First report of two *Chrysoporthe* species, *Chrysoporthe doradensis* and *Chrysoporthe colombiana* sp. nov. from *Henriettea seemannii* pathogenic to *Eucalyptus* in Colombia

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Abstract

Fungi in the *Cryphonectriaceae* include some of the most serious pathogens of trees globally. Amongst these, species of *Chrysoporthe*, previously identified as *Cryphonectria*, are well known and important pathogens of *Eucalyptus* propagated in plantations for the production of various wood products. This study considered *Cryphonectriaceae* isolates collected from stem cankers on *Eucalyptus* and the native tree, *Henriettea seemannii* (*Melastomataceae*) in Colombia. Isolates were identified based on analyses of DNA sequence data and morphology. These were resolved as *Chrysoporthe cubensis*, *C. doradensis* and a new species described here as *Chrysoporthe colombiana*. All three fungi were found on *H. seemannii*, whereas *C. doradensis* was found on *Eucalyptus*. A pathogenicity test on *Eucalyptus* showed that the three *Chrysoporthe* spp. could cause disease on these trees, with *C. doradensis* being the most aggressive. This study provides another interesting example of tree pathogens in the *Cryphonectriaceae* occurring on native *Melastomataceae* and infecting *Eucalyptus* propagated in plantations.

Keywords: Canker disease; *Cryphonectriaceae; Eucalyptus;* Pathogenicity; Taxonomy; One new taxon

Introduction

The fungal genus *Chrysoporthe* includes several pathogens that cause serious canker diseases of *Eucalyptus* spp. (*Myrtales*), mostly occurring in the tropical and sub-tropical areas of the world (Wingfield 2003; Gryzenhout et al. 2009). *Chrysoporthe* was first established when it was recognized that the important canker pathogen of *Eucalyptus*, previously identified as *Cryphonectria cubensis* (Bruner) Hodges, resided in a phylogenetic group very different to the species of *Cryphonectria*, notably *Cryphonectria parasitica* (Murrill) M.E. Barr, the causal agent of the notorious chestnut blight disease (Anagnostakis and Kranz 1987).

Subsequent to their first discovery, numerous species of *Chrysoporthe* and their close relatives have been discovered as pathogens of trees in the *Myrtales*, including members of the

Myrtaceae (Gryzenhout et al. 2004; Chungu et al. 2010; Oliveira et al. 2021) and the *Melastomataceae* and *Combretaceae* (Rodas et al. 2005; Gryzenhout et al. 2006a). Various species of these fungi have also undergone shifts from their native hosts to *Eucalyptus* established in plantations in the tropics and southern hemisphere (Nakabonge et al. 2006; Vermeulen et al. 2013; Gryzenhout et al. 2009; Oliveira et al. 2021).

Species of *Chrysoporthe* have black and globose perithecia, semi-immersed in the bark of infected trees. The ascospores are mostly hyaline and two-celled. Their asexual structures are pycnidia that are usually superficial on the bark, covering cankers and are fuscous-black with attenuated necks. Their conidia are single-celled, hyaline and mostly obovoid. Importantly, as new species of *Chrysoporthe* have been discovered, it has become difficult to identify them based on morphological characteristics. Consequently, identification relies primarily on phylogenetic inference from DNA sequence data, especially of the internal transcribed spacer (ITS) and β-tubulin regions.

Five species of *Chrysoporthe*, *C. cubensis* (Bruner) Gryzenh. & M.J. Wingf.; *C. doradensis* Gryzenh. & M.J. Wingf.; *C. hodgesiana* Gryzenh. & M.J. Wingf. ex Chungu, Gryzenh. & M.J. Wingf.; *C. inopina* Gryzenh. & M.J. Wingf. and *C. puriensis* M.E.S. Oliv., T.P.F. Soar. & M.A.Ferr., have been reported from South America. *Chrysoporthe cubensis* is distributed widely in South American countries, including Brazil, Colombia, Suriname and Venezuela (Gryzenhout et al. 2004; Rodas et al. 2005; Van der Merwe et al. 2013; Soares et al. 2018). This fungus is the best known among these pathogens due to the serious damage that it can cause to *Eucalyptus* spp. established in plantations (Hodges et al. 1979; Wingfield 2003; Soares et al. 2018). *Chrysoporthe doradensis* was first found on *Eucalyptus* trees in plantations in Ecuador (Gryzenhout et al. 2005) and has more recently been found in Brazil (Soares et al. 2018). This fungus is also considered a serious pathogen in South America, having high levels of aggressiveness on *Eucalyptus* spp. (Gryzenhout et al. 2005; Soares et al. 2018). *Chrysoporthe puriensis*, the most recently described species, is known only in Brazil, where it causes a serious canker disease on native *Tibouchina* and on *Eucalyptus* trees in plantations (Oliveira et al. 2021). The remaining two species, *C. hodgesiana* and *C. inopina*, are known only on native species of *Melastomataceae* in Colombia (Rodas et al. 2005; Gryzenhout et al. 2006b) and have never been found on *Eucalyptus* trees under natural conditions. However, in greenhouse pathogenicity tests, *C. hodgesiana* has been shown to have high levels of aggressiveness on *E. grandis* (Wingfield et al. 2001).

Three *Chrysoporthe* spp., *C. cubensis*, *C. hodgesiana* and *C. inopina*, are known in Colombia (Rodas et al. 2005; Gryzenhout et al. 2006b). These fungi have been found on native trees such as *Miconia* spp. and *Tibouchina* spp. (*Melastomataceae*), and *C. cubensis* has evidently moved from these trees through a host shift to infect *Eucalyptus* spp. established in plantations (Slippers et al. 2005; Rodas et al. 2005; Gryzenhout et al. 2006b). During a recent survey of *Chrysoporthe* canker occurring on *Eucalyptus* in the Medellin area of Colombia, structures similar to those in the *Cryphonectriaceae* were found associated with cankers on the stems of *Eucalyptus grandis* W. Hill trees and on the native Colombian tree *Henriettea seemannii* (Naudin) L.O. Williams (*Melastomataceae*). The aims of the study were to identify the resulting isolates and to test their pathogenicity on *Eucalyptus grandis*.

Material and methods

Sampling and isolation

Eucalyptus grandis trees with stem cankers were commonly encountered in plantations on the farm Sonadora near Vegachi, Antioquia, Colombia, which is at 1471 masl and located at 6º 16′ 02″ N, 75º 35′13″W, having an average annual precipitation of 2050 mm/year. Cankers were also occasionally observed on the stems of native *H. seemannii* trees in the same area. Bark pieces bearing fungal structures and associated with cankers on the stems of both *H. seemannii* and *E. grandis* were stripped from the stems, placed in paper bags and transferred to a laboratory for microscopic examination. Fruiting structures on six bark pieces of *H. seemannii* and one of *E. grandis* were dissected to expose spore masses using a scalpel. The spore masses from individual fruiting structures were spread onto the surface of 2% malt extract agar (MEA; 20 g Difco Bacto™ agar, 20 g Biolab malt extract, 1 L deionized water) supplemented with streptomycin. Agar blocks containing spores were then transferred to clean MEA plates using a sterilized hypodermic needle. Cultures were incubated at room temperature for 7 days to allow for growth and further study.

Phylogenetic analysis

Seven isolates (six from *H. seemannii* and one from *E. grandis*) were subjected to phylogenetic analyses (Table 1). Polymerase chain reactions (PCRs) were performed on DNA to amplify the ITS region and two regions within the β-tubulin gene (*TUB1* and *TUB2*) using the primer pairs ITS1/ITS4, Bt1a/Bt1b and Bt2a/Bt2b (White et al. 1990; Glass and Donaldson 1995). PCR reactions followed the methods of Myburg et al. (2002a). PCR products were purified using ExoSAP-IT (Affymetrix). The sequencing reactions were conducted with both the forward and reverse primers using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit v. 3.1 (Applied BioSystems). An ABI PRISM 3100 Autosequencer (Applied BioSystems) was used to generate the sequences. The resulting raw sequences were assembled, and combined datasets of the ITS region; the two regions of the β-tubulin gene were created using MEGA7 (Kumar et al. 2016). Sequences from two isolates of *Amphilogia gyrosa* (Berk. & Broome) Gryzenh., H.F Glen & M.J. Wingf. and eight species of *Chrysoporthe* obtained from GenBank (http://www.ncbi.nlm.nih.gov) were added to datasets (Table 1). Sequences were aligned using MAFFT 7 with the G-INS-I option (http://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019). Substitution models were selected for ITS, as well as both separate and combined datasets for β-tubulin and a combined dataset with the Akaike information criterion (AIC) in jModeltest 2.1.5 (Posada 2008). Sequence alignments were deposited in figshare (https://doi.org/10.6084/m9.figshare.21936807).

Maximum likelihood (ML) analysis was carried out using PhyML 3.1 (Guindon and Gascuel 2003). Confidence levels for nodes were estimated using 1000 replicate bootstrap analyses. Bayesian inference (BI) analyses were performed in MrBayes v.3.2.1 (Ronquist et al. 2012) to estimate the posterior probabilities of tree topologies with Metropolis-coupled Markov Chain Monte Carlo searches. The analyses were performed for 3,000,000 generations with four runs, each consisting of four chains. Trees were sampled every 100 generations, and a 25% burn-in was used to summarize a consensus tree.

Table 1 List of isolates used in this study

Species	Isolate[a]	Location	Host	GenBank accession numbers[b]			References
				ITS	*TUB1*	*TUB2*	
Chrysoporthe austroafricana	CMW2113	South Africa	*Eucalyptus grandis*	AF046892	AF273067	AF273462	Myburg et al. (2002a)
C. austroafricana	CMW9327	South Africa	*Tibouchina granulosa*	AF273473	AF273060	AF273455	Myburg et al. (2002b)
C. cubensis	CMW10026	Colombia	*Miconia rubiginosa*	AY214294	AY214222	AY214258	Rodas et al. (2005)
C. cubensis	CMW14394	Cuba	*Eu. grandis*	DQ368773	AH015642	AH015642	Gryzenhout et al. (2006b)
C. cubensis[c]	CMW55971	Colombia	*Henriettea seemannii*	**OQ298820**	**OQ336265**	**OQ336265**	
C. cubensis[c]	CMW56012	Colombia	*H. seemannii*	**OQ298821**	**OQ336266**	**OQ336266**	
C. deuterocubensis	CMW2631	Australia	*Eu. marginata*	GQ290157	GQ290184	AF543825	Van der Merwe et al. (2010)
C. deuterocubensis	CMW8650	Indonesia	*Syzygium aromaticum*	AY084001	AY084024	GQ290193	Van der Merwe et al. (2010)
C. doradensis	CMW9124	Ecuador	*Eu. deglupta*	DQ224035	DQ224040	DQ224041	Gryzenhout et al. (2005)
C. doradensis	CMW11286	Ecuador	*Eu. deglupta*	JN942331	AY214218	AY214254	Gryzenhout et al. (2005)
C. doradensis[c]	CMW55974	Colombia	*H. seemannii*	**OQ298822**	**OQ336267**	**OQ336267**	
C. doradensis[c]	CMW55975	Colombia	*Eucalyptus* sp.	**OQ298823**	**OQ336268**	**OQ336268**	
C. hodgesiana	CMW10461	Colombia	*T. semidecandra*	AY692322	AY692326	AY692325	Gryzenhout et al. (2005)
C. hodgesiana	CMW10625	Colombia	*M. theaezans*	AY956970	AH014900	AH014900	Rodas et al. (2005)
C. inopina	CMW12727	Colombia	*T. lepidota*	DQ368777	AH015657	AH015657	Gruzenhout et al. (2006b)
C. inopina	CMW12729	Colombia	*T. lepidota*	DQ368778	DQ368808	DQ368809	Gruzenhout et al. (2006b)
C. syzygiicola	CMW29940	Zambia	*S. guinense*	FJ655005	FJ805230	FJ805236	Chungu et al. (2010)
C. syzygiicola	CMW29941	Zambia	*S. guinense*	FJ655006	FJ805231	FJ805237	Chungu et al. (2010)
C. zambiensis	CMW29928	Zambia	*Eu. grandis*	FJ655002	FJ858709	FJ805233	Chungu et al. (2010)
C. zambiensis	CMW29930	Zambia	*Eu. grandis*	FJ655004	FJ858711	FJ805235	Chungu et al. (2010)
C. colombiana[c]	CMW55973	Colombia	*H. seemannii*	**OQ298824**	**OQ336269**	**OQ336269**	
C. colombiana	CMW56173	Colombia	*H. seemannii*	**OQ298825**	**OQ336270**	**OQ336270**	
C. colombiana[c]	CMW56176	Colombia	*H. seemannii*	**OQ298826**	**OQ336271**	**OQ336271**	
Amphilogia gyrosa	CMW10469	New Zealand	*Elaeocarpus dentatus*	AF452111	AF525707	AF525714	Myburg et al. (2004)
A. gyrosa	CMW10470	New Zealand	*El. dentatus*	AF452112	AF525708	AF525715	Myburg et al. (2004)

Sequences newly generated in this study are in bold

[a]CMW: the culture collection of the Forestry & Agricultural Biotechnology Institution (FABI), University of Pretoria, Pretoria

[b]ITS: internal transcribed spacers and intervening 5.8S nrDNA; *TUB1* and *TUB2*: partial β-tubulin gene

[c]Isolates used in pathogenicity tests

Pathogenicity tests

Six isolates (CMW 55971, CMW 56012, CMW 55974, CMW 55975, CMW 55973 and CMW 56176) were screened for pathogenicity in a phytotron on 9-month-old plants of a single *Eucalyptus grandis* clone. The plants were grown in 2-L plastic bags and maintained in the phytotron under natural light at 25 °C. Ten trees were inoculated. An equal number of plants

were inoculated with discs of water agar (WA; 20 g Difco Bacto™ agar, 1 L deionized water) to serve as a negative control.

To inoculate plants, a disc of bark was removed from the stems of plants approximately 30 cm above ground level using a cork borer (5 mm diam) to expose the cambium. Agar discs of the same size were taken from the edges of actively growing cultures and placed into the wounds with the mycelium facing the cambium. The inoculation points were then covered with parafilm to reduce desiccation and contamination of the inoculum. Lesion development was evaluated after 6 weeks by scraping away the bark associated with the inoculation points to expose the cambium allowing for lesion lengths to be measured. Small pieces of discoloured cambium were then removed from the leading edges of the lesions and transferred to MEA plates to determine that the lesions had been caused by the inoculated fungus.

All statistical analyses on the lesion length data were performed using R (R Core Team 2019). A Grubbs test was used to detect outliers with the "outliers" package (Shiffler 1988). Normal distribution of the data and homogeneity of variance were tested using Shapiro–Wilk tests and a Bartlett test. The data were then subjected to one-way ANOVA followed by a Games-Howell post hoc test at a 5% significance level with the "PMCMRplus" package (Pohlert 2020).

Morphological characteristics

Fruiting structures on infected bark samples were located using a dissection microscope and removed for observation. These were placed on microscope slides in water that was later replaced with 85% lactic acid. Measurements were made, and images were captured from preparations in lactic acid. To study the configuration of fruiting structures in the host substrate, pieces of bark containing a fruiting structure were boiled for a few seconds in water to soften the tissue. The pieces were then mounted in a freezing medium and sectioned in 10–12 µm thickness using a Leica CM1100 cryostat microtome (Germany). Up to 50 measurements were made for each morphologically informative character, depending on their availability. Measurements are presented as minimum–maximum (mean ± standard deviation).

Culture characteristics and growth rate of two isolates (CMW 55973 and CMW 56173) were determined to represent the novel taxon and were studied on 90-mm Petri dishes containing 2% MEA. A mycelial disc (5 mm diam.) was taken from the actively growing edges of cultures and placed at the centre of 90-mm Petri dishes. Five replicates were prepared for each isolate and temperature. The cultures were kept in the dark at six temperatures ranging from 10 to 35 °C at 5 °C intervals. The experiment was repeated once. The growth of colonies was marked daily and terminated when the fastest-growing colony reached the edge of the plate. Two measurements of colony diameter perpendicular to each other were made for each plate, and the average daily growth rate at each temperature was calculated. The colour was determined using Rayner's colour chart (Rayner 1970).

Results

Isolations and isolates

Seven isolates were recovered from cankers on *E. grandis* and *H. seemannii* in this study. One was from the former and six were from the latter tree species. All seven isolates are preserved in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Isolates representing the holotype and paratypes of the novel species are maintained in the culture collection of Innovation Africa (CWM-IA), University of Pretoria; the live culture collection of South Africa (PPRI), Roodeplaat, Pretoria, and dried type specimens have been deposited in the H.G.W.J. Scwiekert Herbarium (PRU), University of Pretoria, South Africa.

Phylogenetic analyses

Sequence datasets consisted of 29 taxa, and the aligned sequence including 492 bp for the ITS region, 778 bp for the two regions of the β-tubulin gene and 1 270 bp for the combined dataset. A summary of important parameters applied in the phylogenetic analyses is presented in Supplementary Table 1.

The seven isolates sequenced in this study resided in three distinct clades in the phylogenetic trees based on the *TUB* regions and the combined dataset (Fig. 1B, C). Isolates CMW 55973, CMW 56173 and CMW 56176 from *H. seemannii* grouped with sequences of *Chrysoporthe* but resided in a distinct clade separated from all other *Chrysoporthe* species. The clade was supported by a bootstrap value of 79 to 96% and BI of 0.97 to 1.0. These isolates are thus recognized as representing a new species of *Chrysoporthe*, which is described below.

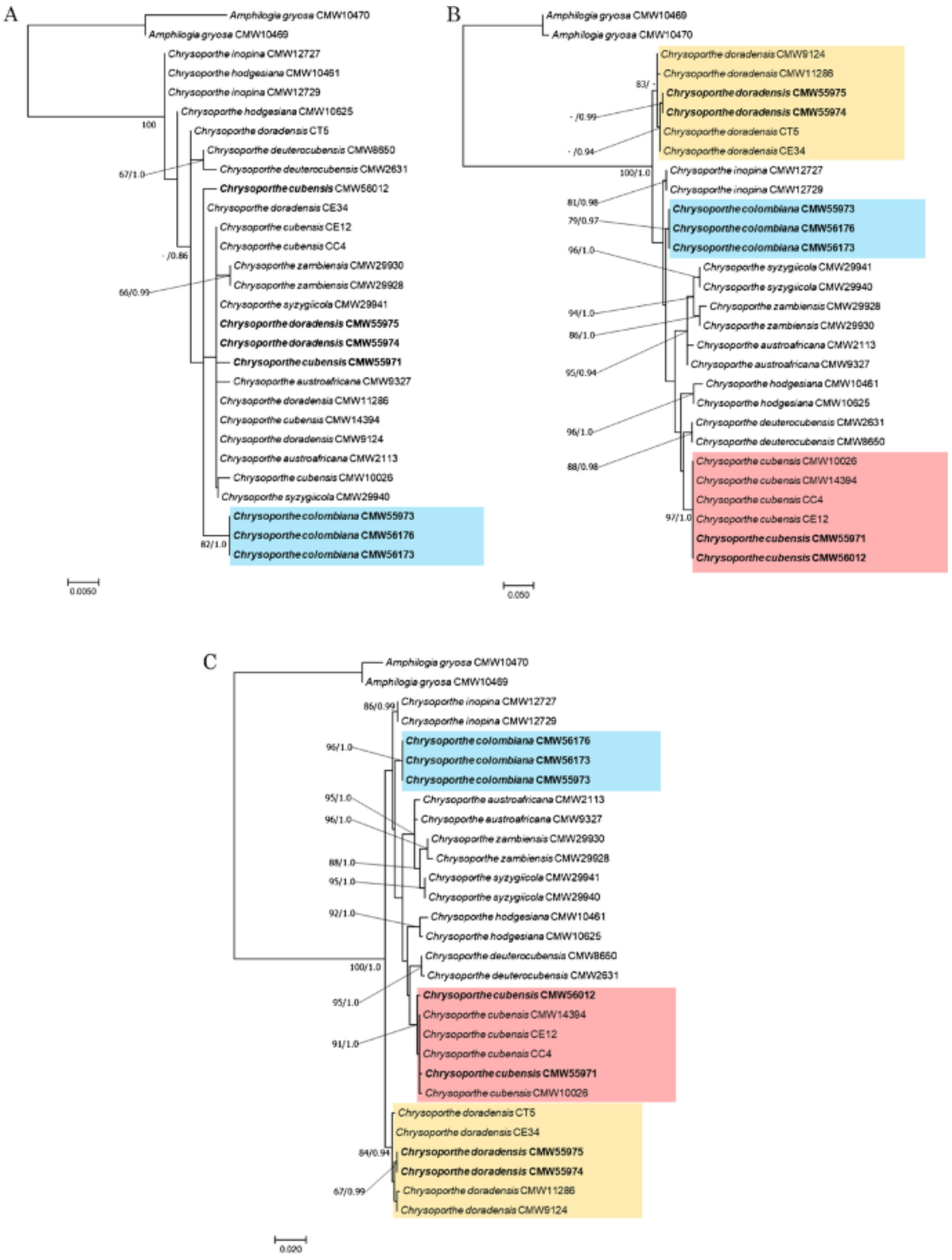

Fig. 1. Phylogenetic tree based on ML analysis of a sequence dataset of ITS (**A**), two regions of β-tubulin gene (**B**) and combined dataset (**C**) of *Chrysoporthe* species. Bootstrap values > 65% for ML and > 0.85 for BI PP are shown as ML/BI PP. The sequences represented in bold were obtained in this study. Clades highlighted in blue, yellow and red indicate those of *C. colombiana*, *C. doradensis*, and *C. cubensis*

Isolates CMW 55974 and CMW 55975, one of which was from *E. grandis* and the other from *H. seemannii*, resided in a clade accommodating *C. doradensis* (Fig. 1B, C). Isolates CMW 55971 and CMW 56012, both of which were from *H. seemannii*, grouped with sequences of *C. cubensis* (Fig. 1B, C).

Taxonomy

***Chrysoporthe colombiana* H. Suzuki, Marinc. & M.J. Wingf.**, sp. nov. Fig. 2.

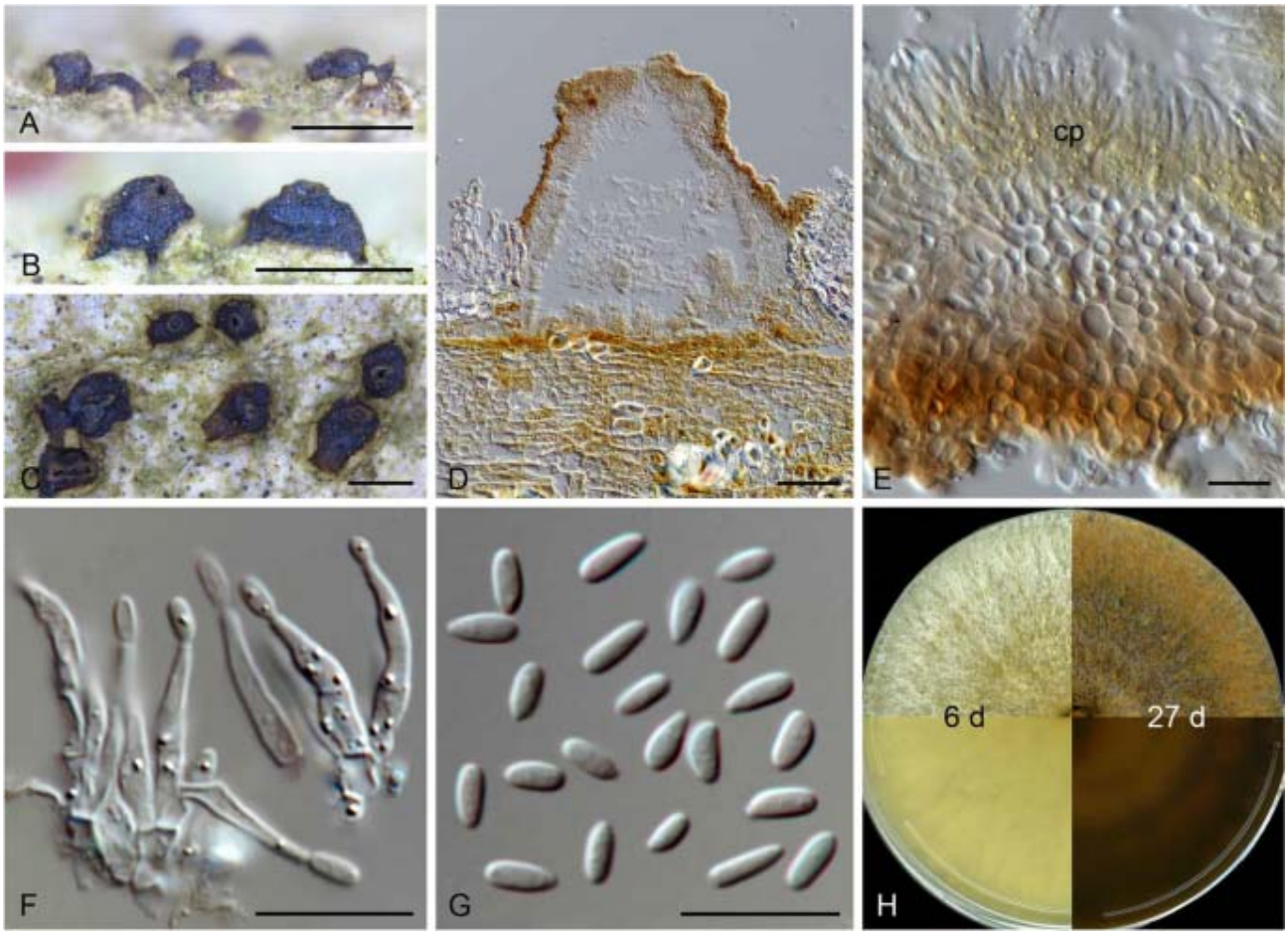

Fig. 2. Micrographs of *Chrysoporthe colombiana* (ex-holotype CMW-IA 157, CMW 55973, holotype PRU(M) 4513). **A**–**C**. Conidiomata on natural substrate. Note that the blue hue in the black structures (**B**) is due to reflected light. **D** Vertical section of semi-immersed conidioma. **E**. Conidiomatal wall consisting of pseudoparenchymatous cells of textura globulosa and conidiophores lining inner locular cells (cp: conidiophores). **F** Conidiophores and conidiogenous cells. **G** Conidia. **H**. Culture grown on 2% MEA in the dark for 6 day and 27 day. Scale bars: **A** 1 mm; **B**, **C** 500 µm; **D** 100 µm; **E**–**G** 10 µm

MycoBank MB847229.

Etymology: Name refers to the country Colombia where this fungus was found and where it is known only on the native tree, *Henrietta seemannii*.

Diagnosis: Differs most obviously from *C. inopina* in having an optimum growth temperature of 30 °C and from *C. doradensis* and *C. inopina* in not having a distinctly attenuated neck.

Type: COLOMBIA: Antioquia Department, Vegachi municipality. Bark of *Henriettea seemannii*. December 2019 *C. A. Rodas*. Holotype PRU(M) 4513; ex-holotype culture CMW-IA 157, PPRI 31291, CMW 55973. GenBank: OQ298824 (ITS); OQ336269 (beta-tubulin).

Description: Sexual morph not observed. Asexual morph on substrate. *Conidiomata* stromatic, immersed, erumpent, conical, globose to pulvinate, ostiolate, 251–488 µm (407.5 ± 80.47) high, 297–616 µm (419.9 ± 105.99) wide, without necks; uniloculate, *locules* even to convoluted; *walls* pseudoparenchymatous, textura globulosa to angularis, 11–63 µm (37.5 ± 12.19) thick, consisting of two tiers, outer tier composed of a few layers of thick-walled, brown cells, inner tier of many layers of sub-hyaline, thin-walled cells, innermost cells giving rise to conidiophores. *Paraphyses* not observed. *Conidiophores* borne on inner cells of locule, consisting of basal cell, supporting structures and conidiogenous cell, supporting structures rarely branched, septate. *Conidiogenous cells* phialidic, lageniform with attenuated apex, 5–15 × 1–3 (9.2 ± 1.93 × 1.9 ± 0.32) µm. *Conidia* hyaline, cylindrical with obtuse apex and pointed base, aseptate, 3–6 × 1–2 µm (4.2 ± 0.56 × 1.7 ± 0.15).

Culture characteristics on 2% MEA in the dark at 15–30 °C after 27 days uniform at all temperatures, showing circular growth with smooth edge, colony elevation flat, texture hairy, medium dense mycelia. Colour varies at each temperature, at 30 °C umber with orange aerial hyphae, at 25 °C fulvous to rust, at 20 °C ochraceous to umber with orange aerial hyphae, at 15 °C pale luteous to luteous with orange tint and showing concentric zonation growth. Optimal growth temperature at 30 °C showing growth of 6.8 mm/day, followed by 25 °C (4.6 mm/day), 20 °C (1.9 mm/day), 35 °C (0.5 mm/day) and 15 °C (0.4 mm/day). No growth was at 10 °C.

Host: *Henriettea seemannii.*

Distribution: Colombia.

Other materials examined: COLOMBIA: Antioquia Department, Vegachi municipality. Bark of *Henriettea seemannii*. December 2019 *C. A. Rodas*. Paratype PRU(M) 4514, ex-paratype culture CMW-IA 158, PPRI 31292, CMW 56173. GenBank: OQ298825 (ITS); OQ336270 (beta-tubulin). Paratype PRU(M) 4515, ex-paratype culture CMW-IA 159, PPRI 31293, CMW 56176. GenBank: OQ298826 (ITS); OQ336271 (beta-tubulin).

Notes: *Chrysoporthe colombiana* is accommodated in a clade clearly separate from all other species in the genus based on phylogenetic analyses of DNA sequence data. Attenuated necks commonly found in the conidiomata of other *Chrysoporthe* spp. were not observed in this new species. *Chrysoporthe colombiana* differs from its closest relatives, C. *inopina* and *C. doradensis*, in its optimal growth temperature (*C. colombiana* and *C. doradensis* at 30 °C, and *C. inopina* at 25 °C), and in its conidial dimensions (*C. colombiana*: 3–6 × 1–2 µm, *C. doradensis*: 3–6.5 × 1.5–2.5 µm and *C. inopina*: 3–4 × 1.5–2.5 µm).

Pathogenicity tests

All inoculated *E. grandis* clonal plants had well-developed lesions after 6 weeks. In contrast, only small wound-associated discolouration was found in the control inoculations (Fig. 3). The lesion length for each isolate showed a normal distribution but with a heterogeneity of variance

($K^2_{(6)} = 74.9$, $P < 0.001$). A significant difference in the lesion lengths among the treatment was seen in ANOVA tests ($F_{(6, 23.6)} = 49.2$, $P < 0.001$). The isolate identified as *C. doradensis* from *Eucalyptus* sp. (CMW 55975) had the highest level of pathogenicity on *E. grandis* plants having a mean lesion length of 74.7 ± 21.5 mm (mean ± SD). The isolate of *C. doradensis* from *H. seemannii* (CMW 55974) developed the second longest lesions (52.9 ± 21.5 mm) after one outlier value (= 192 mm) was excluded from the analyses. According to the Games-Howell post hoc test, the lesion lengths associated with the *C. doradensis* isolates were significantly longer than those of the other isolates (Fig. 4). The *C. cubensis* isolates (CMW 55971 and CMW 56012) resulted in lesions that were 32.8 ± 18.1 and 42.1 ± 16.1 mm long. Isolates of *C. colombiana* (CMW 55973 and CMW 56173) caused lesion lengths of 25.9 ± 8.9 and 25.1 ± 5.9 mm.

Fig. 3. Lesions developed in the xylem of *Eucalyptus* clone (ZG14) in 6 weeks. **A** Control. **B** *C. colombiana* (CMW 55973). **C** *C. colombiana* (CMW 56176). **D** *C. cubensis* (CMW 55971). **E** *C. cubensis* (CMW 56012), **F** *C. doradensis* (CMW 55974), **G** *C. doradensis* (CMW 55975)

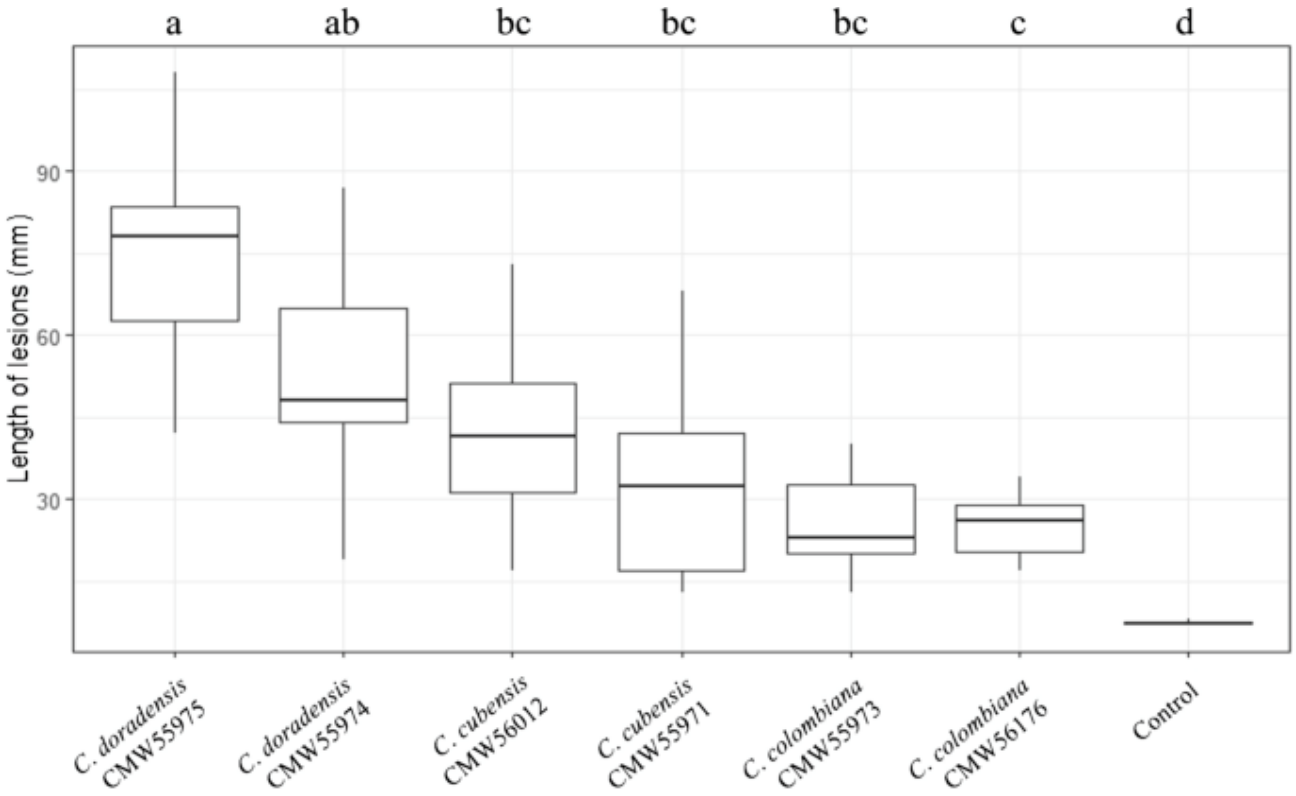

Fig. 4. Lesion length (mm) of *Chrysoporthe doradensis* (CMW 55975 and CMW 55974), *C. cubensis* (CMW 56012 and CMW 55971), and *C. colombiana* (CMW 55973 and CMW 56176) from Colombia on *Eucalyptus* clone (ZG14). Different letters indicate significant differences in pathogenicity based on the Games-Howell post hoc test ($P < 0.05$)

Discussion

A new species of *Chrysoporthe* associated with cankers on *H. seemannii* was discovered and described in this study. The fungus was also shown to be pathogenic to *Eucalyptus*, similar to numerous other *Chrysoporthe* spp. that have been found on native *Myrtales*, including the *Melastomataceae* in previous studies (Myburg et al. 2002a; Chungu et al. 2010; Van der Merwe et al. 2013; Oliveira et al. 2021). Another interesting outcome of this study was the discovery of *C. doradensis* in Colombia for the first time. This fungus, known in other South American countries such as Ecuador and Brazil (Gryzenhout et al. 2005; Soares et al. 2018), was found on cankers collected on both *H. seemannii* and *E. grandis*. Inoculation trials showed that these isolates of *C. doradensis* were pathogenic on *E. grandis*.

Including *C. colombiana*, there are now seven species of *Chrysoporthe* that have been found on native *Melastomataceae*. These include *C. inopina*, which has been found on *Tibouchina lepidota* Baill. in Colombia, *C. cubensis* found on *Miconia* spp. and *Tibouchina* spp. in various countries of South America, *C. deuterocubensis* Gryzenh. & M.J. Wingf. found on *Melastoma* spp. and *Tibouchina urvilleana* Cogn. in various countries of South East Asia, *C. hodgesiana* found on *Tibouchina* spp. and *Miconia theaezans* (Bonpl.) Cogn. in Colombia and *C. doradensis* and *C. puriensis* found on *Tibouchina* spp. in Brazil. With some exceptions, the pathogenicity of these fungi to their native hosts, as well as *Eucalyptus*, has been tested, providing strong evidence that they have undergone host shifts from native to non-native trees. In some cases, species such as *C. austroafricana*, *C. cubensis* and *C. deuterocubensis* have

emerged as important pathogens in *Eucalyptus* plantations and have become a significant constraint to plantation forestry (Gryzenhout et al. 2009; Rauf et al. 2019; Oliveira et al. 2021). It thus seems likely that other species will also emerge as pathogens of *Eucalyptus* in the future.

Chrysoporthe was first recognized as distinct from *Cryphonectria* when the analysis of DNA sequence data showed that isolates previously treated as the well-known *Eucalyptus* pathogen in South Africa were different to those of this species from Brazil (Myburg et al. 2002a). An important subsequent study (Gryzenhout et al. 2004) showed that these fungi, then treated as *Chrysoporthe cubensis* and *Chrysoporthe austroafricana*, occurred on native trees in the *Melastomataceae* and *Myrtaceae* respectively, in Colombia (Rodas et al. 2005) and South Africa (Vermeulen et al. 2013). More recent studies have resulted in the discovery of more species of *Chrysoporthe* and other genera in the *Cryphonectriaceae* and, in various cases, causing diseases on trees in the *Myrtales*, and specifically in the *Myrtaceae*, *Combretaceae* and the *Melastomataceae*. In this regard, the discovery of a new species of *Chrysporthe* on native *Melastomataceae* in Colombia is not unusual. And it seems likely that other species in this genus or its close relatives will be found as pathogens of the *Melastomataceae* in the future.

The discovery of *C. doradensis* on both *Eucalyptus* and *H. seemannii* was surprising. This is given that considerable research has been conducted on diseases of *Eucalyptus* in Colombia during the course of the last 20 years (Rodas and Wingfield 2020). *Chrysoporthe doradensis* was first discovered causing cankers on *Eucalyptus* in Ecuador (Gryzenhout et al. 2005), and it was subsequently found on native *T. granulosa* in Brazil (Soares et al. 2018). The fact that this fungus has emerged as a pathogen of *Eucalyptus* in Colombia suggests that it is relatively widespread in tropical countries of South America. Like *C. cubensis*, it could emerge as an important disease agent in *Eucalyptus* plantations in the future. Pathogenicity tests should be conducted to test this hypothesis.

Chrysoporthe colombiana was found only on *H. seemannii* stem cankers and has as yet not been found on *Eucalyptus*. Pathogenicity to *Eucalyptus* was based only on artificial inoculations under controlled conditions seeking to test its potential threat to these trees. Given the limited occurrence of *H. seemannii* as a native species in natural forests, it was not possible to test the pathogenicity of the new species on what we assume to be its native host. However, other species of *Chrysoporthe* occurring on native *Melastomataceae* in Colombia have been shown to be pathogenic to these plants, for example, *Miconia* spp. and *Tibouchina* spp. (Rodas et al. 2005). We thus feel confident that *C. colombiana*, fruiting on the surface of the cankers on *H. seemannii* trees, was the cause of the disease.

This study was based on a relatively small collection of specimens from cankers on *Eucalyptus* and *H. seemannii*. It was thus interesting that three different species of *Chrysoporthe* were found on *H. seemannii*. This result is somewhat confusing as it raises the question of the relative importance of these three fungi on this native Colombian tree. Finding diseases on these trees in natural forests would likely be difficult, especially as the associated fungi would be under ecological homeostasis and not common. An alternative option could be to attempt inducing endophytic *Chrysoporthe* species from healthy trees to produce fruiting bodies using the method described by Mausse-Sitoe et al. (2016) and Granados et al. (2019).

There are increasing numbers of species in the *Cryphonectriaceae* emerging as pathogens of *Eucalyptus*, where these trees are grown as non-natives in plantations. These host shifts seem likely to threaten the future of *Eucalyptus* plantation forestry in the future (Burgess and Wingfield 2017). Perhaps of greater concern is that these are effectively new diseases of

Eucalyptus, unknown where these trees are native. It is probably only a matter of time before some of these pathogens accidentally find their way to the areas of origin of *Eucalyptus*, including Australia and various Indonesian islands. As has been seen with the notorious chestnut blight caused by *Cry. parasitica*, these can be aggressive novel pathogens if they become invasive in areas native to their naïve hosts. A similar situation has emerged with the myrtle rust pathogen *Austropuccinia psidii*, native to South America and accidentally introduced into Australia relatively recently (Carnegie et al. 2016). Every effort should thus be made to better understand the *Cryphonectriaceae* on the *Myrtales* and to avoid their movement to new areas of the world.

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Contributions

All the authors contributed to the study's conception and design. The first draft of the manuscript was written by H. Suzuki, and all the authors commented on the draft. All the authors read and approved the final manuscript.

Ethics approval

Not applicable.

Consent for publication

The authors consent to the publication of the article.

Competing interests

The authors declare no competing interests.

Data availability

The datasets generated during and/or analyses during the current study are available from the corresponding author upon reasonable request.

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