available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/mycres

# Two new *Fusicoccum* species from *Acacia* and *Eucalyptus* in Venezuela, based on morphology and DNA sequence data

*Sari MOHALI†, Bernard SLIPPERS*, Michael J. WINGFIELD*

*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences, University of Pretoria, 74 Lunnon Road, Hillcrest, Pretoria 0002, South Africa*

ARTICLE INFO

*Article history:*
Received 8 April 2005
Received in revised form
17 December 2005
Accepted 2 January 2006
Published online 18 April 2006
*Corresponding Editor:*
David L. Hawksworth

*Keywords:*
*Botryosphaeria*
Molecular phylogenetics
Tree pathology

ABSTRACT

*Botryosphaeria* spp. are common endophytes of woody plants, and they also include some serious pathogens of *Eucalyptus* and *Acacia* species. Numerous anamorphs have been associated with *Botryosphaeria*, of which the species *Fusicoccum* are amongst the most common. Here, we characterize two new *Fusicoccum* species, isolated from *Eucalyptus* and *Acacia* trees in Venezuela, based on morphological features in culture and comparisons of DNA sequence data. The two taxa named *Fusicoccum andinum* and *F. stromaticum* spp. nov, reside in two well-supported clades (BS values = 100 %) based on a combined data set of the ITS of the rDNA operon and translation elongation factor 1-α (EF1- α) gene sequences. The conidia of *F. andinum* are unusually large amongst *Botryosphaeria* anamorphs, and peripherally resemble those of *B. mamane* and *B. melanops*. *F. stromaticum* is characterized by large conidiomata in cultures, growth at 35 °C and slightly thickened conidial walls, which is different to most other *Fusicoccum* spp. No teleomorphs were observed for these fungi, but DNA sequence data show that they are anamorphs of *Botryosphaeria*.



## Introduction

The genus *Botryosphaeria* is cosmopolitan and its species occur on a wide range of monocotyledonous, dicotyledonous, and gymnosperm hosts. *Botryosphaeria* spp. infect the stems, branches and twigs, leaves of many woody plants, and they have also been found in the stems of grasses and thalli of lichens (Barr 1987). These fungi include opportunistic pathogens that give rise to symptoms such as such shoot blights, stem cankers, fruit rots, die-back, and gummosis (von Arx 1987).

The taxonomy of *Botryosphaeria* has been confused for many years. This is mainly due to the similar morphology of the teleomorphs (Jacobs & Rehner 1998; Slippers *et al.* 2004a). Host association has been used to assign names to species, but this has led to confusion because some species are host specific, whilst others are generalists (Jacobs & Rehner 1998; Crous & Palm 1999; Smith *et al.* 2001; Smith & Stanosz 2001; Slippers *et al.* 2004a).

The anamorphs of *Botryosphaeria* species are generally encountered in culture and on diseased plant parts. For this reason, identification of *Botryosphaeria* spp. has commonly been based on conidial morphology of the anamorphs (Jacobs & Rehner 1998; Smith & Stanosz 2001; Smith *et al.* 2001; Phillips *et al.* 2002; Slippers *et al.* 2004a, d).

Conidial characters considered to be useful for the taxonomic delimitation of *Botryosphaeria* anamorphs are size, colour, septation, wall thickness and texture, as well as the presence of microconidia and mode of conidiogenesis (Sutton

* Corresponding author.
E-mail addresses: bernard.slippers@fabi.up.ac.za, msari@ula.ve
† Present address: Laboratorio Nacional de Productos Forestales, Sección de Patología Forestal, Facultad de Ciencias Forestales y Ambientales, Universidad de los Andes, Mérida 5101-A, Venezuela.
0953-7562/$ – see front matter 
doi:10.1016/j.mycres.2006.01.006

1980; Sivanesan 1984; Pennycook & Samuels 1985). However, these characters require careful interpretation, as there is substantial overlap between many species. Thus, conidial size represents a continuous character and it is also variable between isolates and may change with age or on different substrates and hosts (Pennycook & Samuels 1985; Butin 1993; Crous & Palm 1999; Slippers *et al.* 2004a).

In recent years, analyses of DNA sequence data have contributed substantially towards resolving taxonomic questions in *Botryosphaeria*. Nucleotide sequences of the ITS region have in particular been used to resolve phylogenetic relationships between species, and these have been combined with morphological characters (Jacobs & Rehner 1998; Denman *et al.* 2000; Zhou & Stanosz 2001; Phillips *et al.* 2002; Alves *et al.* 2004; Slippers *et al.* 2004a).

*Botryosphaeria* spp. occur on various forestry and agricultural crops in Venezuela, but very little attention has been given to their identity. *Lasiodiplodia theobromae*, *Diplodia pinea* (syn. *Sphaeropsis sapinea*), *D. mutila*, and a species of *Dothiorella* have been identified as the disease causing agents (Cedeño *et al.* 1994, 1996; Mohali 1997; Mohali & Encinas 2001; Mohali *et al.* 2002).

The aim of this study was to characterize two *Fusicoccum* spp. commonly isolated from *Eucalyptus* and *Acacia* trees in Venezuela, and which appeared to be undescribed. These fungi were thus studied based on morphology and a comparison of DNA sequences data for the ITS rDNA (ITS 1 and ITS 2) and translation elongation factor 1-α (EF1-α).

## Materials and methods

### *Fungal isolation*

A survey was conducted during 2003 in plantations of *Eucalyptus urophylla*, an unidentified *Eucalyptus* sp., a *Eucalyptus* hybrid, and *Acacia mangium*. Isolations were made from twigs, stems and branches displaying symptoms of blue stain, dieback and from dead trees. Single conidial isolates were obtained after cultures were induced to sporulate on water agar to which sterile pine needles had been added.

For isolations, plant tissues were surface disinfested with 70 % ethanol for 30 s and thereafter rinsed in sterile water for 1 min. Small tissue pieces (4–5 mm) were cut from the plant tissue and placed on 2 % malt extract agar (MEA; DIFCO, Becton Dickinson, MD) and incubated at 25 °C. Cultures resembling *Botryosphaeria* spp. were transferred to water agar (WA; 2 % Biolab agar, Midrand, South Africa) with sterilized pine needles placed on the agar surface and these were incubated for 3–6 wk at 25 °C under a combination of near-UV and cool-white fluorescent light to induce sporulation. All isolates used in this study are maintained in the collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and representative isolates have also been deposited in CBS (Utrecht).

### *DNA isolation and amplification*

DNA was extracted from isolates of unknown identity (Table 1) using the technique described by Slippers *et al.* (2004a). The quantification of nucleic acids was made using a spectrophotometer (Eppendorf, Hamburg) with a radio of absorbance at 260 nm and 280 nm.

The DNA extraction was used as template to amplify part of the nuclear rRNA operon in PCR reactions using the primers ITS1 and ITS4 (White *et al.* 1990). The amplified fragments included the 3′ end of the small subunit (SSU) rRNA gene, ITS1 region, the complete 5.8 S rRNA gene, the ITS2 region and the 5′ end of the LSU rRNA gene. A part of the EF1-α was amplified using the primers EF1-728F and EF1-986R (Carbone *et al.* 1999). The PCR reaction mixtures and conditions were described in Slippers *et al.* (2004a), except that the PCR annealing temperatures varied between 52–60 °C for EF 1-α region. Extracted DNA (20–25 ng $\mu l^{-1}$) was used as template in the reactions. PCR amplicons were separated on 1.5 % (w/v) agarose gels, stained with ethidium bromide and visualized under UV-light. The sizes of the PCR amplicons were estimated using DNA molecular weight marker XIV (100 bp ladder) (Roche Molecular Biochemicals, Almeda, California).

### *Sequence analysis*

In all 27 isolates were used in the phylogenetic analysis (Table 1). BLAST searches were done to determine whether any related sequences are present in GenBank, but none were found that were more closely related to the test isolates than those chosen for comparison here. The trees were rooted to sequence data of an isolate of a *Bionectria* sp., which was included as an outgroup taxon in the analysis of 30 ingroup taxa.

PCR amplicons were purified and sequenced as described in Slippers *et al.* (2004a), except that products were run on an ABI PRISM 3100 automated sequencer (Perkin-Elmer Applied BioSystems, Foster City, California, USA). Sequence data were analysed using Sequence Navigator version 1.0.1™ (Perkin-Elmer Applied BioSystems, Foster City, California, USA) and manually aligned by inserting gaps. Gaps were treated as a fifth character and all characters were given equal weight. Phylogenetic analyses were done using PAUP version 4.0b8 (Swofford 1999). Heuristic searches were done using random stepwise addition tree bisection and reconstruction (TBR) as branch swapping algorithm for the construction of maximum parsimonious trees. One thousand BS replicates (Felsenstein 1985) were run to determine the confidence intervals of branching points on the shortest tree. Branches with a length of zero were collapsed and all multiple equally parsimonious trees were saved. Levels of homoplasy (retention and consistency indices) (Hillis & Huelsenbeck 1992) were determined.

### *Morphological characterization*

Conidial morphology was studied using a light microscope with an Axiocam digital camera and software to analyse photographs (Carl Zeiss, Jena). Sections through some of the pycnidia and stromatal structures were made with a Leica CM100 cryostat (Leica, Wetzlar). Length, breadth, shape and colour of the conidia were recorded after mounting in clear lactophenol. At least 50 conidia of each isolate of two different *Fusicoccum* spp. were measured.

The growth of selected isolates was determined by placing mycelial discs (5 mm diam) at the centres of MEA plates, with

**Table 1 – Isolates used in the phylogenetic study**

| Culture[a] | Other[a] | Identity[b] | Host | Location | Isolator | GenBank accession no. | |
|---|---|---|---|---|---|---|---|
| | | | | | | ITS | EF1-α |
| CMW7780 | | *Botryosphaeria dothidea* | *Fraxinus excelsior* | Molinizza, Switzerland | *B. Slippers* | AY236947 | AY236896 |
| CMW7999 | | *B. dothidea* | *Ostrya* sp. | Crosifisso, Switzerland | *B. Slippers* | AY236948 | AY236897 |
| CMW8000 | CBS 115476 | *B. dothidea* | *Prunus* sp. | Crosifisso, Switzerland | *B. Slippers* | AY236949 | AY236898 |
| CMW9077 | ICMP 7924 | *B. parva* | *Actinidia deliciosa* | New Zealand | *S. R. Pennycook* | AY236939 | AY236884 |
| CMW9078 | ICMP 7925 | *B. parva* | *A. deliciosa* | New Zealand | *S. R. Pennycook* | AY236940 | AY236885 |
| CMW9079 | ICMP 7933 | *B. parva* | *A. deliciosa* | New Zealand | *S. R. Pennycook* | AY236941 | AY236886 |
| CMW9080 | ICMP 8002 | *B. parva* | *Populus nigra* | New Zealand | *G. J. Samuels* | AY236942 | AY236887 |
| CMW9081 | ICMP 8003 | *B. parva* | P. nigra | New Zealand | *G. J. Samuels* | AY236943 | AY236888 |
| CMW7772 | CBS 115475 | *B. ribis* | *Ribes* sp. | New York, USA | *B. Slippers & G. Hudler* | AY236935 | AY236877 |
| CMW7773 | | *B. ribis* | *Ribes* sp. | New York, USA | *B. Slippers & G. Hudler* | AY236936 | AY236878 |
| CMW7054 | CBS 121.26 | *B. ribis* | *Ribes* sp. | New York, USA | *N. E. Stevens* | AF241177 | AY236879 |
| CMW9076 | ICMP 7818 | *B. lutea* | *Malus* × *domestica* | New Zealand | *S. R. Pennycook* | AY236946 | AY236893 |
| CMW992 | KJ93.52 | *B. lutea* | *A. deliciosa* | New Zealand | *G. J. Samuels* | AF027745 | AY236894 |
| CMW7801 | | *Fusicoccum mangiferum* | *Mangifera indica* | Australia | *G. I. Johnson* | AY615187 | AY615174 |
| CMW7024 | BRIP 24101 | *F. mangiferum* | *M. indica* | Australia | *G. I. Jonnson* | AY615185 | AY615172 |
| CMW10125 | CBS 115791 | *B. eucalyptorum* | *Eucalyptus grandis* | Mpumalanga, South Africa | *H. Smith* | AF283686 | AY236891 |
| CMW10126 | | *B. eucalyptorum* | *E. grandis* | Mpumalanga, South Africa | *H. Smith* | AF283687 | AY236892 |
| CMW13446 | CBS 117452 | *F. andinum*[b] | *Eucalyptus* sp. | Mérida state, Venezuela | *S. Mohali* | DQ306263 | DQ306264 |
| CMW13455 | CBS 117453 | *F. andinum*[b] | *Eucalyptus* sp. | Mérida state, Venezuela | *S. Mohali* | AY693976 | AY693977 |
| CMW13434 | CBS 117448 | *F. stromaticum*[b] | *Eucalyptus* hybrid | Cojedes state, Venezuela | *S. Mohali* | AY693974 | AY693975 |
| CMW13435 | CBS 117449 | *F. stromaticum*[b] | *Eucalyptus* hybrid | Cojedes state, Venezuela | *S. Mohali* | DQ436935 | DQ436936 |
| CMW7060 | CBS 431.82 | *B. stevensii* | *F. excelsior* | Netherlands | *H. A. van der Aa* | AY236955 | AY236904 |
| CMW7774 | | *B. obtusa* | *Ribes* sp. | New York, USA | *B. Slippers & G. Hudler* | AY236953 | AY236902 |
| CMW7775 | | *B. obtusa* | *Ribes* sp. | New York, USA | *B. Slippers & G. Hudler* | AY236954 | AY236903 |
| CMW9074 | | *B. rhodina* | *Pinus* sp. | Mexico | *T. Burgess* | AY236952 | AY236901 |
| CMW10130 | | *B. rhodina* | *Vitex donniana* | Uganda | *J. Roux* | AY236951 | AY236900 |
| CMW7063 | | *Bionectria* sp. | *Unknown* | Netherlands | *H. A. van der Aa* | AY236956 | AY236905 |

a Culture collections and isolates abbreviations: CMW, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria; CBS, Centraalbureau voor Schimmelcultures, Utrecht; ICMP, International Collection of Microorganisms from Plants, Auckland, NZ; BRIP, Plant Pathology Herbarium, Department of Primary Industries, Ql; KJ, Jacobs and Rehner (1998); isolates CMW 7999 and CMW 7772 are ex-type isolates.
b Identities determined in this study.

three replicate plates for each of three isolates for each of the two morphologically different *Fusicoccum* spp. Plates were incubated at temperatures ranging from 15–40 °C at 5 ° intervals. Two diameter measurements were taken perpendicular to each other after 4 d for each colony, and averages computed. Colony colours were determined using the colour charts of Rayner (1970).

## Results

### *Phylogenetic analyses*

The partition homogeneity test indicated that the ITS-rDNA (547 characters) and EF1-α (340 characters) sequence partitions were congruent and that the data sets could be combined ($P = 0.440$). This resulted in a final data set of 887 characters after alignment of which 284 characters were constant, 165 variable characters were parsimony uninformative and 426 were parsimony informative. Heuristic search analysis in PAUP of the sequence data resulted in one tree (CI = 0.753; RI = 0.857; HI = 0.247).

The isolates in the tree obtained from the combined data sets resided in 13 principal clades (I–XIII)(Fig 1). Isolates in clades I–X all have hyaline and thin-walled conidia and are thus *Fusicoccum*-like anamorphs. In contrast, isolates residing in clades XI–XIII all have pigmented and thick-walled conidia which can be referred to as *Diplodia*-like anamorphs (Denman *et al*. 2000; Slippers *et al*. 2004a). Isolates from Venezuela resided in clade I and clade IX were distinct from all other clades that included known *Botryosphaeria* spp. (Fig 1). The *Fusicoccum* sp. from Venezuela residing in clade I and *B. dothidea* (Slippers *et al*. 2004a) in clade X were each strongly supported (100 % BS) and distinct from other *Botryosphaeria* spp. with *Fusicoccum*-like anamorphs. The *Fusicoccum* sp. from Venezuela residing in clade IX (100 % BS support) was closely related to *B. parva* and *B. ribis* (clade V, II, III), *B. eucalyptorum* (clade IV), *B. eucalypticola* (clade V), *B. australis* (clade VI), *B. lutea* (clade VII) and *F. mangiferum* (clade VIII) (Phillips *et al*. 2002; Slippers *et al*. 2004b, c, d). *B. obtusa*, *B. stevensii* and *B. rhodina* (clades XI, XII, XIII), all with *Diplodia*-like anamorphs, formed a well-defined group (Alves *et al*. 2004; Punithalingam 1976).

### *Morphological characters*

The two unknown *Fusicoccum* spp. from *Acacia* and *Eucalyptus* in Venezuela and residing in distinct clades in the phylogenetic trees, produced conidiomata on sterilized pine needles at 25 °C on WA after 3 wk.

The colonies of *Fusicoccum* sp. isolated from *Eucalyptus* and *Acacia* in Portuguesa and Cojedes state (Table 1), grew rapidly at 30 °C, but produced little or no growth at extremes of 15 °C and 40 °C. This fungus produced few, but large conidiomata, on MEA. The conidia were hyaline, aseptate, bacilliform, and had thin to slightly thickened walls (Fig 2).

Colonies of the *Fusicoccum* sp. isolated from *Eucalyptus* spp. growing on the mountains in Merida state (Table 1), grew at 15 °C with an optimum growth temperature of 20–30 °C. Abundant pycnidia were produced on MEA at 25 °C (Fig 3A–B). Conidia were clavate to slightly navicular and large when compared with other *Fusicoccum* anamorphs (Table 2, Fig 3E–H, see Taxonomy).

## Taxonomy

Based on conidial morphology, cultural characteristics and DNA sequence phylogeny we conclude that the two *Fusicoccum* spp. from *Eucalyptus* and *Acacia* in Venezuela represent undescribed taxa. We thus provide the following descriptions of them here.

**Fusicoccum stromaticum** Mohali, Slippers & M. J. Wingf., **sp. nov.** (Fig 2)

*Etym*.: The name refers to the very large conidiomata on MEA at 25 °C.

*Conidiomata* magna in superficie MEA, multilocularia, eustromatica, cum hyphis tecta; *loculus* omnino inclusus sine ostiolis. *Cellulae conidiogenae* hyalinae, holoblasticae, cylindricae, conidium unicum apicale efferentes, primo holoblastice, dein enteroblastice. *Conidia* hyalina, parietibus tenuibus vel subincrassatis, non septata, granularia, bacilliformia, apice basique obtuse rotundata vel obtusa, (19–)20–23(–24) × (4–)5–6 μm.

*Typus*: **Venezuela**: *Portuguesa State*: Acarigua, Smurfit Company, on branches of *Eucalyptus urophylla*; Feb. 2003, *S. Mohali* (PREM 58237—holotypus; cultura viva CMW 13366).

*Cultures* fluffy, greenish olivaceous (23‘‘‘b) (surface) and olivaceous (21‘‘k) (reverse) after 15 d on MEA at 25 °C (Fig 2A). Colonies reaching 70–75 mm diam on MEA after 4 d in the dark at 25 °C. Cardinal temperatures for growth; min. 15 °C (little or no growth), max. 40 °C (no growth), optimum 30–35 °C. *Conidiomata* large, superficial on MEA (Fig 2A–B), multilocular, eustromatic, covered with hyphae; *locule* totally embedded without ostioles, locule walls consisting of a dark brown *textura angularis*, becoming thinner and hyaline towards the conidiogenous region (Fig 2C). *Conidiogenous cells* hyaline, holoblastic, smooth, cylindrical, producing a single apical conidium, the first conidium produced holoblastically and subsequent conidia produced enteroblastically (Fig 2D), (10–)11–15(–17) × (1.5–)2–3 μm (average of 50 conidiogenous cells 13 × 2.5 μm, l:b 5.33). *Conidia* hyaline, thin to slightly thickened walled, aseptate, granular, bacilliform, straight to slightly curved, apex and base both bluntly rounded or just blunt (Fig 2E), (19–)20–23(–24) × (4–)5–6 μm (average of 50 conidia 21.5 × 5.5 μm, l:b 4.01).

*Teleomorph*. Not observed, but expected to be a *Botryosphaeria* sp. based on phylogenetic analyses.

*Additional specimens examined*: **Venezuela**, *Portuguesa State*: Acarigua, Smurfit Company, on branches of *Eucalyptus urophylla* × *E. grandis* hybrids, Feb. 2003, *S. Mohali* (PREM 58506, 58507, 58508, 58509, 58510, 58511, 58512). *Cojedes State*: San Carlos, DEFORSA Company, on branches of *Eucalyptus* hybrids, Feb. 2003, *S. Mohali* (PREM 58516, 58517). *Portuguesa State*: Acarigua, Smurfit Company, on branches and stems of *Acacia mangium*, Feb. 2003, *S. Mohali* (PREM 58513, 58514, 58515).

**Fusicoccum andinum** Mohali, Slippers & M. J. Wingf., **sp. nov.** (Fig 3)

*Etym*.: Refers to the region where the species was isolated. Cordillera of Los Andes.

*Pycnidia* superficialia, copiose in siperficie MEA ad 25 °C facta, solitaria vel botryosa, stromatica, globosa, pariete pycnidii e textura angulari brunnea. *Cellulae conidiogenae* hyalinae, holoblasticae,

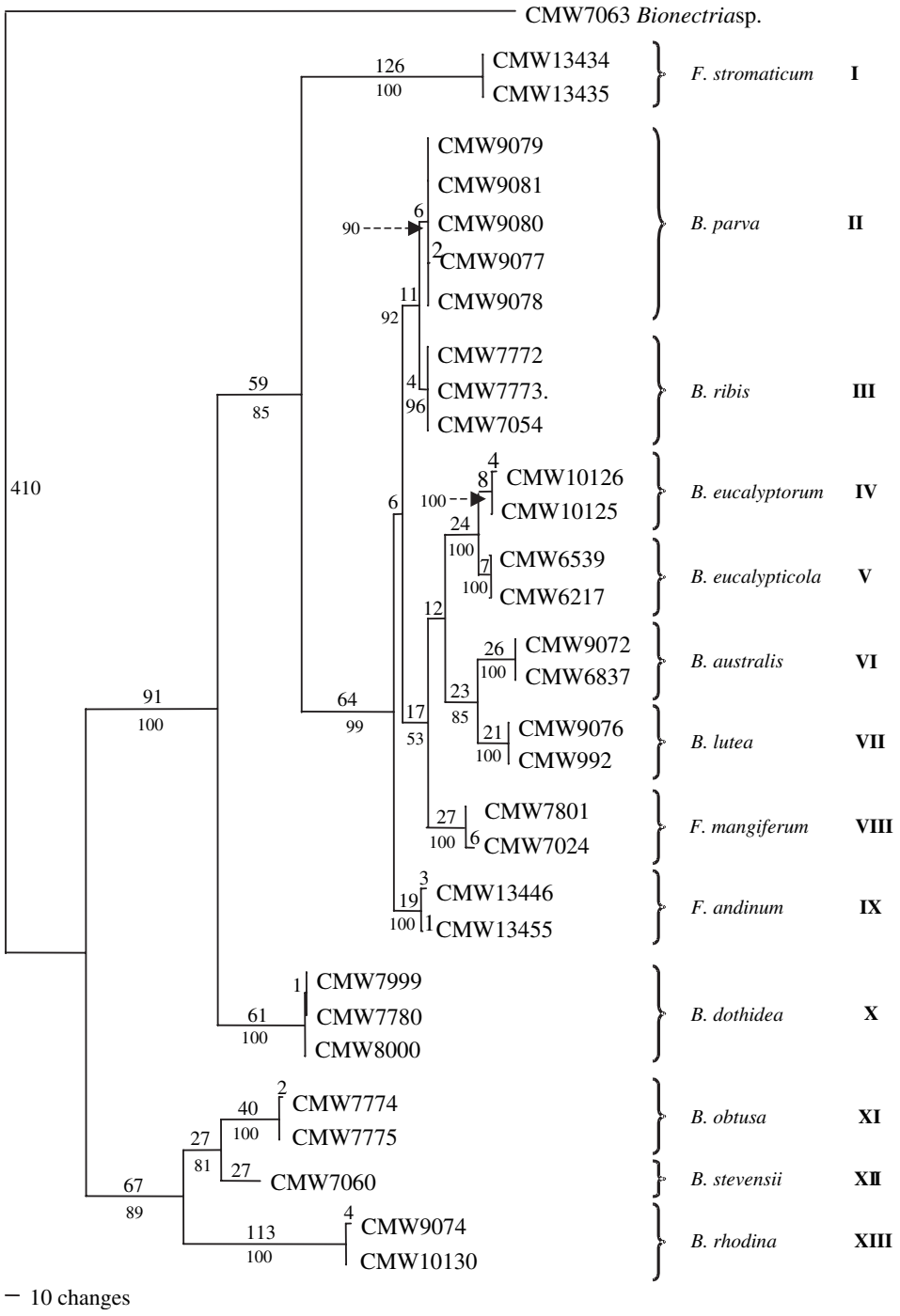


**Fig 1 – Phylogenetic relationships amongst *Fusicoccum andinum* and *F. stromaticum* and related species, based on the most parsimonious tree obtained through heuristic searches of the combined dataset of the ITS rDNA and EF1- α regions. The phylogram is rooted with the outgroup *Bionectria* sp. BS frequencies greater than 50 from 1000 replications of a heuristic search are indicated below internodes. Branch lengths proportional to the number of steps are indicated above internodes. Roman numerals indicate grouping of the different strains.**

cylindricae, conidium unicum apicale efferentes, conidium primum holoblasticum, dein conidia enteroblastica. *Conidia* hyalina, granularia, clavata vel subnavicularia, apice obtusa, basi truncata, sine septis vel cum septo uno, (19–)23–31(–40) × (4–)5–6(–8) μm.

*Typus*: **Venezuela**: *Merida State*: Merida; Mucuchies (3140 m), Cordillera of Los Andes, on branches of *Eucalyptus* sp., Feb. 2003, *S. Mohali* (PREM 58238 - holotypus; cultura viva CBS 117453).

*Cultures* fluffy and flat becoming pale olivaceous grey (21‘‘‘‘d) (surface) and olivaceous buff (21‘‘‘d) (reverse) 15 d after inoculation on MEA at 25 °C (Fig 3A), producing columns of mycelium reaching the Petri dish lid after 30 d at 25 °C. Colonies reaching 80 mm diam on MEA after 4 d in the dark at 25 °C. Cardinal temperatures for growth were min. 15 °C

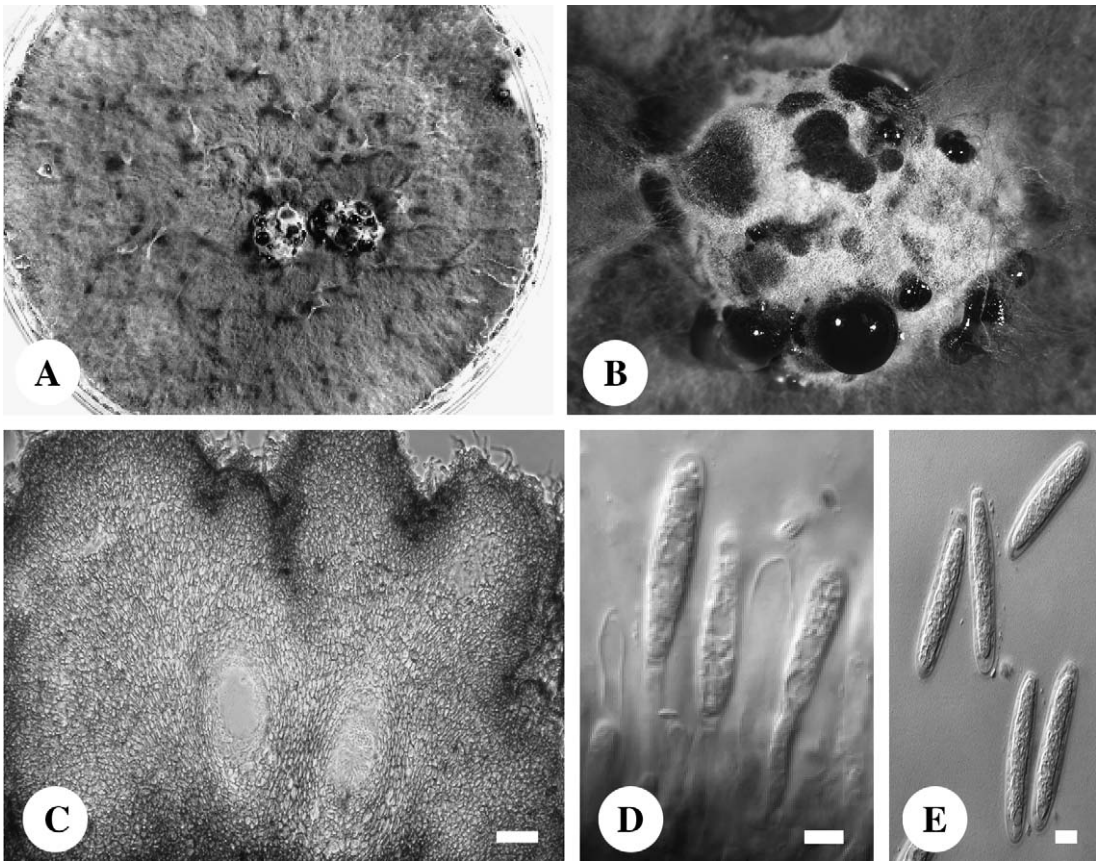


**Fig 2 – *Fusicoccum stromaticum*. A Culture with few conidiomata. B Big conidioma produced on 2 % MEA after 30 d at 25 °C. C Multilocular conidiomata without ostioles and embedded locule. Bar = 50 μm. D Conidiogenous cells and conidia. E Conidia with thin to slightly thickened walls. Bars = 5 μm.**

(reaching an average 24 mm diam), max. 35 °C (no growth), optimum 20–30 °C. *Pycnidia* superficial, produced abundantly on MEA surface at 25 °C (Fig 3A), oozing conida after 30 d at 25 °C on MEA (Fig 3B), solitary or botryose on the colonies, stromatic, globose (Fig 3C–E), (331–)374–597(–740) × (302–)339–557(–671) μm (average of 50 pycnidia 486 × 448 μm, l:b 1.08); pycnidial wall, composed of brown *textura angularis*, 6–8 cell layers thick. *Conidiogenous cells* hyaline, holoblastic, smooth, cylindrical, producing a single apical conidium, the first conidium holoblastic and subsequent conidia enteroblastic (Fig 3E), (8–)11–17(–23) × (1.5–)2–2.5(–3) μm (average of 50 conidiogenous cells 14 × 2 μm, l:b 6.62). *Conidia* hyaline, granular, clavate to slightly navicular, apex obtuse and base truncate, 0–1 septa (Fig 3F–H), (19–)23–31(–40) × (4–)5–6(–8) μm (average of 50 conidia 27 × 5.5 μm, l:b 4.84).

*Teleomorph*. Not observed, but expected to be a *Botryosphaeria* sp. based on phylogenetic analyses.

*Additional specimens examined*: **VENEZUELA**, *Merida State*: Merida, Mucuchies (3140 m) Cordillera of Los Andes, on branches of *Eucalyptus* sp., Feb. 2003, *S. Mohali* (PREM 58518, 58519, 58520, 58521, 58522, 58523, 58524, 58525, 58526, 58527, 58528, 58529, 58530, 58531, 58532).

## Discussion

Two new *Fusicoccum* spp. collected in Venezuela have been characterized here, based on both morphology and their unique DNA sequences. One of these, *F. andinum*, was isolated exclusively from *Eucalyptus* spp. at high altitude sites, whereas *F. stromaticum* was from both *Eucalyptus* spp. and *Acacia* spp. at lower altitude sites in Venezuela. To the best of our knowledge, these are the first two species of *Fusicoccum* to be described from Venezuela.

*F. andinum* was collected from *Eucalyptus* spp. growing in the Cordillera Los Andes mountains of Venezuela at an altitude of approx. 3000 m. The daily mean temperature of this region is 10 °C, and the extreme environmental conditions most probably explain the low optimum growth temperature of *F. andinum* in culture. *F. andinum* grew at 15 °C, had an optimum at 20–30 ° and showed no growth above 35 °C. This is a low optimum temperature for growth when compared with other *Botryosphaeria* species such as *B. dothidea*, *B. parva*, *B. ribis*, *B. mamane*, *B. corticola*, *B. lutea*, *B. eucalyptorum*, *B. eucalypticola*, *B. australis* and *B. protearum* (Morgan-Jones & White 1987; Gardner 1997; Denman *et al.* 1999; Smith *et al.* 2001; Alves *et al.* 2004; Slippers *et al.* 2004a, c, d).

*F. andinum* was isolated from old *Eucalyptus* trees, and mainly from asymptomatic branches, without causing apparent damage to trees. We thus assume that the fungus is an endophyte and that it is not pathogenic. This would be consistent with many *Botryosphaeria* spp. that are known to reside as endophytes in asymptomatic or healthy plant tissues on a non-native host (Fisher *et al.* 1993; Smith *et al.* 1996). In

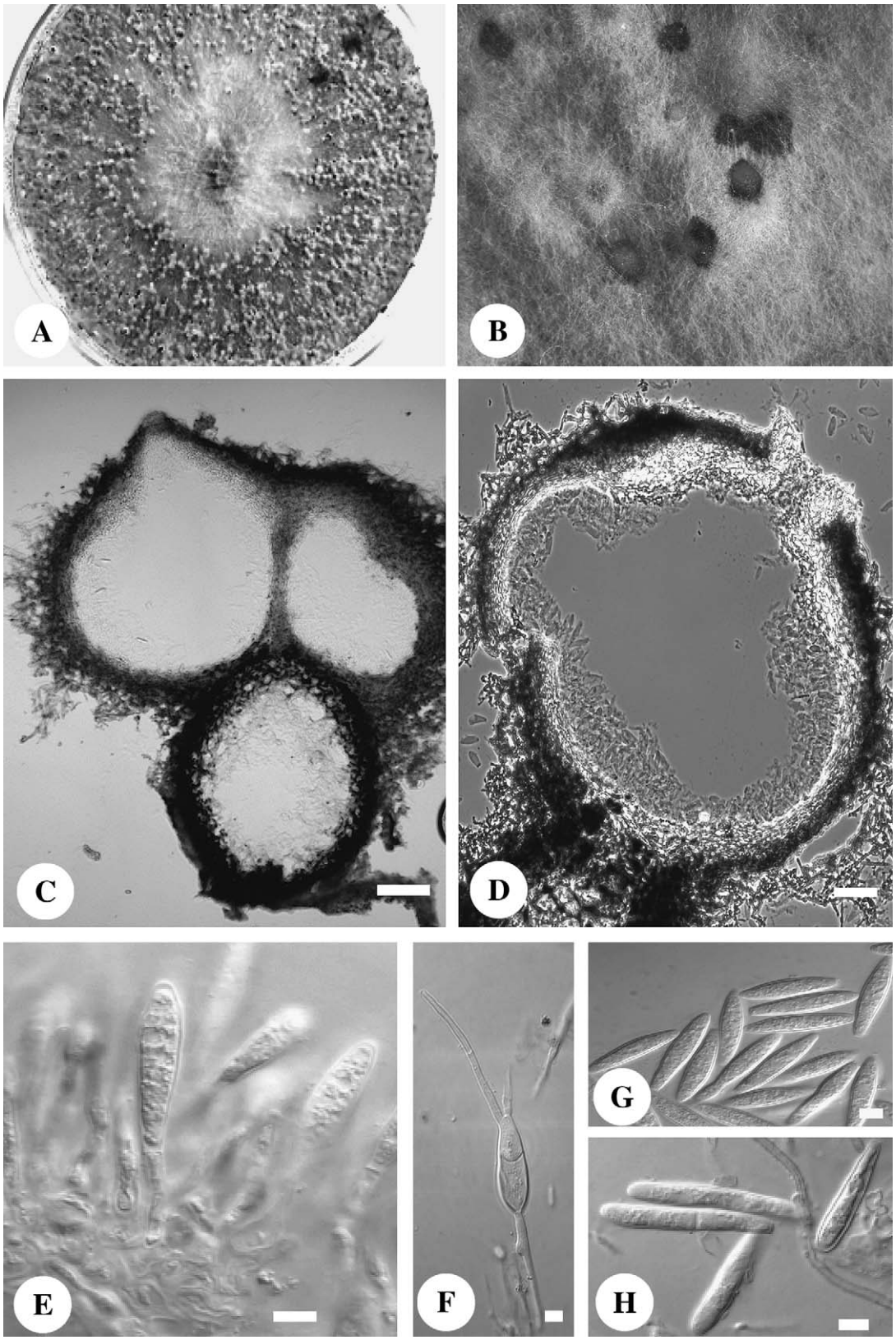


**Fig 3 – *Fusicoccum andinum*. A Abundant pycnidia on 2 % MEA after 30 d at 25 °C. B Pycnidia oozing spore masses. C Botryose pycnidia. Bar = 100 μm. D Solitary pycnidia. Bar = 50 μm. E Conidiogenous cell with germinating conidium. F–H Conidia. Bars = 5 μm.**

**Table 2 – Conidial measurement comparisons of the two new *Fusicoccum* spp. with other *Fusicoccum* anamorphs of *Botryosphaeria***

| Identity | Conidial size in vitro (μm) | L/B | Source |
|---|---|---|---|
| *Botryosphaeria dothidea* | (20–) 23–27 (–30) × 4–5 (–6) [av. 24.7 × 4.9] | 5 | Slippers *et al.* (2004a) |
| *B. parva* | (14.7–) 17–21 (–25.5) × 4.5–6 (–7) [av. 19 × 5.2] | 3.7 | Slippers *et al.* (2004b) |
| *B. ribis* | (16–) 19–23 (–24) × 5–6 (–7) [av. 20.8 × 5.5] | 3.8 | Slippers *et al.* (2004a) |
| *B. lutea* | (15–)18–22.5 (–24) × 4.5–6 (–7.5) [av. 19.7 × 5.6] | 3.6 | Phillips *et al.* (2002) |
| *B. eucalyptorum* | (18–) 20–23 (–25) × 7–8 (–12) | — | Smith *et al.* (2001) |
| *B. eucalypticola* | (20–) 25–27 (–35) × (5–) 7–9 (–10) [av. 26.3 × 7.2] | 3.6 | Slippers *et al.* (2004c) |
| *B. australis* | (18–) 23–26 (–30) × 5–6 (–7.5) [av. 24.7 × 5.1] | 4.8 | Slippers *et al.* (2004d) |
| *Fusioccam mangiferum* | (11–) 12–15 (–17.3) × 5–6.6 [av. 13.6 × 5.4] | 2.5 | Slippers *et al.* (2004b) |
| *B. melanops* | (41–) 47–50 (–53) × (9–) 10–10.5 (–11) | — | Shear and Davidson (1936) |
| *B. mamane* | (19–) 30–44 (–55) × (7–) 8–9 (–10) | — | Gardner (1997) |
| *F. andinum* | (19–) 23–31 (–40) × (4–) 5–6 (–8) [av. 27.1 × 5.6] | 4.84 | This study |
| *F. stromaticum* | (19–) 20–23 (–24) × (4–) 5–6 [av. 21.7 × 5.4] | 4.01 | This study |

different areas or under different environmental conditions, such endophytic species have, however, been considered important pathogens (Fisher *et al.* 1993; Smith *et al.* 2001).

Isolates of *F. andinum* formed a well-defined group based on analyses of sequence data. They are also morphologically distinct. The conidia of this fungus are large when compared with those of other *Fusicoccum* species. Two other *Botryosphaeria* spp. with comparatively large conidia are *B. mamane* (Gardner 1997) and *B. melanops* (Shear & Davidson 1936), although these are larger than those of *F. andinum*. Other than the relatively large conidia found in *F. andinum*, this species can also be distinguished by clavate to slightly navicular conidia. These are different to those of *B. mamane* and *B. melanops*, that both have fusiform conidia.

*F. stromaticum* was isolated from branches and stems of *Eucalyptus* and *Acacia* trees, with and without symptoms. These trees were growing in the Portuguesa and Cojedes states 150–200 m. The annual medium temperatures of these regions ranges between 26–30 °C, and this is also consistent with the fungus having a relatively high optimum temperature for growth in culture of 30–35 °C, compared with many other *Botryosphaeria* spp. (Pennycook & Samuels 1985; Morgan-Jones & White 1987; Gardner 1997; Denman *et al.* 1999; Smith *et al.* 2001; Alves *et al.* 2004).

Isolates of *F. stromaticum* resided in a well-defined group with strong BS support. This confirmed that the fungus represents the anamorph of an undescribed *Botryosphaeria* sp. Furthermore, there were three conspicuous morphological characteristics that distinguished this fungus from other *Fusicoccum* spp. *F. stromaticum* has unusually large conidiomata, it grows at 35 °C, and the conidia have slightly thickened walls.

*F. stromaticum* was isolated from asymptomatic, as well as dead and dying branches and stems of *Eucalyptus* spp. and *A. mangium* trees. The presence of the fungus on asymptomatic tissue suggests that it is an endophyte. In this regard, it is similar to *F. andinum* described here. Whether *F. stromaticum* is pathogenic is unknown, as it may have simply been present on the dying tissue as a saprophyte, without necessarily being the cause of the symptoms observed. *A. mangium* and *Eucalyptus* spp. are important plantation trees in Venezuela, and pathogenicity tests with this fungus should be conducted to determine its relative importance in tree health.

Isolates of *F. andinum* and *F. stromaticum* originated from trees that are not native to Venezuela. These fungi have not been found elsewhere in the world, despite the extensive surveys that have been conducted on *Acacia* and *Eucalyptus* spp. (Keane *et al.* 2000; Slippers *et al.* 2004b, c), suggesting that these newly described species might be native to Venezuela. However, extensive surveys of native woody plants would be necessary to establish this.

## Acknowledgments

We thank members of the Tree Protection Co-operative Programme (TPCP), the National Research Foundation and the THRIP initiative of the Department of Trade and Industry (DTI), South Africa, as well as the University of Los Andes, Facultad de Ciencias Forestales y Ambientales, Mérida, Venezuela for financial support.

## REFERENCES

Alves A, Correia A, Luque J, Phillips AJL, 2004. *Botryosphaeria corticola*, sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* **96**: 598–613.

von Arx JA, 1987. *Plant Pathogenic Fungi* [Beheifte zur Nova Hedwigia No. 87.]. J. Cramer, Berlin.

Barr ME, 1987. *Prodromus to Class Loculoascomycetes*. M.E. Barr, Amherst, MA.

Butin H, 1993. Morphological adaptation and spore pleomorphism in the form-complex *Dichomera–Camarosporium* and *Fusicoccum–Dothiorella*. *Sydowia* **45**: 161–166.

Carbone I, Anderson JB, Kohn LM, 1999. A method for designing primer sets for the speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

Cedeño L, Mohali S, Carrero C, 1994. Primer reporte en Venezuela de *Dothiorella dothidea* como la causa de la podredumbre marrón en frutos del duraznero. *Fitopatologia Venezolona* **7**: 34–36.

Cedeño L, Mohali S, Palacios-Pru E, 1996. Ultrastructure of *Lasiodiplodia theobromae* causal agent of caribbean pine blue stain in Venezuela. *Interciencia* **21**: 264–271.

Crous PW, Palm ME, 1999. Reassessment of anamorph genera *Botryodiplodia*, *Dothiorella* and *Fusicoccum*. *Sydowia* **52**: 167–175.

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. *Studies in Mycology* **45**: 129–140.

Denman S, Crous PW, Wingfield MJ, 1999. A taxonomic reassessment of *Phyllachora proteae*, a leaf pathogen of *Proteaceae*. *Mycologia* **91**: 510–516.

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

Fisher PJ, Petrini O, Sutton BC, 1993. A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**: 338–345.

Gardner DE, 1997. *Botryosphaeria mamane* sp. nov. associated with witches'-brooms on the endemic forest tree *Sophora chrysophylla* in Hawaii. *Mycologia* **89**: 298–303.

Hillis DM, Huelsenbeck JP, 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* **83**: 189–195.

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.

Keane PJ, Kile GA, Podger FD, Brown BN, 2000. *Diseases and Pathogens of Eucalypts*. CSIRO Publishing, Collingwood, Victoria.

Mohali S, 1997. Primer reporte en Venezuela de *Sphaeropsis sapinea*, agente causal del manchado azul del pino caribe. *Fitopatologia Venezolona* **10**: 23.

Mohali S, Encinas O, 2001. Association of *Diplodia mutila* with blue stain of Caribbean pine in Venezuela. *Forest Pathology* **31**: 187–189.

Mohali S, Encinas O, Mora N, 2002. Manchado azul en madera de *Pinus oocarpa* y *Azadirachta indica* en Venezuela. *Fitopatologia Venezolana* **15**: 30–32.

Morgan-Jones G, White JF, 1987. Notes on coelomycetes. II. Concerning the *Fusicoccum* anamorph of *Botryosphaeria ribis*. *Mycotaxon* **30**: 117–125.

Pennycook SR, Samuels GJ, 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. *Mycotaxon* **24**: 445–458.

Phillips AJL, Fonseca F, Povoa V, Castilho R, Nolasco G, 2002. A reassessment of the anamorphic fungus *Fusicoccum luteum* and description of its teleomorph *Botryosphaeria lutea* sp. nov. *Sydowia* **54**: 59–77.

Punithalingam E, 1976. Botryodiplodia theobromae. *CMI Descriptions of Pathogenic Fungi and Bacteria* **519**: 1–2.

Rayner RW, 1970. *A Mycological Colour Chart*. Commonwealth Mycological Institute, Kew.

Shear CL, Davidson RW, 1936. The life histories of *Botryosphaeria melanops* and *Massaria platani*. *Mycologia* **28**: 476–482.

Sivanesan A, 1984. *The Bitunicate Ascomycetes and their Anamorphs*. J. Cramer, Vaduz.

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

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Carnegie AJ, Wingfield MJ, 2004b. Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. *Studies in Mycology* **50**: 343–358.

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ, 2004c. Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. *Mycologia* **96**: 1030–1041.

Slippers B, Johnson GI, Crous PW, Coutinho TA, Wingfields BD, Wingfield MJ, 2004d. Phylogenetic and morphological re-evaluation of the *Botryosphaeria* anamorphs causing diseases of *Mangifera indica* in Australia. *Mycologia* **97**: 102–113.

Smith DR, Stanosz GR, 2001. Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorphs *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. *Mycologia* **93**: 505–515.

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**: 277–285.

Smith H, Wingfield MJ, Petrini O, 1996. *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189–195.

Sutton BC, 1980. *The Coelomycetes*. Commonwealth Mycological Institute, Kew.

Swofford DL, 1999. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, MA.

White TJ, Bruns S, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal genes for phylogenetics. In: Innis M, Gelfand D, Snisky J, White T (eds), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.

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