Chrysoporthe doradensis sp. nov. pathogenic to *Eucalyptus* in Ecuador

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Canker caused by *Chrysoporthe cubensis* is a serious disease of commercially grown *Eucalyptus* in various South American countries. This disease has not previously been recorded from Ecuador. Recent disease surveys in Ecuadorian *Eucalyptus* plantations have led to the discovery of canker symptoms typical of this disease with fruiting bodies resembling *C. cubensis* abundant on diseased tissues. The aim of this study was to characterise the fungus based on morphology and sequences of the ITS1/ITS2 regions of the ribosomal DNA operon and β-tubulin genes. Phylogenetic analyses showed that isolates from Ecuador reside in a clade together with other *Chrysoporthe* spp., but in a clearly distinct group. The distinct phylogenetic position of the Ecuadorian fungus is supported by unique conidial morphology and it is, therefore, described as *Chrysoporthe doradensis* sp. nov. Pathogenicity trials on *Eucalyptus deglupta* showed that the fungus is highly pathogenic on this commonly planted tree as well as on saplings of *Tibouchina urvilleana*.

Key words: *Diaporthales*, *Tibouchina*.

Introduction

Chrysoporthe cubensis (Bruner) Gryzenh. & M.J. Wingf., previously known as *Cryphonectria cubensis* (Bruner) Hodges, causes a serious canker disease of *Eucalyptus* trees in plantations. *Chrysoporthe cubensis* is common in the Neotropics where it has been reported from several countries (Bruner, 1917; Boerboom and Maas, 1970; Hodges *et al*., 1976, 1979; Myburg *et al*., 1999; Van der Merwe *et al*., 2001; Gryzenhout *et al*., 2004). Girdling cankers on the stems of trees by this pathogen has had a substantial impact on

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Eucalyptus propagation in the tropics and sub-tropics, where it has also greatly influenced plantation practices (Wingfield, 2003). The best option for management of this disease has been through breeding and selection of resistant *Eucalyptus* clones, and such programmes have been successfully implemented in various South American countries (Alfenas *et al*., 1983; Wingfield, 2003; Rodas *et al*., 2005).

Chrysoporthe is a recently described genus including the fungus previously known as *Cry*. *cubensis* (Gryzenhout *et al*., 2004). DNA sequence comparisons and detailed morphological studies have shown that specimens and isolates previously identified as *Cry*. *cubensis* from various parts of the world, represent at least three species (Myburg *et al*., 2002a; Gryzenhout *et al*., 2004). The fungus now known as *C*. *cubensis* represents isolates from South and Central America, but also includes isolates from Central Africa, Hawaii, South East Asia and Australia (Gryzenhout *et al*., 2004). In these areas, *C*. *cubensis* not only occurs on *Eucalyptus* but also on other *Myrtaceae* such as *Syzygium aromaticum* (L.) Murr. & Perry (clove) in Brazil, Zanzibar and Indonesia (Hodges *et al*., 1986; Myburg *et al*., 2003), and *Melastomataceae* such as native *Miconia theaezans* (Bonpl.) Cogn. and *Miconia rubiginosa* (Bonpl.) DC. in Colombia (Rodas *et al*., 2005).

Other than *C*. *cubensis*, two other species of *Chrysoporthe* are known and one of these occurs in South America together with *C*. *cubensis*. This species, *Chrysoporthella hodgesiana* Gryzenh. & M.J. Wingf., is recognized as a species of *Chrysoporthe* based on DNA sequence data, but is known only in its asexual state and thus resides in the anamorph genus of *Chrysoporthe*. *Chrysop*. *hodgesiana* is commonly found in Colombia on native *Tibouchina* spp. (Wingfield *et al*., 2001; Gryzenhout *et al*., 2004) and on *M*. *theaezans* (Rodas *et al*., 2005). Other than its unique DNA sequences, it can also be distinguished from *C*. *cubensis* based on its low optimal growth temperature (Gryzenhout *et al*., 2004). Isolates of the fungus previously known as *Cry*. *cubensis* from South Africa represents the third species that has been provided the name *C*. *austroafricana* Gryzenh. & M.J. Wingf. This species is defined by ascospores with rounded apices and is currently known only from South Africa (Gryzenhout *et al*., 2004). All three *Chrysoporthe* species are highly pathogenic to *Eucalyptus* spp. (Wingfield *et al*., 1989; Wingfield *et al*., 2001; Wingfield, 2003).

Forestry in Ecuador includes the planting of native as well as exotic tree species in plantations. Plantations of *Eucalyptus* are not widespread and little is known regarding the diseases that affect them. Cankers caused by *C*. *cubensis* are found in neighbouring countries such as Colombia (Van der Merwe *et al*., 2001), but the disease has not been reported from Ecuador. The presence of

this disease in Ecuador could have a negative impact on forestry in the country, particularly if susceptible species are planted. This provided the motivation for disease surveys in Ecuadorian *Eucalyptus* plantations and the discovery of a canker disease that forms the basis of this study.

Materials and methods

Symptoms and collection of samples

Eucalyptus grandis W. Hill and *E. deglupta* Blume trees of various ages between five and 10-years-old were found with stem cankers (Fig. 1A) in plantations near the town of Buenos Aires. The extent of cankers differed substantially, but in many cases they had girdled and killed trees. Ascostromata and conidiomata were commonly found fruiting around the cankers, and these were collected and transported to the laboratory for further study. Isolates were made, purified and have been lodged in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (Table 1). Representative isolates have also been deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1). The original pieces of bark from which isolates were made were dried and have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Morphology

Fruiting structures were cut from the bark specimens and examined using the methods outlined in Gryzenhout *et al*. (2004). Fifty ascospores, asci, conidia and conidiophores were measured and are presented as (min-)(average - S.D.) - (average + S.D.)(-max) μm. Only minimum and maximum values arising from the smallest and largest structure were obtained for the eustromata and perithecia. Colours were assigned using the notations of Rayner (1970).

Growth in culture was studied for the isolates (CMW 11286, CMW 11287) from *E. grandis* in Ecuador. This is especially important since *Chrysop. hodgesiana* has been distinguished from *C. cubensis* based on growth characteristics in culture (Gryzenhout *et al*., 2004). Growth of cultures was studied in the dark at temperatures ranging from 15 to 35ºC, at 5º intervals. The procedure for assessment of growth in culture was the same as that described by Gryzenhout *et al*. (2004).

Table 1. Isolates included in this study. Isolates in bold were sequenced in this study.

Species identity	Isolate number[a]	Alternative isolate number[a]	Host	Origin	Collector	GenBank accession numbers[b]
Chrysoporthe doradensis	CMW 11286	CBS 115734	*Eucalyptus grandis*	Ecuador	M.J. Wingfield	AY 214289, AY 214217, AY 214253
	CMW 11287	CBS 115735	*E. grandis*	Ecuador	M.J. Wingfield	AY 214290, AY 214218, AY 214254
	CMW 9123	CBS 115717	*E. deglupta*	Ecuador	M.J. Wingfield	DQ 224034, DQ 224038, DQ 224039
	CMW 9124	CBS 115716	*E. deglupta*	Ecuador	M.J. Wingfield	DQ 224035, DQ 224040, DQ 224041
	CMW 9125	CBS 115715	*E. deglupta*	Ecuador	M.J. Wingfield	DQ 224036, DQ 224042, DQ 224043
	CMW 9126	CBS 115723	*E. deglupta*.	Ecuador	M.J. Wingfield	DQ 224037, DQ 224044, DQ 224045
Chrysoporthe cubensis	CMW 1856	-	*Eucalyptus* sp.	Kauai, Hawaii	-	AY 083999, AY 084010, AY 084022
	CMW 9903	-	*Syzygium aromaticum*	Kalimantan, Indonesia	C.S. Hodges	AF 292044, AF 273066, AF 273461
	CMW 11290	CBS 115738	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214304, AY 214232, AY 214268
	CMW 8650	CBS 115719	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084001, AY 084013, AY 084024
	CMW 10774	-	*S. aromaticum*	Zanzibar, Tanzania	-	AF 492130, AF 492131, AF 492132
	CMW 2632	-	*E. marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
	CMW 10453	CBS 505.63	*E. saligna*	Democratic Republic of Congo	-	AY 063476, AY 063478, AY 063480
	CMW 10669	CBS 115751	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 535122, AF 535124, AF 535126
	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
	CMW 9993	CBS 115728	*Miconia theaezans*	Colombia	C.A. Rodas	AY 214298, AY 214226, AY 214262
	CMW 10024	CBS 115739	*M. rubiginosa*	Colombia	C.A. Rodas	AY 262390, AY 262394, AY 262398
	CMW 8757	-	*Eucalyptus* sp.	Venezuela	M.J. Wingfield	AF 046897, AF 273069, AF 273464
	CMW 1853	-	*S. aromaticum*	Brazil	-	AF 046891, AF 273070, AF 273465
	CMW 10778	CBS 115755	*S. aromaticum*	Brazil	C.S. Hodges	AY 084006, AY 084018, AY 084030
	CMW 9432	CBS 115724	*E. grandis*	Mexico	M.J. Wingfield	AY 692321, AY 692324, AY 692323
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
	CMW 8755	-	*E. grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273458
	CMW 9327	CBS 115843	*Tibouchina granulosa*	South Africa	M.J. Wingfield	AF 273473, AF 273060, AF 273455
	CMW 9328	-	*T. granulosa*	South Africa	M.J. Wingfield	AF 273474, AF 273061, AF 273456
Chrysoporthella hodgesiana	CMW 9927	-	*T. urvilleana*	Colombia	C.A. Rodas, M.J. Wingfield	AF 265653, AF 292034, AF 292037
	CMW 9995	CBS 115730	*T. semidecandra*	Colombia	R Arbelaez	AY 956969, AY 956977, AY 956978
	CMW 10641	CBS 115854	*T. semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325
	CMW 10625	CBS 115744	*M. theaezans*	Colombia	C.A. Rodas	AY 956970, AY 956979, AY 956980

Table 1 continued. Isolates included in this study. Isolates in bold were sequenced in this study.

Species identity	Isolate number[a]	Alternative isolate number[a]	Host	Origin	Collector	GenBank accession numbers[b]
Cryphonectria parasitica	CMW 1652	CBS 112914	*Castanea dentata*	U.S.A.	-	AF 046902, AF 273075, AF 273468
Cryphonectria nitschkei	CMW 13742	MAFF 410570	*Quercus grosseserrata*	Japan	T. Kobayashi	AY 697936, AY 697961, AY 697962
Cryphonectria macrospora	CMW 10463	CBS 112920	*Castanopsis cupsidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350
Rostraureum tropicale	CMW 9972	-	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167426, AY 167431, AY 167436
	CMW 10796	CBS 115757	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438

[a] CMW = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

[b] Accession numbers refer to sequence data of the ITS, β-tubulin 1 (primers Bt1a/1b) and β-tubulin 2 (primers Bt2a/2b) regions respectively.

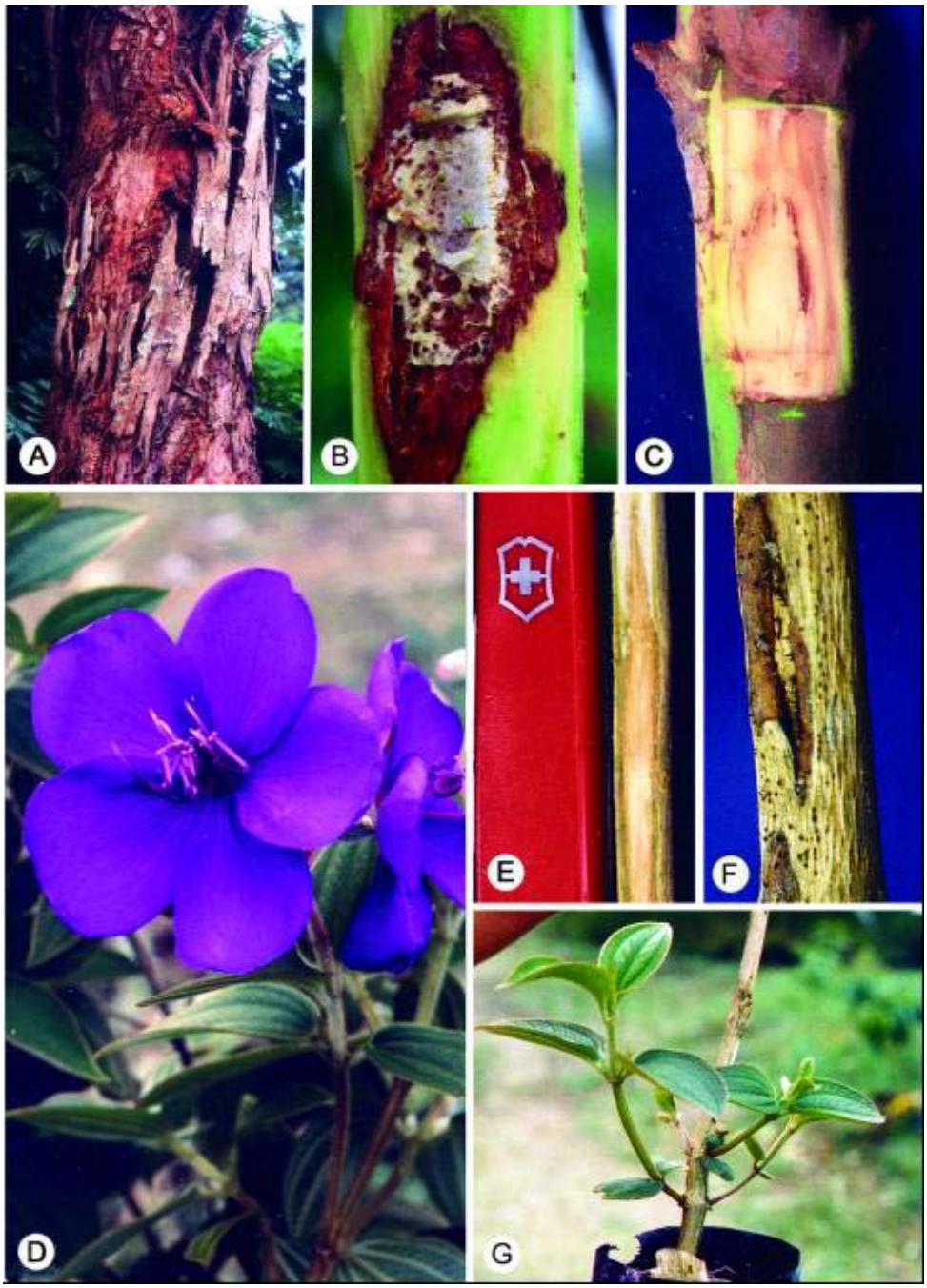

Fig. 1. Symptoms associated with the canker pathogen *Chrysoporthe doradensis* in the field and in inoculation trials. **A.** Stem canker of a dying *Eucalyptus grandis* tree in the field. **B.** Lesion on *Eucalyptus deglupta* associated with inoculations. **C.** Control inoculation on *E. deglupta*. **D.** *Tibouchina urvilleana*. **E.** Lesion associated with inoculations on *T. urvilleana*. **F.** Fruiting structures of *C. doradensis* on *T. urvilleana*. **G.** Formation of epicormic shoots resulting from inoculations on *T. urvilleana*.

DNA sequence comparisons

Sequences were obtained from a number of genic regions of isolates from *E. grandis* in Ecuador. These sequences were compared with those published (Table 1) for *C. cubensis*, *C. austroafricana* and *Chrysop. hodgesiana* from a variety of hosts (Gryzenhout *et al*., 2004; Rodas *et al*., 2005). Isolates of *Rostraureum tropicale* Gryzenh. & M.J. Wingf. were also included (Table 1). This species is closely related to *Chrysoporthe* and was recently described as a pathogen of *Terminalia ivorensis* A. Chev. and *T. superba* Engler & Diels in the same areas of Ecuador where the *Chrysoporthe* sp. was found in this study (Gryzenhout *et al*., 2005). The *R. tropicale* isolates were included as outgroup taxa, together with the closely related *Cryphonectria parasitica* (Murrill) M.E. Barr, *Cry. macrospora* (Tak. Kobay. & Kaz. Itô) M.E. Barr and *Cry. nitschkei* (G.H. Otth) M.E. Barr (Gryzenhout *et al*., 2004; Myburg *et al*., 2004).

DNA was extracted from mycelium grown in Malt Extract Broth [20 g/L Biolab malt extract] following the protocol described in Myburg *et al*. (1999). The internal transcribed spacer (ITS) regions ITS1 and ITS2, and the conserved 5.8S gene of the ribosomal RNA (rRNA) operon, as well as two regions of the β-tubulin gene, were amplified as described in Myburg *et al*. (1999) and Myburg *et al*. (2002a). PCR products were run on 1% agarose (ethidium bromide-stained) gels, and detected under UV light. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced with the same primers that were used to amplify the respective DNA regions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, UK) was used for the sequence reactions on an ABI PRISM 3100™ automated DNA sequencer.

The forward and reverse sequences that were obtained were imported into Sequence Navigator version 1.0.1 software (Perkin-Elmer Applied BioSystems, Foster City, CA). Sequences were manually aligned and inserted, together with those from Rodas *et al*. (2005), in the TreeBASE data matrix (S 1211, M 2095) generated in the study by Gryzenhout *et al*. (2004). Subsequent phylogenetic analyses were done using PAUP version 4.0b (Swofford, 1998). The combinability of the rRNA and β-tubulin gene sequence data sets was determined with a partition homogeneity test (PHT; Farris *et al*., 1994). Parsimony using the heuristic search option with the tree-bisection-reconnection (TBR) branch swapping, MULTREES options (saving all optimal trees) effective and random sequence additions set to 100 was employed to generate trees. During analyses, uninformative sites were excluded and

individual CI values were used to reweight base pairs. Distance analyses were also executed using the distance model determined with MODELTEST version 3.5 (Posada and Crandall, 1998) to confirm results obtained with parsimony. Thus the Transitional model or TIM (Tavaré, 1986) was used (proportion of invariable sites (I) = 0.1840; Base frequency = 0.1952, 0.3262, 0.2408, 0.2379; Rate matrix = 1.0, 3.3491, 1.8115, 1.8115, 5.9357, 1.0). In the heuristic searches, gaps inserted during sequence alignment were treated as fifth character (NEWSTATE), but these were treated as missing data for distance analyses. A 1000 replicate bootstrap analyses was executed to assess the reproducibility levels of the branch nodes of the phylogenetic trees. Individual sequences generated in this study have been deposited in GenBank (Table 1).

Pathogenicity tests

Fifteen one-year-old *E. deglupta* trees were inoculated in November 2001 with isolate CMW 11287 (Table 1) to assess its pathogenicity. The trees were planted on the farm Rio Pitzara near the town of Santa Domingo. An equal number of trees were inoculated with discs from uninoculated MEA plates to serve as negative controls. Inoculation wounds were made with a 10 mm diam. punch to remove the bark and expose the cambium. Agar discs of equal size and bearing the test fungus were inserted into the wounds with the mycelium touching the exposed cambium. Wounds were covered with masking tape to reduce desiccation and contamination. Lesion development was assessed after five weeks by scraping the bark from the lesions and measuring the lesion length. The size of lesions arising from the inoculations was analysed in a Two-Sample t-Test Assuming Equal Variances in Microsoft Excell 2000.

Based on the fact that *Tibouchina* spp. are highly susceptible to *Chrysoporthe* spp. in other countries (Wingfield *et al*., 2001; Myburg *et al*., 2002b; Seisax *et al*., 2004) and are thought to be possible native hosts to *Chrysoporthe* spp. in South America (Gryzenhout *et al*., 2004), susceptibility of these trees, which are native to Ecuador, was also tested. Twenty approximately one-year-old *T. urvilleana* Cogn. plants grown in pots (Fig. 1D) at a nursery near Buenos Aires, were thus inoculated with the *Chrysoporthe* sp. from *Eucalyptus* in Ecuador. Inoculations were done with isolate CMW 11287 in February 2000 using the same method described above and including the same number of negative controls. Data were analysed in a Two-Sample t-Test Assuming Equal Variances in Microsoft Excell 2000.

Results

DNA sequence comparisons

Amplification products of the ITS1, 5.8S and ITS2 rRNA regions of the ribosomal DNA operon were approximately 600 bp, while those of the two regions of the β-tubulin genes approximately 550 bp in length. Based on results from the PHT test (P = 0.04), the two data sets did not differ significantly from each other and could thus be combined. The data set consisted of 34 taxa with the *Cry. parasitica, Cry. macrospora*, *Cry. nitschkei* and *R. tropicale* isolates as the outgroup (Fig. 2). The ribosomal DNA dataset (553 bp) consisted of 399 constant, 50 variable parsimony-uninformative and 104 variable parsimony-informative characters (g1 = -3.186), while the β-tubulin dataset (896 bp) contained 634 constant, 35 variable parsimony-uninformative and 227 variable parsimony-informative characters (g1 = -2.809). The combined data set contained 1449 sequence characters in total.

Thirty phylogenetic trees were generated from the heuristic search (tree length = 474.5 steps, CI = 0.914, RI = 0.946) and only differed in the length of the branches. These trees and the tree obtained through distance analyses, both displayed the sub-clades observed previously for isolates of *Chrysoporthe* (Myburg *et al*., 2002a, 2003; Gryzenhout *et al*., 2004). These sub-clades included the two in which the morphologically identical isolates of *C. cubensis* reside (bootstrap support = 99% for the Asian group, 93% for the South American group), the sub-clade representing *C. austroafricana* (bootstrap support = 97%) and the sub-clade representing *Chrysop. hodgesiana* (bootstrap support = 89%). Isolates from Ecuador did not reside in any of the sub-clades representing existing *Chrysoporthe* spp., but formed a distinct sub-clade with a 100% bootstrap within the greater *Chrysoporthe* clade. This sub-clade was characterised by unique alleles present in all of the isolates (Table 2).

Morphology

Ascostromata and conidiomata (Figs. 3, 4) of the fungus found on *Eucalyptus grandis* in Ecuador were generally similar to those for the descriptions of *Chrysoporthe* spp. (Myburg *et al*., 2003; Gryzenhout *et al*., 2004). Various morphological features were, however, distinct from those of the described *Chrysoporthe* spp. The most important distinguishing feature of this fungus is its conidial morphology. Conidia of the fungus from Ecuador were larger, (3-)3.5-5(-6) µm in length, variable in shape, cylindrical to oblong

Table 2. Summary of polymorphic nucleotides found within sequences of the ribosomal ITS region and two regions in the β-tubulin genes for *Chrysoporte cubensis, C. austroafricana*, *C. doradensis* and *Chrysoporthella hodgesiana*. Only polymorphic nucleotides shown to occur in all of the isolates in this study or previously (Gryzenhout *et al*., 2004), are shown (in bold typeface), and not alleles that occur in a single or small number of individuals per phylogenetic group. Numerical positions of the nucleotides in the DNA sequence alignments are indicated, and those nucleotides occurring in exons are in bold typeface.

Species	**Isolate numbers**	**β-tubulin (Bt1a/b)**													**ITS1/5/8S/ITS2**				
		141	153	161	162	164	167	185	189	201	209	**258**	**276**	**354**	1040	1326	1333	1334	1359
Chrysoporthe cubensis	All isolates in Asian group	C	C	G	G	A	A	A	T	T	T	T	C	C	A	**G**	**T**	**T**	**G**
C. cubensis	All isolates in South American group	**T**	C	G	**A**	A	A	A	T	T	T	T	**T**	C	A	A	C	-	A
C. austroafricana	CMW 9327, CMW 9328	C	C	G	G	**C**	**C**	A	**C**	**C**	**C**	**C**	C	C	A	A	C	-	A
	CMW 2113	C	C	G	G	**C**	**C**	A	**C**	**C**	**C**	**C**	C	C	A	A	**T**	-	A
	CMW 8755	C	C	G	G	A	A	A	**C**	**C**	**C**	**C**	C	C	A	A	C	-	A
Chrysop. hodgesiana	All isolates	C	C	G	G	A	A	**G**	T	T	T	**C**	**T**	C	**G**	A	C	-	**G**
C. doradensis	All isolates	C	**T**	**A**	G	A	A	A	T	**C**	**C**	T	C	**A**	A	A	C	-	A

Species	**Isolate numbers**	**β-tubulin (Bt2a/b)**								
		539	548	561	586	705	**791**	**812**	**821**	**860**
Chrysoporthe cubensis	All isolates in Asian group	C	G	C	**C**	A	**T**	T	**T**	G
C. cubensis	All isolates in South American group	C	G	C	T	A	C	T	C	G
C. austroafricana	CMW 9327, CMW 9328	**T**	G	C	T	A	C	T	C	G
	CMW 2113	**T**	G	C	T	A	C	T	C	G
	CMW 8755	**T**	G	C	T	A	C	T	C	G
Chrysop. hodgesiana	All isolates	C	**A**	C	T	A	C	**C**	C	**A**
C. doradensis	All isolates	C	G	**T**	**C**	**G**	**T**	T	C	G

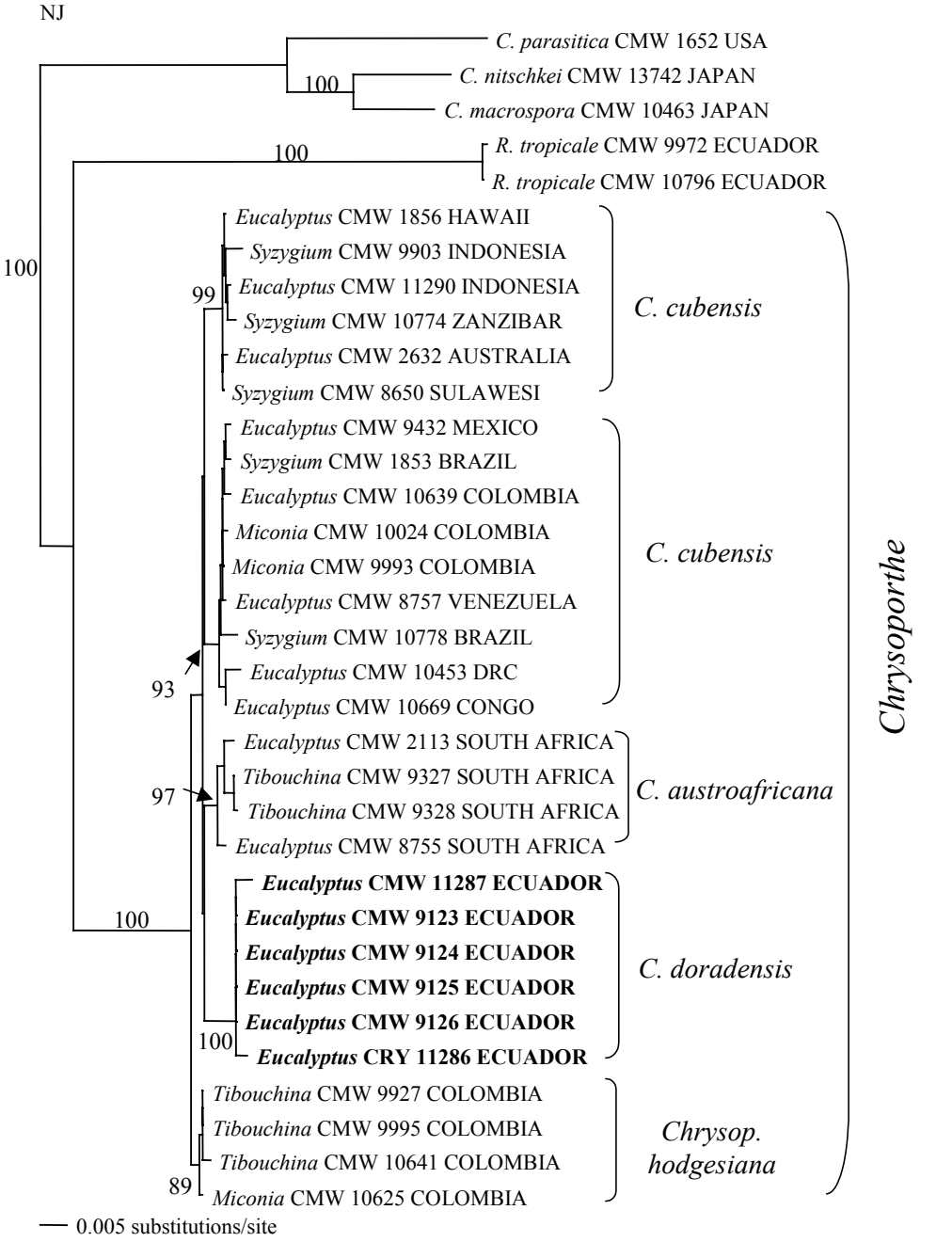

Fig. 2. A phylogram obtained from the combined data set of ribosomal DNA and β-tubulin gene sequences. Distance analyses were done using the Transitional model (proportion of invariable sites (I) = 0.1840; Base frequency = 0.1952, 0.3262, 0.2408, 0.2379; Rate matrix = 1.0, 3.3491, 1.8115, 1.8115, 5.9357, 1.0). Bootstrap values (1000 replicates) of branches are indicated on the branches and isolates representing the new species are in bold type. The outgroups includes *Rostraureum tropicale*, *Cryphonectria parasitica*, *C. nitschkei* and *C. macrospora*.

to ovoid, and occasionally allantoid (Figs. 3K, 4F). Conidia of other *Chrysoporthe* species are oblong to ovoid, and (3-)3.5-4.5(-5) µm in length (Gryzenhout *et al*., 2004). Conidia of the fungus from Ecuador were often also exuded as pale luteous spore drops (Figs. 3G, 3H) rather than the typical luteous to orange spore tendrils common to other *Chrysoporthe* species (Gryzenhout *et al*., 2004). Besides pyriform conidiomata, pulvinate conidiomata with short, thin necks (Figs. 3G, 4D) were also observed, which differ from those of *C. cubensis* (Gryzenhout *et al*., 2004). No obvious differences in ascomatal structure and colony growth were observed for the fungus from Ecuador and those of other *Chrysoporthe* spp. (Gryzenhout *et al*., 2004).

Taxonomy

Results of the DNA sequence and morphological comparisons clearly showed that the fungus associated with cankers on *Eucalyptus* in Ecuador resides in *Chrysoporthe*. Furthermore, it represents a distinct and undescribed species. The following description is provided for this new species.

Chrysoporthe doradensis Gryzenh. & M.J. Wingf., **sp. nov**. (Figs. 3, 4)

Etymology: after El Dorado, legendary South American city of gold and the golden colour of stromatic tissue of the fungus.

Ascostromata in cortice semi-immersa, collis peritheciorum protrudentibus atrofuscis cylindricis, et textura erumpente ascostromatica limitata aurantiaca visibilibus. *Asci* octospori, fusoidei vel ellipsoidei. *Ascosporae* hyalinae, uniseptatae, fusoideae vel ovales. *Conidiomata* superficialia, pyriformia vel pulvinata, 1 collo in quaque structura, atrofusco. *Basis* stromatis e textura globulosa composita, collo e textura porrecta composito. *Conidiophora* hyalina, cellula infima forma irregulari, supra irregulares greges phialidum cylindricarum vel ampulliformium, sursum attenuatarum proferentes; rami ad basim septati an non. *Conidiorum* massa cirrhis vel guttis luteis exudata; conidia hyalina, non septata, oblonga. *Coloniae* in MEA albae cinnamomeo- vel avellaneo-maculatae, celeriter crescentes, temperatura optima 30 ºC.

Ascostromata semi-immersed in bark, recognizable by extending, fuscous-black, cylindrical perithecial necks and in some cases, erumpent, limited ascostromatic tissue appearing orange, 140-380 µm high above the bark, 320-610 µm diam. (Figs. 3A, 3B, 4A, 4B). *Perithecia* valsoid, up to 5 per stroma, bases immersed in the bark, black, globose, 230-400 µm diam., perithecial wall 15-25 µm thick (Figs. 3B, 4B). Top of perithecial bases covered with cinnamon to orange, predominantly prosenchymatous, stromatic tissue, which is occasionally visible above the bark surface (Figs. 3A, 3B, 3C, 4A, 4B). *Perithecial necks* black, periphysate, 90-110 µm wide (Figs. 3B, 4B). Necks emerging through the bark covered in umber, stromatic tissue of *textura porrecta*, thus appearing fuscous-black, extending necks up to 1680 µm long,

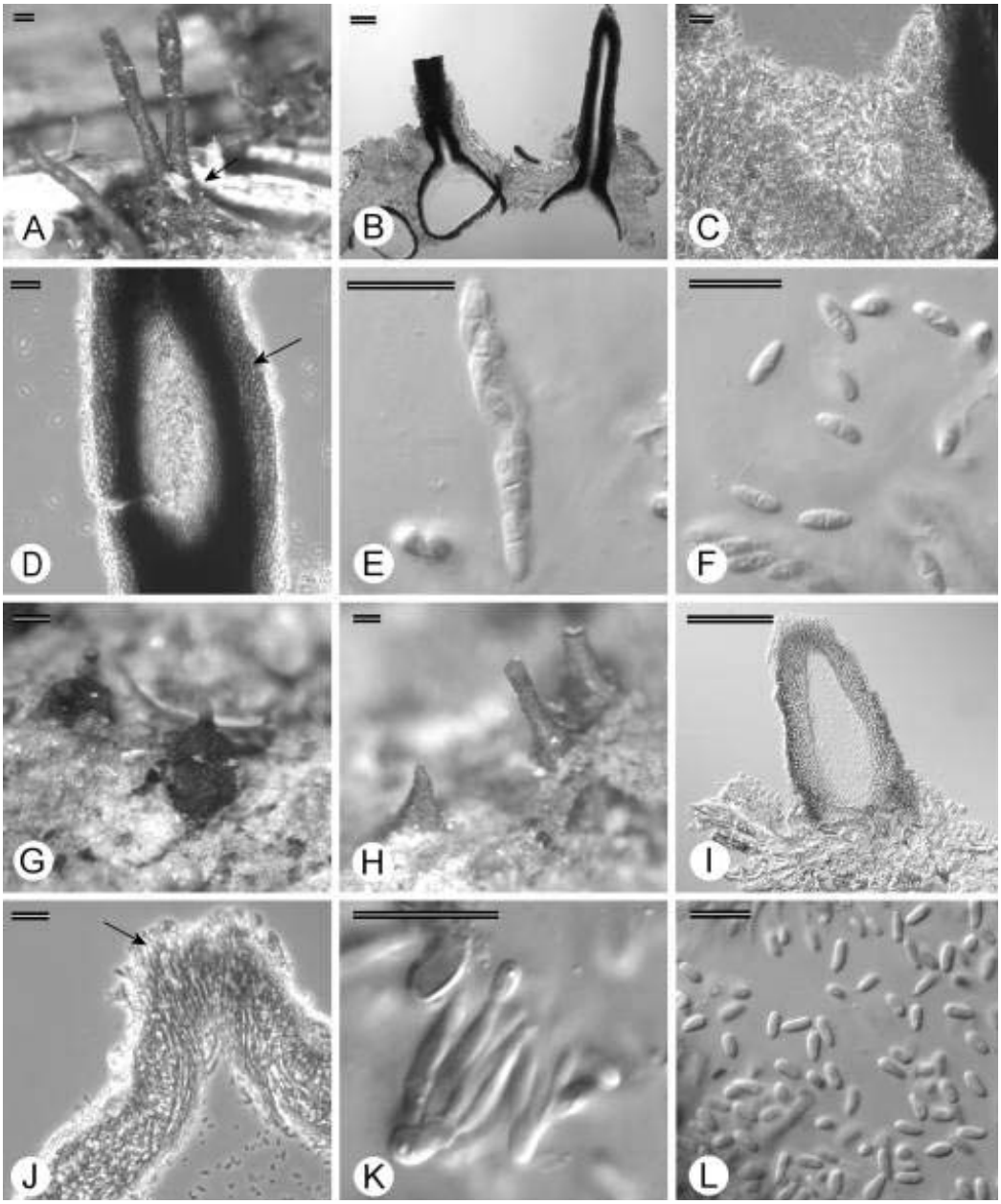

Fig. 3. Micrographs of fruiting structures of *Chrysoporthe doradensis* (from holotype). **A.** Ascostroma on bark showing black perithecial necks and orange stromatic tissue (arrow). **B.** Vertical section through ascostroma. **C.** Stromatic tissue of ascostroma. **D.** Perithecial neck and surrounding tissue (arrow). **E.** Ascus. **F.** Ascospores. **G, H.** Conidiomata. **I.** Vertical section through conidiomata. **J.** Tissue of the conidiomal base and neck (arrow). **K.** Conidiophores. **L.** Conidia. Bars: A, B, G, H, I = 100 µm; C, D, J = 20 µm; E, F, K, L = 10 µm.

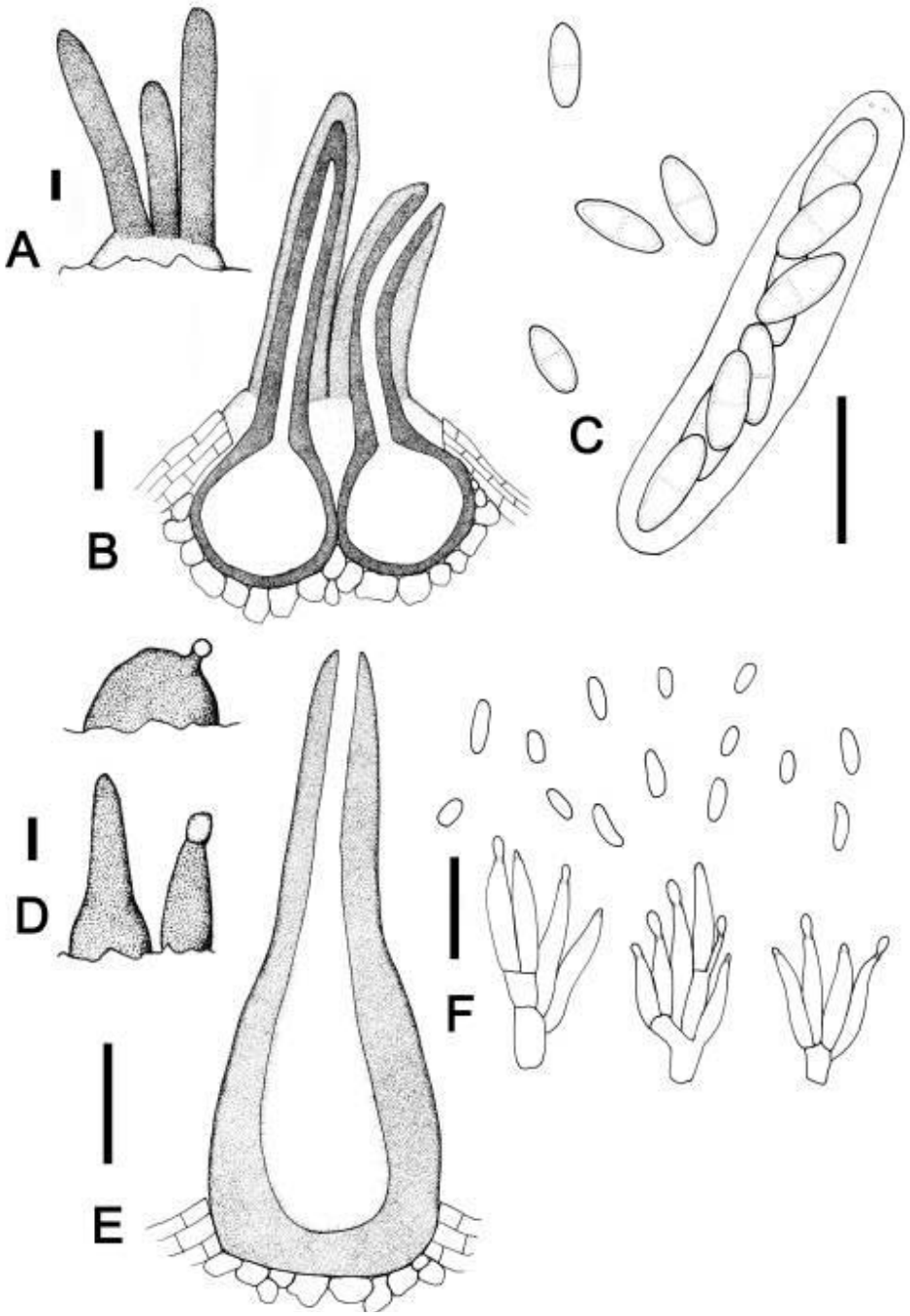

Fig. 4. Schematic drawings of *Chrysoporthe doradensis* (from holotype). **A.** Ascostroma on bark. **B.** Section through ascostroma. **C.** Asci and ascospores. **D.** Conidiomata on bark. **E.** Section through conidioma. **F.** Conidiophores and conidia. Bars: A, B, D, E = 100 μm; C, F = 10 μm.

80-140 µm wide (Figs. 3D, 4B). *Asci* 8-spored, biseriate, unitunicate, free when mature, non-stipitate with a non-amyloid refractive ring, fusoid to ellipsoid, (19.5-)21.5-24(-25) × (4-)4.5-6(-7)µm (Figs. 3E, 4C). *Ascospores* hyaline, one-septate, fusoid to oval, with tapered apices, (4.5-)5.5-7.5(-8.5) × 2-2.5 µm (Figs. 3F, 4C).

Conidiomata eustromatic, superficial to slightly immersed, pyriform to pulvinate, usually with one attenuated neck per structure (Figs. 3G, 3H, 4D, 4E), fuscous-black, inside umber when young, conidiomatal base above the bark surface 70-300 µm high, 100-290 µm diam., necks up to 300 µm long, 20-90 µm wide. *Conidiomatal locules* with even to convoluted inner surface (Figs. 3I, 4E). *Stromatic tissue* of base of *textura globulosa*, the walls of outer cells thickened, neck tissue of *textura porrecta* (Fig. 3J). *Conidiophores* hyaline, with irregular shaped basal cells, (2-)3.5-6(-7.5) × (2-)2.5-4(-5) µm, branched irregularly at the base or above into cylindrical cells, with or without separating septa, total length of conidiophore (9.5-)12.5-18(-22.5) µm (Figs. 3K, 4F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath a septum, cylindrical to flask-shaped with attenuated apices, 1.5-2(-2.5) µm wide, collarette and periclinal thickening inconspicuous (Figs. 3K, 4F). *Conidia* hyaline, non-septate, oblong to ovoid to cylindrical, occasionally allantoid, (3-)3.5-5(-6.5) × 1.5-2(-2.5) µm (Figs. 3L, 4F), masses exuded as pale luteous droplets.

Cultures on MEA white with cinnamon to hazel patches, or completely cinnamon to hazel, fluffy with a smooth margin, fast-growing, covering a 90 mm diam. plate after a minimum of four to five days at the optimum temperature of 30ºC. Cultures rarely sporulating after sub-culturing, teleomorphs not produced.

Habitat: Bark of *Eucalyptus* spp. including *E. grandis* and *E. deglupta*.

Known distribution: Ecuador

Material examined: ECUADOR, Pichincha, Buenos Aires, Buenos Aires nursery, *Eucalyptus grandis*, November 2001, M.J. Wingfield (PREM 58581; **holotype here designated**; CMW 11286/CBS 115734, CMW 11287/CBS 115735 ex-type cultures). Buenos Aires nursery, *Eucalyptus grandis*, July 2004, M.J. Wingfield (PREM 58582). Buenos Aires nursery, ex-type isolate CMW 11287 from *Eucalyptus grandis* inoculated into *Tibouchina urvilleana*, February 2000, M.J. Wingfield (PREM 58583). SOUTH AFRICA, Pretoria, ex-type isolates CMW 11286 and CMW 11287 from *Eucalyptus grandis* inoculated into *Eucalyptus grandis* clone ZG14 in the greenhouse, June 2002, M. Gryzenhout & H. Myburg (PREM 58584).

Pathogenicity tests

Inoculations with *C. doradensis* resulted in significant lesion development (avg. 57.5 mm, max. 72 mm) on the *E. deglupta* trees (Fig. 1B). These lesions were girdling trees and were significantly larger ($P < 0.001$) than

those in the control inoculations (Fig. 1C). Fruiting structures were produced abundantly on the lesions caused by the test fungus and *C. doradensis* could thus easily be re-isolated from the inoculation wounds.

The inoculations on the *T. urvilleana* trees resulted in extremely long lesions (lesions avg. 111.5 mm, max. 260 mm), often resulting in death of the tree parts above the inoculation points (Figs. 1E, 1G) and significantly different ($P < 0.001$) from those of the control inoculations. As was true on the *E. deglupta* trees, the fungus fruited profusely on the lesions (Fig. 1F) and resulted in girdling of the stems and the production of epicormic shoots below the points of inoculation (Fig. 1G).

Discussion

The stem canker disease caused by the *Chrysoporthe* sp. described in this study appears to be a serious and common disease of *E. grandis* and *E. deglupta* in the lowlands of Ecuador. The climate in this area is tropical and thus very similar to that in other parts of the world where *Chrysoporthe* spp. cause disease. Outward symptoms of the disease are similar to those caused by *C. cubensis*, but morphological and DNA based comparisons have shown clearly that the pathogen represents a distinct species. We have thus provided the name *C. doradensis* for this fungus, which is clearly an important pathogen in Ecuador.

Although peripherally similar to *C. cubensis*, *C. doradensis* could clearly be distinguished from *C. cubensis*. Based on DNA sequences differences, *C. doradensis* grouped separately from *C. cubensis*, *C. austroafricana* and *Chrysop. hodgesiana*. The most pronounced morphological feature supporting this phylogenetic distinction is the variable shape of the conidia of *C. doradensis* that is different from the more uniform oblong to oval conidia of other *Chrysoporthe* spp. In addition, *C. doradensis* produces spore masses of a different colour to those of the other species and *C. cubensis*.

Pathogenicity trials showed that *C. doradensis* is highly pathogenic and results resemble those found with the aggressive pathogen *C. austroafricana* in South Africa (Van Heerden and Wingfield, 2002; Roux *et al*., 2003; Rodas *et al*., 2005). Large lesions were produced by *C. doradensis* on *E. deglupta*, which together with *E. grandis*, is also naturally infected by the fungus in field stands. Although no quantitative data are available, and no *E. grandis* trees were available for inoculation at the time of this study, field observations suggest that *E. grandis* is substantially more susceptible to infection than is *E. deglupta*.

Pathogenicity trials with *C. doradensis* on seedlings of *T. urvilleana* showed that this host is highly susceptible to infection by the pathogen. In the

two separate trials involving *E. deglupta* and *T. urvilleana* respectively, lesions on *T. urvilleana* were twice the length of those of *E. deglupta*. Similar results were obtained in pathogenicity trials involving *Chrysop. hodgesiana* (Wingfield *et al*., 2001) and *C. cubensis* (Rodas *et al*., 2005), where the pathogenicity of these species were also compared on *Tibouchina* and *Eucalyptus* species. In these trials, the *Tibouchina* trees were usually considerably more susceptible than the *Eucalyptus* trees, and were often killed during the course of the trials.

The origin of *C. doradensis* is unknown although we suspect that it is native to Ecuador. Despite widespread surveys for *Chrysoporthe* species over the past few years, *C. doradensis* has not been isolated in other parts of South America or the world, indicating that it might be restricted to Ecuador. If *C. doradensis* is native to Ecuador, it would have originated from a host other than *Eucalyptus*, which is a non-native in this country. Other species of *Chrysoporthe* in South and Central America occur on native members of the *Melastomataceae* including species of *Tibouchina* and *Miconia* (Wingfield *et al*., 2001; Gryzenhout *et al*., 2004; Rodas *et al*., 2005). One possibility is that the fungus has originated on native *Melastomataceae* such as *Tibouchina* spp. that are common in Ecuador. The fact that *T. urvilleana* was highly susceptible to infection in this study might argue against this genus of tree being a native host. However, *Tibouchina* spp. in Ecuador are never found in the hot humid lowlands but are rather restricted to cool, high altitude forests of Ecuador. Susceptibilty of these trees to *Chrysoporthe* spp. could thus be associated with hot humid climates.

It is not clear whether *C. cubensis* and *Chrysop. hodgesiana* occur in Ecuador. The wide distribution of *C. cubensis* throughout South and Central America would suggest that the pathogen should also occur in this country. *Chrysop. hodgesiana*, however, has been reported only from Colombia (Gryzenhout *et al*., 2004; Rodas *et al*., 2005) and it is unknown whether it occurs elsewhere in South America. Cankers caused by *C. doradensis* are common on *Eucalyptus* spp. in the Ecuadorian lowlands and some of these may be caused by *Chrysoporthe* spp. other than the newly described *C. doradensis*. Identification of these fungi is difficult and demands robusts tests and ideally DNA sequence comparisons. For the present, it is clear that *C. doradensis* is commonly associated with cankers on *Eucalyptus* spp. in Ecuador, but other species of *Chrysoporthe* may also be present. If other species are present, this would potentially complicate disease management strategies.

The relative susceptibility of *Eucalyptus* spp. world wide to canker caused by species of *Chrysoporthe*, has been based on knowledge of the well

known *C. cubensis*. The recent discovery that there are various other species related to this fungus, and that these are all pathogenic to *Eucalyptus*, should change this view. While *C. cubensis* might be pathogenic to certain species of *Eucalyptus*, others such as the new species described in this study, could have a different host range. For example, the fungus causing cankers on *Eucalyptus* in South Africa, now known as *C. austroafricana*, is highly pathogenic and appears to have a rather different biology to *C. cubensis* (Wingfield, 2003). These fungi, like the well known and devastating chestnut blight pathogen *Cry. parasitica* (Anagnostakis, 1987), are virulent pathogens that can cause substantial damage to trees. Thus great effort should be made to ensure that they are not introduced into new areas of the world, where native *Myrtaceae*, *Melastomataceae*, or possibly hosts in other families, could be highly susceptible to infection by them.

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