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Five new species of the Botryosphaeriaceae from *Acacia karroo* in South Africa

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Abstract – The Botryosphaeriaceae represents an important, cosmopolitan family of latent pathogens infecting woody plants. Recent studies on native trees in southern Africa have revealed an extensive diversity of species of Botryosphaeriaceae, about half of which have not been previously described. This study adds to this growing body of knowledge, by discovering five new species of the Botryosphaeriaceae on *Acacia karroo,* a commonly occurring native tree in southern Africa. These species were isolated from both healthy and diseased tissues, suggesting they could be latent pathogens. The isolates were compared to other species for which DNA sequence data are available using phylogenetic analyses based on the ITS, TEF-1α, β-tubulin and LSU gene regions, and characterized based on their morphology. The morphological data were, however, useful to make comparisons with other species found in the same region and on similar hosts. The five new species were described as *Diplodia allocellula*, *Dothiorella dulcispinae*, *Do. brevicollis*, *Spencermartinsia pretoriensis* and *Tiarosporella urbis-rosarum.* Evidence emerging from this study suggests that many more species of the Botryosphaeriaceae remain to be discovered in the southern Africa.

***Acacia karroo* / *Diplodia* / *Dothiorella* / phylogeny / southern Africa / *Tiarosporella* / *Spencermartinsia* / taxonomy**

INTRODUCTION

The Botryosphaeriaceae (Dothideales) is a cosmopolitan family of fungi with a very wide host range of mostly woody plants. They can be primary or opportunistic pathogens, endophytes or saprobes, as reviewed by Slippers & Wingfield (2007). Many species have been reported from South Africa in recent years, isolated from trees in the Myrtaceae (*Eucalyptus* spp.), Proteaceae (*Protea* spp.), Fabaceae (*Acacia* spp.), Combretaceae (*Terminalia* spp.) and Pinaceae

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(*Pinus* spp.) (Begoude *et al.*, 2010; Denman *et al.*, 2003; Pavlic *et al.*, 2004; Roux & Wingfield, 1997; Slippers *et al.*, 2004; Smith *et al.*, 2001; Smith *et al.*, 1994; Swart & Wingfield, 1991; Van der Walt *et al.*, 2008). Most studies on members of the Botryosphaeriaceae in southern Africa have focused on their association with trees of agricultural or forestry importance.

A few recent studies of Botryosphaeriaceae on native southern African trees, such as *Syzygium cordatum* (Myrtaceae), *Pterocarpus angolensis* (Leguminosae), *Terminalia catappa* (Combretaceae), *Acacia* spp. especially *A. melifera* (Fabaceae), and woody species of *Leucadendron*, *Leucospermum* and *Protea* (Proteaceae), have revealed large numbers of species in these native environments, many of which have represented new species and genera. In total, 32 species of Botryosphaeriaceae have been identified from native woody hosts in South Africa, 12 species of which have been from native African *Acacia* spp., eight species from *S. cordatum,* seven species from *P. angolensis* and five species from *T. catappa*. Among these 32 species, 15 were new taxa (Begoude *et al.*, 2010; Mehl *et al.*, 2011; Pavlic *et al.*, 2007; Van der Walt *et al.*, 2008). The number and distribution of new species in these studies suggest that there probably are many more Botryosphaeriaceae species in South Africa on unsampled hosts and in unexplored regions.

The ecological relevance of the diversity of the Botryosphaeriaceae discovered recently in native environments of South Africa, is not well understood. Endophytism is common to most species of the family. For example *Diplodia pterocarpi* Cooke*, Lasiodiplodia crassispora* T. Burgess & Barber*, L. mahajangana* Begoude, Jol. Roux, Slippers*, L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous*, Neofusicoccum kwambonambiense* Pavlic, Slippers & M.J. Wingf.*, N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips*, N. batangarum* Begoude, Jol. Roux & Slippers*,* and *Pseudofusicoccum olivaceum* J.W.M. Mehl & B. Slippers have been isolated from asymptomatic tissues of *T. catappa* in South Africa (Begoude *et al.*, 2010). Many of the Botryosphaeriaceae are, however, best described as latent pathogens that can cause significant damage to woody agricultural crops and forestry species typically when host plants are under stress (Slippers & Wingfield, 2007). This is also evident from their association with die-back of native trees such as is found for *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not.*, L. gonubiensis* Pavlic, Slippers & M.J. Wingf.*, L. theobromae* (Pat.) Griffon & Maubl.*, N. australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips*, N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips*, N. mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips*, N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips and *N. ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips from *S. cordatum* in South Africa (Pavlic *et al.*, 2004). Pathogenicity studies with species of Botryosphaeriaceae have shown that they often differ in pathogenicity on a particular host, or between hosts. For example, *N. vitifusiforme* causes die-back on *Vitis vinifera* (Van Niekerk *et al.*, 2004), but was isolated as an endophyte from healthy tissue of *Terminalia* spp. in southern Africa (Begoude *et al.*, 2011). *L. pseudotheobromae* was isolated from both healthy tissues and those with symptoms of die-back on *T. catappa* trees, but isolates showed varying levels of pathogenicity on that host (Begoude *et al.*, 2010). These studies have shown that much work will be required to fully understand the ecology of the Botryosphaeriaceae.

Little is known regarding species of the Botryosphaeriaceae on *Acacia karroo* in South Africa. This tree species is the most widespread and

ecologically important native *Acacia* in southern Africa (Barnes *et al.*, 1996; Timberlake *et al.*, 1999). Previous studies on Botryosphaeriaceae species of native *Acacia* have focused mostly on *A. mellifera* (black thorn) and were limited to the Prieska area (Northern Cape province) in South Africa and Namibia (Van der Walt *et al.*, 2008). Furthermore, surveys on non-native *A. mearnsii* or black wattle were conducted in Pietermaritzburg (KwaZulu/ Natal Province), Piet Retief (Mpumalanga Province) and Alexandria (Eastern Cape Province) (Roux & Wingfield, 1997; Roux *et al.*, 1997). Samples of *A. karroo* were limited and yielded only three Botryosphaeriaceae species, namely *B. dothidea,* and a new species in *Phaeobotryon* and *Spencermartinsia*, respectively (Van der Walt *et al.*, 2008). There thus appears to be substantial scope to more thoroughly sample this important and widespread host, especially in previously unsampled areas.

This study is part of a larger effort to document the diversity of the Botryosphaeriaceae associated with South African native trees in general, and on *A. karroo* in particular. Here we report on two previously unsampled regions of its distribution, namely Pretoria (Gauteng Province) and Bloemfontein (Free State Province) in South Africa. As in previous studies on native hosts, surveys revealed a high level of diversity of Botryosphaeriaceae species. The isolated species were characterized here based on morphology and DNA sequences of the ITS, TEF1-α, β-tubulin and large subunit rDNA gene regions.

MATERIALS AND METHODS

Isolates examined

Sixty four asymptomatic branches and 40 branches showing die-back were collected from 48 trees at various sites around the city of Pretoria, Gauteng Province, and from the Glen area, Bloemfontein, Free State Province, South Africa, in June 2008 and February 2010 (Table 1). Plant tissues were surface sterilized in 10% hydrogen peroxide for two minutes, rinsed three times in sterile water and placed on 2% malt extract agar (Biolab, Midrand, South Africa). Cultures showing typical morphology of the Botryosphaeriaceae (fast growing, white to black cultures with aerial hyphae) were isolated after 4-5 days. Single hyphal-tip cultures of these isolates were made and these are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, with duplicates of type cultures deposited in the Centraalbureau voor Schimmelcultures (CBS), The Netherlands.

DNA sequence analyses

Isolates obtained in this study were initially grouped based on culture morphology. DNA was extracted from the mycelium of 5-day-old single hyphal-tip cultures (Lee & Taylor, 1990) of three representatives for each morphological group. Sequence data from the following genome regions were used in phylogenetic analyses, namely the internal transcribed spacer (ITS) region of the

Table 1. Representative isolates from *Acacia karroo* used in the phylogenetic analyses

Isolate No.	*Identity*	*Location*	*Collector*	*GenBank*			
				ITS	*EF1-α*	*LSU*	*β-tubulin*
CMW36477	*Tiarosporella urbis-rosarum*	Bloemfontein, South Africa	M. Gryzenhout	JQ239407	JQ239394	JQ239420	JQ239381
CMW36478	*T. urbis-rosarum*	Bloemfontein, South Africa	M. Gryzenhout	JQ239408	JQ239395	JQ239421	JQ239382
CMW36479	*T. urbis-rosarum*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239409	JQ239396	JQ239422	JQ239383
CMW36468	*Diplodia allocellula*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239397	JQ239384	JQ239410	JQ239378
CMW36469	*D. allocellula*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239398	JQ239385	JQ239411	JQ239379
CMW36470	*D. allocellula*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239399	JQ239386	JQ239412	JQ239380
CMW36460	*Dothiorella dulcispinae*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239400	JQ239387	JQ239413	JQ239373
CMW36461	*Do. dulcispinae*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239401	JQ239388	JQ239414	JQ239374
CMW36462	*Do. dulcispinae*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239402	JQ239389	JQ239415	JQ239375
CMW36463	*Do. brevicollis*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239403	JQ239390	JQ239416	JQ239371
CMW36464	*Do. brevicollis*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239404	JQ239391	JQ239417	JQ239372
CMW36480	*Spencermartinsia pretoriensis*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239405	JQ239392	JQ239418	JQ239376
CMW36481	*S. pretoriensis*	Pretoria, South Africa	F. Jami & M. Gryzenhout	JQ239406	JQ239393	JQ239419	JQ239377

ribosomal RNA (rRNA) operon using primers ITS-1 (Gardes & Bruns, 1993) and ITS-4 (White *et al*., 1990), the translation elongation factor 1-α (TEF-1α) gene using primers EF1-728F and EF1-986R (Carbon & Kohn, 1999), the β-tubulin gene using primers Bt2a and Bt2b (Glass & Donaldson, 1995), and the large subunit rDNA (LSU) gene region using primers LR0 and LR5 (Vilgalys & Hester, 1990).

The 25 µl PCR reaction mixtures contained 2.5 µl of 10 mM PCR buffer (PCR buffer with $MgCl_2$), 1 µl of 25 mM $MgCl_2$, 2.5 µl of 100 mM of each deoxynucleotide triphosphate, 1 µl of 10 nM of each primer, 2 ng DNA template and 1 U Taq polymerase (Biotech International, Needville, TX, USA). Non-template controls of sterile water were used with every PCR reaction. The amplification conditions were as follows: initial denaturation of 5 min at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 56°C, and 1 min at 72°C, and a final extension of 5 min at 72°C. Five µl of each PCR product were separated by electrophoresis in 1% agarose gels in 5% TAE buffer (40 mM Tris, 40 mM acetate, 2 mM EDTA, pH 8.0). The amplified PCR fragments were purified with Sephadex (Sigma, Steinheim, Germany) and sequenced with the BigDye terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems, Foster City, California, USA) in both directions, with the same primers used for the PCR reactions. Products were separated with an ABI 3730 48 capillary sequencer (Perkin-Elmer Applied Biosystems).

Sequences of the isolates were edited using Vector NTI 11 (Lu & Moriyama, 2004). DNA sequences for species previously published were retrieved from GenBank (http://www.ncbi.nlm.gov). The resulting data matrices for each gene were rooted with *Pseudofusicoccum stromaticum* (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf. following the example of Phillips *et al.* (2008). The data matrices were aligned online using MAFFT (http://align.bmr.kyushuu.ac.jp/mafft/online/server/) version 6 (Katoh *et al.*, 2005) and checked manually for alignment errors.

Phylogenetic analyses of sequence data for Maximum Parsimony (MP) and Maximum Likelihood (ML) were made using PAUP* v.4.0b10 (Swofford, 2001). Maximum parsimony (MP) genealogies for single genes were constructed with the heuristic search option (100 random taxa additions, tree bisection and reconstruction (TBR) in PAUP. The uninformative aligned regions within each dataset were removed from the analyses, gaps were treated as fifth character and all characters were unordered and of equal weight. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the tree(s) obtained was evaluated by 1000 bootstrap replications. Congruence between the different datasets was tested using the partition homogeneity test (PHT) in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Farris *et al.*, 1995; Huelsenbeck *et al.*, 1996), with the uninformative characters removed before analysis. Other measures such as tree length (TL), consistency index (CI), rescaled consistency index (RC), and the retention index (RI) (Hillis and Huelsenbeck, 1992) were recorded.

For ML analyses, the best nucleotide substitution models for each dataset separately, were found with Modeltest 3.7 (Posada & Buckley, 2004). The models K81uf+I+G (G=0.528, I=0.476), TrNef+I+G (G=0.819, I=0.759), TrN+I+G (G=1.807, I=0.144), TrN+I+G (G= 1.476, I= 0.562) and GTR+I+G (G=0.2910, I=0.0) were chosen for the ITS, LSU, TEF-1α, β-tubulin and combined datasets, respectively. The analyses were also performed in PAUP 4.0b10 and confidence levels were determined with 1000 bootstrap replications.

Morphological characteristics

To induce sporulation, cultures were inoculated onto sterilized twigs of *A. karroo* placed on the surface of 2% MEA (Biolab), and these were incubated at 25°C under near-UV light. Fifty released conidia, and 20 pycnidia and conidiogenous cells were measured for the isolates chosen to represent holotypes for each putative new species, and the ranges and averages were computed. These measurements were augmented with 20 measurements obtained from additional isolates. Measurements and digital images were made with an HRc Axiocam digital camera and accompanying Axiovision 3.1 software (Carl Zeiss Ltd., Munich, Germany). The specimens were deposited in the National Collection of Fungi (PREM), Pretoria, South Africa.

Colony morphology and colour were determined from cultures grown on MEA at 5-35°C, at 5°C intervals, in the dark. For these, 6 mm diam. mycelial plugs were taken from the edges of actively growing 5-day-old single conidial cultures, and transferred to the centers of 25 ml MEA in 90 mm diam. Petri dishes. Three replicate plates were used for each isolate per temperature. Two perpendicular measurements were taken of the colony diameter daily until the mycelium of the fastest growing isolates had covered the plates and averages were computed. Colony colours were assigned using the designations of Rayner (1970).

RESULTS

Isolates

Several known as well as previously uncharacterized species of Botryosphaeriaceae were recovered from both healthy and diseased *A. karroo* and the focus of this study will only be on those not previously described. Twenty three isolates from all those recovered were determined as belonging to one of several new species. Six isolates of undescribed species were from samples collected in Bloemfontein and 17 were from the Pretoria area. These could be grouped into five morphotypes that were selected for sequencing.

DNA sequence analyses

The datasets for the ITS, TEF-1α, β-tubulin and LSU rDNA sequences were analyzed individually and in combination. The ITS sequence dataset contained 522 characters (4 parsimony-uninformative, 148 parsimony-informative, 370 constant characters) with CI = 0.602, RI = 0.902, RC = 0.543, HI = 0.398 and TL = 321. The TEF-1α dataset contained 362 characters (3 parsimony-uninformative, 224 parsimony-informative, 135 constant characters) with CI = 0.617, RI = 0.891, RC = 0.550, HI = 0.383 and TL = 523. The β-tubulin dataset contained 471 characters (0 parsimony-uninformative, 142 parsimony-informative, 329 constant characters) with CI = 0.698, RI = 0.901, RC = 0.629, HI = 0.302, and TL=304. The LSU dataset contained 848 characters (7 parsimony-uninformative, 74 parsimony-informative and 767 constant characters) with CI = 0.652, RI = 0.885, RC = 0.577, HI = 0.348 and TL = 128. The tree statistics for the combined dataset were CI = 0.487, RI = 0.854, RC = 0.416, HI = 0.513, TL = 2148, TreeBase

Accession No. S12358, and the partition homogeneity test (PHT) on the datasets produced a P-value of 0.01.

The topology of the trees emerging from the ML and MP analyses were similar for the individual gene regions as well as in the combined analysis with regards to the clades representing species isolated in this study. However, clades representing genera occasionally collapsed in individual analyses. Fixed alleles could be identified in the datasets for the clades of the species identified in this study (Tables 2-4). Seven clades were identified in the MP and ML analyses representing *Lasiodiplodia*, *Diplodia*, *Tiarosporella*, *Dothiorella*, *Spencermartinsia*, *Phaeobotryon* and *Botryosphaeria.*

Isolates resided in four genera (Fig. 1). Isolates CMW36468, CMW36469 and CMW36470 (from healthy branches of *A. karroo* in Pretoria) formed a distinct clade together with other *Diplodia* species. *I*solates CMW36477, CMW36478 and CMW36479 (from healthy *A. karroo* in Pretoria and Bloemfontein) formed a distinct clade with *Tiarosporella* species. Isolates from healthy branches of *A. karroo* in Pretoria (CMW36480 and CMW36481) grouped separately with *Spencermartinsia* species, while the clade containing species of *Dothiorella* included two sub-clades (isolates CMW36460, CMW36461 and CMW36462 from branches with die-back from Pretoria, and isolates CMW36463 and CMW36464 from healthy branches in Pretoria, respectively). There was considerable sequence variation in the four gene regions (ITS, TEF-1α, β-tubulin and LSU rDNA) among isolates representing the undescribed species and those of their sister species, which included an undescribed *Dothiorella* species (Van der Walt *et al*. 2008) from *Acacia mellifera* (Table 2), *Dothiorella longicollis* Pavlic, T.I. Burgess & M.J. Wingf. (Table 3), and an undescribed *Spencermartinsia* species (Van der Walt *et al*. 2008) from *Acacia erioloba* (Table 4).

Morphological characteristics

Isolates were divided into five groups based on culture morphology, conidial shape and colour. The isolates in the fifth group corresponded to *Tiarosporella* in the DNA sequence comparisons and had white, raised aerial mycelium at the centers of the cultures with gray edges and undersides of the plates, and produced large hyaline conidia different from those of the other taxa. Isolates in the other four groups had dark gray or olivaceous colonies with aerial hyphae and dark conidia. *Diplodia*, *Dothiorella* and *Spencermartinsia* isolates could be distinguished by differences in growth and colour of the isolates. The isolates residing in *Diplodia* had gray mycelium with dark, regular edges and mostly aseptate conidia while those in *Dothiorella* had dark gray mycelium with irregular edges. The *Spencermartinsia* isolates were similar to the *Dothiorella* isolates and had similar conidia, but the colonies had regular edges. The species distinguished by phylogenetic inference could also be distinguished from other species in the respective genera based on differences in pycnidium shape and conidial size.

The use of *A. karroo* twigs on MEA to induce fruiting structures resulted in only anamorph states that were similar in characteristics between genera. The substantial overlap in these characters allowed only limited comparisons with herbarium specimens and previous descriptions. Differences in ascospore septation distinguish *Spencermartinsia* (2-celled ascospores with an apiculus at either end of the ascospore and *Dothiorella* (1-septate ascospores and apiculi absent) but ascospores were not observed in this study.

Table 2. Polymorphic nucleotides fixed between an undescribed *Dothiorella* species from *Acacia mellifera* and *Dothiorella dulcispinae* based on sequence data of the ITS, TEF-1α, LSU and β-tubulin. Polymorphisms unique to *Dothiorella dulcispinae* are highlighted

Identity	*Isolate no.*	*ITS*	*TEF-1α*	*LSU*					
		11	*9*	*16*	*43*	*342*	*89*	*342*	*356*
Undescribed *Dothiorella* species from *Acacia mellifera*	CBS121764	–	–	T	C	–	–	–	–
Undescribed *Dothiorella* species from *A. mellifera*	CBS121765	–	–	T	C	–	–	–	–
Dothiorella dulcispinae	CMW36460	**T**	**A**	–	–	**T**	**G**	**T**	**G**
Do. dulcispinae	CMW36461	**T**	**A**	–	–	**T**	**G**	**T**	**G**
Do. dulcispinae	CMW36462	–	**A**	–	–	**T**	**G**	**T**	**G**

Identity	*Isolate no.*	*β-tubulin*													
		43	*49*	*56*	*61*	*139*	*157*	*166*	*171*	*180*	*267*	*333*	*345*	*348*	*410*
Undescribed *Dothiorella* species from *Acacia mellifera*	CBS121764	T	A	A	–	G	C	–	C	C	A	G	T	T	–
Undescribed *Dothiorella* species from *A. mellifera*	CBS121765	T	A	A	–	G	C	–	C	C	A	G	T	T	–
Dothiorella dulcispinae	CMW36460	–	–	–	**T**	–	–	**C**	**G**	**T**	**G**	–	–	–	**C**
Do. dulcispinae	CMW36461	–	–	–	**T**	–	–	**C**	**G**	**T**	**G**	–	–	–	**C**
Do. dulcispinae	CMW36462	–	–	–	**T**	–	–	**C**	**G**	**T**	**G**	–	–	–	**C**

Table 3. Polymorphic nucleotides from sequence data of the ITS, TEF-1α, LSU and β-tubulin to show the relationships between *Dothiorella longicollis* and *Dothiorella brevicollis*. Polymorphisms unique to *Dothiorella brevicollis* are highlighted. (There were no differences for LSU sequence data.)

Identity	*Isolate no.*	*ITS*	*TEF-1α*								*β-tubulin*	
		72	*9*	*31*	*39*	*88*	*90*	*244*	*247*	*350*	*56*	*450*
Dothiorella longicollis	CBS122066	T	A	A	T	T	–	–	A	C	–	T
Do. longicollis	CMW26164	T	A	A	T	T	–	–	A	C	–	T
Dothiorella brevicollis	CMW36463	–	**G**	–	–	–	**G**	**A**	–	–	**A**	–
Do. brevicollis	CMW36464	–	**G**	–	–	–	**G**	**A**	–	–	**A**	–

Table 4. Polymorphic nucleotides from sequence data of the ITS, TEF-1α, LSU and β-tubulin showing the relationships between an undescribed *Spencermartinsia* species from *Acacia erioloba* and *Spencermartinsia pretoriensis*. Polymorphisms unique to *Spencermartinsia pretoriensis* are highlighted

Identity	*Isolate no.*	*ITS*																							
		7	*18*	*26*	*77*	*78*	*84*	*85*	*87*	*92*	*98*	*104*	*105*	*107-109*	*145*	*147*	*169-170*	*395*	*406*	*487*	*488*				
Undescribed *Spencermartinsia* species from *Acacia erioloba*	CMW25404	A	T	–	C	–	T	C	C	–	–	–	–	– – –	A	–	A –	T	A	A	–				
Undescribed *Spencermartinsia* species from *A. erioloba*	CMW25405	A	T	–	C	–	T	C	C	–	–	–	–	– – –	A	–	A –	T	A	A	–				
Spencermartinsia pretoriensis	CMW36480	–	–	**T**	–	**T**		–	–	**A**	**G**	**C**	**C**	**C C A**	–	**G**	– **C**	–	–	–	**C**				
S. pretoriensis	CMW36481	–	–	**T**	–	**T**		–	–	**A**	**G**	**C**	**C**	**C C A**	–	**G**	– **C**	–	–	–	**C**				
Identity	*Isolate no.*	*ITS*																							
		8	*20*	*21*	*34*	*36*	*37*	*40*	*46*	*47*	*48*	*59*	*128*	*155*	*160*	*161*	*163*	*164*	*166*	*180*	*193*	*216*	*220*	*244*	*247*
Undescribed *Spencermartinsia* species from *Acacia erioloba*	CMW25404	T	G	C	T	T	C	T	T	C	A	T	–	A	C	A	C	C	C	–	A	C	A	A	A
Undescribed *Spencermartinsia* species from *A. erioloba*	CMW25405	T	G	C	T	T	C	T		C	A	T	–	A	C	A	C	C	C	–	A	C	A	A	A
Spencermartinsia pretoriensis	CMW36480	**C**	**C**	**G**	–	**C**	–	**C**	**C**	**G**	**T**	–	**C**	**G**	**G**	**G**	**T**	–	**T**	**A**	**T**	**G**	**C**	**G**	**C**
S. pretoriensis	CMW36481	**C**	**C**	**G**	–	**C**	–	**C**	**C**	**G**	**T**	–	**C**	**G**	**G**	**G**	**T**	–	**T**	**A**	**T**	**G**	**C**	**G**	**C**

Identity	*Isolate no.*	*β-tubulin*		*LSU*								
		261	*299*	*16*	*41*	*43*	*117*	*146*	*436*	*470*	*475*	*485*
Undescribed *Spencermartinsia* species from *Acacia erioloba*	CMW25404	–	A	T	–	–	–	T	A	T	–	A
Undescribed *Spencermartinsia* species from *A. erioloba*	CMW25405	–	A	T	–	–	–	T	A	T	–	A
Spencermartinsia pretoriensis	CMW36480	**C**	–	–	**C**	**C**	**C**	–	–	–	**C**	–
S. pretoriensis	CMW36481	**C**	–	–	**C**	**C**	**C**	–	–	–	**C**	–

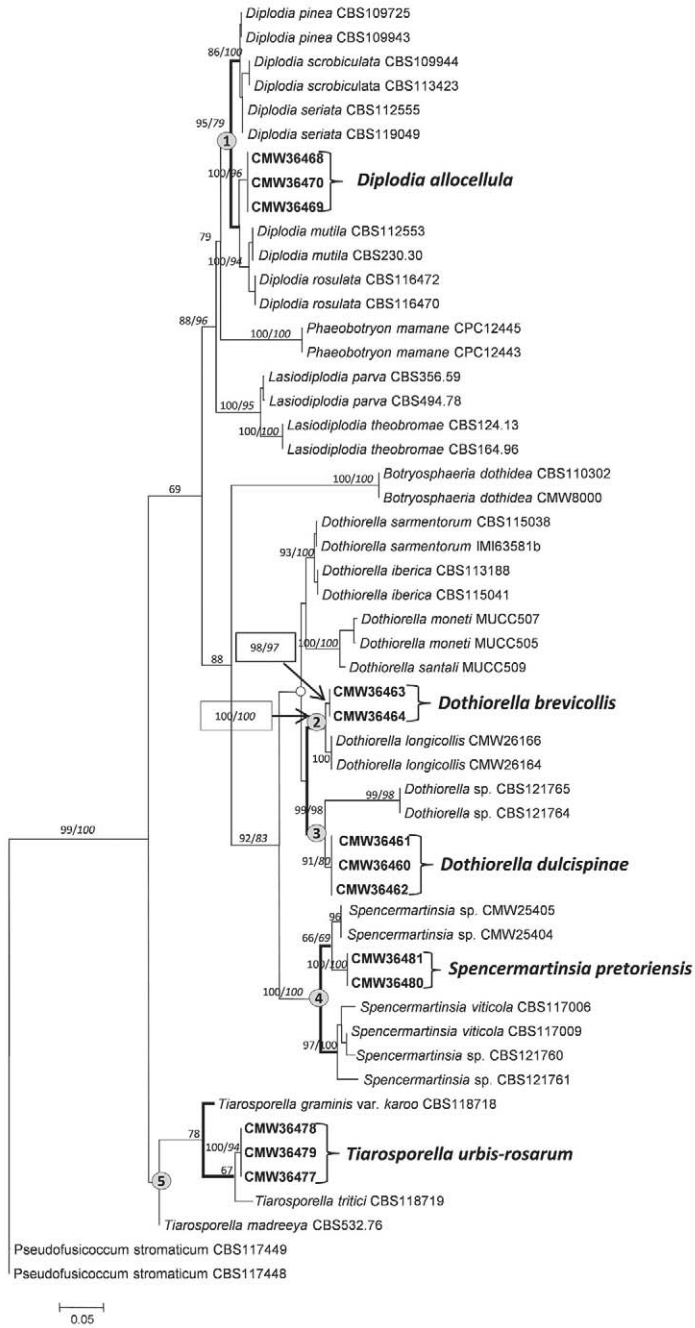

Fig. 1. ML tree of the combined data set of ITS ribosomal DNA, TEF-1α, β-tubulin and LSU gene region sequences. Bootstrap values for ML (roman) and MP (italic) above 60% are given at the nodes. The numbers are shown in bold for isolates newly sequenced in this study. The tree was rooted to *Pseudofusicoccum stromaticum* (CBS117448 and CBS117449).

TAXONOMY

The following are descriptions of new species based on morphology:

Tiarosporella urbis-rosarum Jami, Gryzenh., Slippers & M.J. Wingf. **sp. nov. Fig.2**

Mycobank: MB 564139

Etymology: The name refers to the city, Bloemfontein, where this species was first isolated and popularly called "The city of roses".

No teleomorph produced. *Pycnidia* produced on *Acacia karroo* twigs on MEA in 6-10 weeks, solitary, globose, dark black (29````m), up to 200 µm wide, immersed, unilocular, with long necks (4-9 mm); wall 5-7 cell layers thick, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline towards the inner region. *Conidiogenous cells* holoblastic, hyaline, cylindrical, (5–) 5.5-9.5 (–11) × (3–) 3.2-4 (–5) µm. *Conidia* ovoid, smooth with fine granular content, thin-walled, hyaline, aseptate, apices rounded, (21–) 23.5-29.5 (–34) × (8–) 9-10 (–11) µm.

Colonies on MEA with appressed mycelial mats, pycnidia emerging after 2 months under near-ultraviolet light on *A. karroo* twigs. Mycelium gray, white at the centres, becoming dark gray at the regular edges, reverse dark gray to black. Growth at 5-35°C. Growth rate 14.4 mm per day at an optimal temperature of 25°C; covering the agar surface in a 90 mm diam. Petri dish after 6 days in the dark.

Specimens examined: South Africa, Free State Province, Bloemfontein, June 2008, M. Gryzenhout, from healthy wood section of *Acacia karroo*, holotype PREM 60698 resulting from inoculations of living isolate to *A. karroo* twigs, living cultures CMW 36477 = CBS 130405.

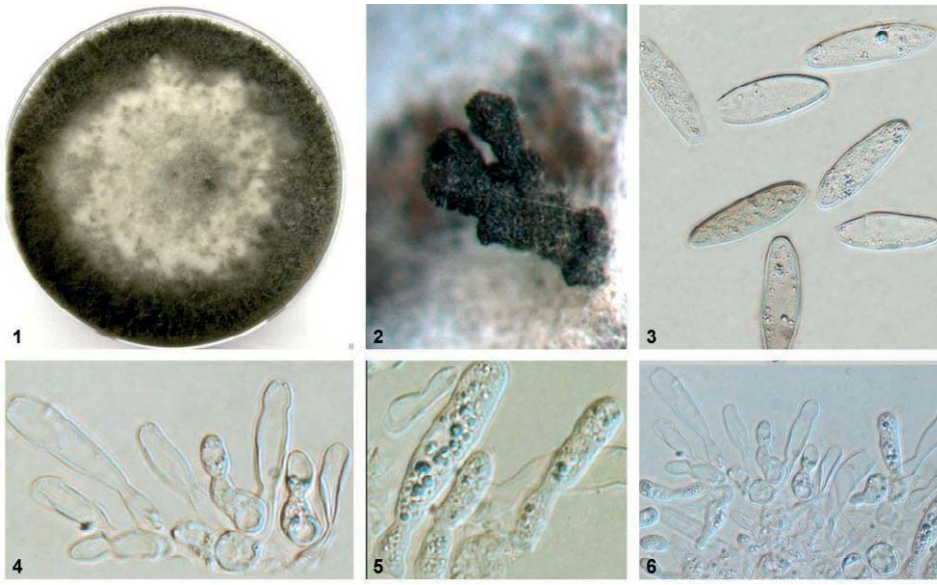

Fig. 2. Micrographs of *Tiarosporella urbis-rosarum.* **1.** Culture morphology on MEA (scale bar = 100 µm); **2.** Pycnidium; **C.** Conidia (scale bar = 10 µm); **4-6.** Conidiogenous cells (scale bar 4,5 = 10 µm & scale bar 6 = 5 µm).

Additional specimens: South Africa, Free State Province, Bloemfontein, June 2008, M. Gryzenhout, from healthy wood section of *Acacia karroo.* Paratype PREM 60699 (living cultures CMW 36478 = CBS 130406). South Africa, Gauteng Province, Pretoria, June 2008, M. Gryzenhout & F. Jami, from healthy wood section of *Acacia karroo*. Paratype PREM 606700 (living cultures CMW 36479 = CBS 130407).

Commentary: Eight species of this genus have been described (Dragan, 2003). DNA sequences of only *Tiarosporella madreeya, T. tritici* and *T. graminis* var. *karroo* are, however, available (Crous *et al.*, 2006) and the DNA sequences of *T. urbis-rosarum* differ from these species. The sizes of conidia of *T. urbis-rosarum* are very similar to those of *T. tritici (*Crous *et al.*, 2006), but conidia of *T. urbis-rosarum* are slightly smaller (23.5-29.5 × 9-10 µm) than those of *T. tritici* (29-38 × 12-17 µm).

Diplodia allocellula Jami, Gryzenh., Slippers & M.J. Wingf. **sp. nov.** **Fig. 3**

Mycobank: MB 564140

Etymology: The name refers to the variability in length of the conidiogenous cell in this fungus.

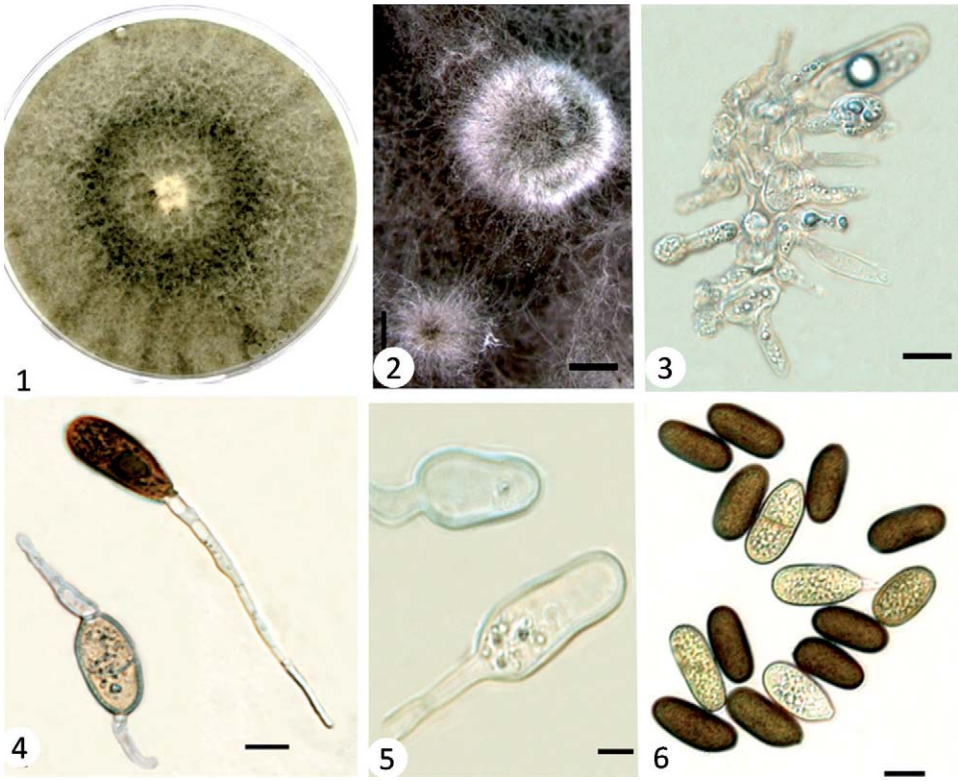

Fig. 3. Micrographs of *Diplodia allocellula* **1.** Culture morphology on MEA; **2.** Pycnidium (scale bar = 100 µm); **3.** Conidiogenous cells (scale bar = 10 µm); **4.** Germinating conidia (scale bar = 10 µm); **5.** Young conidia with attached conidiogenous cells (scale bar = 5 µm; **6.** Maturing conidia at various stages (scale bar = 10 µm).

No teleomorph produced. *Pycnidia* immersed on MEA in 6-10 weeks, solitary, globose, chestnut, covered by hyphal hairs, up to 100 µm diam, without necks. *Conidiogenous cells* holoblastic, smooth with fine granular contents, unicellular, cylindrical to sub-cylindrical, hyaline and truncate at base, (4–) 4.2-5 (–5.5) × (10.3–) 13.4-23.6 (–27.6) µm. *Conidia* ovoid to ellipsoid, smooth with fine granular content, apices rounded and truncated at the base, thick-walled, aseptate, initially hyaline, becoming dark brown or sepia (13``k), aseptate, (9–) 10-12.5 (–14.5) × (20–) 21.5-25 (–30) µm.

Colonies on MEA initially white turning gray from the middle of colonies within 5-7 d, aerial mycelium slightly fluffy, becoming dense, cottony with age, turning smoke gray to dark gray (23````d) toward the edges after 7-9 days, reverse olivaceous-black (33```` m) and with regular edges. Growth in culture from 5-35°C. Growth rate of 18.2 mm per day optimal at 25°C and covering 90 mm diam. Petri dishes after 5 days in the dark.

Specimens examined: South Africa, Gauteng Province, Pretoria (George Storrar avenue, Fountain circle), Nov. 2009, M. Gryzenhout & F. Jami, from branch of *Acacia karroo* showing die-back, holotype PREM 60701 resulting from inoculations of living isolates onto *A. karroo* twigs, living cultures CMW 36468 = CBS 130408.

Additional specimens: South Africa, Gauteng Province, Pretoria (George Storrar avenue, Fountain circle), Nov. 2009, M. Gryzenhout & F. Jami, from branch of *Acacia karroo* showing die-back. Paratype PREM 60702 (living cultures CMW 36469= CBS 130409) and PREM 60703 (living cultures CMW 36470 = CBS 130410).

Commentary: Diplodia allocellula grouped with *D. mutila* (Fr.) Mont., *D. rosulata* Gure, Slippers & Stenlid and *D. africana* Damm & Crous as sister species in the phylogenetic analyses of other *Diplodia* species. *Diplodia allocellula* differ phylogenically from its closest *Diplodia* relatives and morphologically in its smaller conidia (10-12.5 × 21.5-25 µm) compared to those of *D. mutila* (13.2-13.5 × 25.1-25.7 µm), *D. rosulata* (11-17.5 × 25-32 µm) and *D. africana* (10-15 × 25.5-33 µm). *Diplodia rosulata* has been found from seeds of *Prunus africana* in Ethiopia (Gure *et al.*, 2005), *D. mutila* is known from diseased *Vitis vinifera*, *Pyrus communis*, *Quercus suber* and *Malus pumila* in Spain, Portugal and USA (Alves *et al.*, 2004), while *D. africana* has been described from wounds on *Prunus persica* in South Africa (Western Cape Province) (Damm *et al.*, 2007). In contrast, *Diplodia allocellula* was obtained from healthy *A. karroo* tissue in this study in Pretoria, South Africa.

Dothiorella dulcispinae Jami, Gryzenh., Slippers & M.J. Wingf. **sp. nov.** **Fig. 4**

Mycobank: MB 564141

Etymology: The name refers to the host, *Acacia karroo* (Sweet Thorn), dulcis = sweet and spina = a thorn.

No teleomorph produced. *Pycnidia* produced on *Acacia karroo* twigs on MEA after 2-4 weeks, solitary, globose, dark brown, up to 200 µm wide, semi-immersed, unilocular, with a short necks (100-300 µm); wall 6-8 cell layers thick, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiogenous cells* 1-2 celled, holoblastic, hyaline, cylindrical, proliferating percurrently near the apex. *Conidia* ovoid, smooth with fine granular content, rounded apices, thick-walled, initially hyaline and aseptate, becoming dark brown or sepia (13``k) and 1-septate, with 2 cells of unequal length, apices rounded, (6–) 7-10 (–11) × (14–) 16-22 (–24) µm.

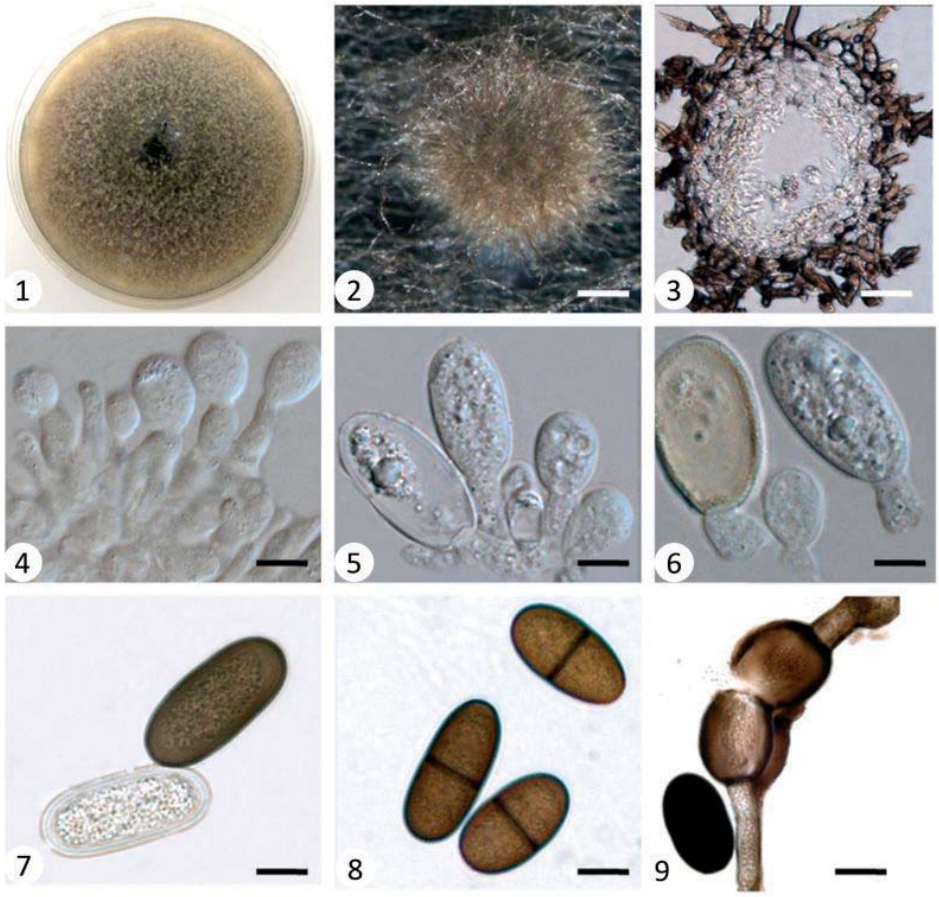

Fig. 4. Micrographs of *Dothiorella dulcispinae*. **1.** Culture morphology on MEA; **2**. Pycnidium (scale bar = 100 µm); **C.** Longitudinal section through pycnidium (scale bar = 100 µm); **4-6.** Conidia and conidiogenous cells (scale bar = 10 µm), **7-8.** Conidia (scale bar = 10 µm); **9.** Chlamydospore (scale bar = 10 µm).

Colonies on MEA developing dense aerial mycelium with age, olivaceous-gray (23````l), surface pale olivaceous-gray (21````i) to olivaceous black (27````m), reverse olivaceous-black, umbonate with irregular zonation and lobate edges. Growth at 5-35°C, 17.9 mm per day and optimal at 25°C, covering the surface of 90 mm diam. Petri dishes after 5 days in the dark.

Specimens examined: South Africa, Gauteng Province, Pretoria, Nov. 2009, F. Jami, from die-back wood section of *Acacia karroo*, holotype PREM 60706 resulting from inoculations of living isolates onto *A. karroo* twigs, living cultures CMW 36460 = CBS 130413.

Additional specimens: South Africa, Gauteng Province, Pretoria, Nov. 2009, F. Jami, from die-back wood section of *Acacia karroo,* paratype PREM 60707 (living cultures CMW 36461 = CBS 130414) and PREM 60708 (living cultures CMW 36462 = CBS 130415).

Commentary: Based on phylogenetic analyses, isolates of *Dothiorella dulcispinae* are most closely related to those of a undescribed species of *Dothiorella*, which is known as an endophyte of *A. mellifera* in South Africa (Van der Walt *et al.*, 2008). It differs from the latter species in the morphology of pycnidia, conidiogenous cells and conidia which are all smaller than those of the undescribed *Dothiorella* species.

Dothiorella brevicollis Jami, Gryzenh., Slippers & M.J. Wingf. **sp. nov.** **Fig. 5**

Mycobank: MB 564142

Etymology: The name refers to the fact that the pycnidia have short necks.

No teleomorph produced. *Pycnidia*, produced on *Acacia karroo* twigs on MEA after 2-4 weeks, brown (7``m), up to 200 µm wide, semi-immersed, unilocular, globose with a short neck; wall 5-7 cell layers thick, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Conidiogenous cells* holoblastic, hyaline, cylindrical, (3–) 3.2-3.7 (–4) × (3–) 3.2-7.5 (–9) µm. *Conidia* ovoid, smooth with fine granular content, rounded apices, thick-walled, initially hyaline and aseptate, becoming dark brown and 1-septate, with 2 cells of equal length, apices rounded, (8–) 9-12 (–13) × (20–) 21.5-26 (–27) µm.

Colonies on MEA appressed, pycnidia emerging after 9–10 d under near-ultraviolet light. Mycelium olivaceous-gray (21````b), surface pale olivaceous-gray to dark olivaceous-gray (23````l), reverse olivaceous-black (29````m) to iron grey (29`````k), irregular edges. Growth observed from 5-35°C, reaching 17.6 mm per day and optimal at 25°C, covering the surface of a 90 mm diam. Petri dish after 6 days in the dark.

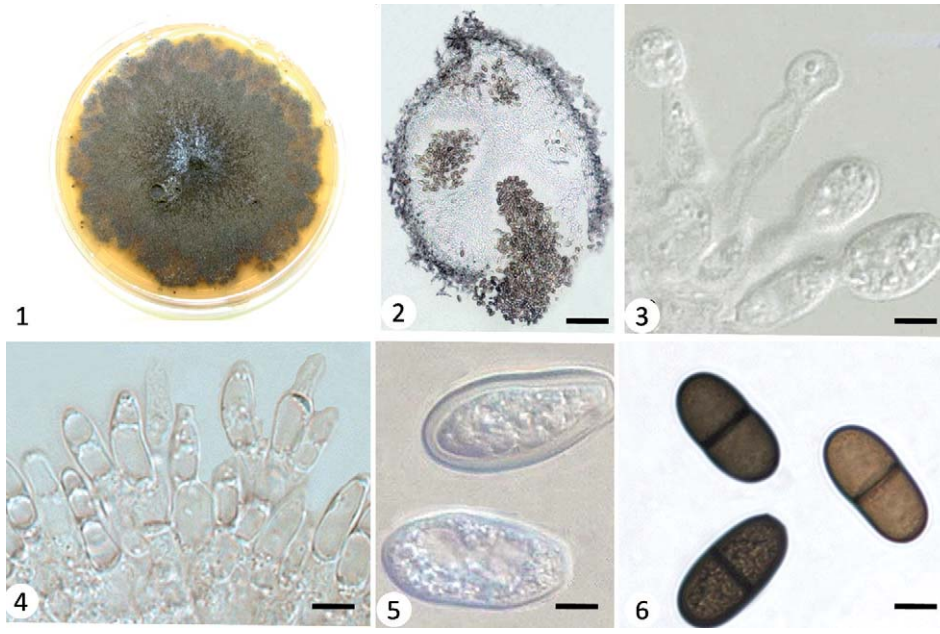

Fig. 5. Micrographs of *Dothiorella brevicollis*. **1.** Culture morphology on MEA; **2.** Longitudinal section through pycnidium (scale bar = 100 µm); **3-4.** Conidiogenous cells (scale bar = 10 µm); **5.** Young Conidia (scale bar = 5 µm); **6.** Conidia at various stages of maturity (scale bar = 10 µm).

Specimens examined: South Africa, Gauteng Province, Pretoria, Nov. 2009, F. Jami, from healthy wood section *Acacia karroo,* holotype PREM 60704 resulting from inoculations of living isolates onto *A. karroo* twigs, living cultures CMW 36463 = CBS 130411.

Additional specimens: South Africa, Gauteng Province, Pretoria, Nov. 2009, F. Jami, from healthy wood section *Acacia karroo,* paratype PREM 60705 (living cultures CMW 36464 = CBS 130412).

Commentary: Do. brevicollis has shorter pycnidial necks than those of *Do. longicollis*. It can also be distinguished from *Do. dulcispinae* described in this study based on phylogenetic analyses and by its larger conidia.

Spencermartinsia pretoriensis Jami, Gryzenh., Slippers & M.J. Wingf. **sp. nov. Fig. 6**

Mycobank: MB 564143

Etymology: The name refers to Pretoria, where samples yielding the species were collected.

No teleomorph produced. *Pycnidia* produced on *Acacia karroo* twigs on MEA after 2-4 weeks, brown, up to 200 µm wide, semi-immersed, unilocular, with short necks; wall 5-7 cell layers thick, outer layers composed of dark-brown *textura angularis*, becoming thin-walled and hyaline towards the inner regions. *Conidiophores* absent and reduced to *conidiogenous cells*, 1-2 celled, hyaline, holoblastic, cylindrical, proliferating percurrently near the apex, (3–) 3.2-7.5 (–9) × (3–) 3.2-3.7 (–4) µm. *Conidia* ovoid, smooth with a fine granular content, rounded apices, thick-walled, initially hyaline and aseptate, becoming dark brown (7``m) and 1-septate, with 2 cells of equal length, apices rounded, (18–) 20-28 (–33) × (6.5–) 7-14 (–11) µm.

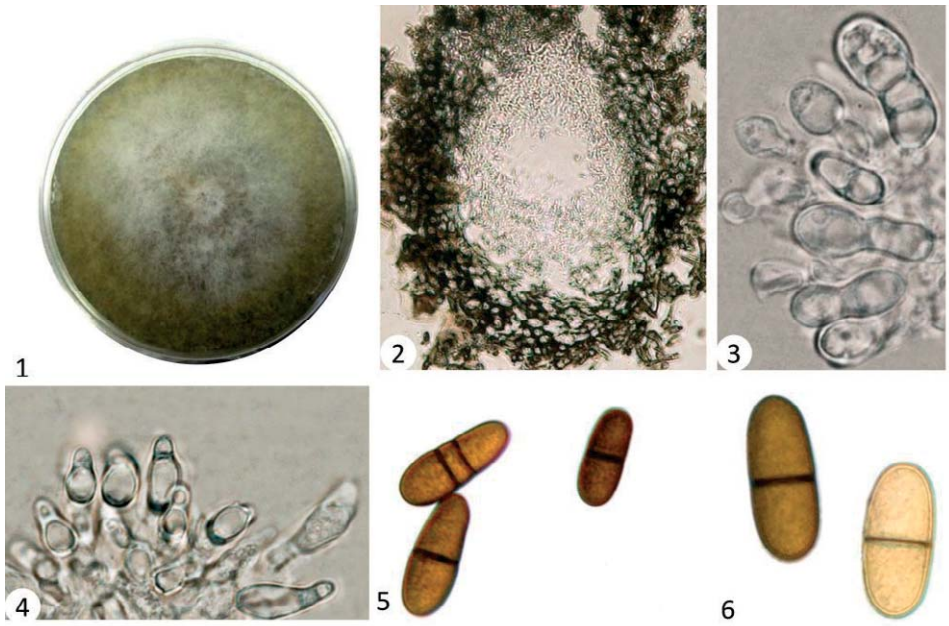

Fig. 6. Micrographs of *Spencermartinsia pretoriensis.* **1.** Culture morphology on MEA; **2.** Longitudinal section through pycnidium (scale bar = 100 µm), **3-4.** Conidiogenous cells (scale bar = 20 µm), **5-6.** Conidia (scale bar = 10 µm).

Colonies on MEA appressed, and pycnidia emerging after 8-10 days. Mycelium pale olivaceous, surface pale olivaceous to dark greenish olivaceous, reverse olivaceous-black (29```m), regular zonation and lobate edges. Growth from 5-35°C; up to 22.5 mm per day and optimal at 25°C, covering the surface of 90 mm diam. Petri dish after 4 days in the dark.

Specimens examined: South Africa, Gauteng Province, Nov. 2009, F. Jami, from die-back wood section *Acacia karroo,* holotype PREM 60709 resulting from inoculations of living isolates onto *A. karroo* twigs, living cultures CMW 36480 = CBS 130404.

Additional specimens: South Africa, Gauteng Province, Nov. 2009, F. Jami, from die-back wood section *Acacia karroo,* paratype PREM 60710 (living cultures CMW 36481 = CBS 130405).

Commentary: Spencermartinsia pretoriensis is not distinguishable from an undesrcibed *Spencermartinsia* species from *Acacia erioloba b*ased on morphology and could be differentiated from this species only with DNA sequence comparisons (Table 4).

DISCUSSION

Extensive sampling for the Botryosphaeriaceae on native trees has been undertaken in South Africa in recent years. Despite this fact, this study yielded a large number of new taxa, which suggests that many undescribed species remain to be isolated and identified. Here we considered only tree species (*A. karroo*), in two areas where were not previously sampled. In the process we discovered five new species in four genera of the Botryosphaeriaceae. Identifications of the species collected were primarily based on DNA sequence comparisons of four loci. It was not possible to compare the species with the plethora of those previously described in these genera and for which no sequence data are available. However, morphological data were useful to distinguish these five species from each other and from other species described from the region.

Three of the five newly discovered species are relatively closely related and reside in the *Spencermartinsia* and *Dothiorella* clades. These two genera have only recently been split and were previously treated in the single genus *Dothiorella*. When *Do. viticola* was described as a new species, it could not be distinguished from *Do. iberica* and *Do. sarmentorum* based on anamorphic features, thus differences in teleomorph characters were used to differentiate the three species (Luque *et al.*, 2005) where *Do. viticola* has 2-celled ascospores with apiculi at either end. Later studies introduced *Do. viticola* as the new genus *Spencermartinsia* (Phillips *et al.*, 2008). The teleomorph for other members of *Spencermartinsia* spp. is still unknown, and their placement in the genus rests only on gene sequence similarities. In this study *S. pretoriensis* was placed in the genus because of its grouping with other *Spencermartinsia* spp. based on DNA sequences alone, and separate from the clade containing *Dothiorella* spp. These analyses, however, also show distinct clades within both the *Dothiorella* and *Spencermartinsia* clades, which might later be interpreted as representing distinct genera. As in the case of *Dothiorella* and *Spencermartinsia*, discovery of a teleomorph may also lead to changes in the taxonomy of this group.

Spencermartinsia species appear to be diverse on *Acacia* in southern Africa and the discovery of *S. pretoriensis* on *A. karroo* in this study adds to this view. All four *Spencermartinsia* spp. that are currently known were recently

isolated from native *Acacia* spp. in southern Africa, such as *A. erioloba, A. mellifera* and *A. tortillis* (Van der Walt *et al.*, 2008). *Spencermartinsia viticola,* the type species of the genus, was described from grape vines in Spain (Luque *et al.*, 2005) and from citrus in California (Adesemoye and Eskalen, 2011). It was also found on *A. melifera* in Pretoria, South Africa (Van der Walt *et al.*, 2008). Interestingly it is the only species thusfar known from hosts other than *Acacia* and from the countries other than South Africa. Further studies of the Botryosphaeriaceae on native *Acacia* will test the emerging hypothesis that this group evolved on this host and in Africa.

Tiarosporella spp. have a distinct culture and conidial morphology compared to other genera of the Botryosphaeriaceae. Species have a woolly mycelium that is white at the center and gray at the colony edges. Pycnidia have long necks and produce ovoid hyaline conidia. Three species, *T. tritici* B. Sutton & Marasas from *Triticum aestiuum* (Poaceae), *T. graminis* var. *karroo* B. Sutton & Marasas, and *T. graminis* var. *gamines* (Piroz. & Shoemaker) Nag Raj from *Tribulus terrestris* (Zygophyllaceae), *Eriocephalus* sp. (Asteraceae) and *Nestlera* sp. (Poaceae) have been described from Heilbron (Free State province) and Middelburg (Eastern Cape province) in South Africa (Sutton & Marasas, 1976). Crous *et al.* (2006) found that *Tiarosporella* grouped in an unresolved clade with *Lasiodiplodia*/*Diplodia.* Subsequent studies using greater numbers of samples showed that *Diplodia* and *Lasiodiplodia* group closely together (Phillips *et al.*, 2008), but the placement of *Tiarosporella* was not addressed. Results of this study support the distinct grouping of *Tiarosporella* from species of *Lasiodiplodia* and *Diplodia*.

Species of the Botryosphaeriaceae are well-known as latent pathogens that can cause disease under conditions of stress (Slippers & Wingfield, 2007). Two of the five species described in this study (*Dothiorella dulcispinae* and *Spencermartinsia pretoriensis*) were isolated from diseased tissue, while three (*Dothiorella brevicollis*, *Tiarosporella urbis-rosarum* and *Diplodia allocellula*) were associated only with healthy *A. karroo* tissue. These patterns of isolation do not allow for deductions to be made regarding the ability of the fungi to cause disease on *A. karroo,* and pathogenicity trials will be needed to better understand the role of these fungi as possible disease agents.

The host ranges of the new species described in this study are unknown. Very few Botryosphaeriaceae appear to be host specific when they are sampled widely. For example, *N. eucalyptorum* (Crous, H. Smith & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips and *N. eucalypticola* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips were thought to be specific to *Eucalyptus* spp. (Slippers *et al.*, 2004), but *N. eucalyptorum* was subsequently found on other Myrtaceae (Pérez *et al.*, 2009). A number of species in the Botryosphaeriaceae such as *L. theobromae, B. dothidea,* and *N. parvum* are known to have wide host ranges (Slippers & Wingfield, 2007). Similarly, of the 12 Botryosphaeriaceae species isolated by Van der Walt *et al.* (2008) from *Acacia*, three species (*B. dothidea* and undescribed species in *Spencermartinsia* and *Pheaobotryon*) were found on *A. karroo* and other *Acacia* species. It is thus expected that the species described here may also be found on other members of *Acacia* and perhaps even other tree genera.

A number of apparently host specific specific species are found among *Dothiorella* species, such as *Do. moneti* K. Taylor, Barber & T.I. Burgess from *Acacia rostellifera*, *Do. santali* K. Taylor, Barber & T.I. Burgess from *Santalum acuminatum* in Western Australia (Taylor *et al.*, 2009), an undescribed species of *Dothiorella* from *A. melifera* in South Africa (Van der Walt *et al.*, 2008), and *Do. casuarini* J. de Wet, Slippers & M.J. Wingf. from a *Casuarina* sp. in Australia

(De Wet *et al.*, 2009). However, very few Botryosphaeriaceae appear to be host specific when they are sampled widely. For example, *N. eucalyptorum* (Crous, H. Smith & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips and *N. eucalypticola* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips were thought to be specific to *Eucalyptus* spp. (Slippers *et al.*, 2004), but *N. eucalyptorum* was subsequently found on other Myrtaceae (Pérez *et al.*, 2009). Other species in Dothiorella have been reported from different hosts such as *Do. longicollis* Pavlic, Burgess & M.J. Wingf. described from both *Lysiphyllum cunninghamii* and a *Terminalia* sp. in Western Australia (Pavlic *et al.*, 2008), *Do. sarmentorum* from *Malus, Ulmus, Pyrus, Prunus, Menispermum* and almond, and *Do. iberica* from *Quercus* and *Malus* in Europe and USA (Inderbitzin *et al.*, 2010; Phillips *et al.*, 2005). A number of species in the Botryosphaeriaceae such as *L. theobromae, B. dothidea,* and *N. parvum* are also known to have wide host ranges (Slippers & Wingfield, 2007). Similarly on *Acacia*, of the 12 Botryosphaeriaceae species isolated by Van der Walt *et al.* (2008) from *Acacia*, three species (*B. dothidea* and undescribed species in *Spencermartinsia* and *Pheaobotryon*) were found on *A. karroo* and other *Acacia* species. It is thus expected that the species described here may also be found on other members of *Acacia* and perhaps even other tree genera and their complete host ranges of the new species described in this study are still unknown.

The two different areas sampled in this study revealed different Botryosphaeriaceae species. Only one of five species was found in Bloemfontein, while all five occurred in Pretoria. Van der Walt *et al.* (2008) found that geography influenced the structure of Botryosphaeriaceae diversity on *Acacia*. In that study, which included samples from diverse areas of southern Africa, only three of 12 species overlapped among the regions sampled, while the others were found at only one or two sites. Other studies on the Botryosphaeriaceae have shown similar patterns. For example, seven species Botryosphaeriaceae from *Adansonia gibbosa* sampled at five different sites in Western Australia (Pavlic *et al.*, 2008), and six Botryosphaeriaceae species from *Prunus dulcis* from seven different sites in California, USA (Inderbitzin *et al.*, 2010) revealed largely different species at the different sites. Thorough and repeated sampling targeting the various Botryosphaeriaceae species on *Acacia karroo* will, however, be needed to delimit geographic boundaries for the various species on this host.

Recent studies on the Botryosphaeriaceae from diverse native trees in South Africa (Begoude *et al.*, 2010; Mehl *et al.*, 2011; Pavlic *et al.*, 2007; Van der Walt *et al.*, 2008) have resulted in the discovery of a large number of Botryosphaeriaceae species in this region. We, therefore, expected that the rate of new species descriptions in the family would decrease with additional sampling. The discovery of five new species in this study from a previously sampled host, and from only a few samples and sites, indicates that this expectation is unfounded and may not be realistic until a greater diversity of hosts have been sampled in many more locations across the region. *A. karroo* might be an ideal host, given its wide incidence across the region, to target for sampling alongside other hosts that are more geographically isolated. Such future work would provide a baseline of data on the geographical and host influence on structuring diversity in this important family of latent tree pathogens.

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