Chrysoporthe puriensis sp. nov. from *Tibouchina* spp. in Brazil: an emerging threat to *Eucalyptus*

M. E. S. Oliveira[1,2], N. A. van der Merwe[3], M. J. Wingfield[3], B. D. Wingfield[3], T. P. F. Soares[1], A. M. Kanzi 3, M. A. Ferreira[1,*]

[1] Department of Plant Pathology, Universidade Federal de Lavras (Federal University of Lavras), Postal Box 3037, Lavras 37200-000, Minas Gerais, Brazil
[2] Forest Pathology Laboratory, Universidade Federal do Tocantins (Federal University of Tocantins), Postal Box 66, Gurupi 77402-970, Tocantins, Brazil
[3] Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

*Correspondence to M. A. Ferreira. Email: ferreirama.ufla@gmail.com

Abstract

The discovery of Cryphonectriaceae and more specifically species related to the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* on shrubs and trees in the Melastomataceae, has deepened our understanding of relevant, and potentially globally threatening tree pathogens. Recent isolations of Cryphonectriaceae associated with cankers on *Tibouchina* spp. in Brazil gave rise to an apparently undescribed species of *Chrysoporthe* associated with stem and branch cankers that lead to tree death. Cultures of this fungus were subjected to phylogenetic studies based on sequences for the ITS and β-tubulin gene regions. These analyses revealed a novel taxon that is described here as *Chrysoporthe puriensis* sp. nov., having both sexual and asexual states. Pathogenicity tests on two species of *Tibouchina* (*T. granulosa*, *T. heteromalla*) and hybrids of *Eucalyptus grandis* x *E. urophylla* showed that *Chr. puriensis* can infect and cause disease on all of these trees. It is clearly not only damaging on native *Tibouchina* spp. where environmental conditions are conducive to disease development, but also potentially threatening to non-native *Eucalyptus* spp., which form the basis of a major **plantation forest industry.**

Keywords: Cryphonectriaceae ; Canker disease ; Tibouchina granulosa ; T. heteromalla ; Phylogeny ; Pathogenicity

Introduction

The Cryphonectriaceace includes some of the most important pathogens of trees. Most notable of these are the chestnut blight pathogen *Cryphonectria parasitica* (Gryzenhout et al. 2006b; Gryzenhout et al. 2009) and the *Eucalyptus* canker species *Chrysoporthe cubensis*, *Chr. deuterocubensis* and *Chr. austroafricana* (Wingfield et al. 2001; Gryzenhout et al. 2009). Of these species *Chr. cubensis* is well known and apparently native in Brazil where it was first discovered (as *Cryphonectria cubensis*) as a threat to plantation-grown *Eucalyptus* (Hodges et al. 1973, 1976, 1986). Intriguingly, this pathogen and some of its relatives were later discovered in various South American countries on native shrubs and trees in the Melastomataceae (Seixas et al. 2004; Rodas et al. 2005; Gryzenhout et al. 2005; Barreto et al. 2006). These fungi have also been found on native trees in the Myrtaceae and Lythraceae in various countries of the world (Gryzenhout et al. 2005, 2006a; Nakabonge et al. 2006;

Chungu et al. 2009; Wingfield et al. 2010; Chen et al. 2010, 2018). In these situations, they have apparently undergone host shifts to infect *Eucalyptus* spp. established as non-natives in plantations. All of these woody plant families including *Eucalyptus* are related in that they reside in the Myrtales.

Chrysoporthe cubensis was first described as, *Diaporthe cubensis,* causing cankers and damage to *Eucalyptus* plantations in Cuba (Bruner 1917). Phylogenetic studies based on DNA sequence data for the β-tubulin and histone H3 gene regions led to the recognition that *Chr. cubensis* was only distantly related to well-known species such as *C. parasitica.* This resulted in the establishment of the new genus *Chrysoporthe* and the new combination *Chr. cubensis* (Gryzenhout et al. 2004). Subsequent studies in various parts of the world have revealed numerous new species of *Chrysoporthe* and related fungi on native Myrtaceae (Rodas et al. 2005; van der Merwe et al. 2013; Soares et al. 2018). Many of these have either undergone host shifts to infect *Eucalyptus* or have the potential to infect these trees, as revealed by artificial inoculation studies. There are few reports of the *Chrysoporthe* spp. on *Eucalyptus* spp. where these trees are native, and in those cases, these fungi are most probably non-natives (Myburg et al. 2002, 2003, 2004; Gryzenhout et al. 2004).

Two species of *Chrysoporthe*, *Chr. cubensis* and *Chr. doradensis*, have been recorded in Brazil (Hodges et al. 1976; Soares et al. 2018). *Chrysoporthe cubensis* has been found associated with canker diseases on *Syzygium aromaticum*, *Plinia edulis*, *Corymbia citriodora* and *Eucalyptus* spp. (Myrtaceae) as well as on species of *Tibouchina* (Melastomataceae). Of these, *P. edulis*, and *Tibouchina* spp. are native in Brazil(Barreto et al. 2006; Soares et al. 2018). *Chrysoporthe doradensis* was first discovered causing cankers on non-native *E. grandis* and *E. deglupta* in Ecuador (Gryzenhout et al. 2005) but its native host remains unknown. This species was later recorded occurring on *Tibouchina* spp. (Seixas et al. 2004; Soares et al. 2018) and *Eucalyptus* spp. (Soares et al. 2018) in Brazil.

While *Eucalyptus* spp. form the basis of the plantation forestry industry in Brazil, *Tibouchina* spp. are widely used for urban afforestation and in the recovery of degraded areas. These trees are seriously damaged and dying due to canker caused by a *Chrysoporthe* species (Barreto et al. 2006; Soares et al. 2018). The implication here is that they provide a substantial source of inoculum for potential infection of *Eucalyptus* spp.

During the course of surveys to collect isolates of *Chr. cubensis* in Brazil and aimed at interrogating the area of origin of that pathogen, cultures of an apparently unknown species of *Chrysoporthe* associated with cankers on *Tibouchina* spp. were observed. The aim of this study was to identify this fungus and to test its pathogenicity to *Tibouchina* spp. as well as *Eucalyptus*.

Material and methods

Symptoms and isolates

Cankers occurring on *Tibouchina* spp. in native forests as well as in urban environments were sampled. The sampled areas included the Brazilian states of Bahia, Minas Gerais, and Rio de Janeiro (Table 1). Bark covering the cankers was examined for fruiting bodies typical of *Chrysoporthe* spp. and isolations were made from these structures.

Table 1. Details of *Chrysoporthe puriensis* isolates used in this study

Isolates	Number of isolates	Host	Location	
			State	City
TGCD01	1	*T. granulosa*	Bahia	Lençóis
TCL01	1	*T. candolleana*	Minas Gerais	Lavras
CT05, TGL02, TGL03, TGL05	4	*T. granulosa*	Minas Gerais	Lavras
TIL01	1	*T. heteromalla*	Minas Gerais	Lavras
CT07, CT10, CT11, CT13	4	*T. granulosa*	Minas Gerais	São João del Rei
TGDR01	1	*T. granulosa*	Minas Gerais	São João del Rei
TGSC01, TGSC03, TGSC04, TGSC07, TGSC09, TGSC11, TGSC13, TGSC14	8	*T. granulosa*	Minas Gerais	São Roque de Minas
THSC01, THSC04	2	*T. heteromalla*	Minas Gerais	São Roque de Minas
TISC02	1	*Tibouchina* sp.	Minas Gerais	São Roque de Minas
TIST01	1	*Tibouchina* sp.	Minas Gerais	São Tomé das Letras
TGS01, TGS02, TGS03, TGS04, TGS06, TGS07, TGS08	7	*T. granulosa*	Minas Gerais	Silveirânia
TGT02, TGT03	2	*T. granulosa*	Minas Gerais	Tiradentes
TGPNI01, TGPNI03, TGPNI04, TGPNI08, TGPNI09, TGPNI10, TGPNI11, TGPNI12, TGPNI13, TGPNI14, TGPNI16, TGPNI19	12	*T. granulosa*	Rio de Janeiro	Itatiaia

Single spore isolates were made from a *Chrysoporthe* pycnidium occurring on each tree sampled, yielding a total of 103 isolates. For this purpose, spore masses exuding from a single structure were transferred to sterile water with a sterilized needle and plated onto 20% *w*/*v* potato dextrose agar (PDA). The plates were incubated in the dark at 28 °C for 24 h after which single germinating conidia were transferred to fresh PDA plates and incubated at 28 °C for 7 days. The isolates were stored in microtubes containing 0.85% NaCl and maintained at room temperature (16–23 °C) as described by Castellani (1939) as well as in microtubes with 15% glycerol and stored at −80 °C.

The resultant cultures were maintained in the culture collection of the Forest Pathology Laboratory (LPF) of Federal University of Lavras, Brazil, and representative cultures have been deposited in culture collection of the Coleção Micológica de Lavras (CML), Lavras, Minas Gerais, Brazil. Isolates TGS06 (= CMW54429), TIS101 (= CMW54437), and TGT02 (= CMW54402) have also been lodged in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. The original bark specimens from which isolations were made have been deposited in the herbarium of the Forest Pathology Laboratory (LPF), Federal University of Lavras, Brazil.

DNA isolations, sequencing and phylogenetic analyses comparisons

For DNA extraction, isolates were grown at 28 °C for 7–10 days in the dark in liquid malt extract (20% *w*/*v*). The mycelium was filtered, and total genomic DNA was extracted using a Wizard Genome DNA Purification Kit (Promega of USA) following the manufacturer's instructions.

Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer (ITS) and the conserved 5.8 S gene of the ribosomal DNA using the primers ITS1 and ITS4 (White et al. 1990) as well as the β-tubulin gene region with two pair primers Bt1a/Bt1b and Bt2a/Bt2b (Glass and Donaldson 1995). Sequencing was performed using Big Dye terminator sequencing kits (Life Technologies) on an ABI 13100 sequencer (Applied Biosystems) following the approach of van der Merwe et al. (2010). Sequences were manually edited when necessary.

Sequences of 45 isolates obtained in this study (Table 1), as well as of 25 representatives for the other known species of *Chrysoporthe* and *Amphilogia gyrosa* (outgroup) were obtained from Genbank (Table 2) and analysed. Sequence alignments were made using the online interface of MAFFT (Katoh et al. 2019). A Partition Homogeneity Test (PHT) described by Farris et al. (1994) was applied using PAUP* 4.0 (Swofford 2002) to the combined rDNA ITS and β-tubulin sequence data, using 1000 replicates, to ascertain whether they could be analysed collectively. The combined gene alignment was subjected to Maximum likelihood (ML) and Maximum parsimony (MP) analyses.

Table 2. Reference sequences for *Chrysoporthe* spp. and *Amphilogia gyrosa* used in the phylogenetic analyses

Species	Isolate number	GenBank accession numbers		
		ITS	BT1	BT2
Chrysoporthe puriensis	**CT10**	MN590028	MN590040[a]	–
	CT13	MN590029	MN590041[a]	–
	TCL01	MN590030	MN590042[a]	–
	TGL02	MN590031	MN590043[a]	–
	TGPNI01	MN590032	MN590044[a]	–
	TGS01	MN590033	MN590045[a]	–
	TGSC01	MN590034	MN590046[a]	–
	TGT03	MN590035	MN590047[a]	–
	CT07	MN590036	MN590048[a]	–
	TGCD01	MN590037	MN590049[a]	–
	TIL01	MN590038	MN590050[a]	–
	THSC01	MN590039	MN590051[a]	–
Chrysoporthe cubensis	CMW10669	GQ290154	GQ290177	AF535126
	CMW10778	GQ290155	GQ290178	GQ290189
	CMW10639	AY263421	AY263419	AY263420
	CMW10028	GQ290153	GQ290175	GQ290186
Chrysoporthe deuterocubensis	CMW12745	DQ368764	GQ290183	DQ368781
	CMW12746	HM142105	HM142121	HM142137
	CMW17178	DQ368766	AH015649	AH015649
	CMW2631	GQ290157	GQ290184	AF543825
	CMW8650	AY084001	AY084024	GQ290193
Chrysoporthe hodgesiana	CMW10641	AY692322	AY692326	AY692325
	CMW9995	AY956969	AH014904	AH014904
Chrysoporthe austroafricana	CMW10192	AY214299	GQ290176	GQ290187
	CMW9327	GQ290158	GQ290185	AF273455
	CMW2113	AF046892	AF273067	AF273462
Chrysoporthe syzygiicola	CMW29940	FJ655005	FJ805230	FJ805236
	CMW29942	FJ655007	FJ805232	FJ805238
Chrysoporthe zambiensis	CMW29928	FJ655002	FJ858709	FJ805233
	CMW29930	FJ655004	FJ858711	FJ805235
Chrysoporthe inopina	CMW12729	DQ368778	AH015656	AH015656
	CMW12727	DQ368777	AH015657	AH015657
	CMW12731	DQ368779	AH015655	AH015655
Chrysoporthe doradensis	CMW11286	AY214290	AY214218	AY214254
	CMW11287	GQ290156	GQ290179	GQ290190
Amphilogia gyrosa	CMW10469	AF452111	AF525797	AF525714
	CMW10470	AF452112	AF535708	AF525715

Isolates presented in bold were sequenced in this study

CMW Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria

[a] These sequences represent sections of BT1/BT2 combined of the gene beta-tubulin

Maximum parsimony analysis was undertaken using PAUP* 4.0 (Swofford 2002). Only parsimony informative characters were used. The Heuristic search option with random stepwise addition and tree bisection reconnection (TBR) was used as the swapping algorithm. Confidence levels of the branching points were determined using 1000 bootstrap replicates.

Maximum likelihood analysis was conducted using MEGA 6 (Tamura et al. 2013), incorporating the Tamura 3-parameter model of evolution as determined by MEGA 6 (Tamura 1992). A discrete Gamma distribution was used to model evolutionary rate differences among sites. The confidence in branches was tested using 1000 bootstrap replicates.

Morphological description

For morphological characterization of the undescribed *Chrysoporthe* sp., fruiting structures from bark specimens were observed and sectioned. These included both perithecia (sexual) and pycnidia (asexual) structures. Sections were mounted in lactophenol and examined using light microscopy (LABOMED Lx400, Labo America, Fremont, Canada) equipped with an iVu 500 camera and Software Capture Pro 2.8.8.5. Fifty measurements were made for all taxonomically informative structures including pycnidia, conidiophores, conidia perithecia, asci, and ascospores. Measurements are presented as (min –)(average – SD) – (average + SD)(− max) µm, where SD is the standard deviation. Micrographs were captured using a Zeiss observer Z.1 motorized inverter microscope using differential interference contrast. For assessment of mycelial growth in culture, 5 mm diam discs were cut from the margins of an actively growing culture and transferred to 90 mm diam. Petri dishes containing Malt Extract Agar (MEA) and incubated at temperatures ranging from 15 °C to 30 °C at 5 °C intervals. Five plates were used per temperature. Colony diameters were measured after 7 days and the averages computed.

Pathogenicity

The pathogenicity of the undescribed *Chrysoporthe* sp. was tested on 18-month-old *T. granulosa* and *T. heteromalla* plants as well as those of a 4-month-old *Eucalyptus grandis* x *E. urophylla* hybrid clone. This study was conducted in a greenhouse maintained at temperatures ranging from 15 to 27 °C. A single isolate (CML3738) was randomly selected for use in the inoculation test.

The isolate for inoculation was grown on 2% MEA and maintained in the dark at 28 °C for 7 days. Ten plants of each host were inoculated with the test isolate and ten additional plants were inoculated with a sterile water agar plug to serve as controls. A 5-mm-diameter cork borer was used to remove a disc of bark from stems of the plants to expose the cambium, and a mycelial plug of equal size was taken from the margins of actively growing cultures and placed into the wounds with the mycelium facing the cambium. Wounds were sealed with Parafilm 'M' (American National Can™ Chicago, USA) to avoid desiccation as described by Chungu et al. (2009).

Lesion lengths were recorded 8 weeks after inoculation (w.a.i.). Re-isolations were made from the areas of inoculation on both the control and treated plants. In the case of treated plants, fruiting structures of the *Chrysoporthe* sp. had formed on the surface of the lesions. Identification of the inoculated fungus was made by considering the morphological characteristics of fruiting structures under a light-microscope. Average lesion lengths were

analysed using the Scott-Knott test (Scott and Knott 1974). To verify the significance among the averages, a t-test was used and values where $P \leq 0.05$ were considered as significant.

Results

Symptoms and isolates

A total of 45 isolates of the purportedly undescribed species of *Chrysoporthe* were collected from trees of *T. granulosa*, *T. candolleana*, *T. heteromalla* and an unknown *Tibouchina* sp. in eight cities of Brazil (Table 1). Symptoms on *Tibouchina* spp. trees included cracked bark, branch dieback (Fig. 1a), cankers on the stems (Fig. 1b) and tree death. Fruiting structures including pycnidia and perithecia were found between the cracks on the cankers and on dead areas of the branches and stems (Fig. 1c).

Fig. 1. Symptoms on *Tibouchina* spp. **a** Tree showing branch dieback caused by *Chrysoporthe puriensis*. **b** Cracked bark and canker on the stem of a tree infected by *Chr. puriensis*. **c** Pycnidia of *Chr. puriensis* on bark of an infected tree

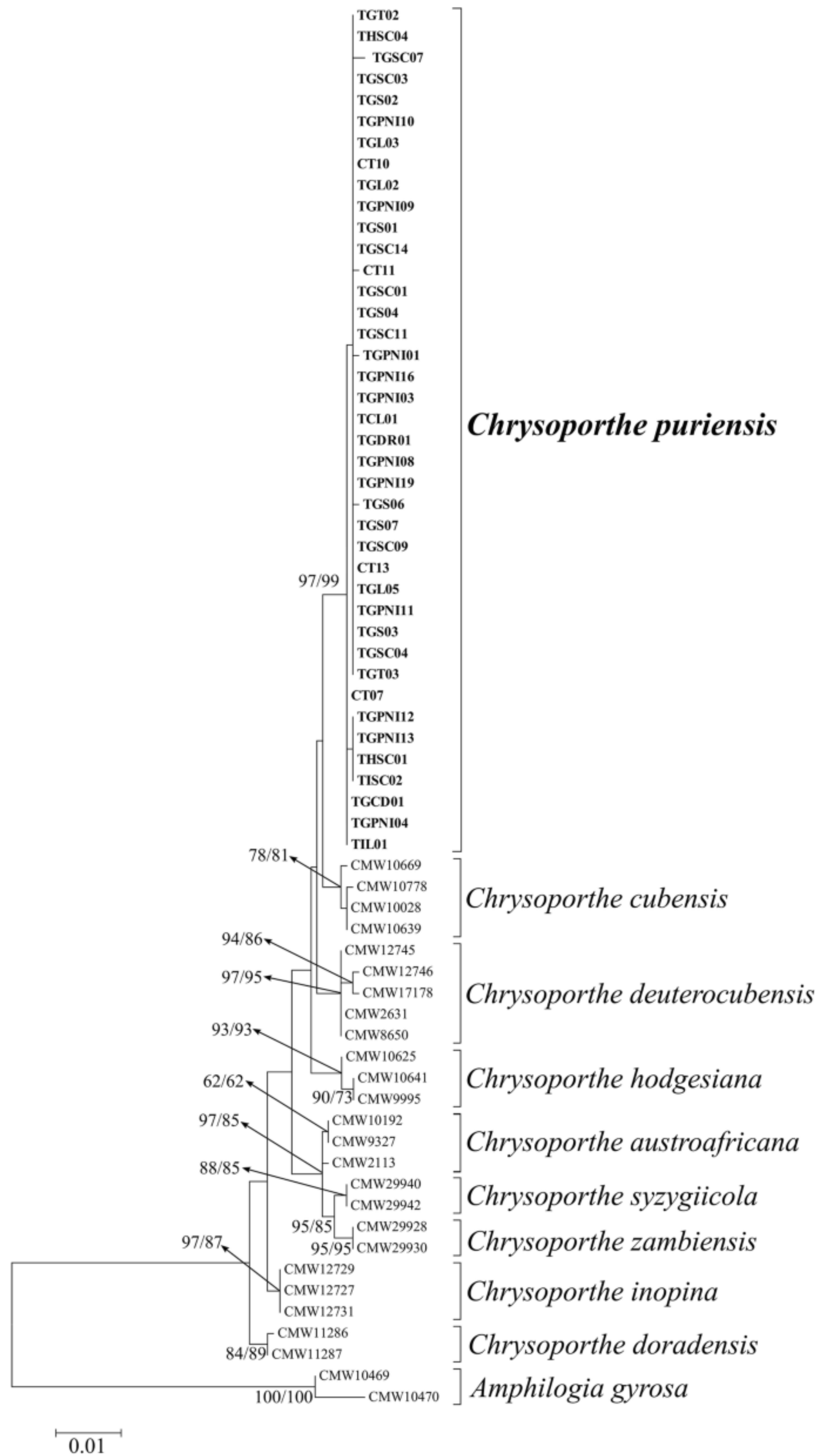

Fig. 2. Molecular Phylogenetic analysis by Maximum Likelihood (ML) combined DNA sequence data set of regions of the Internal Transcribed Spacer of rRNA gene (ITS), and Beta-tubulin (BT1 and BT2 regions). Bootstrap values above 60% are indicated above each branch (ML/MP). The isolates of *Chrysoporthe puriensis* isolated from this study are highlighted in bold

DNA sequence comparisons

PCR products were approximately 438 bp (ITS) and 696 bp (β-tubulin) in size. The combined sequence dataset, of ITS-rDNA including the 5.8S gene and fragments of the β-tubulin gene, produced 1134 sequence-aligned characters, of which 1020 were constant, 12 parsimony non-informative and 102 informative based on parsimony. The partition homogeneity test (PHT) showed that the ITS-rDNA and β-tubulin sequence data sets did not have any significant conflict ($P = 0.01$) and could thus be combined (Gryzenhout et al. 2006a, b; Chungu et al. 2009).

Phylograms obtained by MP and ML analyses were similar and had consistent topologies with well supported branches. Tree statistics from the maximum parsimony analyses were: alignment length in base pairs (length) 124, consistency index (CI) 0.880, retention index (RI) 0.962 and homoplasy index (HI) 0.120. Maximum likelihood analysis was chosen to produce a phylogenetic tree (Fig. 2).

The *Chrysoporthe* isolates in the phylogram (Fig. 2) generated from the combined sequence data set resided in nine sub-clades (1–9), clustering separately from the outgroup taxon represented by *A. gyrosa*. Clades 2–9 represented known species of *Chrysoporthe* that included *Chr. cubensis*, *Chr. deuterocubensis*, *Chr. hodgesiana*, *Chr. austroafricana*, *Chr. syzygiicola*, *Chr. zambiensis*, *Chr. inopina* and *Chr. doradensis*. Each clade was strongly supported by bootstrap values of >70%. Clade 1 represented the undescribed *Chrysoporthe* sp. from *Tibouchina* spp. in Brazil, distinct from those representing known species (>90% bootstrap support), residing in a clade most closely related to *Chr. cubensis* (Fig. 2). The *Chrysoporthe* isolates comprising the nine clades in the phylogenetic analyses could be distinguished by 44 nucleotides in the ITS-rDNA and β-tubulin gene regions (Table 3). Six nucleotides were different between the undescribed *Chrysoporthe* sp. and other species in the genus (Table 3).

Table 3. Summary of polymorphic sites found within sequences of the ribosomal ITS region and two regions in the β-tubulin genes for all known *Chrysoporthe* species, including *Chr. puriensis* described in this study. Polymorphic nucleotides unique to *Chr. puriensis* are highlighted.

Species	Beta-tubulin 2 (Bt2a/Bt2b)														Beta-tubulin 1 (Bt1a/Bt1b)																								ITS1/5.8S/ITS4					
	10	49	58	79	146	196	202	250	251	252	273	281	290	320	404	431	455	458	509	527	561	568	571	582	586	595	598	600	601	605	608	619	620	621	696	716	750	754	779	780	787	840	859	1066
"*Chr. puriensis*"	G	C	T	C	A	C	C	G	T	**T**	C	G	C	**T**	T	C	G	C	C	T	**T**	T	T	T	A	A	A	G	G	C	C	**T**	T	T	-	**T**	C	G	T	**T**	A	-	C	A
Chr. cubensis	G	C	T	C	A	C	C	G	T	C	C	G	C	C	T	C	G	C	T	T	C	T	T	T	A	A	A	A	G	C	C	C	T	C	-	-	C	A	-	C	A	-	C	A
Chr. doradensis	G	C	T	T	G	T	C	G	C	C	T	G	C	C	T	A	G	C	C	T	C	C	C	T	A	A	A	G	A	C	T	C	C	C	-	-	C	A	-	C	A	-	C	A
Chr. hodgesiana	A	C	C	C	A	C	C	G	T	C	C	A	C	C	T	C	G	C	T	C	C	T	T	T	G	A	A	G	G	C	C	C	C	C	-	-	C	G	-	C	A	-	C	G
Chr.inopina	G	C	T	T	A	T	C	A	C	C	T	G	C	C	T	C	G	C	C	C	C	C	C	T	A	A	A	G	G	C	C	C	C	C	-	-	C	G	-	C	A	-	C	G
Chr. austroafricana	G	C	T	C	A	C	C	G	T	C	C	G	T	C	T	C	G	C	C	C	C	C	C	C	A	C	C	G	G	C	C	C	C	C	-	-	C	A	-	C	A	-	T	A
Chr. syzygiicola	G	C	T	C	A	A	C	G	T	C	C	G	T	C	T	C	T	A	C	C	C	C	C	C	A	C	C	G	G	T	C	C	C	C	G	-	C	A	-	C	A	-	C	A
Chr. zambiensis	G	C	T	C	A	A	A	G	T	C	C	G	T	C	A	C	G	C	C	C	C	C	C	C	A	C	C	G	G	T	C	C	C	C	C	-	A	A	-	C	A	-	C	A
Chr. deuterocubensis	G	T	T	T	A	C	C	G	C	C	C	G	C	C	T	C	G	C	C	T	C	T	T	T	A	A	A	G	G	C	C	C	C	C	-	-	C	G	T	T	G	C	C	A

Table 4. Morphological characteristics of *Chrysoporthe puriensis* compared with other *Chrysoporthe* species

Species	Optimal temp. For growth	Conidiomata base width(μm)	Conidium size(μm)		Ascus size (μm)		Ascospore size (μm)	
			Length	Width	Length	Width	Length	Width
Chr. puriensis[a]	28 °C	95–470	(3-)3.5-5(−6,5)	1.5–2(−2.5)	(14-)17.5–24(−28)	(3.5-)4–6 (−7)	(3-)3.5–6(−9)	(1-)1.5–3(−4)
Chr. cubensis[b]	30 °C	100–950	4.5(−5)	(3−)3.5	(19-)22-26.5(−28)	(4.5-)5–6.5(−7)	(5.5-)6.5–7.5(−8)	2–2.5(−3)
Chr. deuterocubensis[c]	30 °C	100–950	(3-)3.5-4.5(−5)	(1.5-)2(−2.5)	(19-)22-26.5(−28)	(4.5-)5–6.5(−7)	(5.5-)6.5–7.5	(−8)2–2.5(−3)
Chr. doradensis[d]	30 °C	100–290	(3-)3–5(−6.5)	1.5–2(−2.5)	(19.5-)21.5-24(−25)	(4-)4.5–6(−7)	(4.5-)5.5–7.5(−8.5)	2–2.5
Chr. hodgesiana[e]	25 °C	145–635	(3-)3.5-5(−5.5)	1.5–2(−2.5)	–	–	–	–
Chr. inopina[e]	25 °C	70–710	(3-)3.5–4	(1.5-)2–2.5	(27.5-)29.5-34(−35)	(4.5-)5.5–6.5(−7)	(4.5-)6–7.5(−8)	2.5–3.5
Chr. austroafricana[f]	25–30 °C	80–120	3–4(−4.5)	1.5–2	(25-)27-32(−34)	(4-)5.5–7(−7.5)	(5.5-)6–7	(2-)2.5
Chr. syzygiicola[g]	30 °C	250–500	(2.1-)2.5-3.5(−4.0)	(1.2-)1.5–2.0	–	–	–	–
Chr. zambiensis[g]	30 °C	208–310	(2.5-)3.0-3.5(−4.0)	(1.0-)1.5–2.0	–	–	–	–

Reference: [a] This work; [b] Roux et al. 2003, Gryzenhout et al. 2004; [c] van der Merwe et al. 2010; [d] Gryzenhout et al. 2005; [e] Gryzenhout et al. 2006a, b; [f] Gryzenhout et al. 2004, Chungu et al. 2009; [g] Chungu et al. 2009

Taxonomy

Phylogenetic analyses in this study provided robust evidence for an undescribed species of *Chrysoporthe* commonly occurring on *Tibouchina* spp. in Brazil. This fungus fruits abundantly on the surface of cankers on infected trees producing both sexual and asexual states. The morphological characteristics of this fungus were very similar to those of other *Chrysoporthe* spp. (Table 4) and the fungus is described here as a novel taxon.

Chrysoporthe puriensis, M.E.S. Oliv., T.P.F. Soar. & M.A. Ferr., sp. nov.

Mycobank: MB 832138.

Etymology

"*Puris*" refers to the name of an extinct indigenous tribe that lived in the areas where this fungus was first found.

Ascostromata

semi-immersed in bark, fuscous-black to cinnamon, cylindrical perithecial necks, and in some cases, erumpent, orange ascostromatic tissue, 130–230 µm high, above level of bark, 140–440 µm diam. **Perithecia** valsoid, bases immersed in bark, fuscous-black, top of perithecial bases covered with cinnamon to orange, limited stromatic tissue present around the structures above the bark surface, extending necks up to 370 µm long emerging through bark covered in umber stromatic tissue of *textura epidermoidea*, appearing fuscous-black (Fig. 3a, b). **Asci** (14-)17.5–24(−28) x (3.5-)4–6 (−7) µm, fusoid to ellipsoidal, 8-spored (Fig. 3c). **Ascospores** (3-)3.5–6(−9) x (1-)1.5–3(−4) µm, hyaline, 1-septate, oval to ellipsoid, ends tapered, with septum central (Fig. 3d).

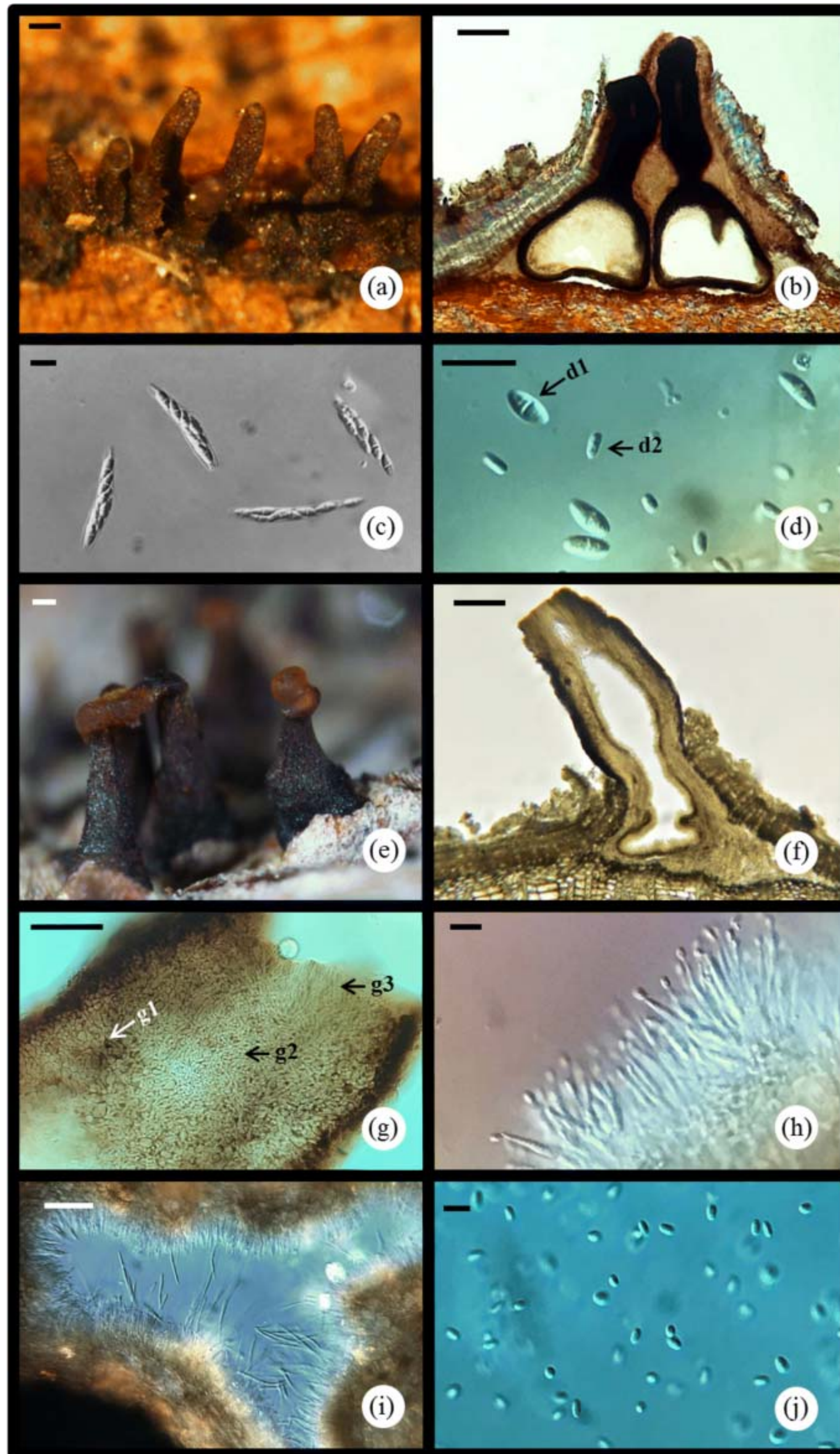

Fig. 3. Fruiting structures of *Chrysoporthe puriensis*. **a** Ascostromata on bark. **b** Longitudinal section through ascostroma. **c** Asci. **d** d1 = Ascospores, d2 = Conidium. **e** Conidioma on bark. **f** Longitudinal section through conidioma. **g** Tissue of *Textura globulosa* (g1), *Textura epidermoidea* (g2) and *Textura porrecta* (g3) for the neck. **h** Conidiophores. **i** Paraphyses. **j** Conidia . Scale bars: a,b,e,f (100 µm); g,i (20 µm); c,d,h,j (10 µm)

Conidiomata

pycnidia occurring on the surface of ascostroma or as separate structures, superficial to slightly immersed, matt black, pyriform to pulvinate, with one to four necks, but usually one, conidial masses exuding as bright luteous droplets. (Fig. 3e). Conidiomatal bases above the bark surface 70–1350 µm height, 95–470 µm width. Conidiomatal locules with flat to rounded inner surfaces, occasionally multilocular (Fig. 3f). Stromatic base tissue of *textura globulosa* and *epidermoidea* and neck tissue of *textura porrecta* and *epidermoidea* (Fig. 3g). **Conidiophores** hyaline, consisting of a basal cell, branched irregular at the base or above into cylindrical cells, with or without septa, 2.3–4.0 × 1.2–2.1 and 9.2–17.0 µm (Fig. 3h). Occasionally long cylindrical paraphyses, occurring between conidiophores (Fig. 3i). Conidiogenous cells phialidic, apical or lateral on branches bellow a septum, cylindrical to flask-shaped with attenuated apices, 1.5–2.5 µm length, collarette and periclinal thickening inconspicuous. **Conidia** hyaline, aseptate, oblong, fusoid to oval, (3-)3.5–5(−6.5) × 1.5–2(−2.5) µm (Fig. 3j).

Culture characteristics

Colonies on MEA with white and fluffy mycelial growth when younger and turning orange when older, smooth margins, showing orange discoloration in the growth medium. Optimum temperature for growth 28 °C, covering 90 mm plates in 7 days.

Substrate

Bark of *Tibouchina* spp. trees, such as *T. granulosa*, *T. heteromalla* and *T. candolleana*.

Distribution

Three states of Brazil: Bahia, Minas Gerais and Rio de Janeiro.

Material examined

Brazil, Minas Gerais State, São João del Rei (21°9′57”S, 43°10′28”W), *Tibouchina granulosa*, October 2016, M.A. Ferreira and M.E.S. Oliveira, Holotype LPFCT13 (branches with mature conidiomata and perithecia), ex-type culture CT13 = CML3738; Brazil, Minas Gerais State, Silveirânia (21°10′41”S, 43°12′36” W), *T. granulosa*, January 2017, M.E.S. Oliveira. Paratype LPFTGS06 (trunk with mature conidiomata and perithecia), living culture TGS06 = CMW 54429); Brazil, Bahia, Lençóis (12°35′11″S, 41°23′22”W), *T. granulosa*, December 2016, M.E.S. Oliveira, Paratype LPFTGCD01 (branches with mature conidiomata), living culture TGCD01. Brazil, Rio de Janeiro, Itatiaia (22°27′33″S, 44°36′23”W), *T. granulosa*, November 2016, M.E.S. Oliveira, Paratype LPFTGPNI08 (branches with mature conidiomata), living culture TGPNI08 = CMW 54426.

Pathogenicity

Well-developed lesions were found on all seedlings inoculated with *Chr. puriensis*. No lesions developed associated with any of the control inoculations. Analysis of variance revealed significant differences among hosts ($P \leq 0.05$). The lesions on the *Tibouchina* spp. were significantly longer than those on the *E. grandis* x *E. urophylla* hybrid plants (Fig. 4). Fruiting bodies produced on the lesions of the inoculated plants were morphologically

identical to those of *Chr. puriensis* and the isolates resembled those of the inoculated fungus. There was no evidence of a *Chrysoporthe* sp. on any of the control plants.

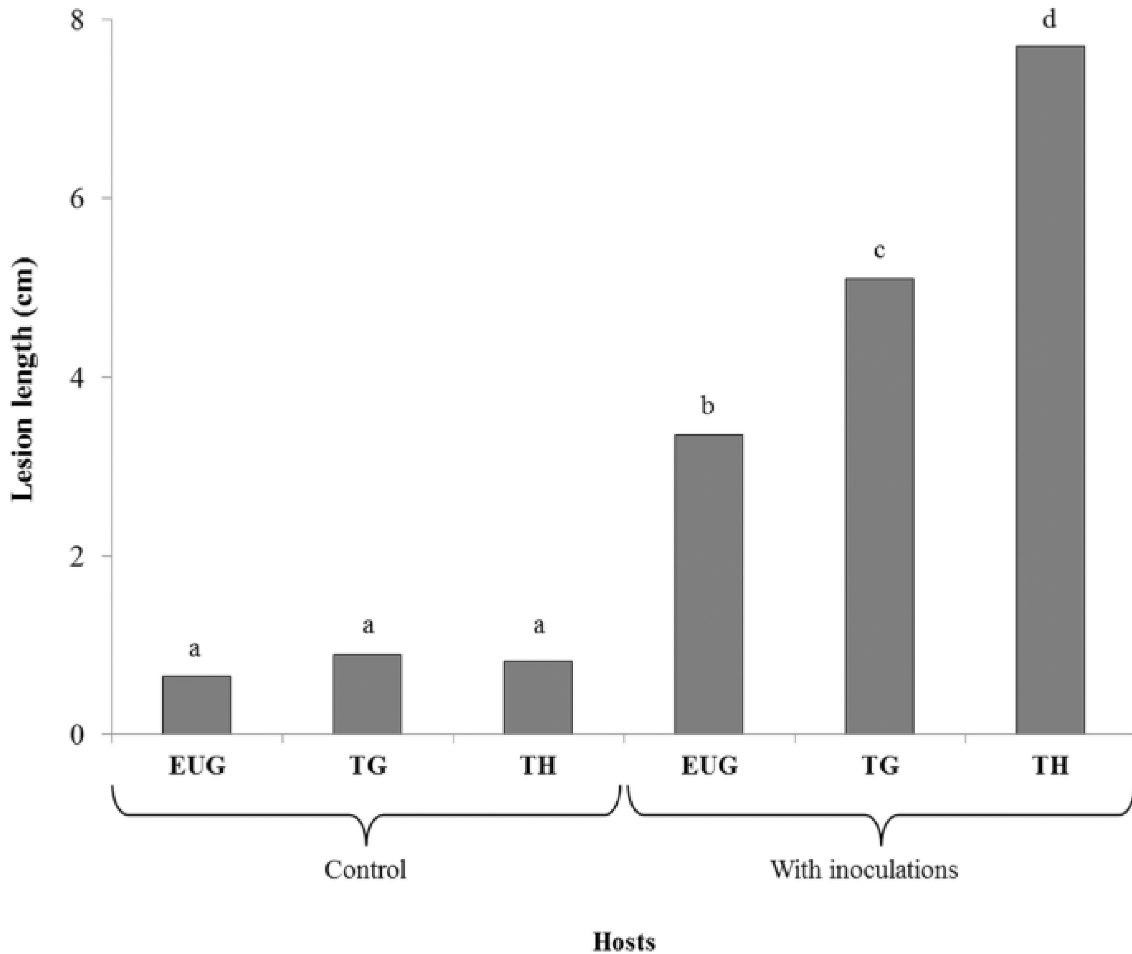

Fig. 4. Mean lesion lengths (cm) associated with *Chrysoporthe puriensis* inoculation on different hosts, 8 weeks after inoculation. Means followed by the different letter were not grouped by the Scott-Knott test ($P \leq 0.05$). EUG = Hybrid of *Eucalyptus grandis* x *E. urophylla,* TG = *Tibouchina granulosa*, TH = *Tibouchina heteromalla*

Discussion

The results of this study revealed a new species of *Chrysoporthe* from *Tibouchina* spp. in Brazil and for which the name *Chr. puriensis* has been provided. *Chrysopothe puriensis* was commonly isolated from numerous native *Tibouchina* spp. where it was associated with severe cankers that appeared to kill trees. Pathogenicity tests on species of this tree confirmed that it can infect plants and give rise to stem cankers.

Chrysoporthe puriensis shares many morphological characteristics with other species of *Chrysoporthe*. However, it has smaller asci and perithecia with *textura epidermoidea* tissue composed of elongated cells, non-parallel hyphae fused together, without inter-hyphal spaces (Kiffer and Morelet 2000), which are not found in other species of this genus. These differences are relatively difficult to distinguish and, as with other species of *Chrysoporthe*, reliance of DNA sequence data is necessary for accurate identification. In this regard, *Chr. puriensis* was most closely related to *Chr. cubensis* but formed a strongly supported branch in

a clade arising from analyses of the ITS-rDNA and β-tubulin sequences, distinct from the latter species.

Pathogenicity tests with *Chr. puriensis* showed that the fungus can cause disease on the trees from which it was isolated. This confirms that the cankers observed under field conditions were caused by the fungus. The fact that *Chr. puriensis* was able to cause symptoms on a *Eucalyptus* hybrid shows that it can cause disease on these important plantation-grown trees. Similar results have been found for numerous members of the Cryphonectriaceae occurring on native Melatomataceae or Myrtaceae (Hodges et al. 1986; Gryzenhout et al. 2006a, b; Seixas et al. 2004; Barreto et al. 2006; van der Merwe et al. 2010; Chen et al. 2010). Some of these species and most notably *Chr. cubensis*, *Chr. deuterocubensis* and *Chr. austroafricana*, native in the areas where they occur under natural conditions, have become significant constraints to *Eucalyptus* plantation forestry (Roux et al. 2003; Gryzenhout et al. 2004; Gryzenhout et al. 2005; Roux et al. 2005; Gryzenhout et al. 2006a, b; Chungu et al. 2009; van der Merwe et al. 2010).

Evidence resulting from this study suggests that *Chr. puriensis* has the capacity to become a relevant *Eucalyptus* pathogen in Brazil in the future. *Chrysoporthe cubensis* was one of the most important pathogens shaping the *Eucalyptus* forestry in Brazil (Ferreira 1989; Alfenas et al. 2009). Considerable effort has been made to establish *Eucalyptus* planting stock with high levels of tolerance to *Chr. cubensis*. This material might not be equally tolerant to infection by *Chr. puriensis*. We thus argue for *Chr. puriensis* to be considered a threat to *Eucalyptus* forestry in Brazil and that it needs to be included in screening programmes.

An intriguing aspect of this study was the fact that *Chr. puriensis* is apparently responsible for disease and even death of *Tibouchina* spp. in Brazil. This is unusual for an apparently native fungus on native trees. But it is consistent with what has been observed previously in countries of South America such as in Colombia (Gryzenhout et al. 2006a, b). In those cases, disease is seldom found on *Tibouchina* trees in native forests. Yet when these trees are established as ornamentals or as amenity plantings, they often succumb to disease. What appears to occur is that these trees are moved to environments less conducive for their growth, and they subsequently become infected and often die (Soares et al. 2018).

A recent discovery has been the fact that *Chrysoporthe* spp. occurs in healthy tissues of the Melastomataceae including species of *Tibouchina* (Maússe-Sitoe et al. 2016). Consequently, moving asymptomatic and apparently healthy material of these trees to new environments provides a concerning pathway for their accidental introduction to new areas. *Tibouchina* spp. produce attractive flowers and have commonly been moved globally as ornamentals. Likewise, there is at least anecdotal evidence that *Eucalyptus* planting stock has been moved globally as part of an important and growing plantation forestry industry (Burgess and Wingfield 2017). Cuttings of these plants root easily, and it is most likely that they have been moved globally as part of the nursery trade. This would account for the appearance of non-native species of Cryphonectriaceae in new environments such as *Chr. cubensis* in Africa (Myburg et al. 2002, 2003, 2004; Gryzenhout et al. 2004).

The global movement of tree pathogens is of growing concern. This includes those that have moved to natural forest environments and that have resulted in irreparable damage to tree species. Classic examples are those of Dutch elm disease (Brasier 2000; Wingfield et al. 2010) and Chestnut blight (Anagnostakis 2001). Canker pathogens of the Myrtales and important to plantation forestry such as those considered in this study are less well known

(Wingfield et al. 2015; Burgess and Wingfield 2017). But they are easily moved globally, and they have the capacity to cause devastation equivalent to Chestnut blight. In this regard, native Myrtales such as in Australia where these trees are hyperdiverse are threatened. The relatively recent emergence of myrtle rust caused by *Austropuccinia psidii* in Australia (Carnegie and Pegg 2018; Winzer et al. 2019) provides a sobering example. Every possible effort should be made to better understand the host range and diversity of *Chrysoporthe* spp. and to avoid their introduction into new environments.

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