Plant Pathology (2017) Doi: 10.1111/ppa.12803

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, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002; and* [b]*Department of Genetics, 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 *RPB2* 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 analyses

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*	*RPB2*	*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		
		CMW14221	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*	*RPB2*	*LSU*	*60S*
Berkeleyomyces rouxiae sp. nov.	*Thielaviopsis basicola*	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 they were thus 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; GTR, generalized time-reversible.

Growth in culture

Optimal temperature for growth of isolates was determined on MEA using the protocols described by Duong *et al.* (2012). The same three 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 analyses

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 (CBS413.52) 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*.

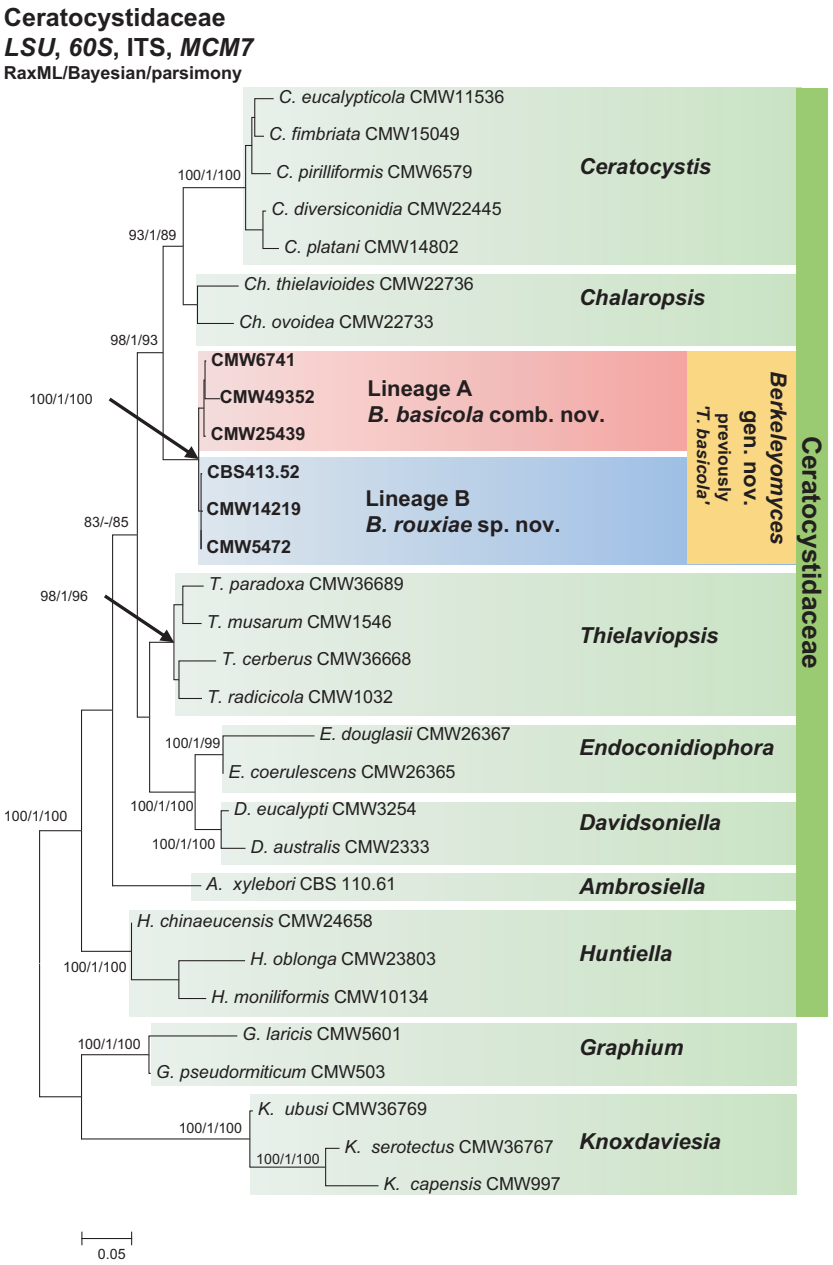

Figure 1 RaxML phylogram derived from maximum likelihood analysis of concatenated dataset (*LSU*, *60S*, ITS and *MCM7*) for selected species in the Ceratocystidaceae. Bootstrap support >75% and posterior probabilities >0.95 is given as ML/BI/MP.

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

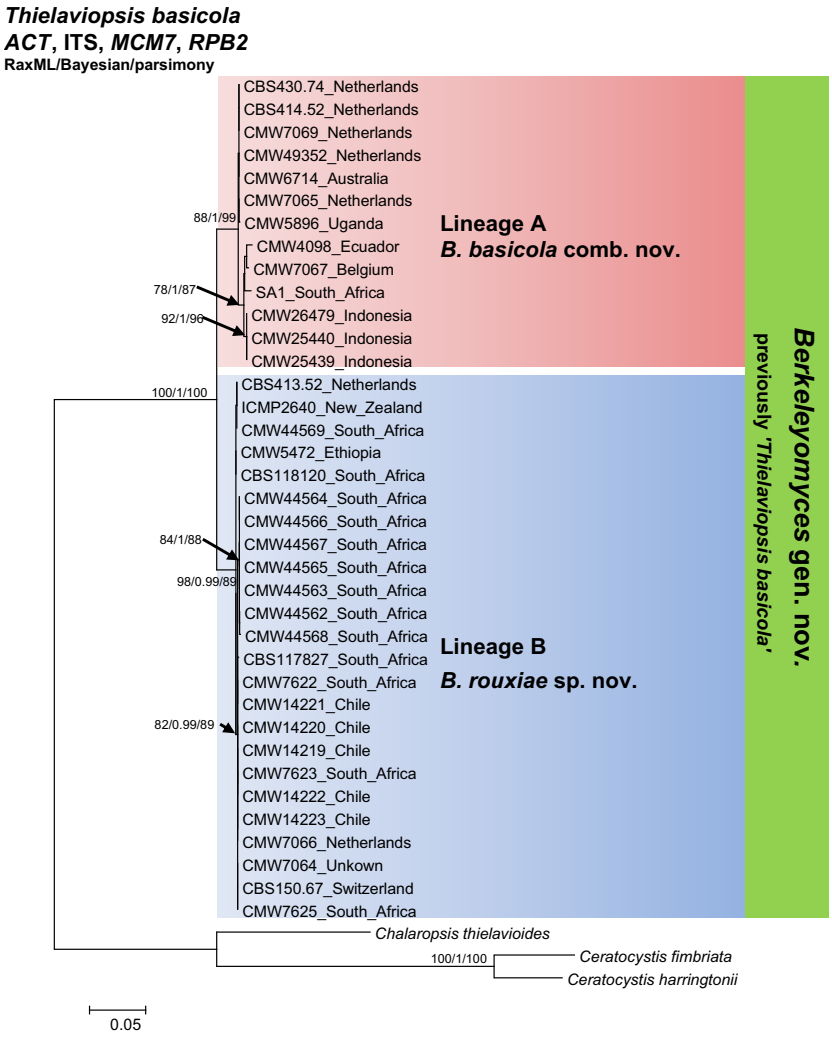

Figure 2 RaxML phylogram derived from maximum likelihood analysis of concatenated dataset (ITS, *ACT*, *MCM7* and *RPB2*) for isolates previously labelled as *Thielaviopsis basicola*. Bootstrap support >75% and posterior probabilities >0.95 is given as ML/BI/MP.

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

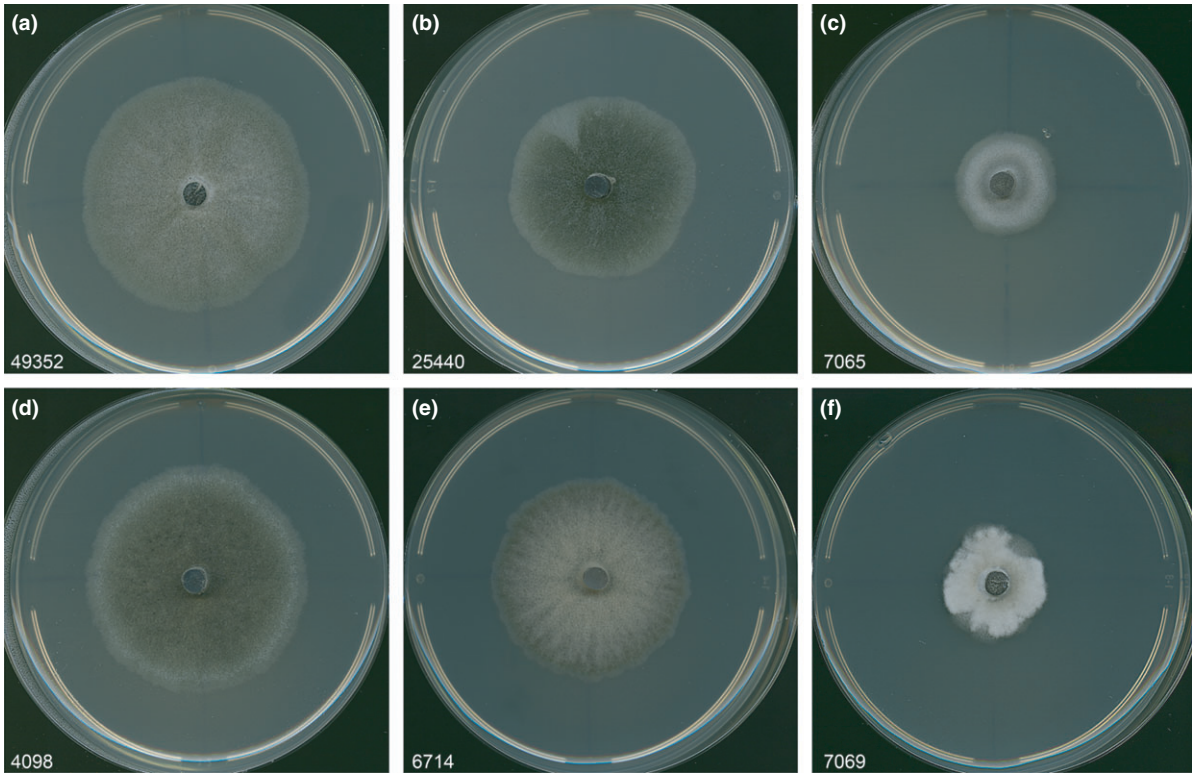

Figure 3 Some of the morphological variation within species of *Berkeleyomyces basicola*. Isolates were grown for 10 days at 25 °C, CMW numbers for the selected isolates are provided in the bottom left corner of each figure.

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

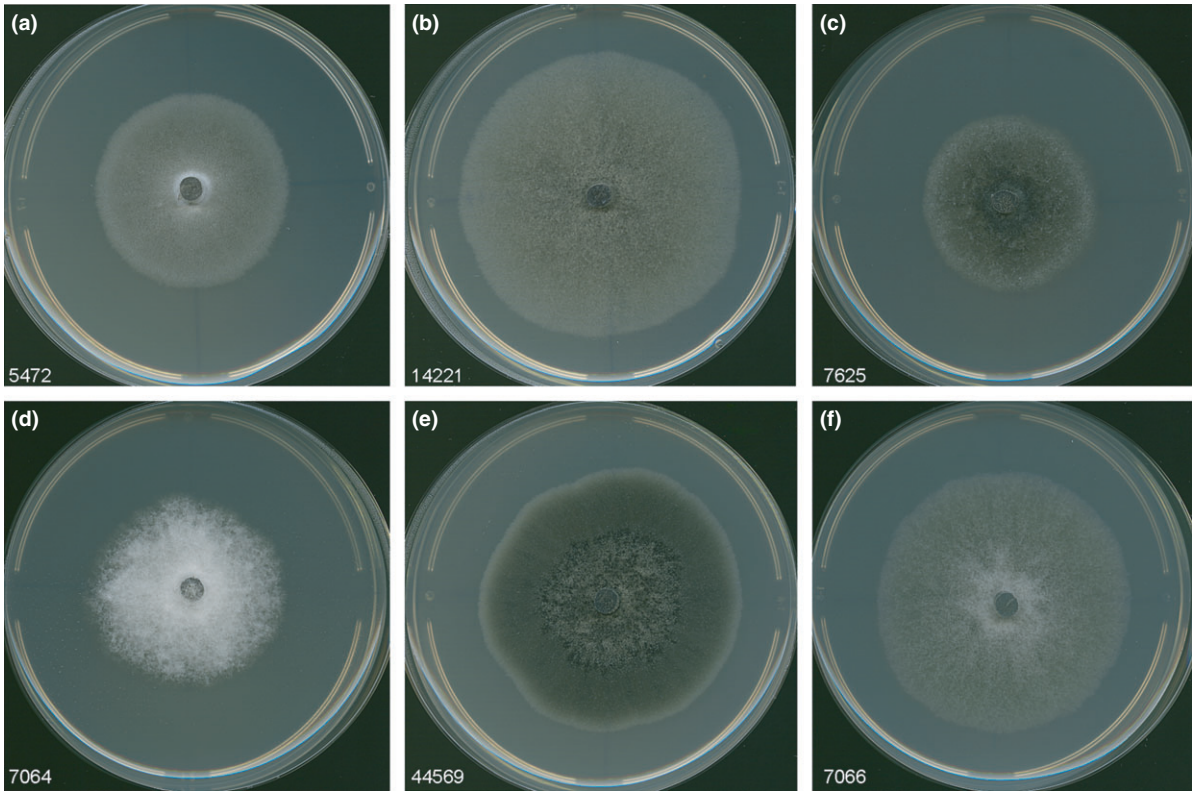

Figure 4 Some of the morphological variation within species of *Berkeleyomyces rouxiae*. Isolates were grown for 10 days at 25 °C, CMW numbers for the selected isolates are provided in the bottom left corner of each figure.

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 are 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

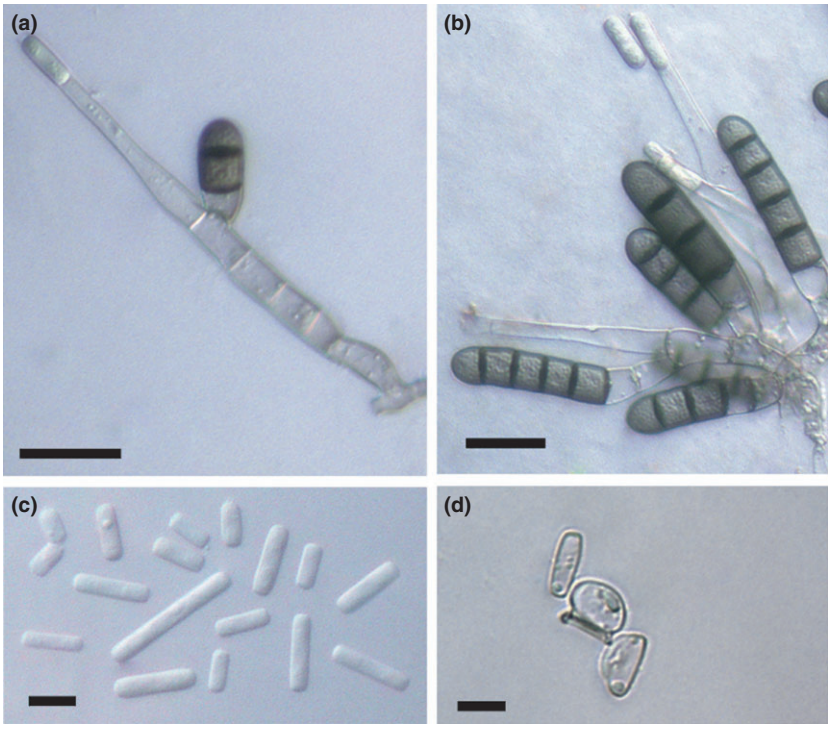

Figure 5 Structures of *Berkeleyomyces basicola*. (a) Terminal phialide giving rise to an endoconidium and a laterally borne chlamydospore containing two segments; (b) a cluster of chlamydospores and phialides; (c) endoconidia; (d) two secondary conidia with one endoconidium. Scale: (a, b) 20 μm; (c,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

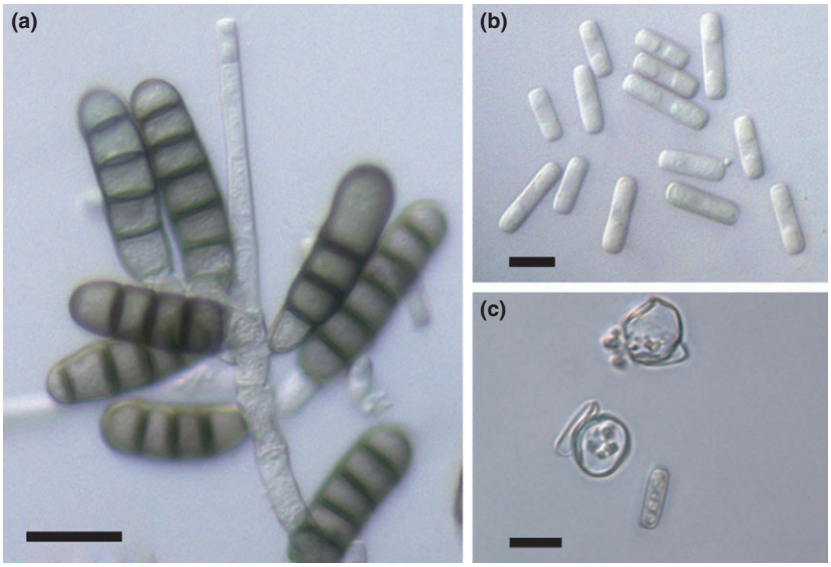

Figure 6 Structures of *Berkeleyomyces rouxiae*. (a) A cluster of chlamydospores and phialides; (b) endoconidia; (c) two secondary conidia with one endoconidium. Scale: (a) 20 μm; (b, c) 10 μm.

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

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: *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**, 105–12.

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.