



Chapter 2

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

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

Botryosphaeria spp. are common endophytes of woody plants and they also include some serious pathogens of *Eucalyptus* and *Acacia* species. In this study, we characterise two new *Botryosphaeria* anamorphs, isolated from *Eucalyptus* and *Acacia* trees in Venezuela. These fungi were characterised based on morphological features in culture and comparisons of DNA sequence data. The two taxa, which have been provided the names *Fusicoccum andinum* and *Fusicoccum stromaticum*, resided in two well-supported clades (bootstrap values = 100 %) based on a combined data set of the internal transcribed spacers (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*. *Fusicoccum stromaticum* is characterized by large conidiomata in culture, growth at 35 °C and slightly thickened conidial walls, characteristics different from most other *Fusicoccum* spp. No teleomorph states were observed for these fungi, but DNA sequence data show that they are anamorphs of *Botryosphaeria*.

INTRODUCTION

Species of *Botryosphaeria* Ces. & De Not have a cosmopolitan distribution and occur on a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts. *Botryosphaeria* spp. infect the stems, branches, twigs and 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 shoot blights, stem cankers, fruit rots, die-back and gummosis (von Arx, 1987; Old, 2000; Old & Davison, 2000).

The taxonomy of *Botryosphaeria* has been confused for many years. This is mainly due to the fact that the morphology of the teleomorphs is very similar and these states are rarely encountered either in nature or under laboratory conditions (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, while others are generalists (Jacobs & Rehner, 1998; Smith *et al.*, 2001; Smith & Stanosz, 2001; Crous & Palm, 1999; Slippers *et al.*, 2004a).

The anamorphs of *Botryosphaeria* species are generally encountered in culture or 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, color, septation, wall thickness and texture, as well as the presence of microconidia and mode of conidiogenesis (Sutton, 1980; Sivanesan, 1984; Pennycook & Samuels, 1985). These characters, however, require careful

interpretation, as there is substantial overlap between the characters of many species. Thus conidial size represents a continuous character and it is also variable between isolates and may change with age or on 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 internal transcribed spacers (ITS) have in particular been used to resolve phylogenetic relationships between species and these have aligned 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). Another approach to characterise *Botryosphaeria* spp. is to use comparisons of multiple gene sequences and restriction fragment length polymorphism (RFLP) of anonymous simple sequence repeat (SSR) loci to distinguish closely related species such as *Botryosphaeria parva* Pennycook & Samuels and *B. ribis* Grossenb. & Duggar (Slippers, 2003; Slippers *et al.*, 2004a).

Botryosphaeria spp. are known to occur on various forestry and agricultural crops in Venezuela, but very little attention has been given to their identity (Cedeño *et al.* 1994, 1996; Mohali, 1997; Mohali & Encinas, 2001; Mohali, Encinas & Mora, 2002). *Lasiodiplodia theobromae* (Pat.) Griffon & Maublanc., *Diplodia pinea* (Desm.) Kickx (= *Sphaeropsis sapinea* (Fr.) Dyko & Sutton), *D. nutila* (Fries) Mont., and a species of *Dothiorella* Sacc. have been identified as the disease causing agents (Cedeño *et al.*, 1994, 1996; Mohali, 1997; Mohali & Encinas, 2001; Mohali *et al.*, 2002; De Wet *et al.*, 2003).

The aim of this study was to characterise 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 sequence data for the ITS rDNA (ITS 1 and ITS 2) and translation elongation factor 1- α (EF1- α).

MATERIALS AND METHODS

Isolates and morphological characterization

A survey was conducted in plantations of *Eucalyptus urophylla* S.T. Blake, an unidentified *Eucalyptus* sp., a *Eucalyptus*-hybrid and *Acacia mangium* Willd., during 2003. Isolations were made from twigs, stems and branches displaying symptoms of blue stain or 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, Detroit, MI, USA) 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. These were incubated for 3-6 weeks at 25 °C under a combination of near-ultraviolet 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. Representative isolates have also been deposited in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands.

Conidial morphology was studied using a light microscope with an Axiocam digital camera and software to analyse photographs (Carl Zeiss, Germany). Sections

through some of the pycnidia and stromatal structures were made with an American Optical Freezing Microtome. Length, width; shape and color 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 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 °C intervals. Two diameter measurements were taken perpendicular to each other after 4 d for each colony, and averages computed. Colony colors were determined using the color charts of Rayner (1970).

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 with a ratio 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, the internal transcribed spacer ITS (ITS1), the complete 5.8S rRNA gene, the second region ITS2 and the 5' end of the large subunit (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 mixture contained 0.02 U *Taq* DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany), 1X PCR buffer containing MgCl₂ (Roche Molecular Biochemicals, Alameda, CA), 0.4 mM of each dNTPs, 0.2 μ M of each primer and 20-25 ng/ μ l of DNA template and made up to a final volume of 25 μ l with

Sabax water. Standard PCR reaction cycles were followed with primer annealing at 58 °C. Due to difficulties in amplifying the EF 1- α region for some isolates it was necessary to vary the PCR annealing temperature between 52 to 60 °C for this region. 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, Mannheim, Germany).

Sequence analysis

A total of twenty-seven isolates were used in the phylogenetic analysis (Table 1). All the sequences used are from isolates maintained in the CMW and CBS culture collection. 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.

All PCR amplicons were purified prior to sequencing using High Pure PCR Product Purification Kit (Roche Molecular Biochemicals, Alameda, California, USA) following the manufacturer's specifications. The PCR products were sequenced in both directions using the primers ITS1, ITS4 and EF1-728F, EF1-986R. Sequencing reactions were performed using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied BioSystems, Foster City, CA) as recommended by the manufacturer and run on an ABI PRISM 3100 automated sequencer (Perkin-Elmer Applied BioSystems, Foster City, CA).

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 (Phylogenetic Analysis Using Parsimony) 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. 1000 bootstrapping 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.

RESULTS

Morphological characters

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

The colonies of *Fusicoccum* sp., isolated from *Eucalyptus* and *Acacia* in the states of Portuguesa and Cojedes (Table I), grew rapidly and covered the surface of the Petri dishes at 30 °C in four days, 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, with thin to slightly thickened walls, aseptate, bacilliform, straight to slightly curved, and their apices and bases were both blunt or bluntly rounded, 21.7 x 5.4 µm, l/w 4.01 (average of 50 conidia) (Figs. 2-8).

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 to 30 °C. Abundant pycnidia were produced on MEA at 25 °C with conidia oozing from these structures (Figs 9-12). Conidia were clavate to slightly navicular and large when compared with other *Fusicoccum* anamorphs, reaching 40 µm in length, 27.1 x 5.6 µm, l/w 4.84 (average of 50 conidia) (Table 2, Figs 13-16).

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 value = 0.440). This resulted in a final data set of 887 characters after alignment of which 289 characters were constant, 163 variable characters were parsimony-uninformative and 435 were parsimony informative. Heuristic search analysis in PAUP of the sequence data resulted in one tree [Consistency Index (CI) = 0.763; Retention Index (RI) = 0.865; Homoplasy Index (HI) = 0.237].

The isolates in the tree obtained from the combined data sets resided in thirteen principal clades (I to XIII)(Fig. 1). Isolates in clades I to X all have hyaline and thin-walled conidia and are thus *Fusicoccum*-like anamorphs. In contrast, isolates residing in clades XI to XIII all have pigmented and thick-walled conidia that can be referred to as *Diplodia*-like anamorphs (Denman *et al.*, 2000; Slippers *et al.*, 2004a). Isolates from Venezuela (VZLA) resided in clade I and clade IX and 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* (Fr.: Mough.) Ces. & De Not. (Slippers *et al.*, 2004a) in clade X were each strongly supported (100% bootstrap) and distinct from other *Botryosphaeria* spp with *Fusicoccum*-like anamorphs. The *Fusicoccum* sp. from

Venezuela residing in clade IX (100 % bootstrap support) was related to all the following species: *B. parva* and *B. ribis* (clade II, III), *B. eucalyptorum* Crous, H. Smith et M. J. Wingf. (clade IV), *B. eucalypticola* Slippers, Crous & M. J. Wingf. (clade V), *B. australis* Slippers, Crous & M. J. Wingf. (clade VI), *B. lutea* A. J. L. Phillips (clade VII) and *Fusicoccum mangiferum* (Syd. & P. Syd.) Johnson, Slippers & M. J. Wingf. (clade VIII) (Phillips *et al.*, 2002; Slippers *et al.*, 2004b, c, d), *Botryosphaeria obtusa* (Schwein.) Shoemaker, *B. stevensii* Shoemaker and *B. rhodina* (Berk. & Curt.) Arx (clades XI, XII, XIII), all with *Diplodia*-like anamorphs, formed a well defined group (Alves *et al.*, 2004; Punithalingam, 1976).

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 for them here.

Fusicoccum stromaticum. Mohali, Slippers & M. J. Wingf., **sp. nov.** (Figs 2-8)

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

Culturae laxae lanuginosae, superficie viride-olivaceae, infra post 15 dies in MEA ad 25 °C olivaceae. Coloniae ad 70-75 mm diametro post 4 dies in tenebris ad 25 °C. Aegre vel non creverunt ad 15 °C, non ad 40 °C, optime inter 30 °C et 35 °C creverunt. *Conidiomata* magna in superficie MEA, multilocularia, eustromatica, cum hyphis tecta; *loculus* omnino inclusus sine ostiolis, parietibus e textura angulari atrobrunnea compositis, ad aream conidiogenam tenuescens hyalinescensque. *Cellulae conidiogenae* hyalinae, holoblasticae, laeves, cylindricae, conidium unicum apicale efferentes, primo

reaching 80 mm diam on MEA after 4 d in the dark at 25 °C. Cardinal temperatures for growth were min 15 °C (reaching an average 24 mm in diam.), max 35°C (no growth), optimum 20 to 30 °C. *Pycnidia* superficial, produced abundantly on MEA surface at 25 °C (Fig. 9), oozing conida after 30 d at 25 °C on MEA (Fig. 10), solitary or botryose on the colonies, stromatic, globose (Figs 11-12), (331-) 374-597 (-740) x (302-) 339-557 (-671) µm (average of 50 pycnidia 486 x 448 µm, l/w 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. 13), (8-) 11-17 (-23) x (1.5-) 2 -2.5 (-3.0) µm (average of 50 conidiogenous cells 13.9 x 2.1 µm, l/w 6.62). *Conidia* hyaline, granular, clavate to slightly navicular, apex obtuse and base truncate, 0-1 septa (Figs 14-16), (19-) 23-31 (-40) x (4-) 5-6 (-8) µm (average of 50 conidia 27.1 x 5.6 µm, l/w 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 characterised in this study, based on both morphology and their unique DNA sequences. One of these fungi, now known as *Fusicoccum 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.

Fusicoccum andinum was collected from *Eucalyptus* spp. growing in the Cordillera Los Andes mountains of Venezuela at an altitude of approximately 3000 m. The daily mean temperature of this region is 10 °C and these extreme environmental conditions most probably explains the lower optimum growth temperature of *F. andinum* in culture. *Fusicoccum andinum* grew at 15 °C, had an optimum at 20 to 30 °C 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* A. J. L. Phillips, Alves et Luque, *B. lutea*, *B. eucalyptorum*, *B. eucalypticola*, *B. australis* and *B. protearum* Denman & Crous (Morgan-Jones & White, 1987; Smith *et al.*, 2001; Gardner, 1997; Denman *et al.*, 1999; Alves *et al.*, 2004; Slippers *et al.*, 2004a, c, d).

Fusicoccum andinum was isolated from old *Eucalyptus* trees 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 (Smith *et al.*, 1996; Fisher *et al.*, 1993). In 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* (Tul.) Wint. (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 its clavate to slightly navicular conidia. These are different to those of *B. mamane* and *B. melanops* that have fusiform conidia. Thus, the combination of relatively large and clavate to slightly navicular conidia, makes *F. andinum* easy to recognise.

Fusicoccum 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 at an altitude of 150 to 200 m. The annual medium temperatures of these regions ranges between 26 to 30 °C and this is also consistent with the fact that the fungus had a relatively high optimum temperature for growth in culture of between 30-35 °C, compared with many other *Botryosphaeria* spp. (Pennycook & Samuels, 1985; Morgan-Jones & White, 1987; Smith *et al.*, 2001; Gardner, 1997; Denman *et al.*, 1999; Alves *et al.*, 2004).

Isolates of *Fusicoccum stromaticum* resided in a well-defined group with strong bootstrap 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. *Fusicoccum stromaticum* has unusually large conidiomata, it grows at 35 °C and its conidia have slightly thickened walls.

Fusicoccum 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 also similar to *F. andinum* described in the present study. Whether *F. stromaticum* is pathogenic is unknown as it may have simply been present on dying tissue as

saprophyte, without necessarily being the cause of the symptoms observed. *Acacia mangium* and *Eucalyptus* species 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. The fact that these fungi have not been found elsewhere in the world, despite the extensive surveys that have been conducted on *Acacia* and *Eucalyptus* spp. (Roux & Wingfield, 1997; Hadi & Nuhamara, 1997; Sharma & Florence, 1997; Old, 2000; Old & Davison, 2000; Slippers *et al.*, 2004b, c), suggests that these newly described species might be native to Venezuela. However, surveys of native woody plants would be necessary to establish this fact.

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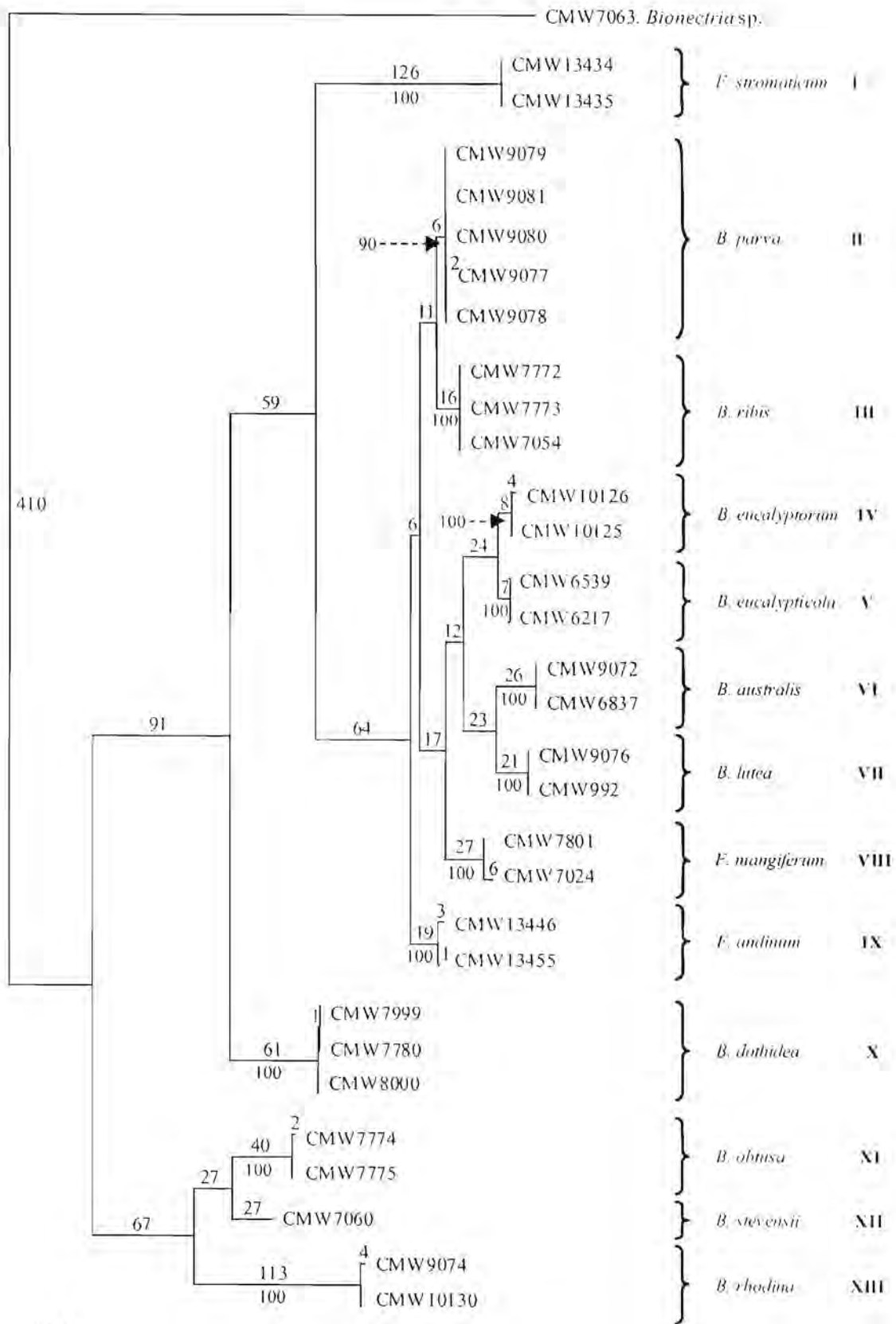
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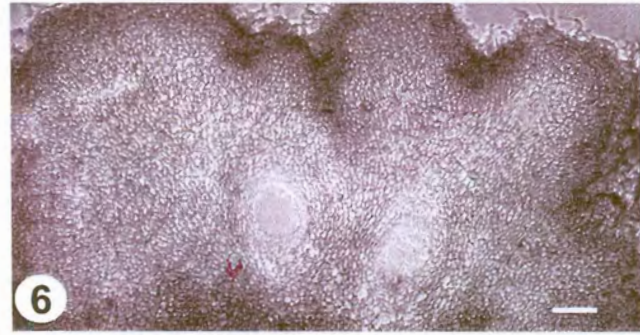
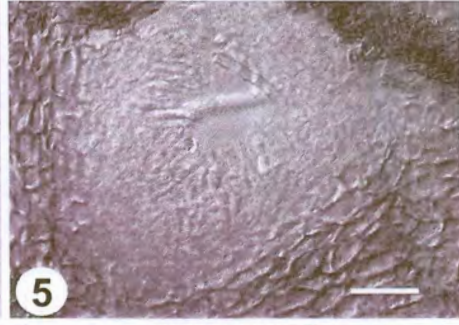
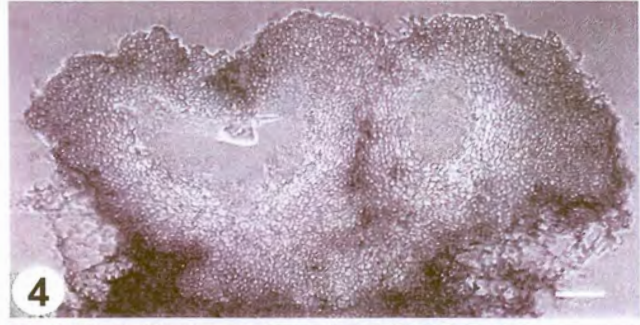
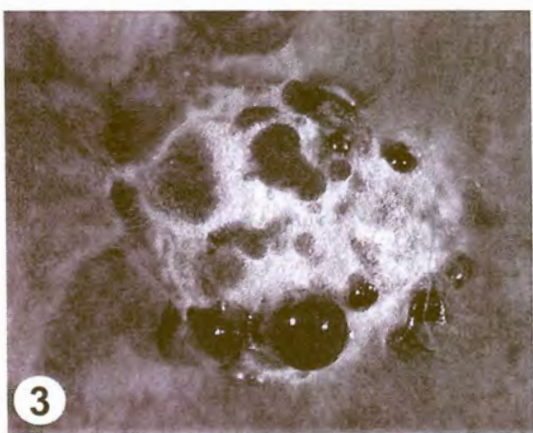
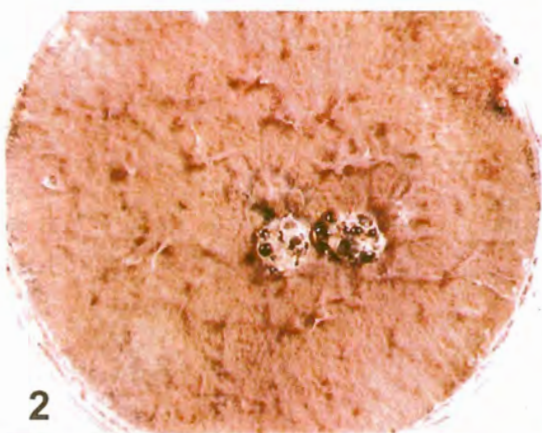
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Fig. 1. Phylogenetic relationships amongst *Fusicoccum andinum* and *F. stromaticum* from Venezuela and related species from elsewhere, based on the most parsimonious tree obtained through heuristic searches of the combined dataset of the ITS rDNA and EF1- α regions. The phylogram is rooted with the outgroup *Bionectria* sp. Bootstrap 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.



Figs 2-8. *Fusicoccum stromaticum*. **Fig. 2.** Culture with few conidiomata. **Fig. 3.** Big conidioma produced on 2% MEA after 30 d at 25 °C. **Figs 4-6.** Multilocular conidiomata without ostioles and embedded locule. **Fig. 5.** Close-up of a locule. Bars = 50 µm. **Fig. 7.** Conidiogenous cells and conidia. **Fig. 8.** Conidia with thin to slightly thickened walls. Bars = 5 µm.



Figs 9-16. *Fusicoccum andinum*. **Fig. 9.** Abundant pycnidia on 2% MEA after 30 d at 25 °C. **Fig. 10.** Pycnidia oozing spore masses. **Fig. 11.** Botryose pycnidia. Bar = 100 μm . **Fig. 12.** Solitary pycnidia. Bar = 50 μm . **Fig. 13.** Conidiogenous cell with conidium. **Fig. 14.** Germinating conidium. **Figs 15-16.** Conidia. Bars = 5 μm .

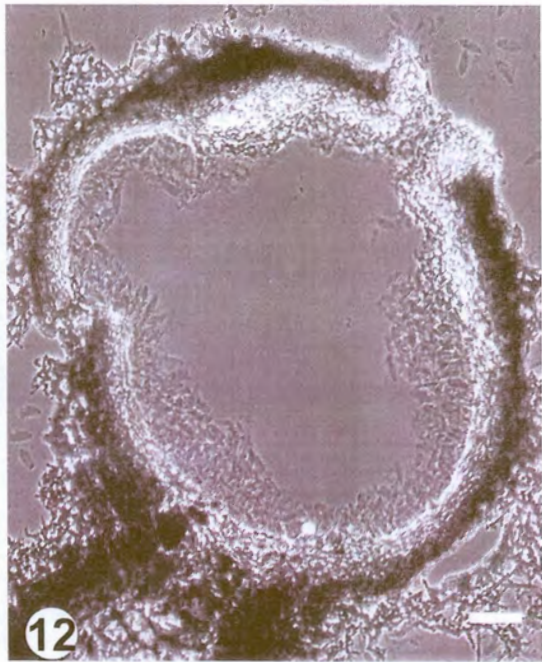
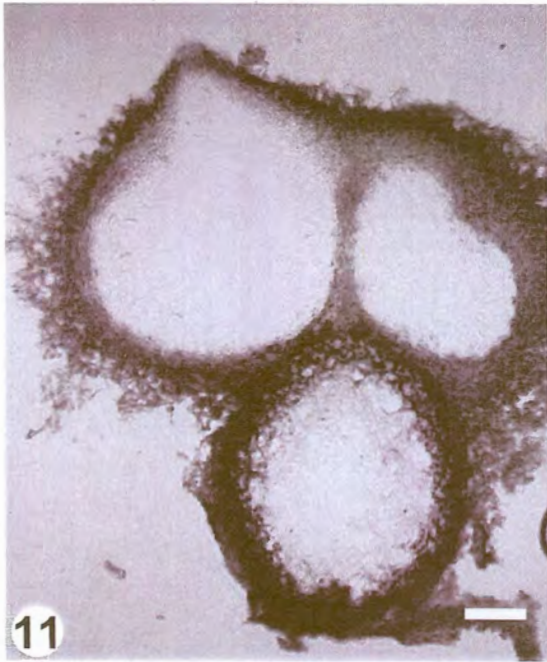
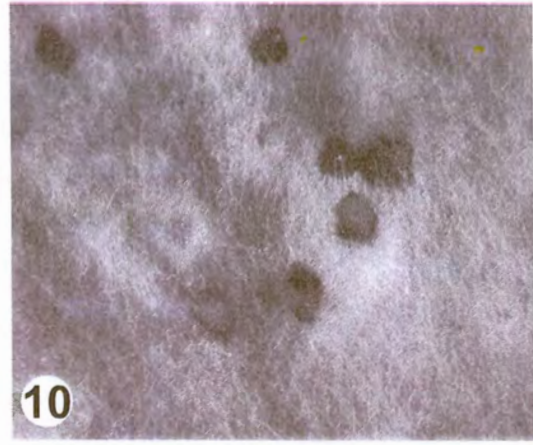


Table 1. Isolates used in the phylogenetic study.

Culture No. ¹	Other No. ¹	Identity ²	Host	Location	Isolator	GenBank ITS	EF1- α
CMW7780		<i>Botryosphaeria dothidea</i>	<i>Fraxinus excelsior</i>	Molinizza, Switzerland	B. Slippers	AY236947	AY236896
CMW7999		<i>B. dothidea</i>	<i>Ostrya</i> sp.	Crosifisso, Switzerland	B. Slippers	AY236948	AY236897
CMW8000		<i>B. dothidea</i>	<i>Prunus</i> sp.	Crosifisso, Switzerland	B. Slippers	AY236949	AY236898
CMW9077	ICMP7924	<i>B. parva</i>	<i>Actinidia deliciosa</i>	New Zealand	S. R. Pennycook	AY236939	AY236884
CMW9078	ICMP7925	<i>B. parva</i>	<i>A. deliciosa</i>	New Zealand	S. R. Pennycook	AY236940	AY236885
CMW9079	ICMP7933	<i>B. parva</i>	<i>A. deliciosa</i>	New Zealand	S. R. Pennycook	AY236941	AY236886
CMW9080	ICMP8002	<i>B. parva</i>	<i>Populus nigra</i>	New Zealand	G. J. Samuels	AY236942	AY236887
CMW9081	ICMP8003	<i>B. parva</i>	<i>P. nigra</i>	New Zealand	G. J. Samuels	AY236943	AY236888
CMW7772		<i>B. ribis</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers/ G. Hudler	AY236935	AY236877
CMW7773		<i>B. ribis</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers/ G. Hudler	AY236936	AY236878
CMW7054	CBS121	<i>B. ribis</i>	<i>Ribes</i> sp.	New York, USA	N. E. Stevens	AF241177	AY236879
CMW9076	ICMP7818	<i>B. lutea</i>	<i>Malus x domestica</i>	New Zealand	S. R. Pennycook	AY236946	AY236893
CMW992	KJ93.52	<i>B. lutea</i>	<i>A. deliciosa</i>	New Zealand	G. J. Samuels	AF027745	AY236894
CMW7801	BRIP23396	<i>Fusicoccum mangiferum</i>	<i>Mangifera indica</i>	Australia	G. I. Johnson		
CMW7024	BRIP24101	<i>F. mangiferum</i>	<i>M. indica</i>	Australia	G. I. Johnson		
CMW10125		<i>B. eucalyptorum</i>	<i>Eucalyptus grandis</i>	Mpumalanga, South Africa	H. Smith	AF283686	AY236891
CMW10126		<i>B. eucalyptorum</i>	<i>E. grandis</i>	Mpumalanga, South Africa	H. Smith	AF283687	AY236892
CMW13446	PREM 58532	<i>F. andinum</i> ²	<i>Eucalyptus</i> sp.	Mérida state, Venezuela	S. Mohali		
CMW13455	PREM 58238	<i>F. andinum</i> ²	<i>Eucalyptus</i> sp.	Mérida state, Venezuela	S. Mohali	AY693976	AY693977
CMW13434	PREM 58516	<i>F. stromaticum</i> ²	<i>Eucalyptus</i> -hybrid	Cójeles state, Venezuela	S. Mohali	AY693974	AY693975

Table 1. Continued.

Culture No. ¹	Other No. ¹	Identity ²	Host	Location	Isolator	GenBank	
						ITS	EF1- α
CMW13435	PREM 58517	<i>F. stromaticum</i> ²	<i>Eucalyptus</i> -hybrid	Cojedes state, Venezuela	S. Mohali		
CMW7060	CBS431	<i>B. stevensii</i>	<i>F. excelsior</i>	Netherlands	H. A. van der Aa	AY236955	AY236904
CMW7774		<i>B. obtusa</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers/ G. Hudler	AY236953	AY236902
CMW7775		<i>B. obtusa</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers/ G. Hudler	AY236954	AY236903
CMW9074		<i>B. rhodina</i>	<i>Pinus</i> sp.	Mexico	T. Burgess	AY236952	AY236901
CMW10130		<i>B. rhodina</i>	<i>Vitex donniana</i>	Uganda	J. Roux	AY236951	AY236900
CMW7063		<i>Bionectria</i> sp.	Unknown	Netherlands	H. A. van der Aa	AY236956	AY236905

¹ Culture collections and isolates abbreviations: CMW = Collection Michael Wingfield, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; CBS = Centraalbureau voor Schimmecultures, Utrecht, Netherlands; ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand; BRIP = Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; KJ = Jacobs and Rehner (1998); Isolates CMW7999, CMW7772 are ex-type isolates.

² Identities determined in this study.

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

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