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Molecular characterization of *Endothia gyrosa* isolates from *Eucalyptus* in South Africa and Australia

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Endothia gyrosa is a canker pathogen best known as the causal agent of pin oak blight in North America, and causes cankers on other woody hosts such as *Castanea* spp. and *Liquidambar* spp. In South Africa, Australia and Tasmania, a fungus identified as *E. gyrosa* has been recorded on *Eucalyptus* spp. Some morphological differences exist between the North American fungus and the isolates from *Eucalyptus*. Phylogenetic relationships between *E. gyrosa* from North America and *E. gyrosa* from South Africa and Australia, as well as that of the related fungi *Cryphonectria parasitica* and *C. cubensis*, were studied using PCR-based restriction fragment length polymorphism (RFLP) and sequences of the internal transcribed spacer (ITS) region of the rRNA operon. *Endothia gyrosa* isolates from South Africa produced the same RFLP banding patterns as those from Australia, which differed markedly from North American isolates of *E. gyrosa*. In a phylogram based on the DNA sequences, the Australian and South African isolates of *E. gyrosa* resided in a single, well resolved clade, distinct from North American isolates. Isolates of *C. parasitica* grouped in the same clade as the South African and Australian isolates of *E. gyrosa*, but *C. cubensis* was distantly related to them. The molecular data suggest that the *E. gyrosa* isolates from South Africa and Australia represent a distinct taxon, and probably belong to the genus *Cryphonectria*.

Keywords: *Cryphonectria cubensis*, *Cryphonectria parasitica*, *Endothia gyrosa*, RFLP, ribosomal DNA sequence

Introduction

Endothia gyrosa is a fungal pathogen best known for its association with pin oak (*Quercus palustris*) blight in North America (Stipes & Phipps, 1971; Roane *et al*., 1974; Appel & Stipes, 1986). This fungus, native to North America, also causes serious cankers on exotic Formosan sweetgum (*Liquidambar formosana*) (Snow *et al*., 1974), as well as on other *Quercus* spp., *Acer saccharinum* (Roane *et al*., 1974), *Liquidambar styraciflua* (Snow *et al*., 1974), *Castanea* spp., *Ilex opaca* (Appel & Stipes, 1986), *Fagus grandiflora*, *Fagus sylvatica*, *Prunus laurocerasus* (Roane, 1986), *Corylus*, *Ulmus* and *Vitis* (Farr *et al*., 1989). *Endothia gyrosa* is reported to occur widely in North America, and is particularly well known in the south-eastern parts of the USA (Shear *et al*., 1917; Stevens, 1917; Roane *et al*., 1974; Snow *et al*., 1974; Hunter & Stipes, 1978; Appel & Stipes, 1986). *Endothia gyrosa* has also been reported from China (Teng, 1934) and Europe (Spaulding, 1961).

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A fungus also identified as *E. gyrosa* has been reported in mainland Australia and Tasmania on various species of *Eucalyptus*, including *E. saligna*, *E. maculata*, *E. delegatensis*, *E. regnans* and *E. grandis* (Walker *et al*., 1985; Old *et al*., 1986; White & Kile, 1993). A similar fungus was recently reported from South Africa, where it was associated with cankers on several species of *Eucalyptus* such as *E. grandis*, *E. nitens*, *E. urophylla*, and hybrids of *E. grandis* with *E. camaldulensis* and *E. urophylla* (Van der Westhuizen *et al*., 1993).

Endothia gyrosa has been known to occur in the USA for a considerable period (Shear *et al*., 1917; Stevens, 1917; Barr, 1978). Its recent discovery in Australia and South Africa, on a very different host to those known in North America, was enigmatic. The identity of the North American fungus and the one from the Southern Hemisphere was discussed by Walker *et al*. (1985), who noted morphological differences between them, namely that stromata in the Australian specimens were less developed and that the perithecial bases were seated in the bark and not in the fungal tissue, as occurred in specimens from North America. The size and shape of the perithecia, asci and ascospores of the two groups, however, were indistinguishable, and this led to the

Table 1 Isolates used in PCR-RFLP analysis and DNA sequencing

Culture number[a]	Alternative designation[b]	Identification	Host	Origin	Collector	GenBank accession number
CRY1	ATCC48192	*Endothia gyrosa*	*Quercus palustris*	USA	R. J. Stipes	AF232874
CRY2	ATCC48192	*E. gyrosa*	*Q. palustris*	USA	R. J. Stipes	
CRY39	CBS 510·76	*E. gyrosa*	*Q. suber*	USA	M. K. Roane	AF232876
CRY70	CBS 510·76	*E. gyrosa*	*Q. suber*	USA	M. K. Roane	
CRY37	CBS 510·76	*E. gyrosa*	*Q. suber*	USA	M. K. Roane	
CRY9		*E. gyrosa*	*Q. palustris*	USA	S. Anagnostakis	AF232875
CRY38		*E. gyrosa*	*Q. palustris*	USA	S. Anagnostakis	
CRY12		*E. gyrosa*	*Q. borealis*	USA	S. Anagnostakis	
CRY21		*E. gyrosa*	*Q. borealis*	USA	S. Anagnostakis	
CRY518		*E. gyrosa*	*Fagus* sp.	USA	C. S. Hodges	
CRY103		*E. gyrosa*	*Eucalyptus*	RSA	I. van der Westhuizen	AF232877
CRY62		*E. gyrosa*	*Eucalyptus*	RSA	I. van der Westhuizen	AF232878
CRY287		*E. gyrosa*	*Eucalyptus*	RSA	H. Smith	AF232879
CRY286		*E. gyrosa*	*Eucalyptus*	RSA	I. van der Westhuizen	
CRY232		*E. gyrosa*	*Eucalyptus*	RSA	I. van der Westhuizen	
CRY45		*E. gyrosa*	*E. delegatensis*	Australia	K. Old	AF232880
CRY909	PREM56217[c]	*E. gyrosa*	*E. globulus*	Australia	M. J. Wingfield	AF232881
CRY66[d]		*Cryphonectria parasitica*	*Castanea dentata*	USA	P. J. Bedker	AF046901
CRY67		*C. parasitica*	*C. dentata*	USA	P. J. Bedker	AF046903
CRY289		*C. cubensis*	*E. grandis*	Indonesia	M. J. Wingfield	AF046896
CRY140		*C. cubensis*	*E. grandis*	RSA	M. J. Wingfield	AF046892
CMW2498	CBS134·42	*Diaporthe ambigua*	*Malus sylvestris*	Netherlands	S. Truter	AF046909

[a]Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa. CRY refers to a collection specifically of *Endothia* and *Cryphonectria* spp., whereas CMW refers to a more general collection of the Institute.
[b]American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA; Centraalbureau voor Schimmelcultures, Fungal and Yeast Collection, PO Box 273, 3240 AG, Baarn, Netherlands.
[c]Deposited as a bark specimen which contains the stromata from which the culture was isolated. PREM refers to the National Collection of Fungi, Pretoria, South Africa.
[d]Sequences of isolates in bold were obtained from Myburg *et al.* (1999).

conclusion that the Australian and South African fungus represents *E. gyrosa* (Walker *et al.*, 1985; Van der Westhuizen *et al.*, 1993).

Members of the genera *Endothia* and *Cryphonectria* have long been regarded as very closely related (Shear *et al.*, 1917; Roane, 1986; Barr, 1990). They also share a common *Endothiella* anamorph (Barr, 1978; Davison & Coates, 1991). *Cryphonectria* was separated from *Endothia* by Barr (1978) based on differences in ascospore and stromatal morphology. *Cryphonectria* has one-septate ascospores and valsoid stromata with ectostromatic and entostromatic areas in predominantly prosenchymatous tissue. This is in contrast to the ascospores of *Endothia* that are nonseptate, and the stromata diatrypoid with predominantly pseudoparenchymatous, entostromatic tissue (Barr, 1978; Micales & Stipes, 1987; Barr, 1990). This distinction was maintained in later studies (Micales & Stipes, 1987; Barr, 1990).

Cryphonectria parasitica, which causes chestnut blight, is one of the best-known and important pathogens of forest trees (Elliston, 1981; Griffin & Elkins, 1986). *Cryphonectria parasitica* and *E. gyrosa* are difficult to distinguish in the absence of a teleomorph, as both produce red-to-orange stromata (Stipes *et al.*, 1982). The fact that *C. parasitica* once resided in *Endothia* as *E. parasitica* (Shear *et al.*, 1917; Roane *et al.*, 1974) probably also led to further confusion. *Endothia gyrosa* and *C. parasitica* have, however, been differentiated by many researchers using molecular and chemotaxonomic techniques (Stipes *et al.*, 1982; Micales & Stipes, 1986; Myburg *et al.*, 1999), and the fact that they reside in distinct genera is unequivocal.

Cryphonectria cubensis is a serious canker pathogen of plantation-grown *Eucalyptus* spp. that occurs in most tropical and subtropical areas of the world (Sharma *et al.*, 1985; Florence *et al.*, 1986; Davison & Coates, 1991) The fungus also causes canker of clove (*Syzigium aromaticum*) in Africa, Brazil and Indonesia, but on this host does not cause serious damage (Hodges *et al.*, 1986). In South Africa it shares the same host and approximately the same geographical distribution as *E. gyrosa* on *Eucalyptus* (Wingfield *et al.*, 1989). The morphology of *C. cubensis* is quite different from that of the other *Cryphonectria* species. On *Eucalyptus* spp., *C. cubensis* lacks the prominent orange stromata typical of other *Cryphonectria* and *Endothia* spp., and forms distinct pycnidia as opposed to pycnidial locules within a stroma, as is the case for *Cryphonectria* and *Endothia* (Hodges, 1980). On clove, *C. cubensis* sometimes forms orange stromata containing both pycnidial locules and

perithecia, but the stromata are mostly embedded in the bark and are not as readily visible as those of other species of *Cryphonectria* (Hodges *et al.*, 1986).

In this study, restriction fragment length polymorphism (RFLP) analysis based on a technique developed by Myburg *et al.* (1999), as well as partial sequence of the rRNA operon for some key isolates, were used to determine the relatedness of *E. gyrosa* isolates from North America, South Africa and Australia, and the relationship of *E. gyrosa* with *C. parasitica* and *C. cubensis*.

Materials and methods

Source of isolates

Isolates of *E. gyrosa* from North America, South Africa and Australia (Table 1), deposited in the culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa were maintained on 2% malt extract agar (Biolab, Merck, Midrand, South Africa) at 4°C.

DNA isolation

Mycelia from isolates were grown in 250 mL malt extract broth (20 gL^{-1} malt extract, Biolab) in the light at 25°C. After 2 weeks the mycelia were harvested by filtration (Whatman no. 1 filter paper) and dried between sterilized paper towels. DNA was extracted from the dried mycelium with a modified version of the DNA extraction method developed by Raeder & Broda (1985). Dried mycelium was transferred to sterile Eppendorf tubes with 100 μL extraction buffer (200 mM Tris–HCl pH 8·5, 250 mM NaCl, 25 mM EDTA, 0·5% SDS). The mixture was frozen in liquid nitrogen, ground to a fine powder using a mortar and pestle and incubated at 65°C for 5 min. The freezing, grinding and incubation steps were repeated with an added 400 μL extraction buffer until a homogeneous mixture was obtained. The suspension was mixed twice with phenol and chloroform (3 : 1) and centrifuged at 18000 ***g***. All centrifugations were conducted at 4°C. One volume of chloroform was then added to the aqueous phase, followed by centrifugation at 18000 ***g*** for 10 min. This step was repeated until the interphase was clean. The DNA in the aqueous phase was precipitated overnight at −20°C with 0·54 and 0·1 of the total volume of the mixture of isopropanol and 3 M sodium acetate (pH 8), respectively. This mixture was subsequently centrifuged for 30 min at 13850 ***g***. The resulting pellet was rinsed with 100 μL ice-cold 70% ethanol, centrifuged for 10 min at 18000 ***g***, and dried in a SpeedVac SC100 (Savant Instruments Inc., Farmingdale, NY, USA). The dried pellet was resuspended in 100 μL double-distilled H_2O and stored at −20°C.

DNA amplification and RFLP analysis

The variable ITS1 (internal transcribed spacer) and ITS2 regions, and conserved 5·8S rRNA gene of the ribosomal RNA operon, were amplified with primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.*, 1990) using the polymerase chain reaction (PCR). The PCR reaction consisted of 0·25 μL (2·5 units μL^{-1}) of *Taq* polymerase (Boehringer Mannheim, Indianapolis, IN, USA), 10 × PCR buffer (10 μL, PCR buffer supplied with the enzyme), 0·3 mM dNTP, 0·5 μL of each primer (500 $\mu g\ mL^{-1}$) and 20–120 ng μL^{-1} template DNA. The reaction mix was made up to 100 μL and overlaid with mineral oil. The PCR conditions were as follows: 95°C for 5 min (denaturation), followed by 30 cycles of 45 s each (annealing), 72°C for 2 min (polymerization) and 45 s at 95°C (denaturation) using a Hybaid Touch Down thermal cycler (Hybaid Ltd, Ashford, UK). An annealing temperature of 59°C was used for the American and Australian isolates, and 56°C was used for the South African isolates. The lower temperature was used for the latter isolates because of a 2 bp deletion for the South African isolates in the area where the ITS1 primer bound to the template DNA. A final elongation step was conducted at 72°C for 7 min. The PCR products were separated on a 1·4% agarose (Promega, Madison, CT, USA) gel stained with ethidium bromide (10 $mg\ mL^{-1}$), and visualized under UV illumination. PCR products were purified using the High Pure PCR Product Purification Kit (Boehringer Mannheim, Germany) to remove excess primers and dNTPs.

Restriction enzymes *Cfo*I and *Eco*RI were used to cut the amplified PCR products. The digested DNA fragments were separated on a 3% agarose gel containing ethidium bromide (10 $mg\ mL^{-1}$), and visualized under UV light.

DNA sequencing

DNA sequences of the amplified PCR products were determined using an automated sequencer (ABI Prism, model 377, Perkin Elmer Corporation, Foster City, CA, USA). The sequences of the *C. parasitica* (CRY66, CRY67), *C. cubensis* (CRY289, CRY140) and *Diaporthe ambigua* (CMW2498) isolates were obtained from Genbank based on Myburg *et al.* (1999) (Table 1). Primers ITS1 and ITS4 (White *et al.*, 1990), and internal primers CS2 (5′-CAATGTGCGTTCAAAGATTCG-3′) and CS3 (5′-CGAATCTTTGAACGCACATTG-3′) (Wingfield *et al.*, 1996), which binds within the 5·8S rRNA gene, were used to sequence both strands of the amplified DNA. The sequencing reactions were done with the Big Dye sequencing system (ABI Advanced Biotechnological Institute, Perkin-Elmer) according to the manufacturer's instructions.

Phylogenetic analysis

The sequences obtained were manually aligned with Sequence Navigator version 1·01 (ABI Prism, Perkin

Elmer, 1986) by inserting gaps. Aligned sequences were analysed with PAUP* version 4·0b2 (Phylogenetic Analysis Using Parsimony; Swofford, 1998). All characters were treated as unordered and were equally weighted. Gaps were treated as missing data. *Diaporthe ambigua* was defined as a monophyletic outgroup with respect to the other isolates as it belongs to the same family as *Cryphonectria* and *Endothia* according to the latest classification of the families of the Diaporthales (Hawksworth *et al*., 1996).

Both the branch and bound algorithm ('as is' addition sequence, MAXTREES set to prompt for new value), and the tree bisection–reconnection (TBR) swapping option of the heuristic search algorithm, were used to search for the most parsimonious tree. The confidence intervals for each of the branches were estimated by bootstrap analyses (1000 replications). A total of 69 ambiguous characters (bases 1–22, 31–40, 63–76, 111–118, 231–236 and 506–514) were also excluded in order to determine whether these ambiguities would have an influence on the topology of the tree. The consistency index (CI) and retention index (RI) were also calculated using PAUP* to establish the phylogram that best reflected the true phylogeny of this group.

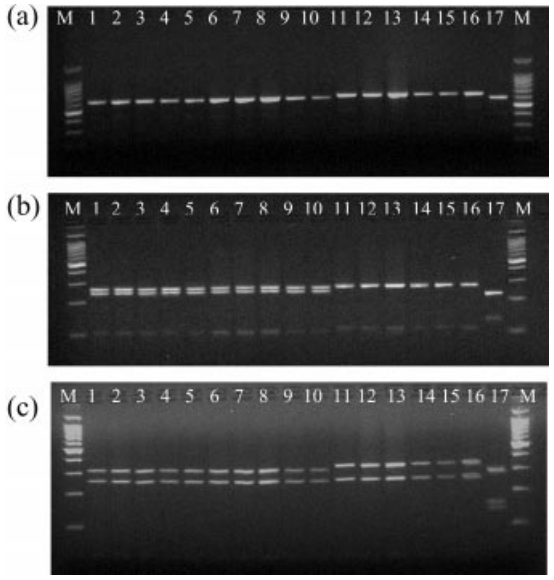

Figure 1 Agarose gels containing PCR products and PCR–RFLP products of the ITS1, ITS2 and 5·8S rRNA gene of the rRNA operon. Lanes 1–10 represent North American isolates of *Endothia gyrosa* (CRY1, CRY2, CRY70, CRY37, CRY39, CRY12, CRY21, CRY38, CRY9, CRY518); lane 11 an Australian *E. gyrosa* isolate (CRY45) and lanes 12–16 South African isolates of *E. gyrosa* (CRY286, CRY232, CRY103, CRY62, CRY287). Lane 17 represents *Diaporthe ambigua* (CMW2498) which was used as outgroup. Lanes M are a 100 bp molecular weight marker (Promega, Madison, CT, USA) with the following band sizes: 100, 200, 300, 400, 500 (brightest band), 600, 700, 800, 900, 1000 bp. (a) PCR amplification products; (b) Restriction profiles generated by *Cfo*I; (c) Restriction profiles generated by *Eco*RI.

Results

DNA amplification and RFLP analysis

Differences in size were observed for the amplification products obtained for the North American (607 bp) and South African (640 bp) isolates, while the PCR product of the Australian isolate (644 bp) differed by only 4 bp from the South African isolates (Fig. 1a). The fragment size of the PCR product of the *D. ambigua* isolate was estimated to be ≈600 bp, and is therefore different in size to the *E. gyrosa* isolates.

The Australian and South African isolates had the same RFLP banding patterns when either *Cfo*I (Fig. 1b) or *Eco*RI (Fig. 1c) was used to digest the PCR product. These patterns differed from those of *E. gyrosa* isolates from North America and from *D. ambigua* for both enzymes (Fig. 1b,c). The restriction maps (Fig. 2) generated from the DNA sequence reflect these differences.

DNA sequencing and analysis

The length of the sequences aligned to those of

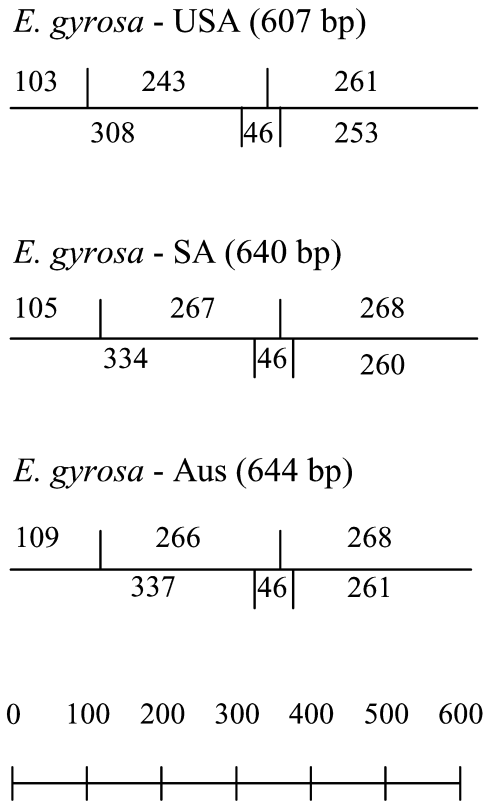

Figure 2 Restriction maps based on RFLP profiles and complete DNA sequences of PCR amplification products of the ITS1, ITS2 and 5·8S rRNA gene. PCR products were cut with restriction enzymes *Cfo*I and *Eco*RI. North American, Australian and South African isolates of *Endothia gyrosa* were used and a *Diaporthe ambigua* isolate chosen as outgroup. *Cfo*I restriction sites are indicated above the line; *Eco*RI restriction sites below the line.

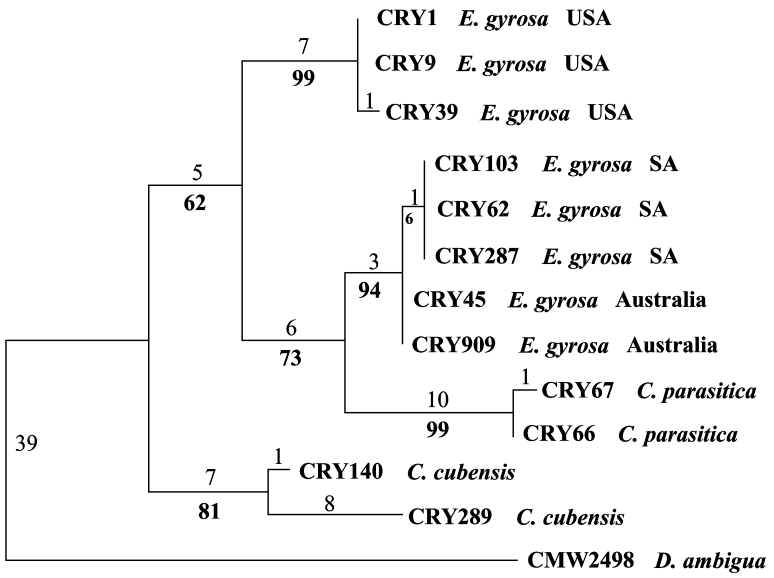

Figure 3 The most parsimonious tree obtained from sequences of the ITS1, ITS2 and 5·8S rRNA gene of the ribosomal operon for isolates of *Endothia gyrosa* (USA, Australia and South Africa), *Cryphonectria parasitica, C. cubensis* and the outgroup *Diaporthe ambigua*. The tree was obtained using the branch and bound algorithm of PAUP* 4·0b2 without the exclusion of ambiguous regions (tree length = 99; CI = 0·9495; RI = 0·9367; g1 = 0·860094). Percentage confidence levels (1000 bootstrap replications) are indicated in bold below the branches; the numbers of steps are indicated above the branches.

C. parasitica, *C. cubensis* and *D. ambigua*, obtained from Genbank, was 474 bp for the North American isolates, 506 bp for the South African isolates, and 509 bp for the Australian isolates. A total of 563 characters for each isolate was aligned after the inclusion of gaps. Trees identical regarding CI and RI values (0·9495 and 0·9367, respectively), number of constant and parsimonious informative characters (476 and 38, respectively), and number of base changes per branch and tree length (99 steps), were obtained with both the TBR swapping option and the branch and bound option of PAUP. The phylogenetic signal was also significant, as indicated by the g1 value (0·860094) for all of the trees. Only the number of trees (two for branch and bound option, three for TBR option), bootstrap values and branch lengths differed between the trees obtained with the different options. These differences were due to a few single base differences that existed between isolates of the same species.

Exclusion of ambiguous regions did not have any influence on the phylogenetic groupings of the isolates. The tree obtained with the TBR swapping option when data were excluded was identical to the tree obtained with the branch and bound option. The CI and RI values (0·9438 and 0·9324) and g1 value (0·853969) were slightly lower when ambiguous regions were excluded than when such regions were included. Fewer trees (three when ambiguous regions were not excluded, one when they were excluded) with a lower number of steps (89) were also obtained.

Gaps in the sequence were also treated as newstate (as a fifth character) to determine whether this might have an effect. Using this approach, the South African and Australian isolates still grouped separately from the North American isolates and together with the *C. parasitica* isolates. One difference in the grouping of the isolates was observed from trees generated with gaps treated as 'missing data'. Here the *C. cubensis* isolates did not group separately from the *Endothia* and *Cryphonectria* isolates, but formed a subclade in the greater *C. parasitica*, Australian and South African clade. Trees were much longer (301 and 240 steps when ambiguous bases were excluded), and CI and RI values were lower than when gaps were treated as missing data (0·8272 and 0·8729, respectively, and 0·825 and 0·8743 when ambiguous bases were excluded). Treating gaps in the sequence as missing, and not as newstate, was therefore preferred as the resulting trees had higher CI and RI values, and fewer steps were needed to obtain the trees.

The phylogram obtained using the branch and bound option of PAUP without the exclusion of ambiguous regions was chosen to illustrate the relationships between the taxa (Fig. 3). The topology of the tree reflected the same similarities and differences seen in the restriction digests. The Australian and South African isolates of *E. gyrosa* resided in a single, well resolved clade (bootstrap support 94%). In contrast, North American isolates of *E. gyrosa* resided in a different and distinct clade (bootstrap support 99%). The *C. parasitica* isolates grouped in the same clade as the *E. gyrosa* isolates from South Africa and Australia (bootstrap support 73%), while *C. cubensis* did not group in this particular clade. The *C. cubensis* isolates had a basal grouping with respect to all the *C. parasitica* and different *E. gyrosa* isolates (bootstrap support 62%), while *C. parasitica* and the South African and Australian isolates grouped with *E. gyrosa* from North America in a more general clade.

Discussion

Results of this study have shown that the South African and Australian isolates identified as *E. gyrosa* are different from those from North America. This suggests that the morphological differences observed by Walker *et al.* (1985), are taxonomically relevant. Different hosts

sometimes influence the variability of stromatal morphology (Micales & Stipes, 1987; Micales *et al*., 1987; Fernández & Hanlin, 1996). Therefore the differences observed between the Australian and North American specimens could have been due to different hosts. For example *C. cubensis* (*E. eugeniae*) from clove, and *C. cubensis* from eucalypts, were classified as two species based on their different morphology, but later were shown to be conspecific by means of cross-inoculations, cultural studies and electrophoretic studies on proteins (Hodges *et al*., 1986; Micales *et al*., 1987). The molecular data described here indicate that the South African and Australian fungus represents a taxon distinct from the North American fungus. Thus the morphological differences are not only due to the different hosts on which the fungus occurs.

If additional morphological evidence can be found to support the molecular evidence, the South African and Australian species of *Endothia* appear to represent a new taxon. If this is necessary, choosing the correct genus in which to place the new species poses an interesting dilemma. DNA evidence suggests that the South African and Australian fungus (which has nonseptate, cylindrical to allantoid ascospores; Walker *et al*., 1985; Van der Westhuizen *et al*., 1993) is more closely related to *C. parasitica* (one-septate, elliptical ascospores) than to *E. gyrosa* from North America (nonseptate, cylindrical to allantoid ascospores; Barr, 1978). It is possible that the South African and Australian fungus represents a species of *Cryphonectria* rather than *Endothia*, but this ignores ascospore septation and shape as valid morphological characters for generic separation.

Barr (1978) separated *Cryphonectria* from *Endothia* and moved them into two families in the Diaporthales, with *Endothia* eventually placed in the Valsaceae and *Cryphonectria* in the Gnomoniaceae (Barr, 1990). Separation into these families was based mainly on ascospore and stromatal morphology (Barr, 1990). The results presented here support the view of others (Roane, 1986; Chen *et al*., 1996) that these two genera are closely related to each other and are insufficiently different to be placed in separate families. This would also support the views of Cannon (1988) and Hawksworth *et al*. (1996), who afforded Gnomoniaceae *nomen conservandum* status to the Valsaceae.

The phylogenetic relationships between members of the genera *Cryphonectria* and *Endothia* require additional study. For instance, *C. havanensis* and *C. cubensis* have been repeatedly confused in the past (Hodges, 1980), and *C. havanensis* and *C. gyrosa* are also thought to be synonymous (Kobayashi, 1970; Hodges, 1980). Furthermore, the phylogenetic relationship of *C. cubensis* with other species of *Cryphonectria* is unclear. The basal grouping of *C. cubensis* to the other *Endothia* and *Cryphonectria* isolates suggests that *C. cubensis* may reside in a genus other than *Cryphonectria*, although it is still closely related to *Cryphonectria* and *Endothia*.

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