
Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker

Alan J.L. Phillips^{1*}, Peter V. Oudemans², António Correia³ and Artur Alves³

¹Centro de Recursos Microbiológicos, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

²P.E. Marucci Center for Blueberry and Cranberry Research, Rutgers University, 125a Lake Oswego Road, Chatsworth, New Jersey 08019, USA

³Centro de Biologia Celular, Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

Phillips, A.J.L., Oudemans, P.V., Correia, A. and Alves, A. (2006). Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker. *Fungal Diversity* 21: 141-155.

Botryosphaeria corticis was collected from a commercial field of *Vaccinium corymbosum* cv. Bluecrop in New Jersey, USA. The connection between the anamorph and the teleomorph was confirmed through isolations made from single ascospores. The fungus was characterised in terms of morphology of the teleomorph on the host, the anamorph in pure culture, and sequences of the ITS1/ITS2 regions of the ribosomal DNA operon. Morphologically the fungus compared well with the protologue of *B. corticis*. Phylogenetic analyses showed that the isolates from New Jersey reside in a clade together with isolates of the same species from North Carolina and this clade is sister to *B. dothidea*. The specimen from New Jersey is designated as epitype.

Key words: *Botryosphaeriaceae*, *Fusicoccum*, ITS, phylogeny, taxonomy

Introduction

Botryosphaeria Ces. & De Not. is a species rich genus of plant parasites, saprobes and endophytes with a worldwide distribution on a wide range of mainly woody hosts (Denman *et al.*, 2000). The genus is well circumscribed morphologically and its features have been documented in detail (Denman *et al.*, 2000; Slippers *et al.*, 2004a). Morphological features of the teleomorphs vary little from one species to another, but the anamorphs display a wide range of characters that discriminate the species.

*Corresponding author: A.J.L. Phillips; email: alp@mail.fct.unl.pt

Up to 18 anamorph genera have been associated with *Botryosphaeria* (Denman *et al.*, 2000). Phylogenetic studies based on ITS sequences led Jacobs and Rehner (1998) and Denman *et al.* (2000) to recognise two main clades, one that corresponded to species with anamorphs in *Diplodia* Fr. and the other to species with anamorphs in *Fusicoccum* Corda. Subsequent studies by Zhou and Stanosz (2001), Slippers *et al.* (2004a) and Alves *et al.* (2004) with more species and additional markers supported this view. Nevertheless, some authors (e.g., Pavlic *et al.*, 2004) have continued to separate *Lasioidiplodia* Ellis & Everh. from *Diplodia* because of their phylogenetic (ITS) and morphological (striate conidia and paraphyses) distinctions. *Dothiorella* Sacc. was recently reinstated for species with thick-walled conidia that become brown and septate at an early stage of development (Phillips *et al.*, 2005).

In ITS phylogenies the *Fusicoccum* clade is composed of two sub-clades. *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not., the type species of *Botryosphaeria*, lies in one of these together with *B. mamane* D.E. Gardener, *B. corticis* (Demaree & M.S. Wilcox) Arx & E. Müll. (Slippers *et al.*, 2004a; Zhou and Stanosz, 2001; Alves *et al.*, 2004; Phillips *et al.*, 2005) and *Fusicoccum dimidiatum* (Penz.) D.F. Farr (Farr *et al.*, 2005). All other species with *Fusicoccum* anamorphs fall within a second sub-clade. Several new species have recently been described in *Botryosphaeria* and many of these appear in the larger *Fusicoccum* sub-clade that does not contain *B. dothidea* (Smith *et al.*, 2001; Denman *et al.*, 2003; van Niekerk *et al.*, 2004; Slippers *et al.*, 2004b; Slippers *et al.*, 2005a,b; Farr *et al.*, 2005). All species in the non-*B. dothidea* sub-clade are well-characterised by detailed morphological and molecular data, and ex-type or ex-epitype cultures are available for all of them.

In contrast, the sub-clade bearing *B. dothidea* is less well characterised. Slippers *et al.* (2004a) clarified the status of *B. dothidea*, proposed a neotype and an epitype and provided a corresponding ex-epitype culture. However, the other two species that fall within this sub-clade are less well studied and none of the cultures available for them can be linked to their corresponding type specimens. To stabilise these names and provide authentic cultures that can be used as standards, epitype specimens should be selected and cultures derived from them made available in long-term culture collections. The two known ex-type cultures of *B. mamane* are no longer extant (G. Stanosz pers. comm.). Recent attempts to re-collect and isolate *B. mamane* from the type location were unsuccessful (P.W. Crous, pers. comm.). Few cultures of *B. corticis* are available in publicly accessible culture collections. Thus, no isolates are available in IMI or CBS. Although six cultures are available in ATCC, none of these can be connected to the type specimen (BPI 598729).

The objectives of the present study were to collect a specimen of *B. corticis* that is suitable as epitype, to prepare ex-epitype cultures that can be made available for future studies, to fully characterise the species in terms of morphology and assess its phylogenetic relationships with known species.

Materials and methods

Isolates

Canes of *Vaccinium corymbosum* with typical symptoms of infection by *B. corticis* were collected from Hammonton, New Jersey, USA (-74.756, 39.639). Asci and ascospores were dissected from ascomata and spread over the surface of plates of Difco potato dextrose agar (PDA). After incubating overnight at 25°C, single germinating ascospores were transferred to fresh plates of ½ strength PDA and checked microscopically to ensure that a single spore had been transferred. Cultures were stored on ½ strength PDA slopes at 5°C.

Morphology

To induce sporulation, cultures were grown on water agar supplemented with pieces of autoclaved poplar twigs and incubated on the laboratory bench (20-25°C) where they received indirect daylight. Culture characteristics were recorded on Difco corn meal agar (CMA) plates incubated as for sporulation.

For microscopy, the contents of ascomata were dissected out and mounted in 100% lactic acid. For observations of conidiogenesis, the conidiogenous layer was dissected out and mounted in 100% lactic acid. Measurements of ascospores and conidia were made with the Leica IM 500 measurement module from images recorded on a Leica DFC 320 digital camera. From measurements of 50 conidia and ascospores the mean, standard deviation and 95% confidence intervals were calculated. Spore dimensions are given as the range of dimensions with extremes in parentheses. Dimensions of other structures are given as the range of at least 20 measurements.

DNA isolation, amplification and phylogeny

The procedures described by Alves *et al.* (2004) were used to extract genomic DNA and to amplify part of the nuclear rRNA cluster with the primers ITS1 and ITS4 (White *et al.*, 1990). PCR reactions were carried out with *Taq* polymerase, nucleotides and buffers supplied by MBI Fermentas

(Vilnius, Lithuania) and PCR reaction mixtures were prepared according to Alves *et al.* (2004). The amplified PCR fragments were purified with the JETQUICK PCR Purification Spin Kit (GENOMED, Löhne, Germany). Both strands of the PCR products were sequenced with the ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase (PE Applied Biosystems, Foster City, California, USA) in a Bio-Rad iCycler Thermal Cycler. The cycle sequencing procedure has already been described elsewhere (Alves *et al.*, 2004). The sequences of the ITS (partial 18S, ITS1, 5.8S gene, ITS2, and partial 28S sequences) were read and edited with Chromas 1.45 (<http://www.technelysium.com.au/chromas.html>). All sequences were checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences. Sequences were deposited in GenBank. Nucleotide sequences of additional *Botryosphaeria* species were retrieved from GenBank (Table 1).

The ITS sequences were aligned with ClustalX version 1.83 (Thompson *et al.*, 1997), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25%). Alignments were checked and manual adjustments were made where necessary. Phylogenetic information contained in indels (gaps) was incorporated into the phylogenetic analysis using simple indel coding as implemented by GapCoder (Young and Healy, 2003).

Phylogenetic analyses were carried out using PAUP* version 4.0b10 (Swofford, 2003) for maximum-parsimony (MP) analysis and Mr Bayes v3.0b4 (Ronquist and Huelsenbeck, 2003) for the Bayesian analysis. The aim of this study was to determine the phylogenetic position of *B. corticis* within the species with *Fusicoccum* anamorphs. For this reason the trees were rooted to two species in the sister group of *Botryosphaeria* spp. with *Diplodia* anamorphs (Denman *et al.*, 2000; Alves *et al.*, 2004), namely *B. stevensii* Shoemaker and *B. obtusa* (Schwein.) Shoemaker. Trees were visualised with TreeView (Page, 1996).

Maximum-parsimony analysis was performed using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis and Bull, 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI).

Table 1. Isolates studied.

Isolate number	Identity	Host	Locality	Collector	ITS GenBank
CBS 119046	<i>B. australis</i>	<i>Rubus</i> sp.	Alentejo, Portugal	E. Diogo	DQ 299244
CMW 9072	<i>B. australis</i>	<i>Acacia</i> sp.	Melbourne, Australia	J. Roux	AY 339260
CBS 119047	<i>B. corticis</i>	<i>Vaccinium corymbosum</i>	New Jersey, USA	P.V. Oudemans	DQ 299245
CBS 119048	<i>B. corticis</i>	<i>Vaccinium corymbosum</i>	New Jersey, USA	P.V. Oudemans	DQ 299246
ATCC 22927	<i>B. corticis</i>	<i>Vaccinium</i> sp.	North Carolina, USA	R.D. Millholland	DQ 299247
ATCC 22928	<i>B. corticis</i>	<i>Vaccinium</i> sp.	North Carolina, USA	R.D. Millholland	DQ 299248
CBS 115476	<i>B. dothidea</i>	<i>Prunus</i> sp.	Crocifisso, Switzerland	B. Slippers	AY 236949
CBS 110300	<i>B. dothidea</i>	<i>Populus nigra</i>	Braga, Portugal	A.J.L. Phillips	AY 640253
CBS 110302	<i>B. dothidea</i>	<i>Vitis vinifera</i>	Montemor-O-Novo	A.J.L. Phillips	AY 259092
CBS 116741	<i>B. dothidea</i>	<i>Olea europaea</i>	Thessalia, Greece	I. Rumbos	AY 640254
CBS 115766	<i>B. eucalypticola</i>	<i>Eucalyptus rossii</i>	Tidinbilla, NSW, Australia	M.J. Wingfield	AY 615143
CMW 6539	<i>B. eucalypticola</i>	<i>Eucalyptus rossii</i>	Orbost, Victoria, Australia	M.J. Wingfield	AY 615141
CBS 115791	<i>B. eucalyptorum</i>	<i>Eucalyptus grandis</i>	South Africa	H. Smith	AF 283686
CMW 10126	<i>B. eucalyptorum</i>	<i>Eucalyptus grandis</i>	South Africa	H. Smith	AF 283687
CBS 110299	<i>B. lutea</i>	<i>Vitis vinifera</i>	Oeiras, Portugal	A.J.L. Phillips	AY 259091
CMW 9076	<i>B. lutea</i>	<i>Malus × domestica</i>	New Zealand	S.R. Pennycook	AY 236946
GS-97-59	<i>B. mamane</i>	<i>Sophora chrysophylla</i>	Hawaii	D. Gardner	AF 246930
GS-97-58	<i>B. mamane</i>	<i>Sophora chrysophylla</i>	Hawaii	D. Gardner	AF 246929
CBS 112555	<i>B. obtusa</i>	<i>Vitis vinifera</i>	Montemor-o-Novo, Portugal	A.J.L. Phillips	AY 259094
CBS 110301	<i>B. parva</i>	<i>Vitis vinifera</i>	Montemor-o-Novo, Portugal	A.J.L. Phillips	AY 259098
CMW 9081	<i>B. parva</i>	<i>Populus nigra</i>	New Zealand	G.J. Samuels	AY 236943
STE-U 1775	<i>B. protearum</i>	<i>Leucadendron</i> sp.	South Africa	S. Denman	AF 452539
STE-U 4398	<i>B. protearum</i>	<i>Leucadendron</i> sp.	Portugal	S. Denman	AF 452531
CMW 7054	<i>B. ribis</i>	<i>Ribes rubrum</i>	New York, USA	N.E. Stevens	AF 241177
CBS 115475	<i>B. ribis</i>	<i>Ribes</i> sp.	New York, USA	B. Slippers	AY 236935
CBS 112553	<i>B. stevensii</i>	<i>Vitis vinifera</i>	Montemor-o-Novo, Portugal	A.J.L. Phillips	AY 259093
UAMH 6800	<i>F. arbuti</i>	<i>Arbutus menziesii</i>	BC, Canada	A. Funk	AY 819725
CBS 116131	<i>F. arbuti</i>	<i>Arbutus menziesii</i>	Washington, USA	M. Elliott	AY 819720

Table 1 continued. Isolates studied.

Isolate number	Identity	Host	Locality	Collector	ITS GenBank
CBS 204.33	<i>F. dimidiatum</i>	<i>Prunus</i> sp.	Egypt	R.M. Nattrass	AY 819728
CBS 251.49	<i>F. dimidiatum</i>	<i>Juglans regia</i>	California, USA	E.E. Wilson	AY 819726
CBS 118531	<i>F. mangiferum</i>	<i>Mangifera indica</i>	Australia	G.I. Johnson	AY 615185
CBS 118532	<i>F. mangiferum</i>	<i>Mangifera indica</i>	Australia	G.I. Johnson	AY 615186
CBS 112878	<i>F. viticlavatum</i>	<i>Vitis vinifera</i>	South Africa	F. Halleen	AY 343381
CBS 112977	<i>F. viticlavatum</i>	<i>Vitis vinifera</i>	South Africa	F. Halleen	AY 343380
CBS 110887	<i>F. vitifusiforme</i>	<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	AY 343383
CBS 110880	<i>F. vitifusiforme</i>	<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	AY 343382

Bayesian analyses employing a Markov Chain Monte Carlo (MCMC) method were performed. The general time-reversible model of evolution (Rodriguez *et al.*, 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+ Γ) was used. Four MCMC chains were run simultaneously, starting from random trees, for 10^6 generations. Trees were sampled every 100th generation for a total of 10^4 trees. The first 10^3 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang, 1996) were determined from a majority-rule consensus tree generated from the remaining 9000 trees. The analysis was repeated three times starting from different random trees to ensure trees from the same tree space were being sampled during each analysis.

Results

Phylogenetic analysis

The ITS dataset consisted of 34 ingroup and 2 outgroup taxa. New sequences were deposited in GenBank (Table 1) and the alignments in TreeBase (S1443). The alignment contained 554 characters including coded alignment gaps. Of these 554 characters 413 were constant and 16 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 125 parsimony informative characters resulted in 28 equally parsimonious trees with TL = 202, CI = 0.728, RI = 0.930 and HI = 0.213. The Bayesian analysis resulted in the tree shown in Fig. 1, which is essentially the same as the trees resulting from MP analysis. Two major clades (A and B) supported by high statistical support (MP bootstrap values of 98% and 94% respectively, and posterior probabilities of 1.00) were distinguished. Clade A consists of 7 species of *Botryosphaeria* and 4 species of *Fusicoccum* anamorphs. Not all species in this clade could be fully differentiated by the ITS sequence data. Thus, *B. parva* Pennycook & Samuels could not be distinguished unambiguously from *B. ribis* Grossenb. & Duggar and the *F. viticlavatum* Niekerk & Crous clade did not receive any significant bootstrap or posterior probability support. Nevertheless, all these species can be differentiated when ITS sequence data are combined with protein coding genes such as the translation elongation factor 1- α or β -tubulin genes (van Niekerk *et al.*, 2004).

Clade B consisted of four clearly differentiated and well supported sub-clades corresponding to four known species including the type species of the genus, *B. dothidea*. Anamorphs of these four species are known to be *Fusicoccum*-like, but species names have been applied to only two of them,

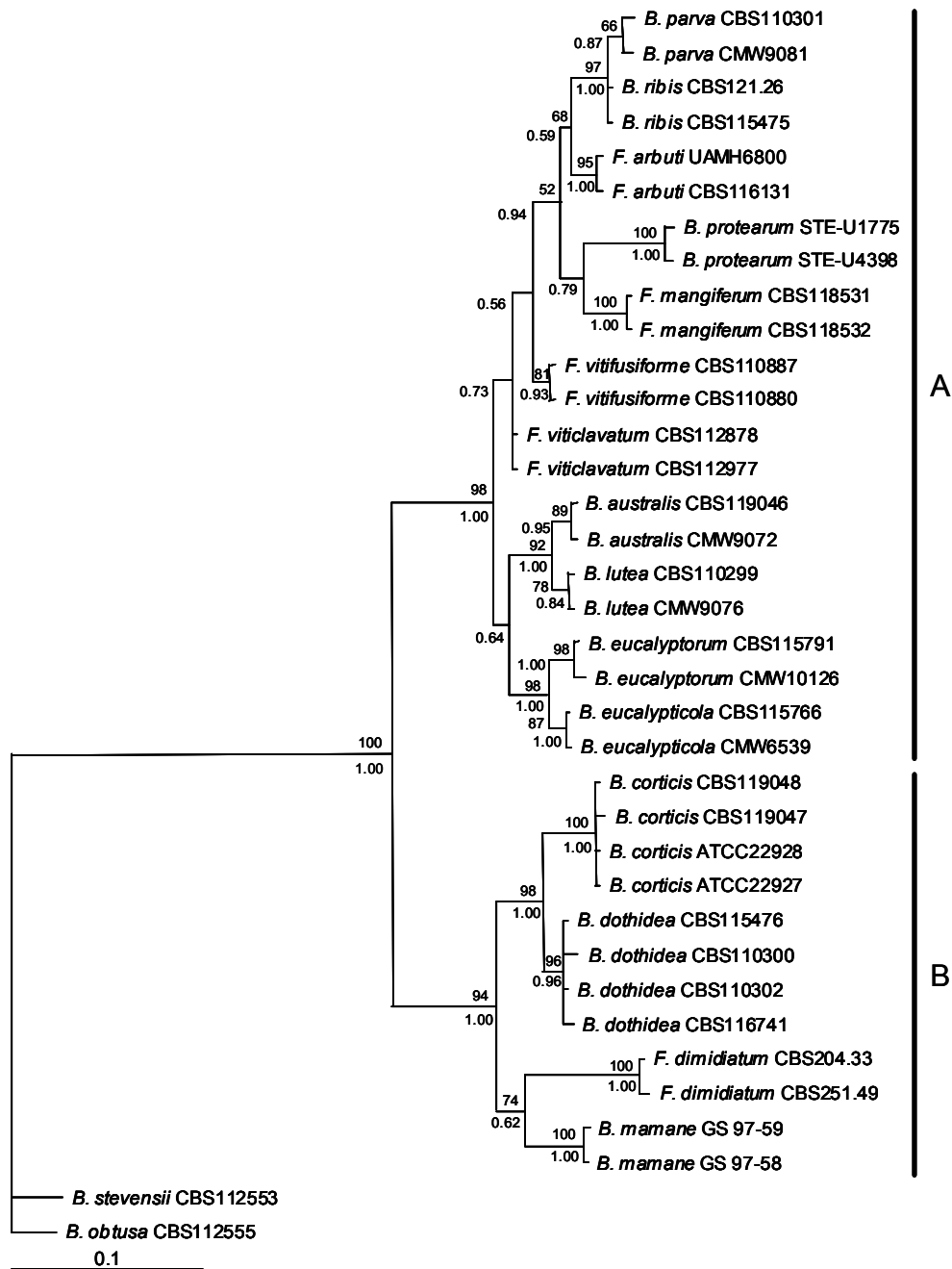


Fig. 1. Bayesian tree resulting from analysis of ITS sequence data for *Botryosphaeria* species with *Fusicoccum* anamorphs. The numbers above the branches indicate pooled posterior percentages from three independent Bayesian analyses, each consisting of 10^6 Markov Chain Monte Carlo generations (GTR+I+ Γ model), with a burn-in of 10^3 generations. Numbers below the branches indicate MP bootstrap support percentages from 10^3 pseudoreplicates. The tree was rooted to *B. stevensii* and *B. obtusa*.

namely *F. aesculi* Corda (*B. dothidea*) and *F. dimidiatum* (Penz.) D.F. Farr (teleomorph unknown). MP and Bayesian analyses placed *B. corticis* in a well-supported clade sister to *B. dothidea*.

Taxonomy

The holotype of *B. corticis* (BPI 598729) was collected from *V. corymbosum* growing at Atkinson, North Carolina, USA in February 1940 by J.B. Demaree. The host was reported as *V. australe*, but this is now generally regarded as a synonym of *V. corymbosum* (VanderKloet, 1988). Since no cultures ex-type are available it was recollected and the fungus cultured. This collection (CBS-H 19706) is designated as epitype below.

Botryosphaeria corticis (Demaree & M.S. Wilcox) Arx & E. Müll., *Beitr. Kryptfl. Schweiz* 11 (1): 43 (1954). (Figs. 2-8)

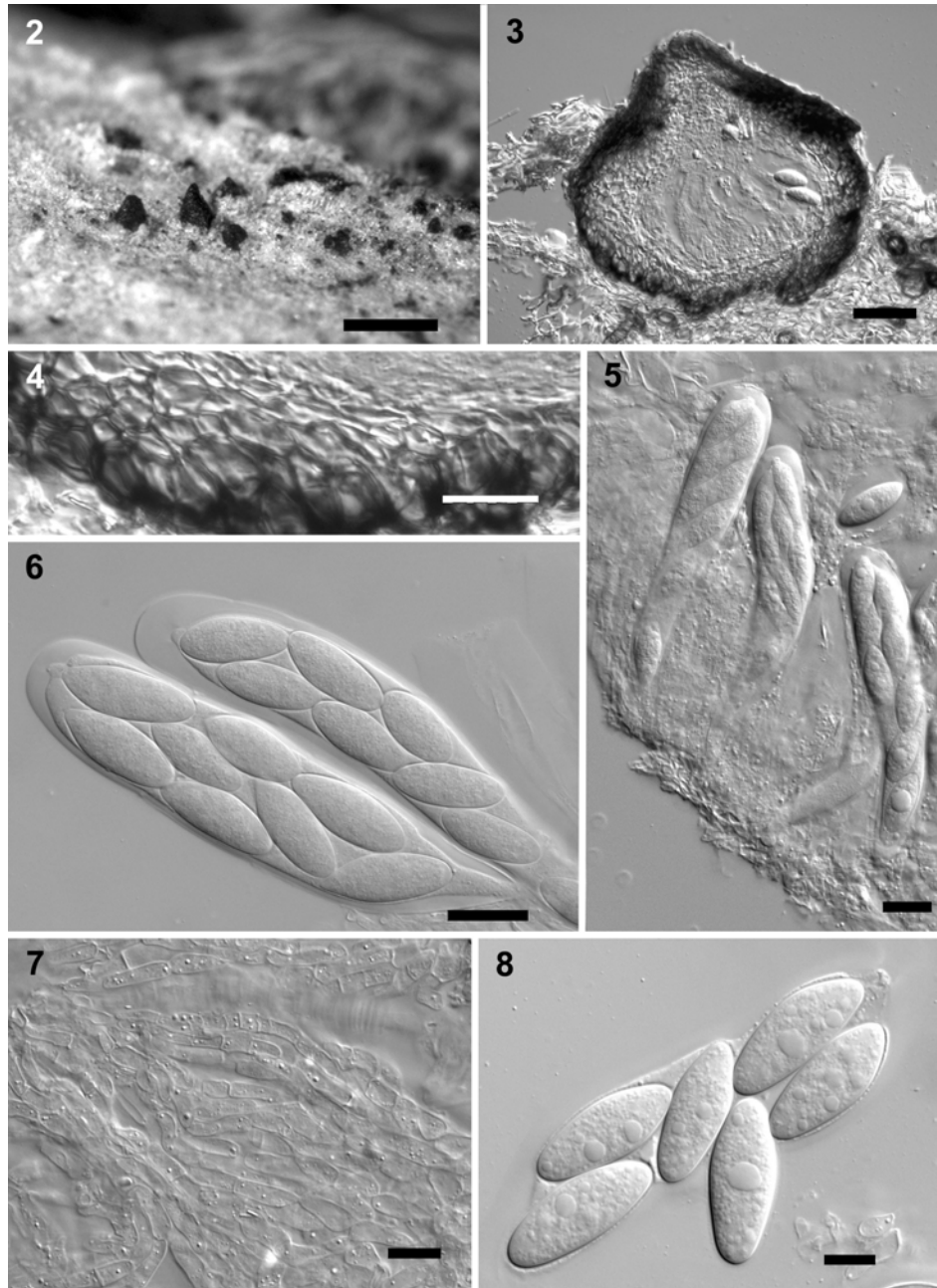
≡ *Physalospora corticis* Demaree & M.S. Wilcox, *Phytopathology* 32: 1074 (1942).

Anamorph: A *Fusicoccum* species. (Figs. 9-17)

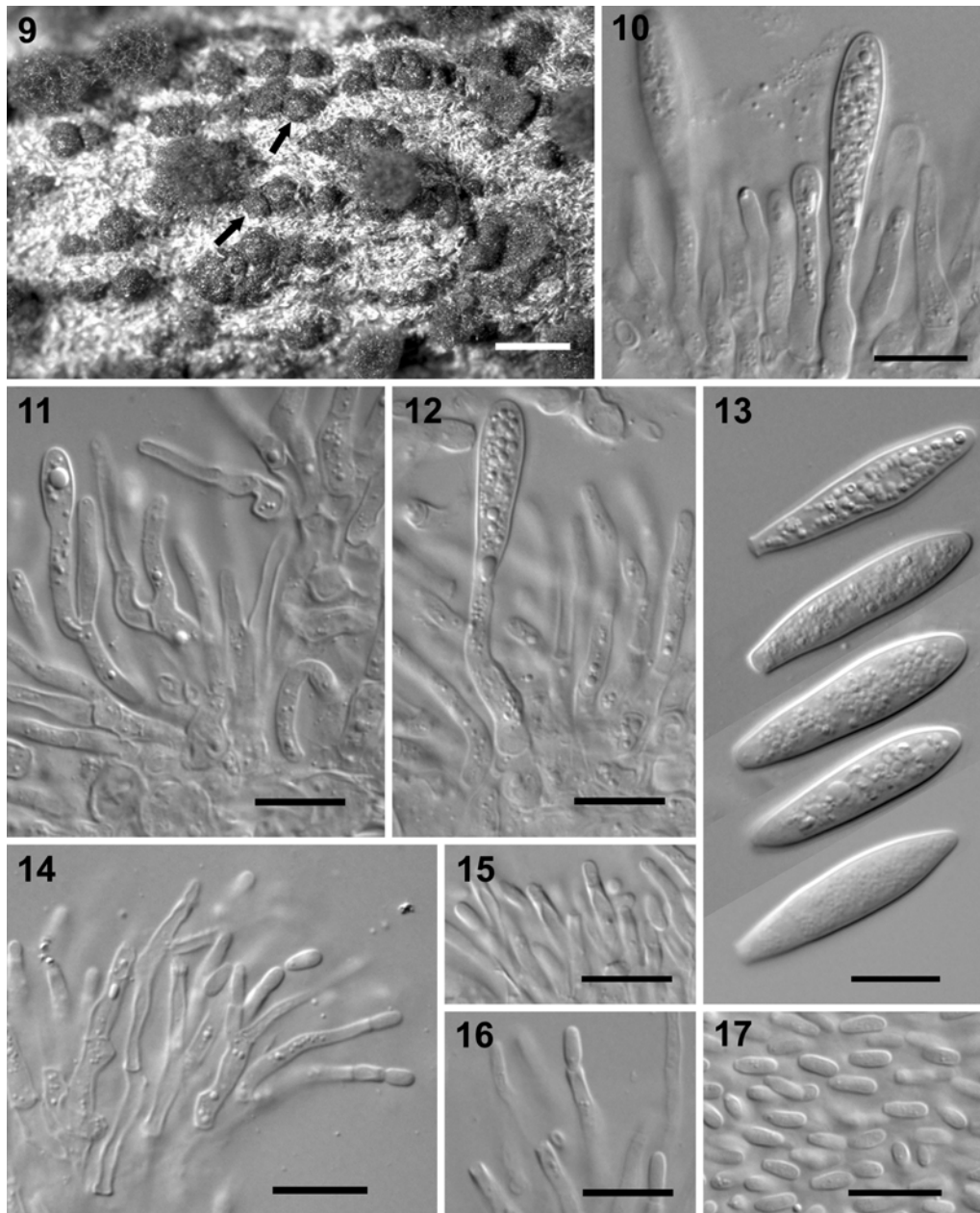
Ascomata were abundant on the host, but conidiomata were not seen on any of the samples examined. *Ascomata* (Figs. 2, 3) embedded in the host becoming partially erumpent at maturity, up to 250 µm diam., conical with a dark brown to black wall composed of up to six cell layers of thick-walled *textura angularis* giving way to hyaline, thinner-walled cells lining the ascomata (Fig. 4). *Asci* (Figs. 5, 6) 145-165 × 25-28 µm, hyaline, clavate and stipitate, bitunicate with a thick endotunica and well-developed apical chamber, eight-spored, irregularly biserial, formed amongst hyaline, thin-walled, septate *pseudoparaphyses* (Fig. 7). *Ascospores* (Fig. 8) ellipsoid to fusoid, (24-)25.5-33(-34.5) × (9.5-)10-12.5(-13.5) µm, 95% confidence limits = 28.5-30.1 × 11.2-11.9 µm ($\bar{x} \pm \text{S.D. of } 32 = 29.3 \pm 2.4 \times 11.6 \pm 1 \mu\text{m}$, L/W = 2.5 ± 0.23), aseptate, hyaline, thin-walled, widest in the middle to upper third.

Ascospores germinated within 24 h at 25°C and formed unbranched germ tubes. Colonies on CMA 28-40 mm diam. after 7 days at 25°C, initially white becoming olive green with clumps of loosely aggregated hyphae.

Conidiomata (Fig. 9) developing in culture on pieces of poplar twigs after 14 days and producing conidia after 28 days, solitary to aggregated, dark brown to black, globose, up to 450 µm diam. *Conidiophores* (Fig. 11) cylindrical, 7.5-14 × 3.5-4.5 µm, hyaline, smooth, thin-walled, septate, branched in the upper parts, lining the entire inner surface of the conidiomata. *Conidiogenous cells* (Figs. 10-12) lageniform, 12.5-17.5 × 2.5-4.5 µm, hyaline,



Figs. 2-8. *Botryosphaeria corticis* Herb CBS-H 19706 (epitype). 2. Ascomata on a cane of *Vaccinium corymbosum*. 3. Longitudinal section through an ascoma. 4. Section of an ascoma wall. 5. Asci. 6. Asci with eight ascospores and well-developed apical chamber. 7. Pseudoparaphyses. 8. Ascospores. Bars: 2 = 500 µm; 3 = 50 µm; 4, 6 = 20 µm; 5, 7, 8 = 10 µm.



Figs. 9-17. *Fusicoccum* anamorph of *Botryosphaeria corticis* CBS 119047 (culture ex-epitype of *B. corticis*). 9. Conidiomata (arrowed) formed in culture on a piece of Poplar twig. 10-12. Conidiogenous cells. 13. Conidia. 14. Microconidiophores. 15. Microconidiogenous cells showing periclinal thickenings. 17. Microconidia. Bars: 9 = 500 μ m; 10-17 = 10 μ m.

thin-walled, smooth, holoblastic producing a single conidium at the tip, rarely proliferating at the same level giving rise to periclinal thickenings. *Conidia* (Fig. 13) fusiform, (20.5-)23.5-32.5(-34.5) \times (5-)5.5-7(-7.5) μm , 95% confidence limits = 27.7-30.2 \times 6.2-6.7 μm ($\bar{x} \pm \text{S.D. of } 26 = 28.9 \pm 3.4 \times 6.4 \pm 0.7 \mu\text{m}$, L/W = 4.5 \pm 0.46), widest in the middle to upper third, hyaline, thin-walled, smooth, apex acute, base truncate with a minute marginal frill. *Microconidiomata* globose, dark brown to black. *Microconidiophores* (Fig. 14) cylindrical, 11-14 \times 2-3 μm , hyaline, branched. *Microconidiogenous cells* (Figs. 15, 16) 14.5-20.5 \times 1.5-2.3 μm , hyaline, thin-walled, smooth, producing conidia at their tips, proliferating internally to form periclinal thickenings. *Microconidia* (Fig. 17) rod-shaped with obtuse ends, 4.1-6 \times 1.5-2 μm , hyaline, thin-walled, smooth.

Habitat: On stems of *Vaccinium* species.

Known distribution: USA.

Material examined: USA, New Jersey, Hammonton, on stems of *Vaccinium corymbosum*, cv. Bluecrop, May 2005, P.V. Oudemans (CBS-H 19706; **epitype designated here**; cultures ex-epitype CBS 119047, CBS 119048; holotype in BPI 598729). USA, North Carolina, on stems of *V. corymbosum*, R.D. Millholland (ATCC 22927, ATCC 22928)

Discussion

Botryosphaeria corticis was first described by Demaree and Wilcox (1942) as *Physalospora corticis* Demaree & M.S. Wilcox. Von Arx and Müller (1954) considered this to be a species of *Botryosphaeria* and made the new combination *Botryosphaeria corticis* (Demaree & M.S. Wilcox) Arx & E. Müll. The morphology of ascomata, asci and ascospores of the specimens examined in this study correlated well with the description of *Physalospora corticis* provided by Demaree and Wilcox (1942). These features are commonly associated with *Botryosphaeria* and the transfer by von Arx and Müller (1954) to *Botryosphaeria* is supported. Phylogenetic analysis of the isolates from *V. corymbosum* placed them within the genus *Botryosphaeria* and within the clade containing *B. dothidea*. The slightly larger ascospores and conidia of *Botryosphaeria corticis* distinguish it from its closest relative, *B. dothidea*.

The specimen of *B. corticis* examined here correlated well with the protologue and is proposed herein as epitype. The type of *B. corticis* was collected from a North Carolina field of *V. corymbosum* while the epitype proposed here was collected from the same host in New Jersey. Two isolates of *B. corticis* from North Carolina were included in the phylogenetic study (ATCC 22927 and ATCC 22928) and the ITS sequences of these did not differ significantly from cultures prepared from the proposed epitype. Thus, the

species in New Jersey and the one in North Carolina can be considered to be the same. For this reason we consider that the proposed epitype is representative of the species.

Demaree and Wilcox (1942) did not apply a name to the anamorph of *B. corticis*, and they made no comments on what form genus it could be allied to. The hyaline, fusoid, aseptate conidia produced holoblastically on conidiogenous cells that proliferate internally to form periclinal thickenings, and the eustromatic, pycnidial conidiomata indicate that this is a species of *Fusicoccum*. We have chosen not to provide a name for this element of the holomorph since the teleomorph is well known, well characterised and appears to be common in nature. Nevertheless, the distinguishing features of this and other *Botryosphaeria* species are seen in the anamorphs.

The *Botryosphaeria* species with *Fusicoccum* anamorphs included in this study clustered in two clades in both the Bayesian and the MP analyses. Both clades were supported by high posterior probabilities and bootstrap values. It thus appears as if *Fusicoccum* consists of two phylogenetic groups. Morphological differences between the two groups are, however, less distinct. Nonetheless, the group consisting of *B. dothidea*, *B. corticis*, *B. mamane* and *F. dimidiatum* have conidia that are more fusoid than the other group in which the conidia tend to be more ellipsoid.

Acknowledgements

This work was financed by the European Regional Development Fund and the Portuguese foundation for science and technology (Fundação para a Ciência e a Tecnologia) under project POCTI/AGR/44348/2002. A. Alves was supported by grant number SFRH/BD/10389/2002 from Fundação para a Ciência e a Tecnologia.

References

- Alves, A., Correia, A., Luque, J. and Phillips, A.J.L. (2004). *Botryosphaeria corticola* sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph *Diplodia mutila*. *Mycologia* 96: 598-613.
- Arx, J.A. von, and Müller, E. (1954). Die Gattungen der Amerosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* 11(1): 1-434.
- Demaree, J.B. and Wilcox, M.S. (1942). Blueberry cane canker. *Phytopathology* 32: 1068-1075.
- Denman, S., Crous, P.W., Taylor, J.E., Kang, J.-C., Pascoe, I. and Wingfield, M.J. (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, P.W., Groenewald, J.Z., Slippers, B., Wingfield, B.D. and Wingfield, M.J. (2003). Circumscription of *Botryosphaeria* species associated with Proteaceae based on morphology and DNA sequence data. *Mycologia* 95: 294-307.

- Farr, D.F., Elliot, M.A., Rossman, A.Y. and Edmonds, R.L. (2005). *Fusicoccum arbuti* sp. nov. causing cankers on Pacific madrone in western North America with notes on *Fusicoccum dimidiatum*, the correct name for *Scytalidium dimidiatum* and *Nattrassia mangiferae*. Mycologia 97: 730-741.
- Hillis, D.M., and Bull, J.J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182-192.
- Jacobs, K.A., and Rehner, S.A. (1998). Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. Mycologia 90: 601-610.
- Page, R.D. (1996). TreeView: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.
- Pavlic, D., Slippers, B., Coutinho, T.A., Gryzenhout, M. and Wingfield, M.J. (2004). *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. Studies in Mycology 50: 313-322.
- Phillips, A.J.L., Alves, A., Correia, A. and Luque, J. (2005). Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. Mycologia 97: 513-529.
- Rannala, B. and Yang, Z. (1996). Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43: 304-311.
- Rodriguez, F., Oliver, J.F., Marin, A. and Medina, J.R. (1990). The general stochastic model of nucleotide substitutions. Journal of Theoretical Biology 142: 485-501.
- Ronquist, F. and Huelsenbeck, J.P. (2003). MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.
- Slippers, B., Crous, P.W., Denman, S., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2004a). Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96: 83-101.
- Slippers, B., Vermeulen, G., Crous, P.W., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2004b). Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. as a sister species to *B. lutea*. Mycologia 96: 1030-1041.
- Slippers, B., Fourie, G., Crous, P.W., Coutinho, T.A., Wingfield, B.D., Carnegie, A.J. and Wingfield, M.J. (2005a). 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., Johnson, G.I., Crous, P.W., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2005b). Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica*. Mycologia 97: 99-110.
- Smith, H., Crous, P.W., Wingfield, M.J., Coutinho, T.A. and Wingfield, B.D. (2001). *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. Mycologia 93: 277-285.
- Swofford, D.L. (2003). *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods) Version 4*. Sunderland, Massachusetts: Sinauer Associates.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876-4882.
- VanderKloet, S.P. (1988). *The Genus Vaccinium in North America*. Agriculture Canada Publication 1828. Ottawa.

- Van Niekerk, J.M., Crous, P.W., Groenewald, J.Z., Fourie, P.H. and Halleen, F. (2004). DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* 96: 781-798.
- White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White). Academic Press San Diego, California: 315-322.
- Young, N.D. and Healy, J. (2003). GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* 4: art. 6.
- Zhou, S. and Stanosz, G.R. (2001). Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8s rDNA sequences. *Mycologia* 93: 516-527.

(Received 5 December 2005; accepted 10 January 2006)