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Lasiodiplodia gonubiensis sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa

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Abstract: *Botryosphaeria* spp. are common and widely distributed pathogens on many economically important crops, including forest tree species. These fungi cause a wide variety of symptoms on trees of all ages, but are mostly associated with canker and die-back of branches and main stems. As disease agents, *Botryosphaeria* spp. are often encountered in their anamorph state, namely species of *Fusicoccum*, *Diplodia* or *Lasiodiplodia*. During a recent survey of botryosphaeriaceous fungi from native *Syzygium cordatum* in South Africa, an unfamiliar *Lasiodiplodia* sp. was isolated. The aim of this study was to compare this apparently undescribed species with other species of *Botryosphaeria* using morphological characteristics and DNA sequence data of the rDNA internal transcribed spacers, ITS1 and ITS2. Based on sequence data, the isolates from *S. cordatum* were more closely related to *B. rhodina* (anamorph *Lasiodiplodia theobromae*) than to other *Botryosphaeria* spp., but also phylogenetically distinct from this species. Conidia of the species from *S. cordatum* were also different to those of *L. theobromae*. We conclude that the isolates from *S. cordatum* represent an undescribed *Lasiodiplodia* sp. and provide the name *Lasiodiplodia gonubiensis* for it.

Taxonomic novelty: *Lasiodiplodia gonubiensis* Pavlic, Slippers & M.J. Wingf. sp. nov.
Key words: *Botryosphaeria*, *Diplodia*, endophyte, *Fusicoccum*, *Lasiodiplodia*, systematics.

INTRODUCTION

Botryosphaeria Ces. & De Not. (*Dothideales*) contains species that have a cosmopolitan distribution and wide host range, including gymnosperms and angiosperms (von Arx & Müller 1954, Barr 1972). These fungi are common endophytes and latent, opportunistic pathogens on many woody plants such as *Eucalyptus* spp. (Fisher *et al*. 1993, Smith *et al*. 1996a, b). Typical disease symptoms associated with *Botryosphaeria* spp. are canker and die-back, followed by kino exudation, and in severe cases tree death (Davison & Tay 1983, Webb 1983, Sharma 1984, Shearer *et al*. 1987, Smith *et al*. 1994, Old & Davison 2000, Roux *et al*. 2000, 2001, Smith *et al*. 2001a).

Eucalyptus belongs to one of the oldest plant families, namely the *Myrtaceae* (Johnson & Briggs 1981). It is largely a Southern Hemisphere family with more than 3000 species and is particularly well represented in the tropical and temperate regions of Australasia and Central and South America (Johnson & Briggs 1981). Myrtaceous species are also an integral part of Southern African indigenous flora (Palgrave 1977). The most common and widely distributed myrtaceous tree in South Africa is *Syzygium cordatum* Hochst. (Palgrave 1977).

Most *Eucalyptus* spp. are native to Australia (Poynton 1979), but they are the most widely grown trees in exotic plantations in other parts of the world (Ciesla *et al*. 1996). These exotic plantations are often planted in close association with native myrtaceous trees that are closely related to *Eucalyptus* (Burgess & Wingfield 2001). A danger in such cases is that pathogens from either of these related native or introduced hosts could cross-infect the other host group and cause serious diseases (Crous & Swart 1995, Wingfield 1999, Burgess & Wingfield 2001). An example of this is the rust fungus *Puccinia psidii* G. Winter that occurs on native *Myrtaceae* in South America, and has become one of the most important pathogens on exotic *Eucalyptus* in this region (Coutinho *et al*. 1998).

Because of its wide distribution, and the fact that this tree often grows alongside plantations of *Eucalyptus*, we conducted a survey of botryosphaeriaceous fungi occurring on native *Syzygium cordatum* in South Africa. This survey resulted in isolates of a *Lasiodiplodia* sp. *Lasiodiplodia* spp. are anamorphs of *Botryosphaeria* and a very common species, particularly in tropical areas, is *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (von Arx 1974, Punithalingam 1976, 1979), teleomorph *B. rhodina* (Berk. & M.A. Curtis) Arx (von Arx 1974). The fungus from *S. cordatum* is similar to *L. theobromae* but has distinctly larger conidia and no teleomorph has been found. The aim of this study was to identify the unknown *Lasiodiplodia* sp. using both morphological characteristics and comparisons of DNA sequence

data of the Internal Transcribed Spacer region (ITS) of the rDNA operon.

MATERIALS AND METHODS

Isolates

Isolates of an unknown *Lasiodiplodia* sp. were collected in the Eastern Cape Province, South Africa in July 2002 (Table 1). Isolations were made from asymptomatic twigs and leaves of naturally growing *S. cordatum*. Leaf and twig portions (5 cm in length) were washed in running tap water, surface disinfected by submerging them for 1 min sequentially in 96 % ethanol, undiluted bleach (3.5–5 % available chlorine) and 70 % ethanol, and rinsed in sterile water. The disinfected twig portions were halved and pieces from the pith tissue (2 mm²) and segments of the leaves (3 mm²) were placed on 2 % malt extract agar (MEA) (2 % malt extract, 1.5 % agar; Biolab, Midrand, Johannesburg, S.A.). Plates were incubated at 20 °C under continuous near-UV light for two weeks and colonies resembling *Botryosphaeria* spp. were selected. These colonies were maintained on 2 % MEA at 25 °C and stored at 5 °C. Isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and in the collection of the Centraalbureau voor Scimmelcultures (CBS), Utrecht, The Netherlands.

Morphology and cultural characteristics

To induce sporulation, isolates were grown on 2 % water agar (WA) (Biolab, S.A.) with sterilized pine needles placed onto the medium, at 25 °C under near-UV light. Herbarium specimens were also sought for *L. theobromae* to compare with the fungus from *S. cordatum*. In the original descriptions of the species (Patouillard 1892) and genus (Clendinin 1896), no reference is made to type material. CBS, ATCC and IMI do not have cultures from the original host and location (*Theobroma cacao* L. in Ecuador), and no herbarium material from the same origin could be located in BPI. Until original material can be located or an epitype specimen assigned, it is necessary to rely on descriptions from the literature. For comparative purposes, we thus compiled a table from previous descriptions, to provide conidial dimensions for this species, as well as many species that have been reduced to synonymy with *L. theobromae* (Table 2).

Released conidia and squash mounts of pycnidia formed on the pine needles, were mounted in lactophenol on microscope slides and examined microscopically. Sections of pycnidia were made by hand and mounted in lactophenol to observe conidiophore morphology. Fifty measurements were taken of pycnidia, conidia, conidiogenous cells and paraphyses for each isolate, and the ranges and averages were computed. Measurements and digital photographs were made using a HRc Axiocam digital camera and accompanying Axiovision 3.1 software (Carl Zeiss Ltd., München, Germany).

Colony growth rate for isolates CMW 14077 and CMW 14078 were studied at temperatures ranging from 5 to 35 °C, at 5 ° intervals in the dark. Mycelial plugs, 6 mm diam, were transferred to 2 % MEA in 90 mm diam Petri dishes from the edges of 7-d-old, single-conidial cultures. Four plates were used for each isolate at each temperature. Two perpendicular measurements were taken of the colony diameter daily until the mycelium of the fastest growing isolates had covered the plates. Average colony diameter of each isolate was calculated from the eight readings per isolate. Colony morphology and colour were determined from cultures grown on 2 % MEA at 25 ºC in the dark. Colony colours (upper surface and reverse) were described by comparison with the colour charts of Rayner (1970).

DNA extraction and ITS rDNA amplification

For DNA extraction, single conidial cultures were grown on 2 % MEA for 7 d at 25 °C in the dark. The mycelium was scraped directly from the medium and transferred to Eppendorf tubes (1.5 mL). DNA was extracted using a modified phenol:chloroform DNA extraction method of Raeder & Broda (1985). The resulting DNA pellets were resuspended in 50 µL sterile SABAX water. RNAse (1 mg/mL) was added to DNA samples and incubated overnight at 37 °C to degrade residual protein or RNA. DNA was separated by electrophoresis on a 1.5 % agarose gel, stained with ethidium bromide and visualized under ultraviolet light. DNA concentrations were estimated against λ standard size markers.

Using the primer pair ITS1 and ITS4 (White *et al*. 1990), the ITS1 and ITS2 regions, and the 5.8S gene of the ribosomal RNA (rRNA) operon were amplified using the PCR protocol of Slippers *et al.* (2004). PCR products were separated as described above and sizes of PCR products were estimated against a 100 bp molecular weight marker XIV (Roche Diagnostics, Johannesburg, S.A.). The PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany).

DNA sequencing and analysis

The purified PCR products were sequenced in both directions using the same primers used for the PCR reactions. The ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, U.K.) was used for sequencing reactions as specified by the manufacturers.

Table 1. Isolates of *Botryosphaeria*, *Guignardia* and *Mycosphaerella* species considered in the phylogenetic study.

Culture no.[1]	Other no.[1]	Identity	Host	Location	Collector	GenBank no.
CMW 9081	ICMP 8003	*Botryosphaeria parva*	*Populus nigra*	New Zealand	G.J. Samuels	AY236943
CMW 10124	BOT 681	*B. parva*	*Heteropyxis natalensis*	KwaZulu-Natal, S. Africa	H. Smith	AF283676
CMW 9075	ICMP 8019	*Botryosphaeria dothidea*	*P. nigra*	New Zealand	G.J. Samuels	AY236950
CMW 8000		*B. dothidea*	*Prunus* sp.	Crocifisso, Switzerland	B. Slippers	AY236949
CMW 10125	BOT 24	*Botryosphaeria eucalyptorum*	*Eucalyptus grandis*	Mpumalanga, S. Africa	H. Smith	AF283686
CMW 10126	BOT 16	*B. eucalyptorum*	*E. grandis*	Mpumalanga, S. Africa	H. Smith	AF283687
CMW 992	KJ 93.52	*Botryosphaeria lutea*	*Actinidia deliciosa*	New Zealand	G.J. Samuels	AF027745
CMW 9076	ICMP 7818	*B. lutea*	*Malus domestica*	New Zealand	S.R. Pennycook	AY236946
CMW 7774		*Botryosphaeria obtusa*	*Ribes* sp.	New York, U.S.A.	B. Slippers & G. Hudler	AY236953
	KJ 93.56	*B. obtusa*	Hardwood shrub	New York, U.S.A.	G.J. Samuels	AF027759
	KJ 93.27	*Botryosphaeria rhodina*	*Quercus* sp.	California, U.S.A.	E. Hecht-Poinar	AF027761
	ZS 96-112	*B. rhodina*	*Pinus radiata*	S. Africa	W. Swart	AF243401
	ZS 96-172	*B. rhodina*	*Theobroma cacao*	Sri Lanka	E. Müller	AF243400
CMW 10130	BOT 977	*B. rhodina*	*Vitex donniana*	Uganda	J. Roux	AY236951
CMW 9074		*B. rhodina*	*Pinus* sp.	Mexico	T. Burgess	AY236952
CMW 7060	CBS 431	*Botryosphaeria stevensii*	*Fraxinus excelsior*	Netherlands	H.A. van der Aa	AY236955
	ZS 94-6	*B. stevensii*	*Malus pumila*	New Zealand	N. Tisserat	AF243407
	CBS 112545	*Botryosphaeria corticola*	*Quercus ilex*	Spain	M.A. Sanchez & A. Trapero	AY259089
	CBS 112551	*B. corticola*	*Q. suber*	Portugal	A. Alves	AY259101
	CBS 418.64	*Botryosphaeria tsugae*	*Tsuga heterophylla*	Canada	A. Funk	AF243405
	KJ 94.07	*Diplodia pinea*	*Pinus resinosa*	Wisconsin, U.S.A.	D.R. Smith	AF027758
CMW 14077	CBS 115812	*Lasiodiplodia gonubiensis*	*Syzygium cordatum*	Eastern Cape, S. Africa	D. Pavlic	AY639595
CMW 14078	CBS 116355	*L. gonubiensis*	*S. cordatum*	Eastern Cape, S. Africa	D. Pavlic	AY639594
CMW 3025		*Mycosphaerella africana*	*Eucalyptus viminalis*	Stellenbosch, S. Africa	P.W. Crous	AF 283690
CMW 7063	CBS 447	*Guignardia philoprina*	*Taxus baccata*	Netherlands	H.A. van der Aa	AY236956

[1]Culture collections: BOT and CMW = Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria; KJ = Jacobs & Rehner (1998); CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand; ZS = Zhou & Stanosz (2001).

Sequence reactions were run on an ABI PRISM 3100™ automated DNA sequencer (Perkin-Elmer, Warrington, U.K.). The nucleotide sequences were analyzed using Sequence Navigator v. 1.0.1. (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software and manually aligned by inserting gaps. Sequence data for isolates of the unknown species have been deposited in GenBank (Table 1).

The DNA sequences of the isolates of the unknown species were compared with those of other *Botryosphaeria* spp. These included twenty one ITS rDNA sequences of *B. parva* Pennycook & Samuels, *B. dothidea* (Moug. : Fr.) Ces. & De Not., *B. eucalyptorum* Crous, H. Smith & M.J. Wingf., *B. lutea* A.J.L. Phillips, *B. obtusa* (Schwein.) Shoemaker, *B. stevensii* Shoemaker, *B. tsugae* Funk, *Diplodia pinea* (Desm.) J. Kickx (= *Sphaeropsis sapinea* (Fr. : Fr.) Dyko & B. Sutton), *B. corticola* A.J.L. Phillips, Alves & Luque and *B. rhodina* obtained from GenBank (Table 1), arising from previous studies (Jacobs & Rehner 1998, Smith *et al*. 2001a, Zhou & Stanosz 2001, Alves *et al*. 2004, Slippers *et al*. 2004). The trees were rooted using the GenBank sequence of *Guignardia philoprina* (Berk. & M.A. Curtis) Aa and *Mycosphaerella africana* Crous & M.J. Wingf.

The DNA sequence data were manually aligned in PAUP version 4.0b10 (Swofford 1999) by insertion of gaps. Gaps were treated as missing data and all characters included in the analyses were unordered and of equal weight. Most parsimonious trees were found using the heuristic search function with 1000 random addition replicates and tree bisection and reconstruction (TBR) selected as branch-swapping algorithm.

Table 2. Conidial size and septation for *Lasiodiplodia theobromae* described under different synonyms.

Species	Host	Origin	Conidia size	No. of septa	Reference
Diplodia gossypina Cooke	*Gossypium* sp.	India	22 ×12 µm	–	Cooke 1879
Botryodiplodia theobromae Pat.	*Theobroma cacao*	Ecuador	25–35 × 12–15 µm	1	Patouillard & De Lagerheim 1892
Macrophoma vestita Prill. & Delacr.	*T. cacao*	Equatorial America	25–28 × 13 µm	1	Prillieux & Delacroix 1894
Lasiodiplodia tubericola Ellis & Everh.	*Ipomoea batatas*	Java	18–22 ×11–14 µm	1	Clendinin 1896
Diplodia cacaoicola P. Henn.	*T. cacao*	Kamerun	22–28 × 12–14 µm	1	Hennings 1897
Botryodiplodia gossypii Ellis & Barthol.	*Gossypium herbaceum*	U.S.A.	15–22 × 12 µm	1	Ellis & Bartholomew 1902
Lasiodiplodia nigra K.R. Appel & Laubert	*T. cacao, Carica papaya*	Samoa	28–32 × 18–21 µm	1	Appel & Laubert 1907
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	*T. cacao*	Equatorial America	20–30 × 11–15 µm	1	Griffon & Maublanc 1909
Diplodia rapax Massee	*Hevea brasiliensis*	Singapore, Ghana	32–35 × 15–16 µm	1	Massee 1910
Diplodia natalensis Pole-Evans	*Citrus* sp.	South Africa	24–15 µm	1	Pole Evans 1910
Lasiodiplodia triflorae B.B. Higgins	*Prunus* sp.	U.S.A.	22–25 × 13–16.5 µm	1	Higgins 1916
Diplodia maniothi Sacc.	*Manihot utilissima*	–	16–22 × 10–12 µm	1	Sydow *et al*. 1916
Diplodia musae Died.	*Musa sapientium*	–	17–20 ×10–13 µm	1	Sydow *et al*. 1916
Diplodia ananassae Sacc.	*Ananas sativus*	Philippines	23–25 ×11–12 µm	1	Saccardo 1917
Diplodia theobromae (Pat.) W. Nowell	*T. cacao*	–	25–30 × 12–15 µm	1	Nowell 1923

Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Branch support was determined using 1000 bootstrap replicates (Felsenstein 1985). The data set was also analysed by distance analyses using the Kimura-2 parameter (Kimura 1980). The sequence alignment and phylogenetic tree have been deposited in TreeBASE as S1133, M1944.

RESULTS

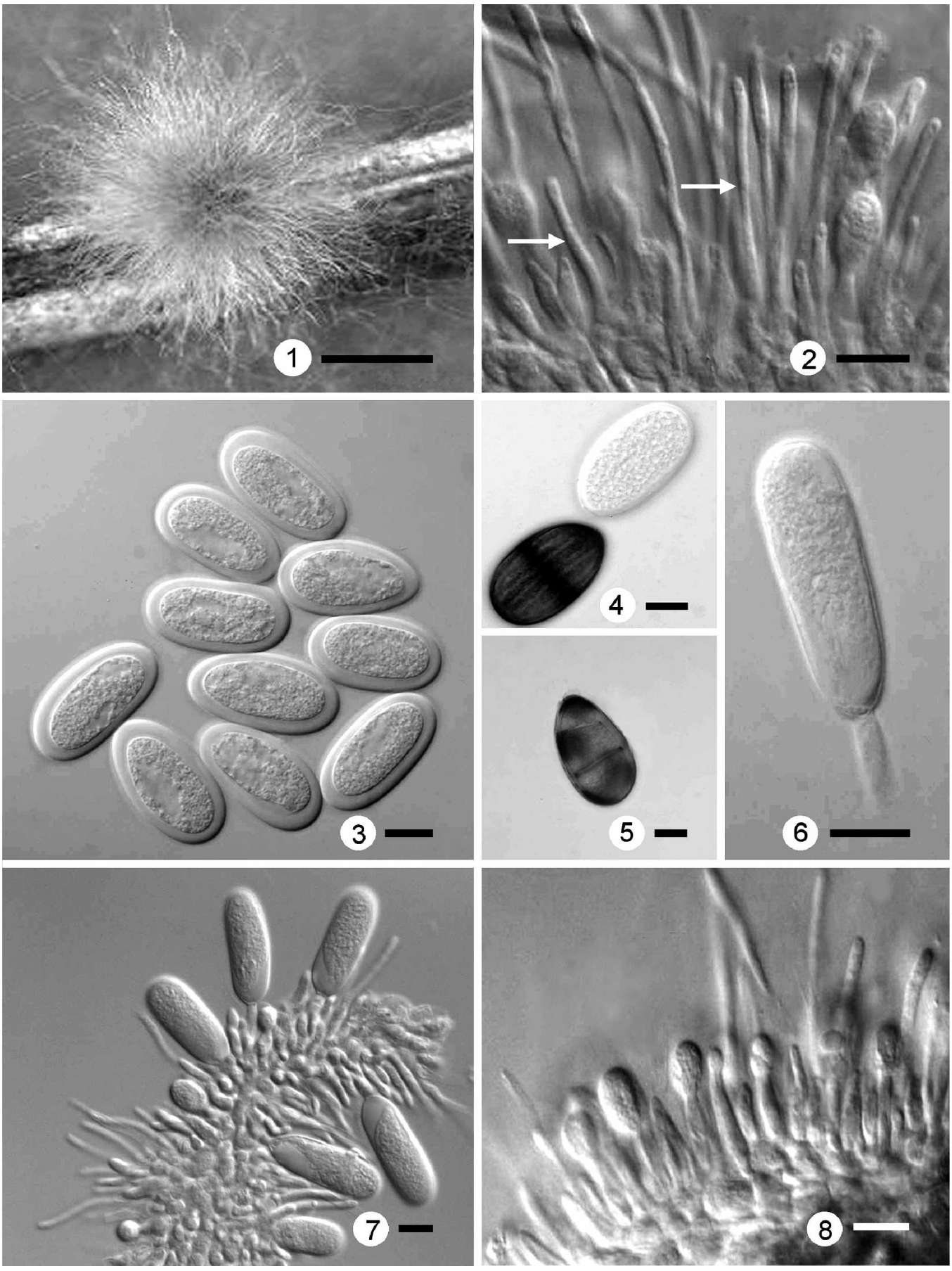
Morphology and cultural characteristics

The isolates from *S. cordatum* produced anamorph structures on the pine needles on WA within 2–3 wk. No sexual (teleomorph) structures were observed during this study. The conidia (Figs 3, 4) were similar to those described for *L. theobromae* in shape, colour and striation (Clendinin 1896, Punithalingam 1976, Sivanesan 1984). These isolates, however, differed from *L. theobromae* in having markedly longer and wider conidia (28−)32−36(−39) × (14−)16−18.5(−21) µm, while those of *L. theobromae* are mostly 18−30 × 10−15 µm (Table 2). Furthermore, aging conidia of the strains from *S. cordatum* become 1–3-septate, (Figs 4, 5, 11), which is different to the single septate conidia that are typical of *L. theobromae* (Table 2).

DNA sequence comparisons

PCR products of approximately 560 base pairs (bp) were amplified. Unreliable sequence data from the ends of sequences were excluded. Alignment of the sequences resulted in a total of 534 characters, of which 386 uninformative characters were excluded, and 148 parsimony informative characters were used in the analyses. The parsimony analysis (using heuristic searches) produced six most parsimonious trees of 318 steps (CI = 0.758, RI = 0.869) that only differed in the length of the internal branches, and one of these trees was chosen for presentation (Fig. 12). A bootstrap search of 1000 replicates (Fig. 12) and distance analyses produced a tree of the same topology as the most parsimonious trees.

The species included in this comparison formed eleven terminal groupings, designated as groups I to XI (Fig. 12). Groups I to IV include *Botryosphaeria* spp. with *Fusicoccum*-like anamorphs: *B. parva*, *B. lutea*, *B. eucalyptorum* and *B. dothidea*. Groups V to IX (Fig. 12) include *Botryosphaeria* spp. with *Diplodia*-like anamorphs: *B. obtusa*, *Diplodia pinea*, *B. stevensii*, *B. tsugae* and *B. corticola*. Isolates of the unnamed species from *S. cordatum* grouped most closely to *B. rhodina* (anamorph *L. theobromae*) (group X), but also resided in a clearly distinct group (group XI) with 95 % bootstrap support (Fig. 12). These two groups were more closely related to isolates that have *Diplodia*-like anamorphs (groups V to IX), but also clearly separated from them with a 78 % bootstrap value.



Figs 1–8. Micrographs of fruiting structures of *Lasiodiplodia gonubiensis*. 1. Pycnidium formed in culture on pine needles, covered with mycelium. 2. Paraphyses (arrows). 3. Conidia. 4. Brown conidium with one septum. 5. Brown conidium with two septa. 6. Conidium attached to conidiogenous cell. 7, 8. Conidia, conidiogenous cells and paraphyses. Bars 1 = 500 µm; 2–8 = 10 µm.

Taxonomy

Based on morphological characteristics and DNA sequence comparisons, we conclude that the fungus isolated from native *S. cordatum* in South Africa is distinct from *L. theobromae* and other *Botryosphaeria* anamorph spp. examined in our study. Our data also indicate that this fungus should reside in *Lasiodiplodia* as a new taxon. We provide the following description for this new species.

Lasiodiplodia gonubiensis Pavlic, Slippers & M.J. Wingf., **sp. nov.** MycoBank MB500079. Figs 1–11.

Etymology: Referring to the town Gonubie, South Africa from where the fungus was collected.

Pycnidia subimmersa, solitaria, globosa, papillata, atroplumbea, mycelio tecta, usque ad 460 µm diametro. Paraphyses cylindricae, non septatae, hyalinae. Cellulae conidiogenae holoblasticae, cylindricae, hyalinae. Conidia primaria hyalina, unicellulares, ellipsoidea vel obovoidea, parietibus crassitunicati, contentu granulari, apice rotundata, interdum basi truncata. Conidia senia cinnamomescentia vel brunnescentia, longitudinaliter striata, unum ad tria septa formantia.

Pycnidia (formed on WA on sterilized pine needles within 7−21 d) semi-immersed, solitary, globose, papillate, leaden-black, covered by mycelium, up to 460 µm diam (Fig. 1). *Paraphyses* cylindrical, aseptate, hyaline, (14−)26.5−47(−65) × (1.5−)2–2.5(−3) µm (Figs 2, 7, 9). *Conidiogenous cells* holoblastic, cylindrical, hyaline, (6.5−)10−15(−18) × (1−)2−4(−4.5) µm (Figs 7–9). *Conidia* initially hyaline, unicellular, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base (Figs 3, 6, 7, 9, 10). Aging conidia becoming cinnamon to sepia with longitudinal striations, forming one to three septa, (28−)32−36(−39) × (14−)16−18.5(−21) µm (av. 33.8 × 17.3 µm, n = 100, l/w 1.9) (Figs 4, 5, 11).

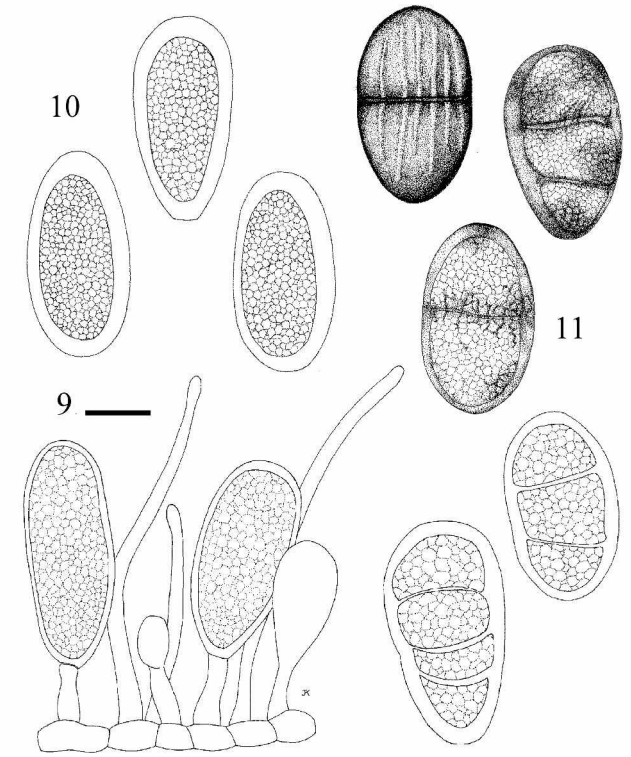
Typus: PREM 58127 **holotype**, fruiting structures induced on needles of *Pinus* sp. on WA, South Africa, Eastern Cape Province, Gonubie, *Syzygium cordatum*, Jul. 2002, D. Pavlic (culture ex-type CMW 14077 = CBS 115812).

Cultural characteristics: Cultures initially white to smoke-grey with fluffy, aerial mycelium, becoming olivaceous-grey on the surface after 3−4 d, with thick aerial mycelium, margins slightly irregular; reverse side of the colonies dark slate-blue. Optimum temperature for colony growth 25 °C, covering the medium surface (90 mm diam Petri dishes) after 5 d in the dark. Isolates growing at 35 °C produced a coral-red pigment within 4 d.

Substrate: Symptomless leaves and branches of *S. cordatum*.

Distribution: Eastern Cape Province (Gonubie), South Africa.

Specimens examined: **South Africa**, Eastern Cape Province, Gonubie, *Syzygium cordatum*, Jul. 2002, D. Pavlic, **holotype** PREM 58127, fruiting structures induced on needles of *Pinus* sp. on WA; culture ex-type CMW 14077 = CBS 115812; Eastern Cape Province, Gonubie, *Syzygium cordatum*, Jul. 2002, D. Pavlic, **paratype** PREM 58128, fruiting structures induced on needles of *Pinus* sp. on WA, culture ex-paratype CMW 14078 = CBS 116355.



Figs 9–11. Line drawings of *Lasiodiplodia gonubiensis*. 9. Conidia, conidiogenous cells and paraphyses. 10. Aseptate conidia. 11. 1–3-septate conidia. Bar = 10 µm.

DISCUSSION

In this study we have identified and described the new species *Lasiodiplodia gonubiensis*, that grows endophytically on native *S. cordatum* in South Africa. Based on its phylogenetic relationships, we expect that the teleomorph of this fungus will be a species of *Botryosphaeria*. Despite careful examination of dead branches and twigs of *S. cordatum*, we have not been able to find a sexual state for this fungus. Ideally, we would provide a name in *Botryosphaeria* for it, but this is not recommended by the ICBN (Art. 59.2, Greuter *et al.* 2000).

Lasiodiplodia gonubiensis was identified as a species of *Lasiodiplodia* based on conidial shape and striation, which are characters typical for this genus (von Arx 1974).

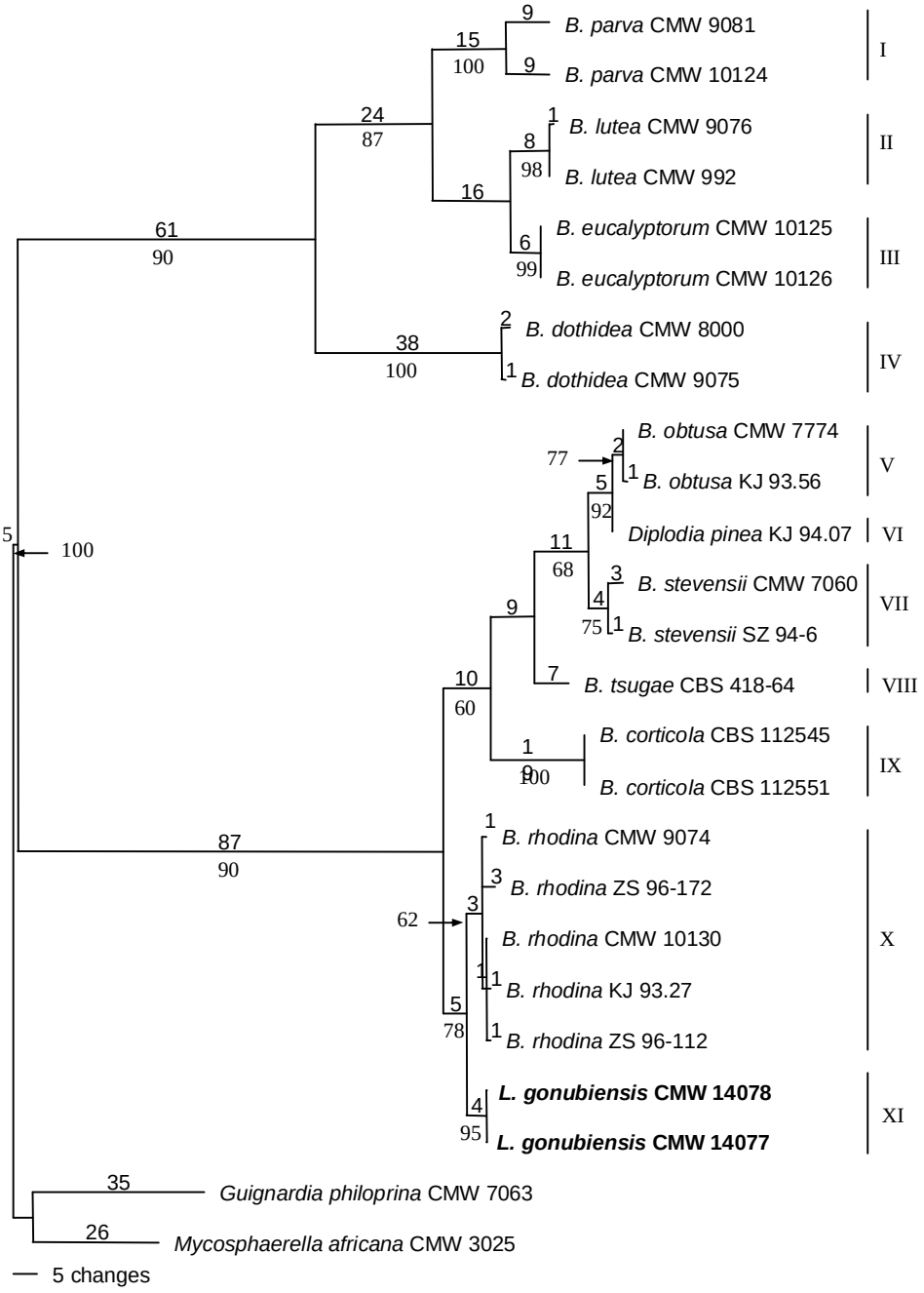


Fig. 12. Phylogram showing relationships amongst *Botryosphaeria* spp. based on parsimony analysis of the ITS1, 5.8 S and ITS2 rDNA sequence data (tree length = 318 steps, CI = 0.758, RI = 0.869). The tree is rooted to the outgroups *Guignardia philoprina* and *Mycosphaerella africana*. Bootstrap values (1000 replicates) are indicated below the internodes, and branch lengths, proportional to the number of steps, are indicated above the internodes.

Conidia of *L. gonubiensis* are similar in appearance to those of *L. theobromae* (Clendinin 1896, Griffon & Maublanc 1909, Goos 1961, Punithalingam 1976, 1979, Sivanesan 1984). However, *L. gonubiensis* can be distinguished from *L. theobromae* by its substantially larger and multiseptate conidia. These conidial characters have also been useful to distinguish other closely related *Botryosphaeria* anamorphs, such as *B. ribis* and *B. parva* (Slippers *et al.* 2004).

Lasiodiplodia gonubiensis grouped separately from other *Botryosphaeria* spp. based on comparison of partial nrDNA ITS sequence data. The results of the phylogenetic study further showed that *L. gonubiensis* was closely related, but clearly distinct from isolates of *L. theobromae*. This is another example where ITS rDNA sequence data were useful to distinguish a new botryosphaeriaceous species. Recent studies have used this region extensively, combined with morphological data, to describe new *Botryosphaeria* spp. and to re-evaluate the placement of their anamorphs (Jacobs & Rehner 1998, Denman *et al*. 1999, 2000, Smith *et al*. 2000b, Smith & Stanosz 2001, Zhou & Stanosz 2001, Denman *et al.* 2003, Slippers 2003, Alves 2004). Despite the general phylogenetic usefulness of this region of the genome, there are cryptic species that cannot be separated based solely on ITS rDNA se-

quence data (De Wet *et al*. 2003, Slippers *et al*. 2004). In these cases sequence data of multiple gene regions have revealed the cryptic species.

The teleomorph of *L. gonubiensis* was not observed in this study. *Lasiodiplodia* spp. are, however, well-known as anamorphs of *Botryosphaeria*. This is confirmed in this study, because *L. gonubiensis* groups significantly more closely to other *Botryosphaeria* spp. than even the closely related genus *Guignardia.* Due to the rarity of *Botryosphaeria* teleomorphs and their overlapping morphological features, species are often identified based on morphological characteristics of associated anamorphs (Shoemaker 1964, Laundon 1973, Sivanesan 1984, Jacobs & Rehner 1998, Slippers 2003). This has been true for *L. gonubiensis*, which could easily be distinguished from other closely related species based on conidial morphology.

In this study, *L. gonubiensis* and *L. theobromae* (teleomorph *B. rhodina*) grouped together as a subclade, within a greater clade that contains *Botryosphaeria* spp. with anamorphs in *Diplodia.* Previous phylogenetic re-evaluations have shown that *Botryosphaeria* anamorphs can be separated into two groups, namely *Diplodia*-like anamorphs with ellipsoid, thick-walled dark conidia, and *Fusicoccum*-like anamorphs with hyaline conidia (Denman *et al.* 2000, Zhou & Stanosz 2001). *Lasiodiplodia* has, however, always grouped separately within the *Diplodia* clade (Denman *et al.* 2000, Zhou & Stanosz 2001, Slippers 2003, Slippers *et al.* 2004), as was the case in our study. It has been proposed that all *Botryosphaeria* anamorphs might either be placed in *Fusicoccum* or *Diplodia*, with *Lasiodiplodia* residing in *Diplodia* (Denman *et al.* 2000). Because it is morphologically distinct, especially based on its obvious and unique conidial striations (von Arx 1974), there seemed little reason from our data to change the name of this important tree pathogen. *Lasiodiplodia* has also not formally been reduced to synonymy with *Diplodia* and we have thus chosen to assign the new species from *S. cordatum* to *Lasiodiplodia* rather than *Diplodia.*

Lasiodiplodia gonubiensis is the first species in this genus to be found on native trees in South Africa. The closely related *L. theobromae* is an important opportunistic pathogen recorded from more than 500 host plants, mostly in tropical and subtropical regions (Punithalingam 1976). *Lasiodiplodia theobromae* has not been reported from native trees in South Africa, but it occurs on exotic *Acacia*, *Eucalyptus* and *Pinus* spp. in South Africa (Cilliers *et al.* 1993, Crous *et al*. 2000, Burgess *et al.* 2003).

Lasiodiplodia gonubiensis was discovered as an endophyte in asymptomatic twigs and leaves of *S. cordatum*. Other *Botryosphaeria* spp. are common endophytes and latent, opportunistic pathogens on *Eucalyptus* (Fisher *et al.* 1993, Smith *et al*. 1996a, b). For these fungi, disease symptoms typically develop when trees are exposed to unfavourable environmental conditions. *Lasiodiplodia gonubiensis* might thus also be a latent pathogen although we have not found it in association with disease symptoms.

Lasiodiplodia gonubiensis could become a pathogen of commercial *Eucalyptus* spp. in South Africa. Both *S. cordatum* and *Eucalyptus* reside in the *Myrtaceae* and they are sufficiently related that they could share pathogens. This would be consistent with the fact that *B. parva* has been shown to infect both hosts (Smith *et al.* 2000a, Slippers *et al*. 2004). Although *B. parva* has been found as a pathogen on *Eucalyptus*, its pathogenicity on *S. cordatum* is not known. Future studies will consider the pathogenicity and potential threat of *L. gonubiensis* and other *Botryosphaeria* spp. to both *Syzygium* and *Eucalyptus* spp.

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