Chapter 5

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

---

Published as: Pavlic D, Wingfield MJ, Barber P, Slippers B, Hardy GEStJ, Burgess TI. 2008. Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. Mycologia 100:851–866.

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 predominant endophytes isolated from apparently healthy sapwood and bark of endemic trees; others were isolated from dying branches. Phylogenetic analyses of ITS and EF-1α sequence data revealed seven new species: *Dothiorella longicollis*, *Fusicoccum ramosum Lasiodiplodia margaritacea*, *Neoscytalidium novaehollandiae*, *Pseudofusicoccum adansoniae*, *P. ardesiacum* and *P. kimberleyense*.

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 were also 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) summarised this work and represented several lineages in the Botryosphaeriaceae that were identified with generic names based on large sub-unit (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 EF-1α 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 characterised and described based on ITS and EF-1α sequence data combined with anamorph morphology.

MATERIALS AND METHODS

Isolates

Isolates used in this study were collected from *A. gibbosa* and surrounding native tree in northwestern Australia in June and July of 2006 (TABLE I). Asymptomatic and dying twigs of *A. gibbosa* were collected from 26 locations approximately 20 km apart along the Gibb

River Road. At three locations asymptomatic twigs were also collected from eight other tree species. The other tree 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 were also made from pycnidia formed on dying branches. When pycnidia were found on dying branches, masses of conidia were directly transferred to 2 % malt extract agar (MEA) (Biolab, S.A.). Single-conidial cultures of all isolates used 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 has also been deposited in the collection of the Centraalbureau voor Schimmelcultures (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 using 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 1α (EF-1α) gene was amplified using 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, using 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 data set after partition homogeneity tests (PHT) was performed in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2000) to determine whether sequence data from the ITS and EF-1α gene regions were statistically congruent (Farris et al 1995, Huelsenbeck et al 1996). Non-informative characters were removed prior to 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 using 1000 bootstrap replicates (Felsenstein 1985). The tree is rooted to a *Guignardia* sp.

Bayesian analysis was conducted on the same individual and combined dataset 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) using 4 chains were run over 1 000 000 generations. Trees were saved each 1000 generation, 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 ºC 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 sterilised streaking loop. Plates were incubated at 25 ºC under near-UV light for approximately 12 h and single germinating conidia were transferred on the MEA, using sterilised 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 using 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 using 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 using the method described by Barber and Keane (2007). Colony morphology and color were determined from cultures grown on MEA at 25 ºC in the dark. Colony colors (upper surface and reverse) were determined by comparison to the color charts of Rayner (1970).

Growth rates at temperatures ranging from 5 to 35 ºC, at 5 ºC intervals were determined from 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 EF-1α data sets was significant (P = 0.007) indicating that the individual data sets were not congruent and produced trees with differing topology. These differences were not due to the relationships among species in a genus, but rather 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 EF-1α 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 data set contained significant phylogenetic signal compared to 1000 random trees (P < 0.01, g1 = -0.43). Heuristic searches resulted in 2 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 *Lysiphyllum cunninghamii* and one from a *Terminalia* sp., formed a well-supported lineage in the *Dothidotthia/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 EF-1α) 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 sub-clade within the *Neoscytalidium* clade (FIG. 1). Although support for this sub-clade 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* (CMW26160), all the isolates of the Botryosphaeriaceae obtained from *A. gibbosa* and other native trees in northwestern Australia produced pycnidia on the pine needles and eucalyptus twigs on WA within two to three weeks. 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 diametro. Cellulae conidiogenae holoblasticae laeves cylindricae hyalinae, conidio

primo holoblastico, posteriora enteroblastica. Conidia mediocriter 22.5 × 5.2 µm, 4.3 plo longiora quam latiora, hyalinae, parietibus tenuibus, viscida strato persistenti muci tecta, laeves contentu tenue granulari, 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) × (1.5–) 2–3 (–3.5) µm (av. 12.7 × 2.4 µm). *Conidia* ellipsoid, occasionally slightly bent or irregularly shaped, (19–) 21–24 (–26) × (3.5–) 4.5–6 (–6.5) µm (av. 22.5 × 5.2 µm, 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 prior to germination. *Cultural characteristics*. Colonies initially white with moderately dense, appressed mycelial mat. Submerged mycelium, turning grey 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 grey (21’’’’f), usually turning pale olivaceous grey (21’’’’’d) within 7 d and becoming olivaceous grey (21’’’’’i) to iron grey 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 7–10 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 temperature 30 ºC, 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, mycelio tecta, usque ad 500 µm diametro. Cellulae conidiogenae holoblasticae laeves cylindricae vel subcylindricae hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter 30.7 × 7.4 µm, 4.1 plo longiora quam latiora, hyalinae, parietibus tenuibus, viscida strato persistenti muci tecta, laeves contentu tenue granulari, 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 µm diam. *Conidiogenous cells* holoblastic, smooth, cylindrical to subcylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (7–) 8.5–11 (–14) × (2.5–) 3–3.5 (–4) µm (av. 9.8 × 3.3 µm). *Conidia* ellipsoid, straight or slightly curved, (24–) 28–33 (–34) × (6.5–) 7–8 (–8.5) µm (av. 30.7 × 7.4 µm, 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 prior to germination. *Cultural characteristics*. Colonies initially white, hyphae forming a moderately dense, appressed mycelial mat. Submerged mycelium citrine (21k) to grey 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 grey (21’’’’f) to pale olivaceous grey (21’’’’’d). Colonies slightly irregular with sinuate edges. Optimum growth temperature 30 ºC, covering the 90 mm diam Petri dish after 4 d in the dark.

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 µm diametro. Cellulae conidiogenae holoblasticae laeves cylindricae vel subcylindricae hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter 25 × 7.5 µm, 3.3 plo longiora quam latiora, hyalinae, parietibus tenuibus, viscida strato persistenti muci tecta, laeves contentu tenue granulari, 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 µm diam. *Conidiogenous cells* holoblastic, smooth, cylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically, (6–) 7.5–10 (–11) × (2.7–) 3–4 (–4.3) µm (av. 8.6 × 3.5 µm). *Conidia* ellipsoid to rod-shape, straight or slightly bent, (17.5–) 21–29 (–32) × (6.3–) 7–8 (–9) µm (av. 25 × 7.5 µm, 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 prior to germination. *Cultural characteristics*. Colonies initially white with sparse to moderately dense appressed mycelial mat. Submerged mycelium dark violet (59m) to dark blue (47m) (middle of the colony) and smoke grey (21’’’’f) to grey olivaceous (21’’’’b) towards edges within 3–5 days, becoming violaceous grey (59’’’’i) to slate blue (47’’’k) with age. Aerial mycelium slightly fluffy, becoming dense, cottony with age, turning smoke grey (21’’’’f) to pale purplish grey (71’’’’’d) in the middle of colony and smoke grey (21’’’’f) to grey olivaceous (21’’’’b) towards edges after 5–7 days, becoming lavender grey (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 days. Optimum growth temperature 30 ºC, 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: Mt 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 µm diametro, 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 × 8.7µm, 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.

Pycnidia semi-immersed, mostly solitary, with globose base (up to 550 µm 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) × (2.5–) 3–4 (–4.5) µm (av. 7.3 × 3.4 µm). *Conidia* oval to ovoid, (17–) 19–22 (–23) × (7–) 8–9.5 (–10.5) µm (av. 20.4 × 8.7 µm, l/w 2.3), apices rounded and truncate base, initially hyaline, unicellular, becoming cinnamon (13'') to sepia (13''k) and one-septate while still attached to conidiogenous cells. *Cultural characteristics*. Colonies initially white to olivaceous-buff (21'''d), becoming greenish-olivaceous (23''') to citrine (21k) from the middle of colonies within 7 d, iron grey (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 days, 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 days after incubation. Optimum growth temperature 25 ºC, covering the 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 *Lysiphyllum cunninghamii* (Caesalpiniaceae) and *Terminalia* sp. (Combretaceae).

Known distribution. Western Australia.

HOLOTYPE. AUSTRALIA. WESTERN AUSTRALIA: Tunnel 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 FIG. 10, 11.

Pycnidia subimmersa solitaria globosa papillata nigra mycelio tecta, usque ad 520 µm diametro. Paraphyses cylindricae, 1–2-septatae, hyalinae. Cellulae conidiogenae holoblasticae, cylindricae vel subcylindricae, hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia mediocriter 15.3 × 11.4µm, 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 µm diam. *Paraphyses* cylindrical, 1–2 septate, hyaline, (19–) 28–46 (–54) × (1.5–) 2–2.5 (–3) µm (av. 37.1 × 2.2 µm), 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) × (2–) 3–4 (–4.5) µm (av. 10.3 × 3.3 µm). *Conidia* globose to subglobose to obovoid, (12–) 14–17 (–19) × (10–) 11–12 (–12.5) µm (av. 15.3 × 11.4 µm, l/w 1.3), with granular content, thick-walled (1–2 µm), 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 grey (21''''f) with woolly aerial mycelium, becoming pale olivaceous grey (21'''''d) within 5–7 d, olivaceous grey (21'''''i) to iron grey (23'''''k) with age, margins regular. Submerged mycelium dense, reverse grey olivaceous (21''''b) to olivaceous black (27''''m) after 7 d, becoming black with age. Optimum growth temperature 30 ºC, 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 *Adansonia 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 µm diametro, 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 µm latae 50 µm longae, simplices vel ramosae. Conidia mediocriter 13.4 × 5.7 µm, 2.3 plo longiora quam latiora, hyalinae, parietibus tenuibus vel subcrassis, laeves contentu tenue granulari, fusiformia ellipsoidea vel ovalia apicibus rotundatis basibus truncatis, unicellularia vel 1-septata.

Pycnidia semi-immersed, solitary, globose, papillate, chestnut, covered by hyphal hairs, up to 510 µm 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) × (2–) 2–3 (–3.5) µm (av. 8.7 × 2.5 µm). *Conidiophores* smooth, cylindrical, septate, up to 2 µm wide and 50 µm long, simple or branching. *Conidia* fusiform to ellipsoid to oval, (11–) 12–15 (–16) × (4.7–) 5–6 (–7) µm (av. 13.4 × 5.7 µm, l/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 grey olivaceous (21''''b) from the middle of colonies within 5–7 days, with appressed mycelial mat and white moderately dense, cottony aerial mycelium towards the edge of the colony, becoming smoke grey (21''''f) to olivaceous grey (21'''''i) (surface) and iron grey

(23’’’’’k) (beneath) within 10–14 days. Optimum growth temperature 25 ºC, 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 some isolates of the type species *Botryosphaeria dothidea* (Barber et al 2005). The conidia of *Fusicoccum ramosum* are significantly shorter then those of known species in this genus.

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

Pycnidia ad dimidium immersa vel superficiales, solitaria vel in stromata multilocularia, nigra, cum basim globosa, diametrus usque ad 300 µm, collis usque ad 600 µm longis. Cellulae conidiogenae holoblasticae, cylindricae vel subcylindricae, hyalinae, conidio primo holoblastico, posteriora enteroblastica. Conidia (1) mediocriter 11.5 × 4.4 µm, 2.6 plo longiora quam latiora, apices rotundati, primo hyalina, evadentes cinnamomeo- vel sepiaceo-brunnescentia cum maturitate, sive ellipsoidea vel ovoidea et 0–1–2-septata cum maturitate; (2) mediocriter 10.6 × 6.9 µm, 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 aerio, mediocriter 6.5 × 4 µm, 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 µm diam and long neck, up to 600 µm long. *Conidiogenous*

cells holoblastic, cylindrical to subcylindrical, 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: (1) 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; (2) 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 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 (21k) from the middle of colonies within 7 d, and black (surface and beneath) with age, with suppressed, moderately fluffy mycelium, edges smooth. Optimum growth temperature 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 species *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 *PSEUDOFUSICOCCUM* SPECIES

1. Blue-violet pigment in cultures visible after 3–5 days; conidia averaging 25 µm long, l/w 3.3, aseptate, forming 1–3 septa prior to 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 1or 2 septa prior to germination, l/w 4.3	***P. adansoniae***

DISCUSSION

Seven new species of Botryosphaeriaceae were isolated from endemic trees in Western Australia. Combined ITS and EF-1α sequence data distributed these isolates among the 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 *Pseudofusiccum* spp. occured 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 Australia.

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 analysed. These variations could indicate that *P. kimberleyense* is comprised of 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*. High numbers of dead and dying baobabs (*A. digitata*) have been reported in Southern Africa, particularly in Zimbabwe (Anonymous 1991, Piearce et al 1994). The symptoms identified

on the trees in Zimbabwe were originally reported as "sooty bark disease" caused by species of sooty mould fungi (Calvert 1989, Anonymous 1991). However, Piearce 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 then 500 hosts world-wide (Punithalingham 1976), and as such, could also be involved in the decline of baobab trees in African countries (Roux 2002). Since *Lasiodiplodia margaritacea* was found only on *A. gibbosa* that shows die-back symptoms, this fungus could be pathogenic to this host.

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). Except for the pycnidia with long necks, which are 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 EF-1α phylogenies. *Dothiorella longicollis* occured as an endophyte in asymptomatic branches of two unrelated hosts, *Lysiphyllum 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 were recently described for *Botryosphaeria dothidea*, *Neofusicoccum parvum, N. ribis* and *N. australe* (Barber et al 2005), however this is the first time that *Dichomera*-like synanamorph is identified for *Neoscytalidium*. *Neoscytalidium dimidiatum* has been isolated from different substrates including plant tissues, soil, human skin and nails, and is known as plant pathogen (Punithalingam and Waterston 1970, Crous et

al 2006). The isolates examined in this study were collected as endophytes from plant tissues. This is the first report of *Neoscytalidim* sp. on *A. gibbosa.*

Fusicoccum ramosum was isolated as endophyte from asymptomatic twigs of *Eucalyptus camaldulensis*. Numerous species of Botrosphaeraceae with '*Fusicoccum*' anamorphs identified from *Eucalyptus* have now 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 and 2007), however no *Neofusicoccum* spp. were isolated from *Euclyptus* 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 also 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,* reflects 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.

LITERATURE CITED

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

Anonymous. 1991. Africa's favourite tree falls ill. New Scientist 1784.

Barber PA, Keane PJ. 2007. A novel method of illustrating microfungi. Fungal Divers 27:1–10.

Barber PA, Burgess TI, Hardy GEStJ, Slippers B, Keane PJ, Wingfield MJ. 2005. *Botryosphaeria* species from *Eucalyptus* in Australia are pleoanamorphic, producing *Dichomera* synanamorphs in culture. Mycol Res 109:1347–1363.

Baum DA, Small RL, Wendel JF. 1998. Biogeography and floral evolution of baobabs (*Adansonia,* Bombacaceae) as inferred from multiple data sets. Syst Biol 47:181–207.

Bowman DMJS. 1997. Observations on the demography of the Australian Baobab (*Adansonia gibbosa*) in the north-west of the Northern Territory, Australia. Aust J Bot 45:893–904.

Burgess TI, Barber PA, Hardy GESJ. 2005. *Botryosphaeria* spp. associated with eucalypts in Western Australia including description of *Fusicoccum macroclavatum* sp. nov. Aust Plant Path 34:557–567.

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

Burgess TI, Sakalidis M, Hardy GEStJ. 2006b. Gene flow of the canker pathogen *Botryosphaeria australis* between *Eucalyptus globulus* plantations and native eucalypt forests in Western Australia. Austral Ecol 31:559–566.

Calvert GM.1989. Dying baobabs.The Zimbabwe Science News 23:21. Letter to the editor.

Carbone I, Kohn LM. 1999. A method for designing primer sets for speciations studies in filamentous ascomycetes. Mycologia 91:553–556.

Crisp M, Cook L, Steane D. 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? Phil Tran Roy Soc B: Biol Sci 359:1551–1571.

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

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

Denman S, Crous PW, Taylor JE, Kang JC, Pascoe I, Wingfield MJ. 2000. An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Stud Mycol 45:129–140.

de Wet J, Slippers B, Preisig O, Wingfield BD, Wingfield MJ. 2008. Phylogeny of Botryosphaeriaceae reveals patterns of host association. Mol Phylogenet Evol 46:116–126.

Farris JS, Kallersjo M, Kluge AG, Bult C. 1995. Testing significance of incongruence. Cladistics 10:315–319.

Felsenstein J. 1985. Confidence intervals on phylogenetics: an approach using bootstrap. Evolution 39:783–791.

Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118.

Hillis DM, Huelsenbeck JP. 1992. Signal, noise and reliability in molecular phylogenetic analysis. J Hered 83:189–195.

Huelsenbeck JP, Bull JJ, Cunningham CV. 1996. Combining data in phylogenetic analysis. Trends Ecol Evol 11:152–158.

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

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

Mohali S, Slippers B, Wingfield MJ. 2006. Two new *Fusicoccum* spp. from *Eucalyptus* and *Acacia* in Venezuela, based on morphology and DNA sequence data. Mycol Res 110:405–413.

Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pavlic D, Slippers B, Coutinho TA, Gryzenhout M, Wingfield MJ. 2004. *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. Stud Mycol 50:313–322.

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

Piearce GD, Calvert GM, Sharp C, Shaw P. 1994. Sooty Baobabs–Disease or Drought? Zimbabwe Forestry Commission Research Paper No. 6:1–13.

Punithalingam E. 1976. *Botryodiplodia theobromae.* CMI descriptions of pathogenic fungi and bacteria. No. 519. Kew, Surrey, England: Commonwealth Mycological Institute. 2 p.

Punithalingam E, Waterston JM. 1970. *Hendersonula toruloidea.* CMI descriptions of pathogenic fungi and bacteria. No. 274. Kew, Surrey, England: Commonwealth Mycological Institute. 2 p.

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

Ronquist F, Huelsenback JP. 2003. MrBayes: bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Roux J. 2002. Report on baobab mortality in Messina nature reserve. Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, from http://www.fabinet.up.ac.za

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

Slippers B, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biology Reviews 21: 90–106.

Swofford DL. 2000. PAUP* 4.0: Phylogenetic Analysis Using Parsimony (*and other methods). Sunderland, Massachusetts: Sinauer Associates.

von Arx JA. 1987. Plant Pathogenic Fungi. Berlin, Germany: J. Cramer. 288 p.

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

Zhou S, Stanosz GR. 2001. Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. Mycologia 93:516–527.

TABLE I. Isolates included in the phylogenetic study

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

TABLE I. Continued

Culture no.[1]	Other no. [1]	Identity	Host	Location [2]	Genbank no. [3]	
					ITS	EF-1α
CMW26165	CBS122067	*Do. longicollis*	*Lysiphyllum cunninghamii*	WA, Tunnel Creek NP	EU144053	EU144068
CMW26166	CBS122068	*Do. longicollis*	*L. cunninghamii*	WA, Tunnel Creek NP	EU144054	EU144069
CBS117008		*Do. viticola*	*Vitis vinifera*	Spain, Sant Sadurní d'Anoia	*AY905557*	AY905560
CBS117010		*Do. viticola*	*V. vinifera*	Spain, Sant Esteve Sesrovires	*AY905558*	AY905561
CBS115041		*Do. iberica*	*Quercus ilex*	Spain, Aragon	*AY573202*	*AY573222*
CBS115035		*Do.iberica*	*Q. ilex*	Spain, Aragon	*AY573213*	*AY573228*
CBS115038		*Do.sarmentorum*	*M. pumila*	Netherlands, Delft	*AY573206*	*AY573223*
IMI63581		*Do.sarmentorum*	*Ulmus* sp.	England, Warwickshire	*AY573212*	*AY573235*
CMW26167	CBS122069	*Fusicoccum ramosum*	*E. camaldulensis*	WA, Bell Gorge	EU144055	EU144070
CMW991	ATCC58188	*B. dothidea*	*Prunus nigra*	New Zealand	*AF241175*	*AY236895*
CMW8000		*B. dothidea*	*Prunus* sp.	Switzerland, Crocifisso	*AY236949*	AY236898
ATCC22928		*B. corticis*	*Vaccinium* sp.	U.S.A., North Carolina	*DQ299248*	EF614932
ATCC22927		*B. corticis*	*Vaccinium* sp.	U.S.A., North Carolina	*DQ299247*	EF614931
MUCC531		*Macrophomina phaseolina*	*Sesbania formosa*	Western Australia, Kununurra	*EF585505*	*EF585560*
MUCC532		*M. phaseolina*	*S. formosa*	Western Australia, Kununurra	*EF585506*	*EF585561*
CMW6837		*Neofusicoccum australe*	*Acacia* sp.	New South West, Batermans Bay	*AY339262*	*AY339270*
CMW7054	CBS121.26	*N. ribis*	*Ribis rubrum*	U.S.A., New York	*AF236908*	*AY236879*
CMW9081	ICMP8003	*N. parvum*	*Populus nigra*	New Zealand	*AY236943*	*AY236888*
CMW26171	MUCC534, CBS122072	*Neoscytalidium novaehollandiae*	*Ad. gibbosa*	WA, Gibb River Rd, 50 km E of Derby	EF585535	EF585581
CMW26168	MUCC535, CBS122610	*N. novaehollandiae*	*Ac. synchronica*	WA, Gibb River Rd, near Meda	EF585536	EF585578
CMW26169	MUCC536, CBS122070	*N. novaehollandiae*	*Grevillia agrifolia*	WA, Gibb River Rd, near Meda	EF585539	EF585579
CMW26170	MUCC537, CBS122071	*N. novaehollandiae*	*Crotalaria medicaginea*	WA, Bell Gorge	EF585540	EF585580
CBS499.66		*Neoscytalidium dimidiatum*	*Mangifera indica*	Mali	*AY819727*	EU144063
CBS204.33		*N. dimidiatum*	*Prunus* sp.	Egypt	*AY819728*	EU144064
	MUCC684	*Guignardia* sp.	*Agonis flexuosa*	Western Australia, Yalgorup	*EU675682*	EU686573
	MUCC685	*Guignardia* sp.	*Ag. flexuosa*	Western Australia, Yalgorup	*EU675681*	EU686572

[1] Abbreviations of isolates and culture collections: CBS = Centraalbureau voor Schimmelcultures Utrecht, The 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, VA, U.S.A; 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.

FIG. 1. One of 2 most parsimonious trees of 1566 steps resulting from the analysis of the combined ITS–EF-1α 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 Botryosphaeriacea according to Crous et al. (2006) are indicated by circles at the nodes.

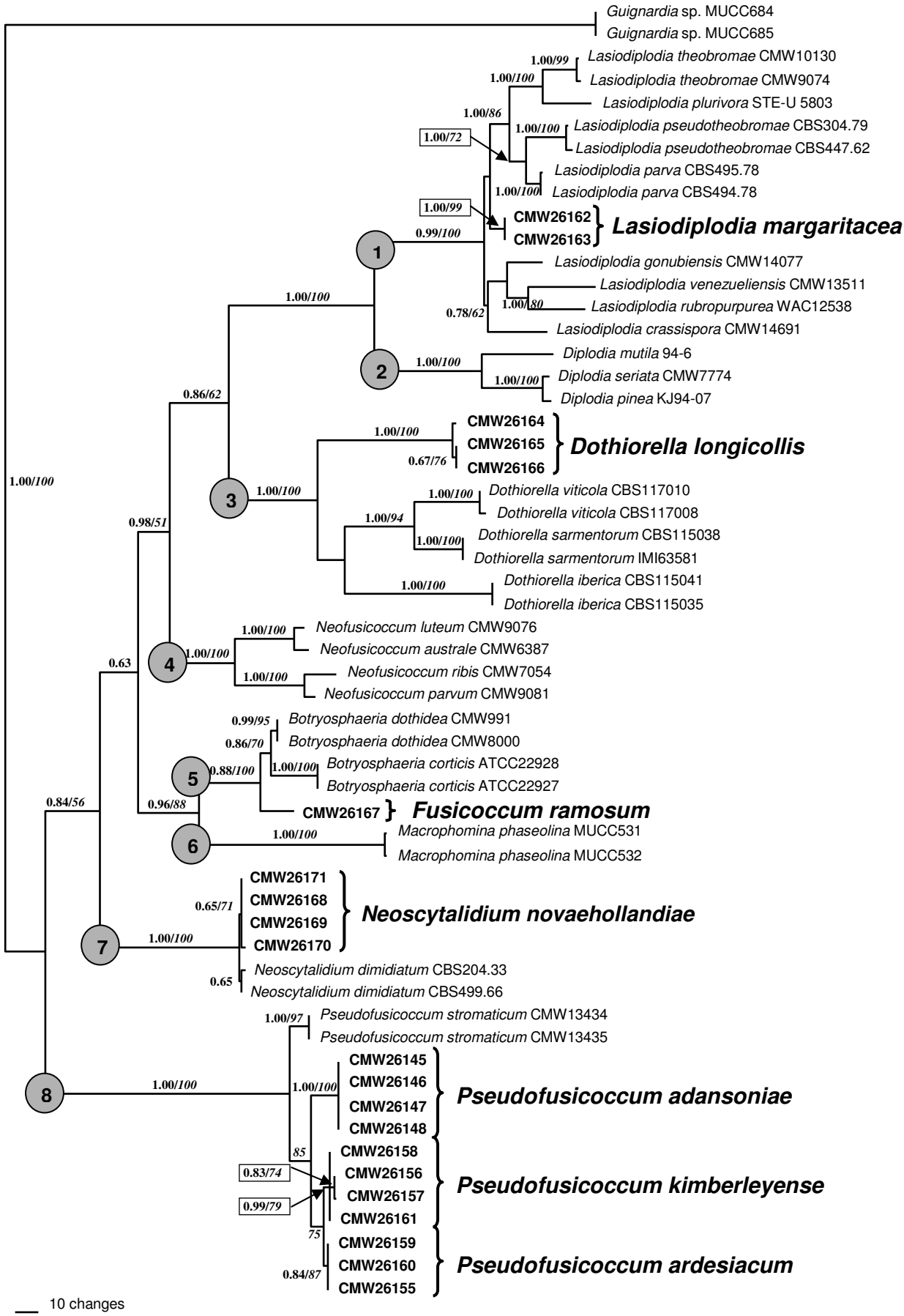

Guignardia sp. MUCC684
Guignardia sp. MUCC685
1.00/99
Lasiodiplodia theobromae CMW10130
1.00/100
Lasiodiplodia theobromae CMW9074
Lasiodiplodia plurivora STE-U 5803
1.00/86
1.00/100
Lasiodiplodia pseudotheobromae CBS304.79
1.00/72
Lasiodiplodia pseudotheobromae CBS447.62
Lasiodiplodia parva CBS495.78
1.00/100
Lasiodiplodia parva CBS494.78
1.00/99
CMW26162
CMW26163
Lasiodiplodia margaritacea
0.99/100
1
Lasiodiplodia gonubiensis CMW14077
Lasiodiplodia venezueliensis CMW13511
1.00/100
1.00/80
Lasiodiplodia rubropurpurea WAC12538
0.78/62
Lasiodiplodia crassispora CMW14691
2
1.00/100
Diplodia mutila 94-6
1.00/100
Diplodia seriata CMW7774
Diplodia pinea KJ94-07
0.86/62
1.00/100
CMW26164
CMW26165
CMW26166
Dothiorella longicollis
0.67/76
1.00/100
3
1.00/100
1.00/100
Dothiorella viticola CBS117010
Dothiorella viticola CBS117008
0.98/51
1.00/94
1.00/100
Dothiorella sarmentorum CBS115038
Dothiorella sarmentorum IMI63581
1.00/100
Dothiorella iberica CBS115041
Dothiorella iberica CBS115035
1.00/100
Neofusicoccum luteum CMW9076
0.63
4
1.00/100
Neofusicoccum australe CMW6387
1.00/100
Neofusicoccum ribis CMW7054
Neofusicoccum parvum CMW9081
0.99/95
Botryosphaeria dothidea CMW991
0.86/70
Botryosphaeria dothidea CMW8000
0.88/100
1.00/100
Botryosphaeria corticis ATCC22928
5
Botryosphaeria corticis ATCC22927
0.84/56
0.96/88
CMW26167
Fusicoccum ramosum
6
1.00/100
Macrophomina phaseolina MUCC531
Macrophomina phaseolina MUCC532
CMW26171
CMW26168
CMW26169
CMW26170
Neoscytalidium novaehollandiae
0.65/71
7
1.00/100
Neoscytalidium dimidiatum CBS204.33
0.65
Neoscytalidium dimidiatum CBS499.66
1.00/97
Pseudofusicoccum stromaticum CMW13434
Pseudofusicoccum stromaticum CMW13435
CMW26145
CMW26146
CMW26147
CMW26148
Pseudofusicoccum adansoniae
1.00/100
8
1.00/100
85
CMW26158
0.83/74
CMW26156
CMW26157
Pseudofusicoccum kimberleyense
0.99/79
CMW26161
75
CMW26159
CMW26160
CMW26155
Pseudofusicoccum ardesiacum
0.84/87
10 changes

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

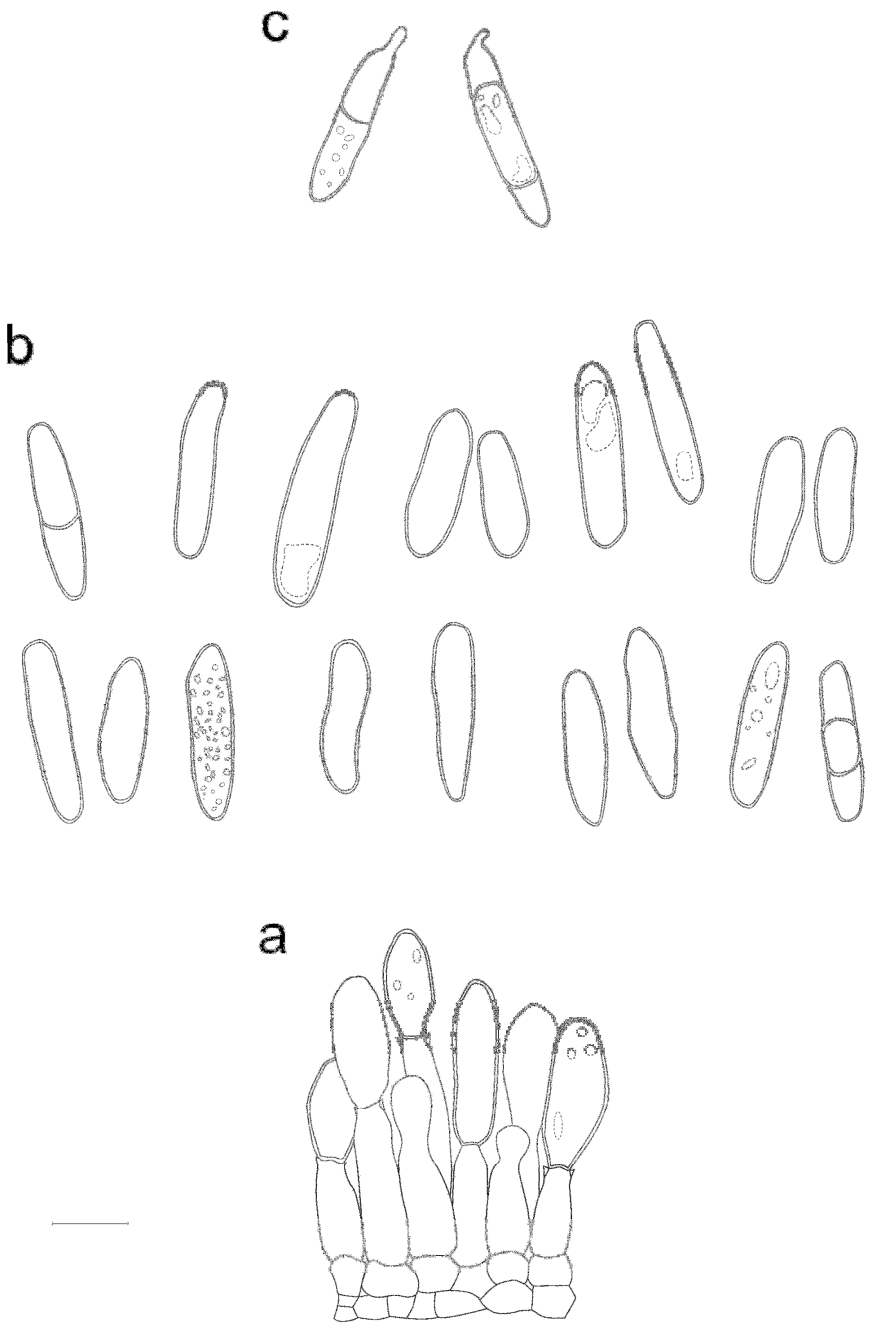

c
b
a

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

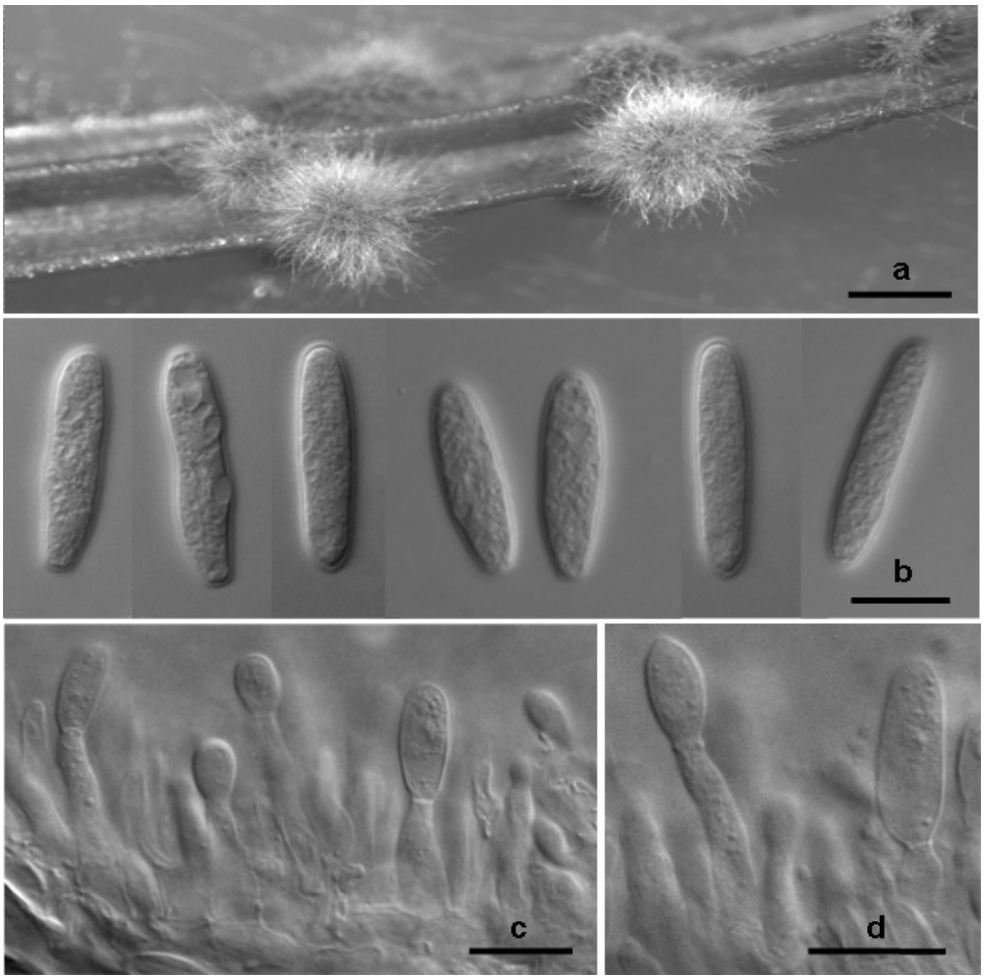

a
b
c
d

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

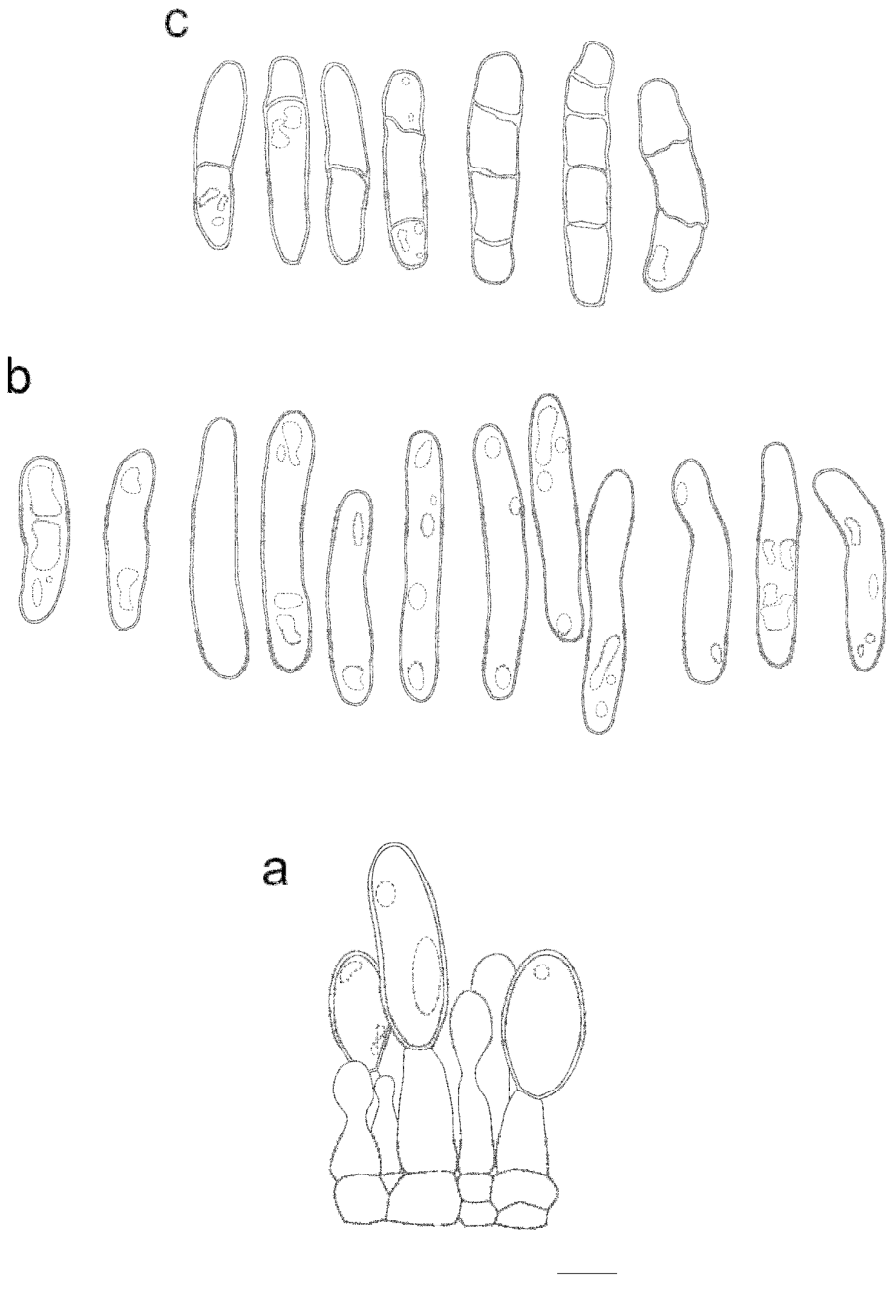

c
b
a

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). Scale bars: a = 500 µm, b–h = 10 µm.

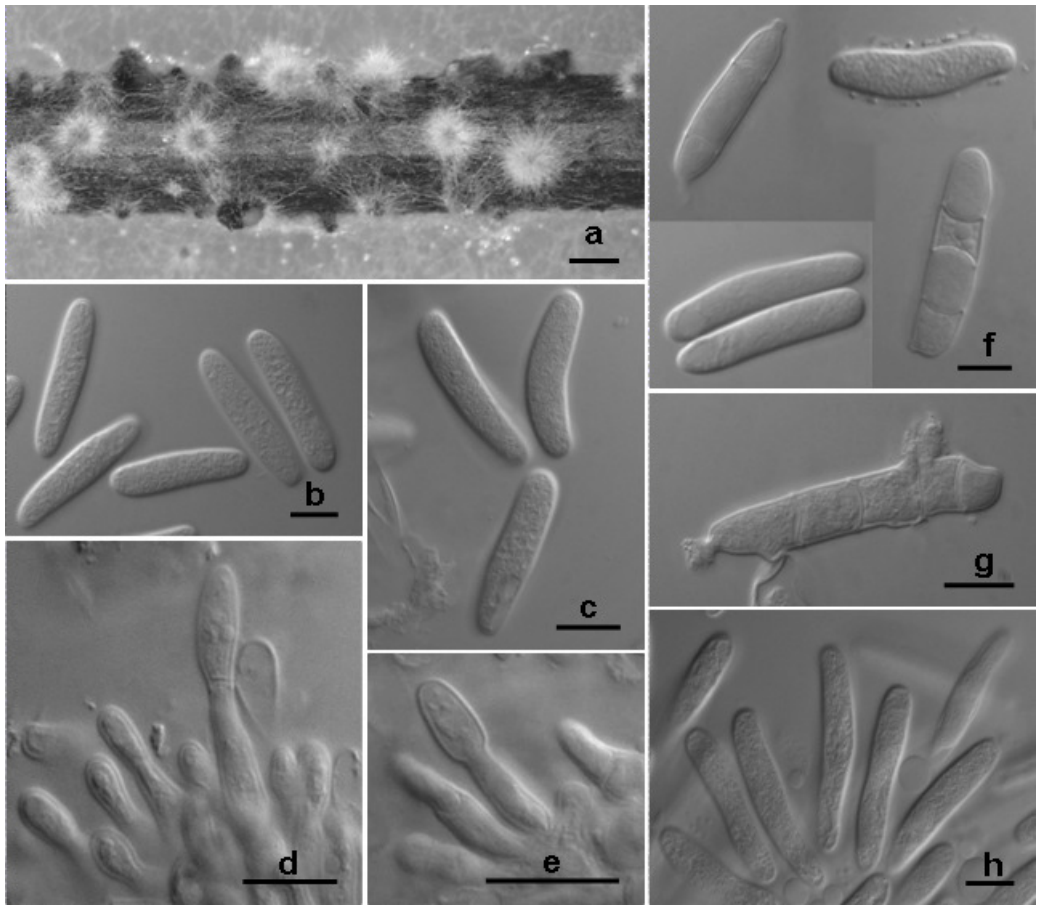
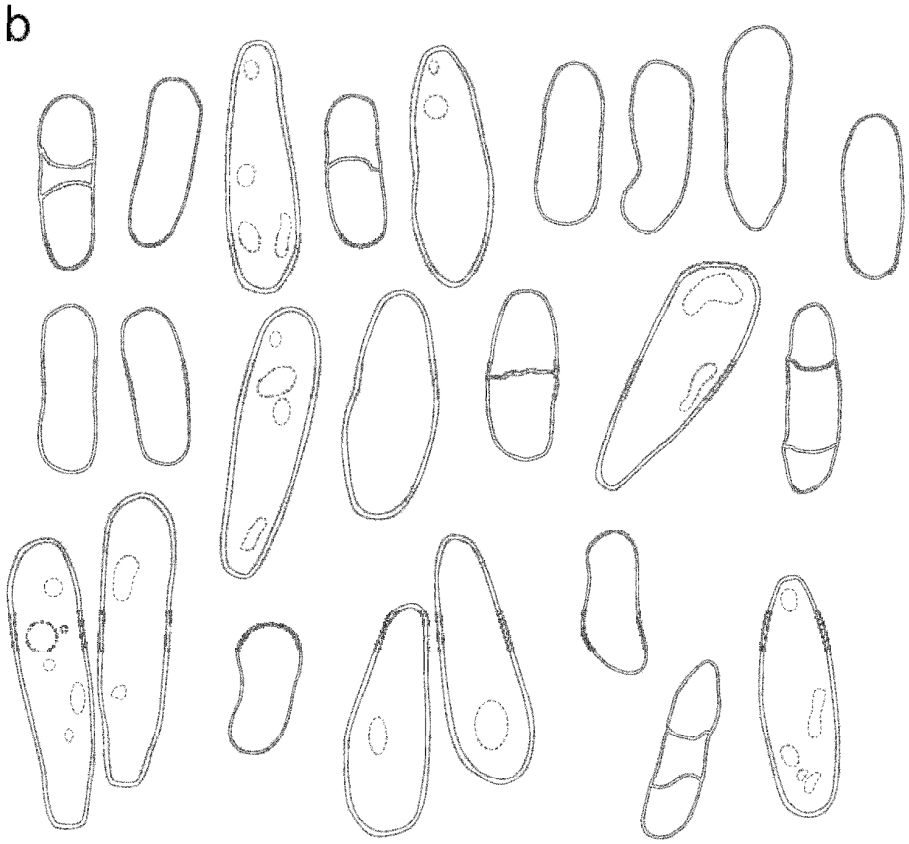

a
b
c
d
e
f
g
h

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

b



a

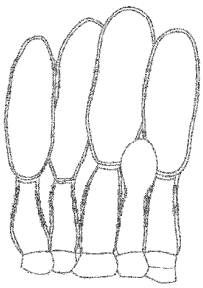


FIG. 7. *Pseudofusicoccum ardesiacum*. a. Pycnidia formed in culture on eucalypt twig (CBS122063). b, c. Conidiogenous cells (CBS122063). d. Conidium attached to conidiogenous cell (CBS122062). e. Aseptate conidia (CBS122062). f. Two-septate conidium (CBS122062). g. Aseptate conidia covered with mucus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

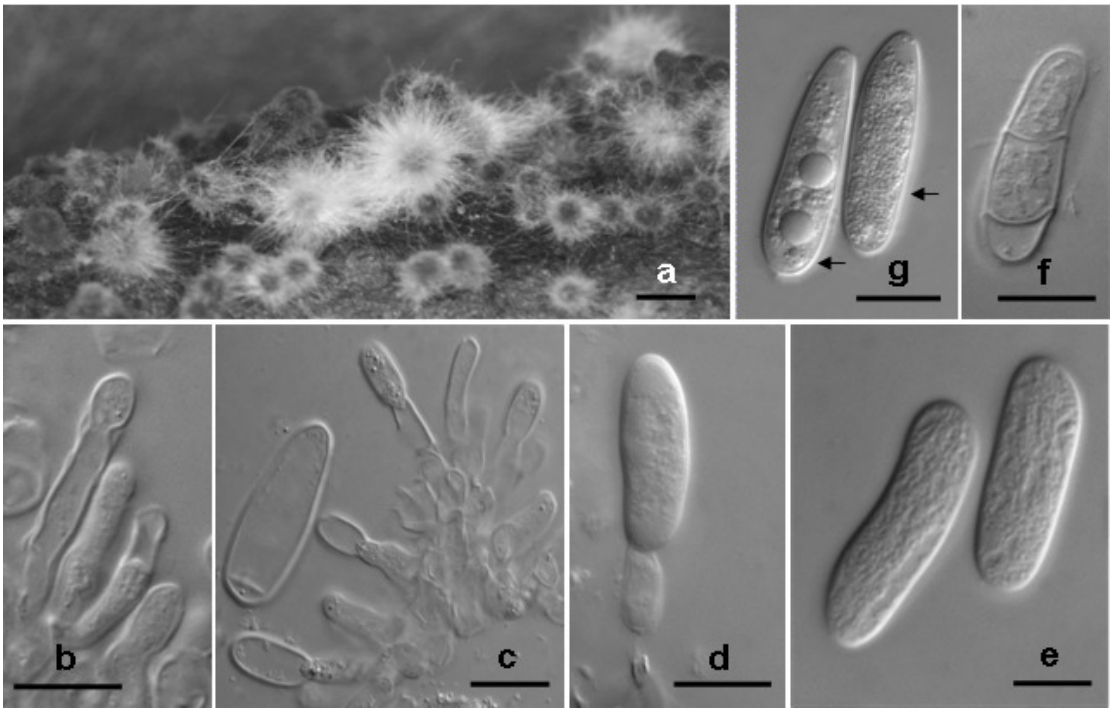

a
g
f
b
c
d
e

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

b

a

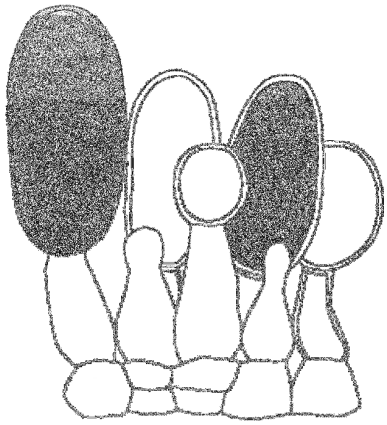


FIG. 9. *Dothiorella longicollis*. a. Pycnidia formed in culture on water agar (CBS122068). b. Pycnidium formed in culture releasing dark one-septate conidia (CBS122068). c. Cross section through a pycnidium showing outer layers of dark brown cells and inner layers of hyaline cells with conidiogeneous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

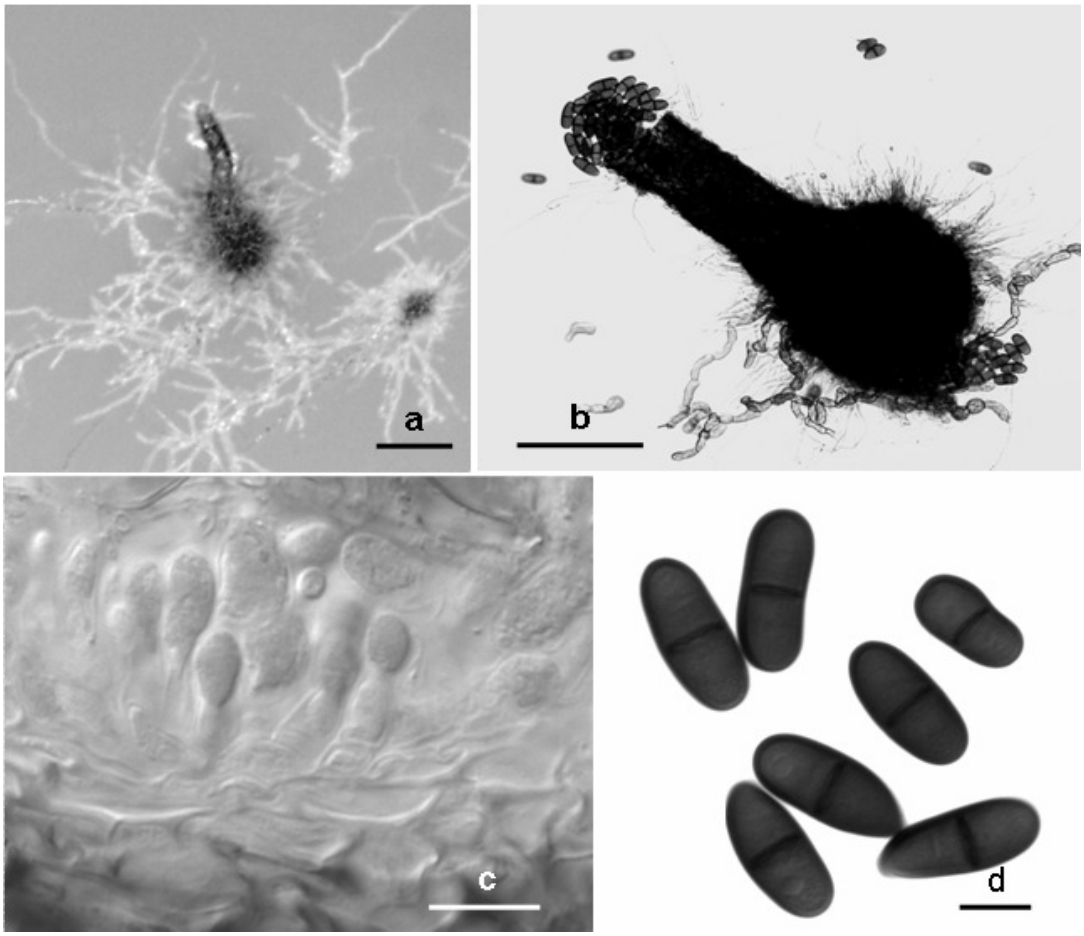

a
b
c
d

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

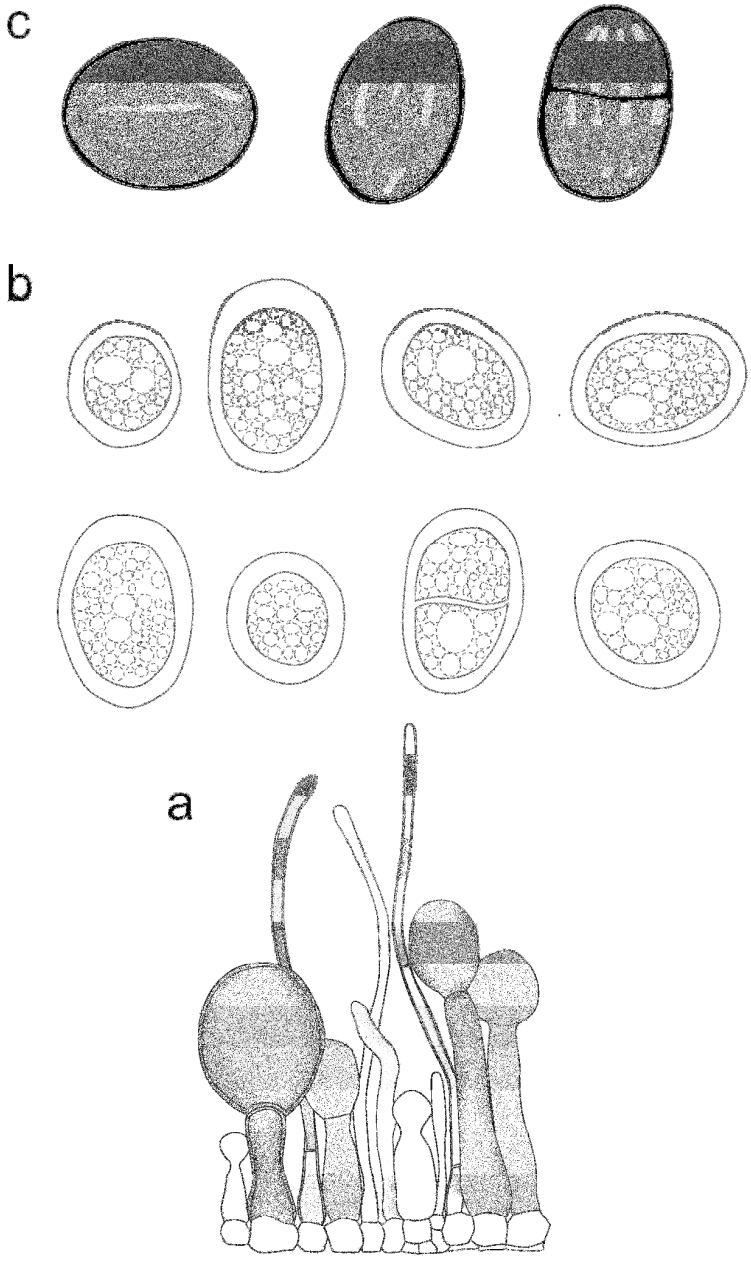

c
b
a

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). Scale bars: a = 500 µm, b–h = 10 µm.

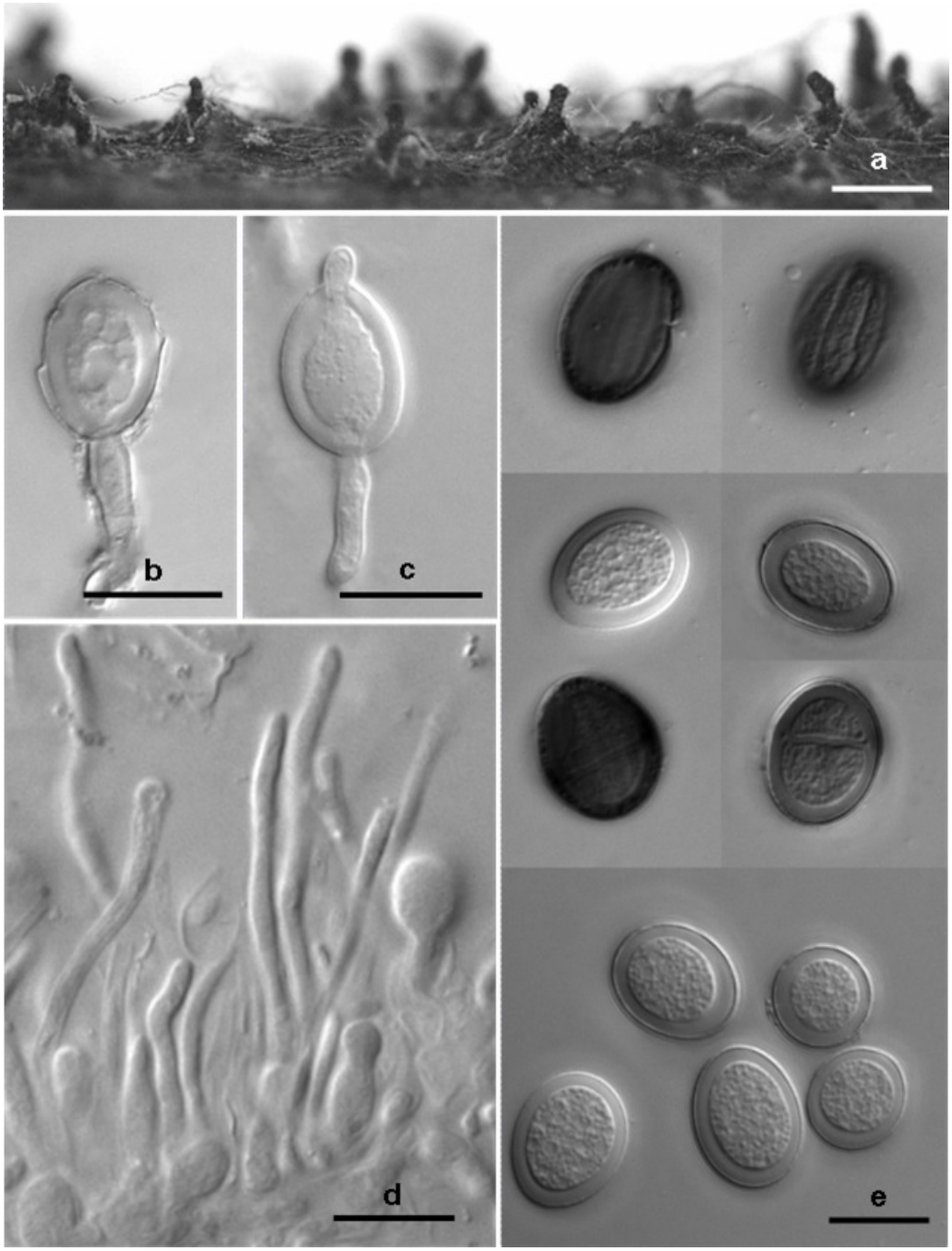

a
b
c
d
e

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

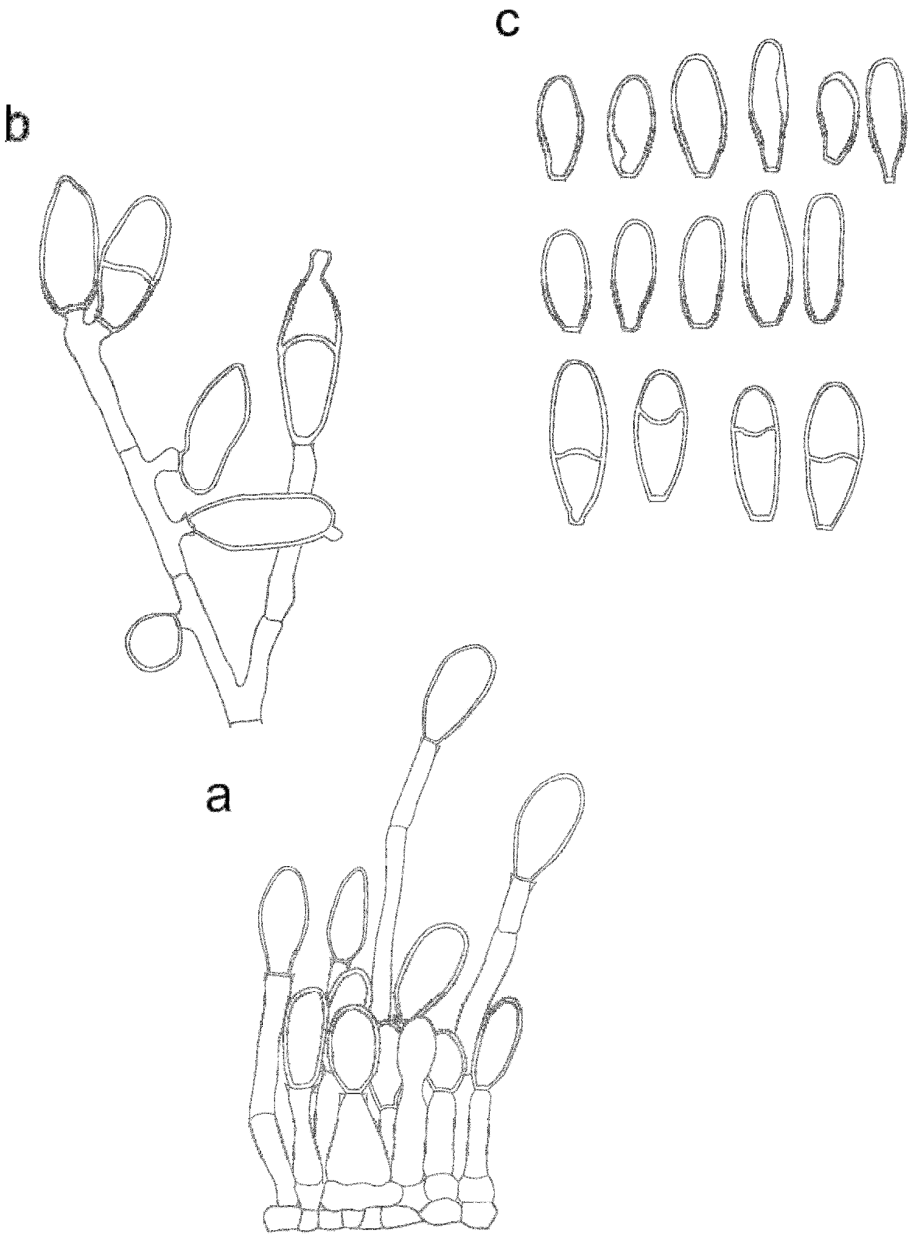

c
b
a

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 one-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). Scale bars: a = 500 µm, b–g = 10 µm.

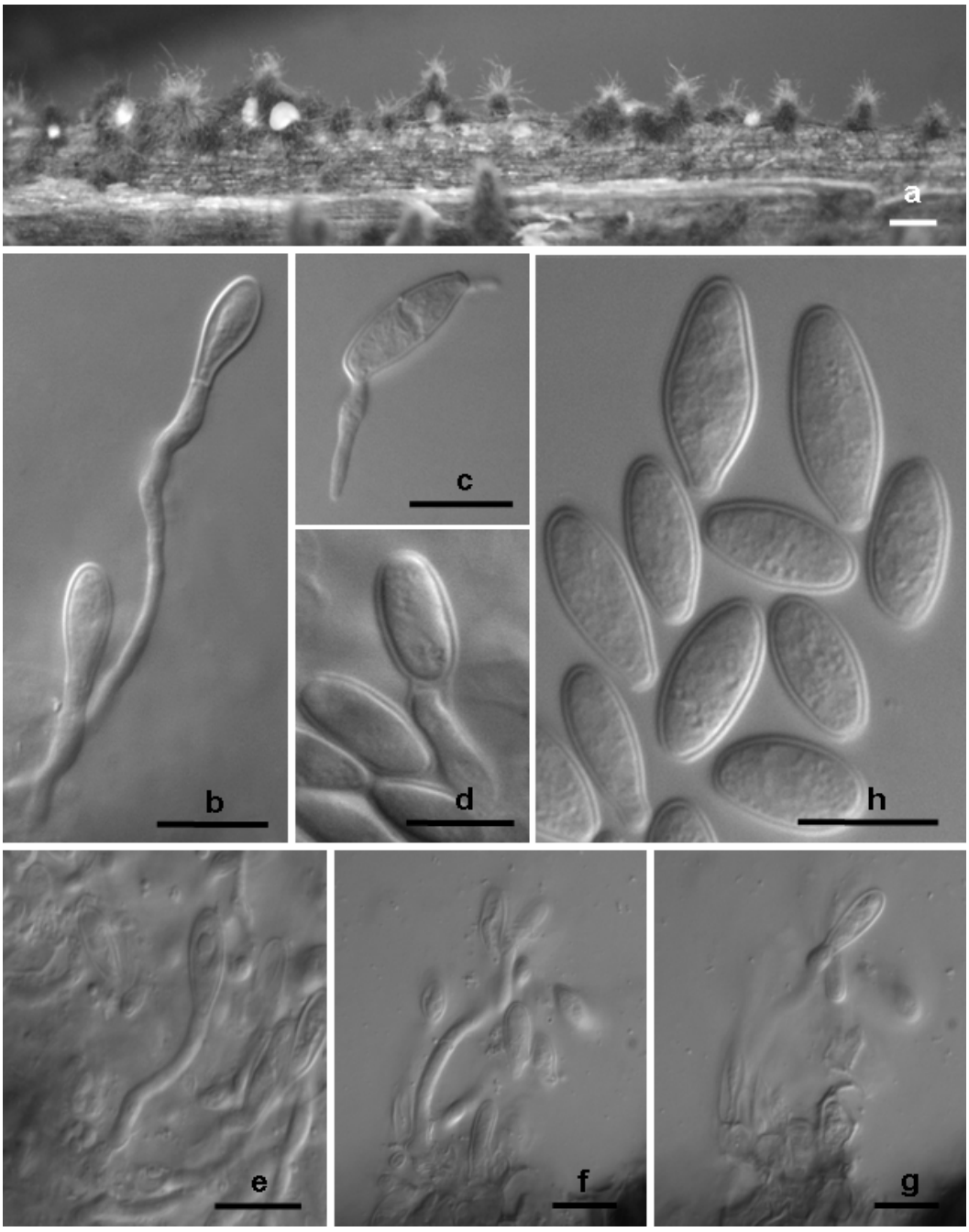

a
b
c
d
h
e
f
g

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

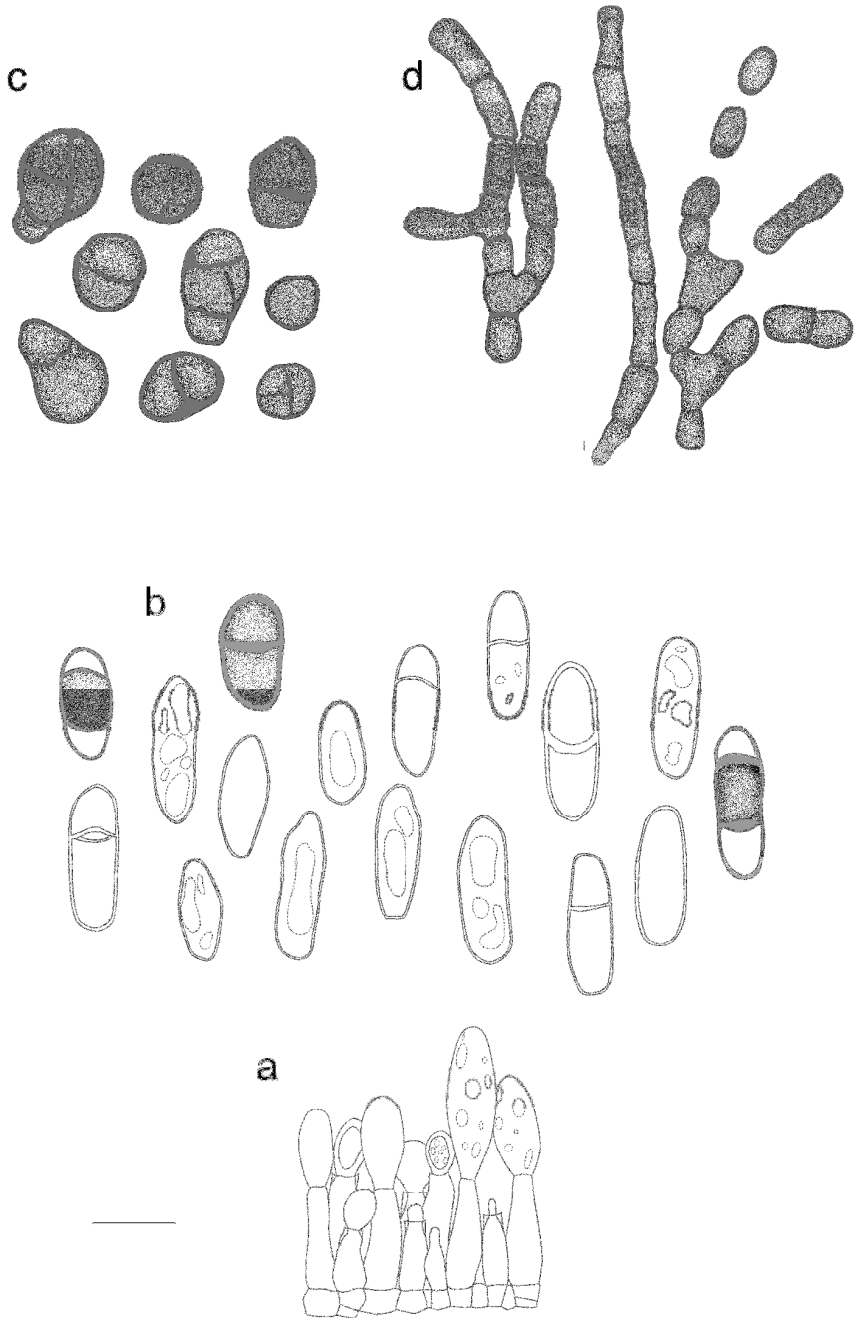
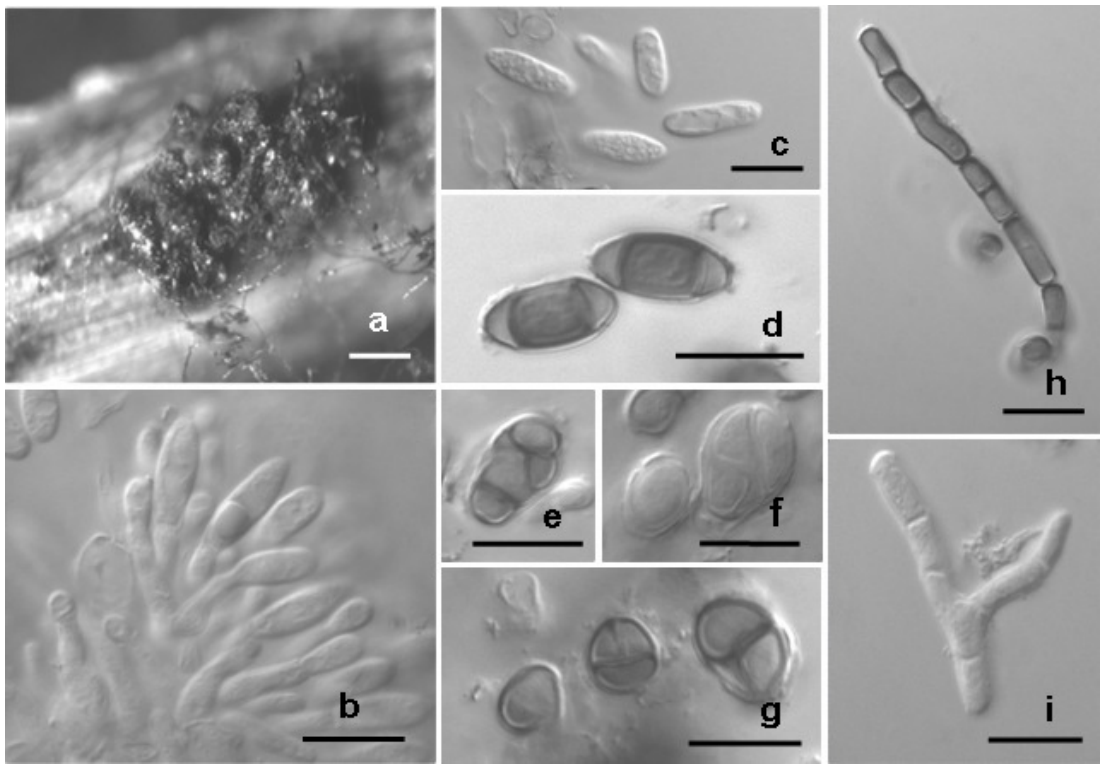

c
d
b
a

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–h CBS122071). Bars: a = 500 µm, b–h = 10 µm.


a
b
c
d
e
f
g
h
i