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


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 Schimmelcultures (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 Axioskop 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 °C 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 °C 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 a 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.

<table>
<thead>
<tr>
<th>Culture no.</th>
<th>Other no.</th>
<th>Identity</th>
<th>Host</th>
<th>Location</th>
<th>Collector</th>
<th>GenBank no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMW 9081</td>
<td>ICMP 8003</td>
<td><em>Botryosphaeria parva</em></td>
<td><em>Populus nigra</em></td>
<td>New Zealand</td>
<td>G.J. Samuels</td>
<td>AY236943</td>
</tr>
<tr>
<td>CMW 10124</td>
<td>BOT 681</td>
<td><em>B. parva</em></td>
<td><em>Heteropyxis natalensis</em></td>
<td>KwaZulu-Natal, S. Africa</td>
<td>H. Smith</td>
<td>AF283676</td>
</tr>
<tr>
<td>CMW 9075</td>
<td>ICMP 8019</td>
<td><em>Botryosphaeria dothisidea</em></td>
<td><em>P. nigra</em></td>
<td>New Zealand</td>
<td>G.J. Samuels</td>
<td>AY236950</td>
</tr>
<tr>
<td>CMW 8000</td>
<td></td>
<td><em>B. dothisidea</em></td>
<td><em>Prunus sp.</em></td>
<td>Crocifisso, Switzerland</td>
<td>B. Slippers</td>
<td>AY236949</td>
</tr>
<tr>
<td>CMW 10125</td>
<td>BOT 24</td>
<td><em>Botryosphaeria eucalyptorum</em></td>
<td><em>Eucalyptus grandis</em></td>
<td>Mpumalanga, S. Africa</td>
<td>H. Smith</td>
<td>AF283686</td>
</tr>
<tr>
<td>CMW 10126</td>
<td>BOT 16</td>
<td><em>B. eucalyptorum</em></td>
<td><em>E. grandis</em></td>
<td>Mpumalanga, S. Africa</td>
<td>H. Smith</td>
<td>AF283687</td>
</tr>
<tr>
<td>CMW 992</td>
<td>KJ 93.52</td>
<td><em>Botryosphaeria latea</em></td>
<td><em>Actinidia delicosa</em></td>
<td>New Zealand</td>
<td>G.J. Samuels</td>
<td>AF027745</td>
</tr>
<tr>
<td>CMW 9076</td>
<td>ICMP 7818</td>
<td><em>B. latea</em></td>
<td><em>Malus domestica</em></td>
<td>New Zealand</td>
<td>S.R. Pennycook</td>
<td>AY236946</td>
</tr>
<tr>
<td>CMW 7774</td>
<td></td>
<td><em>Botryosphaeria obtusa</em></td>
<td><em>Ribes sp.</em></td>
<td>New York, U.S.A.</td>
<td>B. Slippers &amp; G. Hudler</td>
<td>AY236953</td>
</tr>
<tr>
<td>KJ 93.56</td>
<td></td>
<td><em>B. obtusa</em></td>
<td>Hardwood shrub</td>
<td>New York, U.S.A.</td>
<td>G.J. Samuels</td>
<td>AF027759</td>
</tr>
<tr>
<td>KJ 93.27</td>
<td></td>
<td><em>Botryosphaeria rhodina</em></td>
<td><em>Quercus sp.</em></td>
<td>California, U.S.A.</td>
<td>E. Hecht-Poinar</td>
<td>AF027761</td>
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<tr>
<td>ZS 96-112</td>
<td></td>
<td><em>B. rhodina</em></td>
<td><em>Pinus radiata</em></td>
<td>S. Africa</td>
<td>W. Swart</td>
<td>AF243401</td>
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<tr>
<td>ZS 96-172</td>
<td></td>
<td><em>B. rhodina</em></td>
<td><em>Theobroma cacao</em></td>
<td>Sri Lanka</td>
<td>E. Müller</td>
<td>AF243400</td>
</tr>
<tr>
<td>CMW 10130</td>
<td>BOT 977</td>
<td><em>B. rhodina</em></td>
<td><em>Vitex donniana</em></td>
<td>Uganda</td>
<td>J. Roux</td>
<td>AY236951</td>
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<tr>
<td>CMW 9074</td>
<td></td>
<td><em>B. rhodina</em></td>
<td><em>Pinus sp.</em></td>
<td>Mexico</td>
<td>T. Burgess</td>
<td>AY236952</td>
</tr>
<tr>
<td>CMW 7060</td>
<td>CBS 431</td>
<td><em>Botryosphaeria stevensii</em></td>
<td><em>Fraxinus excelsior</em></td>
<td>Netherlands</td>
<td>H.A. van der Aa</td>
<td>AY236955</td>
</tr>
<tr>
<td>ZS 94-6</td>
<td>B. stevensii</td>
<td><em>Malus pumila</em></td>
<td>New Zealand</td>
<td>N. Tisserat</td>
<td>AY236947</td>
<td></td>
</tr>
<tr>
<td>CBS 112545</td>
<td></td>
<td><em>Botryosphaeria corticola</em></td>
<td><em>Quercus ilex</em></td>
<td>Spain</td>
<td>M.A. Sanchez &amp; A. Trapero</td>
<td>AY259089</td>
</tr>
<tr>
<td>CBS 112551</td>
<td>B. corticola</td>
<td><em>Q. suber</em></td>
<td>Portugal</td>
<td>A. Alves</td>
<td>AY259101</td>
<td></td>
</tr>
<tr>
<td>CBS 418.64</td>
<td><em>Botryosphaeria tsugae</em></td>
<td><em>Tsuga heterophylla</em></td>
<td>Canada</td>
<td>A. Funk</td>
<td>AF243405</td>
<td></td>
</tr>
<tr>
<td>KJ 94.07</td>
<td><em>Diplodia pinea</em></td>
<td><em>Pinus resinosa</em></td>
<td>Wisconsin, U.S.A.</td>
<td>D.R. Smith</td>
<td>AF027758</td>
<td></td>
</tr>
<tr>
<td>CMW 14077</td>
<td>CBS 115812</td>
<td><em>Lasiodiplodia goniobiensis</em></td>
<td><em>Syzygium cordatum</em></td>
<td>Eastern Cape, S. Africa</td>
<td>D. Pavlic</td>
<td>AY639595</td>
</tr>
<tr>
<td>CMW 14078</td>
<td>CBS 116355</td>
<td><em>L. goniobiensis</em></td>
<td><em>S. cordatum</em></td>
<td>Eastern Cape, S. Africa</td>
<td>D. Pavlic</td>
<td>AY639594</td>
</tr>
<tr>
<td>CMW 3025</td>
<td>CBS 447</td>
<td><em>Mycosphaerella africana</em></td>
<td><em>Eucalyptus viminalis</em></td>
<td>Stellenbosch, S. Africa</td>
<td>P.W. Crous</td>
<td>AF283690</td>
</tr>
<tr>
<td>CMW 7063</td>
<td>CBS 447</td>
<td><em>Guignardia philoprina</em></td>
<td><em>Taxus baccata</em></td>
<td>Netherlands</td>
<td>H.A. van der Aa</td>
<td>AY236956</td>
</tr>
</tbody>
</table>

1Culture 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 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.
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.

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

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

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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 (formed on WA on sterilized pine needles within 7–21 d) semi-immersed, solitary, globose, papillate, lea...
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 & Stanisz 2001, Zhou & Stanisz 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-
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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