

Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica*

Bernard Slippers¹

Department of Microbiology and Plant Pathology,
Forestry and Agricultural Biotechnology Institute,
Faculty of Natural and Agricultural Sciences,
University of Pretoria, Pretoria, South Africa

Greg I. Johnson

ACIAR, P.O. Box 1571, Canberra, ACT 101,
Australia

Pedro W. Crous

Centraalbureau voor Schimmelcultures, Uppsalaalaan
8, 3584 CT Utrecht, The Netherlands

Teresa A. Coutinho

Brenda D. Wingfield

Michael J. Wingfield

Department of Microbiology and Plant Pathology, and
Department of Genetics, Forestry and Agricultural
Biotechnology Institute, Faculty of Natural and
Agricultural Sciences, University of Pretoria, Pretoria,
South Africa

Abstract: Species of *Botryosphaeria* are among the most serious pathogens that affect mango trees and fruit. Several species occur on mangoes, and these are identified mainly on the morphology of the anamorphs. Common taxa include *Dothiorella dominicana*, *D. mangiferae* (= *Natrassia mangiferae*), *D. aromatica* and an unidentified species, *Dothiorella* 'long'. The genus name *Dothiorella*, however, is acknowledged as a synonym of *Diplodia*. This study aimed to characterize and name the *Botryosphaeria* spp. associated with disease symptoms on mangoes. To achieve this isolates representing all four *Dothiorella* spp. mentioned above were compared with the anamorphs of known *Botryosphaeria* spp., based on conidial morphology and DNA sequence data. Two genomic regions were analyzed, namely the ITS rDNA and β -tubulin regions. The morphological and molecular results confirmed that the fungi previously identified from mango as species of *Dothiorella* belong to *Fusicoccum*. *Dothiorella dominicana* isolates were identical to isolates of *F. parvum* (teleomorph = *B. parva*). A new epithet, namely *F. mangiferum*, is proposed for isolates previously treated as *D. mangiferae* or *N. mangiferae*. Isolates of *D. aromatica* were

identified as *F. aesculi* (teleomorph = *B. dothidea*). A fourth *Fusicoccum* sp. also was identified as those isolates previously known as *Dothiorella* 'long'. A key is provided to distinguish these species based on anamorph morphology in culture. This study provides a basis for the identification of *Botryosphaeria* species from mango, which is important for disease control and to uphold quarantine regulations.

Key words: Conidia, dieback, *Fusicoccum*, identification, mango, phylogeny, soft rot, stem-end rot, taxonomy

INTRODUCTION

Stem-end rot of mango (*Mangifera indica* L.) fruit is one of the most serious post harvest diseases affecting this industry worldwide (Prakash and Srivastava 1987, Cappellini et al 1988, Prusky 1991, Mitra and Baldwin 1997). This disease is caused by a complex of fungal pathogens, of which various *Botryosphaeria* spp. dominate (Darvas 1991; Johnson et al 1991a, b, 1992; Sangchote 1991). Apart from fruit diseases, *Botryosphaeria* spp. also cause tip- and branch dieback and cankers on mango trees (Stevens 1926, Ramos et al 1991). These fungi live endophytically in healthy tissue of all parts of mango plants and mostly cause disease after stress to the trees or fruit after harvest (Johnson et al 1991a, 1992; Sangchote 1991).

Botryosphaeriaceous fungi considered as pathogens of mango trees and fruit are best known by their anamorph states. Some of the most commonly encountered species are *Dothiorella dominicana* Petr. & Cif., *D. mangiferae* Syd. & P. Syd., *D. aromatica* (Sacc.) Petr. & Syd. and an unnamed species, *Dothiorella* 'long' (Johnson 1992). These names, however, are in need of revision. *Dothiorella mangiferae* has been reduced to synonymy under *Natrassia mangiferae* (Syd. & P. Syd.) Sutton & Dyko (Sutton and Dyko 1989). This synonymy has been recognized by some researchers (Londsdale 1992, Roux 1993) but disputed by others (Johnson 1991a, b, 1992). In addition the type species of *Dothiorella* was synonymized recently under *Diplodia*, raising questions about the correct generic affinities of all species presently placed in *Dothiorella* (Crous and Palm 1999).

Not all *Dothiorella* spp. are of equal importance as pathogens of mango. *Dothiorella dominicana* is the

Accepted for publication 18 Aug 2004.

¹ Corresponding author. E-mail: bernard.slippers@fabi.up.ac.za

most common pathogen and causes significant losses annually (Darvas 1991, Johnson et al 1991a). *Dothiorella mangiferae* is found on mango trees worldwide, especially in Australia and Thailand (Sydow et al 1916; Johnson et al 1991a, 1992; Mitra and Baldwin 1997). *Dothiorella aromatica* (Sacc.) Petr. & Syd., and an unnamed species, *Dothiorella* 'long', occasionally have been recorded from mango in Thailand and Australia but are of less importance (Johnson et al 1991a, Johnson 1992). *Dothiorella aromatica* has been reported from mango but is better known as a pathogen of avocado (Johnson 1992, Johnson et al 1992, Hartill 1991).

It has been suggested that all *Dothiorella* spp. occurring on mango should be accommodated in the genus *Fusicoccum* (Johnson 1992). In that study it is suggested that *D. dominicana* is a synonym of *F. aesculi* Corda (*B. dothidea* [Fr. : Moug.] Ces. & De Not.), and that *D. aromatica* should be placed in *Fusicoccum* with *F. luteum* Pennycook & Samuels as a synonym. He also suggested that *D. mangiferae* should be recombined in *Fusicoccum* as the anamorph of *B. parva* Pennycook & Samuels, and that *Dothiorella* 'long' is *F. cajani* (Syd., P. Syd. & E.J. Butler) Samuels & Singh (teleomorph *B. xanthocephala* [Syd., P. Syd. & E.J. Butler] Theissen). The new taxonomic combinations, however were, not formally proposed.

Other researchers have reported species of *Fusicoccum* from mango and avocado. Hartill (1991) examined botryosphaeriaceous fungi from avocado in New Zealand that had been described previously as *Dothiorella* species. He concluded that they were either *F. aesculi*, *F. parvum* Pennycook & Samuels or *F. luteum*. In California Ramos et al (1991) observed the *Fusicoccum* anamorph of *B. ribis* Grossenb. & Duggar from mango plants.

Anamorph morphology is used commonly to identify species of *Botryosphaeria* (Shoemaker 1964, Pennycook and Samuels 1985, Jacobs and Rehner 1998, Slippers et al 2004). The morphological distinctions of the anamorphs of some of the closely related species, however, are not clear. Recent studies using DNA sequence data have highlighted taxonomic groups and relationships in *Botryosphaeria* (Jacobs and Rehner 1998, Denman et al 2000, Smith et al 2001, Smith and Stanosz 2001, Zhou and Stanosz 2001, Slippers et al 2004). These data combined with morphological characteristics could clarify the current taxonomic confusion. There is a clear need to use the same approach to clarify the relationships and identities of the stem-end rot pathogens of mango.

The aim of this study was to re-evaluate the status of the anamorph names of *Botryosphaeria* species from mango in Australia and determine their rela-

tion to other *Botryosphaeria* spp. DNA sequence data from the internal transcribed spacer regions (ITS1 and ITS2) and 5.8S gene of the rRNA operon and the β -tubulin gene were used in combination with morphological characteristics to characterize and name the different '*Dothiorella*' spp. The taxonomy of *B. rhodina* (Berk. & Curt.) von Arx (anamorph = *Lasiodiplodia theobromae* [Pat.] Griffon & Maubl.), another *Botryosphaeria* sp. that commonly occurs on mango in Australia, is not considered in this study.

MATERIALS AND METHODS

Isolates and morphological characterization.—A total of 14 single-spore isolates from stem-end rot lesions on mango fruit or from necrotic twigs were used in this study (TABLE I). These isolates previously had been characterized based on morphology by Johnson (1992). In the current study the isolates were induced to sporulate on water agar amended with pine needles as substratum and exposed to near UV light for a 12 h cycle at 20–25 C for up to 1 mo. Pycnidia and conidia were mounted in lactophenol. At least 50 conidia were measured for each species.

Molecular characterization.—A phenol : chloroform DNA extraction technique was used to isolate the genomic DNA, as described in Raeder and Broda (1985) and Smith et al (2001). Partial sequences from two housekeeping gene regions were used for phylogenetic comparisons between isolates. First, the region spanning the 3' end of the 16S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the complete 5.8S rRNA gene, the second ITS (ITS2) and the 5' end of the 26S (large subunit) rRNA gene, was amplified using the primers ITS1 (5'TCCGTAGGTGAACCTGCGG 3') and ITS4 (5'TCCTCCGCTTATTGATATGC 3') (White et al 1990). Second, a part of the β -tubulin gene was amplified using the primers Bt2a (5'GGTAACCAATCGGTGCTGCTTTC 3') and Bt2b (5'ACCCTCAGTGTAGTGACCCTTGGC 3') (Glass and Donaldson 1995). PCR reaction mixtures, PCR conditions and visualization of amplicons were as described in Slippers et al (2004). ITS and β -tubulin PCR amplicons were purified and sequenced as described in Slippers et al (2004).

To compare the sequence data determined in this study with those of known taxa, 15 ITS rDNA sequences and 15 β -tubulin sequences obtained from GenBank were included in the analyses (TABLE I). These sequence data included those of *B. dothidea*, *B. ribis* and *B. parva* from a study of type material and ex-type cultures (Slippers et al 2004), as well as other sequence data of related *Botryosphaeria* spp. (Jacobs and Rehner 1998, Smith et al 2001, Smith and Stanosz 2001, Zhou and Stanosz 2001). BLAST searches were done to identify any other related sequence data to the fungi studied here. *Bionectria* sp. was included as an outgroup taxon in the analyses. Despite the relationship between outgroup and ingroup taxa, unambiguous alignment of intron regions of the outgroup sequence with the ingroup was not always possible, due to the high degree of sequence variation within these regions. Analysis with and

TABLE I. Isolates from mango fruit and trees considered in the phylogenetic study

Culture No.	Other No. ¹	Identity ²	Host	Location	Collector	GenBank ³	
						ITS	β -tubulin
CMW7772		<i>Botryosphaeria ribis</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers/G. Hudler	AY236935	AY236906
CMW7773		<i>B. ribis</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers/G. Hudler	AY236936	AY236907
CMW7054	CBS121	<i>B. ribis</i>	<i>Ribes</i> sp.	New York, USA	N.E. Stevens	AF241177	AY236908
CMW9078	ICMP7925	<i>B. parva</i>	<i>Achimidia delicatosa</i>	New Zealand	S.R. Pennycook	AY236940	AY236914
CMW0981	ICMP8003	<i>B. parva</i>	<i>Populus nigra</i>	New Zealand	G.J. Samuels	AY236943	AY236917
CMW7025	24083	<i>B. parva</i>	<i>Mangifera indica</i>	Australia	G.I. Johnson	AY615181	AY615168
CMW7026	BRIP19684	<i>B. parva</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY615182	AY615169
CMW7798	BRIP19488	<i>B. parva</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY516183	AY615170
CMW7799	BRIP19486	<i>B. parva</i>	<i>Persea americana</i>	Australia	K.G. Pegg	AY615184	AY615171
CMW7024	BRIP24101 α	<i>Fusicoccum mangiferum</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY615185	AY615172
CMW7797	BRIP24083	<i>F. mangiferum</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY615186	AY615173
CMW7801	23396 α	<i>F. mangiferum</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY615187	AY615174
CMW7022	BRIP19782 β	<i>Fusicoccum</i> sp.	<i>M. indica</i>	Australia	A.W. Cooke	AY615188	AY615175
CMW7023	BRIP19560	<i>Fusicoccum</i> sp.	<i>M. indica</i>	Australia	G.I. Johnson	AY615189	AY615176
CMW7802	23491 β	<i>Fusicoccum</i> sp.	<i>M. indica</i>	Australia	A.W. Cooke	AY615190	AY615177
CMW7020	BRIP24286	<i>B. dothidea</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY615191	AY615178
CMW7027	BRIP24172 γ	<i>B. dothidea</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY615192	AY615179
CMW7803	BRIP23750 γ	<i>B. dothidea</i>	<i>M. indica</i>	Australia	G.I. Johnson	AY615193	AY615180
CMW7780		<i>B. dothidea</i>	<i>Fraxinus excelsior</i>	Switzerland	B. Slippers	AY236947	AY236925
CMW8000		<i>B. dothidea</i>	<i>Prunus</i> sp.	Switzerland	B. Slippers	AY236949	AY236927
CMW9076	ICMP7818	<i>B. lutea</i>	<i>Mahoe</i> \times <i>domestica</i>	New Zealand	S.R. Pennycook	AY236946	AY236922
CMW992	K.J.93.52	<i>B. lutea</i>	<i>A. delicatosa</i>	New Zealand	G.J. Samuels	AF027745	AY236923
CMW10125	BOT24	<i>B. eucalyptorum</i>	<i>Eucalyptus grandis</i>	South Africa	H. Smith	AF283686	AY236920
CMW10126	BOT16	<i>B. eucalyptorum</i>	<i>E. grandis</i>	South Africa	H. Smith	AF283687	AY236921
CMW7774		<i>B. obtusa</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers/G. Hudler	AY236953	AY236931
CMW7060	CBS431	<i>B. stevensii</i>	<i>F. excelsior</i>	Netherlands	H.A. van der Aa	AY236955	AY236933
CMW10130	BOT977	<i>B. rhodina</i>	<i>Vitex donniana</i>	Uganda	J. Roux	AY236951	AY236929
CMW9074		<i>B. rhodina</i>	<i>Pinus</i> sp.	Mexico	T. Burgess	AY236952	AY236930
CMW7063		<i>Bionectria</i> sp.	<i>Taxus baccata</i>	Netherlands	H.A. van der Aa	AY236956	AY236934

¹ Abbreviations for culture collections and isolates: BRIP = Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CBS = Centraal bureau voor Schimmelcultures, Utrecht, Netherlands; CMW = Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; BOT = *Botryosphaeria* subcollection of CMW; ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand; K.J. = Jacobs and Rehner (1998). Isolates marked with α , β , or γ are duplicates.

² Identities are given as determined in this study. Isolates from *M. indica* were previously known by the anamorph names *D. dominicana* (*B. parva*), *D. mangiferae* (*F. mangiferum*), *D. 'long'* (*Fusicoccum* sp.) and *D. aromatica* (*B. dothidea*).

³ Sequences obtained from GenBank are from Jacobs and Rehner (1998), Smith et al (2001) and Slippers et al (2004).

without these regions did not affect the relationships of isolates of the ingroup taxa and thus were left as is.

Sequence data determined in this study were analyzed using Sequence Navigator 1.0.1[®] (Perkin Elmer Applied Biosystems, Foster City, California). These data were aligned manually with each other and with the data obtained from GenBank by inserting gaps. Gaps were treated as a fifth character, and all characters were unordered and of equal weight. Partition homogeneity tests (Farris et al 1995, Huelßenbeck et al 1996) were run in PAUP (Phylogenetic Analysis Using Parsimony) 4.0b8 (Swofford 1999) to determine whether the ITS rDNA and β -tubulin sequence datasets were congruent and, therefore, combinable. These data then were analyzed together to determine possible phylogenetic relationships among the taxa using parsimony in PAUP. To construct maximum parsimonious trees from the data, heuristic searches were done using informative characters and stepwise (random) addition and tree bisection and reconstruction (TBR) as branch-swapping algorithm. MaxTrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Levels of homoplasy and phylogenetic signal (retention and consistency indices and g1-value) (Hillis and Huelßenbeck 1992) were determined. Branch and branch node supports were determined using 1000 bootstrap replicates (Felsenstein 1985) and decay analysis of the branch nodes using Autodecay (Eriksson 1998).

RESULTS

Isolates and morphological characterization.—The isolates used in this study had been maintained in culture for an extended period of time and subcultured extensively. Many of the cultures grew poorly and isolates did not sporulate as readily on pine needles as observed previously with freshly isolated strains (Slippers et al 2004). Nevertheless, all species sporulated on the needles in 1–4 wk. Pycnidia were spherical (150–400 μm), with an apical papilla, with or without a conical neck (50–200 μm), semi-immersed to superficial on the needle surfaces and mostly occurred singly (FIG. 1). The apical papilla often was inconspicuous due to dense growth of gray mycelium covering the pycnidia.

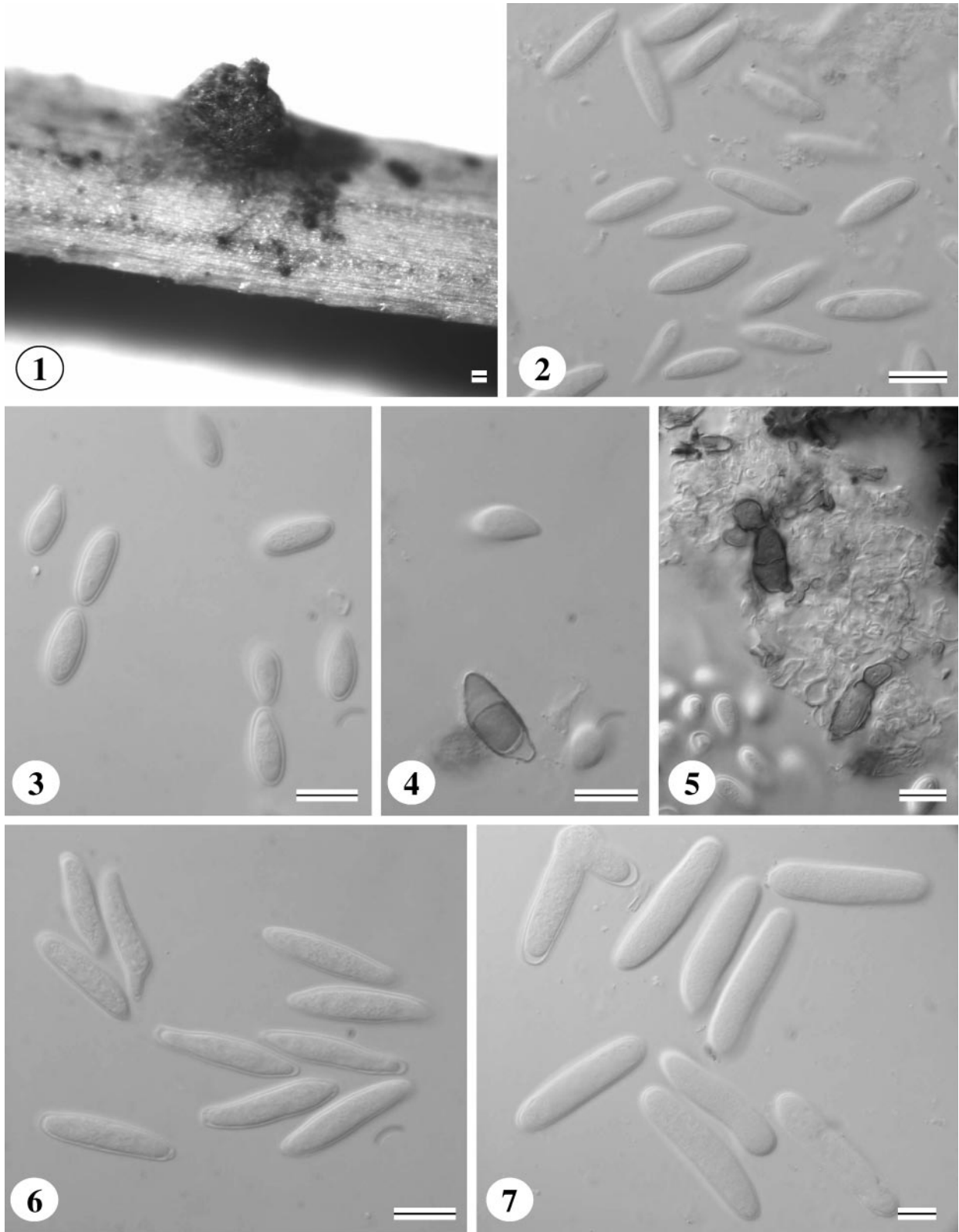
The four taxa represented by the isolates were distinguished by conidial size and shape (TABLE II) (KEY) (FIGS. 2–7). Isolates previously identified as *D. dominicana* had fusiform to ellipsoid, hyaline conidia (average of 56 conidia = $19 \times 5.2 \mu\text{m}$). These conidia were observed infrequently to become 1–2 euseptate, often with a darker brown middle cell. Such septate, versicolored spores usually were observed after discharge from the pycnidia or on material that had been left to dry. These conidia are similar to those reported to be *F. parvum* (TABLE II). Conidia of isolates previously identified as *D. mangiferae* were similar in shape, septation and color to those of *D.*

dominicana but were smaller (average of 54 conidia = $13.6 \times 5.4 \mu\text{m}$). Dark brown mycelial, toruloid cells were observed infrequently on pine needles or in culture. Isolates identified as *D. aromatica* produced long, fusiform conidia (average of 59 conidia = $23 \times 5.1 \mu\text{m}$). These isolates were similar to those reported as *F. aesculi*, the anamorph of *B. dothidea* (TABLE II). Isolates previously identified as *D.* ‘long’ also produced long conidia but differed from the last named taxon by their broader, rod-shaped conidia (average of 59 conidia = $26.6 \times 6 \mu\text{m}$).

Molecular characterization.—Amplicons of about 550 bp were obtained using the primers ITS1 and ITS4 and approximately 450 bp using the primers Bt2a and Bt2b. Approximately 25 bp of the terminal end sequence data were excluded in each case in the final alignments (GenBank 1216767–1216792, TABLE I). The total aligned sequence dataset had 1016 characters (TreeBASE matrix accession number = M1883). Only the 260 parsimony informative characters were included in the analysis.

A partition homogeneity test showed that the ITS rDNA and β -tubulin datasets were congruent (P value = 0.2). Evaluation of random trees showed that the combined datasets contained significant phylogenetic signal ($P < 0.01$; $g1 = -0.72$) (Hillis and Huelßenbeck 1992). Heuristic searches found two equal, most parsimonious trees (tree length = 544 steps; CI = 0.746; RI = 0.885) (TreeBASE Study accession number = S1101) (FIG. 8). The nine clades in these trees were identified as: clade I = *B. ribis*, clade II = *B. parva*, clade III = *F. mangiferum*, clade IV = *B. eucalyptorum* Crous, H. Smith & M.J. Wingf., clade V = *B. lutea* A.J.L. Phillips, clade VI = *Fusicoccum* sp., clade VII = *B. dothidea*, clade VIII = *B. obtusa* (Schwein.) Shoemaker and *B. stevensii* Shoemaker, and clade IX = *B. rhodina*. All were supported by high bootstrap values (>99%).

Clades I–VII all represent *Botryosphaeria* spp. with *Fusicoccum* anamorphs and formed a monophyletic group supported by a 100% bootstrap value. Within this group, clades I–II (*B. ribis*, *B. parva*) grouped together (100% bootstrap), and these two clades were related most closely to clades III–V (*F. mangiferum*, *B. eucalyptorum* and *B. lutea*) with 85% bootstrap support. Clade VI (undescribed *Fusicoccum* sp.) and clade VII (*B. dothidea*) grouped apart from the other groupings. There was sequence variation among isolates within each of clades VI and VII. The variation in clade VI is in the β -tubulin region in only one isolate (CMW7023). The variation within clade VII is in two bases located in a repetitive G (nine repeats) and C (10 repeats) rich area in the ITS1



FIGS. 1–7. Anamorphs of *Botryosphaeria* spp. formed in culture. 1. *Fusicoccum mangiferum* (CMW7024) pycnidium forming on a pine needle. Bar = 100 μ m. 2. *Fusicoccum parvum* (CMW7025) conidia and spermatia. 3, 4. Young, aseptate conidia of *F. mangiferum* (CMW7024), become septate and discolored after discharge. 5. Toruloid cells of *F. mangiferum* (CMW7024). 6. Fusiform conidia of *F. aesculi* (CMW7803). 7. Rod-shaped conidia of a *Fusicoccum* sp. (CMW7022) Bars = 10 μ m.

TABLE II. Conidial measurements for *Botryosphaeria* spp. and their *Fusicoccum* anamorphs associated with mango

Identity ¹	Previously used name ²	Conidial size <i>in vitro</i> (µm)	L/W	Source of data ³
<i>B. parva</i> / <i>F. parvum</i>	<i>D. dominicana</i>	(12-)15-19(-24) × 4-6 [Ave. 16.9 × 5.4] 14.5-18.7(-17.1) × 4.6-7 [Ave. 17.1 × 4.7]	3.1 3.6	Pennycook and Samuels 1985 Johnson 1992
<i>B. dothidea</i> / <i>F. aesculi</i>	<i>D. aromatica</i>	(14.7-)17-21(-25.5) × 4.5-6(-7) [Ave. 19 × 5.2] (20-)23-27(-30) × 4-5(-6) [Ave. 24.7 × 4.9] 19.9-26.2 × 3.9-5.5 [Ave. 22.8 × 4.6] (18.8-)21-24(-30.4) × 4.5-6(-7) [Ave. 23 × 5.1] 9-13 × 3.5-5 10-16 × 3.5-6.5	3.7 5 4.9 4.5	This study Slippers et al 2004 Johnson 1992 This study Sydow et al 1916 Sutton and Dyko 1989
<i>F. mangiferum</i>	<i>D. mangiferum</i> or <i>N. mangiferum</i>	11-14 × 4.5-5.5 [Ave. 12.8 × 5] (11-)12-15(-17.3) × 5-6.6 [Ave. 13.6 × 5.4] 18.6-37.2 × 4.6-7 [Ave. 22.4 × 5] (20.2-)23-30(-35.5) × (4.7-)5.5-7 [Ave. 26.6 × 6]	N/a 2.6 2.5 4.5 4.4	Johnson 1992 This study Johnson 1992 This study
<i>Fusicoccum</i> sp.	<i>D. 'long'</i>			

¹ Teleomorph (where known) and anamorph names as used in this study.

² Common anamorph name used for this taxon from mango in earlier literature.

³ The isolates studied in this study and by Johnson (1992) are the same, but measurements from both studies are given because of the difference in the technique used to induce sporulation.

region. Clade VIII and IX represent *Botryosphaeria* spp. with *Diplodia*-like conidia.

TAXONOMY

Four botryosphaeriaceous fungi were identified in this study from mango. Two species have known teleomorphs, *B. parva* and *B. dothidea*. Johnson (1992) provisionally suggested a new combination in the genus *Fusicoccum* for the fungus reported as *Dothiorella mangiferae* or *Natrassia mangiferae* from mango and other hosts. That proposal is supported by molecular and morphological data obtained in this study and a new combination is proposed formally here. The fourth distinct species is identified only as a species of *Fusicoccum*.

1. *Botryosphaeria parva* Pennycook & Samuels, Mycotaxon 24:455. 1985.

Anamorph. Fusicoccum parvum Pennycook & Samuels, Mycotaxon 24:455. 1985.

FIG. 2

Notes. The anamorph state of *B. parva* has been identified commonly from mango as *D. dominicana*. The conidia from putative *D. dominicana* isolates collected from mango in Australia (Johnson 1992) are identical to those reported from the type of *F. parvum* (Pennycook and Samuels 1985, Slippers et al 2004). The data from these studies (TABLE II) show that this taxon can be distinguished from other botryosphaeriaceous fungi on mango by conidial characteristics. The most recognizable characteristics of these conidia are that they are aseptate, hyaline, granular, broadly ellipsoid to fusoid, on average 17-19 × 5-6 µm (see KEY). Older, discharged conidia sometimes become 1-2-septate and light brown with darker middle cells. Septate conidia with distinctly darker middle cells in this fungus have been confused with *N. mangiferae* and *D. mangiferae* (Sutton and Dyko 1989, Roux 1993).

Although the mango isolates identified as *D. dominicana* are here conspecific with *B. parva*, the true identity of the type of *D. dominicana* remains unclear. The dimensions reported in the original description of *D. dominicana* from mango leaves by Petrak and Ciferri (1930) fall within the range of *F. parvum*. Johnson (1992) re-examined and described the type material of *D. dominicana*, which he considered to be synonymous with *B. dothidea*. The conidia reported by Johnson (1992) from the *D. dominicana* type material, however, are smaller than the anamorphs of either *B. dothidea* or *B. parva*. Despite this uncertainty, it is clear that the name *D. dominicana* is not appropriate for isolates associated with stem-end rot and other diseases of mango in Australia.

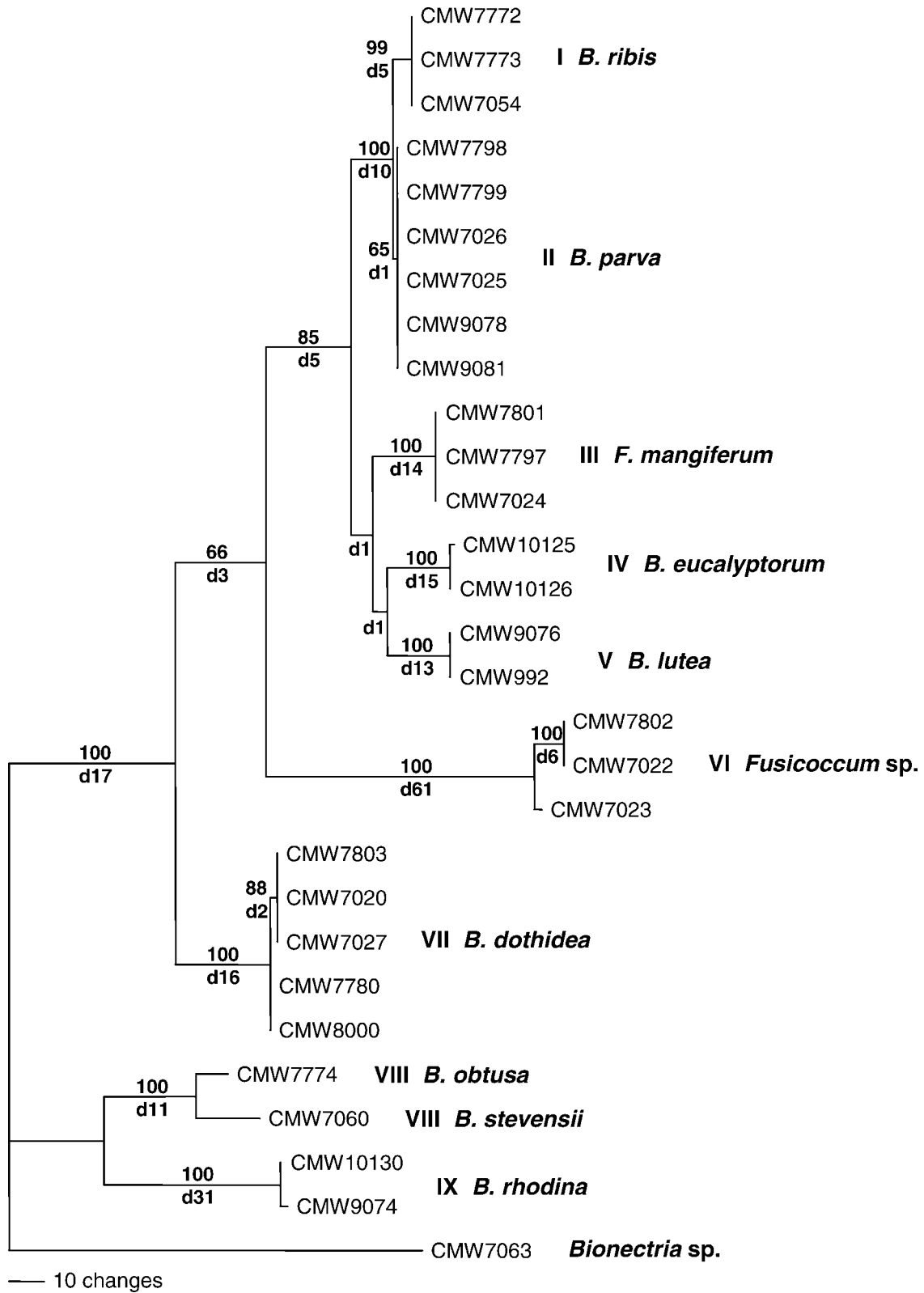


FIG. 8. One of the two equal, most parsimonious trees obtained by heuristic searches of the ITS rDNA and β -tubulin sequence datasets in PAUP. Branch supports are indicated by decay indices below and bootstrap values above the branches. Nine clades or taxa are identified. Clade VI represents an unknown *Fusicoccum* sp. (previously identified as *Dothiorella* 'long').

2. **Fusicoccum mangiferum** (Syd. & P. Syd.) Johnson, Slippers & M.J. Wingf. comb. nov. FIGS. 3–5

Basionym. *Dothiorella mangiferae* Syd. & P. Syd., In H. Sydow, P. Sydow & Butler, Ann. Mycol. 14:192. 1916.

Synonyms. *Natrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko, Mycol. Res. 93:484. 1989.

Hendersonula toruloidea Natrass, Trans. Br. Mycol. Soc. 18: 197. 1933.

Hendersonula cypria Natrass, Cypress fungi, Nicosia: 43. 1937.

Synanamorph. *Scytalidium dimidiatum* (Penz.) B. Sutton & Dyko, Mycol. Res. 93:484. 1989.

Teleomorph. **Botryosphaeria** sp.

Notes. Various morphological descriptions have been given for this taxon. The species was described first by Sydow et al (1916) from *M. indica* in India. Sutton and Dyko (1989) studied the holotype and the types of *H. toruloidea* connected with this taxon, including the holotype, and provided a very thorough description. Other clear descriptions are found in Natrass (1933), Punithalingam and Waterston (1970) and Johnson (1992).

Duplication of previous descriptions is avoided here, but the most distinctive features are highlighted. The conidia of *F. mangiferum* are distinct from other *Fusicoccum* spp. by their shorter average length (~13–14 µm) and smaller length/width ratio (2–2.5) (see KEY). The conidia often become 1- or 2-septate, light brown with distinctly darker middle cells. This feature is shared also with *F. parvum*. *Fusicoccum mangiferum* produces vegetative, toruloid cells in culture and in nature. This species also produces fluffy, evenly gray-colored aerial mycelium, lacking the white tufts found in other similar species such as *F. parvum*.

Sydow et al (1916) described *D. mangiferae* from mango but noted only the aseptate conidia. Re-examination of the type material, however, confirmed the presence of 1–2-septate, pigmented conidia (Sutton and Dyko 1989). It is possible that the spores on the type material aged and became septate after the description by Sydow. Natrass (1933) studied the taxon from pome and stone fruit trees and first noticed the pigmented conidia, which led him to describe it as *Hendersonula toruloidea* Natrass. He also studied the fungus in culture and noted the characteristic brown 1- or 2-celled, toruloid, vegetative cells. Sutton and Dyko (1989) synonymized both *D. mangiferae* and *H. toruloidea* with *Natrassia mangiferae*.

Natrass (1933) and Sutton and Dyko (1989) reported fragmented mycelial cells or toruloid cells in culture and in nature. In the last named study, this form was described as the synanamorph *Scytalidium dimidiatum*. Johnson (1992) reported no toruloid state but referred only to these cells as fragmented

mycelia and he did not use the last named epithet. The cells described by Natrass (1933) and Sutton and Dyko (1989) rarely were observed in this study and when seen, resembled fragmented, thick-walled hyphae.

Sutton and Dyko (1989) reduced *Fusicoccum eucalypti* Sousa da Câmara and *H. agathi* to synonymy with *F. mangiferum* (as *N. mangiferae*). These synonymies are not accepted here because the conidia of both taxa differ from those of *F. mangiferum* in length and in length/width ratio (Young 1948, Sutton and Davison 1983, Sutton and Dyko 1989). The conidial sizes reported in these studies for *F. eucalypti* and *H. agathi* were more similar to those of *F. parvum*.

The teleomorph of *F. mangiferum* is a *Botryosphaeria* sp. The DNA sequence data presented here group this species with the type species *B. dothidea* and other *Botryosphaeria* sp. Johnson (1992) also reported *Botryosphaeria* ascomata and ascospores forming in cultures of *F. mangiferum* (Johnson 1992). Sufficient material, however, was not available to describe formally a specific name for the teleomorph.

3. **Botryosphaeria dothidea** (Moug. : Fr.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1:212. 1863.

Anamorph. **Fusicoccum aesculi** Corda in Sturm, Deutschl. Fl., Abth. 3, 2:111. 1829. FIG. 6

Notes. Previous reports of this fungus from mango and avocado were misidentified as *D. aromatica*. Conidial morphology of this species as described from mango is similar to that described for the anamorph of *B. dothidea* (Pennycook and Samuels 1985, Slippers et al 2004). The most distinctive feature of this taxon is its conidia, which are aseptate, hyaline, fusiform to narrowly fusiform and on average 23–25 × 4–5 µm (see KEY).

The type specimen of *D. aromatica* (Sacc.) Petr. & Syd. (= *Macrophoma aromatica* Sacc.) (Saccardo 1915, Petrak and Sydow 1927) was obtained from PAD (497) on *Persea gratissima* (avocado) leaves. The conidia are fusiform, aseptate and 20–22 × 6–7 µm and most closely resemble those of *F. luteum* (Pennycook and Samuels 1985, Phillips et al 2002). *Fusicoccum luteum* also has been reported from avocado (Hartill 1991, Johnson 1992). We, therefore, consider *D. aromatica* to be a synonym of *F. luteum*. Although the type specimen of *D. aromatica* does not resemble *F. aesculi* (Crous and Palm 1999, Slippers et al 2004), the fungus occurring as a pathogen of mango and generally misidentified as *D. aromatica* is *F. aesculi*.

4. *Fusicoccum* sp.

FIG. 7

Notes. Johnson et al (1991a) and Johnson (1992) identified an unknown *Dothiorella* or *Fusicoccum* sp. from mango in Australia and Thailand, which was referred to only as *Dothiorella* 'long'. DNA sequence and morphological data presented here confirm that isolates defined as *Dothiorella* 'long' represent one, probably undescribed *Fusicoccum* sp.

Johnson (1992) considered this *Fusicoccum* sp. as possibly synonymous with *F. cajani* (teleomorph = *B. xanthocephala*). Samuels and Singh (1986) described *F. cajani* causing stem canker in *Cajanus* spp. (pigeon pea) from Fiji, India and the USA. Conidial measurements of *F. cajani* [(17–)21.6–27.8(–32) × (5–)6.5–8(–9) μm] and the *Fusicoccum* sp. (average = 26.6 × 6 μm, see TABLE II) considered here, overlap. This alone, however, is not sufficient evidence for synonymy. For example, the measurements and the shape of the conidia of *F. cajani* overlap with the anamorphs of a number of other *Botryosphaeria* spp., such as *B. lutea* (Pennycook and Samuels 1985, Phillips et al 2002), *B. eucalyptorum* (Smith et al 2001), *B. protearum* Denman and Crous (Denman et al 2003) and others. Given differences in hosts and diseases, it is unlikely that *Dothiorella* 'long' is *F. cajani*. No isolates of *B. xanthocephala* could be located to further test this hypothesis, using molecular or cultural characters.

KEY TO BOTRYOSPHAERIA SPP. AND THEIR ANAMORPHS FROM MANGO IN AUSTRALIA

Conidial characters are used to separate the botryosphaeriaceous fungi treated here. The anamorph is encountered most frequently in nature and also is induced readily in vitro on nutrient-poor medium (e.g., water agar) supplemented with sterilized pine needles. Differences among the species are more pronounced in anamorph than teleomorph features. Teleomorphs have not been described or observed for all the species treated here, but teleomorph names are used preferentially where they are known. The unnamed species of *Fusicoccum* refers to the fungus previously known as *Dothiorella* 'long'.

1. Conidia in culture on average <20 μm in length, l/w 2–3.5, occasionally becoming light brown and 1–2-septate with a darker brown middle cell after discharge, colony on MEA or PDA thick felt of gray aerial mycelium 2
1. Conidia in culture on average >20 μm in length, l/w >4, colony on MEA or PDA appressed with only occasional tufts of gray to buff aerial mycelium 3
2. No toruloid cells; conidia 12–23 × 4–6 μm (average 19 × 5.2 μm), l/w 3–3.5 *B. parva*
2. Toruloid cells; conidia 12–14 × 4–6 μm (average 13.6 × 5.4 μm), l/w 2–3 *F. mangiferum*
3. Conidia rod-shaped, 20–32 × 5–7 μm (average 26.6 × 6 μm), l/w 3.5–4.5 *Fusicoccum* sp.

3. Conidia narrowly fusiform, 19–30 × 4–6 μm (average 23 × 5.1 μm), l/w 4–5 *B. dothidea*

DISCUSSION

Four *Fusicoccum* spp. were identified as endophytes and pathogens of Australian mango fruit and trees in the current study. Identification of these species is based on a combination of morphological and molecular phylogenetic analyses. These species are *F. parvum* (teleomorph *B. parva*), *F. mangiferum*, *F. aesculi* (teleomorph *B. dothidea*) and an undescribed *Fusicoccum* sp. They all were identified previously from mango as species of *Dothiorella* or *Natrassia*. This study shows that all are species of *Fusicoccum* and that their teleomorphs are most likely *Botryosphaeria*.

The description of *Dothiorella* spp. from mango as *Fusicoccum* spp. is in accordance with recent proposals for the correct use of these two generic names (Crous and Palm 1999, Denman et al 2000). *Fusicoccum* and *Dothiorella* often have been confused because both have been used commonly to describe anamorphs of *Botryosphaeria* (Saccardo 1882, Petrak 1922, von Arx and Müller 1954). The common use of the name *Dothiorella* for suspected *Botryosphaeria* anamorphs from mango follows Sydow et al (1916) and Petrak (1922). *D. pyrenophora* Sacc., the type species of *Dothiorella*, recently was redescribed as *Diplodia pyrenophora* (Sacc.) Crous & M.E. Palm (Crous and Palm 1999). These authors suggested that all *Botryosphaeria* anamorphs that are placed in *Dothiorella* should be re-examined. Denman et al (2000) argued that all hyaline, thin-walled fusiform conidial *Botryosphaeria* anamorphs are *Fusicoccum*.

Results of this study and those of Johnson (1992) show clearly that *B. parva* (reported as *D. dominicana*) is one of the most common pathogens of mango causing fruit stem-end rot, dieback and blossom blight. The species first was described by Pennycook and Samuels (1985) from *Populus*, *Malus* and *Actinidia* species in New Zealand. It subsequently was shown that this species occurs worldwide on a number of hardwood species, including native Australian flora such as *Eucalyptus* spp. (Slippers et al 2004). *Botryosphaeria parva* often has been misidentified as *B. ribis* and *B. dothidea* due to overlapping host ranges, morphological similarities and taxonomic confusion over the use of the names (Slippers et al 2004). Thus it also is likely that the fungus described as *B. ribis* from mango in Florida (Ramos et al 1991) is *B. parva*. These identifications from Florida were based on conidial dimensions, which overlap between *B. ribis* and *B. parva* (Slippers et al 2004).

The name *F. mangiferum* has been proposed in this study for the mango pathogen that previously was

identified as *D. mangiferae* and *Natrassia mangiferae*. Johnson (1992) first suggested that *D. mangiferae* and *N. mangiferae* should be placed in *Fusicoccum*. This proposal is supported in the current study by the phylogenetic monophyly of this taxon with the type species *F. aesculi* and other *Fusicoccum* spp. Isolates used for sequence analyses were not ex-type cultures. The conidia of these isolates were, however, morphologically indistinguishable from the type specimens of *D. mangiferae* (Sutton and Dyko 1989, Sydow et al 1916).

The septation and pigmentation of conidia of *F. mangiferum* and *F. parvum* have obvious similarities. This has led to confusion between these taxa in the past. These species, however, can be separated by conidial size because conidia of the former species are smaller in average length and width. Moreover in culture *F. parvum* has fluffier aerial mycelium than the appressed gray aerial mycelium of *F. mangiferum*.

Botryosphaeria dothidea is of little importance as a pathogen of mango in Australia or other parts of the world. It is less common on mango than *B. parva* and *F. mangiferum* and is omitted often from lists of important pathogens of this host (Johnson et al 1991a, b, 1992; Johnson 1992). This name, however, is one of the most commonly used for *Botryosphaeria* pathogens on a wide variety of other hosts (McGlohon 1982, Pennycook and Samuels 1985, Brown and Britton 1986, Hartill 1991, Jacobs and Rehner 1998, Smith et al 2001). Some of these identifications need to be viewed with care because many species have been relegated incorrectly to the name *B. dothidea*. This followed the extensive synonymy of many species with *B. dothidea* by von Arx and Müller (1954). Due to this synonymy, *B. ribis* was treated as a synonym of *B. dothidea*. *Botryosphaeria parva* often was not distinguished from *B. ribis* and consequently was treated also under *B. dothidea* (Slippers et al 2004).

Reports of *B. dothidea* from Australasia and other Southern Hemisphere countries are from exotic hosts (Pennycook and Samuels 1985, Hartill 1991, Slippers et al 2004). Studies of pathogens of native hosts in Australasia have not reported this pathogen (Denman et al 2003). This species, however, is common on both cultivated and indigenous hosts in the Northern Hemisphere (Zhou and Stanosz 2001, Slippers et al 2004). This suggests a Northern Hemisphere origin for this fungus and implies that it was introduced into the Southern Hemisphere with planting material of agricultural crops.

The taxon previously known from mango as *Dothiorella* 'long' is identified in this study as an undescribed species of *Fusicoccum*. This species was found rarely in extensive surveys during previous studies and is not considered important in causing pre- or

postharvest diseases of mango (Johnson et al 1991a, Johnson 1992). This species of *Fusicoccum* is not known from any other hosts. Further collections and studies are needed to understand the distribution and biology of this fungus.

Johnson (1992) suggested that *F. luteum* (teleomorph *B. lutea*) occurs on mango in Australasia. None of the *Fusicoccum* spp. from mango in Australia group with this taxon, based on DNA sequence data produced in this study. This finding is surprising because *F. luteum* seems to be common in Australasia. *Fusicoccum luteum* initially was described from *Actinidia*, *Malus* and *Pyrus* in New Zealand (Pennycook and Samuels 1985) and subsequently also from avocado (Hartill 1991).

Sequence variation was observed among isolates of clade VI (*Fusicoccum* sp.) and clade VII (*B. dothidea*) that was not phylogenetically informative. Among the three isolates identified in clade VI one isolate had sequence variation only in the β -tubulin region. In clade VII the three isolates from mango grouped together based on two variable bases in the ITS region. In both cases these variable characters thus were found only in one of the two sequenced regions. Additional data and a larger number of isolates are required to determine the extent of variation and its phylogenetic relevance to populations of the above clades.

The many misidentifications of botryosphaeriaceous fungi from mango illustrate aptly how confusing morphological characterization of these fungi has been. This problem resulted from the fact that continuous characters for these species overlap. The confusion was amplified by differences in morphological characters from nature and from culture (Slippers et al 2004, Johnson 1992). Furthermore, conidial septation and color, which have been used to characterize species, was not always consistent. Conidia tend to age only after discharge from the pycnidia and their color and septation changes with age.

This study provides a basis on which future identifications of *Botryosphaeria* and its anamorphs from mango can be made. The combination of molecular data and average conidial size and shape, as well as cultural characteristics, has been used successfully here to identify these fungi from mango. Correct identifications of these pathogens are crucial due to increased quarantine requirements. These data also will help studies for a better understanding of the epidemiology of the different fungal species.

ACKNOWLEDGMENTS

We thank the National Research Foundation (NRF), Mellon Foundation, members of the Tree Pathology Co-operative

- Shoemaker RA. 1964. Conidial states of some *Botryosphaeria* species on *Vitis* and *Quercus*. *Can J Bot* 42:1297–1301.
- 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.
- Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD. 2001. *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* 93:277–284.
- Smith DR, Stanosz GR. 2001. Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. *Mycologia* 93:505–515.
- Stevens NE. 1926. Occurrence of the currant cane blight fungus on numerous hosts in the southern states. *Mycologia* 18:278–282.
- Sutton BC, Davison EM. 1983. Three stromatic Coelomycetes from Western Australia. *Trans Br Mycol Soc* 81:291–301.
- Sutton BC, Dyko BJ. 1989. Revision of *Hendersonula*. *Mycol Res* 93:466–488.
- Swofford DL. 1999. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Sydow H, Sydow P, Butler EJ. 1916. Fungi Indiae Orientalis. V. *Ann Mycol* 14:177–220.
- von Arx JA, Müller E. 1954. Die Gattungen der amerosporen Pyrenomyceten. *Beitr Kryptogamenfl Schweiz* 11(1):1–434.
- White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press. p 315–322.
- Young HE. 1948. *Hendersonula agathi* n. sp., the cause of leaf cast of Kauri pine in Queensland. *Queensland J Agric Sci* 5:9–12.
- 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.