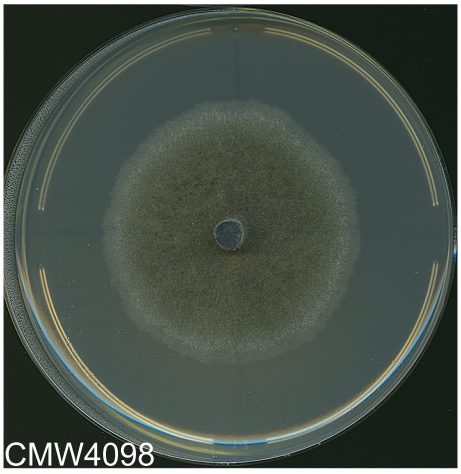

CMW7065


CMW26479


CMW5896


CMW7067


CMW26479


CMW4098

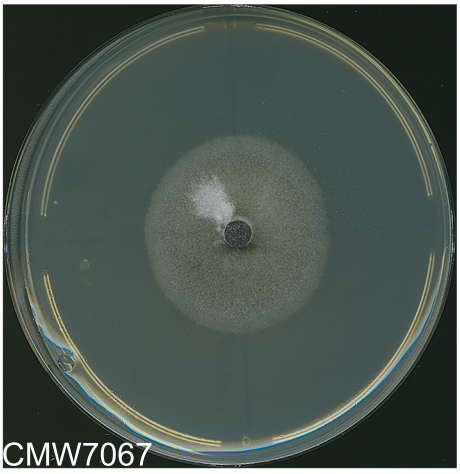

CMW7067

Figure 10: Cultures containing sectors photographed during the present study (Chapter 2). (a, c) cultures containing single albino sectors and (b) a culture containing two albino sectors.

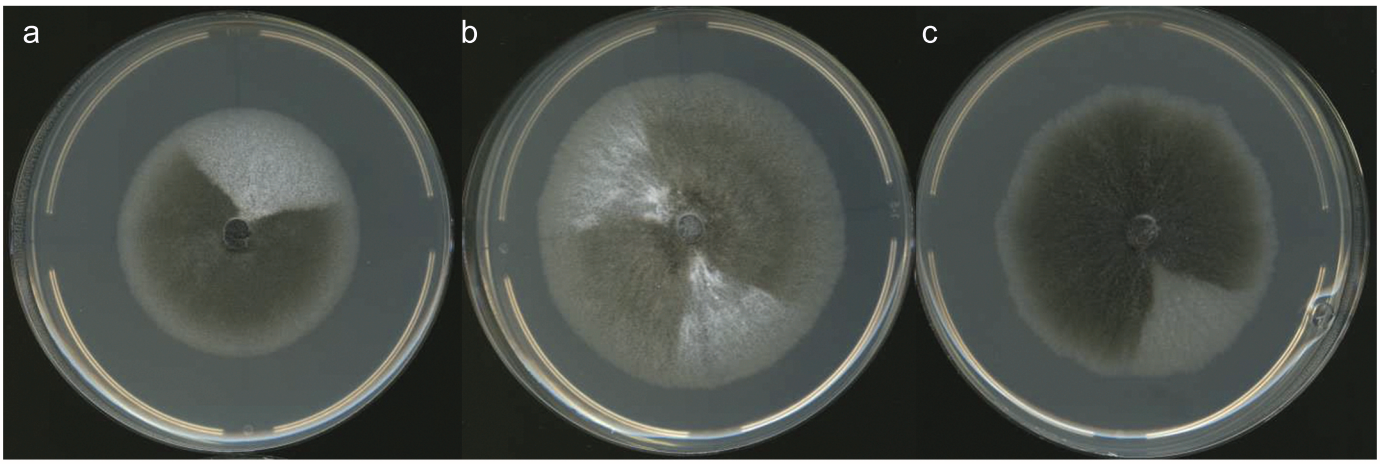
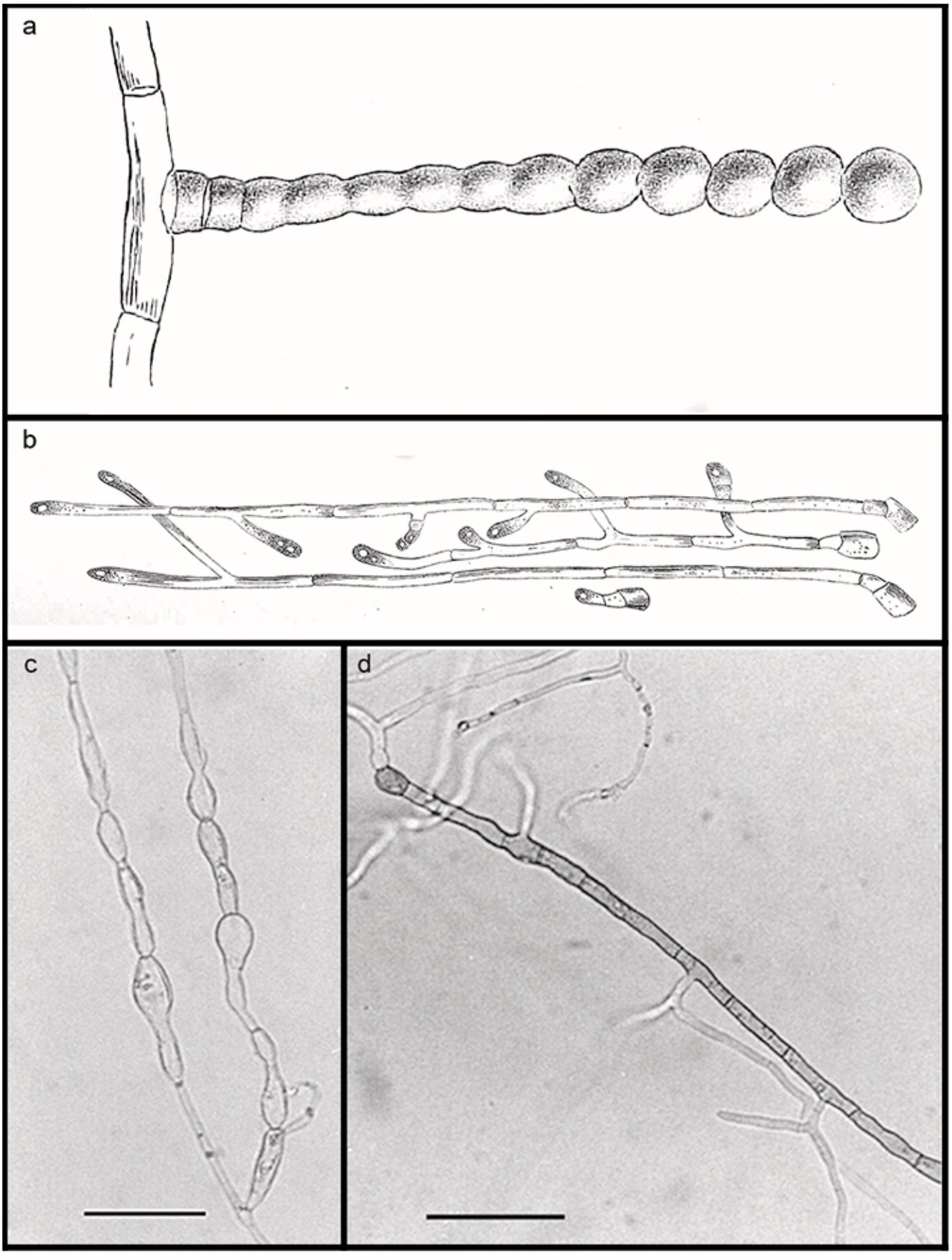

a
b
c

Figure 11: The resemblance between structures drawn by Massee (1884) during his original description of *Milowia nivea* (a, b) and those of nutrient stressed isolates of *Thielaviopsis basicola* (c,d) photographed by Hood and Shew (1997b).


a
b
c
d

1.7.2. Tables

Table 1: Plant species susceptible to infection by *Thielaviopsis basicola.*

Family	Species	Common name	References
Adoxaceae	*Sambucus nigra*	Elderberry	Michel (2009)
Amaranthaceae	*Amaranthus retroflexus*	Redroot pigweed	Gayed (1972)
	Beta vulgaris	Beet	Aderhold (1905)
	Chenopodium album	Lamb's quarters	Gayed (1972)
Apiaceae	*Apium graveolens*	Celery	Aderhold (1905)
	Cryptotaenia japonica	Japanese hornwort	Kasuyama & Tanimei (2008)
	Daucus carota	Wild carrot	Aderhold (1905)
	Pastinaca sativa	Parsnip	Taubenhaus (1914)
Apocynaceae	*Catharanthus roseus*	Madagascar periwinkle	McGovern & Seijo (1999)
Aquifoliaceae	*Ilex aquifolium*	English holly	Lambe & Wills (1978)
	Ilex aquipernyi	Dragon lady holly	Lambe & Wills (1978)
	Ilex crenata	Japanese holly	Lambe & Wills (1978)
	Ilex cornuta	Chinese holly	Lambe & Wills (1978)
	Ilex opaca	American holly	Lambe & Wills (1978)
Araceae	*Elaeis guineensis*	Oil palm	Stover (1950a)
	Epipremmum aureus (as *Scindapsus aureus*)	Devil's ivy	Keller & Potter (1954)
Araliaceae	*Panax quinquefolia* (as *Aralia quinquefolia*)	Ginseng	Selby (1904)
Asteraceae	*Aster* sp.	-	Massee (1912)
	Cichorium intybus	Chicory	Prinsloo (1986)
	Erigeron canadensis (as *Conyza canadensis*)	Horseweed	Gayed (1972)
	Gerbera jemsonii	Baberton daisy	Keller & Potter (1954)
	Lactuca sativa	Lettuce	O'Brien & Davis (1994)
	Scorzonera hispanica	Black salsify	Aderhold (1905)
	Senecio cruentus	-	Keller & Potter (1954)
	Senecio elegans	Wild cineraria	Zopf (1876b)
	Sonchus oleraceus	Common sowthistle	O'Brien & Davis (1994)
Begoniaceae	*Begonia perflorens (semperflorens?)*	Wax begonia	Johnson (1916)
	Begonia rubra	Orange begonia	Selby (1896)
	Begonia tuberhybrida	Tuberous begonia	Aderhold (1905)
	Catalpa speciosa	Northern catalpa	Selby (1896)
Brassicaceae	*Brassica oleracae*	Cabbage	Yarwood (1981)
	Capsella bursa-pastoris	Shapherd's purse	Massee (1912)
	Cochlearia armoracia	Horseradish	Sorokïn (1876)
Caprifoliaceae	*Lonicera* sp.	Honeysuckle	Baker *et al.* (1953)
	Valerianella locusta	Corn-salad plants	Stanghellini & Barta (1990)
	Valerianella olitoria	Lamb's lettuce	Garibaldi *et al.* (2005)
Convolvulaceae	*Ipomoea coccinea*	Red morning glory	Johnson (1916)
Cucurbitaceae	*Citrullus lanatus*	Watermelon	O'Brien & Davis (1994)

Family	Species	Common name	References
	Citrullus vulgaris	African melon	O'Gara (1915)
	Cucumis flexuosus	Armenian cucumber	Johnson (1916)
	Cucumis sativus	Cucumber	Johnson (1916)
	Cucurbita maxima	Arikara squash	Johnson (1916)
	Cucurbita moschata	Crookneck pumpkin	Johnson (1916)
	Cucurbita pepo	Field pumpkin	Johnson (1916)
	Luffa acutangula (as *Cucumis acutangulus*)	Angled luffa	Johnson (1916)
	Melilotus indica	Muskmelon	Johnson (1916)
Cyperaceae	*Blysmus compressus*	Flat sedge	Massee (1884)
Equisetaceae	*Equisetum arvense*	Horsetail	Gayed (1972)
Euphorbiaceae	*Euphorbia pulcherrima*	Poinsettia	Keller & Shanks (1955)
	Ricinus communis	Castorbean	Thomas & Papavizas (1965)
Fabaceae	*Arachis hypogaea*	Peanut	Johnson (1916)
	Astragalus sinicus	Chinese milkvetch	Johnson (1916)
	Cassia chamaecrista	Partridge pea	Johnson (1916)
	Cicer arietinum	Chickpea	Bowden *et al.* (1985)
	Cytisus scoparius	Common broom	Johnson (1916)
	Desmodium tortuosum	Dixie ticktrefoil	Johnson (1916)
	Lablab purpureus (as *Dolichos lablab*)	Hyacinthbean	Johnson (1916)
	Galactia sp.	Milk peas	Johnson (1916)
	Glycine hispida	Soybean	Johnson (1916)
	Glycine max	Soybean	Lockwood *et al.* (1970)
	Kummerowiai striata (as *Lespedeza striata)*	Japanese clover	Johnson (1916)
	Lathyrus odoratus	Sweet pea	Chittenden (1911)
	Lens esculenta	Lentils	Johnson (1916)
	Lotus corniculatus	Birds foot-trefoil	Johnson (1916)
	Lotus halophilus (as *Lotus villosus*)	Greater bird's-foot trefoil	Johnson (1916)
	Lupinus albus	White lupin	Zopf (1891)
	Lupinus angustifolius	Blue lupin	Zopf (1891)
	Lupinus luteus	Annual yellow lupin	Zopf (1891)
	Lupinus micranthus (as *Lupinus hirsutus*)	Small flowered lupin	Johnson (1916)
	Lupinus thermis	White lupin	Zopf (1891)
	Medicago lupulina	Black medick	Gayed (1972)
	Medicago sativa	Alfalfa	Burkholder (1916)
	Medicago polymorpha (as *Medicago denticulate*)	California burclover	Johnson (1916)
	Melilotus alba	Bokhara clover	Johnson (1916)
	Melilotus indica	Sweet clover	Johnson (1916)
	Onobrychis crista-galli	Cockscomb sainfoin	Zopf (1891)
	Onobrychis viciaefolia	Common sainfoin	Johnson (1916)
	Ornithopsus sativus	Seradella	Johnson (1916)
	Phaseolus acutifolius	Tepary bean	Johnson (1916)
	Phaseolus coccineus (as *Phaseolus multiflorus*.)	Runner bean	Kirchner (1906)

Family	Species	Common name	References
	Phaseolus vulgaris	Common bean	Aderhold (1905)
	Pisum sativum	Pea	Berkeley & Broome (1850)
	Robinia pseudoacacia	Black locust	Johnson (1916)
	Sclotis chinensis	-	Johnson (1916)
	Strophostyles helvola	Amberique-bean	Johnson (1916)
	Tephrosia virginiana	Goat-rue	Johnson (1916)
	Trifolium hybridum	Alsike clover	Burkholder (1916)
	Trifolium incarnatum	Crimson clover	Johnson (1916)
	Trifolium pratense	Red clover	Burkholder (1916)
	Trifolium repens	White clover	Gilbert (1909)
	Trigonella caerulea	Blue fenugreek	Zopf (1891)
	Trigonella foenum-graecum	Fenugreek	Johnson (1916)
	Ulex europaeus	Gorse	Johnson (1916)
	Vicia faba	Broad bean	Johnson (1916)
	Vicia villosa	Hairy vetch	Johnson (1916)
	Vigna sinensis	Cowpea	Smith (1899)
Gentianaceae	*Eustoma grandiflorum*	Lisianthus	Michel (2015)
	Pelargonium tricolor x P. ovale sp. ovale	Pansy geranium	Doroszewska & Przybys (2007)
Hydrohyllaceae	*Nemophila auriculata*	-	Berkeley & Broome (1850)
	Nemophila aurita	Blue fiesta flower	Johnson (1916)
	Nemophila insignis	Baby blue eyes	Johnson (1916)
Lamiaceae	*Tectona grandis*	Teak	Borges *et al.* (2014)
Malvaceae	*Gossypium herbaceum*	Levant cotton	Smith (1899)
	Gossypium hirsutum	Mexican cotton/upland cotton	Staffeldt (1959)
	Malva neglecta	Round leaved mallow	Gayed (1972)
Onagraceae	*Clarkia elegans*	Elegant clarkia	Rieuf (1970)
Orchidaceae	*Cypripedium* sp	-	Massee (1912)
	Paphiopedilum grossianum	-	Johnson (1916)
Oxalidaceae	*Oxalis corniculata*	Creeping woodsorrel	Gilbert (1909)
Paeoniaceae	*Paeonia suffruticosa*	Moutan peony	Baker *et al.* (1953)
Papaveraceae	*Papaver nudicaule*	Iceland poppy	Johnson (1916)
Passifloraceae	*Passiflora edulis*	Passionfruit vine	Young (1970)
Pedaliaceae	*Sesamum indicum*	Sesame	Thomas & Papavizas (1965)
Pinaceae	*Pinus radiata*	Radiata pine	Allen (1990)
	Pinus pinaster	Pinaster pine	Allen (1990)
Plantaginaceae	*Cymbalaria muralis* (as *Linaria cymbalaria*))	Ivy leaved toadflax	Johnson (1916)
	Linaria maroccana	Moroccan toadflax	Johnson (1916)
Poaceae	*Triticum aestivum*	Wheat	Pereg (2013)
Polemoniaceae	*Phlox drummondii*	Annual phlox	Johnson (1916)
	Phlox paniculata	Perennial phlox	Peterson (1967)
Polygonaceae	*Polygonum persicaria*	Lady's thumb	Gayed (1972)

Family	Species	Common name	References
Portulacaceae	*Portulaca oleracea*	Purslane	Johnson (1916)
Primulaceae	*Cyclamen* sp	-	Sorauer (1893)
	Cyclamen persicum	Persian cyclamen	Keller & Potter (1954)
Rosaceae	*Prunus armeniaca*	Apricot	Bosshard *et al.* (2007)
	Prunus avium	Sweet cherry	Hoestra (1965)
	Prunus pissardii nigra	Purple leaved plum	Hoestra (1965)
Rutaceae	*Citrus sinensis*	Sweet orange	Tsao (1963)
	Citrus limon X Poncirus trifoliata	Citrimon	Tsao (1963)
	Citrus taiwanica	Nansho daidai	Tsao (1963)
	Citrus reticulata	Cleopatra mandarin	Tsao (1963)
	Citrus aurantium	Keen sour orange	Tsao (1963)
	Citrus jambhiri	Limoneira rough lemon	Tsao (1963)
	Citrus sinensis X Citrus renticulata	Troyer citrange	Tsao (1963)
	Citrus limonia	Rangpur lime	Tsao (1963)
	Citrus ichangensis X Citrus grandis	Ichang pummelo	Tsao (1963)
	Poncirus trifoliata	Towne trifoliate orange	Tsao (1963)
	Citrus reticulata X Citrus paradisi	Siamelo	Tsao (1963)
	Citrus aurantifolia	West indian lime	Tsao (1963)
	Citrus renticulata X citrus sinensis	Carrizo citrange	Tsao (1963)
Scrophulariaceae	*Nuttallanthus canadensis* (as *Linaria canadensis*)	Blue toadflax	Gilbert (1909)
	Nemesia sp.	-	O'Brien & Davis (1994)
Solanaceae	*Datura cornucopia*	Devil's trumpet	Johnson (1916)
	Datura metel	Metel	Johnson (1916)
	Datura stramonium	Jimsonweed	Johnson (1916)
	Datura tatula	Moon flower	Johnson (1916)
	Nicotiana atropurpurea	-	Johnson (1916)
	Nicotiana alata	Jasmine tobacco	Johnson (1916)
	Nicotiana calyciflorae	-	Johnson (1916)
	Nicotiana glauca	Tree tobacco	Johnson (1916)
	Nicotiana glutinosa	-	Johnson (1916)
	Nicotiana langsdorffii	Langdroff's tobacco	Johnson (1916)
	Nicotiana latterrima	-	Johnson (1916)
	Nicotiana longiflora	Longflower tobacco	Johnson (1916)
	Nicotiana repanda	Fiddleleaf tobacco	Johnson (1916)
	Nicotiana sanderae	Flowering tobacco	Johnson (1916)
	Nicotiana sylvestris	Woodland tobacco	Johnson (1916)
	Nicotiana tabacum	Cultivated tobacco	Peglion (1891)
	Nicotiana tabacum (as *Nicotiana chinensis*)	-	Johnson (1916)
	Nicotiana tabacum (as *Nicotiana macrophylla*)	-	Johnson (1916)
	Nicotiana rustica	Aztec tobacco	Kirchner 1906
	Nicotiana rustica (as *Nicotiana angustifolia*)	-	Johnson (1916)
	Petunia hybrida	Petunia	Johnson (1916)
	Solanum carolinense	Carolina horsenettle	Johnson (1916)

Family	Species	Common name	References
	Lycopersicon esculentum	Tomato	Koike & Henderson (1998)
Styraceae	*Styrax benzoin*	Gum benjamin tree	Van Wyk *et al.* (2009)
Theaceae	*Camellia japonica*	Japanese camellia	Baker *et al.* (1953)
Thymelaeaceae	*Daphne cneorum*	Rose daphne	Noshad *et al.* (2006)
Ulmaceae	*Ulmus americana*	American elm	Keller & Potter (1954)
Violaceae	*Viola odorata*	Wood violet	Thaxter (1892)
	Viola tricolor	Johnny jump up	Johnson (1916)
	Viola carnula	Pansy	Copes & Hendrix (1996)
	Viola × Wittrockiana	Garden pansy	Kasuyama & Tanimei (2008)
Vitaceae	*Vitis rupestris*	Grapevine: Rupestris st. George	Canter-Visscher & Over De Linden (1972)
	Vitis rupestris X Vitis mourvedre	Rupestris 1202	Canter-Visscher & Over De Linden (1972)
	Vitis berlandieri X Vitis riparia	Kober 5BB	Canter-Visscher & Over De Linden (1972)
	Vitis berlandieri X Vitis riparia	Oppenhein SO4	Canter-Visscher & Over De Linden (1972)
	Vitis solonis X Vitis othello	Othello 1613	Canter-Visscher & Over De Linden (1972)
	Vitis champinii	Dog ridge	Canter-Visscher & Over De Linden (1972)

Table 2: Countries/Regions where *Thielaviopsis basicola* has been reported to occur.

Continent	Country	Region	Reference
Africa	Morocco		Rieuf (1970)
	South Africa		Doidge (1950)
Asia	China		Huang & Kang (2010)
	Indonesia		Van Wyk *et al.* (2009)
	Japan	Okayama	Kasuyama & Tanimei (2008)
	Japan	Shizuoka Prefecture	Doroszewska & Przybys (2007)
	Japan	Tokushima	Yoshinari *et al.* (2010)
	Korea		Rosenbaun (1912)
	Pakistan		Abbas *et al.* (2007)
	Russia	Moscow	Yarwood (1981)
	Saudi-Arabia		AbdAllah *et al.* (2011)
	Tadjikistan		Popov & Zdrojevskaya (1969)
	Taiwan		Huang & Chen (2010)
	Uzbekistan		Kamyshko (1968)
Australasia	Australia	Brisbane	O'Brien & Davis (1994)
	Australia	NSW	Young (1970)
	Australia	Queensland	Simmonds (1966)
	New Zealand	Auckland	Young (1970)
Europe	Belgium		Rosenbaun (1912)
	Budapest		Vörös (1958)
	Croatia	Kutjevo	Bodgan (2000)
	Denmark		Bodker *et al.* (1993)
	Germany	Munich	Rosenbaun (1912)
	Italy		Tosi *et al.* (1988)
	Netherlands		Van der Ven & Van der Want (1948)
	Poland	Pulawy	Miczyńska & Jeziorska (1970)
	Poland		Bodker *et al.* (1993)
	Spain		Delgado *et al.* (2005)
	Switzerland		Ramette *et al.* (2003)
	United Kingdom	England	Yarwood (1981)
North America	Canada		Gayed (1970)
	Canada	Alberta	Yarwood (1981)
	Canada	British Colombia	Punja *et al.* (1992)
	Canada	Ontario	Anderson & Patrick (1978)
	Canada	Quebec	Punja *et al.* (1992)
	Cuba		Arnold (1986)
	Jamaica		Larter & Martyn (1943)
	Panama		Farrow (1954)
	USA	Alabama	Lambe & Wills (1978)
	USA	Arizona	Yarwood (1981)
	USA	Arkansas	Walker *et al.* (1999b)
	USA	California	Tsao (1962)
	USA	Connecticut	Johnson & Hartman (1919)
	USA	Florida	Feder & Ford (1963)
	USA	Illinois	Rosenbaun (1912)
	USA	Indiana	Rosenbaun (1912)

Continent	Country	Region	Reference
	USA	Kentucky	Johnson & Hartman (1919)
	USA	Maryland	Rosenbaun (1912)
	USA	Michigan	Lloyd & Lockwood (1963)
	USA	Minneapolis	Yarwood (1981)
	USA	Minnesota	Yarwood (1981)
	USA	Mississippi	Walker *et al.* (1999b)
	USA	Missouri	Yarwood (1946)
	USA	Nebraska	Rosenbaun (1912)
	USA	New Mexico	Staffeldt (1959)
	USA	New York	Rosenbaun (1912)
	USA	North Carolina	Lambe & Wills (1978)
	USA	Ohio	Rosenbaun (1912)
	USA	Pennsylvania	Rosenbaun (1912)
	USA	Tennessee	Lambe & Wills (1978)
	USA	Utah	O'Gara (1915)
	USA	Vermont	Rosenbaun (1912)
	USA	Virginia	Lambe & Wills (1978)
	USA	Wisconsin	Rosenbaun (1912)
	USA	Texas	Wheeler *et al.* (2000)
South America	Brazil		Borges *et al.* (2014)
	Brazil	Maranhao	Da Silva & Costa (2013)
	Brazil	Rio Grande do Sul	Dalbosco *et al.* (2004)

Table 3: List of authors that investigated the life cycle of *Thielaviopsis basicola*, the aspect of the life cycle they investigated and the substrate they were working on.

Aspect of life cycle studied	Substrate	Author
Chlamydospore and endoconidia germination	Carrot extract agar and cotton root extract agar	Mathre & Ravenscroft (1966)
Chlamydospore chain separation	Chitinase solution or cellulase solution	Christias & Baker (1967)
Chlamydospore germination	Plant residue	Patrick *et al.* (1965)
Endoconidia and phialide development	Banana or potato agar	Brierley (1915)
Endoconidial development	N/A	Hawes & Beckett (1977)
Infection cycle	Tobacco	Conant (1927)
Infection cycle	Tobacco	Stover (1950a)
Infection cycle	Bean	Christou (1962)
Infection cycle	Citrus	Tsao & Van Gundy (1962)
Infection cycle	Cotton	Mathre *et al.* (1966)
Infection cycle	Cotton	Linderman & Toussoun (1967)
Infection cycle	Bean	Pierre & Wilkinson (1970)
Infection cycle	Japanese holly	Wick & Moore (1983)
Infection cycle	White clover	Lim & Cole (1984)
Infection cycle	Groundnuts	Baard & Laubscher (1985)
Infection cycle	Cotton	Mauk & Hine (1988)
Infection cycle	Carrot	Punja *et al.* (1992)
Infection cycle	Red Clover	Nan *et al.* (1992)
Infection cycle	Chicory	Prinsloo *et al.* (1992)
Infection cycle	Tobacco	Hood & Shew (1996)
Infection cycle	Tobacco	Hood & Shew (1997c)
Infection cycle	Pansy	Mims *et al.* (2000)
Infection cycle	Bean leaves	Punja (2004)
Infection cycle	Lupin	Mosma & Struck (2013)
Ultrastructure of chlamydospores and chlamydospore chain development	Potato dextrose agar	Riggs & Mims (2000)
Ultrastructure of chlamydospores and chlamydospore chain separation	Cornmeal agar	Tsao & Tsao (1970)
Ultrastructure of chlamydospores and chlamydospore chain separation	Potato dextrose agar	Christias & Baker (1970)
Ultrastructure of hyphae, endoconidia and chlamydospores	2% malt extract agar	Delvecchio *et al.* (1969)

Table 4: List of authors investigating the existence of host specificity and the existence of different physiologic races of *Thielaviopsis basicola* and their important findings.

Reference	Findings
Rosenbaun (1912)	*Thielaviopsis basicola* isolated from tobacco, cotton and ginseng could cause disease on tobacco and ginseng plants.
Johnson (1916)	A single isolate of *T. basicola* was used to induce black root rot symptoms on more than 100 different plant species. He concluded that no specialized races of the fungus exist and its ability to parasitize a host is dependent on the resistance or susceptibility of the plant.
Johnson & Hartman (1919)	Found *T. basicola* to be relatively constant in its pathogenicity, no matter the culture age or strain.
Tiddens (1934)	A *T. basicola* isolate from *Primula obconica* caused more severe infection on *P. obconica* than *P. malacoides* Authors concluded that different pathological races of *T. basicola* exist. Isolates from Primula, tobacco and poinsettia were most pathogenic on the host from which they occur
Sattler (1936)	Tobacco plants were susceptible to American *T. basicola* isolates from tobacco and not susceptible to *T. basicola* isolates from Germany and Holland isolated from *Phaseolus multiflorus, Cyclamen* and *Primula obconica.* Bean and lupin plants were susceptible to the European isolates and not to the American isolates.
Allison (1938)	Separated *T. basicola* into four different physiological races each having a different level of pathogenicity to different lines of tobacco
Rawlings (1940)	Found that *T. basicola* isolates from Tennessee grown tobacco, Texan grown cotton and Dutch grown Primula differed in their ability to cause infection in cotton, tobacco, primula, peanut and watermelon plants. The tobacco isolate was most virulent causing infection on all hosts tested. The cotton isolate was moderately virulent causing infection on watermelon and *Primula* and some symptoms on cotton and peanuts in the first experiment. When repeated, the isolate could cause infection on all four these hosts. The isolate was never able to infect tobacco. The *Primula* isolate was least virulent causing some symptoms on cotton and peanuts in the first experiment and on cotton and watermelon when the experiment was repeated.
Stover (1950a)	Brown wild-type isolates were more pathogenic than grey wild-type isolates.
Keller & Shanks (1955)	Poinsettia *T. basicola* isolates could cause disease on poinsettia plants but not tobacco plants and tobacco *T. basicola* isolates could cause disease on tobacco plants but not poinsettia plants. Found that isolates maintained for long periods in culture because less virulent with time.
King & Presley (1942)	Both cotton and tobacco isolates of *T. basicola* could cause disease on both cotton and tobacco plants.
Maier & Staffeldt (1960)	Authors could group 11 cotton isolates of *T. basicola* into four different pathogenicity groups based on their ability to infect Pima 32 Cotton.
Lloyd & Lockwood (1963)	Poinsettia, orange and pea *T.basicola* isolates were moderate to severely pathogenic on bean and pea and not on tobacco plants. Tobacco isolates were pathogenic toward tobacco and not bean plants. One tobacco isolate was moderately pathogenic to pea plants but the others were not. Concluded that *T. basicola* has a large host range but host specificity exists for each individual isolate.
Thomas & Papavizas (1965)	Sesame *T. basicola* isolates could cause disease symptoms on Baker 296, Hale, and Nebraska 145-4 castorbean but not Mississippi Wild-a castorbean.
Linderman & Toussoun (1968)	*Thielaviopsis basicola* exists as clones that vary in their virulence and reaction toward different hosts. The more virulent clones have chlamydospores that germinate more readily in the response to host exudates than those of non-virulent clones.
Gayed (1969)	Leaf disks of different species and varieties of tobacco show varying degrees of resistance and susceptibility when inoculated with *T. basicola*.
Gayed (1972)	*Thielaviopsis basicola* mixed in with soil resulted in more severe infection on cowpea and bean plants than on tobacco plants. The author concluded that cowpea and bean are more susceptible to *T. basicola* infection.
Lambe & Wills (1978)	*Thielaviopsis basicola* from tobacco, sesame and holly were all moderate pathogens of holly. One isolate from holly was only mildly pathogenic to holly and bean and soil isolates were only weak pathogens.
Wills & Lambe (1978)	A single *T. basicola* isolate showed varying degrees of pathogenicity toward legumous and woody ornamental plants, as well as tobacco, tomato, pansy, and eggplant

Reference	Findings
Blume & Harman (1979)	14 isolates of *T. basicola* isolated from various fields of infected pea plant were all able to cause disease when inoculated onto pea plants.
Bowden *et al.* (1985)	*Thielaviopsis basicola* isolates from pea and chickpea could cause disease in chickpea and pea plants, but only caused minor symptoms on lentil roots.
O'Brien & Davis (1994)	*Thielaviopsis basicola* isolates from lettuce caused severe infection on different lettuce cultivars and bean plants. Disease was less severe on watermelon, cucumber and rockmelon plants and no disease was caused on capsicum, celery, cotton, eggplant, parsley, radish, tomato and watercress.
Punja & Sun (2000)	Molecular analyses suggested that genetically distinct strains of *T. basicola* exist that may be adapted to their specific host.
Cilliers (2001)	Various groundnut cultivars were found to be susceptible to *T. basicola* infection in varying degrees. The cultivars Sellie and Anel were found to most susceptible and the cultivars Billy and Makatini were found to be least susceptible.
Punja (2004)	Morphological groups of *T. basicola* show variation in their virulence toward bean leaves. Kentucky Wonder leaves were most susceptible to *T. basicola* and Royal Burgundy and Kentucky blue leaves appeared much more resistant
Coumans *et al.* (2011)	Proteome data separate isolates of *T. basicola* into distinct groups based on the host from which they were isolated suggesting host specialization on a protein level

Table 5: Different cultural characteristics of Brown- and Grey-type isolates. (Reproduced from Stover (1950a))

Characters	**Cultural type**	
	Brown	**Grey**
Colour	Colonies cinnamon to light brownish olive or greyish olive	Colonies pallid neutral grey to various shades of grey and black. No trace of olivaceous or brown colours
Average diameter after eight days at room temperature	23 mm	17mm
Average range of endoconidia length	7.37-21.58 µm	8.52-29.01 µm
Colony perimeter	Mycelial growth near surface	Mycelium often becomes submerged and filamentous

A new genus and species for the globally important, multihost root pathogen *Thielaviopsis basicola*

W. J. Nel[a], T. A. Duong[b], B. D. Wingfield[b], M. J. Wingfield[a] and Z. W. de Beer[a]*

[a]*Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002;* [b]*Department of Genetics, the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*

The plant pathogenic asexual fungus *Thielaviopsis basicola* (Ascomycota) causes black root rot on many important agricultural and ornamental plant species. Since its first description in 1850, this species has had a tumultuous taxonomic history, being classified in many different genera. Thus far, DNA-based techniques have not played a significant role in identification of *T. basicola* and have been used only to confirm its placement in the Microascales. This investigation reconsidered the phylogenetic placement of *T. basicola*, using DNA sequence data for six different gene regions. It included 41 isolates identified as *T. basicola* from 13 geographical locations worldwide. Phylogenetic analyses showed that these isolates grouped in a well-supported lineage distinct from other genera in the Ceratocystidaceae, here described as *Berkeleyomyces* gen. nov. The data also provided robust evidence that isolates of *T. basicola* include a cryptic sister species. As a result, this report provides a new combination as *B. basicola* comb. nov. and introduces a new species as *B. rouxiae* sp. nov.

Keywords: black root rot, phylogenetics, reference specimen, taxonomy

Introduction

The globally distributed fungus *Thielaviopsis* (*T.*) *basicola* (Berk. & Broome) Ferraris (= *Chalara elegans* Nag Raj & W.B. Kendr.) is a serious root pathogen of many important plant species including cotton, tobacco, groundnut and chicory (Geldenhuis *et al.*, 2006; Coumans *et al.*, 2011). The disease caused by this fungus, commonly known as black root rot, is characterized by necrotic lesions on various parts of the host roots (King & Presley, 1942). This root necrosis leads to stunting, reduced vigour and yield loss (King & Presley, 1942; Coumans *et al.*, 2011), resulting in this pathogen posing a serious threat to some agricultural industries.

During the latter half of the 1800s, *T. basicola* was described independently under three different names by three different authors (Berkeley & Broome, 1850; Sorokïn, 1876; Massee, 1884). The first description of the fungus as *Torula* (*To.*) *basicola* Berk. & Broome was in 1850 based on isolates from pea (*Pisum sativum*) and '*Nemophila auriculata*' in England (Berkeley & Broome, 1850). The species was later subjected to numerous taxonomic treatments regarding an appropriate genus for it. The result has been that, along with its first description, *T. basicola* has been described in seven different genera with four different epithets (Berkeley & Broome, 1850; Sorokïn, 1876; Massee, 1884; Saccardo, 1886b; Ferraris, 1912; Nag Raj & Kendrick, 1975; Carmichael *et al.*, 1980).

*E-mail: wilhelm.debeer@fabi.up.ac.za

In the late 1800s a sexual state, *Thielavia* (*Th.*) *basicola* Zopf, was described for *T. basicola* (Zopf, 1876). Because the name for the sexual state had preference in literature under the dual nomenclature system for fungi (Article 59, Vienna Code and earlier), most authors referred to *Th. basicola* when discussing the species (Nag Raj & Kendrick, 1975). However, McCormick (1925) showed in a series of experiments involving single spore cultures of *T. basicola* and *Th. basicola* that the two species were unrelated. This has led to substantial confusion in the literature, especially where authors have failed to include the state of the fungus with which they were working. At present the fungus is only known from its asexual state, as subsequent to the study of McCormick (1925), no other authors have described a sexual state for *T. basicola*.

Despite the very substantial impact that DNA sequence data and phylogenetic inference have had on fungal taxonomy, very little attention has been given to *T. basicola* in this regard. Identification of the pathogen has relied heavily on the characteristic morphology of chlamydospores that are formed profusely on infected tissues, and sometimes the root symptoms (King & Presley, 1942).

Prior to the application of DNA sequence data for species identification, the names *T. basicola* and *Chalara* (*C.*) *elegans* were often used interchangeably in the literature. The name *C. elegans* was introduced by Nag Raj & Kendrick (1975) to describe a second, endoconidial asexual state of the fungus. These authors suggested that the name *To. basicola*, originally suggested by Berkeley & Broome (1850), was applicable only to the

chlamydospore-producing asexual state of the pathogen as it was the only spore form mentioned in their description. Because the endoconidial state of *T. basicola* is produced abundantly in culture, and the chlamydospore state can sometimes be completely lost, Nag Raj & Kendrick (1975) preferred to provide that state with its own name. However, in their argument, they bound the epithet '*basicola*' to the chlamydospore state and then suggested that the new epithet '*elegans*' should only be applied to the endoconidial state.

One of the first phylogenetic investigations including *T. basicola* and other species classified at the time in the genus *Chalara* was performed by Paulin & Harrington (2000). These authors showed that ribosomal large subunit (LSU) data grouped several of these species, including *T. basicola*, in the Microascales, together with the asexual states of several *Ceratocystis* species. Although Paulin & Harrington (2000) did not have a culture of *C. fusidiodes* (Corda) Rabenh., the type species of the genus *Chalara*, they suggested that based on its description it would best reside in the *Leotiales*. *Thielaviopsis* was consequently considered a preferable genus to accommodate *Chalara* species with *Ceratocystis* affinities, including *C. elegans*. Consequently, Paulin-Mahady *et al.* (2002)formally adopted the name *T. basicola* for the root pathogen.

de Beer *et al.* (2014) undertook a comprehensive multigene phylogenetic evaluation of the Ceratocystidaceae, applying the newly introduced one fungus–one name principles (Hawksworth *et al.*, 2011). Results of *LSU*, *60S* and *MCM7* phylogenetic analyses corroborated the placement of *T. basicola* in the Ceratocystidaceae, but also showed that it was not related to species of *Thielaviopsis*, but rather formed a lineage separate from other genera in the family. However, because sequences from the holotype or an ex-type culture were not available, they were unable to formally revise the generic placement of the fungus.

The aim of this investigation was to extend the study of de Beer *et al.* (2014) and to revise the generic placement of *T. basicola* in the Ceratocystidaceae. This included a relatively large global collection of isolates of the root pathogen that made it possible to evaluate the characteristics that define the species.

Materials and methods

Isolates

Cultures used in this study were obtained from various collections including the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands (CBS), the Belgian co-ordinated collections of microorganisms (BCCM/MUCL), CABI (IMI), and the International Collection of Microorganisms from Plants (ICMP), New Zealand. The databases of these collections were interrogated for isolates deposited under any of the names previously applied to *T. basicola*, as well as for isolates deposited under the sexual name *Th. basicola*. Cultures (Table 1) were maintained on 2% malt extract agar (MEA; 2% malt extract, 2% Difco agar; Biolab).

DNA extraction, PCR and sequencing

DNA was extracted from all isolates using the technique described by de Beer *et al.* (2014). The gene regions for the ribosomal large subunit (*LSU*), the 60S ribosomal protein RPL10 (*60S*), the internal transcribed spacer region (ITS), actin (*ACT*), the minichromosome maintenance complex component 7 (*MCM7*), and the RNA polymerase II second largest subunit (*RPB2*) were amplified and sequenced. The *LSU* region was amplified and sequenced using the primers LR0R and LR5, while the primers 60S-506F and 60S-908R were used for the *60S* region, and MCM7-for and MCM7-rev for the *MCM7* region (de Beer *et al.*, 2014). Primers ITS1F and ITS4 were used for the ITS region, and RPB2-5Fb and RPB2-7Rb for the *PRB2* region (Fourie *et al.*, 2015). The *ACT* gene region was amplified using the newly designed primers CeractF1 (5′-AGYTCCGG-CATGTGCAA-3′) and CeractR2 (5′-GRTGCCARATCTTCTC-CAT-3′). PCR and sequencing reactions were carried out following the protocols described by de Beer *et al.* (2014).

Phylogenetic analysis

DNA sequence alignments of the individual sequence datasets were made using the online version of MAFFT v. 7 (Katoh & Standley, 2013) and maximum likelihood phylogenetic analyses was performed using MEGA v. 6.06 (Tamura *et al.*, 2013). The GTR model was used and 1000 bootstrap repeats were tested. Based on the results of these analyses, the sequence data were concatenated into two datasets for final analysis.

The first concatenated sequence dataset contained the *LSU*, *60S*, *MCM7* and ITS sequence data for six selected isolates of *T. basicola*, and of 24 species representing seven genera in the Ceratocystidaceae. Two out-group genera were chosen based on the phylogenies of de Beer *et al.* (2014) and the sequence data were downloaded from the NCBI GenBank database (Table S1). This dataset was used to determine the generic placement of *T. basicola* within the Ceratocystidaceae. The second dataset included the ITS, *MCM7*, *RPB2* and *ACT* sequence data for isolates of *T. basicola* acquired from the various culture collections along with three out-group species. The sequences of the three out-group species [*Chalaropsis thielavioides* Peyronel (GenBank: BCGU01000001.1), *Ceratocystis fimbriata* Ellis & Halst. (Wilken *et al.*, 2013), and *Ceratocystis harringtonii* Z.W. de Beer & M.J. Wingf. (Wingfield *et al.*, 2016)] were extracted from their genomes available on NCBI GenBank. This dataset was used to determine the genetic variation within isolates of *T. basicola*. Alignments, as well as maximum likelihood, Bayesian inference and parsimony analyses of these datasets were carried out as described by de Beer *et al.* (2016).

Morphology

Morphological structures for three isolates residing in each of two lineages representing *T. basicola* were examined using an Axioskop light microscope (Zeiss) using differential interference contrast (DIC) microscopy. Images were captured using an AxioCam ICc3 (Zeiss) and were analysed and measured using AxioVision SE64 v. 4.9.1 software. At least 50 measurements were taken for chlamydospore segments, phialides and conidia. Values are presented as minimum – (average minus standard deviation) – average – (average plus standard deviation) – maximum.

Table 1 Origin, host, accession numbers and other information on isolates sequenced during this study

Current name	Previous name	Collection number	Other collection numbers	Host	Country	Collector	NCBI accession number					
							ITS	*MCM7*	*ACT*	*RPBII*	*LSU*	*60S*
Taxon A												
Berkeleyomyces basicola comb. nov.	*Thielaviopsis basicola*	CMW4098		Unknown	Ecuador	M. J. Wingfield	MF952421	MF967078	MF967131	MF967174		
		CMW5896		Carrot	Uganda	J. Roux	MF952422	MF967100	MF967141	MF967185		
		CMW6714		Carrot	Australia	M. J. Wingfield	MF952423	MF967079	MF967142	MF967186	MF948658	MF967072
		CMW7065	CBS341.33/ MUCL9545	*Primula* sp.	Netherlands	B. A. Tiddens	MF952424	MF967082	MF967144	MF967188		
		CMW7067	CBS487.48/ MUCL9542	*Paphiopedilum* sp.	Belgium	A. Mees	MF952425	MF967084	MF967146	MF967190		
		CMW7069		*Primula* sp.	Netherlands	G. A. van Arkel	MF952420	MF967085	MF967147	MF967191		
		CMW49352	CBS142796	*Betula* sp.	Netherlands	Unknown	MF952429	MF967102	MF967148	MF967183	MF948659	MF967075
		CBS414.52	MUCL8363	*Primula* sp.	Netherlands	G. A. van Arkel	MF952431	MF967104	MF967121	MF967163		
		CMW25439		*Styrax* sp.	Indonesia	M. J. Wingfield, M. van Wyk	MF952427	MF967099	MF967128	MF967171		
		CMW25440	CBS142829	*Styrax* sp.	Indonesia	M. J. Wingfield, M. van Wyk	MF952428	MF967088	MF967129	MF967172	MF948661	MF967073
		CMW26479		*Styrax* sp.	Indonesia	M. J. Wingfield	MF952426	MF967105	MF967130	MF967173		
		CBS430.74	CMW7071	*Betula* sp.	Netherlands	G. S. de Hoog	MF952432	MF967101	MF967122	MF967164		
		SA1		Carrot	South Africa	W. J. Nel	MF952430	MF967108	MF967115	MF967198		
Taxon B												
Berkeleyomyces rouxiae sp. nov.	*Thielaviopsis basicola*	CBS118120		Groundnut	South Africa	N. Geldenhuis	MF952413	MF967098	MF967117	MF967159		
		CMW5472	CBS117825	Groundnut	Ethiopia	N. Geldenhuis	MF952406	MF967080	MF967140	MF967184	MF948657	MF967074
		CMW7064	CBS194.26/ MUCL9544	Unknown	Unknown	W. W. Gilbert	MF952407	MF967081	MF967143	MF967187		
		CMW7066	CBS342.33/ MUCL9456	*Euphorbia pulcherrima*	Netherlands	B. A. Tiddens	MF952408	MF967083	MF967145	MF967189		
		CBS413.52		*Lathyrus odoratus*	Netherlands	G. A. van Arkel	MF952417	MF967106	MF967120	MF967162	MF948662	MF967077
		CBS150.67	IHEM3832	*Nicotiana tabacum*	Switzerland	R. Corbaz	MF952416	MF967107	MF967118	MF967160		
		CMW7622	CBS117826	Chicory	South Africa	N. Geldenhuis	MF952410	MF967109	MF967149	MF967192		
		CMW7623	CBS118119	Chicory	South Africa	N. Geldenhuis	MF952411	MF967110	MF967150	MF967193		
		CBS117827		Chicory	South Africa	N. Geldenhuis	MF952415	MF967114	MF967116	MF967158		
		CMW7625	CBS117828	Chicory	South Africa	N. Geldenhuis	MF952412	MF967112	MF967151	MF967194		
		CMW14219		Carrot/*Eucalyptus regnans*	Chile	J. Roux, R. Ahumada	MF952402	MF967086	MF967123	MF967165	MF948660	MF967076
		CMW14220		Carrot/*Eucalyptus globulus*	Chile	J. Roux, R. Ahumada	MF952403	MF967103	MF967124	MF967166		
		CM 14221	CBS142830	Carrot/*E. globulus*	Chile	J. Roux, R. Ahumada	MF952404	MF967113	MF967125	MF967167		
		CMW14222		Carrot/*Eucalyptus nitens*	Chile	J. Roux, R. Ahumada	MF952405	MF967087	MF967126	MF967168		

(continued)

Table 1 (continued)

Current name	Previous name	Collection number	Other collection numbers	Host	Country	Collector	NCBI accession number					
							ITS	*MCM7*	*ACT*	*RPBII*	*LSU*	*60S*
		CMW14223		Carrot/*Eucalyptus nitens*	Chile	J. Roux, R. Ahumada	MF952393	MF967111	MF967127	MF967169		
		CMW44562		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952395	MF967089	MF967132	MF967175		
		CMW44563		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952396	MF967090	MF967133	MF967176		
		CMW44564		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952397	MF967091	MF967134	MF967177		
		CMW44565		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952398	MF967092	MF967135	MF967178		
		CMW44566		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952399	MF967093	MF967136	MF967179		
		CMW44567		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952400	MF967094	MF967137	MF967180		
		CMW44568		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952401	MF967095	MF967138	MF967181		
		CMW44569		*Chamaecytisus* 'Aura'	South Africa	J. Roux	MF952394	MF967096	MF967139	MF967182		
		IMI125845[a]		*Citrus* sp.	Israel	H. Harin	MF952419					
	Trichocladium basicola	ICMP2460		*Pisum sativum*	New Zealand	J. M. Dingley	MF952414	MF967097	MF967153	MF967196		
		ICMP13276[a]		*Ipomoea batatas*	New Zealand	P. G. Broadhurst	MF952409		MF967152	MF967195		
	Thielavia basicola	CBS178.86[a]	MUCL40417	*Phaseolus vulgaris*	Canada	A. Carter	MF952418		MF967119			
Other												
Chalaropsis thielavioides		JCM1933							MF967154	MF967197		
Ceratocystis fimbriata		CBS114723							MF967156	MF967157		
Ceratocystis harringtonii		CMW14789							MF967155	MF967170		
Thielavia basicola	*Thielavia basicola*	CBS229.82[b]		*Arctostaphylus uva-ursi*	Switzerland	B. Widler	MF952433					

[a]Unable to obtain amplification for any other gene regions besides ITS for these isolates and it was excluded from further analysis.
[b]Initial sequencing showed that this isolate was distinct from *B. basicola* and it was excluded from the rest of the study.

Table 2 Number of characters and substitutional models used in phylogenetic analyses

	Dataset	Ceratocystidaceae	*Berkeleyomyces*
Number of characters	Total	2083	2505
	VPUC	69	196
	Constant	1379	1909
MP	PIC	635	400
	Tree length	2361	737
	CI	0.442186	0.938942
	RI	0.657210	0.960630
ML	Substitution model	GTR + GAMMA	GTR + GAMMA
BI	Substitution model	GTR	GTR

MP, maximum parsimony; ML, maximum likelihood; BI, Bayesian inference; VPUC, variable parsimony uninformative characters; PIC, parsimony informative characters; CI, consistency index; RI, retention index.

Growth in culture

Optimal temperature for growth of isolates was determined on MEA using the protocols described by Duong *et al.* (2012). The same four isolates for each lineage used for the morphological comparisons were used to determine optimal growth temperature. Three replications at each temperature were carried out and mean colony diameter (± standard deviation) was determined.

Results

Fungal isolates

A total of 41 isolates from five continents and 13 geographical locations were obtained from the various culture collections. ITS sequences were obtained for all isolates but problems in amplification or sequencing of some of the other gene regions resulted in exclusion of four isolates from further study (Table 1).

Phylogenetic analysis

Separate phylogenetic trees derived from the maximum likelihood analyses for each of the individual sequence datasets all resulted in similar topologies (Figs S1–S9), supporting their concatenation in subsequent analyses. The number of characters, the substitution models used and other statistical information for the concatenated sequence datasets are presented in Table 2.

The phylogenetic tree derived from the analyses of the dataset consisting of *LSU*, *60S*, ITS and *MCM7* gene regions (Fig. 1) supported the known phylogenetic relationships of genera in the Ceratocystidaceae. The six selected isolates of *Thielaviopsis basicola* (Berk. & Broome) Ferraris grouped separately from the other species described in *Thielaviopsis*, forming a well supported lineage, distinct from all other genera in the Ceratocystidaceae (Fig. 1). The genera most closely related to the lineage in which *T. basicola* resided were *Chalaropsis* and *Ceratocystis*. These genera formed a polyphyletic sister lineage to the isolates of *T. basicola*. The six *T. basicola* isolates also formed two well-supported lineages (A and B) with the isolate (CBS 413.52) originally included in the study of de Beer *et al.* (2014) residing in lineage B.

The phylogenetic tree derived from the analyses of the dataset consisting of ITS, *MCM7*, *RPB2* and *ACT* gene regions (Fig. 2) strongly supported the separation of *T. basicola* into two distinct lineages. Based on these analyses, 13 isolates formed lineage A and 24 isolates formed lineage B. Based on the initial ITS analysis, four additional isolates were identified as residing in lineage B (Table 1).

Morphology

The morphologies of the six isolates residing in the two lineages of *T. basicola* selected for examination were very similar. The phialides and endoconidia produced by isolates in the two lineages closely resembled those found in other species of the Ceratocystidaceae. However, the morphology of the chlamydospores and secondary conidia produced by the isolates were distinct from all other genera in the Ceratocystidaceae. The morphologies of the isolates in lineages A and B were so similar that they could not be separated with confidence in the absence of phylogenetic data.

It is known from the literature that *T. basicola* is very variable in culture and that two distinct morphotypes of the species, a brown type and a grey type, can be found (Stover, 1950). Amongst the large collection of cultures acquired for this study, variation was observed in growth rate, mycelial type, colony colour (including brown and grey cultures), and abundance of endoconidial or chlamydospore production. To try to distinguish between the two lineages morphologically, an attempt was made to determine whether the brown and grey type isolates could represent the two lineages. However, both of these morphotypes were found to occur in the two lineages and had similar levels of variation in growth patterns (Figs 3 & 4).

Taxonomy

Based on phylogenetic analyses and morphology, the results showed that isolates previously identified as *T. basicola* formed a monophyletic lineage representing a new genus in the Ceratocystidaceae, described below. In addition, the isolates separated into two well-supported sublineages representing two distinct species, one being *T. basicola* for which a new combination is provided, the other representing a new species, also described here. Several synonyms have been listed over the years for *T. basicola*, all of which are discussed below. One of these synonyms, *Milowia nivea* Massee, was the type species for a genus and family. The reasons why these names are not being adopted are provided in the discussion of the synonyms of *T. basicola*.

COLOR

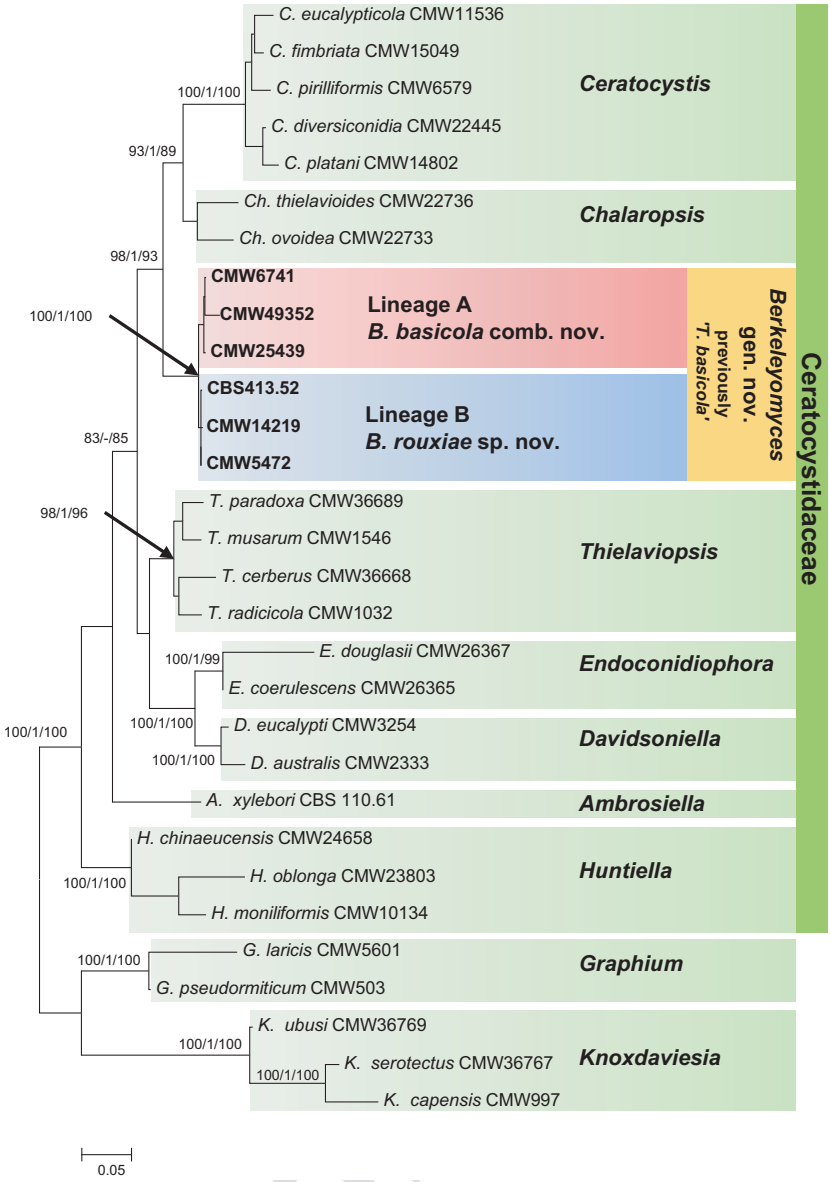


Figure 1 RaxML phylogram derived from maximum likelihood analysis of concatenated dataset (*LSU*, *60S*, ITS and *MCM7*) for selected species in the Ceratocystidaceae. Bootstrap support >75% and posterior probabilities >0.95 is given as ML/BI/MP.

Berkeleyomyces W.J. Nel, Z.W. de Beer, T.A. Duong, M.J. Wingf. **gen. nov.**

Mycobank no: MB822838.

Etymology: The name recognizes the Reverend M. J. Berkeley who originally described the species and honours his considerable contributions to fungal taxonomy.

Sexual state not observed. *Asexual state* mycelial. *Conidiophores* borne terminally or laterally on vegetative hyphae. *Conidiogenous cells* phialidic, cylindrical, tapering towards the apex, hyaline to subhyaline. *Conidia* unicellular, cylindrical, hyaline, produced singly or in chains. *Secondary conidia* occasionally observed, rounded, initially hyaline, thick-walled, with veil on one side. *Chlamydospores* unicellular, dark brown, club-shaped chains of spores held together by outer membrane, borne terminally or laterally on hyphal branches, singly or in clusters, chains are able to separate into multiple viable individual cylindrical segments, terminal segment obtuse.

Type species: ***Berkeleyomyces basicola*** (Berk. & Broome) W.J. Nel, Z.W. de Beer, T.A. Duong, M.J. Wingf.

Notes: The most distinctive features of the genus that separate it from other genera in the Ceratocystidaceae are the septate chlamydospores and secondary conidia with veil-like structures. In both species described below, the septate chlamydospores are regularly observed in culture. However, the secondary conidia are only observed

COLOR

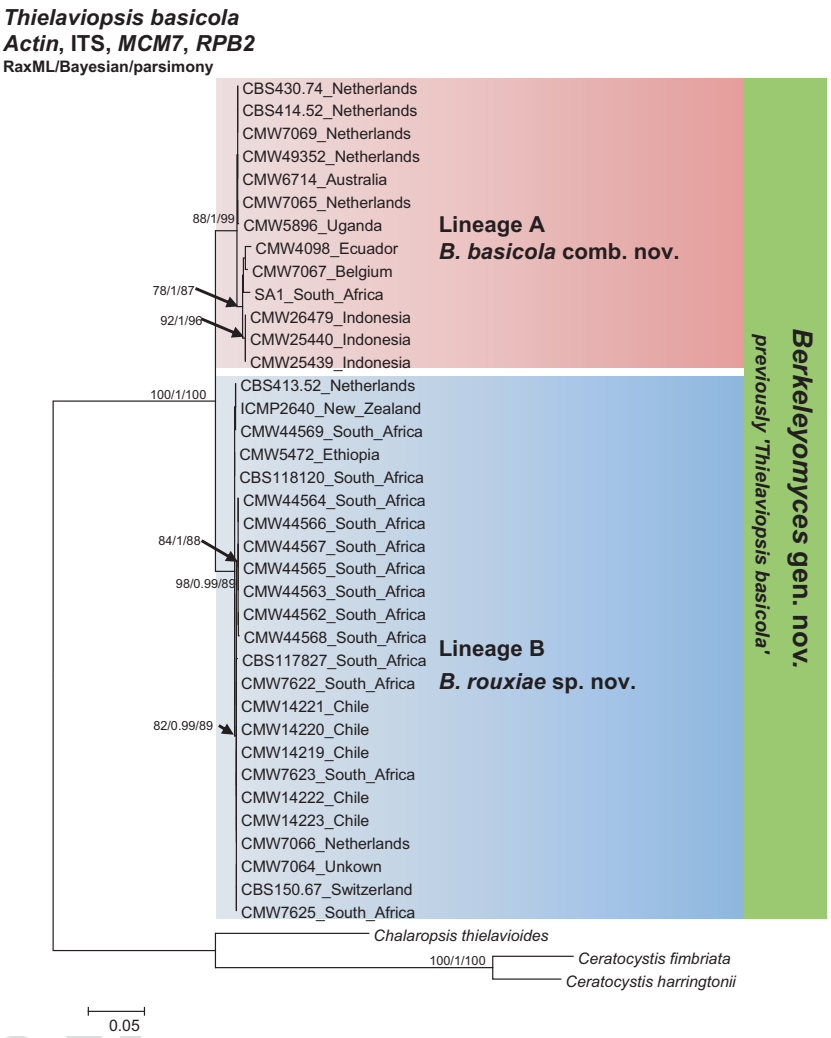


Figure 2 RaxML phylogram derived from maximum likelihood analysis of concatenated dataset (ITS, *ACT*, *MCM7* and *RPB2*) for isolates previously labelled as *Thielaviopsis basicola*. Bootstrap support >75% and posterior probabilities >0.95 is given as ML/BI/MP.

with careful examination under special conditions. Stover (1950) was the first to describe the secondary conidia as 'secondary chlamydospores' that he observed on soil agar. Schippers (1970) later induced the formation of secondary conidia in phosphate buffer supplemented with glucose and asparagine at pH 5 and 7 after 25 days. In the present study, secondary conidia were observed on MEA approximately 4 weeks after isolates were transferred from surface-sterilized carrot slices on which they were incubated in an attempt to measure pathogenicity.

Lineage A

Berkeleyomyces basicola (Berk. & Broome) W.J. Nel, Z.W. de Beer, T.A. Duong, M.J. Wingf. **comb. nov.** (Figs 3 & 5) Mycobank no: MB822839.

= *Torula basicola* Berk. & Broome, XL-Notices of British Fungi, Ann. Mag. Nat. Hist. Ser. 2. 5(30): 461 (1850) (Basionym).

= *Thielaviopsis basicola* (Berk. & Broome) Ferraris, Fl. Ital. Crypt., Fungi 6: 113. (1912).

= *Trichocladium basicola* (Berk. & Broome) J.W. Carmich., Genera of Hyphomycetes, p. 185. (1980).

= *Helminthosporium fragile* Sorokïn, Hedw. 15: 113. (1876).

= *Clasterosporium fragile* (Sorokïn) Sacc., Syll. Fung. 4: 386. (1886).

? = *Milowia nivea* Massee, J. R. Microsc. Soc., IV 2: 841-845. (1884) [*nom. dub.*].

= *Chalara elegans* Nag Raj & W.B. Kendr., A Monograph of *Chalara* and Allied Genera, p. 111. (1975).

Sexual state not observed. *Asexual state: Conidiogenous cells* phialidic 13.8 – (33.7) – 51.7 – (69.6) – 89.0 µm long; cylindrical; tapering toward apex; 2.9 – (3.3) – 3.8 – (4.3) – 5.6 µm wide at tip and 3.2 – (4.2) – 5.1 – (6.0) – 7.0 µm wide at base; hyaline to subhyaline. *Conidia* unicellular; cylindrical; 7.2 – (10.0) – 14.0 – (17.9) – 29.7 µm long and 3.0 – (3.7) – 4.1 – (4.7) – 5.0 µm wide at centre; hyaline; produced singly or in chains. *Secondary conidia* 8.5 – (9.8) – 11.9 – (14.0) – 15.7 µm high and 12.2 – (11.7) – 14.5 – (17.3) – 18.0 µm wide in side view, rarely observed in culture. *Chlamydospores* unicellular; dark-brown; segments

COLOR

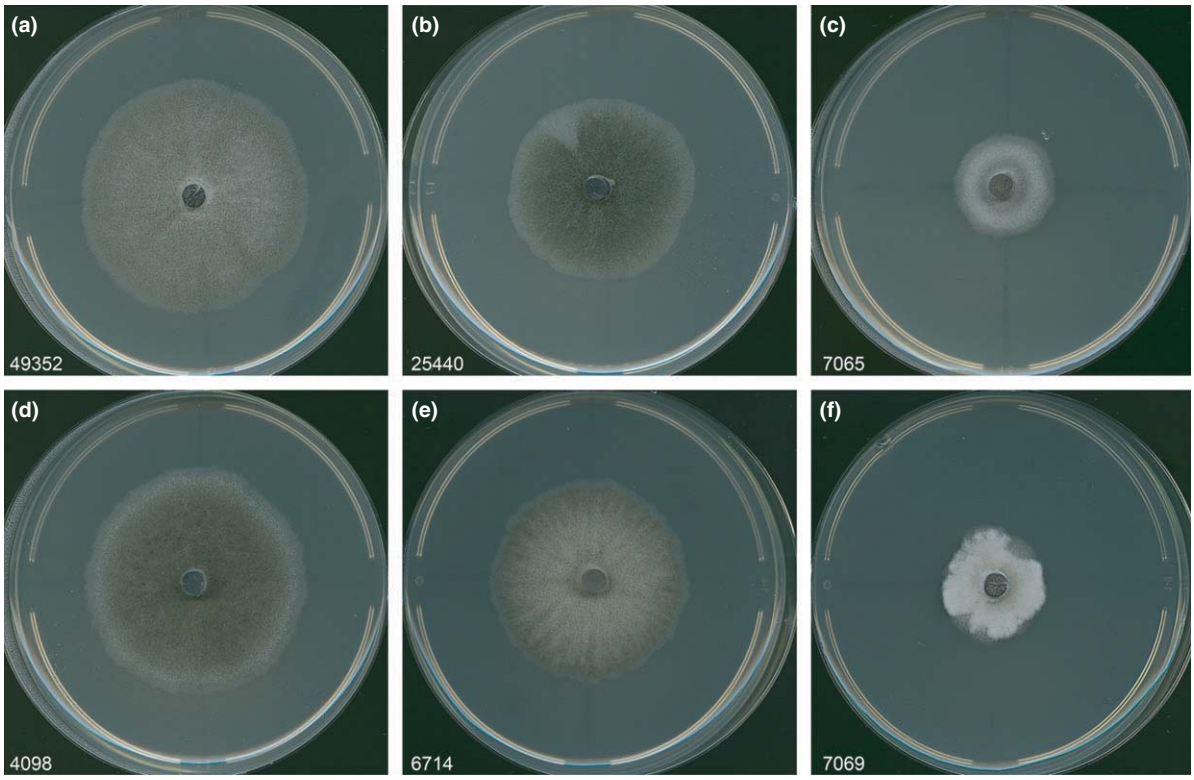


Figure 3 Some of the morphological variation within species of *Berkeleyomyces basicola*. Isolates were grown for 10 days at 25 °C, CMW numbers for the selected isolates are provided in the bottom left corner of each figure.

cylindrical; 6.2 – (8.4) – 9.9 – (11.4) – 14.3 µm long and 7.8 – (9.1) – 10.4 – (11.6) – 13.8 µm wide at centre. Cultures grow optimally at 25 °C reaching on average 44 mm (±10 mm) in 10 days.

Type specimen (not seen). UNITED KINGDOM, England, King's Cliffe, 20 June 1846. From *Pisum sativum*, M.J. Berkeley (HOLOTYPE IMI165190).

Specimens examined. NETHERLANDS, Boskoop, June 1974, From *Betula* sp., S.G. de Hoog (PREM 62125 REFERENCE SPECIMEN designated here, living culture CMW49352 = CBS142796, the latter culture is a single spore isolate obtained from CBS430.74 = CMW7071). NETHERLANDS, Bussum, 1933, From *Primula* sp., B.A. Tiddens (PREM 62128, living culture CMW7065 = CBS341.33 = MUCL9545). INDONESIA, March 2007, From *Styrax benzoin*, M.J. Wingfield & M. van Wyk (PREM 62127, living culture CMW25440 = CBS142829).

Notes. The holotype of *Torula basicola* from Berkeley & Broome (1850) was not available for study. However, the morphological similarity of the isolates in the two lineages of *T. basicola* emerging from this study was such that this material would not have been useful in determining which lineage might represent the fungus described by Berkeley & Broome (1850). Furthermore, no isolates from the same hosts in the UK were available, which means an epitype could not be designated. The recommendations of Ariyawansa *et al.* (2014) were therefore followed, and a reference specimen designated for *T. basicola* instead, selected based on its geographic location in the Netherlands, which was the country closest to the UK, and with morphology that corresponded well with the original description.

Synonyms: Berkeleyomyces basicola was initially described in the genus *Torula*, which at the time had a broad definition accommodating many species with chains of pigmented conidia (Crane & Schoknecht, 1977; Crane & Miller, 2016). The type species for *Torula* is *To. herbarum* (Pers.) Link, for which a neotype was designated recently, the sequences of which placed the genus in the Torulaceae (Pleosporales, Dothideomycetes) (Crous *et al.*, 2015). *Berkeleyomyces basicola* can thus not be treated in *Torula*.

Ferraris (1912) was the first to treat *B. basicola* in *Thielaviopsis*, described by Went (1893) to accommodate an asexual fungus (*T. ethacetica* Went) causing disease on sugarcane. The genus was listed as a synonym of *Chalara* by Nag Raj & Kendrick (1975), but *Chalara*, typified by *Ch. fusioides*, groups in the Helotiales (Leotiomycetes) (Gernandt *et al.*, 2001). Paulin-Mahady *et al.* (2002) reinstated the name *Thielaviopsis* for all asexual states of *Ceratocystis* spp. and several '*Chalara*' species known only from asexual states. Mbenoun *et al.* (2014) resolved confusion between the types of *T. ethacetica* and *C. paradoxa* (Dade) Moreau and other species in the *C. paradoxa* complex. de Beer *et al.* (2014) restricted

COLOR

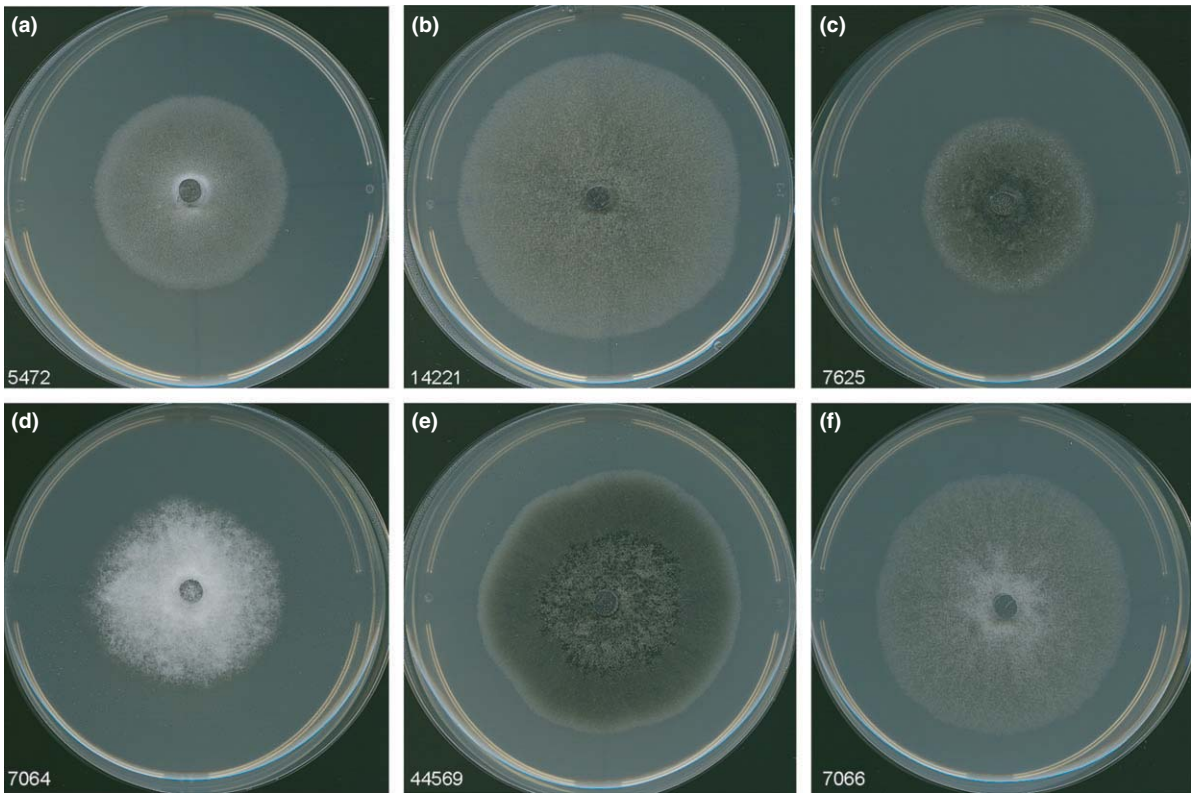


Figure 4 Some of the morphological variation within species of *Berkeleyomyces rouxiae*. Isolates were grown for 10 days at 25 °C, CMW numbers for the selected isolates are provided in the bottom left corner of each figure.

Thielaviopsis to include only species previously treated in the *C. paradoxa* complex, and redefined the genus based on one fungus–one name principles to encompass sexual states as well. *Berkeleyomyces basicola* thus cannot be treated in *Thielaviopsis* as it groups outside this genus as defined by de Beer *et al.* (2014).

Carmichael *et al.* (1980) treated *B. basicola* in *Trichocladium* (*Tr.*), arguing that the spores of this species more closely resembled those produced by *Trichocladium* than those of *Thielaviopsis*. However, the type species for *Trichocladium* is *Tr. asperum* Harz, which groups in the Sordariales (Sordariomycetidae, Sordariomycetes) (Hambleton *et al.*, 2005; Hibbett *et al.*, 2007), which means the genus is distinct from *Berkeleyomyces*.

Helminthosporium fragile was described by Sorokïn (1876) from horseradish. The species was transferred to the genus *Clasterosporium* by Saccardo (1886b), before being synonymized with *T. basicola* by Ferraris (1912). In the absence of the holotype, this synonymy is accepted based on drawings produced by Sorokïn (1876) that closely resemble the structures of *B. basicola*. In addition, neither *Clasterosporium* nor *Helminthosporium* are appropriate genera to accommodate *B. basicola*. The type species for *Helminthosporium* is *H. velutinum* Link, of which sequences from the recently designated epitype place the genus in the Pleosporales (Pleosporomycetidae, Dothideomycetes) (Voglmayr & Jaklitsch, 2017). The type species for *Clasterosporium* is *C. caricinum* Schwein. This genus is listed in both MycoBank and IndexFungorum as legitimate, but no sequences for any species in this genus is available in GenBank, which makes its placement in the Magnaporthaceae (Magnaporthales, Sordariomycetes) as listed on both websites questionable. The description and image in the protologue of *C. caricinum* (Schweinitz, 1832) do not correspond at all with *B. basicola*.

The genus and species, *Milowia nivea*, was described by Massee in 1884, who mysteriously referred to some of the structures in his illustrations as 'octosporous asci' (Massee, 1884). Saccardo (1886a) described the tribe Milowieae to accommodate the genus, referring to some structures as 'basidia 3-locularia'. Almost 20 years after his initial description, Massee (1912) himself synonymized *M. nivea* with *T. basicola*. However, ignoring Massee's (1912) synonymy, Nannizzi (1934) elevated the tribe to the family level, describing the Milowiaceae. According to Nag Raj & Kendrick (1975), several attempts to trace the type specimen of the species were futile. The drawings from Massee's (1884) protologue for *M. nivea* do not closely resemble structures produced by *B. basicola*, but his modified drawings (Massee, 1912) much more accurately depict the species. In view of Massee's own modification of the drawings and the lost type specimen, as well the confusing interpretations of some of the structures as asci and basidia, the present study concurs with Nag Raj & Kendrick (1975) and

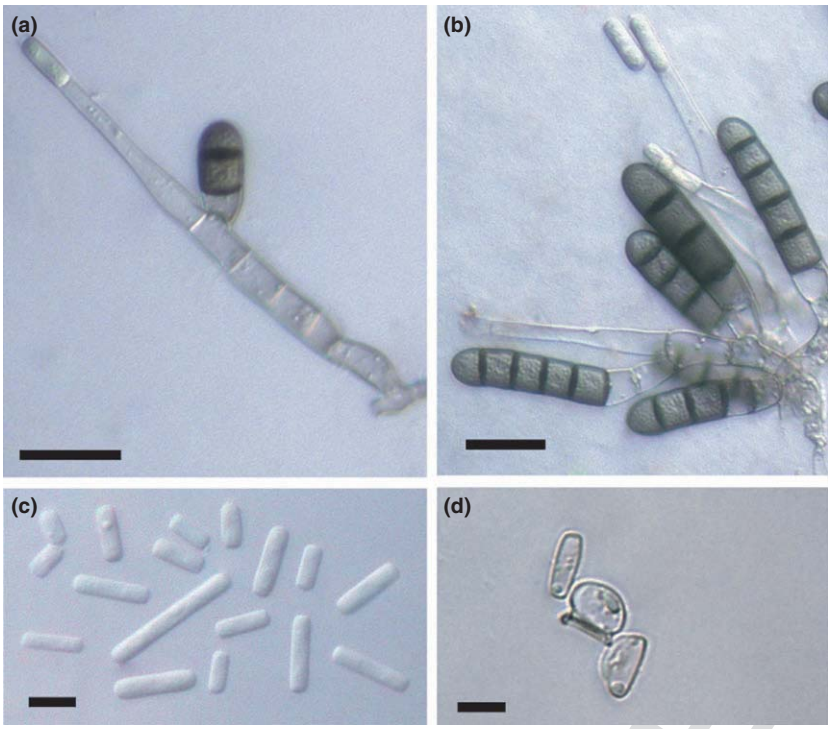


Figure 5 Structures of *Berkeleyomyces basicola*. (a) Terminal phialide giving rise to an endoconidium and a laterally borne chlamydospore containing two segments; (b) a cluster of chlamydospores and phialides; (c) endoconidia; (d) two secondary conidia with one endoconidium. Scale: (a, b) 20 μm; (c, d) 10 μm.

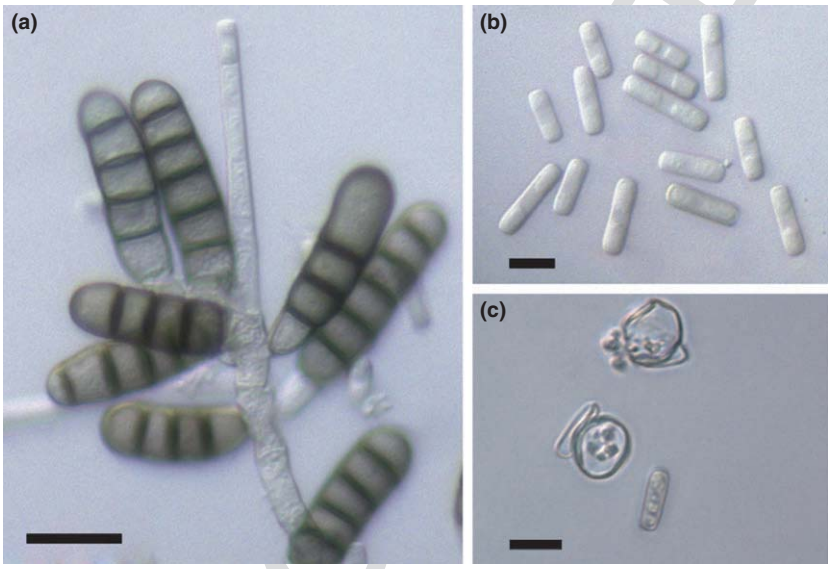


Figure 6 Structures of *Berkeleyomyces rouxiae*. (a) A cluster of chlamydospores and phialides; (b) endoconidia; (c) two secondary conidia with one endoconidium. Scale: (a) 20 μm; (b, c) 10 μm.

considers the species as dubious. The genus name, and the tribe and family names based on it, are also considered as *nomen dubia*, and the authors have submitted a proposal to formally reject *Milowia nivea*. If not formally rejected, the name *Milowia* will be available for the new genus revealed by the present results. This will result in the seldom used family name, Milowiaceae, taking priority over the much younger, but well-established name, Ceratocystidaceae (Réblová *et al.*, 2011; de Beer *et al.*, 2014).

The reasons why *C. elegans* was described as distinct from *T. basicola* by Nag Raj & Kendrick (1975) were discussed in the introduction of this paper. As mentioned, the type species of *Chalara* groups in the Helotiales (Leotiomycetes) (Gernandt *et al.*, 2001) and the genus is not available to accommodate *B. basicola*.

Lineage B

Berkeleyomyces rouxiae W.J. Nel, Z.W. de Beer, T.A. Duong, M.J. Wingf., **sp. nov.** (Figs 4 & 6)

Mycobank no: MB822840.

Etymology: Named for Professor Jolanda Roux in recognition of her considerable contributions to the study of the taxonomy and biology of species in the Ceratocystidaceae including *T. basicola*.

Sexual state not observed. *Asexual state - Conidiogenous cells* phialidic; 16.7 – (45.7) – 61.0 – (67.4) – 81.5 μm long; cylindrical; tapering toward apex; 3.0 – (3.5) – 4.0 – (4.4) – 5.3 μm wide at the tip and 3.5– (4.7) – 5.4– (6.0) – 6.9 μm wide at the base; hyaline to subhyaline. *Conidia* unicellular; cylindrical; 8.4 – (10.9) – 15.6 – (20.3) – 28.9 μm long and 3.5 – (3.9) – 4.4 – (4.8) – 5.7 μm wide at centre; hyaline; produced singly or in chains. *Secondary conidia* 9.0 – (10.1) – 11.5 – (12.8) – 14.3 μm high and 11.5 – (12.4) – 14.1 – (15.7) – 17.8 μm wide in side view, rarely observed in culture. *Chlamydospores* unicellular; dark-brown; segments cylindrical; 6.0 – (7.7) – 9.0 – (10.2) – 12.6 μm long and 8.4 – (10.1) – 11.0 – (12.0) – 13.6 μm wide at centre. Cultures grow optimally at 25 °C reaching 47 mm (±9 mm) in 10 days.

Specimens examined. SOUTH AFRICA, Eastern Cape, Fish River. From chicory, J. Roux & N. Geldenhuis (HOLOTYPE PREM 62135, ex-holotype culture CMW7625 = CBS117828). ETHIOPIA, Wondo. From groundnut, J. Roux (PARATYPE PREM 62131, ex-paratype culture CMW5472 = CBS117825). CHILE, 2004 From *Eucalyptus globulus*, J. Roux & R. Ahumada (PARATYPE PREM 62133, ex-paratype culture CMW14221 = CBS142830).

Notes. *Berkeleyomyces basicola* cannot be distinguished from *B. rouxiae* morphologically.

Discussion

Results of this study, which included analyses of sequences for six gene regions and a large collection of isolates, confirmed the previous findings of de Beer *et al.* (2014) that the important root pathogen *T. basicola* is unrelated to species of *Thielaviopsis* in the Ceratocystidaceae. This provided adequate support for the establishment of a new genus for these isolates, and this study consequently introduces *Berkeleyomyces* for them. Furthermore, a relatively large global collection of isolates of *T. basicola* was shown to represent two distinct phylogenetic lineages. Based on a decision relating to the geographical occurrence of the fungus first described by Berkeley & Broome (1850), one of these taxa represents *T. basicola sensu stricto*. It is now treated as *B. basicola* and a reference specimen has been assigned for it. The lineage accommodating the remaining isolates has been described as the new species *B. rouxiae*.

Although very little recent attention has been given to the taxonomy of the fungus previously known as *T. basicola*, the results of some previous studies considering populations of the fungus have shown that isolates reside in two distinct groups (Punja & Sun, 2000; Harvey *et al.*, 2002; Geldenhuis *et al.*, 2006; Coumans *et al.*, 2011). These now support the findings of the present study and the decision to establish a second species in the genus *Berkeleyomyces*. The morphological characteristics of the cryptic species *B. basicola* and *B. rouxiae* are inordinately similar and do not allow their separation in the absence of DNA sequence data. In this regard, it was also necessary to select a lineage in the new genus *Berkeleyomyces* to represent the fungus originally described as *Torula basicola* by Berkeley & Broome (1850). In this case, a geographic location closest to the area in which the original authors collected the isolate chosen to represent the species has been relied on.

The fungus previously known as *T. basicola* is a well-known and important plant pathogen. It is consequently surprising that it has only now been recognized as representing two distinct taxa. This illustrates not only the relevance of DNA sequence-based identification protocols but also the fact that even commonly occurring plant pathogenic fungi can still include cryptic taxa. The study also illustrates the importance to plant pathology of the decision to apply a single name to all fungi (Hawksworth *et al.*, 2011; Wingfield *et al.*, 2012) and how confusion arises from applying different names to different states of fungi.

Recognition of two species of *Berkeleyomyces* for the fungal pathogen previously known as *T. basicola* could have significant plant pathology implications. Based on the results of this study, both fungi have wide host ranges and there is no indication of host specificity. However, pathogenicity tests should be undertaken to determine whether there might be differences in host susceptibility for the different species. Such knowledge will also impact strongly on quarantine decisions and attempts to limit further global distribution of these important plant pathogens.

Acknowledgements

The authors acknowledge the University of Pretoria, the Tree-Protection Co-operative Programme (TPCP), the DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB) and the National Research Foundation (NRF) for financial support.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ariyawansa HA, Hawksworth DL, Hyde KD *et al.*, 2014. Epitypification and neotypification: guidelines with appropriate and inappropriate examples. *Fungal Diversity* **69**, 57–91.

de Beer ZW, Duong TA, Barnes I, Wingfield BD, Wingfield MJ, 2014. Redefining *Ceratocystis* and allied genera. *Studies in Mycology* **79**, 187–219.

de Beer ZW, Duong TA, Wingfield MJ, 2016. The divorce of *Sporothrix* and *Ophiostoma*: solution to a problematic relationship. *Studies in Mycology* **83**, 165–91.

Berkeley MJ, Broome CE, 1850. Notices of British fungi XL. *Annals and Magazine of Natural History Series* **2** (5), 455–66.

Carmichael JW, Kendrick WB, Conners IL, Sigler L, 1980. *Genera of Hyphomycetes*. Edmonton, Canada: University of Alberta Press.

Coumans JW, Harvey J, Backhouse D *et al.*, 2011. Proteomic assessment of host-associated microevolution in the fungus *Thielaviopsis basicola*. *Environmental Microbiology* **13**, 576–88.

Crane JL, Miller AN, 2016. Studies in genera similar to *Torula*: *Bahusaganda*, *Bahusandhika*, *Pseudotorula*, and *Simmonsiella* gen. nov. *IMA Fungus* **7**, 29–45.
Crane JL, Schoknecht JD, 1977. Revision of *Torula* species. *Rutola*, a new genus for *Torula graminis*. *Canadian Journal of Botany* **55**, 3013–9.
Crous PW, Carris LM, Giraldo A *et al.*, 2015. The genera of fungi – fixing the application of the type species of generic names – G 2: *Allantophomopsis*, *Latorua*, *Macrodiplodiopsis*, *Macrohilum*, *Milospium*, *Protostegia*, *Pyricularia*, *Robillarda*, *Rotula*, *Septoriella*, *Torula*, and *Wojnowicia*. *IMA Fungus* **6**, 163–98.
Duong TA, de Beer ZW, Wingfield BD, Wingfield MJ, 2012. Phylogeny and taxonomy of species in the *Grosmannia serpens* complex. *Mycologia* **104**, 715–32.
Ferraris TA, 1912. Fungi: Hyphales. In: ???? ???? ed. *Flora Italica Cryptogamica Pars I: Fungi. Rocca S*. Casciano, Italy: Stabilimento Tipografico Cappelli, 233–4.
Fourie A, Wingfield MJ, Wingfield BD, Barnes I, 2015. Molecular markers delimit cryptic species in *Ceratocystis sensu stricto*. *Mycological Progress* **14**, 1–18.
Geldenhuis MM, Roux J, Cilliers AJ, Wingfield BD, Wingfield MJ, 2006. Clonality in South African isolates and evidence for a European origin of the root pathogen *Thielaviopsis basicola*. *Mycological Research* **110**, 306–11.
Gernandt DS, Platt JL, Stone JK *et al.*, 2001. Phylogenetics of Helotiales and Rhytismatales based on partial small subunit nuclear ribosomal DNA sequences. *Mycologia* **93**, 915–33.
Hambleton S, Nickerson NL, Seifert KA, 2005. *Leohumicola*, a new genus of heat-resistant hyphomycetes. *Studies in Mycology* **53**, 29–52.
Harvey JA, Aitken EAB, Nehl DB, 2002. Genetic diversity of *Thielaviopsis basicola*. In: ???? ???? ed. *Proceedings of Field to Fashion 11th Australian Cotton Conference, Brisbane, Australia*. Orange, NSW, Australia: Australian Cotton Growers Research Association, 685–7.
Hawksworth DL, Crous PW, Redhead SA *et al.*, 2011. The Amsterdam declaration on fungal nomenclature. *IMA Fungus* **2**, 105.
Hibbett DS, Binder M, Bischoff JF *et al.*, 2007. A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**, 509–47.
Katoh K, Standley D, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**, 772–80.
King JC, Presley JT, 1942. A root rot of cotton caused by *Thielaviopsis basicola*. *Phytopathology* **32**, 752–61.
Massee GE, 1884. Description and life-history of a new fungus, *Milowia nivea*. *Journal of the Royal Microscopical Society* **4**, 842–5.
Massee GE, 1912. A disease of sweet peas, asters, and other plants (*Thielavia basicola* Zopf). *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)* **1912**, 44–52.
Mbenoun M, de Beer ZW, Wingfield MJ, Wingfield BD, Roux J, 2014. Reconsidering species boundaries in the *Ceratocystis paradoxa* complex, including a new species from oil palm and cacao in Cameroon. *Mycologia* **106**, 757–84.
McCormick FA, 1925. Perithecia of *Thielavia basicola* Zopf in culture and the stimulation of their production by extracts from other fungi. *Connecticut Agricultural Experimental Station* **269**, 539–54.
Nag Raj TR, Kendrick WB, 1975. *A Monograph of Chalara and Allied Genera*. Waterloo, Canada: Wilfrid Laurier University Press.
Nannizzi A, 1934. *Repertorio Sistematico dei Miceti Dell' Uomo e Degli Animali*. Siena, Italy: Siena Poligrafica Meini.
Paulin AE, Harrington TC, 2000. Phylogenetic placement of anamorphic species of *Chalara* among *Ceratocystis* and other ascomycetes. *Studies in Mycology* **45**, 169–86.
Paulin-Mahady AE, Harrington TC, McNew D, 2002. Phylogenetic and taxonomic evaluation of *Chalara*, *Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. *Mycologia* **94**, 62–72.
Punja ZK, Sun LJ, 2000. Morphological and molecular characterization of *Chalara elegans* (*Thielaviopsis basicola*), cause of black root rot on diverse plant species. *Canadian Journal of Botany* **77**, 1801–12.
Réblová M, Gams W, Seifert KA, 2011. *Monilochaetes* and allied genera of the *Glomerellales*, and a reconsideration of families in the Microascales. *Studies in Mycology* **68**, 163–91.
Saccardo PA, 1886a. Sect. 3 Phragmosporae Sacc. *Sylloge Fungorum* **4**, 188.
Saccardo PA, 1886b. *Clasterosporium fragile* (Sorok.) Sacc. *Sylloge Fungorum* **4**, 386.
Schippers B, 1970. Survival of endoconidia of *Thielaviopsis basicola* in soil. *Netherlands Journal of Plant Pathology* **76**, 206–11.
Schweinitz LD, 1832. Synopsis fungorum in America Boreali media degentium. Secundum observationes. *Transactions of the American Philosophical Society* **4**, 141–316.
Sorokïn NV, 1876. Über Helminthosporium fragile sp. n. *Hedwigia* **15**, 113.
Stover RH, 1950. The black root rot disease of tobacco: I. Studies on the causal organism *Thielaviopsis basicola*. *Canadian Journal of Research* **28**, 445–70.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–9.
Voglmayr H, Jaklitsch WM, 2017. *Corynespora*, *Exosporium* and *Helminthosporium* revisited – new species and generic reclassification. *Studies in Mycology* **87**, 43–76.
Went FAFC, 1893. De ananasziekte van het suikerriet. *Mededeelingen van het Proefstation West-Java* **V**, 1–8.
Wilken PM, Steenkamp ET, Wingfield MJ, de Beer ZW, Wingfield BD, 2013. Draft nuclear genome sequence for the plant pathogen, *Ceratocystis fimbriata*. *IMA Fungus* **4**, 357–8.
Wingfield MJ, de Beer ZW, Slippers B *et al.*, 2012. One fungus, one name promotes progressive plant pathology. *Molecular Plant Pathology* **13**, 604–13.
Wingfield BD, Duong TA, Hammerbacher A *et al.*, 2016. Draft genome sequences for *Ceratocystis fagacearum*, *C. harringtonii*, *Grosmannia penicillata*, and *Huntiella bhutanensis*. *IMA Fungus* **7**, 317–23.
Zopf W, 1876. Über *Thielavia basicola*, einen endophytischen Parasiten in den Wurzeln des *Senecio elegans*. *Verhandlunegn des Botanischen Vereins fur die Provinz Brandenburg* **18**, 101–5.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. MEGA 6.0. phylogram derived from maximum likelihood analysis of *60S* dataset for selected species in the Ceratocystidaceae. Bootstrap support >75% is shown.

Figure S2. MEGA 6.0. phylogram derived from maximum likelihood analysis of ITS dataset for selected species in the Ceratocystidaceae. Bootstrap support >75% is shown.

Figure S3. MEGA 6.0. phylogram derived from maximum likelihood analysis of *MCM7* dataset for selected species in the Ceratocystidaceae. Bootstrap support >75% is shown.

Figure S4. MEGA 6.0. phylogram derived from maximum likelihood analysis of *LSU* dataset for selected species in the Ceratocystidaceae. Bootstrap support >75% is shown.

Figure S5. MEGA 6.0. phylogram derived from maximum likelihood analysis of ITS dataset for isolates of *Thielaviopsis basicola*. Bootstrap support >75% is shown.

Figure S6 MEGA 6.0. phylogram derived from maximum likelihood analysis of *MCM7* dataset for isolates of *Thielaviopsis basicola*. Bootstrap support >75% is shown.

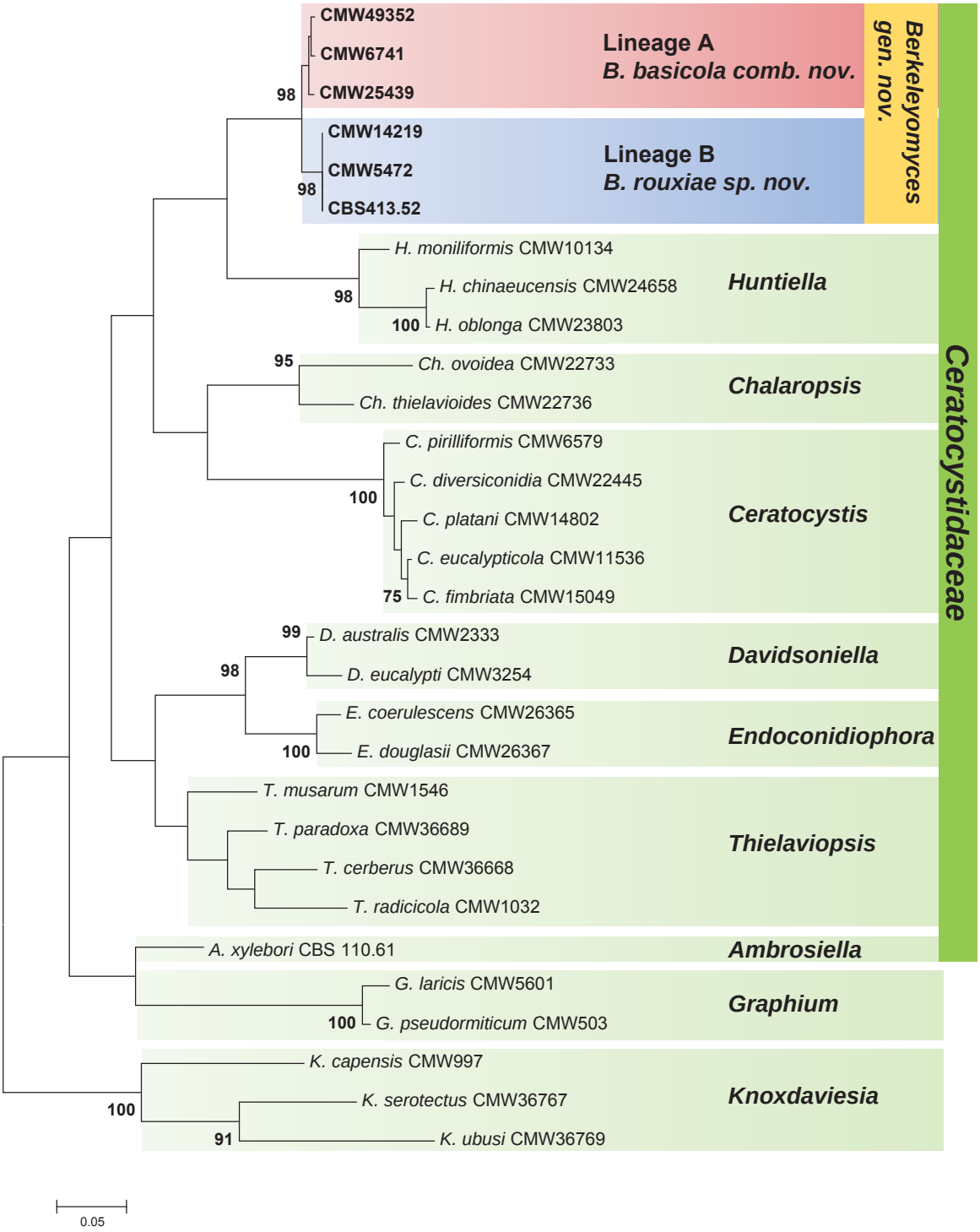
Figure S7 MEGA 6.0. phylogram derived from maximum likelihood analysis of *ACT* dataset for isolates of *Thielaviopsis basicola*. Bootstrap support >75% is shown.

Figure S8 MEGA 6.0. phylogram derived from maximum likelihood analysis of *RPB2* dataset for isolates of *Thielaviopsis basicola*. Bootstrap support >75% is shown.

Figure S9 MEGA 6.0. phylogram derived from maximum likelihood analysis of ITS dataset for all sequenced *Thielaviopsis basicola*. Bootstrap support >75% is shown.

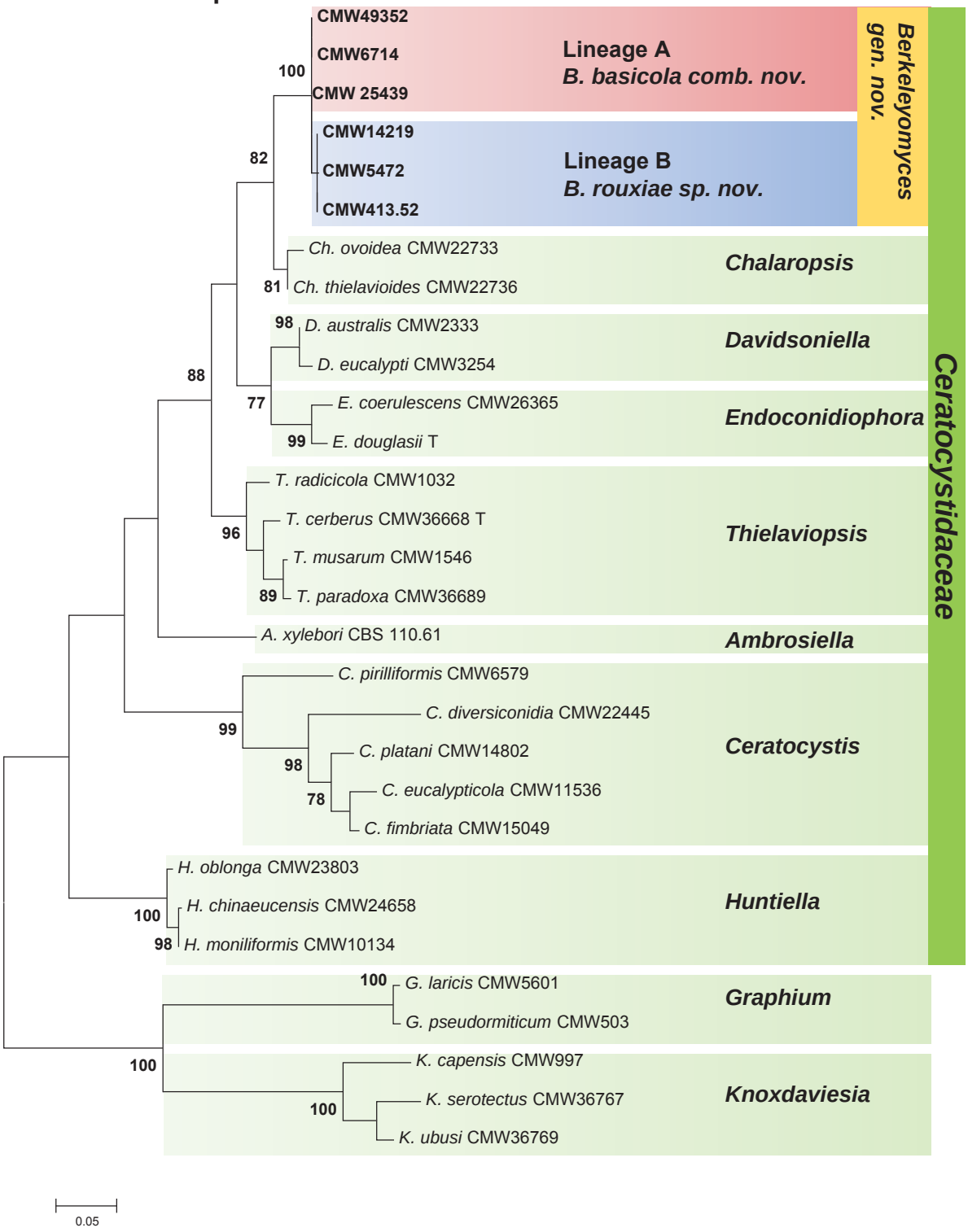
Table S1 Accession numbers for selected Ceratocystidaceae species included in the phylogenetic analyses.

Supplementary figure 1
Ceratocystidaceae
60S – MEGA 6.0 Maximum Likelihood
1000X Bootstrap



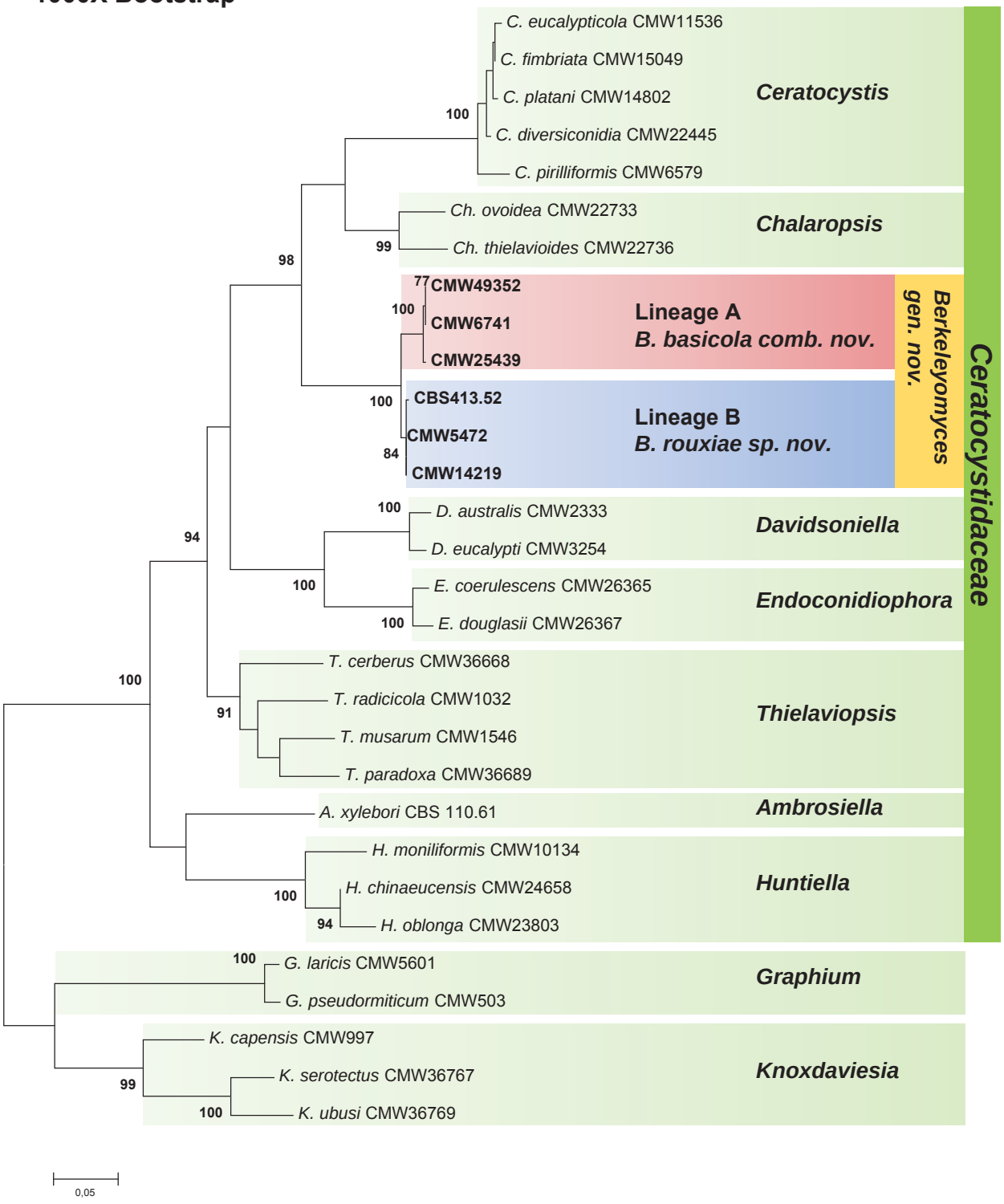
Supplementary Figure 1: Mega 6.0 phylogram derived from maximum likelihood analysis of the 60S sequence data for selected species in the Ceratocystidaceae. Isolates for which sequences were generated in this study is indicated in bold. Bootstrap support >75% is shown.

Supplementary figure 2
Ceratocystidaceae
ITS – MEGA 6.0 Maximum Likelihood
1000X Bootstrap



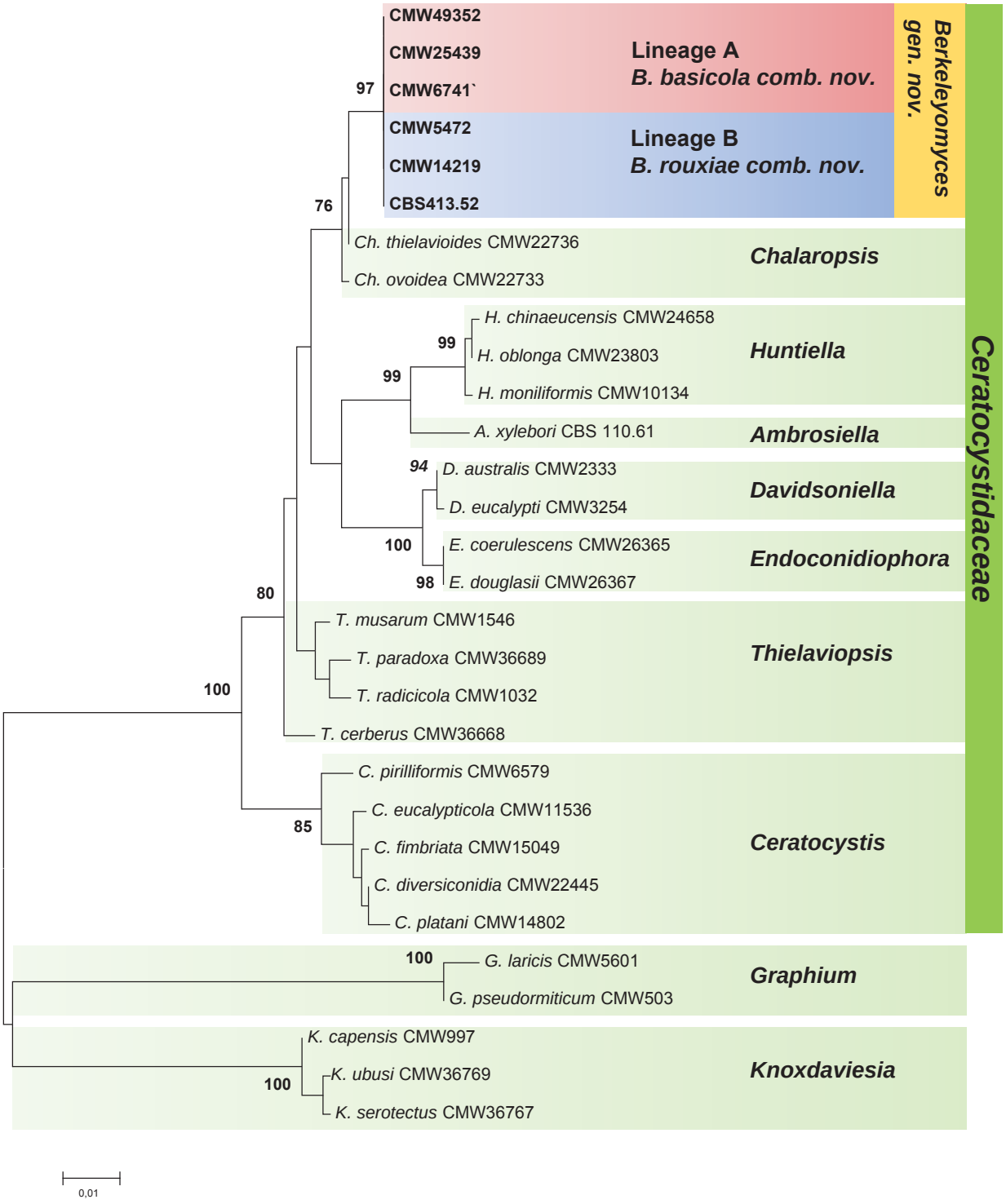
Supplementary Figure 2: Mega 6.0 phylogram derived from maximum likelihood analysis of the ITS sequence data for selected species in the Ceratocystidaceae. Isolates for which sequences were generated in this study is indicated in bold. Bootstrap support >75% is shown.

Supplementary figure 3
Ceratocystidaceae
MCM7 – MEGA 6.0 Maximum Likelihood
1000X Bootstrap



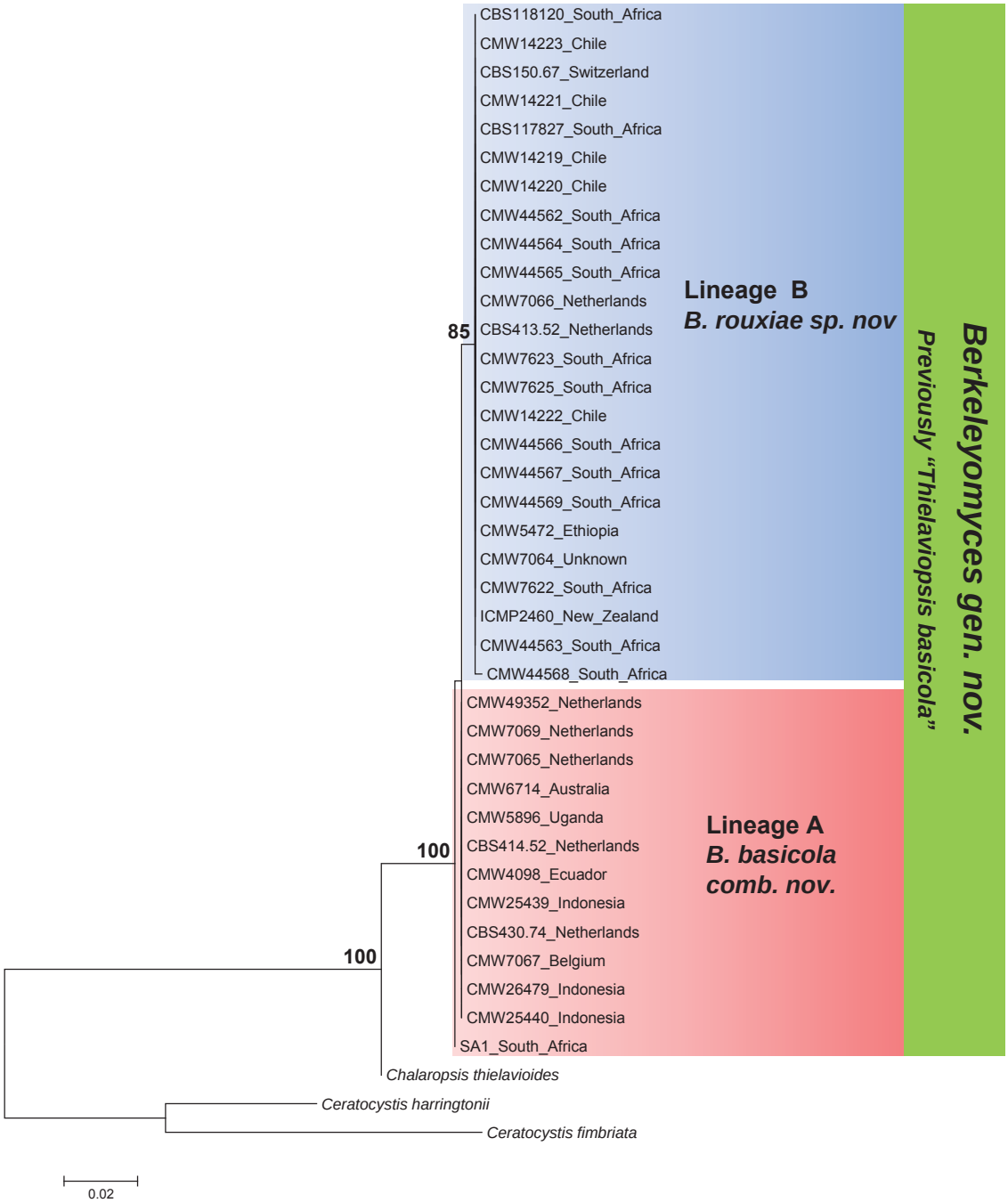
Supplementary Figure 3: Mega 6.0 phylogram derived from maximum likelihood analysis of the MCM7 sequence data for selected species in the Ceratocystidaceae. Isolates for which sequences were generated in this study is indicated in bold. Bootstrap support >75% is shown.

Supplementary figure 4
Ceratocystidaceae
LSU – MEGA 6.0 Maximum Likelihood
1000X Bootstrap



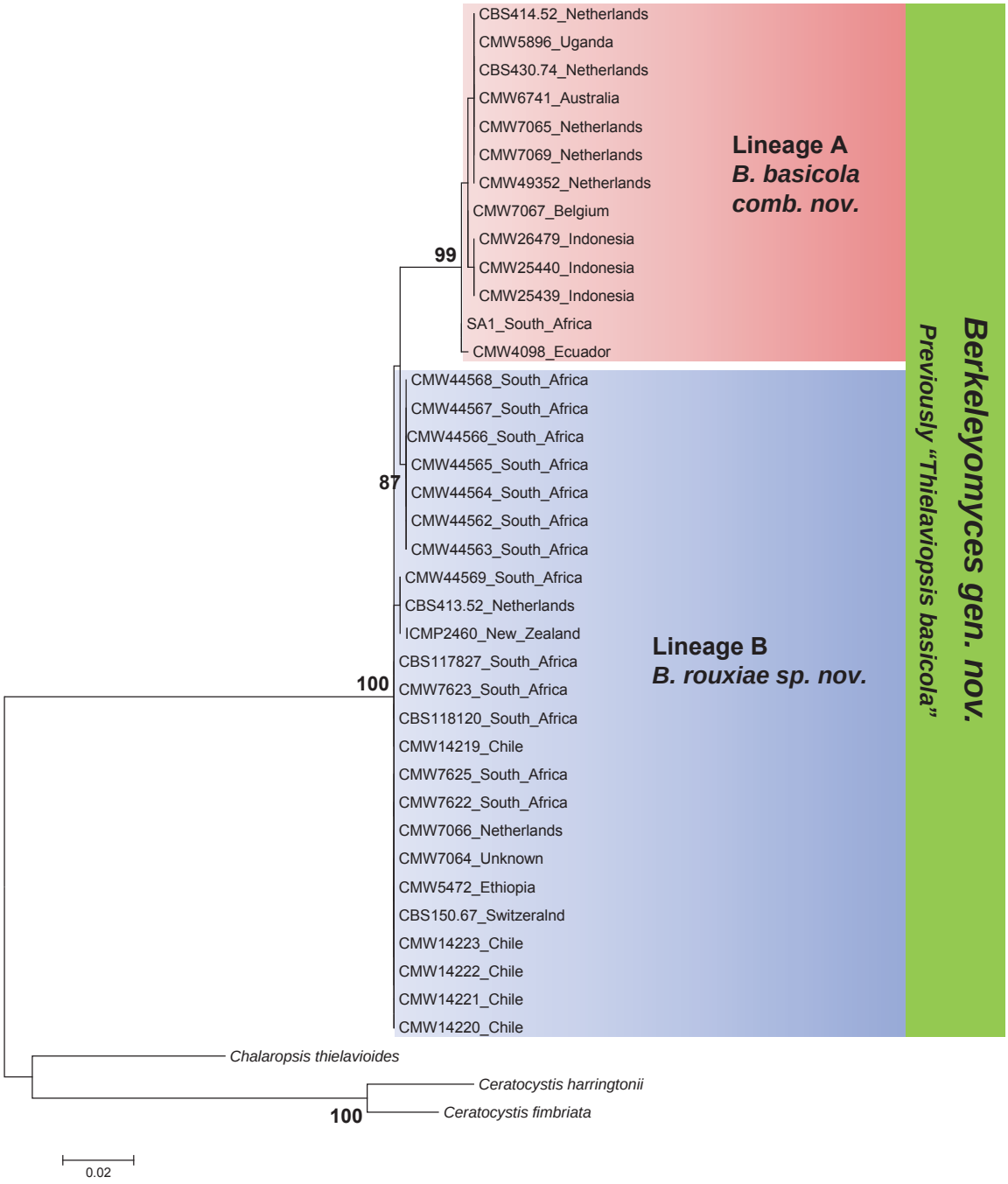
Supplementary Figure 4: Mega 6.0 phylogram derived from maximum likelihood analysis of the LSU sequence data for selected species in the Ceratocystidaceae. Isolates for which sequences were generated in this study is indicated in bold. Bootstrap support >75% is shown.

Supplementary figure 5
Thielaviopsis basicola
ITS – MEGA 6.0 Maximum likelihood
1000x Bootsrap



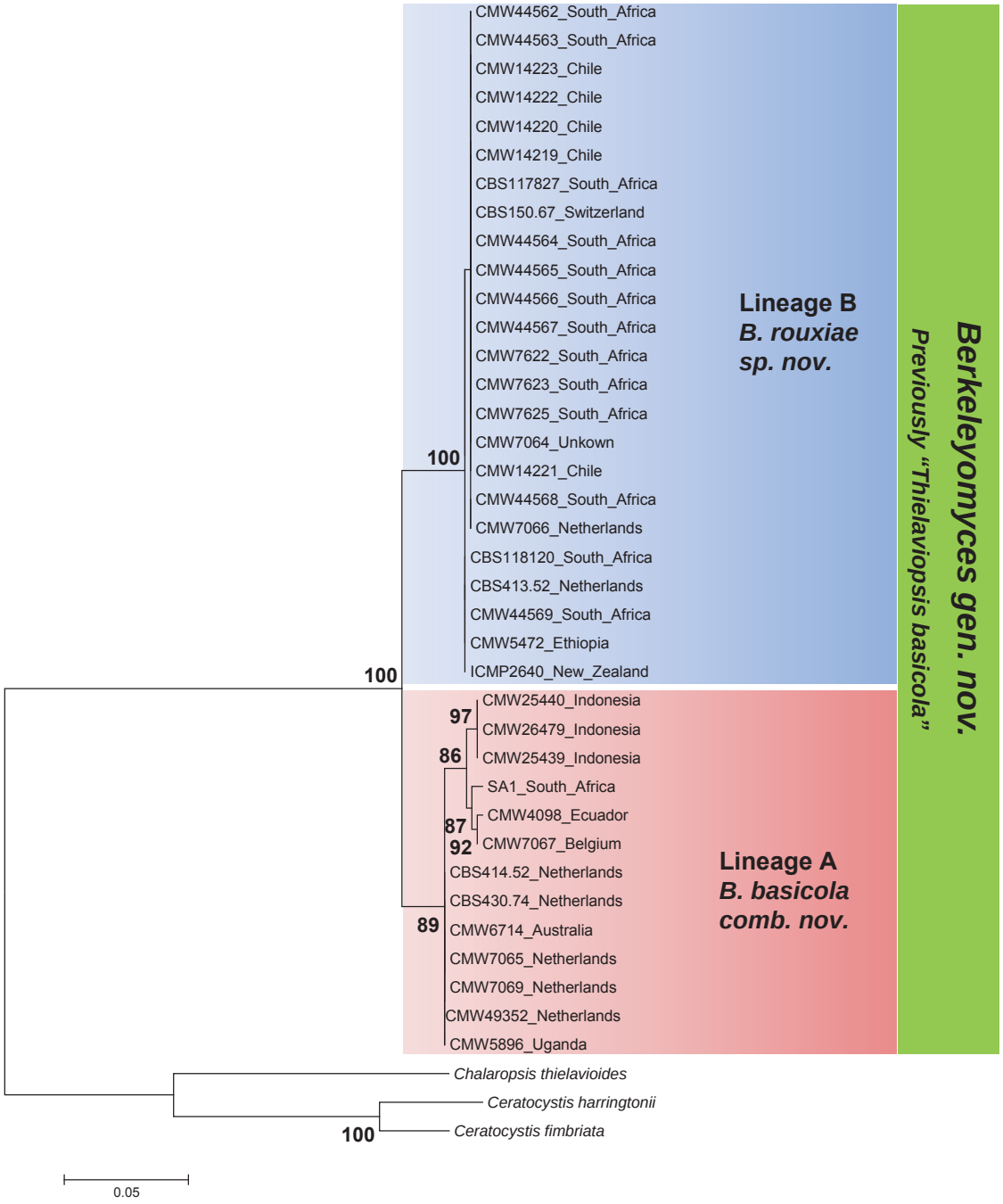
Supplementary Figure 5: Mega 6.0 phylogram derived from maximum likelihood analysis of the ITS sequence data for isolates previously known as *Thielaviopsis basicola*. Bootstrap support >75% is shown

Supplementary figure 6
Thielaviopsis basicola
MCM7 – MEGA 6.0 Maximum Likelihood
1000x bootstrap



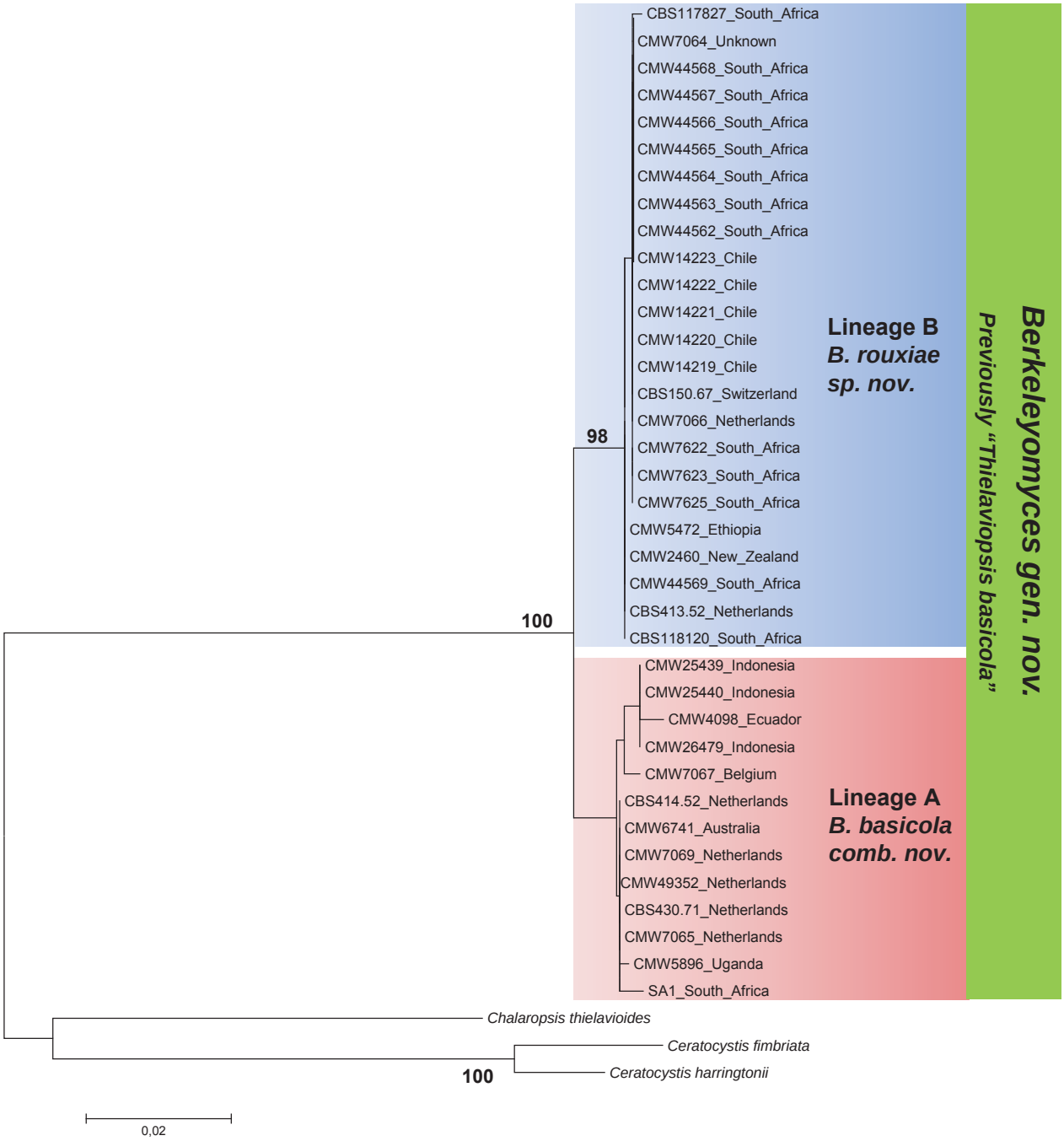
Supplementary Figure 6: Mega 6.0 phylogram derived from maximum likelihood analysis of the MCM7 sequence data for isolates previously known as *Thielaviopsis basicola.* Bootstrap support >75% is shown.

Supplementary figure 7
Thielaviopsis basicola
Actin – MEGA 6.0 Maximum likelihood
1000x Bootstrap



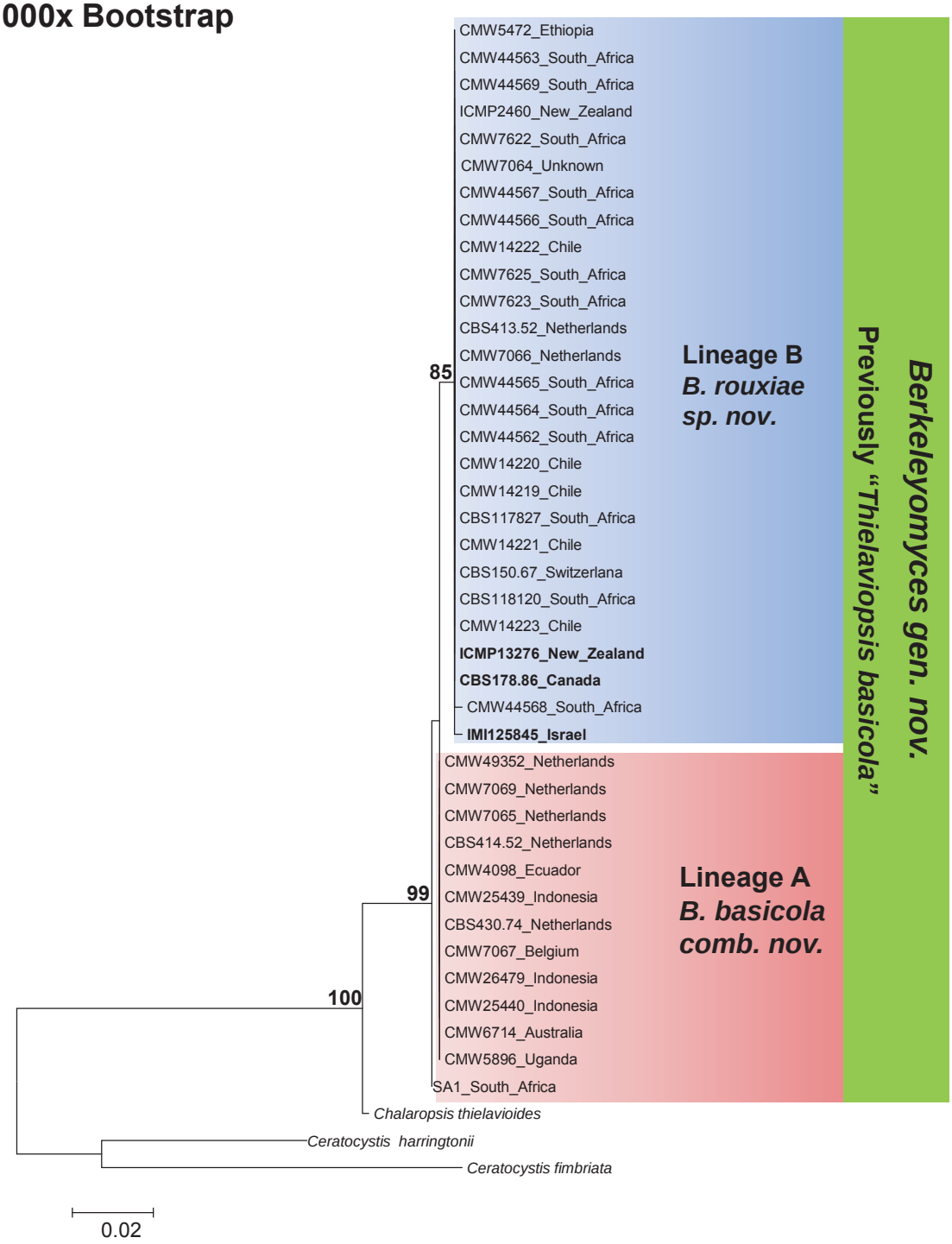
Supplementary Figure 7: Mega 6.0 phylogram derived from maximum likelihood analysis of the Actin sequence data for isolates previously known as *Thielaviopsis basicola*. Bootstrap support >75% is shown.

Supplementary figure 8
Thielaviopsis basicola
RPB2 – MEGA 6.0 Maximum likelihood
1000x Bootstrap



Supplementary Figure 8: Mega 6.0 phylogram derived from maximum likelihood analysis of the RPB2 sequence data for isolates previously known as *Thielaviopsis basicola*. Bootstrap support >75% is shown.

Supplementary figure 9
All sequenced *Thielaviopsis basicola* isolates
ITS – MEGA 6.0 Maximum likelihood
1000x Bootstrap



Supplementary Figure 9: Mega 6.0 phylogram derived from maximum likelihood analysis of the ITS sequence data for all isolates of *Thielaviopsis basicola* obtained during this study. Isolates where only the ITS sequences could be obtained are indicated in bold. Bootstrap support >75% is shown.

Supplementary Table 1: Accession numbers for selected *Ceratocystidaceae* species included in the phylogenetic analyses

Species	Gene region accession number			
	ITS	**LSU**	**60S protein**	**MCM7**
Ambrosiella xylebori	NR_144921	KM495318	KM495495	KM495407
Ceratocystis diversiconidia	FJ151440	KM495334	KM495511	KM495423
C. eucalypticola	FJ236723	KM495339	KM495516	KM495428
C. fimbriata	AY953378	KM495343	KM495520	KM495432
C. pirilliformis	AF427105	KM495365	KM495542	KM495453
C. platani	DQ520630	KM495366	KM495543	KM495454
Chalaropsis ovoidea	FJ411343	KM495400	KM495577	KM495487
Ch. thielavioides	FJ411342	KM495402	KM495579	KM495489
Davidsoniella australis	FJ411325	KM495396	KM495573	KM495483
D. eucalyppti	FJ411327	KM495338	KM495515	KM495427
Endoconidiophora coerulescens	FJ411322	KM495329	KM495506	KM495418
E. douglasii	NR_120295	KM495335	KM495512	KM495424
Graphium laricis	AY148183	KM495389	KM495566	KM495476
G. pseudormiticum	AY148186	KM495390	KM495567	KM495477
Huntiella chinaeucensis	JQ862729	KM495327	KM495504	KM495416
H. moniliformis	FJ151422	KM495355	KM495532	KM495443
H. oblonga	EU245019	KM495359	KM495536	KM495447
Knoxdaviesia capensis	EU660439	KM495391	KM495568	KM495478
K. serotectus	JF947182	KM495394	KM495571	KM495481
K. ubusi	JF947186	KM495395	KM495572	KM495482
Thielaviopsis cerberus	NR_145225	KM495326	KM495503	KM495415
T. musarum	JX518325	KM495357	KM495534	KM495445
T. paradoxa	JX518342	KM495363	KM495540	KM495451
T. radicicola	KF612023	KM495371	KM495548	KM495459

(0000) Proposal to reject the name *Milowia nivea* (*Sordariomycetes: Microascales*)

Wilma J. Nel[1], Michael J. Wingfield[1] & Z. Wilhelm de Beer[1]

[1] *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*

Author for correspondence: *Wilhelm de Beer, wilhelm.debeer@fabi.up.ac.za*

DOI

(0000) ***Milowia nivea*** Massee in J. R. Microsc. Soc. IV 2: 841. 1884, nom. rej. prop.

Typus: non designatus.

(0000) ***Milowia*** Massee in J. R. Microsc. Soc. IV 2: 841. 1884, nom. rej. prop.

(0000) ***Milowieae*** Sacc. in Syll. Fung. 4: 188. 1886, nom. rej. prop.

(0000) ***Milowiaceae*** Nann. in Tratt. Micopat. Um. 4: 471. 1934, nom. rej. prop.

The ascomycete genus and species, *Milowia nivea* Massee, were first described by Massee (in **J. R. Microsc. Soc.** 4: 842–845. 1884). He referred to some of the structures in his illustrations as 'octosporous asci' and provided a detailed illustrated description of how these structures arose from a 'carpogonium' fertilized by a 'pollinodium' resulting in the production of 'sporidia'. In addition to this stage, he illustrated a 'branch bearing globose, moniliform conidia'. Cooke (in Grevillea 14: 1–7. 1885) questioned the nature of the 'asci' and suggested that they might be 'more completely developed hyphae with dissilient joints'. A year later Saccardo (in Syll. Fung. 4: 188. 1886) interpreted Massee's 'carpogonium' as 'sporophora' or 'basidia 3-locularia', and based a new monotypic tribe, *Milowieae* Sacc., on *Milowia*. In 1893, Massee (in British Fungus-Flora Vol. 111: 312, 348. 1893), published a different drawing and new description of the fungus, showing and mentioning only one type of spore and stated that 'the conidia present the appearance of spores in an ascus.' In 1909 Massee (in Naturalist 629: 235–238. 1909) yet again produced a substantially modified drawing of the fungus, showing 'three markedly different stages in its complete life cycle'. He now referred to his initial 'branch bearing globose, moniliform conidia', that were not mentioned or illustrated in his 1893 (l.c.) drawings of *Milowia nivea*, as the 'first conidial (*Milowia*) stage'. The 'sporidia' from 'octosporous asci' of 1884 (l.c.) that was illustrated as *Milowia nivea* conidia in 1893 (l.c.), was now referred to as 'the second conidial (*Torula*) stage', based on similarity with *Torula basicola* Berk. & Broome (in Ann. Mag. Nat. Hist. Ser. 2, 5: 455–466. 1850). The third stage discussed and illustrated (Massee, l.c., 1909) was the

‘ascigerous stage’ with an illustration of an eight-spored ascus and perithecium that he named *Thielavia basicola* Zopf, the sexual state for *Torula basicola* described earlier by Zopf (in Sber. bot. Ver. Prov. Brandenb. 18: 100–105. 1876). Almost 20 years after his initial description, Massee (in Bull. Misc. Inform. 1912: 44–52. 1912) produced a fourth illustration showing more details of the three stages he distinguished in 1909 (l.c.). In this version of the illustration the ‘carpogonium’ and ‘pollinodium’ that were visible in five drawings of his 1884 (l.c.) illustrations, and in a reduced form in the 1893 (l.c.) illustration, were now completely absent. In addition, Massee (1912, l.c.) formally listed *M. nivea*, together with *Torula basicola* and *Helminthosporium fragile* Sorokïn (in Hedw. 15: 113. 1876), as synonyms of *Thielavia basicola.*

Also in 1912, Ferraris (in Flor. Ital. Cryptogam. 1: 233–234. 1912) treated *Torula basicola* for the first time in the asexual genus *Thielaviopsis* Went (in Meded. Proefstn SuikRiet W. Java 5: 4. 1893), as *Thielaviopsis basicola* (Berk. & Broome) Ferraris, listing *H. fragile* as synonym, but not mentioning *M. nivea*. He also considered *Thielavia basicola* to represent the sexual state. However, McCormick (in Bull. Connecticut Agric. Exp. Stat. 269: 539–554. 1925) later showed in a thorough set of laboratory experiments that the sexual *Thielavia basicola* and asexual *Thielaviopsis basicola* were distinct species. Although the name *Thielavia basicola* had been used often in subsequent literature, the true sexual state of *Thielaviopsis basicola* remains to be discovered and the name *Thielavia basicola* should not be used, as that species belongs to the Chaetomiaceae (in Stchigel et al. Mycol. Res. 106: 975–983. 2002).

Nag Raj & Kendrick (in Monogr. Chalara Allied Genera: 43. 1975) recognized two asexual states for *T. basicola*. They argued that Berkeley & Broome (1850, l.c.) only mentioned the chlamydospore-producing asexual state in their description of *Torula basicola*, and therefore that that name was applicable only to that form of the pathogen. They described Massee’s (1909, l.c.) ‘first conidial (*Milowia*) stage’ as ‘endoconidial’, and treated that state in the genus *Chalara* (Corda) Rabenh. (in Rabenh., Deutschl. Krypt.-Fl. Leipzig 1: 38. 1844). However, Nag Raj & Kendrick (1975, l.c.) provided this endoconidial state with a unique epithet (*Chalara elegans* Nag Raj & W.B. Kendr.) based on the argument that the endoconidial state is produced abundantly in culture, while the chlamydospore state (*Thielaviopsis basicola*) can sometimes be completely lost. They formally reduced *Thielaviopsis* to synonymy with *Chalara*, resulting in *Chalara elegans* as the preferred name. Nag Raj & Kendrick (1975, l.c.) could have chosen *Milowia nivea* as basionym for the species they assigned to *Chalara*, but Massee (1884, l.c.) did not mention a type specimen, and several attempts by Nag Raj & Kendrick (1975, l.c.) to locate Massee’s specimen were futile. Based on the varying and changing descriptions and illustrations of Massee, they also did not consider designating one of his illustrations as lectotype, and preferred to treat the species as dubious.

Paulin-Mahady et al. (in Mycologia 94: 62–72. 2002), suggested that the type species of *Chalara* would best reside in the Leotiales, and redefined *Thielaviopsis* to accommodate *Chalara* species with *Ceratocystis* affinities, including *C. elegans*. The name *Thielaviopsis basicola* was thus re-instated, with *C. elegans* as synonym, without mentioning other synonyms. However, in a comprehensive multigene study, De Beer et al. (in Stud. Mycol. 79: 187–219. 2014) showed that *T. basicola* and the respective type species of *Ceratocystis* and *Thielaviopsis* grouped in three distinct, well-supported lineages. They distinguished between and redefined *Ceratocystis* and *Thielaviopsis*, but because a sequence representing the type of *T. basicola* was not available, they refrained from assigning *T. basicola* to a genus. In a study focusing on the generic placement of *T. basicola*, Nel et al. (in Plant Pathol. doi: 10.1111/ppa.12803) determined and analysed sequences for seven gene regions of 41 isolates of *T. basicola* and confirmed that the species consisted of two cryptic sister species in a genus distinct from *Thielaviopsis* and other genera in the Ceratocystidaceae. Again, the name *Milowia* was available for this genus if the synonymy of *T. basicola* and *M. nivea* could be confirmed. However, like Nag Raj & Kendrick (l.c.), Nel et al. (l.c.) also could not locate any of Massee's authentic material for *M. nivea*. In view of Massee's repeated modifications of his own drawings and terminology, and the confusing interpretations of some of the structures as asci and basidia, they concurred with Nag Raj & Kendrick (l.c.) and treated the species as dubious. A new genus, *Berkeleyomyces* W.J. Nel, Z.W. de Beer, T.A. Duong, M.J. Wingf., was thus described (in Nel et al. l.c.) with *B. basicola* (Berk. & Broome) W.J. Nel, Z.W. de Beer, T.A. Duong, M.J. Wingf. as type species, together with a second novel species, *B. rouxiae* W.J. Nel, Z.W. de Beer, T.A. Duong, M.J. Wingf..

Even though the status of *M. nivea* is considered dubious, the name and those for which it serves as basionym, remain available for use. It would also be permissible under the Code to designate Massee's illustrations as lectotype, or to designate a neotype for the species. However, Nannizzi (in Tratt. Micopat. Um. 4: 471. 1934) elevated Saccardo's (1886, l.c.) *Milowieae* tribe to the family level, as *Milowiaceae* Nann. If a neo- or lectotype was to be designated for *M. nivea*, and Massee's synonymy of *M. nivea* with *T. basicola* was to be accepted, the implications will be as follows: (1) *Milowia* will have priority over the new name *Berkeleyomyces*. Much more significantly, (2) the well-established epithet *basicola* of the root pathogen will be replaced with the almost unknown *nivea*, and (3) the family name *Milowiaceae* will have priority over the much younger, but well-established name, *Ceratocystidaceae*, in the *Microascales* (Réblová & al. in **Stud. Mycol.** 68: 163–191. 2011, De Beer & al. l.c.). Online searches on 30 November 2017 for '*Milowiaceae*' in Google Scholar and Google, yielded only 0 and 9 hits respectively, while '*Ceratocystidaceae*' yielded 835 and ~20000 hits, respectively. This provides strong evidence that *Ceratocystidaceae* is the more widely used name (see Hawksworth in IMA Fungus 3: 15–24. 2012).

In view of Massee's repeated modifications of his drawings and terminology, it is doubtful whether any fungus resembles the drawings of the protologue (in Massee l.c. 1884.). Misguided interpretations of his descriptions led to the confused diagnoses of the tribe *Milowieae* (in Saccardo, l.c. 1886) and the family *Milowiaceae* (in Nannizzi, l.c. 1934). In order to protect the *Ceratocystidaceae* against the lesser known *Milowiaceae* (Art. 56.1), we propose that *Milowia nivea* Massee, and the names for which it is the basionym, should be formally rejected.

[Note: This chapter was prepared as a proposal to reject for submission to the journal Taxon. Unlike a regular article, proposals do not follow standard referencing styles. We have followed their recommended style that can be found at: http://www.iapttaxon.org/files/guidelines_proposals.pdf under the heading "Format and contents of proposals"]

Chapter 4: Draft genome sequence of the root pathogen *Berkeleyomyces basicola* (=*Thielaviopsis basicola)*

W.J. Nel[a], T.A. Duong[b], A. Hammerbacher[c], B.D. Wingfield[b], M.J. Wingfield[a], Z.W. de Beer[a]

[a] Department of Microbiology and Plant Pathology, [b] Department of Genetics, [c] Department of Zoology Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

Abstract

The complete genome of the newly designated reference specimen (CBS 142796) of *Berkeleyomyces basicola* (=*Thielaviopsis basicola*) was sequenced and annotated. Assembly produced a genome with a total size of approximately 25.1 Mb with a GC content of 52% and just over 10 000 putative coding regions. This genome will facilitate future genome comparison studies for species of *Ceratocystidaceae.*

[Note: This chapter has been prepared in the style required to be submitted to IMA fungus]

Introduction

Berkeleyomyces basicola (Ascomycota: Microascales), previously known as *Thielaviopsis basicola* (Nel *et al.*, 2017) is an important plant pathogen responsible for root rot of many important agricultural and ornamental plants (Johnson, 1916, Stover, 1950, Nehl *et al.*, 2004, Pereg, 2013). Since its description in the mid 1800's (Berkeley & Broome, 1850), there has been considerable debate surrounding its appropriate taxonomic placement resulting in numerous name changes. The phylogenetic re-evaluation of the *Ceratocystidaceae* by De Beer *et al.* (2014) raised new questions regarding the appropriate taxonomic placement of the species. Their results suggested that *T. basicola* did not group in *Thielaviopsis* or any other genus described in the family. Because the authors included only the sequence data of a single isolate in their analyses they concluded that no taxonomic changes could be made without further study. In a recent investigation, Nel *et al.* (2017) confirmed that *T. basicola* represented a distinct generic lineage in the *Ceratocystidaceae* and introduced the new genus *Berkeleyomyces*. In addition, they showed that isolates of *Berkeleyomyces* represented two cryptic sister species for which they provided the names *B. basicola* and *B. rouxiae.*

The aim of this study was to generate a high-quality genome sequence for *Berkeleyomyces basicola*. This would allow for comparisons to be made with the available genomes of other species in the Ceratocystidaceae including those in the genera *Ceratocystis, Huntiella, Davidsoniella, Thielaviopsis, Chalaropsis, Endoconidiophora* and the recently described *Bretziella* (De Beer et al. 2017). Here we report the complete genome sequence of isolate CBS 142796, the designated reference specimen for *B. basicola* logged in the collection (CBS) of the Westerdijk Institute, Utrecht, the Netherlands, and the culture collection of the Forestry and Agricultural Biotechnology Institute (CMW), University of Pretoria, South Africa.

Sequenced strain:

NETHERLANDS, *South Holland,* Boskoop. *Betula* sp. June 1974. SG de Hoog (CMW 49352 = CBS 142796; PREM 62125 = dried culture).

Nucleotide sequence accession number: The draft genome sequence of *Berkeleyomyces basicola* (CMW 49352 = CBS 142796) has been deposited at DDBJ/EMBL/NCBI GenBank under the accession number PENDING. The version presented here is PENDING.

Materials and Methods

Genome sequencing

Genomic DNA was extracted from lyophilized mycelium of *B. basicola* isolate CMW 49352 grown in malt yeast broth (2% Malt extract, 0.5% yeast extract, Biolab, Midrand, South Africa) using the method described by Duong *et al.* (2013). A paired-end library was prepared (350 bp average insert sizes) and

sequenced using the Illumina HiSeqX Platform. A mate-pair library was prepared (10 Kb average insert size) and sequenced using the Illumina HiSeq2500 platform. Long reads were also generated using one cell of the Single-molecule real time (SMRT or PacBio) sequencing platform (Pacific BioScience). All sequencing was conducted at Macrogen Inc. (Seoul, Korea). Quality and adapter trimming of pair-end and mate-pair reads was carried out using Trimmomatic v. 0.36 (Bolger *et al.*, 2014). *De novo* assembly of the genome was carried out using SPAdes v. 3.9 (Bankevich *et al.*, 2012) using all pair-end, mate-pair and PacBio data. Contigs smaller than 500 bp were removed from the dataset. Initial scaffolding was done using SSPACE-standard v. 3.0 (Boetzer *et al.*, 2011) with the paired-end and mate-pair reads. A second round of scaffolding was done using SSPACE-Longread with the PacBio reads. Assembly gaps were filled using GapFiller v. 1.10 (Boetzer & Pirovano, 2012) with the paired-end and mate-pair reads, and using PBJelly (English *et al.*, 2012) with PacBio reads. Final genome polishing was done using Pilon (Walker *et al.*, 2014). Genome completeness was assessed with the Benchmarking Universal Single-Copy Orthologs (BUSCO v. 1.1b1) tool using the Ascomycota dataset (Simão *et al.*, 2015). The number of protein coding genes was determined using Augustus v. 3.3.2 (Stanke *et al.*, 2004) using pre-optimised species models for *Fusarium graminearum*.

Phylogenetic analyses

The genome of *B. basicola* isolate CMW 49352 was interrogated to identify the MCM7 containing contig. The BLASTn function of CLCbio Genomics Workbench v. 9.5 (CLCbio, QIAGEN, Aarthus, Denmark) was used to screen the assembled genome for the gene sequence using an available reference sequence for the gene from *Berkeleyomyces basicola* (Accession: MF967102). A dataset was prepared based on the phylogenies of Nel *et al.* (2017) and sequences were downloaded from NCBI GenBank. DNA sequence alignments of the dataset were done using the online version of MAFFT v. 7 (Katoh & Standley, 2013) and Maximum Likelihood phylogenetic analyses was performed using MEGA v. 6.06 (Tamura *et al.*, 2013). The GTR model was used and 1000 bootstrap repeats were tested.

Results and Discussion

The paired-end, mate-pair, and PacBio sequencing yielded 431 141 384, 60 673 400 and 42 422 reads, respectively. Final assembly consisted of 81 contigs, with the largest around 3.8 Mb and an N50 of 1.2 Mb. The estimated size of the genome is around 25.1 Mb with a GC content of 52%. This estimated size is similar to that of other species in the *Ceratocystidaceae*, which range between 25.4 Mb for *Huntiella moniliformis* and 33.6 Mb for *Davidsoniella virescens* (Wilken et al., 2013; Van der Nest et al., 2014a, b; Wingfield et al., 2015a, b; 2016a, b).

BUSCO analysis predicted an assembly completeness of 97.4 %. The assembly contained 1280 complete single-copy BUSCOs, one complete and duplicated BUSCOs, 10 fragmented BUSCOs and 24 missing BUSCOs out of a total 1315 BUSCO groups searched. AUGUSTUS annotation predicted 10074 putative coding regions, corresponding to around 401 ORFs/Mb.

The MCM7 sequence extracted from the genome was identical to the sequence produced for the same isolate using Sanger sequencing in the investigation by Nel *et al.* (2017). Both isolates also grouped together with an additional isolate of *B. basicola* included in the analyses, and these isolates formed a sister group to an isolate of *B. rouxiae* as expected. This provides confidence that the genome sequenced during this investigation is correct.

The availability of the genome for *B. basicola* will make possible genome comparisons with other species in the *Ceratocystidaceae* and it will facilitate investigations into factors involved in pathogenicity, ecology, mating, and evolution of this important plant pathogen.

Acknowledgements

We would like the thank The University of Pretoria, the Tree Protection Co-operative Programme (TPCP), the DST-NRF Centre of Excellence in Tree Health Biotechnology, the National Research Foundation, the Genomics Research Institute (GRI) for their financial support of this project.

References

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M (2012) SPAdes: a new genome assembly alogrith and its applications to single-cell sequencing. *Journal of Computational Biology* **19**: 455-477.

Berkeley MJ, Broome CE (1850) Notices of British fungi. *Annals and Magazine of Natural History Series 2* **5:** 455-465.

Boetzer M, Pirovano W (2012) Toward almost closed genomes with GapFiller. *Genome Biology* **13**: R56.

Boetzer M, Henkel C, Jansen H, Butler D, Pirovano W (2011) Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics* **27**: 578-579.

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114-2120.

De Beer ZW, Duong TA, Barnes I, Wingfield BD, Wingfield MJ (2014) Redefining *Ceratocystis* and allied genera. *Studies in Mycology* **79**: 187-219.

De Beer ZW, Marincowitz S, Duong TA, Wingfield MJ (2017) *Bretziella*, a new genus to accommodate the oak wilt fungus, *Ceratocystis fagacearum* (Microascales, Ascomycota). *MycoKeys* **27**: 1-19.

Duong TA, De Beer ZW, Wingfield BD, Wingfield MJ (2013) Characterization of the mating-type genes in *Leptographium procerum* and *Leptographium profanum*. *Fungal Biology* **117**: 411-421.

English AC, Richards S, Han Y, Wang M, Vee V *et al.* (2012) Mind the gap: Upgrading genomes with Pacific Biosciences RL long-read sequencing technology. *PLoS ONE* **7**: e47768.

Johnson J (1916) Host plants of *Thielavia basicola*. *Journal of Agricultural Research* **7:** 289-300.

Katoh K, Standley D (2013) MAFFT multiple seqeunce alignment software version 7: Improvements in performance and usablility. *Molecular Biology and Evolution* **30**: 772-780.

Nehl DB, Allen SJ, Mondal AH, Lonergan PA (2004) Black root rot: A pandemic in Australian cotton. *Australasian Plant Pathology* **33**: 87-95.

Nel WJ, Duong TA, Wingfield MJ, Wingfield BD, De Beer ZW (2017) A new genus and species for the globally important, multi-host root pathogen *Thielaviopsis basicola*. *Plant Pathology In Press*.

Pereg LL (2013) Black root rot of cotton in Australia: The host, the pathogen and disease management. *Crop and Pasture Science* **64**: 1112-1126.

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**: 3210-3212.

Stanke M, Steinkamp R, Waack S, Morgenstern B (2004) AUGUSTUS: A web server for gene finding in Eukaryotes. *Nucleic Acids Research* **32**: W309-W312.

Stover RH (1950) The black rootrot disease of tobacco: I. Studies on the causal organism *Thielaviopsis basicola*. *Canadian Journal of Research* **28**: 445-470.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.

Van der Nest MA, Beirn LA, Crouch JA, Demers JE, De Beer ZW, *et al.* (2014a) Draft genomes of *Amanita jacksonii, Ceratocystis albifundus, Fusarium circinatum, Huntiella omanensis, Leptographium procerum, Rutstroemia sydowiana,* and *Sclerotinia echinophila*. *IMA Fungus* **5:** 473–486.

Van der Nest MA, Bihon W, De Vos L, Naidoo K, Roodt D *et al.* (2014b) Draft genome sequences of *Diplodia sapinea, Ceratocystis manginecans,* and *Ceratocystis moniliformis*. *IMA Fungus* **5**: 135-140.

Walker BJ, Abeel T, Shea T, Priest M, Abouselliel A *et al.* (2014) Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE* **9:** e112963.

Wilken PM, Steenkamp ET, Wingfield MJ, De Beer ZW, Wingfield BD (2013) Draft nuclear genome sequence for the plant pathogen, *Ceratocystis fimbriata*. *IMA Fungus* **4:** 357-358.

Wingfield BD, Ades PK, Al-Naemi FA, Beirn LA, Bihon W, *et al.* (2015a) Draft genome sequences of *Chrysoporthe austroafricana, Diplodia scrobiculata, Fusarium nygamai, Leptographium lundbergii, Limonomyces culmigenus, Stagonosporopsis tanaceti,* and *Thielaviopsis punctulata*. *IMA Fungus* **6:** 233-248.

Wingfield BD, Barnes I, De Beer ZW, De Vos L, Duong TA *et al.* (2015b) Draft genome sequences of *Ceratocystis eucalypticola, Chrysoporthe cubensis, C. deuterocubensis, Davidsoniella virescens, Fusarium temperatum, Graphilbum fragrans, Penicillium nordicum,* and *Thielaviopsis musarum*. *IMA Fungus* **6**: 493-506

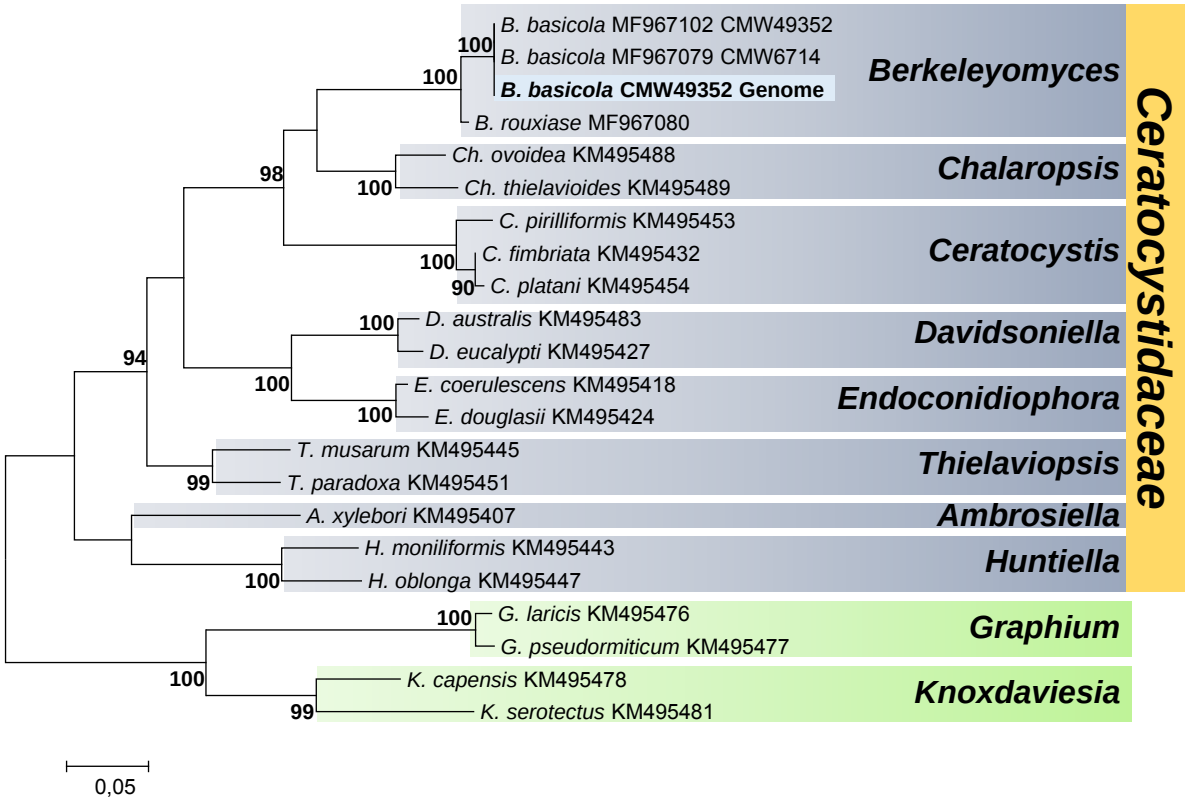
Wingfield BD, Ambler JM, Coetzee MPA, De Beer ZW, Duong TA *et al.* (2016a) Draft genome sequences of *Armillaria fuscipes, Ceratocystiopsis minuta, Ceratocystis adiposa, Endoconidiophora laricicola, E. polonica* and *Penicillium freii* DAOMC 242723. *IMA Fungus* **7:** 217-227.

Wingfield BD, Duong TA, Hammerbacher A, Van der Nest MA, Wilson A *et al.* (2016b) IMA Draft genome sequences for *Ceratocystis fagacearum, C. harringtonii, Grosmannia penicillata*, and *Huntiella bhutanensis*. *IMA Fungus* **7:** 317-323.

Figures:

Figure 1: Phylogram derived from maximum likelihood analysis of the MCM7 sequence data for species in the Ceratocystidaceae. Bootstrap support >75% is shown at nodes.

MCM7


B. basicola MF967102 CMW49352
B. basicola MF967079 CMW6714
B. basicola CMW49352 Genome
B. rouxiase MF967080
Berkeleyomyces
Ch. ovoidea KM495488
Ch. thielavioides KM495489
Chalaropsis
C. pirilliformis KM495453
C. fimbriata KM495432
C. platani KM495454
Ceratocystis
D. australis KM495483
D. eucalypti KM495427
Davidsoniella
E. coerulescens KM495418
E. douglasii KM495424
Endoconidiophora
T. musarum KM495445
T. paradoxa KM495451
Thielaviopsis
A. xylebori KM495407
Ambrosiella
H. moniliformis KM495443
H. oblonga KM495447
Huntiella
G. laricis KM495476
G. pseudormiticum KM495477
Graphium
K. capensis KM495478
K. serotectus KM495481
Knoxdaviesia
Ceratocystidaceae
100
100
100
98
100
100
90
94
100
100
100
99
100
100
100
99
0,05

Chapter 5: Heterothallism revealed in the root rot fungi *Berkeleyomyces basicola* and *B. rouxiae* (previously *Thielaviopsis basicola*)

Abstract

Berkeleyomyces basicola and *B. rouxiae*, two cryptic sister species previously treated collectively as *Thielaviopsis basicola*, reside in the *Ceratocystidaceae* (Microascales, Ascomycota). Both species are important plant pathogens that infect the roots of many important agricultural and ornamental species. Although *T. basicola* had been known for more than 150 years, no sexual state has been described for it and it was assumed to be an asexual pathogen. The aim of this study was to determine the mating strategy of the two *Berkeleyomyces* species. Interrogation of the genome sequences of two individual *B. basicola* isolates allowed the complete characterization of its *MAT-1* locus and revealed that this species has a typical heterothallic mating system with the *MAT1-1* and *MAT1-2* idiomorphs occurring in different isolates of the species. PCR amplification using newly developed mating type primers showed that the *MAT1-1-1* and *MAT1-2-1* gene regions are present in different isolates of *B. rouxiae*. Pairing of isolates representing the two mating types in both *B. basicola* and *B. rouxiae* using a variety of techniques failed to result in sexual structures. While these fungi are clearly heterothallic and both mating types occur in some countries, we have found no direct evidence that they actually reproduce sexually.

Introduction

Berkeleyomyces basicola and its sister species *B. rouxiae* are filamentous Ascomycete fungi that reside in the *Ceratocystidaceae* (Nel *et al.*, 2017; De Beer *et al*., 2014). The two species are plant pathogens, causing a disease commonly known as black root rot on many important crops such as tobacco (Stover, 1950), cotton (Pereg, 2013) and carrots (AbdAllah *et al.*, 2011). Until recently, these two pathogens were treated under the single name *Thielaviopsis basicola*, originally introduced by Ferraris (1912) based on morphological species classification. De Beer *et al.* (2014), while investigating the taxonomy of the *Ceratocystidaceae*, suggested that the generic level placement of the species required revision because the isolate included in their study grouped outside *Thielaviopsis* and all other known genera in the family. Based on phylogenetic and clear biological and morphological differences with other genera in the family, Nel *et al.* (2017) described the new genus *Berkeleyomyces* and showed that isolates previously described as *T. basicola* represent two cryptic species. They retained the epithet *basicola* for the type species of the new genus, and described the second species as *B. rouxiae*.

Although *T. basicola* was known for more than 150 years (Berkeley & Broome, 1850) nothing is known regarding its mating strategy. Soon after the original description of the fungus, Zopf (1876) described a sexual state that he named *Thielavia (Th.) basicola* for the species. However, during the early 1900's multiple experiments involving single spore cultures were used to show that the sexual structures of *Th. basicola* and the asexual structures of *T. basicola* belonged to two unrelated species (McCormick, 1925, Lucas, 1948, Lucas, 1949, Stover, 1950). Subsequently, there has only been a single report attempting to induce sexual structures in *T. basicola*. Johnson & Valleau (1935) performed co-culture experiments using multiple-single spore isolates of the fungus but no sexual structures were observed over a period of more than one year. The absence of a sexual state has led to a view that the species represents an exclusively asexual pathogen (Tabachnik & DeVay, 1980, Paulin & Harrington, 2000, Paulin-Mahady *et al.*, 2002).

Mating and sexual reproduction in filamentous Ascomycetes, including the *Ceratocystidaceae*, is controlled by a single region in the genome known as the mating type or *MAT-1* locus (Yoder *et al.*, 1986, Wilken *et al.*, 2017a). This region can be present as two non-homologous idiomorphs known as *MAT1-1* and *MAT1-2* (Yoder *et al.*, 1986, Turgeon & Yoder, 2000, Wilken *et al.*, 2017a). The *MAT1-1* idiomorph contains at least the *MAT1-1-1* open reading frame (ORF) encoding a protein with an α-box domain. Similarly, the *MAT1-2* idiomorph contains at least the *MAT1-2-1* ORF encoding a protein with an HMG-box domain (Butler, 2007, Wilken *et al.*, 2017a). When both the *MAT1-1-1* and *MAT1-2-1* genes are found in the genome of a single isolate, the species is considered homothallic and sexual reproduction can take place independent of a second isolate (Butler, 2007, Wilson *et al.*, 2015a, Wilken *et al.*, 2017a). However, when the *MAT1-1-1* and *MAT1-2-1* genes occur in different isolates of a species, the species is considered heterothallic and at least two isolates, each containing one of the *MAT*

idiomorphs, are required for sexual reproduction to take place. Although the overall genetic organization at the *MAT-1* locus can vary among species, there are some gene regions, such as those encoding the cytoskeleton assembly control protein (*SLA2*), the AP endonuclease (*APN2*), the anaphase promoting complex (*APC*), and subunit VIa of cytochrome c oxidase (*COX13*), that are frequently found adjacent to the *MAT* locus (Butler, 2007, Wilken *et al.*, 2017a).

The *Ceratocystidaceae* includes examples of a wide variety of sexual reproductive strategies. These include species that are heterothallic (fungal species that require outcrossing between isolates of opposite mating type to sexually reproduce) (Wilson *et al.*, 2015b, Wilken *et al.*, 2017b, Mayers *et al.*, 2017) and primary homothallic (self-fertile species that can sexually reproduce without a partner) (Mbenoun *et al.*, 2014). Some species have secondary homothallic (genetically heterothallic but present as homothallic in culture) reproductive strategies including mating type switching (Harrington & McNew, 1997, Witthuhn *et al.*, 2000, Wilken *et al.*, 2014) and unisexuality (Wilson *et al.*, 2015b). Against the background of this diverse array of reproductive strategies in the *Ceratocystidaceae*, we questioned whether the newly described *Berkeleyomyces* species could be exclusively asexual, as has been previously suggested. The aim of this study was thus to characterize the mating locus in these fungi and to consider their possible mating strategy.

Materials and Methods

Isolates

Cultures used in this study were obtained from various collections including the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands, and the International Collection of Microorganisms from Plants (ICMP), New Zealand. Cultures (**Table 1**) were maintained on 2% Malt Extract Agar (MEA - 2% malt extract, 2% Difco® agar Biolab, Midrand, South Africa).

Characterization of the MAT1-2 *idiomorph*

The genome of *B. basicola* isolate CMW49352 (Nel *et al.*, 2017b) was interrogated to identify the contig containing the *MAT-1* locus. To do this, the tBLASTx algorithm of CLCbio Genomics Workbench 9.5 (CLCbio, QIAGEN, Aarthus, Denmark) was used to screen the assembled genome for *MAT* idiomorph sequences using reference sequences for *MAT* genes from the *Ceratocystis fimbriata* genome (GenBank accession number: APWK02000000; Wilken *et al.*, 2013). The contig containing the highest similarity to the reference sequences was extracted and annotated using Augustus 3.3.2 (Stanke *et al.*, 2004). A BLASTp analysis was done using the NCBI database to identify each of the predicted protein sequences.

Identification of putative **MAT1-1** *idiomorphic isolates*

CLCbio Genomics Workbench 9.5 (CLCbio, QIAGEN, Aarthus, Denmark) was used to design primers for the two *MAT1-2* (*MAT1-2-1, MAT1-2-7*) genes identified during the previous step, along with two additional *MAT* locus associated genes (*SLA2* and an Unknown gene (*UNK*)) (**Table 2**). Thirteen isolates of *B. basicola* and 26 isolates of *B. rouxiae* were screened by PCR, using these primers, to identify isolates putatively harbouring the *MAT1-1* and *MAT1-2* idiomorphs. DNA extraction and PCR amplification were done using the methods described by De Beer *et al.* (2014) and the annealing temperatures for the different primer pairs are indicated in **Table 2**. Based on the amplification results for the different isolates, *B. basicola* isolate CMW25440 (putatively identified as containing the *MAT1-1* idiomorph) was selected for additional genome sequencing.

Genome sequencing and characterization of the **MAT1-1** *idiomorph*

A single spore culture of isolate CMW25440 (= CBS142829 collected in Indonesia from *Styrax benzoin*) was grown in YM broth (2% malt extract, 0.5% yeast extract, Biolab, Midrand, South Africa) for 3-5 days. Mycelia were collected in 1.5 mL Eppendorf tubes by centrifugation and lyophilized. The tubes containing the lyophilized mycelia were submerged in liquid nitrogen and the mycelium ground to a fine powder using a sealed sterile pipette tip. Total genomic DNA was extracted from the powdered mycelia using the method described by Duong *et al.* (2013). A paired-end library (100bp insert size) was prepared and sequenced using the Illumina HiSeq2500 Platform at Macrogen Inc. (Seoul, Korea). The reads obtained were assembled into contigs using SPAdes 3.9 (Bankevich *et al.*, 2012). The *MAT1-1* region was identified and annotated using the same method described above to identify the *MAT1-2* region, but using the flanking genes of the *MAT1-2* isolate and the *MAT1-1-1* gene from *C. fimbriata*.

Development of multiplex PCR-based mating type markers for **Berkeleyomyces**

A primer set targeting the *MAT1-1-1* gene was designed based on the *MAT1-1* sequences identified from the genome of *B. basicola* isolate CMW25440 (**Table 2**). This allowed for multiplex PCR amplification together with the *MAT1-2-1* primer set designed from the *MAT1-2* isolate. The primer set was selected to produce a longer amplicon size but with the same annealing temperature as the *MAT1-2-1* set. These primers were combined in a multiplex PCR reaction following the protocol described by Duong *et al.* (2013) with an annealing temperature of 56°C for all isolates of *B. basicola* and *B. rouxiae*. From the PCR result, three putative *MAT1-1-1* and three *MAT1-2-1* amplicons from *B. rouxiae* isolates were selected for Sanger sequencing to confirm the identity of the amplified products. Sequencing PCR reactions were carried out using the BigDye® Terminator 3.1 cycle sequencing premix kit (Applied Biosystems) for both the forward and reverse primer of either the *MAT1-1-1* or *MAT1-2-1* gene. Products were purified using sodium acetate and ethanol precipitation and sent for sequencing at the University of Pretoria sequencing facility.

Comparison and phylogenetic analyses of the HMG and α-box amino acid sequences

The *MAT* locus and its flanking regions were extracted and annotated from the available genomes of various species in the *Ceratocystidaceae* (**Table 3**) in the same manner as described above for *B. basicola*. The amino acid sequences of the HMG and α-box were identified using the conserved domain function of BLASTp. Datasets were compiled and aligned to the HMG and α-box amino acid sequences of *B. basicola* and *B. rouxiae* using the online version of MAFFT 7.0 (Katoh & Standley, 2013). Neighbour-joining analyses were performed using MEGA 6.0 (Tamura *et al.*, 2013) using the poisson method with 1000 bootstrap replicates.

Pairing of mating types

Pairing on carrot slices

Three isolates of *B. basicola* identified as *MAT1-1* and ten isolates identified as *MAT1-2* were crossed with each other in every possible combination on carrot slices. The same was done for 18 *MAT1-1* and eight *MAT1-2* isolates of *B. rouxiae*.

Carrots were surface sterilized with 70% ethanol and sectioned into 5cm thick sections. These sections were halved, rinsed in a solution of streptomycin (0.3g Streptomycin, 500mL distilled water) and allowed to dry. The surface of one of the halves was then inoculated with one *MAT1-1* and one *MAT1-2* isolate (±20mm from each other). The uninoculated half of the carrot was placed on top of the inoculated half and the two halves were wrapped with parafilm to form a tight "sandwhich". The experiment included three replicates of each combination of isolates representing different MAT isolates of *B. basicola* and *B. rouxiae*. The carrot "sandwiches" were placed in a sealed sterile plastic container and incubated for two weeks at 20°C (±2°C).

After two weeks, the carrot halves were separated and inspected for the presence of sexual structures. The separated carrot halves were then returned to the containers and incubated for a further two weeks under the same conditions. After this period, the pieces were again inspected for sexual structures. The containers were then placed in a 4°C incubator and the carrot surfaces insepected fortnightly for three months, after which the experiment was terminated.

Mating in culture

- *Single crosses*

Three isolates of *B. basicola* having the *MAT1-1* idiomorph and ten isolates with the *MAT1-2* idiomorph were crossed with each other in every possible combination on 0.2% MEA, 10% Carrot agar (900mL MEA and 100mL organic carrot juice, Rugani, South Africa), and 2% MEA supplemented with Thiamine (200mg/L, Sigma Aldrich). The same was done for nine *MAT1-1* and six *MAT1-2* isolates of *B. rouxiae*. Inoculum (3mm x 3mm) of each isolate was placed 25mm from each other at the centre of

the plates (**Supplementary figure 1A**). Three replicate plates of each of the combinations of paired isolates were prepared, resulting in a total of 90 combinations of *B. basicola* and 162 combinations of *B. rouxiae* on each medium. The paired isolates were incubated for one month at 20°C (±2°C) and the Petri dishes monitored regularly for sexual structure development. After one month, the plates were moved to a 10°C incubator and they were inspected fortnightly for the appearance of sexual structures for one month.

- *Multi-isolate crosses*

Two *MAT1-1* isolates of *B. basicola* and three *MAT1-2* isolates were co-cultured with each other in combinations of four (two *MAT1-1* and two *MAT1-2* individuals on each agar plate (**Supplementary figure 1B**)) in every possible combination on 0.2% MEA, 10% Carrot agar (900mL MEA and 100mL organic carrot juice, Rugani, South Africa), and 2% MEA supplemented with Thiamine (200mg/L, Sigma Aldrich). The same experiment was performed with three *MAT1-1* and three *MAT1-2* isolates of *B. rouxiae*. Three replicate plates of each of the combinations of paired isolates were perfomed resulting in 9 combinations of *B. basicola* and 27 combinations of *B. rouxiae* on each of the different media. The paired isolates were incubated for one month at 20°C (±2°C) and the Petri dishes monitored regularly for sexual structure development. After one month, the plates were moved to a 10°C incubator and they were inspected fortnightly for the appearance of sexual structures for one month.

Results

Genome sequencing

Illumina sequencing of the genome of the putative *MAT1-1* isolate (CMW25440), produced approximately 39.6 million paired reads. *De novo* assembly of the genome using SPAdes 3.9 produced 6606 scaffolds. The assembly produced a genome of about 25.1 Mb in size with an N50 of 90Kb and a GC content of 52%. This draft assembly was similar in size to that available for *B. basicola* (Nel *et al.* 2017b). BUSCO analysis predicted an assembly completeness of 97%. The assembly contained 1276 complete single-copy BUSCOs, 1 complete and duplicated BUSCO, 10 fragmented BUSCOs and 28 missing BUSCOs from a total of 1315 BUSCO groups searched.

***Identification of the* MAT1-1 *and* MAT1-2 *loci of* B. basicola**

tBLASTx searches using the reference *Ceratocystis fimbriata MAT* gene sequences identified highly similar sequences to *MAT1-2-1* in contig 18 of the genome of isolate CMW49352 (Nel *et al.* 2017b). Annotation of this 2 Mb contig using Augustus 3.3.2 identified 1001 putative open reading frames (ORF's). BLASTp of the predicted protein sequences identified the structure of the *MAT1-2* idiomorph. A *MAT1-2-1* and a *MAT1-2-7* encoding ORF was identified, flanked on the one side by Cytochrome C oxidase subunit VIa (*COX13*), *APN*2, *APC*, and *SLA2* encoding genes, and on the other side by a gene of unknown function (*UNK*) and an importin gene (*IMP*) (**Fig. 1A**).

The tBLASTx searches using the identified *MAT* flanking genes from isolate CMW49352 identified highly similar sequences to *MAT1-1-1* from *C. fimbriata* in contig 63 of the genome of isolate CMW25440 (sequenced in this study). Annotation of the 100Kb contig identified 41 putative ORF's. BLASTp of the predicted protein sequences allowed for the identification of the *MAT1-1* idiomorph. The organization of the genes flanking the *MAT1-1* from CMW49352 and *MAT1-2* from CMW25440 were almost identical (**Fig. 1B**) and only the presence of the different *MAT* genes allowed for delineation between the two isolates.

In both the *MAT1-1* and *MAT1-2* isolates, the region from the *COX13* encoding region to the *IMP* encoding region spanned about 31Kb and closely resembled that of the *MAT* idiomorphs in other *Ceratocystidaceae* species (**Supplementary figure 2**). The predicted *MAT1-2-1* gene was 909 bases long, composed of three exons with a combined sequence length of 798 bases and two introns (53 bases and 55 bases). The predicted protein sequence included 265 amino acids and harboured the characteristic HMG-box domain. The predicted *MAT1-2-7* gene was 857 bases long, composed of three exons with a sum sequence length of 603 bases and two introns (53 bases and 201 bases). The predicted *MAT1-2-7* protein sequence included 200 amino acids and contained no known conserved structural domains. The predicted *MAT1-1-1* gene was 1233 bases long, composed of two exons with a total sequence length of 1176 bases with a single intron of 56 bases. The predicted protein sequence included 391 amino acids and harboured the characteristic α-box. The predicted *MAT1-1-2* gene region was 1518 bases long, composed of five CDSs of 1284 bases in total and four introns (59 bases, 57 bases, 141 bases and 59 bases). The predicted protein sequence included 427 amino acids and contained the HPG/PFF pfam domain (accession pfam17043). These sequences, together with their *MAT* gene annotations, were deposited to NCBI GenBank (**Table 1**).

Multiplex and other PCRs

The *MAT1-1-1* and *MAT1-2-1* primer pairs were successfully used in multiplex amplification of the *MAT* genes from both *Berkeleyomyces* species. Multiplex amplification of 13 *B. basicola* isolates identified three isolates containing the *MAT1-1-1* gene region and ten isolates containing the *MAT1-2-1* gene region (**Fig. 2A**). Multiplex amplification of 26 *B. rouxiae* isolates identified 18 isolates containing the *MAT1-1-1* gene region and eight isolates containing the *MAT1-2-1* gene region (**Fig. 2B**).

Additional support for the identity of *MAT1-1* and *MAT1-2* idiomorphic isolates was provided by PCR amplification of the *MAT1-2-7* gene region. This region was successfully amplified in all the isolates where the *MAT1-2-1* region was amplified, and no amplification was observed in isolates where the *MAT1-1-1* region was amplified (**Supplementary figure 3A**). The *SLA2* gene and the conserved *UNK* region were successfully amplified for all *B. basicola* and *B. rouxiae* isolates included in this study (**Supplementary figure 3B & 3C**).

From the multiplex PCR result, we selected three *MAT1-1-1* and three *MAT1-2-1* containing isolates for MAT amplicon sequencing. Sequencing of the *MAT1-1-1* region produced a product of around 810 base-pairs spanning the entire α-box region (**Fig. 3A**). Sequencing of the *MAT1-2-1* region produced a product of around 500 base-pairs containing the intron region and encoding 53 of the 81 amino acids of the HMG-box domain (**Fig. 3B**).

Phylogenetic analyses

Neighbour joining analyses of the HMG-box and α-box amino acid sequences for various isolates of the *Ceratocystidaceae* produced phylogenetic trees with similar topology (**Fig. 4 A & B**). In both trees, *B. basicola* and *B. rouxiae* grouped together with high bootstrap support. The included *Ceratocystis* species formed a strongly supported sub-group to the *Berkeleyomyces* species, except for *C. adiposa* that grouped together with *Huntiella* species in both trees.

Mating experiments

None of the pairings between the identified *MAT1-1* and *MAT1-2* idiomorphic isolates gave rise to sexual structures under any of the conditions tested. All isolates produced abundant mycelium, asexual endoconidia and chlamydospores. However, in many cases a zone of vegetative incompatibility developed between the isolates preventing the mycelium of the different isolates colonizing a plate from making contact. However, this should not necessarily affect the ability of the isolates to mate (Glass & Kaldau, 1992).

Discussion

Whole genome sequencing and interrogation of the genomes of two *B. basicola* isolates allowed for the identification and characterization of the *MAT-1* locus for *Berkeleyomyces.* From the genome of *B. basicola* isolate CMW49352 (Nel *et al*., 2017b) we were able to identify and characterize the *MAT1-2* idiomorph of the *MAT-1* locus. From genome sequencing results of *B. basicola* isolate CW25440 in this study, we were further able to identify and characterize the *MAT1-1* idiomorph of the *MAT-1* locus. The presence of the *MAT1-1* and *MAT1-2* idiomorphs in different isolates of *B. basicola* is similar to that of other species of *Ceratocystidaceae* and Ascomycete fungi that utilize a heterothallic mating system. This study revealed that both *B. basicola* and *B. rouxiae* contain the genes necessary for a typical heterothallic life style. Although no sexual state is currently known for either species, these results suggest that cryptic sexual reproduction might be taking place in nature (Kuck & Poggeler, 2009, Dyer & Kuck, 2017).

Characterization of the genes located at the *MAT-1* locus of the two isolates of *B. basicola* allowed us to elucidate the structure of both the *MAT1-1* and *MAT1-2* idiomorphs for the fungus. Each idiomorph was flanked on one side by the *COX13*, *APN2*, *APC*, and *SLA2* genes and on the other side by a gene of unknown function (here referred to as *UNK*) and an *IMP* gene. This arrangement is very similar to

the one known for *Ceratocystis fimbriata* (Wilken *et al.*, 2014) and appears to be conserved among the various genera described in the family (Aylward *et* al., 2016, Wilken *et al.*, 2017b, Wilson *et al*., 2015b). Our results indicated that *B. rouxiae*, the cryptic sister species to *B. basicola*, is also heterothallic. Although the *MAT-1* locus of this species was not fully characterised, using PCR it was possible to confirm that the *MAT1-2* idiomorph in *B. rouxiae* contains homologs to the *MAT1-2-1* and *MAT1-2-7* genes and that the *MAT1-1* idiomorph contains a homolog of the *MAT1-1-1* gene. We also confirmed the presence of homologs to the *SLA2* and *UNK* genes.

The *MAT1-1* idiomorph of *B. basicola* included the additional *MAT* gene, *MAT1-1*-2 originally described from *Neurospora crassa* (Wilken *et al.*, 2017a). Like many other heterothallic *Ceratocystidaceae* species the gene was located adjacent to the *MAT1-1-1* gene. In this gene, we were able to identify the conserved HPG/PFF domain. Like the *MAT1-1-2* gene, its conserved domain was also first identified in *Neurospora crassa* (Wilken *et al.*, 2017a). The *MAT1-2* idiomorph included the *MAT1-2-7* originally described in *Huntiella omanensis* (Wilken *et al.*, 2017a, Wilson *et al*., 2015b). This gene was located adjacent to *MAT1-2-1*, but is in the opposite orientation. Prior to this investigation, a full length *MAT1-2-7* gene had been characterized only in *Huntiella omanensis* and a truncated version of this gene had been characterized in *Huntiella moniliformis* (Wilson *et al.*, 2015b). The present study revealed homologs to this region in both *Berkeleyomyces* species

Alignment of the α-box amino acid sequences in *B. basicola, B. rouxiae* and of the various other genera in the *Ceratocystidaceae* showed relatively low conservation of this region. Alignment of the HMG-box amino acid sequences, showed that the region is more conserved than that of the α-box region, but overall conservation between the genera was low. A similar situation was seen when aligning the HPG/PFF domain amino acid sequences. The low level of conservation of these conserved domains among genera was not unexpected and has for example been found in the *Ophiostomatales* (Duong *et al.*, 2013) and *Pleosporales* (Yun *et al.*, 2013).

Phylogenetic analyses of the α-box amino acid sequences and HMG-box amino acid sequences grouped *B. basicola* and *B. rouxiae* distinct from the other *Ceratocystidaceae* genera. This region had little resolution and the two species were not distinguishable in these analyses. Additionally, the trees generated were not congruent, which is not unexpected (Goodwin *et al.*, 2003, Yokoyama *et al.*, 2006, Bihon *et al.*, 2014). Mating type genes are under selective and evolutionary pressure (Duong *et al.*, 2013, Bihon *et al.*, 2014), which makes them unsuitable for phylogenetic analyses (Schmitt *et al.*, 2009, Stielow *et al.*, 2015).

It was not possible to induce sexual structures in cultures of either *B. basicola* or *B. rouxiae*, even though isolates of known opposite mating type were paired in many different combinations and grown under a relatively wide range of conditions. While these experiments provide some evidence that sexual reproduction does not easily occur in *B. basicola* or *B. rouxiae*, many different biological, ecological

and environmental factors could influence the ability of these fungi to reproduce sexually. Various other fungi known to include isolates of two different mating types typical of heterothallism have yet to reveal sexual states (Gordon 2017). In some cases such as *Aspergillus fumigatus*, many years passed before sexual structures were found even though the fungus was known to be heterothallic (Paoletti *et al.*, 2005, O'Gorman *et al.*, 2009). For the present, it is not possible to know whether sexual reproduction can occur in the two cryptic species of *Berkeleyomyces*.

PCR amplification of the *MAT* genes using the mating type markers developed in this study identified three *MAT1-1* and ten *MAT1-2* isolates of *B. basicola*, and 18 *MAT1-1* and eight *MAT1-2* isolates of *B. rouxiae* in our collection of isolates. From the number of isolates we had available of the two species, we found that both *B. basicola* and *B. rouxiae* occurs in South Africa and the Netherlands. For the isolates of *B. basicola* we only had isolates of a single mating type from each country. However, for the isolates of *B. rouxiae* both *MAT1-1* and *MAT1-2* isolates were identified in collections from South Africa and New Zealand. The size of our populations is inordinately small to use the frequencies of the *MAT* loci to make conclusions regarding the population structure and its diversity. However, as both mating types of *B. rouxiae* are present in some countries the potential for sexual reproduction for the species exists.

Acknowledgements

We thank the University of Pretoria, members of the Tree-Protection Co-operative Programme (TPCP), the DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB) and the National Research Foundation (NRF) for financial support. The authors declare that they have no conflict of interest.

References

AbdAllah EF, Hashem A, Bahkali AH & Al-Huqail A (2011) First report of black root rot disease (*Thielaviopsis basicola*) of carrot in Saudi Arabia. *African Journal of Microbiology Research* **5**: 2867-2869.

Aylward J, Steenkamp ET, Dreyer LL, Roets F, Wingfield MJ & Wingfield BD (2016) Genetic basis for high population diversity in *Protea*-associated *Knoxdaviesia*. *Fungal Genetics and Biology* **96**: 47-57.

Bankevich A, Nurk S, Antipov D, Gurevich AA & Dvorkin M (2012) SPAdes: a new genome assembly alogrith and its applications to single-cell sequencing. *Journal of Computational Biology* **19**: 455-477.

Berkeley MJ & Broome CE (1850) Notices of British fungi XL. *Annals and Magazine of Natural History Series 2*. **5**: 455-466.

Bihon W, Wingfield MJ, Slippers B, Duong TA & Wingfield BD (2014) *MAT* gene idiomorphs suggest a heterothallic sexual cycle in a predominantly asexual and important pine pathogen. *Fungal Genetics and Biology* **62**: 55-61.

Butler G (2007) The evolution of MAT: the Ascomycetes. In Heitman J, Kronstad JW & Taylor JW, eds. *Sex in Fungi : Molecular Determination and Evolutionary Implications*, ASM Press, Washington.

De Beer ZW, Duong TA, Barnes I, Wingfield BD & Wingfield MJ (2014) Redefining *Ceratocystis* and allied genera. *Studies in Mycology* **79**: 187-219.

Duong TA, De Beer ZW, Wingfield BD & Wingfield MJ (2013) Characterization of the mating-type genes in *Leptographium procerum* and *Leptographium profanum*. *Fungal Biology* **117**: 411-421.

Dyer PS & Kuck U (2017) Sex and the imperfect fungi. *Microbiology Spectrum* **5**: FUNK-0043-2017.

Ferraris TA (1912) *Pars 1: Fungi, Hyphales, Dematiaceae*. *Flora Italica Cryptogamica Vol. 8.* Stabilimento tipografico L. Cappelli, Rocca San Casciano, Italy.

Glass NL & Kuldau GA (1992) Mating type and vegetative incompatibility in filamentous ascomycetes. *Annual Review of Phytopathology* **30**: 201-224.

Goodwin SB, Waalwijk C, Kema GHJ, Cavaletto JR & Zhang G (2003) Cloning and analysis of the mating-type idiomorphs from the barley pathogen *Septorai passerinii*. *Molecular Genetics and Genomics* **269**: 1-12.

Gordon TR (2017) *Fusarium oxysporum* and the *Fusarium* wilt syndrome. *Annual Review of Phytopathology* **55**: 23-39.

Harrington TC & McNew DL (1997) Self-fertility and uni-directional mating-type switching in *Ceratocystis coerulescens*, a filamentous ascomycete. *Current Genetics* **32**: 52-59.

Johnson EM & Valleau WD (1935) Cultural variations of *Thielaviopsis basicola*. *Phytopathology* **25**: 1011-1018.

Katoh K & Standley D (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772-780.

Kuck U & Poggeler S (2009) Cryptic sex in fungi. *Fungal Biology Reviews* **23**: 86-90.

Schmitt I, Crespo A, Divakar PK, *et al.* (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* **23**: 35-40.

Lucas GB (1948) Comparative cultural studies with *Thielavia basicola* and *Thielaviopsis basicola.* Abstract. *Phytopathology* **38**: 16.

Lucas GB (1949) Studies on the morphology and cytology of *Thielavia basicola* Zopf. *Mycologia* **41**: 553-560.

Mayers CG, Harrington TC & Ranger CM (2017) First report of a sexual state of an ambrosia fungus: *Ambrosiella cleistominuta* sp. nov. associated with the ambrosia beetle *Anisandrus maiche*. *Botany* **95**: 503-512.

Mbenoun M, De Beer ZW, Wingfield MJ, Wingfield BD & Roux J (2014) Reconsidering species boundaries in the *Ceratocystis paradoxa* complex, including a new species from oil palm and cacao in Cameroon. *Mycologia* **106**: 757-784.

McCormick FA (1925) Perithecia of *Thielavia basicola* Zopf in culture and the stimulation of their production by extracts from other fungi. *Connecticut Agricultural Experimant Station* **269**: 539-554.

Nel WJ, Duong TA, Wingfield BD, Wingfield MJ, Hammerbacher A & De Beer ZW (2017b) The draft genome sequence for the important root rot pathogen previously known as *Thielaviopsis basicola*. *IMA Fungus In Press*.

Nel WJ, Duong TA, Wingfield MJ, Wingfield BD & De Beer ZW (2017) A new genus and species for the globally important, multi-host root pathogen *Thielaviopsis basicola*. *Plant Pathology* DOI: 10.1111/ppa.12803

O'Gorman CM, Fuller HT & Dyer PS (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* **457**: 471-474.

Paoletti M, Rydholm C, Schwier EU, Anderson MJ, Szakacs G, *et al.* (2005) Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Current Biology* **15**: 1242-1248.

Paulin-Mahady AE, Harrington TC & McNew D (2002) Phylogenetic and taxonomic evaluation of *Chalara, Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. *Mycologia* **94**: 62-72.

Paulin AE & Harrington TC (2000) Phylogenetic placement of anamorphic species of *Chalara* among *Ceratocystis* and other ascomycetes. *Studies in Mycology* **45**: 169-186.

Pereg LL (2013) Black root rot of cotton in Australia: The host, the pathogen and disease management. *Crop and Pasture Science* **64**: 1112-1126.

Stanke M, Steinkamp R, Waack S & Morgenstern B (2004) AUGUSTUS: A web server for gene finding in Eukaryotes. *Nucleic Acids Research* **32**: W309-312.

Stielow JB, Levesque CA, Seifert KA, *et al.* (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia* **35**: 242-263

Stover RH (1950) The black rootrot disease of tobacco: I. Studies on the causal organism *Thielaviopsis basicola*. *Canadian Journal of Research* **28**: 445-470.

Tabachnik M & DeVay JE (1980) Black root rot development in cotton roots caused by *Thielaviopsis basicola* and the possible role of methyl acetate in pathogenesis. *Physiological Plant Pathology* **16**: 109.

Tamura K, Stecher G, Peterson D, Filipski A & Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729

Turgeon BG & Yoder OC (2000) Proposed nomenclature for mating type genes of filamentous ascomycetes. *Fungal Genetics and Biology* **31**: 1-5.

Van der Nest MA, Bihon W, De Vos L, *et al.* (2014b) Draft genome sequences of *Diplodia sapinea, Ceratocystis manginecans,* and *Ceratocystis moniliformis*. *IMA Fungus* **5**: 135-140.

Van der Nest MA, Steenkamp ET, McTaggart AR, *et al.* (2015) Saprophytic and pathogenic fungi in the Ceratocystidaceae differ in their ability to metabolize plant-derived sucrose. *BMC Evolutionary Biology* **15**: 273.

Van der Nest MA, Beirn LA, Crouch JA, *et al.* (2014) Draft genomes of *Amanita jacksonii, Ceratocystis albifundus, Fusarium circinatum, Huntiella omanensis, Leptographium procerum, Rutstroemia sydowiana,* and *Sclerotinia echinophila. IMA Fungus* **5**: 473-486.

Wilken PM, Steenkamp ET, Wingfield MJ, De Beer ZW & Wingfield BD (2014) DNA loss at the *Ceratocystis fimbriata* mating locus results in self-sterility. *PLoS ONE* **9**: e92180.

Wilken PM, Steenkamp ET, Wingfield MJ, De Beer ZW & Wingfield BD (2017a) Which MAT gene? *Pezizomycotina* (Ascomycota) mating-type gene nomenclature reconsidered. *Fungal Biology Review* **35:** 242-263.

Wilken PM, Steenkamp ET, Van der Nest MA, Wingfield MJ, De Beer ZW & Wingfield BD (2017b) Unexpected placement of the *MAT1-1-2* gene in the *MAT1-2* idiomorph of *Thielaviopsis*. *Fungal Genetics and Biology In press.*

Wilken PM, Steenkamp ET, Wingfield MJ, De Beer ZW & Wingfield BD (2013) Draft nuclear genome sequence for the plant pathogen, *Ceratocystis fimbriata*. *IMA Fugnus* **4**: 357-358.

Wilson AM, Wilken PM, van der Nest MA, Steenkamp ET, Wingfield MJ & Wingfield BD (2015a) Homothallism: an umbrella term for describing diverse sexual behaviours. *IMA Fungus* **6**: 207-214.

Wilson AM, Godlonton T, van der Nest MA, Wilken PM, Wingfield MJ & Wingfield BD (2015b) Unisexual reproduction in *Huntiella moniliformis*. *Fungal Genetics and Biology* **80**: 1-9.

Wingfield BD, Duong TA, Hammerbacher A, *et al.* (2016) IMA Genome-F 7 Draft genome sequences for *Ceratocystis fagacearum, C. harringtonii, Grosmannia penicillata,* and *Huntiella bhutanensis*. *IMA Fungus* **7**: 317-323.

Wingfield BD, Barnes I, De Beer ZW, *et al.* (2015) Draft genome sequences of *Ceratocystis eucalypticola, Chrysoporthe cubensis, C. deuterocubensis, Davidsoniella virescens, Fusarium temperatum, Graphilbum fragrans, Penicillium nordicum,* and *Thielaviopsis musarum. IMA Fungus* **6**: 493-506.

Wingfield BD, Ambler JM, Coetzee MPA, *et al.* (2016) IMA Genome-F 6: Draft genome sequences of *Armillaria fuscipes, Ceratocystiopsis minuta, Ceratocystis adiposa, Endoconidiophora laricicola, E. polonica* and *Penicillium freii* DAOMC 242723. *IMA Fungus* **7**: 217-227.

Wingfield BD, Ades PK, Al-Naemi FA, *et al.* (2015b) IMA Genome-F 4. Draft genome sequences of *Chrysoporthe austroafricana, Diplodia scrobiculata, Fusarium nygamai, Leptographium lundbergii, Limonomyces culmigenus, Stagonosporopsis tanaceti,* and *Thielaviopsis punctulata*. *IMA Fungus* **6**: 233-248.

Witthuhn RC, Harrington TC, Wingfield BD, Steimel JP & Wingfield MJ (2000) Deletion of the *MAT-2* mating-type gene during uni-directional mating-type switching in *Ceratocystis*. *Current Genetics* **38**: 48-52.

Yoder OC, Valent B & Chumley F (1986) Genetic nomenclature and practice for plant pathogenic fungi. *Phytopathology* **76**: 383-385.

Yokoyama E, Arakawa M, Yamagishi K & Hara A (2006) Phylogenetic and structural analyses of the mating-type loci in *Clavicipitaceae*. *FEMS Microbiology Letters* **264**: 182-191.

Yun SH, Yoder OC & Turgeon BG (2013) Structure and function of the mating-type locus in the homothallic Ascomycete, *Didymella zeae-maydis*. *Journal of Microbiology* **51**: 814-820

Zopf W (1876) *Thielavia basicola* Zopf. Genus Novum Perisporiacearum. *Verhandlungen des Botanischen Vereins für die Provinz Brandenburg* **18**: 101-105.

Figures:

Figure 1: The structure of the *MAT1-2* and *MAT1-1* idiomorphs of *B. basicola* elucidated from its genomes. (**A**) The structure of the *MAT1-2* idiomorph and its flanking genes was identified from the genome of CMW49352; (**B**) The structure of the *MAT1-1* idiomorph and its flanking genes was identified from the genome of isolate CMW25440.

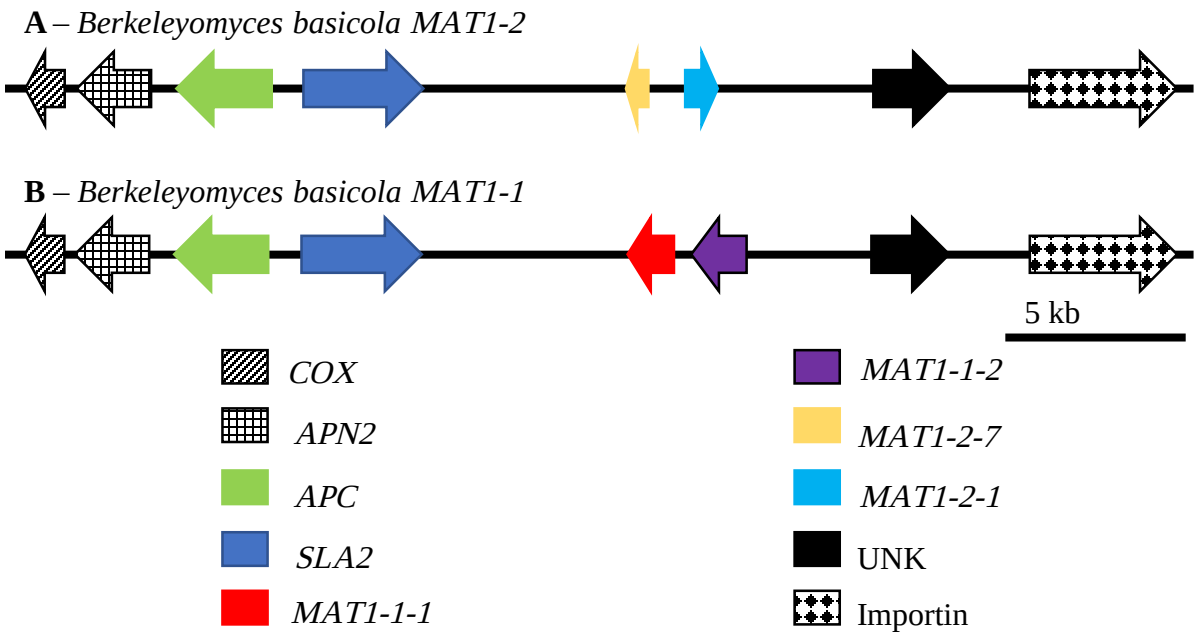
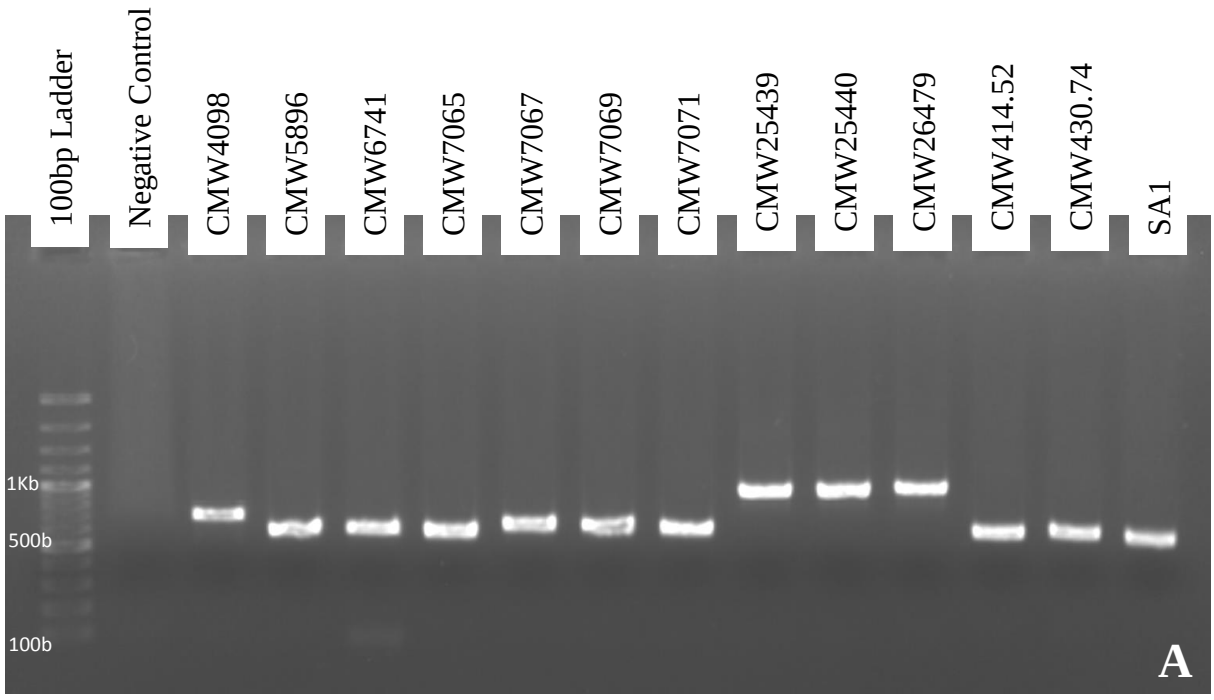

A – *Berkeleyomyces basicola MAT1-2*
B – *Berkeleyomyces basicola MAT1-1*
5 kb
COX
APN2
APC
SLA2
MAT1-1-1
MAT1-1-2
MAT1-2-7
MAT1-2-1
UNK
Importin

Figure 2: Results obtained from a multiplex PCR assay to determine the *MAT-1* genotype of isolates of *B. basicola* and *B. rouxiae*. (**A**) Electrophoresis gel for 13 isolates of *B. basicola;* (**B**) Electrophoresis gel for 13 isolates of *B. rouxiae*. The molecular weight maker used was a GeneRuler 100bp DNA Ladder (Fermentas). Fragments were separated on a 2% agarose gel stained with GelRed and visualized under UV light. The band sizes of 1kb, 500 and 100bp are indicated for the molecular weight marker lane. Amplification of the *MAT1-1-1* gene region produced an amplicon size of 845bp and amplification of the *MAT1-2-1* gene region produced an amplicon size of 530bp.


100bp Ladder
Negative Control
CMW4098
CMW5896
CMW6741
CMW7065
CMW7067
CMW7069
CMW7071
CMW25439
CMW25440
CMW26479
CMW414.52
CMW430.74
SA1
1Kb
500b
100b
A

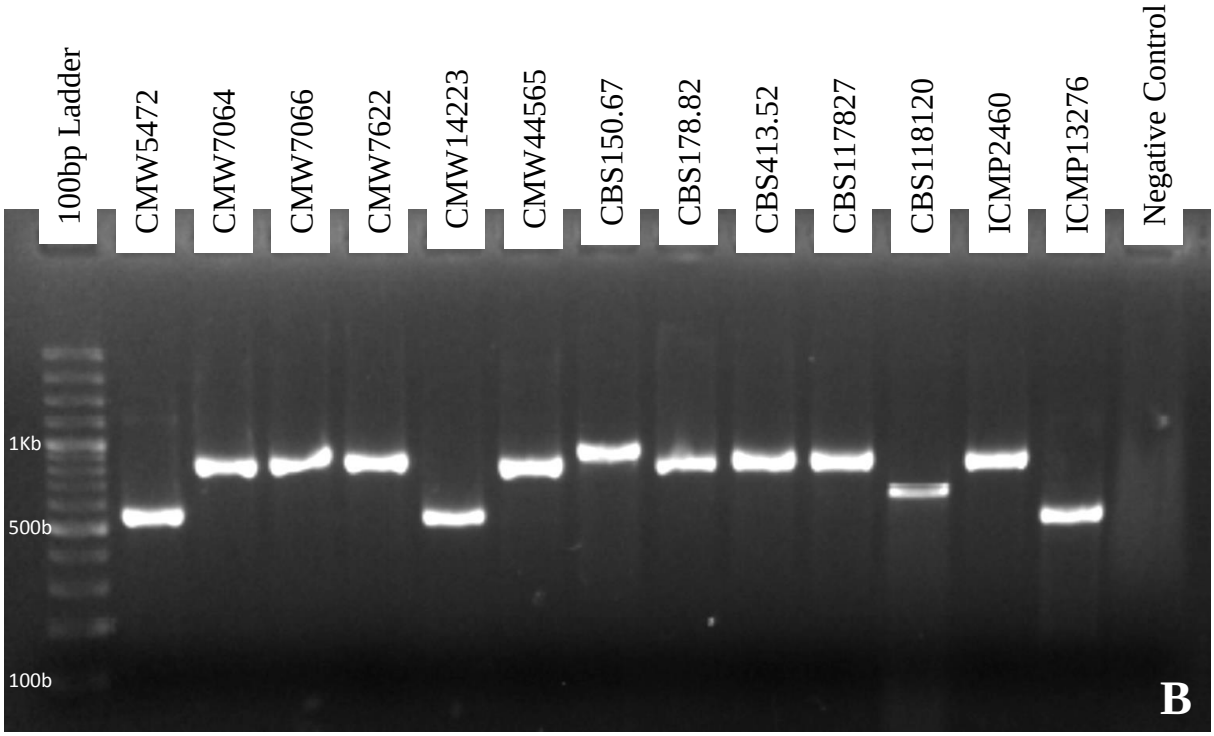
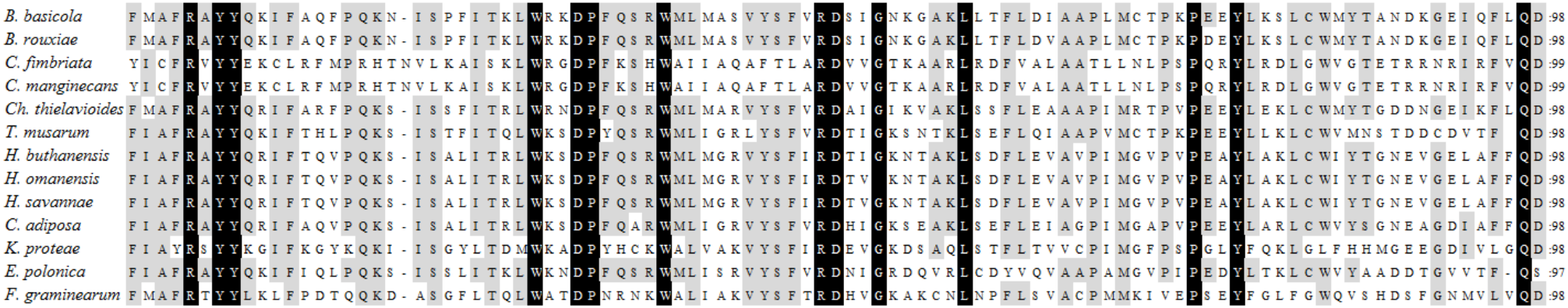

100bp Ladder
CMW5472
CMW7064
CMW7066
CMW7622
CMW14223
CMW44565
CBS150.67
CBS178.82
CBS413.52
CBS117827
CBS118120
ICMP2460
ICMP13276
Negative Control
1Kb
500b
100b
B

Figure 3: Alignment of the amino acid sequences of the (**A**) α-domain and the (**B**) HMG-box domain (**B**) from *B. basicola*, *B. rouxiae* and other *Ceratocystidaceae* species.

A

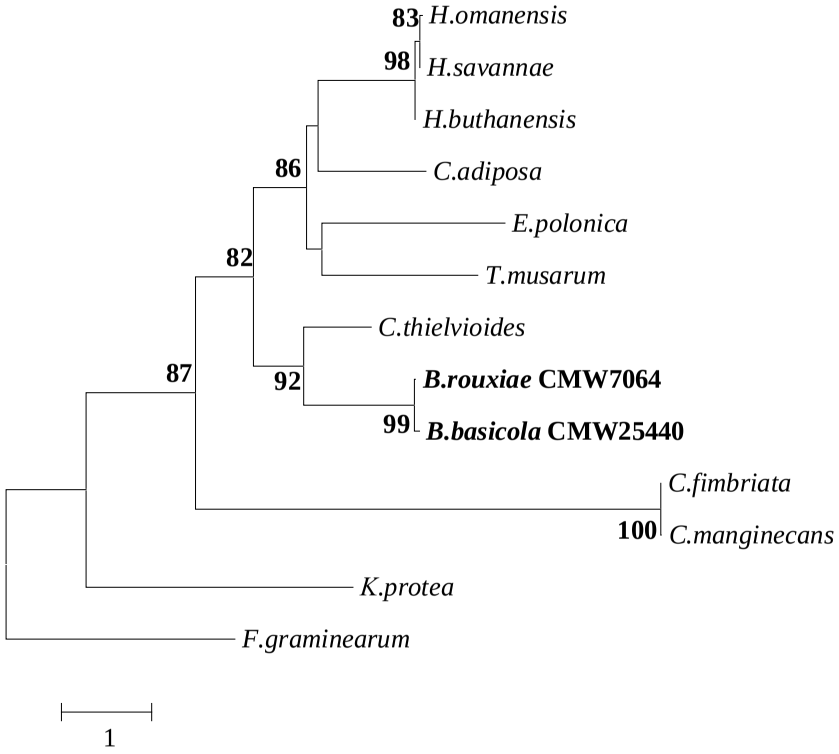

B. basicola
B. rouxiae
C. fimbriata
C. manginecans
Ch. thielavioides
T. musarum
H. buthanensis
H. omanensis
H. savannae
C. adiposa
K. proteae
E. polonica
F. graminearum

B

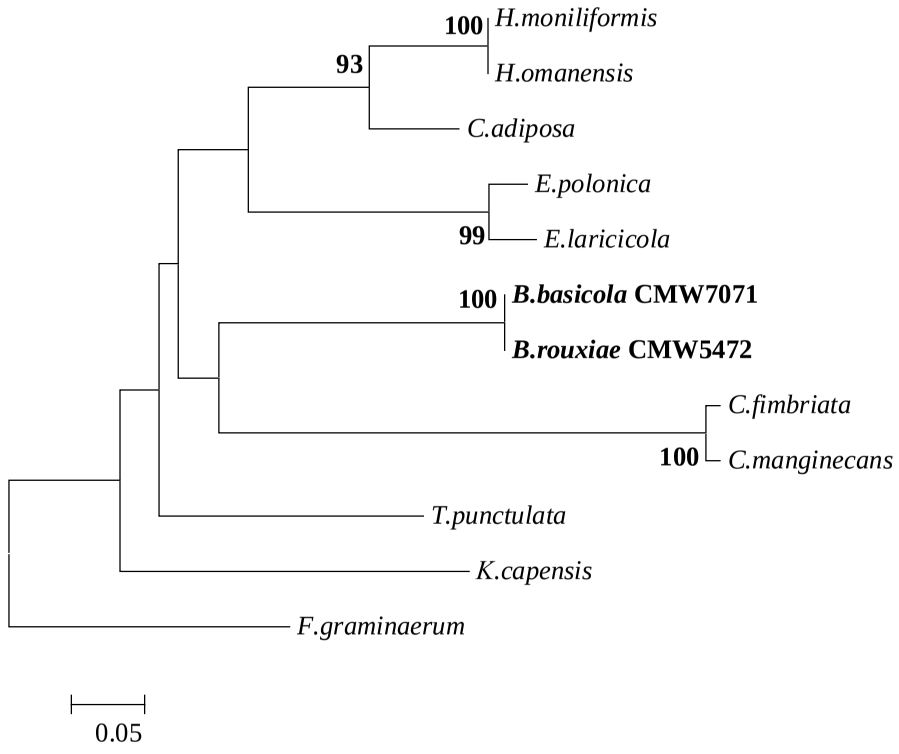

B. basicola
B. rouxiae
C. fimbriata
C. manginecans
T. punctulata
H. moniliformis
H. omanensis
C. adiposa
K. capensis
E. polonica
E. laricicola
F. graminearum

Figure 4: Neighbour joining trees derived from phylogenetic analyses of the (**A**) α-domain and (**B**) HMG-box amino acid sequences for various *Ceratocystidaceae* species including *B. basicola* and *B. rouxiae*. Bootstrap values above 75% are indicated at nodes.

A
***MAT1-1-1* α-domain**
Neighbour-Joining
1000 bootstraps

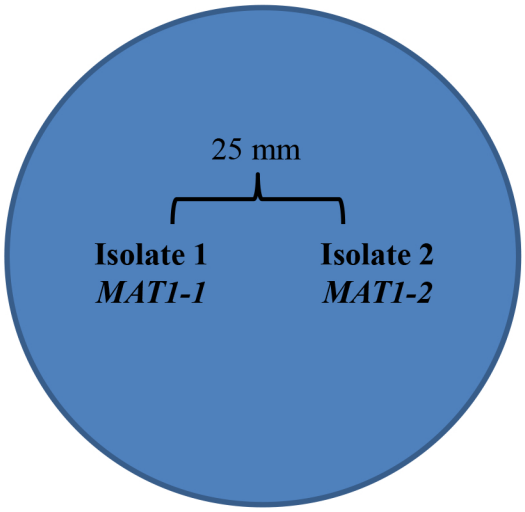


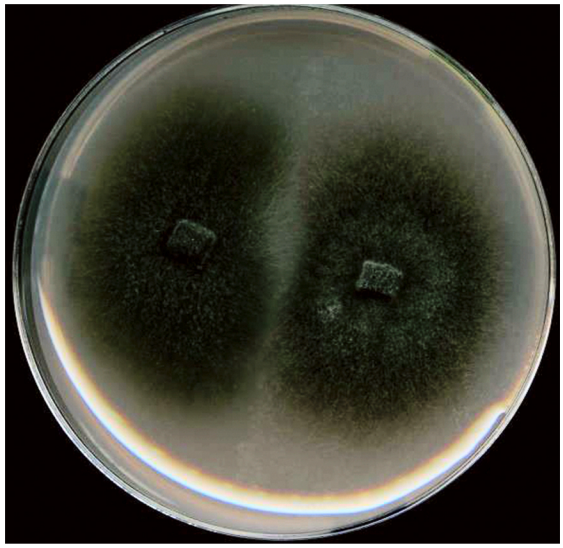
B
***MAT1-2-1* HMG-box**
Neighbour-Joining
1000 bootstraps



Supplementary Figure 1: Experimental design of the co-inoculation mating experiments. (**A**) Single crosses; (**B**) Multi-isolate crosses.

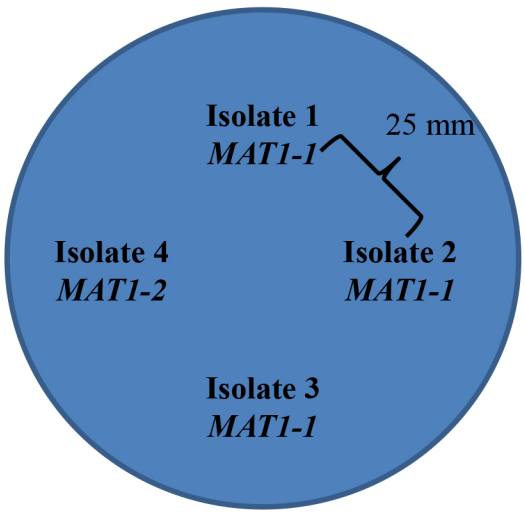
A Single crosses

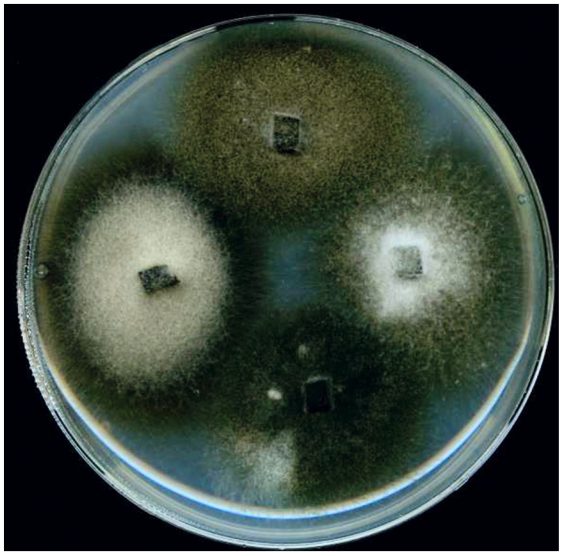




Species	No of MAT1-1 isolates	No of MAT1-2 isolates	No of crosses	3x replicates
B. basicola	3	10	30	90
B. rouxiae	9	6	54	162

B Multi-isolate crosses





Species	No of MAT1-1 isolates	No of MAT1-2 isolates	Possible combinations	3x replicates
B. basicola	2	3	3	9
B. rouxiae	3	3	9	27

Supplementary Figure 2: Putative *MAT* locus regions for isolates in the *Ceratocystidaceae* determined from their available genome sequences. * Indicates published *MAT* loci. Figures are not drawn to scale.

**Ceratocystis fimbriata* - homothallic

Ceratocystis manginecans - homothallic

Endoconidiophora polonica- homothallic

Chalaropsis thielavioides – heterothallic MAT1-1

**Thielaviopsis punctulata*– heterothallic MAT1-2

**Huntiella omanensis*– heterothallic MAT1-1

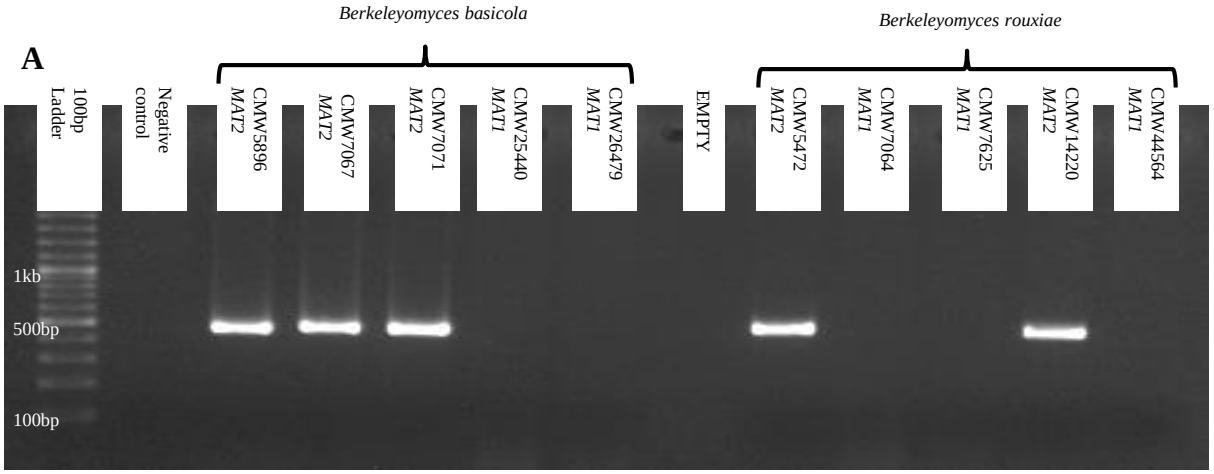
**Huntiella omanensis*– heterothallic MAT1-2

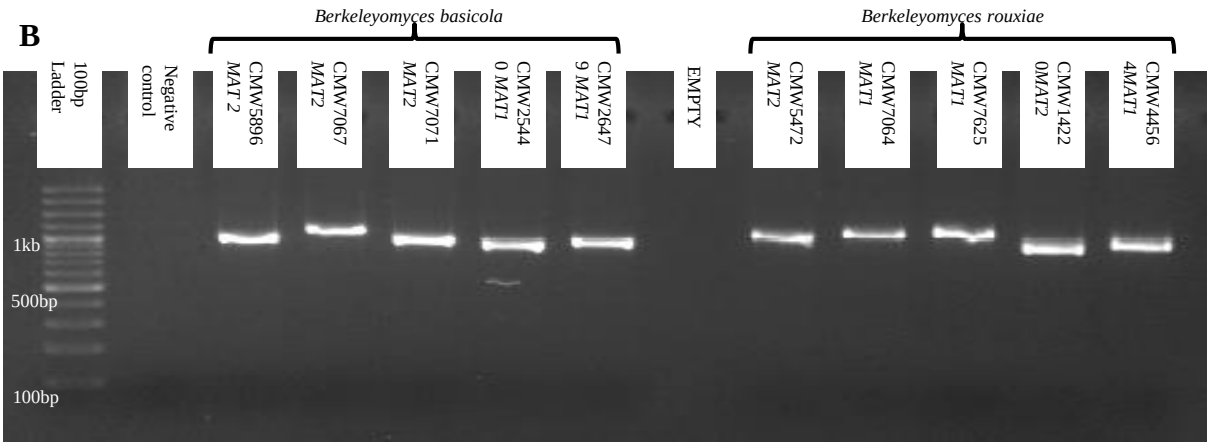
**Huntiella moniliformis*– homothallic

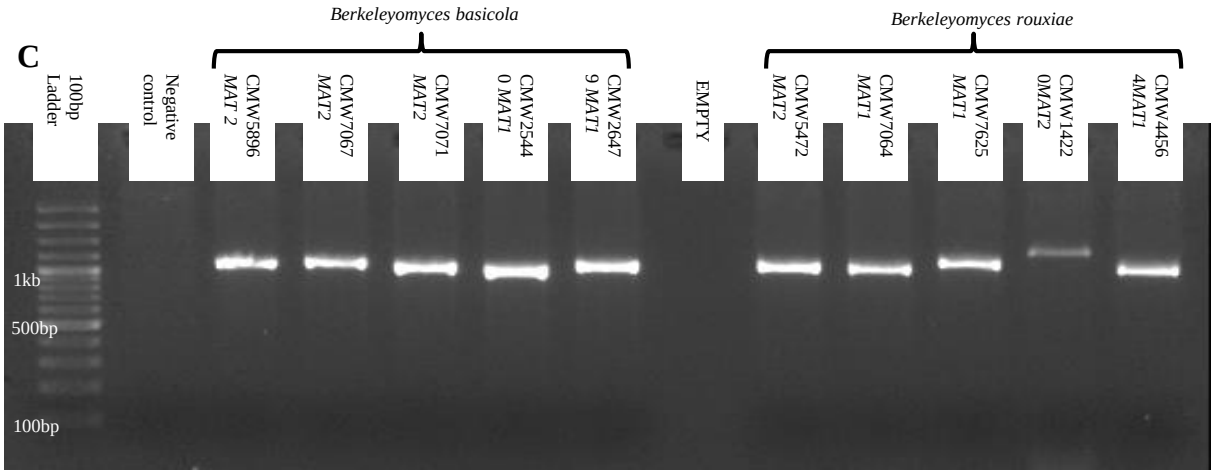
**Gondwanamyces capensis*– heterothallic MAT1-1

**Gondwanamyces proteae*– heterothallic MAT1-2

Supplementary Figure 3: Results obtained from PCR amplification using primers designed for the *MAT1-2-7*, *SLA2*, and *UNK* gene regions, for five selected isolates of both *B. basicola* and *B. rouxiae.* (**A**) Electrophoresis gel for the *MAT1-2-7* gene region (**B**); Electrophoresis gel for the *SLA2* gene region; **(C)** Electrophoresis gel for the *UNK* gene region. The molecular weight maker used was a GeneRuler 100bp DNA Ladder (Fermentas). Fragments were separated on a 2% agarose gel stained with GelRed and visualized under UV light. The band sizes of 1kb, 500 and 100bp are indicated for the molecular weight marker lane.


A
Berkeleyomyces basicola
Berkeleyomyces rouxiae
100bp Ladder
Negative control
CMW5896 MAT2
CMW7067 MAT2
CMW7071 MAT2
CMW25440 MAT1
CMW26479 MAT1
EMPTY
CMW5472 MAT2
CMW7064 MAT1
CMW7625 MAT1
CMW14220 MAT2
CMW44564 MAT1
1kb
500bp
100bp


B
Berkeleyomyces basicola
Berkeleyomyces rouxiae
100bp Ladder
Negative control
CMW5896 MAT 2
CMW7067 MAT2
CMW7071 MAT2
CMW25440 MAT1
CMW26479 MAT1
EMPTY
CMW5472 MAT2
CMW7064 MAT1
CMW7625 MAT1
CMW14220MAT2
CMW44564MAT1
1kb
500bp
100bp


C
Berkeleyomyces basicola
Berkeleyomyces rouxiae
100bp Ladder
Negative control
CMW5896 MAT 2
CMW7067 MAT2
CMW7071 MAT2
CMW25440 MAT1
CMW26479 MAT1
EMPTY
CMW5472 MAT2
CMW7064 MAT1
CMW7625 MAT1
CMW14220MAT2
CMW44564MAT1
1kb
500bp
100bp

Tables

Table 1: Isolates included in this study with the GenBank accession numbers of the respective *MAT* gene sequences obtained for the different isolates

					GenBank accession numbers									
Current name	**Collection number**	**Other collection numbers**	**Country**	**Idiomorph**	***MAT 1-1-1***	***MAT 1-1-2***	***MAT 1-2-1***	***MAT 1-2-7***	***SLA2***	***APN***	***APC***	***COX***	***UNK***	***IMP***
Berkleyomyces basicola	CMW4098*		Ecuador	*MAT1-2*										
	CMW5896*		Uganda	*MAT1-2*										
	CMW6714*#		Australia	*MAT1-2*										
	CMW7065*	CBS341.33/MUCL9545	Netherlands	*MAT1-2*										
	CMW7067*	CBS487.48/MUCL9542	Belgium	*MAT1-2*										
	CMW7069*		Netherlands	*MAT1-2*										
	CMW49352*#	CBS142796	Netherlands	*MAT1-2*			PND	PND	PND	PND	PND	PND	PND	PND
	CBS414.52*	MUCL8363	Netherlands	*MAT1-2*										
	CBS430.74*	CMW7071	Netherlands	*MAT1-2*										
	CMW25439*		Indonesia	*MAT1-1*										
	CMW25440*#	CBS142829	Indonesia	*MAT1-1*	PND	PND			PND	PND	PND	PND	PND	PND
	CMW26479*#		Indonesia	*MAT1-1*	PND									
	SA1*#		South Africa	*MAT1-2*										
Berkeleyomyces rouxii	CBS118120*		South Africa	*MAT1-2*										
	CMW5472*	CBS117825	Ethiopia	*MAT1-2*			PND							
	CMW7064*	CBS194.26/MUCL9544	Unknown	*MAT1-1*	PND									
	CMW7066*	CBS342.33/MUCL9456	Netherlands	*MAT1-1*										
	CBS413.52*		Netherlands	*MAT1-1*										
	CBS150.67*	IHEM3832	Switzerland	*MAT1-1*										

					GenBank accession numbers									
Current name	**Collection number**	**Other collection numbers**	**Country**	**Idiomorph**	***MAT 1-1-1***	***MAT 1-1-2***	***MAT 1-2-1***	***MAT 1-2-7***	***SLA2***	***APN***	***APC***	***COX***	***UNK***	***IMP***
	CMW7622	CBS117826	South Africa	*MAT1-1*										
	CMW7623	CBS118119	South Africa	*MAT1-1*	PND									
	CBS117827		South Africa	*MAT1-1*										
	CMW7625*	CBS117828	South Africa	*MAT1-1*										
	CMW14219*		Chile	*MAT1-2*										
	CMW14220		Chile	*MAT1-2*										
	CMW14221	CBS142830	Chile	*MAT1-2*			PND							
	CMW14222		Chile	*MAT1-2*										
	CMW14223		Chile	*MAT1-2*										
	CMW44562		South Africa	*MAT1-1*										
	CMW44563		South Africa	*MAT1-1*										
	CMW44564		South Africa	*MAT1-1*										
	CMW44565		South Africa	*MAT1-1*										
	CMW44566*		South Africa	*MAT1-1*	PND									
	CMW44567		South Africa	*MAT1-1*										
	CMW44568		South Africa	*MAT1-1*										
	CMW44569		South Africa	*MAT1-1*										
	ICMP2460*		New Zealand	*MAT1-1*										
	ICMP13276*		New Zealand	*MAT1-2*			PND							
	CBS178.86*	MUCL40417	Canada	*MAT1-1*										

*Isolates used in single mating crosses

#Isolates used in double mating crosses

PND – submission to GenBank pending

Table 2: Newly designed primer sets for amplification of *Berkeleyomyces* mating genes

Name	Sequence	Annealing Temperature	Fragment length
Tbas_SLA_F	CAACACCAAGGGTCTACTCCG	59	887
Tbas_SLA_R	TCCACCTGCTGCTCCATCTC		
Tbas_M127_F	TGAAGGAAGTAAGTCCGCACAG	59	445
Tbas_M127_R	GGGAAACTCAACCCAGAAGC		
Tbas_Unk_F	GACTGCCTACATCGCCTACC	57	1088
Tbas_Unk_F	TTGCCGTCACTACCAACCTG		
Tbas_M121_F	AAGACTTTACTCCGTGACTTTAGG	56	530*
Tbas_M121_R	CCAATTCTTGATAGTGGGTGC		
TBAS_M111_F	GCTGAAATGGGTGGTGTT	56	845*
TBAS_M111_R	CTTGGTTTTGGTTGGGTTG		

* Allowing for multiplexing of *MAT1-1-1* and *MAT1-2-1* amplification

Table 3: Accession numbers of Ceratocystidaceae genomes used in this study

Species	Genome accession number	Reference
Ceratocystis adiposa	LXGU01000000	Wingfield *et al.*, 2016
C. fimbriata	APWK00000000	Wilken *et al.*, 2013
C. manginecans	JJRZ00000000	Van der Nest *et al.*, 2014b
Chalaropsis thielavioides	BCGU00000000	Unpublished
Endoconidiophora laricicola	LXGT00000000	Wingfield *et al.*, 2016
E. polonica	LXKZ01000000	Wingfield *et al.*, 2016
Huntiella buthanensis	MJMS00000000	Wingfield *et al.*, 2016
H. moniliformis	JMSH00000000	Van der Nest *et al.*, 2014b
H. omanensis	JSUI00000000	Van der Nest *et al.*, 2014
H. savannaea	LCZG00000000	Van der Nest *et al.*, 2015
Thielaviopsis musarum	LKBB00000000	Wingfield *et al.*, 2015
T. punctulata	LAEV00000000	Wingfield *et al.*, 2015b
Knoxdaviesia capensis	LNGK00000000	Aylward *et al*, 2016
K. proteae	LNGL00000000	Aylward *et al*, 2016

Summary

The Ascomycete species *Thielaviopsis basicola* is a well-known pathogen of multiple important crop and ornamental plant species. This pathogen has been known for more than 150 years and has been extensively studied during this time, but important questions surrounding its taxonomy and mating behaviour remained to be answered. The first aim of this project was to resolve the taxonomic placement of the species using a multi-gene phylogenetics approach. Our research to address this aim revealed that the species represented a lineage distinct from all other genera in the Ceratocystidaceae, that we proceeded to describe as the new genus *Berkeleyomyces*. Our phylogenetic analyses also separated the collection of *T. basicola* isolates into two well-supported lineages within the genus, that we recognized as two distinct species. The first of these represented *T. basicola,* which was provided with a new combination, namely *B. basicola.* The second was described as a new species, named *B. rouxiae.* From the range of genera in which *T. basicola* was treated over the years, the name *Milowia* was technically available to accommodate the two species. However, we submitted a formal proposal to reject the name of the type species of this genus, *M. nivea*, and all names for which it serves as basionym, due to the absence of a type specimen and its dubious identity based on varying descriptions and illustrations by the same author across different publications. The second aim of our study was to determine the sexuality of these species, as some researches had suggested that *T. basicola* might exist exclusively asexually. To address this aim, we sequenced and assembled the whole genome sequence of the reference specimen of *Berkeleyomyces basicola*, designated when we described the genus. Using information from this genome we were able to determine the mating strategy of both species in the genus. Our results showed that both *B. basicola* and *B. rouxiae* contain all the typical genes required for heterothallic mating, but despite our efforts to obtain the sexual state in laboratory crosses, the sexual state of both species remains unknown. As a whole, the research conducted as part of this thesis has contributed to the global knowledge of these important pathogens by clarifying and settling the confusing taxonomy of the species, and by elucidating the mating strategy of these fungi. This will no doubt assist in improved diagnosis of the disease and a better understanding of the risks posed by introduced populations of these pathogens.