

Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia

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Abstract: In this study seven new species of the Botryosphaeriaceae are described from baobab (*Adansonia gibbosa*) and surrounding endemic tree species growing in the Kimberley region of northwestern Australia. Members of the Botryosphaeriaceae were predominantly endophytes isolated from apparently healthy sapwood and bark of endemic trees; others were isolated from dying branches. Phylogenetic analyses of ITS and EF1- α sequence data revealed seven new species: *Dothiorella longicollis*, *Fusicoccum ramosum*, *Lasiodiplodia margaritacea*, *Neoscytalidium novaehollandiae*, *Pseudofusicoccum adansoniae*, *P. ardesiacum* and *P. kimberleyense*.

Key words: *Adansonia gibbosa*, biodiversity, *Botryosphaeria*, *Dothiorella*, *Fusicoccum*, *Lasiodiplodia*, *Neoscytalidium*, *Pseudofusicoccum*, systematics, taxonomy

INTRODUCTION

Only eight species of baobabs (*Adansonia* spp.) are known. *Adansonia gibbosa* is the only baobab species endemic to Australia and is restricted to the northwestern part of the country (Crisp et al 2004). *Adansonia digitata* has a wide natural distribution

throughout tropical parts of Africa and the six other species are found on Madagascar (Bowman 1997, Baum et al 1998). A recent biogeographical study on baobabs presented the intriguing view that the distribution of these unusual trees between Africa and Australia occurred after the division of Gondwana (Baum et al 1998). The same study revealed that *A. gibbosa* in Australia is more closely related to *A. digitata* from Africa than it is to species from Madagascar.

In this first study of fungi associated with *A. gibbosa* and surrounding endemic tree species in northwestern Australia members of the Botryosphaeriaceae were found as non sporulating endophytes in apparently healthy sapwood and bark of branches collected from all tree species sampled; they also were found sporulating and releasing conidia on dying branches of baobabs. Numerous studies have combined phenotype with DNA sequence analyses in defining genera and species in the Botryosphaeriaceae (Jacobs and Rehner 1998, Denman et al 2000, Zhou and Stanosz 2001, Slippers et al 2004, Phillips et al 2005). Crous et al (2006) summarized this work and represented several lineages in the Botryosphaeriaceae that were identified with generic names based on large subunit (LSU) sequence data, including *Botryosphaeria*, *Dothidotthia*, *Macrophomina*, *Neofusicoccum*, *Neoscytalidium*, *Pseudofusicoccum*, *Saccharata* and *Guignardia*. The identity and generic placement of the numerous species included in *Diplodia* and *Lasiodiplodia* were unclear in the study of Crous et al (2006), but they are clearly separated in ITS and EF1- α phylogenies (Burgess et al 2005, Phillips et al 2005, Damm et al 2007, Alves et al 2008).

In this study we describe seven new species of Botryosphaeriaceae associated with *A. gibbosa* and other native trees in the northwestern Australia. The new taxa are characterized and described based on ITS and EF1- α sequence data combined with anamorph morphology.

MATERIALS AND METHODS

Isolates.—Those used in this study were collected from *A. gibbosa* and surrounding native trees in northwestern Australia in Jun and Jul 2006 (TABLE I). Asymptomatic and dying twigs of *A. gibbosa* were collected from 26 locations approximately 20 km apart along Gibb River Road. At three locations asymptomatic twigs also were collected from eight other tree species. The other tree

TABLE I. Isolates included in the phylogenetic study

Culture no.	Other no. ¹	Identity	Host	Location ²	Genbank no. ³	
					ITS	EF1- α
CMW26145	MUCC520 / CBS122053	<i>Pseudofusicoccum adansoniae</i>	<i>Acacia synchronica</i>	WA, Tunnel Creek NP	EF585525	EF585569
CMW26148	MUCC521 / CBS122056	<i>P. adansoniae</i>	<i>Ficus opposita</i>	WA, Tunnel Creek NP	EF585524	EF295489
CMW26147	MUCC522 / CBS122055	<i>P. adansoniae</i>	<i>Adansonia gibbosa</i>	WA, Derby	EF585523	EF585571
CMW26146	MUCC533 / CBS122054	<i>P. adansoniae</i>	<i>Eucalyptus</i> sp.	WA, Tunnel Creek NP	EF585532	EF585570
CMW26161	CBS122061	<i>P. kimberleyense</i>	<i>F. opposita</i>	WA, Tunnel Creek NP	EU144059	EU144074
CMW26156	CBS122058	<i>P. kimberleyense</i>	<i>Ac. synchronica</i>	WA, Tunnel Creek NP	EU144057	EU144072
CMW26157	CBS122059	<i>P. kimberleyense</i>	<i>Eucalyptus</i> sp.	WA, Tunnel Creek NP	EU144056	EU144071
CMW26158	CBS122060	<i>P. kimberleyense</i>	<i>Ad. gibbosa</i>	WA, Tunnel Creek NP	EU144058	EU144073
CMW26155	CBS122063	<i>P. ardesiacum</i>	<i>Ad. gibbosa</i>	WA, Derby	EU144061	EU144076
CMW26159	CBS122062	<i>P. ardesiacum</i>	<i>Ad. gibbosa</i>	WA, Mt Hardman, Great North Hwy	EU144060	EU144075
CMW26160	CBS122064	<i>P. ardesiacum</i>	<i>Eucalyptus</i> sp.	WA, Tunnel Creek NP	EU144062	EU144077
CMW13434		<i>P. stromaticum</i>	<i>Eucalyptus</i> hybrid	Venezuela, Cojedes state	AY693974	AY693975
CMW13435		<i>P. stromaticum</i>	<i>Eucalyptus</i> hybrid	Venezuela, Cojedes state	DQ436935	DQ436936
CMW26162	CBS122519	<i>Lasiodiplodia margaritacea</i>	<i>Ad. gibbosa</i>	WA, Tunnel Creek Gorge	EU144050	EU144065
CMW26163	CBS122065	<i>L. margaritacea</i>	<i>Ad. gibbosa</i>	WA, Tunnel Creek Gorge	EU144051	EU144066
CMW10130		<i>L. theobromae</i>	<i>Vitex donniana</i>	Uganda	AY236951	AY236900
CMW9074		<i>L. theobromae</i>	<i>Pinus</i> sp.	Mexico	AY236952	AY236901
CMW14077	CBS115812	<i>L. gonubiensis</i>	<i>Syzygium cordatum</i>	South Africa, Gontubie	AY639595	DQ103566
WAC12538		<i>L. rubropurpurea</i>	<i>E. grandis</i>	Queensland, Tully	DQ103556	DQ103574
CMW13511	WAC12539	<i>L. venezuelensis</i>	<i>Ac. mangium</i>	Venezuela, Acarigua	DQ103547	DQ103568
CMW14691	WAC12533	<i>L. crassispora</i>	<i>Santalum album</i>	Western Australia, Kununurra	DQ103550	DQ103557
	STE-U5803 / CBS120832	<i>L. plurivora</i>	<i>Prunus salicina</i>	South Africa, Stellenbosch	EF445362	EF445395
	CBS304.79	<i>L. pseudotheobromae</i>	<i>Rosa</i> sp.	Netherlands	EF622079	EF622061
	CBS447.62	<i>L. pseudotheobromae</i>	<i>Citrus aurantium</i>	Suriname	EF622081	EF622060
	CBS495.78	<i>L. parva</i>	<i>Cassava</i> -field soil	Colombia	EF622085	EF622065
	CBS494.78	<i>L. parva</i>	<i>Cassava</i> -field soil	Colombia	EF622084	EF622064
ZS94-6		<i>Diplodia mutila</i>	<i>Malus pumila</i>	New Zealand	AF243407	AY236904
CMW7774		<i>Di. seriata</i>	<i>Ribes</i> sp.	USA, New York	AY236953	AY236902
KJ94-07		<i>Di. pinea</i>	<i>Pinus resinosa</i>	USA, Wisconsin	AF027758	AY624251
CMW26164	CBS122066	<i>Dothiorella longicollis</i>	<i>Terminalia</i> sp.	WA, Bell Gorge	EU144052	EU144067
CMW26165	CBS122067	<i>Do. longicollis</i>	<i>Lysiphyllum</i> <i>cunninghamii</i>	WA, Tunnel Creek NP	EU144053	EU144068
CMW26166	CBS122068	<i>Do. longicollis</i>	<i>L. cunninghamii</i>	WA, Tunnel Creek NP	EU144054	EU144069
CBS117008		<i>Do. viticola</i>	<i>Vitis vinifera</i>	Spain, Sant Sadurn d'Anoia	AY905557	AY905560

TABLE I. Continued

Culture no.	Other no. ¹	Identity	Host	Location ²	Genbank no. ³	
					ITS	EF1- α
CBS117010		<i>Do. viticola</i>	<i>V. vinifera</i>	Spain, Sant Esteve Sesrovires	<i>AY905558</i>	<i>AY905561</i>
CBS115041		<i>Do. iberica</i>	<i>Quercus ilex</i>	Spain, Aragon	<i>AY573202</i>	<i>AY573222</i>
CBS115035		<i>Do. iberica</i>	<i>Q. ilex</i>	Spain, Aragon	<i>AY573213</i>	<i>AY573228</i>
CBS115038		<i>Do. sarmentorum</i>	<i>M. pumila</i>	Netherlands, Delft	<i>AY573206</i>	<i>AY573223</i>
IMI63581		<i>Do. sarmentorum</i>	<i>Ulmus</i> sp.	England, Warwickshire	<i>AY573212</i>	<i>AY573235</i>
CMW26167	CBS122069	<i>Fusicoccum ramosum</i>	<i>E. camaldulensis</i>	WA, Bell Gorge	EU144055	EU144070
CMW991	ATCC58188	<i>B. dothidea</i>	<i>Prunus nigra</i>	New Zealand	<i>AF241175</i>	<i>AY236895</i>
CMW8000		<i>B. dothidea</i>	<i>Prunus</i> sp.	Switzerland, Crocifisso	<i>AY236949</i>	<i>AY236898</i>
ATCC22928		<i>B. corticis</i>	<i>Vaccinium</i> sp.	USA, North Carolina	<i>DQ299248</i>	EF614932
ATCC22927		<i>B. corticis</i>	<i>Vaccinium</i> sp.	USA, North Carolina	<i>DQ299247</i>	EF614931
MUCC531		<i>Macrophomina phaseolina</i>	<i>Sesbania formosa</i>	Western Australia, Kununurra	<i>EF585505</i>	<i>EF585560</i>
MUCC532		<i>M. phaseolina</i>	<i>S. formosa</i>	Western Australia, Kununurra	<i>EF585506</i>	<i>EF585561</i>
CMW6837		<i>Neofusicoccum australe</i>	<i>Acacia</i> sp.	New South West, Baternans Bay	<i>AY339262</i>	<i>AY339270</i>
CMW7054	CBS121.26	<i>N. ribis f. chromagena</i>	<i>Ribis rubrum</i>	U.S.A., New York	<i>AF236908</i>	<i>AY236879</i>
CMW9081	ICMP8003	<i>N. parvum</i>	<i>Populus nigra</i>	New Zealand	<i>AY236943</i>	<i>AY236888</i>
CMW26171	MUCC534 / CBS122072	<i>Neoscytalidium</i> <i>novaehollandiae</i>	<i>Ad. gibbosa</i>	WA, Gibb River Rd, 50 km E of Derby	EF585535	EF585581
CMW26168	MUCC535 / CBS122610	<i>N. novaehollandiae</i>	<i>Ac. synchronica</i>	WA, Gibb River Rd, near Meda	EF585536	EF585578
CMW26169	MUCC536 / CBS122070	<i>N. novaehollandiae</i>	<i>Grevillia agrifolia</i>	WA, Gibb River Rd, near Meda	EF585539	EF585579
CMW26170	MUCC537 / CBS122071	<i>N. novaehollandiae</i>	<i>Crotalaria</i> <i>medicaginea</i>	WA, Bell Gorge	EF585540	EF585580
CBS499.66		<i>Neoscytalidium dimidiatum</i>	<i>Mangifera indica</i>	Mali	<i>AY819727</i>	EU144063
CBS204.33		<i>N. dimidiatum</i>	<i>Prunus</i> sp.	Egypt	<i>AY819728</i>	EU144064
	MUCC684	<i>Guignardia</i> sp.	<i>Agonis flexuosa</i>	Western Australia, Yalgorup	<i>EU675682</i>	EU686573
	MUCC685	<i>Guignardia</i> sp.	<i>Ag. flexuosa</i>	Western Australia, Yalgorup	<i>EU675681</i>	EU686572

¹ Abbreviations of isolates and culture collections: CBS = Centraalbureau voor Schimmelcultures Utrecht, Netherlands; CMW = Forestry and Agricultural Biotechnology Institute, University of Pretoria South Africa; MUCC = Murdoch University Culture Collection, Perth, Australia; KJ = Jacobs and Rehner (1998); ATCC = American Type Culture Collection, Manassas, Virginia; ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI = CABI Bioscience, Egham, U.K; ZS = Zhou and Stanosz (2001); WAC = Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia.

² WA = Western Australia, NP = National park.

³ Sequence numbers in italics were obtained from the GenBank public database. All others were obtained in this study.

species were different at the three locations but included *Acacia synchronica*, *Crotalaria medicaginea*, *Eucalyptus camaldulensis*, an unidentified *Eucalyptus* sp., *Ficus opposita*, *Grevillia agrifolia*, *Lysiphyllum cunninghamii* and a *Terminalia* sp. (TABLE I). Isolations were made from visually healthy sapwood and bark collected from branches following Burgess et al (2006b). Collections also were made from pycnidia formed on dying branches. When pycnidia were found on dying branches masses of conidia were transferred directly to 2% malt extract agar (MEA) (Biolab, S.A.). Single-conidial cultures of all isolates in this study are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, and the Murdoch University Culture Collection (MUCC). A representative set of isolates also has been deposited in the collection of the Centraalbureau voor Schimmelfcultures (CBS), Utrecht, The Netherlands.

DNA sequence comparisons.—DNA was extracted from fungal mycelium from 7 d old single-conidial cultures as described by Burgess et al (2005). DNA was purified with the Ultrabind® DNA purification kit following the instructions given by the manufacturer (MO BIO Laboratories). Two gene regions were used for phylogenetic analyses. The internal transcribed spacer (ITS) region of the ribosomal RNA (rRNA) operon was amplified for all isolates using primers ITS-1F (Gardes and Bruns 1993) and ITS-4 (White et al 1990). For selected isolates a part of the elongation factor 1- α (EF1- α) gene was amplified with primers EF1-728F and EF1-986R (Carbon and Kohn 1999). The PCR reaction mixture, PCR conditions and visualization were as described by Pavlic et al (2004) except that 0.5 U of Taq polymerase (Biotech International, Needville, Texas) was used. PCR products were cleaned with the Ultrabind® DNA purification kit and sequenced with the BigDye terminator cycle sequencing kit (PE Applied Biosystems) in both directions, with the same primers used for the PCR reactions. Products were separated with an ABI 3730 48 capillary sequencer (Applied Biosystems, Foster City, California). Data were collected with ABI data collection software.

Sequence data for isolates of the unknown species were deposited in GenBank (TABLE I). Sequences of known species were obtained from GenBank, and the isolate code, identity and accession numbers for sequence data used are given in TreeBASE (<http://www.treebase.org/treebase/index.html>, accession number SN3768). Parsimony analysis was performed on individual datasets (individual trees are not illustrated) and on the combined dataset after partition homogeneity test (PHT) was performed in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2000) to determine whether sequence data from the ITS and EF-1 α gene regions were statistically congruent (Farris et al 1995, Huelsenbeck et al 1996). Noninformative characters were removed before analysis and characters were unweighted and unordered. The most parsimonious trees were obtained using heuristic searches with random stepwise addition in 100 replicates, with the tree bisection-reconnection branch-swapping option on and the steepest-

descent option off. MAXTREES were unlimited, branches of zero length were collapsed and all multiple equally parsimonious trees were saved. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indices) were determined (Hillis and Huelsenbeck 1992). Branch and branch node supports were determined with 1000 bootstrap replicates (Felsenstein 1985). The tree was rooted to a *Guignardia* sp.

Bayesian analysis was conducted on the same individual and combined datasets as that used in the parsimony analysis. First MrModeltest v.2.5 (Nylander 2004) was used to determine the best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v. 3.1 (Ronquist and Huelsenbeck 2003) applying a general time reversible (GTR) substitution model with gamma (G) and proportion of invariable site (I) parameters to accommodate variable rates across sites. Two independent runs of Markov chain Monte Carlo (MCMC) with four chains were run 1 000 000 generations. Trees were saved each 1000 generations, resulting in 10 001 trees. Burn-in was set at 50 001 generations (i.e. 51 trees), well after the likelihood values converged to stationery, leaving 9950 trees from which the consensus trees and posterior probabilities were calculated.

Morphological characteristics.—To induce sporulation, cultures were inoculated onto sterilized pine needles and/or eucalypt twigs placed on the surface of 2% water agar (WA) (Biolab, S.A.) and incubated at 25 C under near-UV light. To obtain single-conidial cultures, releasing conidia from pycnidia formed on pine needles and/or eucalypt twigs were transferred on WA and spread on the medium surface by sterilized streaking loop. Plates were incubated at 25 C under near-UV light for approximately 12 h and single germinating conidia were transferred on the MEA with sterilized needle and incubated under the same conditions. A single pycnidium was placed in a drop of lactoglycerol on a microscope slide and cut in pieces with a sterile medical needle before adding the cover slip. Fifty released conidia and 30 of pycnidia, conidiogenous cells and paraphyses were measured for each isolate, and the ranges and averages were computed. Measurements and digital images were made with an HRc Axiocam digital camera and accompanying Axiovision 3.1 software (Carl Zeiss Ltd., Munich, Germany). Drawings were prepared with a drawing tube and finalized with the method described by Barber and Keane (2007). Colony morphology and color were determined from cultures grown on MEA at 25 C in the dark. Colony colors (upper surface and reverse) were determined with the charts of Rayner (1970).

Growth rates at 5–35 C, at 5 C intervals were determined for cultures grown in the dark. To determine growth rate, mycelial plugs, 6 mm diam, were taken from the actively growing edges of 7 d old single-conidial cultures and transferred to the centers of MEA in 90 mm diam Petri dishes. Three replicate plates were used for each isolate at each temperature. Two perpendicular measurements were taken of the colony diameter daily until the mycelium of the fastest growing isolates had covered the plates.

RESULTS

DNA sequence comparisons.—The partition homogeneity test comparing the ITS and EF1- α datasets was significant ($P = 0.007$) indicating that the individual datasets were not congruent and produced trees with differing topology. These differences were not due to the relationships among species in a genus but instead were due to the relationship of the genera to each other. Thus when the data were combined support for the placement of species within a genus was high but the support for the deeper branches, indicating relationships between genera, was low. Similar discrepancies were found when comparing the phylogeny obtained from parsimony and Bayesian analyses. Thus the analyses for the individual ITS and EF1- α datasets are available from TreeBASE (SN3768), while the results emerging from the combined dataset are presented here.

The combined dataset consisted of 996 characters of which 546 were parsimony informative. The dataset contained significant phylogenetic signal compared to 1000 random trees ($P < 0.01$, $g1 = -0.43$). Heuristic searches resulted in two most parsimonious trees of 1566 steps (CI = 0.60, RI = 0.90) (FIG. 1, TreeBASE SN3768). In the Bayesian analysis the positions of the genera in relation to each other differed, but within each genus the topology was similar to the parsimony tree (TreeBASE SN3768). Eight clades were identified, each corresponding to a separate genus and each supported with bootstrap values of 100% and Bayesian probabilities of 1.00. These were Clade 1 (*Lasiodiplodia*), Clade 2 (*Diplodia*), Clade 3 (*Dothiorella*), Clade 4 (*Neofusicoccum*), Clade 5 (*Botryosphaeria*), Clade 6 (*Macrophomina*), Clade 7 (*Neoscytalidium*) and Clade 8 (*Pseudofusicoccum*). Isolates obtained in this study resided in Clades 1, 3, 5, 7 and 8.

Within the *Lasiodiplodia* clade two isolates were found to be distinct from the known species in this genus (FIG. 1). Three isolates, two from *Lysiphylum cunninghamii* and one from a *Terminalia* sp., formed a well supported lineage in the *Dothidothia/Dothiorella* clade (FIG. 1). Within the *Botryosphaeria* clade a single isolate from *Eucalyptus camaldulensis* was phylogenetically distinct from the two previously sequenced (for ITS and EF1- α) species, *B. dothidea* and *B. corticis* (FIG. 1). Four isolates obtained in the present study from *Acacia synchronica*, *Adansonia gibbosa*, *Crotalaria medicaginea* and *Grevillia agrifolia* formed a separate subclade within the *Neoscytalidium* clade (FIG. 1). Although support for this subclade was low, these isolates produce *Dichomera*-like synanamorphs that distinguish them from known *Neoscytalidium* species and are described in this study as a new

Neoscytalidium species. *Pseudofusicoccum* is currently monotypic for *P. stromaticum*. In this study three new species were found that phylogenetically reside in this genus (FIG. 1).

Morphology.—With exception of one isolate of *Pseudofusicoccum ardesiacum* (CMW 26160) all isolates of the Botryosphaeriaceae obtained from *A. gibbosa* and other native trees in northwestern Australia produced pycnidia on the pine needles and eucalypt twigs on WA within 2–3 wk. No ascomata were observed. Based on culture and conidial morphology, isolates were separated into seven species: three in *Pseudofusicoccum* and one species each in *Dothiorella*, *Fusicoccum*, *Lasiodiplodia* and *Neoscytalidium*. These species are described as follows.

TAXONOMY

Pseudofusicoccum adansoniae Pavlic, Burgess, M.J. Wingfield, sp. nov. MB512048 FIGS. 2, 3

Pycnidia subimmersa solitaria globosa papillata castanea, mycelio tecta, usque ad 500 μm diam. Cellulae conidiogenae holoblasticae laeves cylindricae hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter $22.5 \times 5.2 \mu\text{m}$, 4.3 plo longiora quam latiora, hyalinae, parietibus tenuibus, viscida strato persistenti mucitecta, laeves contentu tenue granulicis, raro subflexa vel irregularia, apicibus rotundatis, unicellularia, ante germinationem 1–2 septa formantia.

Pycnidia semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 500 μm diam. *Conidiogenous cells* holoblastic, smooth, cylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (9–) 10–15(–16) \times (1.5–)2–3(–3.5) μm (av. $12.7 \times 2.4 \mu\text{m}$). *Conidia* ellipsoid, occasionally slightly bent or irregularly shaped, (19–)21–24(–26) \times (3.5–)4.5–6(–6.5) μm (av. $22.5 \times 5.2 \mu\text{m}$, l/w 4.3), apices rounded, smooth with fine granular content, hyaline, thin-walled, covered with a persistent mucus layer, unicellular, forming 1 or 2 septa before germination. *Cultural characteristics.* Colonies initially white with moderately dense, appressed mycelial mat. Submerged mycelium turning gray olivaceous (21''''b) to olivaceous black (27''''m) from the middle of colony after 3–5 d and becoming dark slate-blue (39''''k) with age. Aerial mycelium slightly fluffy, becoming dense, cottony with age, sometimes remaining white to smoke gray (21''''f), usually turning pale olivaceous gray (21''''d) within 7 d and becoming olivaceous gray (21''''i) to iron gray 23''''k) with age. Colonies slightly irregular, occasionally radially striated with lobate edges and/or forming concentric, irregular circles. Conidiomata readily formed from the middle of colony within 7–

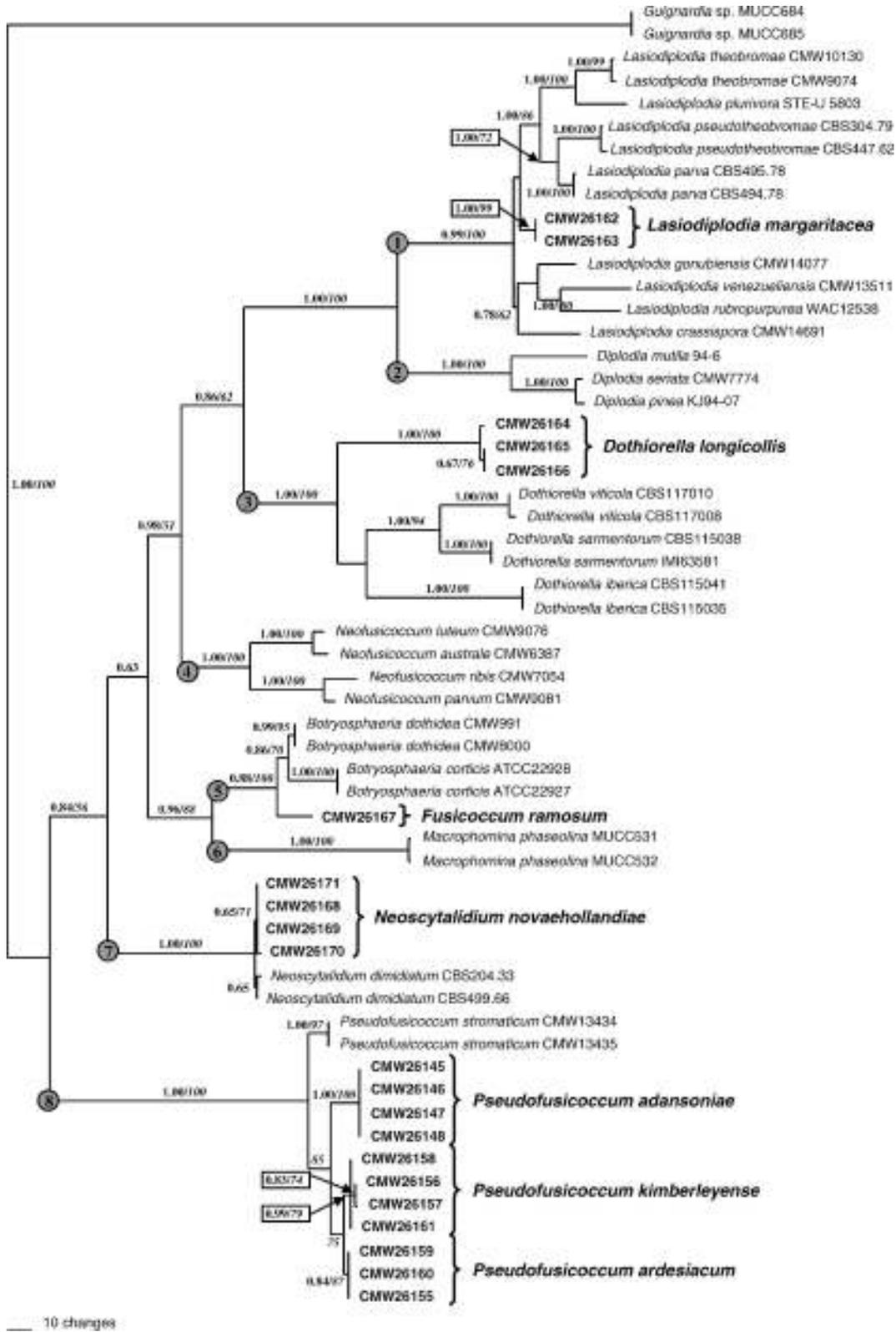


FIG. 1. One of two most parsimonious trees of 1566 steps resulting from the analysis of the combined ITS-EF1 α sequence data. Bootstrap values of the branch nodes are given in italics and the posterior probabilities resulting from Bayesian analysis are indicated in bold. Isolates from this study are in bold. Tree is rooted to a *Guignardia* sp. The strongly supported clades that represent different genera within the Botryosphaeriaceae according to Crous et al. (2006) are indicated by circles at the nodes.

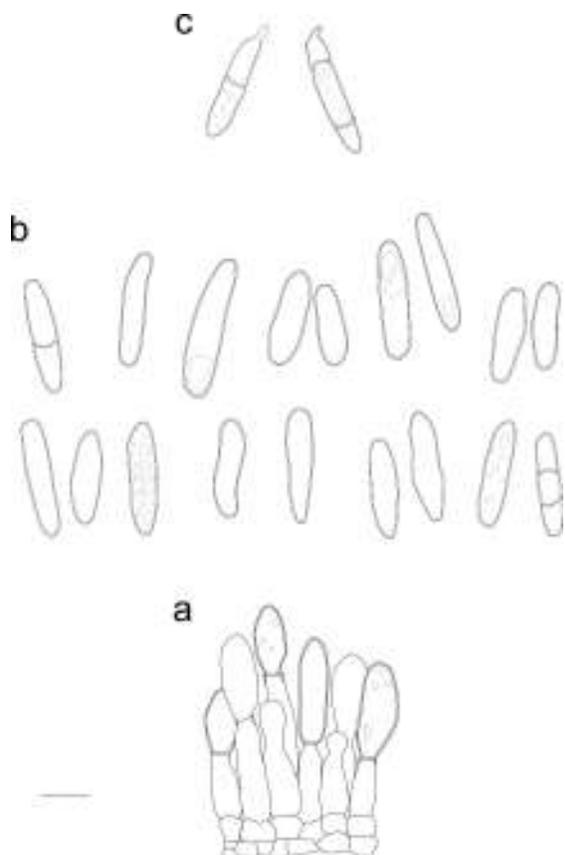


FIG. 2. *Pseudofusicoccum adansoniae*. a. Conidiogenous cells (CBS122056). b. Conidia (CBS122054, CBS122055, CBS122056). c. Germinating conidia (CBS122056). Bar = 10 μ m.

10 d, covering the entire surface of the colony and immersed in the medium (seen as a round black structures on the reverse side of Petri dishes) 14 d after incubation. Optimum growth at 30 C, covering the 90 mm diam Petri dish after 4 d in the dark.

Teleomorph. Not known.

Etymology. Refers to the host from which the type specimen was isolated.

Habitat. Dying branches of *Adansonia gibbosa* and asymptomatic branches of *Acacia synchronica*, *Eucalyptus* sp. and *Ficus opposita*.

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Derby (17°21'03.150S, 123°40'07.578E), on *Adansonia gibbosa*, Jul 2006, T.I. Burgess (PREM 59841, a dry culture ex CMW 26147 on pine needles; ex-type culture CMW 26147 = CBS 122055).

Additional specimens examined. See TABLE I.

Pseudofusicoccum kimberleyense Pavlic, Burgess, M.J. Wingfield, sp. nov. MB512049 FIGS. 4, 5
Pycnidia subimmersa solitaria globosa papillata castanea,

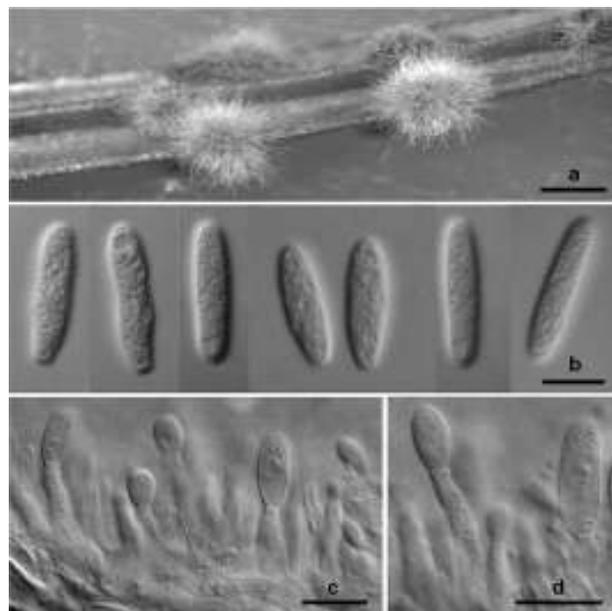


FIG. 3. *Pseudofusicoccum adansoniae*. a. Pycnidia formed in culture on pine needles (CBS122054). b. Aseptate conidia (CBS122055). c, d. Conidiogenous cells (CBS122053). Bars: a = 500 μ m, b–d = 10 μ m.

mycelio tecta, usque ad 500 μ m diam. Cellulae conidiogae holoblasticae laeves cylindricae vel subcylindricae hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter $30.7 \times 7.4 \mu$ m, 4.1 plo longiora quam latiora, hyalinae, parietibus tenuibus, viscida strato persistenti mucis tecta, laeves contentu tenue granulati, ellipsoidea, recta vel subfalcata, apicibus rotundatis, unicellularia, ante germinationem 1–4 septa formantia.

Pycnidia semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 500 μ m diam. *Conidiogenous cells* holoblastic, smooth, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (7–)8.5–11(–14) \times (2.5–)3–3.5(–4) μ m (av. 9.8 \times 3.3 μ m). *Conidia* ellipsoid, straight or slightly curved, (24–)28–33(–34) \times (6.5–)7–8(–8.5) μ m (av. 30.7 \times 7.4 μ m, l/w 4.1), apices rounded, smooth with fine granular content, hyaline, thin-walled, covered with a persistent mucus layer, unicellular, forming 1–4 septa before germination. *Cultural characteristics.* Colonies initially white, hyphae forming a moderately dense, appressed mycelial mat. Submerged mycelium citrine (21''''b) to gray olivaceous (21''''b) from the middle of colony after 3–5 d, becoming olivaceous black (27''''m) to black with age. Aerial mycelium slightly fluffy, becoming dense, cottony with age, smoke gray (21''''f) to pale olivaceous gray (21''''d). Colonies slightly irregular with sinuate edges. Optimum growth at 30 C, covering the 90 mm diam Petri dish after 4 d in the dark.

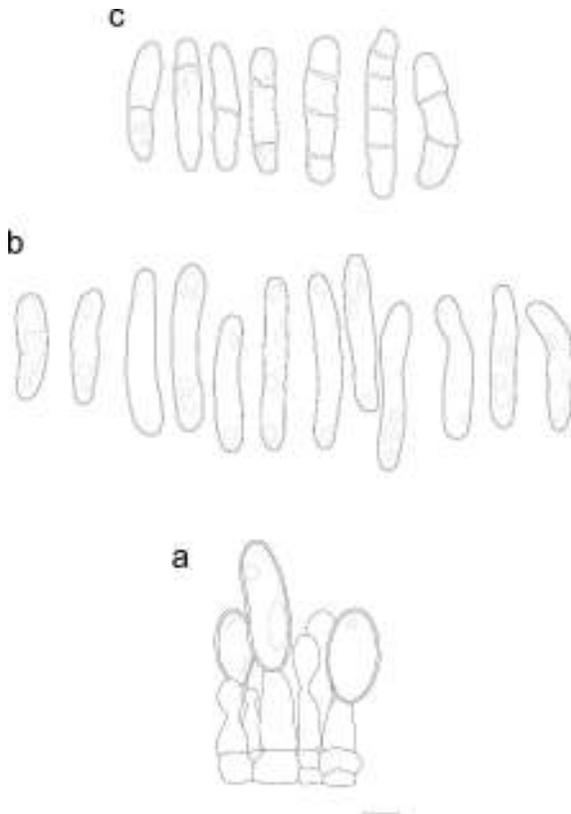


FIG. 4. *Pseudofusicoccum kimberleyense*. a. Conidiogenous cells (CBS122059). b. Aseptate conidia (CBS122058, CBS122060, CBS122061). c. 1–4-septate conidia (CBS122059, CBS122060, CBS122061). Bar = 10 μ m.

Teleomorph. Not known.

Etymology. Refers to Kimberley region, Western Australia, where the substratum was collected from which the fungus was isolated.

Habitat. Dying branches of *Adansonia gibbosa* and asymptomatic branches of *Acacia synchronica*, *Eucalyptus* sp. and *Ficus opposita*.

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Tunnel Creek National Park (17°54'33.342S, 125°17'01.686E), on *Acacia synchronica*, Jul 2006, T.I. Burgess (PREM 59842, a dry culture on pine needles ex CMW 26156; ex-type culture CMW 26156 = CBS 122058).

Additional specimens examined. See TABLE I.

Pseudofusicoccum ardesiacum Pavlic, Burgess, M.J. Wingfield, sp. nov. MB512051 FIGS. 6, 7

Pycnidia subimmersa solitaria globosa papillata castanea, mycelio tecta, usque ad 510 μ m diam. Cellulae conidiogenae holoblasticae laeves cylindricae vel subcylindricae hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter 25 \times 7.5 μ m, 3.3 plo longiora quam latiora, hyalinae, parietibus tenuibus, viscida strato persistenti mucis tecta, laeves contentu tenue granulati,

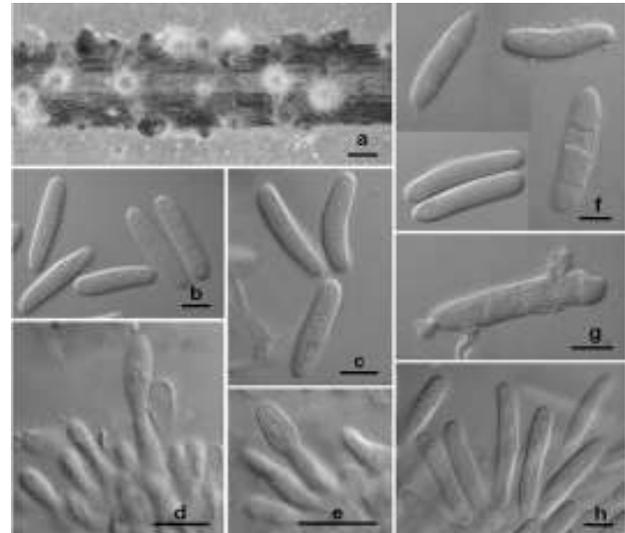


FIG. 5. *Pseudofusicoccum kimberleyense*. a. Pycnidia formed in culture on pine needles (CBS122058). b, c. Aseptate conidia (CBS122058). d, e. Conidiogenous cells (CBS122058). f, g. Aseptate and 2–4-septate conidia (CBS122060). h. Aseptate conidia (CBS122061). Bars: a = 500 μ m, b–h = 10 μ m.

ellipsoidea vel vergata, recta vel subflea, apicibus rotundatis, unicellularia, ante germinationem 1–3 septa formantia.

Pycnidia semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 510 μ m diam. *Conidiogenous cells* holoblastic, smooth, cylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–) 7.5–10(–11) \times (2.7–) 3–4(–4.3) μ m (av. 8.6 \times 3.5 μ m). *Conidia* ellipsoid to rod-shape, straight or slightly bent, (17.5–) 21–29(–32) \times (6.3–) 7–8(–9) μ m (av. 25 \times 7.5 μ m, l/w 3.3), apices rounded, smooth with fine granular content hyaline, thin-walled, covered with a persistent mucus layer, unicellular, forming 1–3 septa before germination. *Cultural characteristics.* Colonies initially white with sparse to moderately dense appressed mycelial mat. Submerged mycelium dark violet (59 m) to dark blue (47 m) (middle of the colony) and smoke gray (21''''f) to gray olivaceous (21''''b) toward edges within 3–5 d, becoming violaceous gray (59''''i) to slate blue (47''''k) with age. Aerial mycelium slightly fluffy, becoming dense, cottony with age, turning smoke gray (21''''f) to pale purplish gray (71''''d) in the middle of colony and smoke gray (21''''f) to gray olivaceous (21''''b) toward edges after 5–7 d, becoming lavender gray (45''''f) with age; occasional columns of aerial mycelium in the middle of colony, reaching the lid. Colonies slightly irregular with sinuate edges. Conidiomata readily formed and immersed in aerial mycelia on the entire colony surface within 7–10 d.

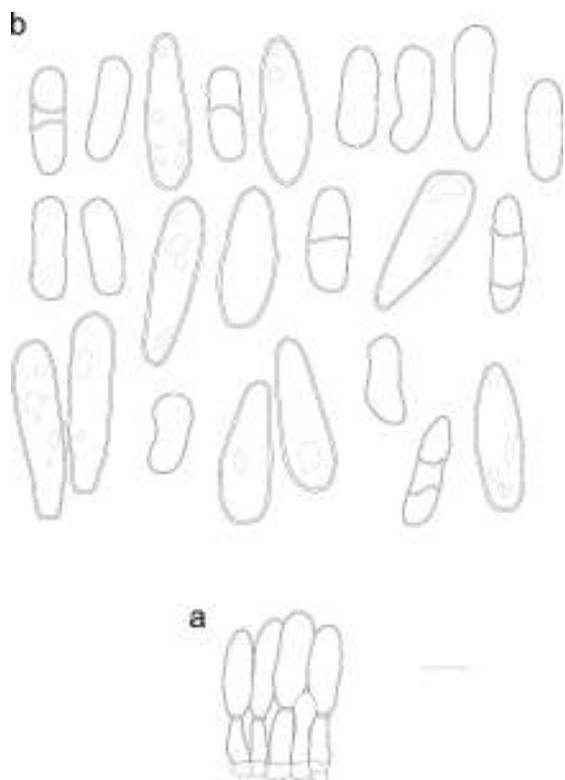


FIG. 6. *Pseudofusicoccum ardesiacum*. a. Conidiogenous cells (CBS122062). b. Conidia (CBS122062, CBS122063). Bar = 10 μ m.

Optimum growth at 30 C, covering the 90 mm diam Petri dish after 4 d in the dark.

Teleomorph. Not known.

Etymology. Refers to the slate blue-violet pigment found in cultures.

Habitat. Dying branches of *Adansonia gibbosa* and asymptomatic branches of *Eucalyptus* sp.

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Mount Hardman, Great Northern Highway (17°16'05.952S, 123°45'26.930E), on *Adansonia gibbosa*, Jul 2006, T.I. Burgess (PREM 59843, a dry culture ex CMW 26159 on pine needles; ex-type culture CMW 26159 = CBS 122062).

Additional specimens examined. See TABLE I.

Dothiorella longicollis Pavlic, Burgess, M.J. Wingfield, sp. nov. MB512053 FIGS. 8, 9

Pycnidia subimmersa plerumque solitaria, basi globosa usque ad 550 μ m diam, collis longis, interdum ramosis, usque ad 1.5 mm longis, e substrato orientia. Cellulae conidiogenae holoblasticae cylindricae vel subcylindricae hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter 20.4 \times 8.7 μ m, 2.3 plo longiora quam latiora, primo hyalina unicellularia, dum etiam ad cellulas conidiogenas affixa cinnamomeo- vel sepiaceo-brunnescentia, uniseptata, ovalia vel ovoidea apice rotundata basi truncata.

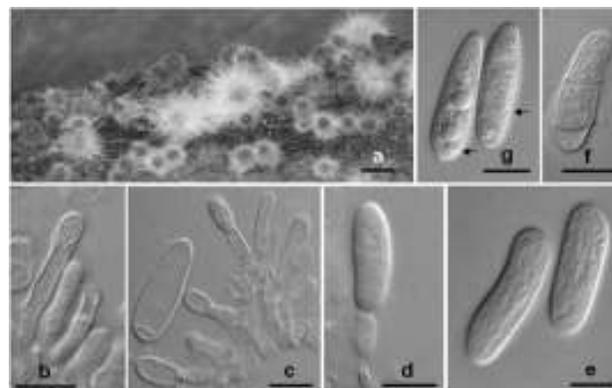


FIG. 7. *Pseudofusicoccum ardesiacum*. a. Pycnidia formed in culture on eucalypt twig (CBS122063). b, c. Conidiogenous cells (CBS122063). d. Conidium attached to conidiogenous cell (CBS122062). e. Aseptate conidia (CBS122062). f. Two-septate conidium (CBS122062). g. Aseptate conidia covered with mucus layer (indicated by arrow) (CBS122063). Bars: a = 500 μ m, b–g = 10 μ m.

Pycnidia semi-immersed, mostly solitary, with globose base (<550 μ m diam) and long neck (sometimes branching), up to 1.5 mm long, arising from the substrate. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (5–)6–8(–10) \times (2.5–)3–4(–4.5) μ m (av. 7.3 \times 3.4 μ m). *Conidia* oval to ovoid, (17–)19–22(–23) \times (7–)8–9.5(–10.5) μ m (av. 20.4 \times 8.7 μ m, l/w 2.3), apices rounded and truncate base, initially hyaline, unicellular, becoming cinnamon (13''') to sepia (13''k) and 1-septate while still attached to conidiogenous cells. *Cultural characteristics.* Colonies initially white to olivaceous-buff (21''d), becoming greenish-olivaceous (23''') to citrine (21 k) from the middle of colonies within 7 d, iron gray (23''''') (surface) and black (beneath) with age, with suppressed, moderately fluffy mycelium, edges smooth appearing sinuate as the colony darkens with age. Conidiomata readily formed from the middle of colony within 7–10 d, covering the entire surface of the colony and immersed in the medium (seen as round black structures on the reverse side of Petri dishes) 14 d after incubation. Optimum growth at 25 C, covering 90 mm diam Petri dish after 4 d in the dark.

Teleomorph. Not known.

Etymology. Refers to the fact that the pycnidia have long necks.

Habitat. Asymptomatic branches of *Lysiphylum cunninghamii* (Caesalpinaceae) and *Terminalia* sp. (Combretaceae).

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Tunnel

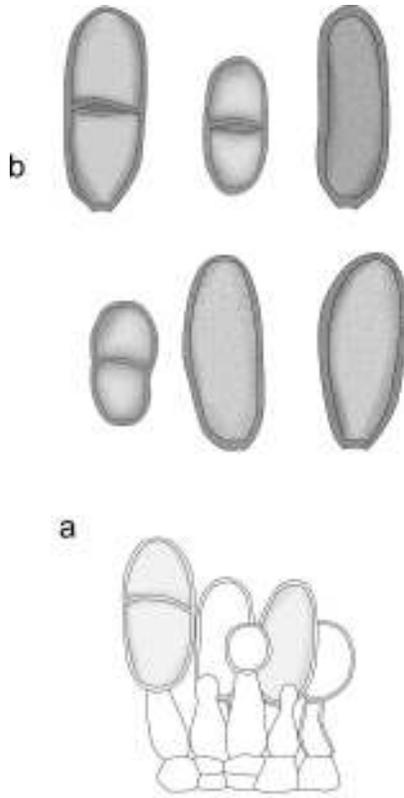


FIG. 8. *Dothiorella longicollis*. a. Conidiogenous cells (CBS122067, CBS122068). b. Conidia (CBS122067, CBS122068). Bar = 10 μ m.

Creek National Park (17°54'33.342S, 125°17'01.686E), on *Lysiphyllum cunninghamii*, Jul 2006, T.I. Burgess (PREM 59845, a dry culture ex CMW 26166 on pine needles; ex-type culture CMW 26166 = CBS 122068).

Additional specimens examined. See TABLE I.

Note. Cultures transferred onto WA with pine needles formed numerous pycnidia on the surface and immersed in the medium. *Dothiorella longicollis* conforms well to morphological concept of the genus proposed by Phillips et al (2005).

Lasiodiplodia margaritacea Pavlic, Burgess, M.J. Wingfield, sp. nov. MB512052 FIGS. 10, 11

Pycnidia subimmersa solitaria globosa papillata nigra mycelio tecta, usque ad 520 μ m diam. Paraphyses cylindricae, 1–2-septatae, hyalinae. Cellulae conidiogenae holoblasticae, cylindricae vel subcylindricae, hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter 15.3 \times 11.4 μ m, 1.3 plo longiora quam latiora primo unicellularia, hyalina globosa subglobosa vel obovoidea, parietibus crassis, contentu granuloso, cinnamomeo- vel sepiaceo-brunnescentia, cum maturitate 1-septata longitudinaliter striata.

Pycnidia semi-immersed, solitary, globose, papillate, black, covered by hyphal hairs, up to 520 μ m diam. *Paraphyses* cylindrical, 1–2-septate, hyaline,

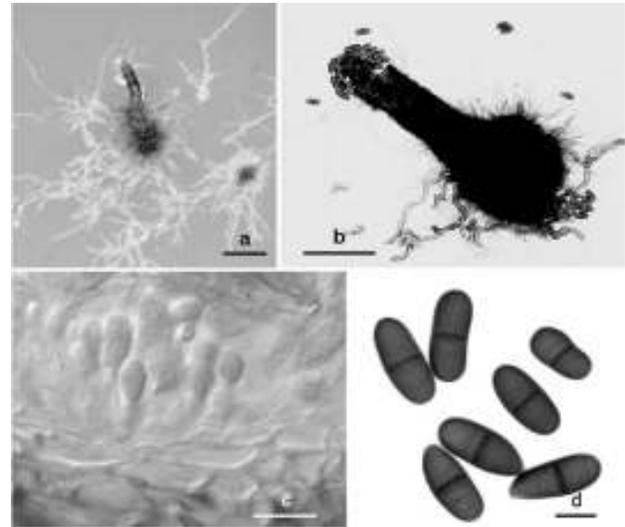


FIG. 9. *Dothiorella longicollis*. a. Pycnidia formed in culture on water agar (CBS122068). b. Pycnidium formed in culture releasing dark 1-septate conidia (CBS122068). c. Cross section through a pycnidium showing outer layers of dark brown cells and inner layers of hyaline cells with conidiogenous cells arising from the pycnidial wall (CBS122067). d. Dark 1-septate conidia (CBS122067). Bars: a = 500 μ m, b–d = 10 μ m.

(19–)28–46(–54) \times (1.5–)2–2.5(–3) μ m (av. 37.1 \times 2.2 μ m), formed among conidiogenous cells. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–)10–11(–19.5) \times (2–)3–4(–4.5) μ m (av. 10.3 \times 3.3 μ m). *Conidia* globose to subglobose to obovoid, (12–)14–17(–19) \times (10–)11–12(–12.5) μ m (av. 15.3 \times 11.4 μ m, l/w 1.3), with granular content, thick-walled (1–2 μ m), initially unicellular, hyaline, becoming cinnamon (13'') to sepia (13''k), forming one septum and longitudinal striations with maturation. *Cultural characteristics.* Colonies initially white to smoke gray (21''''f) with woolly aerial mycelium, becoming pale olivaceous gray (21''''d) within 5–7 d, olivaceous gray (21''''i) to iron gray (23''''k) with age, margins regular. Submerged mycelium dense, reverse gray olivaceous (21''''b) to olivaceous black (27''''m) after 7 d, becoming black with age. Optimum growth at 30 C, covering the 90 mm diam Petri dish after 3 d in the dark.

Teleomorph. Not known.

Etymology. The name refers to the conidia that have a pearl-like appearance

Habitat. Asymptomatic branches of *Adansonia gibbosa*.

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Tunnel Creek Gorge (17°36'22.884S, 125°108'46.056E), on *Adan-*

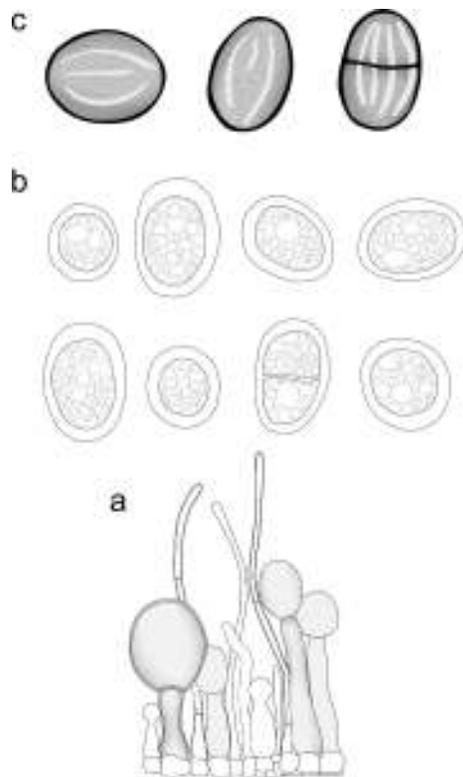


FIG. 10. *Lasiodiplodia margaritacea*. a. Conidiogenous cells and paraphyses. b. Immature conidia. c. Mature conidia. (a–c CBS122519). Bar = 10 μ m.

sonia gibbosa, Jul 2006, T.I. Burgess (PREM 59844, a dry culture ex CMW 26162 on pine needles; ex-type culture CMW 26162 = CBS 122519).

Additional specimens examined. See TABLE I.

Notes. Isolates of *L. margaritacea* clustered with other *Lasiodiplodia* species with high bootstrap support (100%). The septate conidia with striations that darken with age, as well as paraphyses, are typical of the genus (Punithalingham 1976, Pavlic et al 2004, Burgess et al 2006a). However the smaller, subglobose conidia clearly distinguish this species from previously described species (Punithalingham 1976, Pavlic et al 2004, Burgess et al 2006a, Damm et al 2007, Alves et al 2008).

Fusicoccum ramosum Pavlic, Burgess, M.J. Wingfield, sp. nov. MB512054 FIGS. 12, 13

Pycnidia subimmersa solitaria globosa papillata castanea, mycelio tecta, usque ad 510 μ m diam, interdum collis ad 1.7 mm longis, e substrato orientia. Cellulae conidiogenae holoblasticae, cylindricae vel subcylindricae, hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidiophorae laeves cylindricae septatae usque ad 2 μ m latae 50 μ m longae, simplices vel ramosae. Conidia mediocriter $13.4 \times 5.7 \mu$ m, 2.3 plo longiora quam latiora, hyalinae, parietibus tenuibus vel subcrassis, laeves contentu tenue granulati, fusiformia ellipsoidea vel ovalia apicibus rotundatis basibus truncatis, unicellularia vel 1-septata.

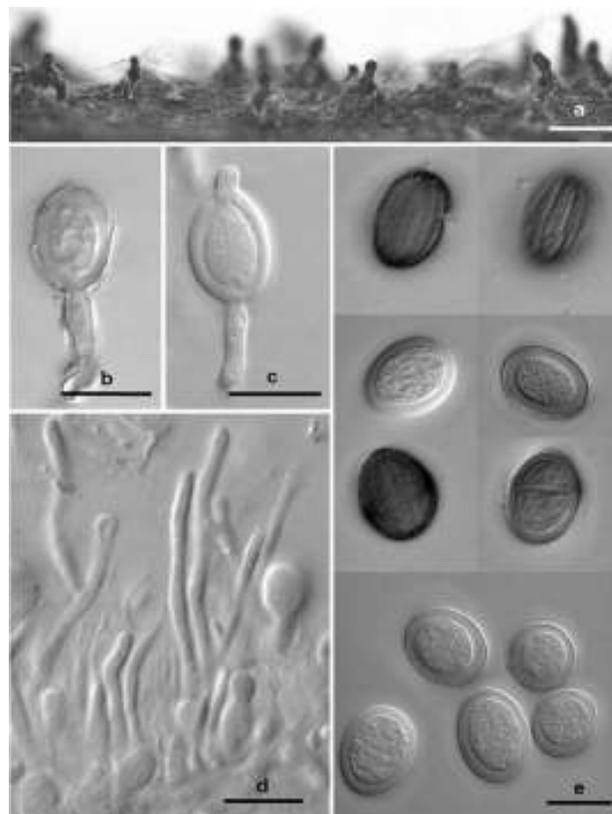


FIG. 11. *Lasiodiplodia margaritacea*. a. Pycnidia emerging through the eucalypt bark in culture. b. Conidium attached to conidiogenous cell. c. Germinating conidium. d. Paraphyses. e. Conidia in various stages of development, including young, hyaline, aseptate conidia, unicellular conidia with developing pigmentation with and without septation, mature striate conidia. (a–e CBS122519). Bars: a = 500 μ m, b–e = 10 μ m.

Pycnidia semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 510 μ m diam, sometimes with a neck to 1.7 mm long, arising from the substrate. *Conidiogenous cells* smooth, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, $(6-7.5-10(-11) \times (2-2.3(-3.5) \mu$ m (av. $8.7 \times 2.5 \mu$ m). *Conidiophores* smooth, cylindrical, septate, up to 2 μ m wide and 50 μ m long, simple or branching. *Conidia* fusiform to ellipsoid to oval, $(11-12-15(-16) \times (4.7-5-6(-7) \mu$ m (av. $13.4 \times 5.7 \mu$ m, 1/w 2.3), apices rounded or round at apex and truncate at base, smooth with fine granular contents, hyaline, wall thin to slightly thickened, unicellular or 1-septate. *Cultural characteristics.* Colonies initially white turning gray olivaceous (21''''b) from the middle of colonies within 5–7 d, with appressed mycelial mat and white moderately dense, cottony aerial mycelium toward the edge of colony, becoming smoke gray (21''''f) to olivaceous gray

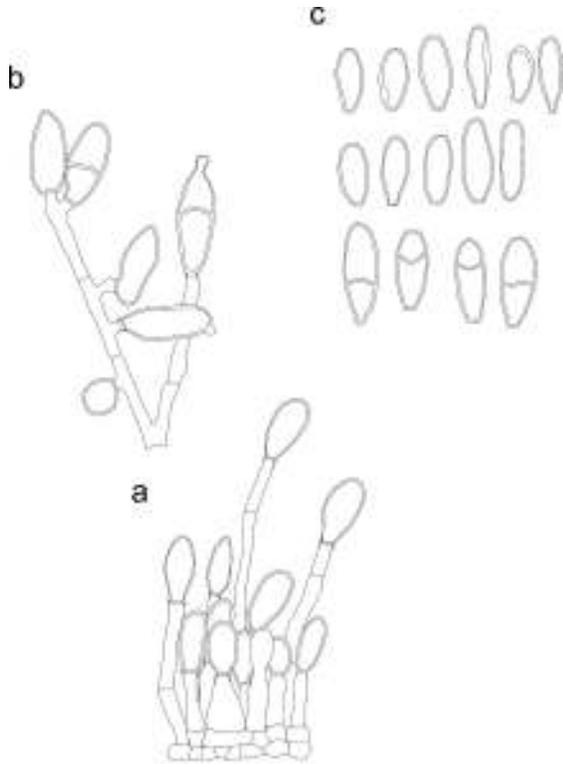


FIG. 12. *Fusicoccum ramosum*. a. Conidiogenous cells and conidiophores. b. Branching conidiophores. c. Conidia. (a–c CBS122069). Bar = 10 μ m.

(21''''i) (surface) and iron gray (23''''k) (beneath) within 10–14 d. Optimum growth at 25 C, covering the 90 mm diam Petri dish after 4 d in the dark.

Teleomorph. Not known.

Etymology. Name refers to the branched conidiophores of this species.

Habitat. Asymptomatic branches of *Eucalyptus camaldulensis*.

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Bell Gorge (17°00'58.584S, 125°13'47.866E), on *Eucalyptus camaldulensis*, Jul 2006, T.I. Burgess (PREM 59846, a dry culture ex CMW 26167 on pine needles; ex-type culture CMW 26167 = CBS 12206).

Notes. The only known culture of “*Botryosphaeria*”, anamorph *Fusicoccum ramosum*, is distinguished from other species in the genus by its long, simple or branching conidiophores. Its conidia develop a single septum before germinating, as is typical of *Botryosphaeria* (Slippers et al 2004). It did not produce a *Dichomera* synanamorph, which is reported for type species *Botryosphaeria dothidea* (Barber et al 2005). The conidia of *Fusicoccum ramosum* are significantly shorter than those of known species in this genus.

Neoscytalidium novaehollandiae Pavlic, Burgess, M.J. Winfield, sp. nov. MB512103 FIGS. 14, 15

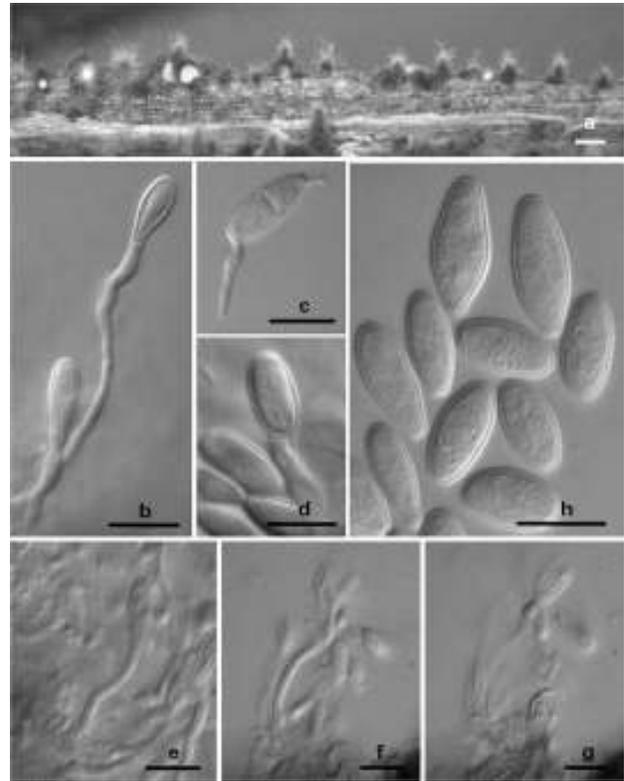


FIG. 13. *Fusicoccum ramosum*. a. Pycnidia emerging through the eucalypt bark in culture releasing white masses of conidia. b. Conidiophores with attached conidia. c. Germinating 1-septate conidium. d. Conidium attached to conidiogenous cell. e. Conidiophores arising from the pycnidial wall. f, g. Conidiophore with attached conidium, at two different focuses. h. Aseptate conidia. (a–h CBS122069). Bars: a = 500 μ m, b–h = 10 μ m.

Pycnidia ad dimidium immersa vel superficiales, solitaria vel in stromata multilocularia, nigra, cum basim globosa, diametrus usque ad 300 μ m, collis usque ad 600 μ m longis. Cellulae conidiogenae holoblasticae, cylindricae vel subcylindricae, hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia (1) mediocriter 11.5 \times 4.4 μ m, 2.6 plo longiora quam latiora, apices rotundati, primo hyalina, evadentes cinnamomeo- vel sepiaceo-brunnescentia cum maturitate, sive ellipsoidea vel ovoidea et 0–2-septata cum maturitate; (2) mediocriter 10.6 \times 6.9 μ m, 1.5 plo longiora quam latiora, primo hyaline, evadentes cinnamomeo- vel sepiaceo-brunnescentia cum maturitate, sive in forma variabilia, irregularia, globosa, subglobosa vel obpyriformia, cum septis muriformibus, Arthroconidia catenulata in mycelio aereo, mediocriter 6.5 \times 4 μ m, 1.6 plo longiora quam latiora, pulveriformia, disarticulantia, cylindrica, oblonga, obtusa vel doliiformia, crasse tunicata, primo hyalina et unicellularia, cinnamomeo- vel sepiaceo-brunnescentia et 0–1-septata cum maturitate.

Pycnidia semi-immersed or superficial, solitary or in multilocular stromata, black, with globose base, up to 300 μ m diam and long neck, up to 600 μ m long. *Conidiogenous cells* holoblastic, cylindrical to subcy-

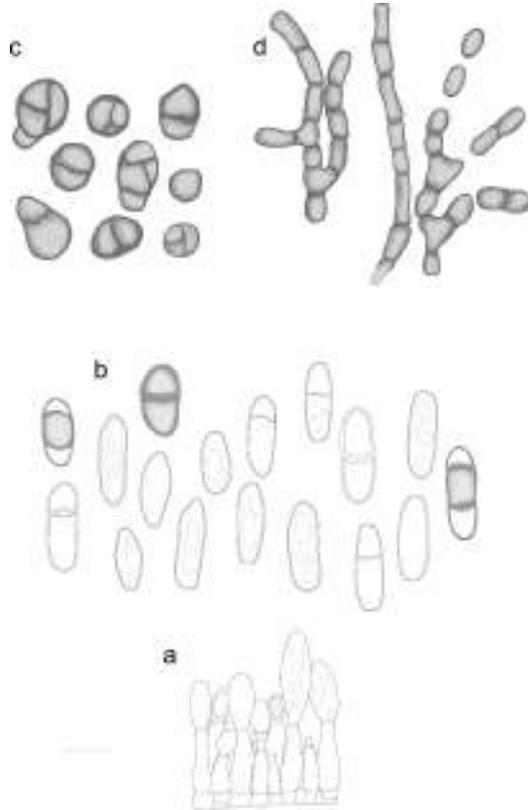


FIG. 14. *Neoscytalidium novaehollandiae*. a. Conidiogenous cells (CBS122610). b. Conidia. c. Muriform conidia. d. Chains of arthroconidia. (b–d CBS122071). Bar = 10 µm.

lindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–)7–10(–11) × (2–)2–3(–4) µm (av. 8.6 × 2.5 µm). Conidia of two distinct types: (i) ellipsoidal to oval, (8–)10.5–12.5(–14) × (3–)4–5(–5) µm (av. 11.5 × 4.4 µm, l/w 2.6), apices rounded, initially hyaline, unicellular, becoming cinnamon (13'') to sepia (13''k), and 0–1-septate or 2-septate with darker central cell; (ii) variable in shape, globose, subglobose to obpyriform with muriform septa, (8–)8.5–12.5(–15.5) × (5–)5.5–7.5(–8) µm (av. 10.6 × 6.9 µm, l/w 1.5), initially hyaline becoming cinnamon (13'') to sepia (13''k). Aerial mycelium forms chains of arthroconidia, (5–)5.5–7.5(–9.5) × (3–)3.5–4.5(–5) µm (av. 6.5 × 4 µm, l/w 1.6), unicellular, powdery to the touch, disarticulating, cylindrical, oblong to obtuse to doliiform, thick-walled, initially hyaline becoming becoming cinnamon (13'') to sepia (13''k) and 0–1-septate. Cultural characteristics. Colonies initially white to olivaceous-buff (21''d), becoming greenish-olivaceous (23''') to citrine (21 k) from the middle of colonies within 7 d, and black (surface and beneath) with age, with suppressed, moderately fluffy mycelium, edges smooth.

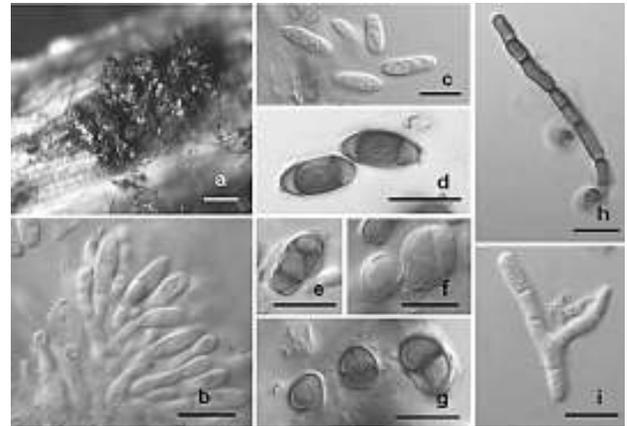


FIG. 15. *Neoscytalidium novaehollandiae*. a. Pycnidia emerging from a pine needle in culture. b. Conidiogenous cells (CBS122610). c. Hyaline aseptate conidia. d. Two-septate dark conidia. e–g. Muriform conidia. h, i. Chains of arthroconidia. (a, c–i CBS122071). Bars: a = 500 µm, b–i = 10 µm.

Optimum growth at 35 C, covering the 90 mm diam Petri dish after 3 d in the dark.

Teleomorph. Not known.

Etymology. Name refers to original Dutch name for Western Australia, where the substratum was collected from which the fungus was isolated.

Habitat. Asymptomatic branches (sapwood) of *Acacia synchronica*, *Adansonia gibbosa*, *Crotalaria medicaginea* and *Grevillia agrifolia*.

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Bell Gorge (17°00'58.584S, 125°13'47.866E), on *Crotalaria medicaginea*, Jul 2006, T.I. Burgess (PREM 60069, a dry culture ex CMW 26170 on pine needles; ex-type culture CMW 26170 = CBS 122071).

Additional specimens examined. See TABLE I.

Note. Isolates of *Neoscytalidium novaehollandiae* are similar in morphological characteristics to those of the type specimen *N. dimidiatum* (Punithalingam and Waterston 1970, Crous et al 2006). However isolates obtained in this study produce muriform, *Dichomera*-like conidia that distinguish this species from known *Neoscytalidium* spp.

KEY TO PSEUDOFUSICOCOCUM SPECIES

1. Blue-violet pigment in cultures visible after 3–5 d; conidia averaging 25 µm long, l/w 3.3, aseptate, forming 1–3 septa before germination . . . *P. ardesiacum*
1. Blue-violet pigment absent in cultures 2
2. Conidia on average >30 µm long. *P. kimberleyense*
2. Conidia on average <25 µm long. 3
3. Conidia aseptate, l/w 4 *P. stromaticum*
3. Conidia aseptate, forming 1 or 2 septa before germination, l/w 4.3 *P. adansoniae*

DISCUSSION

Seven new species of Botryosphaeriaceae were isolated from endemic trees in Western Australia. Combined ITS and EF1- α sequence data distributed these isolates among genera *Botryosphaeria*, *Dothiorella*, *Lasiodiplodia*, *Neoscytalidium* and *Pseudofusicoccum*. Teleomorphs were not observed for any of the species identified in this study.

Three of the seven new fungi are species of *Pseudofusicoccum*, a genus previously monotypic for *P. stromaticum* (Crous et al 2006, Mohali et al 2006). *Pseudofusicoccum* is separated from *Fusicoccum* by the presence of persistent mucous sheaths surrounding the conidia (Crous et al 2006). *Pseudofusicoccum stromaticum* was described on non-native *Acacia* and *Eucalyptus* spp. in Venezuela (Mohali et al 2006). Strains of *P. adansoniae* and *P. kimberleyense* described in this study were obtained from four unrelated hosts (*Acacia* sp., *Eucalyptus* sp., *Ficus* sp. and *A. gibbosa*) residing in four families all native to Western Australia. Isolates of *P. ardesiacum* were obtained from two of these native hosts, *Eucalyptus* and *A. gibbosa*. The fact that all *Pseudofusicoccum* spp. occurred on native hosts in a relatively undisturbed area of Australia or in the case of *P. stromaticum* on Australian plants suggests that the species are most likely native to the country.

Isolates of *P. adansoniae* came from different hosts but were morphologically uniform. This is in contrast to isolates of *P. kimberleyense*, which displayed differences in conidial morphology and variation in DNA sequences in both gene regions analyzed. These differences could indicate that *P. kimberleyense* comprises more than one species. *Pseudofusicoccum ardesiacum* was easily distinguished from other species in the genus by its smaller conidia and the distinct slate blue-violet pigment that it produces in culture.

Two species with dark conidia were identified in this study. Based on phylogenetic analyses and phenotype, they have been placed in *Lasiodiplodia* and *Dothiorella*. *Lasiodiplodia margaritacea* was identified only from dying branches of *A. gibbosa*. This was interesting because high numbers of dead and dying baobabs (*A. digitata*) have been reported in southern Africa, particularly in Zimbabwe (Anonymous 1991, Pearce et al 1994). The symptoms identified on the trees in Zimbabwe originally were reported as "sooty bark disease" caused by species of sooty mold fungi (Calvert 1989, Anonymous 1991). However Pearce et al (1994) reported that the "sooty" baobabs were dying due to drought, related to climatic change, rather than being caused by fungal pathogens. A recent study on diseases of baobabs in South Africa, showing symptoms of die-back and death of branches

followed by sap exudation, revealed that *Lasiodiplodia theobromae* was the most abundant fungus present (Roux 2002). This fungus is a well known latent, stress-associated pathogen on more than 500 hosts worldwide (Punithalingam 1976) and as such could be involved in the decline of baobab trees in African countries (Roux 2002). Because *Lasiodiplodia margaritacea* was found only on *A. gibbosa* that shows die-back symptoms this fungus could be pathogenic to this tree.

Dothiorella longicollis is another species with dark conidia described in this study. This species is morphologically similar to the other species with *Dothiorella* anamorphs, *D. iberica*, *D. sarmentorum* and *D. viticola* (Luque et al 2005, Phillips et al 2005). Other than the pycnidia with long necks, which are a distinct feature of *D. longicollis*, other morphological characteristics, such as conidial shape and size, overlap among these species and cannot be used to separate them with confidence. However their distinction is well supported in the ITS and EF1- α phylogenies. *Dothiorella longicollis* occurred as an endophyte in asymptomatic branches of two unrelated hosts, *Lysiphylum cunninghamii* (Caesalpiniaceae) and a *Terminalia* sp. (Combretaceae) endemic to Western Australia and nothing is known regarding its ecology.

A number of isolates obtained from asymptomatic branches on different hosts, including *Acacia*, *Adansonia*, *Crotalaria* and *Grevillia*, were identified as *Neoscytalidium novaehollandiae*. *Neoscytalidium*, with *N. dimidiatum* as a type, accommodates species with *Scytalidium*-like synanamorphs (Crous et al 2006). These are characterized by conidia held in arthric chains in the aerial mycelium. In addition to arthroconidia the cultures produce *Fusicoccum*-like conidia in pycnidia. Isolates of *N. novaehollandiae* identified in this study produce a *Dichomera*-like synanamorph, which is not known for other species in this genus. *Dichomera*-like synanamorphs recently were described for *Botryosphaeria dothidea*, *Neofusicoccum parvum*, *N. ribis* and *N. australe* (Barber et al 2005), however this is the first time that a *Dichomera*-like synanamorph has been found in *Neoscytalidium*. *Neoscytalidium dimidiatum* has been isolated from different substrates including plant tissues, soil, human skin and nails, and is known as a plant pathogen (Punithalingam and Waterston 1970, Crous et al 2006). The isolates examined in this study were collected as endophytes from plant tissues, and they seem unlikely to be important pathogens. *Fusicoccum ramosum* was isolated as endophyte from asymptomatic twigs of *Eucalyptus camaldulensis*.

Numerous species of Botryosphaeriaceae with "*Fusicoccum*" anamorphs identified from *Eucalyptus*

now have been placed in a new genus *Neofusicoccum* (Crous et al 2006). *Neofusicoccum* spp. are the most common endophytes and latent pathogens of *Eucalyptus* (Burgess et al 2006b, Slippers and Wingfield 2007), however no *Neofusicoccum* spp. were isolated from *Eucalyptus* in this study.

Species of Botryosphaeriaceae are well known as endophytes and latent, opportunistic canker and die-back pathogens on numerous woody hosts worldwide (von Arx 1987, Slippers and Wingfield 2007, de Wet et al 2008). However this is the first detailed study to consider these fungi on *Adansonia gibbosa* and other endemic trees in Western Australia, including *Acacia synchronica*, *Crotalaria medicaginea*, *Eucalyptus camaldulensis*, *Eucalyptus* sp., *Ficus opposita*, *Grevillia agrifolia*, *Lysiphyllum cunninghamii* and *Terminalia* sp. The seven new species emerging from this study, of which five were recorded on *A. gibbosa*, reflect a lack of knowledge regarding the fungi on *A. gibbosa* and of the Botryosphaeriaceae on native plants in this region. The role of these fungi in the ecology of the trees from which they were collected will be considered in future studies.

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