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A new genus of Cryphonectriaceae isolated from *Lagerstroemia speciosa* in southern China

S. F. Chen[ab]*, Q. L. Liu[a], G. Q. Li[a], M. J. Wingfield[b] and J. Roux[c]

[a]*China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), ZhanJiang 524022, GuangDong Province, China;* [b]*Department of Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028; and* [c]*Department of Plant and Soil Sciences, FABI, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa*

Numerous species in the Cryphonectriaceae have been recorded on the Myrtales and many of these are economically important pathogens of *Eucalyptus*. Some species have also recently been shown to be endophytes of native Myrtaceae and to have undergone host jumps to infect *Eucalyptus* species established as exotics in plantations. Recent surveys in the GuangDong and HaiNan Provinces of South China reveal the presence of a species of Cryphonectriaceae associated with cankers on trees of *Lagerstroemia speciosa* (Lythraceae, Myrtales). Fungal structures were observed on the surface of dead bark covering cankers and on branch stubs. Multigene phylogenetic analyses were conducted based on DNA sequence comparisons of the partial *LSU* gene, ITS region of the nuclear ribosomal RNA gene and two regions of the β-tubulin (*BT*) gene. The results revealed the presence of a previously undescribed genus and species in the Cryphonectriaceae. The fungus is described here as *Chrysomorbus lagerstroemiae* gen. et sp. nov. Inoculation tests showed that it is an aggressive pathogen on *L. speciosa* and that it can also infect *Eucalyptus*.

Keywords: Lythraceae, Myrtaceae, Myrtales, pathogenicity, tree pathogens

Introduction

The Cryphonectriaceae (Diaporthales) was described by Gryzenhout *et al.* (2006a) and includes numerous important tree pathogens of global significance. The best known of these is *Cryphonectria parasitica*, the causal agent of the notorious chestnut blight that has devastated *Castanea* spp. (Fagaceae, Fagales) in Europe and North America (Anagnostakis, 1987). Several species of *Chrysoporthe* (previously *Cryphonectria*) are economically important pathogens of *Eucalyptus* (Myrtaceae, Myrtales) species where these trees are grown as non-natives in plantations in Africa (Wingfield *et al.*, 1989), South America (Hodges *et al.*, 1976) and southeastern Asia (Old *et al.*, 2003).

Host plants of the Cryphonectriaceae include more than 100 tree species in over 14 families (Gryzenhout *et al.*, 2009). In recent years, species infecting *Eucalyptus* trees and other hosts in the Myrtales have received attention because of the economic importance of these trees globally (Gryzenhout *et al.*, 2009). It has been shown that species of *Chrysoporthe* have undergone host shifts (Slippers *et al.*, 2005; Wingfield *et al.*, 2015) to infect multiple tree/plant species within the Myrtales (Rodas *et al.*, 2005; Heath *et al.*, 2006; van der Merwe *et al.*, 2013). *Chrysoporthe austroafricana* has been shown to be a native African fungus (Heath *et al.*, 2006; Vermeulen *et al.*, 2013a), occurring in the absence of disease on native African Myrtaceae (Mausse-Sitoe *et al.*, 2016). However, it has expanded its host range to infect non-native *Eucalyptus* species (Myrtaceae) and *Tibouchina* species (Melastomataceae) in southern African countries, causing disease and death of these trees (Myburg *et al.*, 2002a; Heath *et al.*, 2006). *Chrysoporthe cubensis* is native on plants in the Melastomataceae in South American countries but has spread to cause diseases on *Eucalyptus* trees (Rodas *et al.*, 2005; van der Merwe *et al.*, 2013).

Twenty-two genera have been identified and described in the Cryphonectriaceae (Cheewangkoon *et al.*, 2009; Gryzenhout *et al.*, 2009, 2010; Begoude *et al.*, 2010; Vermeulen *et al.*, 2011, 2013b; Crous *et al.*, 2012a,b, 2015; Chen *et al.*, 2013a,b, 2016; Crane & Burgess, 2013; Beier *et al.*, 2015). Four of these have been reported from China including *Celoporthe*, *Chrysoporthe*, *Corticimorbus* and *Cryphonectria*, which occur on cankers of trees such as *Castanea mollissima*, *Quercus* sp. (Fagales, Fagaceae), as well as *Eucalyptus* species, *Rhodomyrtus tomentosa* and *Syzygium cumini* (Myrtales, Myrtaceae) in China (Fairchild, 1913; Myburg *et al.*, 2004b; Chen *et al.*, 2010, 2011, 2013a,b, 2016). Inoculation tests have also shown that species isolated from Myrtaceae are pathogenic to *Eucalyptus* (Chen *et al.*, 2010, 2011, 2016) and they threaten industries based on these trees (Burgess & Wingfield, 2017).

At least two species in the Cryphonectriaceae have been reported from the Lythraceae (Myrtales). These are

*E-mail: shuaifei.chen@gmail.com

C. cubensis from *Lagerstroemia indica* in Cuba (Gryzenhout *et al.*, 2006b) and *Latruncellus aurorae* from *Galpinia transvaalica* in Swaziland (Vermeulen *et al.*, 2011). *Lagerstroemia* is a genus of approximately 80 species of deciduous and evergreen trees and shrubs native to the Indian subcontinent, southeastern Asia, northern Australia, and parts of Oceania (Cabrera, 2004). These trees have beautifully coloured flowers and are planted as ornamentals. The two most popular ornamental species are *Lagerstroemia speciosa* and *L. indica*, which are grown as non-natives globally (Pounders *et al.*, 2007). In southern China, *L. speciosa* is commonly grown as an ornamental in parks and gardens, or as a shade tree along the roadsides in FuJian, GuangDong, GuangXi, HaiNan and YunNan Provinces (Zhong *et al.*, 2005).

During the course of tree disease surveys in South China, cankers were observed on the stems of *L. speciosa*. The aims of this study were to identify the fungus isolated from the cankers and to test its pathogenicity on *L. speciosa*. Its potential to impact other trees in the Myrtales was investigated by conducting inoculation studies on a commercially planted *Eucalyptus* clone that forms the backbone of forestry plantations in the region.

Materials and methods

Sample collection and isolations

Lagerstroemia speciosa trees planted as ornamentals in gardens and parks in the GuangDong and HaiNan Provinces of South China were observed with cankers, dead trunks and branches (Fig. 1a,b,d,e). Orange to black fruiting structures characteristic of the Cryphonectriaceae were common on the surface of the dead and dying tissues (Fig. 1c,f). Surveys were conducted in November 2015 and March to April 2016. Dead bark and branch sections were collected in brown paper bags and transported to the laboratory for fungal isolations and further study.

Fruiting structures were cut open horizontally using a sterile scalpel blade under a dissecting microscope and the spore masses were transferred onto 2% malt extract agar (MEA) (20 g malt extract, 20 g agar per litre water) and incubated at 25 °C. Single germ tubes developing from the spores were transferred to 2% MEA to obtain pure cultures. Cultures were deposited in the culture collection of the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), ZhanJiang, China, and representative cultures are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1). Isolates linked to type specimens have been deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1). Original bark and branch specimens bearing fruiting structures connected to representative isolates were deposited in the Collection of Central South Forestry Fungi of China (CSFF), GuangDong Province, China.

DNA extraction, PCR and sequence reactions

Isolates collected from *L. speciosa* trees were identified based on DNA sequence comparisons (Table 1). Isolates were grown on 2% MEA at 25 °C for 1 week and actively growing mycelium for each isolate was scraped from the surface of the medium using sterile scalpel blades and transferred to 1.5 mL Eppendorf tubes for DNA extraction. DNA was extracted using 'method 5' described by van Burik *et al.* (1988). The concentration of resulting DNA was checked using a NanoDrop 2000 spectrometer (Thermo Fisher Scientific Inc.).

Three gene regions including the conserved nuclear large subunit (*LSU*) ribosomal RNA gene, the internal transcribed spacer (ITS) regions including the 5.8S gene of the ribosomal RNA operon, and the β-tubulin gene 1 (*BT1*) and β-tubulin gene 2 (*BT2*) were amplified as described by Chen *et al.* (2011, 2016). Nucleotide sequences were edited using MEGA v. 4 software (Tamura *et al.*, 2007). All sequences obtained in this study were deposited in GenBank (Table 1).

Phylogenetic analysis

To identify the isolates, sequences of the *LSU* gene as well as a combination of the sequences of 5.8S rRNA and the exon regions of the *BT1* and *BT2* genes (including partial exon 4, exon 5, partial exon 6 and partial exon 7) of all the described genera in the Cryphonectriaceae were compared to sequences generated in the current study (Table 1). The datasets of *LSU*, 5.8S rRNA and exons of *BT1* and *BT2* gene sequences were not combined for analysis, because sequences of some Cryphonectriaceae isolates were not available for these datasets.

Prior to conducting the phylogenetic analysis for the combined 5.8S rRNA and the exon regions of the *BT* gene, a partition homogeneity test was performed in PAUP v. 4.0b10 (Swofford, 2003) to determine whether conflict existed between the two datasets (Huelsenbeck *et al.*, 1996). For phylogenetic analyses of the *LSU* sequences, *Phaeoacremonium aleophilum*, *Togninia fraxinopennsylvanica* and *Togninia minima* were used as outgroup taxa. For analyses of the 5.8S rRNA and the exon regions of the *BT* gene, *Diaporthe ambigua* was used as the outgroup taxon. Sequences for the datasets were aligned using the iterative refinement method (FFT-NS-i settings) of the online platform of MAFFT v. 5.667 (Katoh *et al.*, 2002). The alignments were further edited manually in MEGA v. 4.

Two different phylogenetic analyses were conducted for each of the *LSU*, the combined 5.8S rRNA and *BT* exons datasets. Maximum parsimony (MP) analyses were performed with PAUP v. 4.0b10 and maximum likelihood (ML) tests were conducted with PhyML v. 3.0 (Guindon & Gascuel, 2003). The phylogenetic analyses were conducted as described in Chen *et al.* (2016). The phylogenetic trees were viewed using MEGA v. 4.

Morphology

Only asexual fruiting structures were observed on the bark of *L. speciosa* stems and branches. The structures were excised from the bark under a dissecting microscope and heated in water for 2 min to rehydrate the cells. The structures were embedded in tissue freezing medium (Leica Biosystems Nussloch GmbH) and sectioned (10 μm thick) using a cryostat HM550 microtome (Microm International GmbH) at −20 °C to observe stromata and stromatic tissue. Additionally, conidiophores, conidiogenous cells and conidia were measured after crushing fruiting structures on microscope slides in sterile water. Fifty measurements were made for each morphological feature for the holotype specimen, 25 measurements per character were made for the remaining specimens used in the descriptions. The structures were examined and recorded using an Axio Imager A1 microscope and an AxioCam MRc digital camera with AxioVision v. 4.8 software (Carl Zeiss Ltd). Characteristics of

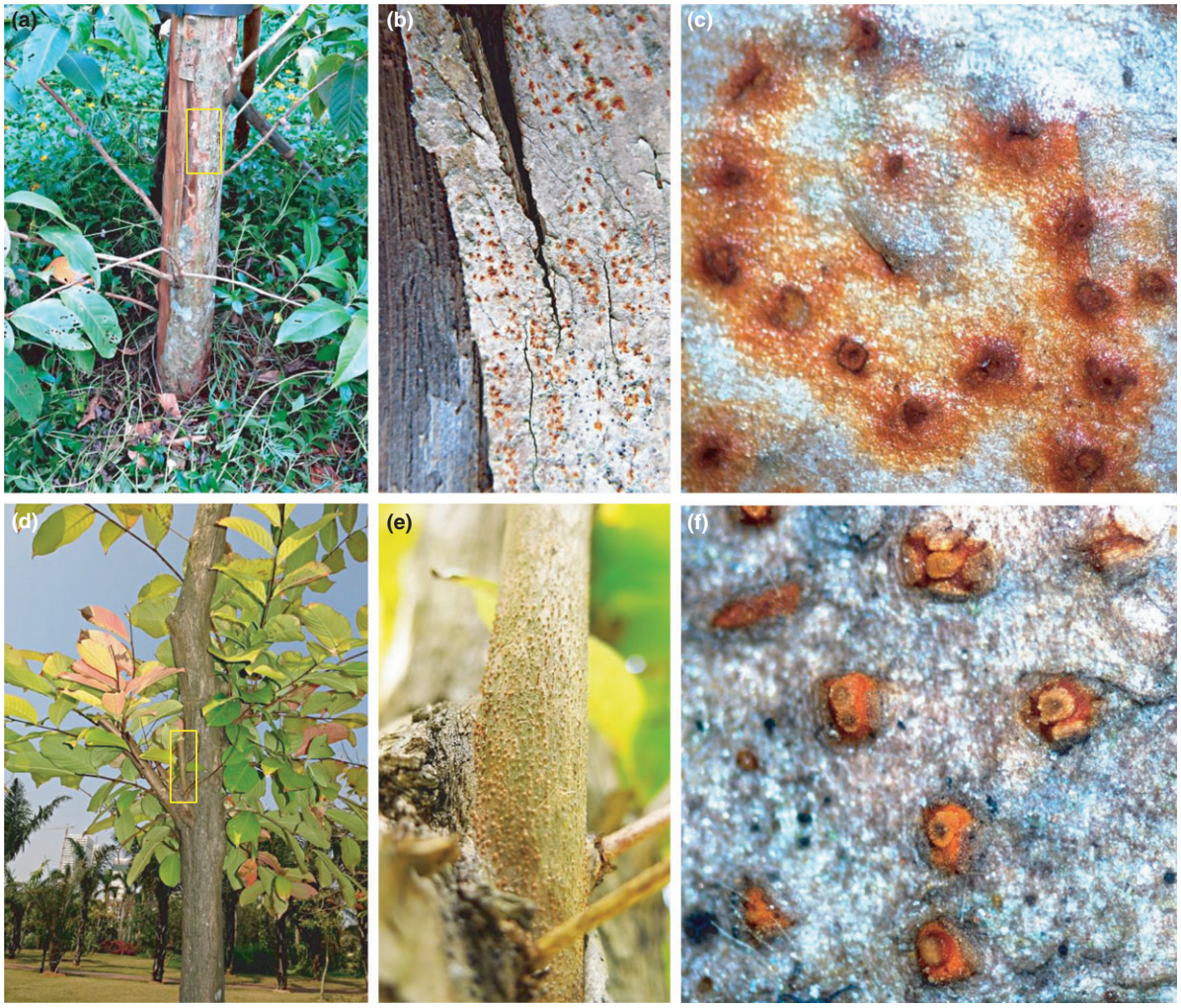

Figure 1 Symptoms of infection by *Chrysomorbus lagerstroemiae* on *Lagerstroemia speciosa* trees. (a, b) Dead trunk, (d, e) dead branch; (c, f) orange fruiting structures of *C. lagerstroemiae*. (a)–(c) from a park in HaiNan Province, and (d)–(f) from a park in GuangDong Province.

specimens were compared with those published for genera and species in the Cryphonectriaceae (Table 1). Results are presented as (minimum–) (mean – standard deviation) – (mean + standard deviation) (–maximum).

To study culture characteristics, four representative isolates (CERC8780/CMW49281, CERC8805/CMW49286, CERC8807/CMW49287 and CERC8810/CMW49289) from four different *L. speciosa* trees were used. Growth in culture, at seven different temperatures, was determined as described by Chen *et al.* (2016). The entire experiment was repeated once. Averages of measurements were calculated for each temperature using EXCEL 2003 (Microsoft). For the descriptions of fruiting bodies and cultures, colour designations were obtained using the colour charts of Rayner (1970).

Pathogenicity tests

Six isolates (CERC8780/CMW49281, CERC8782/CMW49282, CERC8784/CMW49283, CERC8805/CMW49286, CERC8807/CMW49287 and CERC8810/CMW49289) were selected for inoculation studies. Inoculations were conducted on *L. speciosa* trees growing in a park in the ZhanJiang region, GuangDong Province. Isolates were inoculated into the branches (1–2-year-old, approximately 0.8 cm diameter) of healthy *L. speciosa* trees. The inoculations were conducted using the methods described in Chen *et al.* (2013a). Fourteen branches on each of five *L. speciosa* trees (70 branches in total) were selected for inoculations. Two branches on each of five trees were inoculated with each of the six selected isolates or sterile MEA that served as controls. The five inoculated trees were randomly distributed at one site in the park.

To test whether the fungus from *L. speciosa* is pathogenic to *Eucalyptus*, the six isolates were inoculated onto the stems of saplings of a *Eucalyptus urophylla* × *Eucalyptus grandis* clone (CEPT-10). Clone CEPT-10 was chosen because it is widely used in commercial plantations in South China. The saplings were approximately 2 m tall and their main stems were approximately 10 mm in diameter. The *Eucalyptus* saplings were inoculated using the method described in Chen *et al.* (2010). Ten saplings were inoculated for each of the six isolates, and 10

Table 1 Isolates used for phylogenetic studies and pathogenicity tests in this study

Identity	Isolate no.[a]	Host	Location	Collector	GenBank accession no.[b]				Reference
					LSU	ITS	*BT1*	*BT2*	
Amphilogia gyrosa	CMW10469	*Elaeocarpus dentatus*	New Zealand	G. J. Samuels	AY194107	AF452111	AF525707	AF525714	Gryzenhout *et al.* (2005a, 2006a)
	CMW10470	*E. dentatus*	New Zealand	G. J. Samuels	AY194108	AF452112	AF525708	AF525715	Gryzenhout *et al.* (2005a, 2006a)
Aurantioporthe corni	ATCC66834	*Cornus alternifolia*	USA	N/A[c]	AF277133	N/A	N/A	N/A	Zhang & Blackwell (2001)
	CMW10526	*C. alternifolia*	USA	S. Redlin	AF408343	DQ120762	DQ120769	DQ120770	Gryzenhout *et al.* (2006a)
	MES1001	N/A	USA	W. Cullina	N/A	KF495039	KF495069	N/A	Beier *et al.* (2015)
	CTS1001	N/A	USA	K. Kitka	N/A	KF495033	KF495063	N/A	Beier *et al.* (2015)
Aurantiosacculus acutatus	CBS132181	*Eucalyptus viminalis*	Australia	B. A. Summerell & P. Summerell	Q685520	JQ685514	N/A	N/A	Crous *et al.* (2012a)
Aurantiosacculus eucalyptorum	CBS130826	*Eucalyptus globulus*	Australia	C. Mohammed & M. Glen	JQ685521	JQ685515	N/A	N/A	Crous *et al.* (2012a)
Aurapex penicillata	CMW10030	*Miconia theaezans*	Colombia	C. A. Rodas	AY194103	AY214311	AY214239	AY214275	Gryzenhout *et al.* (2006c, 2009)
	CMW11295	*M. theaezans*	Colombia	C. A. Rodas	AY194089	N/A	N/A	N/A	Gryzenhout *et al.* (2009)
	CMW10035	*M. theaezans*	Colombia	C. A. Rodas	N/A	AY214313	AY214241	AY214277	Gryzenhout *et al.* (2006c, 2009)
Aurifilum marmelostoma	CMW28285	*Terminalia mantaly*	Cameroon	D. Begoude & J. Roux	HQ171215	FJ882855	FJ900585	FJ900590	Begoude *et al.* (2010); Vermeulen *et al.* (2011)
	CMW28288	*Terminalia ivorensis*	Cameroon	D. Begoude & J. Roux	HQ171216	FJ882856	FJ900586	FJ900591	Begoude *et al.* (2010); Vermeulen *et al.* (2011)
Celoporthe dispersa	CMW9976	*Syzygium cordatum*	South Africa	M. Gryzenhout	HQ730853	DQ267130	DQ267136	DQ267142	Nakabonge *et al.* (2006); Chen *et al.* (2011)
	CMW9978	*S. cordatum*	South Africa	M. Gryzenhout	HQ730854	AY214316	DQ267135	DQ267141	Nakabonge *et al.* (2006); Chen *et al.* (2011)
Celoporthe eucalypti	CMW26900	*Eucalyptus* clone EC48	China	X. D. Zhou & S. F. Chen	HQ730862	HQ730836	HQ730816	HQ730826	Chen *et al.* (2011)
	CMW26908	*Eucalyptus* clone EC48	China	X. D. Zhou & S. F. Chen	HQ730863	HQ730837	HQ730817	HQ730827	Chen *et al.* (2011)
Celoporthe fontana	CMW29375	*Syzygium guineense*	Zambia	M. Vermeulen & J. Roux	N/A	GU726940	GU726952	GU726952	Vermeulen *et al.* (2013b)
	CMW29376	*S. guineense*	Zambia	M. Vermeulen & J Roux	N/A	GU726941	GU726953	GU726953	Vermeulen *et al.* (2013b)

(continued)

Table 1 (continued)

Identity	Isolate no.[a]	Host	Location	Collector	GenBank accession no.[b]				Reference
					LSU	ITS	*BT1*	*BT2*	
Celoporthe guangdongensis	CMW12750	*Eucalyptus* sp.	China	T. I. Burgess	HQ730856	HQ730830	HQ730810	HQ730820	Chen *et al.* (2011)
Celoporthe indonesiensis	CMW10781	*Syzygium aromaticum*	Indonesia	M.. Wingfield	HQ730855	AY084009	AY084033	AY084021	Myburg *et al.* (2003); Chen *et al.* (2011)
Celoporthe syzygii	CMW34023	*Syzygium cumini*	China	S. F. Chen	HQ730857	HQ730831	HQ730811	HQ730821	Chen *et al.* (2011)
	CMW24912	*S. cumini*	China	M. J. Wingfield & X. D. Zhou	HQ730859	HQ730833	HQ730813	HQ730823	Chen *et al.* (2011)
Celoporthe woodiana	CMW13936	*Tibouchina granulosa*	South Africa	M. Gryzenhout	N/A	DQ267131	DQ267137	DQ267143	Vermeulen *et al.* (2013b)
	CMW13937	*T. granulosa*	South Africa	M. Gryzenhout	N/A	DQ267132	DQ267138	DQ267144	Vermeulen *et al.* (2013b)
Chrysocrypta corymbiae	CBS132528	*Corymbia* sp.	Australia	P. W. Crous & B. A. Summerell	JX069851	JX069867	N/A	N/A	Crous *et al.* (2012b)
Chrysofolia colombiana	CBS139909	*Eucalyptus urophylla* × *Eucalyptus grandis*	Colombia	M. J. Wingfield	KR476771	KR476738	N/A	N/A	Crous *et al.* (2015)
Chrysomorbus lagerstroemiae	CERC8780[d] = CMW49281	*Lagerstroemia speciosa*	China	J. Roux & S. F. Chen	**KY929320**	**KY929330**	**KY929350**	**KY929340**	This study
	CERC8782[d] = CMW49282	*L. speciosa*	China	J. Roux & S. F. Chen	**KY929321**	**KY929331**	**KY929351**	**KY929341**	This study
	CERC8784[d] = CMW49283	*L. speciosa*	China	J. Roux & S. F. Chen	**KY929322**	**KY929332**	**KY929352**	**KY929342**	This study
	CERC8786 = CMW49284	*L. speciosa*	China	J. Roux & S. F. Chen	**KY929323**	**KY929333**	**KY929353**	**KY929343**	This study
	CERC8804 = CMW49285	*L. speciosa*	China	S. F. Chen	**KY929324**	**KY929334**	**KY929354**	**KY929344**	This study
	CERC8805[d] = CMW49286	*L. speciosa*	China	S. F. Chen	**KY929325**	**KY929335**	**KY929355**	**KY929345**	This study
	CERC8807[d] = CMW49287	*L. speciosa*	China	S. F. Chen	**KY929326**	**KY929336**	**KY929356**	**KY929346**	This study
	CERC8809 = CMW49288	*L. speciosa*	China	S. F. Chen	**KY929327**	**KY929337**	**KY929357**	**KY929347**	This study
	CERC8810[d] = CMW49289	*L. speciosa*	China	S. F. Chen	**KY929328**	**KY929338**	**KY929358**	**KY929348**	This study
	CERC8812 = CMW49290	*L. speciosa*	China	S. F. Chen	**KY929329**	**KY929339**	**KY929359**	**KY929349**	This study

(continued)

Table 1 (continued)

Identity	Isolate no.[a]	Host	Location	Collector	GenBank accession no.[b]				Reference
					LSU	ITS	*BT1*	*BT2*	
Chrysoporthe austroafricana	CMW62	*E. grandis*	South Africa	M. J. Wingfield	AY194097	AF292041	AF273063	AF273458	Myburg *et al.* (2002b); Gryzenhout *et al.* (2006a)
	CMW9327	*T. granulosa*	South Africa	J. Roux	N/A	AF273473	AF273060	AF273455	Myburg *et al.* (2002a)
	CMW2113	*E. grandis*	South Africa	M. J. Wingfield	N/A	AF046892	AF273067	AF273462	Myburg *et al.* (1999, 2002b)
Chrysoporthe cubensis	CBS101281	*E. urophylla*	Cameroon	I. A. S. Gibson	AF408338	N/A	N/A	N/A	Castlebury *et al.* (2002)
	CMW10453	*Eucalyptus saligna*	Democratic Republic of the Congo	N/A	AF408339	AY063476	AY063478	AY063480	Castlebury *et al.* (2002); Gryzenhout *et al.* (2004)
	CMW10669	*Eucalyptus* sp.	Republic of the Congo	J. Roux	N/A	AF535122	AF535124	AF535126	Gryzenhout *et al.* (2004)
	CMW10639	*E. grandis*	Colombia	C. A. Rodas	N/A	AY263421	AY263419	AY263420	Gryzenhout *et al.* (2004)
Chrysoporthe deuterocubensis	CMW11290	*Eucalyptus* sp.	Indonesia	M. J. Wingfield	N/A	AY214304	AY214232	AY214268	Gryzenhout *et al.* (2004)
	CMW8758	*Eucalyptus* sp.	Venezuela	M. J. Wingfield	AY194098	AF046898	AF273068	AF273463	Myburg *et al.* (2002b); Gryzenhout *et al.* (2006a)
	CMW8651	*S. aromaticum*	Indonesia	M. J. Wingfield	N/A	AY084002	AY084026	AY084014	Myburg *et al.* (2003)
Chrysoporthe doradensis	CMW11287	*E. grandis*	Ecuador	M. J. Wingfield	N/A	AY214289	AY214217	AY214253	Gryzenhout *et al.* (2005b)
	CMW11286	*E. grandis*	Ecuador	M. J. Wingfield	N/A	AY214290	AY214218	AY214254	Gryzenhout *et al.* (2005b)
Chrysoporthe hodgesiana	CMW10625	*M. theaezans*	Colombia	C. A. Rodas	N/A	AY956970	AY956979	AY956980	Rodas *et al.* (2005)
Chrysoporthe inopina	CMW12727	*Tibouchina lepidota*	Colombia	R. Arbelaez	N/A	DQ368777	DQ368806	DQ368807	Gryzenhout *et al.* (2006b)
	CMW12729	*T. lepidota*	Colombia	R. Arbelaez	N/A	DQ368778	DQ368808	DQ368809	Gryzenhout *et al.* (2006b)
Corticimorbus sinomyrti	CERC3629	*Rhodomyrtus tomentosa*	China	S. F. Chen & G. Q. Li	KT167179	KT167169	KT167189	KT167189	Chen *et al.* (2016)
	CERC3631	*R. tomentosa*	China	S. F. Chen & G. Q. Li	KT167180	KT167170	KT167190	KT167190	Chen *et al.* (2016)
Cryphonectria decipiens	CMW10436	*Quercus suber*	Portugal	B. d'Oliviera	JQ862750	AF452117	AF525703	AF525710	Myburg *et al.* (2004a); Chen *et al.* (2013a)
	CMW10484	*Castanea sativa*	Italy	A. Biraghi	N/A	AF368327	AF368349	AF368349	Venter *et al.* (2002); Myburg *et al.* (2004a)

(continued)

Table 1 (continued)

Identity	Isolate no.[a]	Host	Location	Collector	GenBank accession no.[b]				Reference
					LSU	ITS	*BT1*	*BT2*	
Cryphonectria japonica	CMW10527	*Quercus mongolica*	Russia	L. Vasilyeva	AF408341	DQ120761	DQ120767	DQ120768	Castlebury *et al.* (2002); Gryzenhout *et al.* (2006a)
	CMW10528	*Q. mongolica*	Russia	L. Vasilyeva	AF408340	DQ120760	DQ120765	DQ120766	Castlebury *et al.* (2002); Gryzenhout *et al.* (2006a)
	CMW13742	*Quercus grosseserrata*	Japan	T. Kobayashi	N/A	AY697936	AY697961	AY697962	Myburg *et al.* (2004b)
	CMW13747	*Quercus serrata*	Japan	T. Kobayashi	N/A	AY697937	AY697963	AY697964	Myburg *et al.* (2004b)
Cryphonectria macrospora	CMW10463	*Castanea cuspidata*	Japan	T. Kobayashi	N/A	AF368331	AF368351	AF368350	Gryzenhout *et al.* (2006a)
	CMW10914	*C. cuspidata*	Japan	T. Kobayashi	JQ862749	AY697942	AY697973	AY697974	Gryzenhout *et al.* (2006a); Chen *et al.* (2013a)
Cryphonectria parasitica	N/A	*Castanea* sp.	N/A	N/A	AF277132	N/A	N/A	N/A	Zhang & Blackwell (2001)
	CMW7048	*Quercus virginiana*	USA	R. Stipes	AY194100	AF368330	AF273076	AF273470	Venter *et al.* (2002); Gryzenhout *et al.* (2006a)
	CMW13749	*Castanea mollisima*	Japan	N/A	N/A	AY697927	AY697943	AY697944	Myburg *et al.* (2004b)
Cryphonectria radicalis	CMW10455	*Q. suber*	Italy	A. Biraghi	AY194101	AF452113	AF525705	AF525712	Gryzenhout *et al.* (2006a)
	CMW10477	*Q. suber*	Italy	A. Biraghi	AY194102	AF368328	AF368347	AF368347	Venter *et al.* (2002); Gryzenhout *et al.* (2006a)
	CMW13754	*Fagus japonica*	Japan	T. Kobayashi	N/A	AY697932	AY697953	AY697954	Myburg *et al.* (2004b)
Cryptometrion aestuescens	CMW18790	*E. grandis*	Indonesia	M. J. Wingfield	HQ171211	GQ369458	GQ369455	GQ369455	Gryzenhout *et al.* (2010); Vermeulen *et al.* (2011)
	CMW18793	*E. grandis*	Indonesia	M. J. Wingfield	HQ171212	GQ369459	GQ369456	GQ369456	Gryzenhout *et al.* (2010); Vermeulen *et al.* (2011)
Diversimorbus metrosiderotis	CMW37321	*Metrosideros angustifolia*	South Africa	J. Roux	JQ862827	JQ862870	JQ862911	JQ862952	Chen *et al.* (2013b)
	CMW37322	*M. angustifolia*	South Africa	J. Roux	JQ862828	JQ862871	JQ862912	JQ862953	Chen *et al.* (2013b)
Endothia gyrosa	N/A	*Quercus* sp.	USA	N/A	AF362555	N/A	N/A	N/A	Gryzenhout *et al.* (2009)
	CMW2091	*Quercus palustris*	USA	R. J. Stipes	AY194114	AF368325	AF368337	AF368336	Venter *et al.* (2002); Gryzenhout *et al.* (2006a)
	CMW10442	*Q. palustris*	USA	R. J. Stipes	AY194115	AF368326	AF368339	AF368338	Venter *et al.* (2002); Gryzenhout *et al.* (2006a)

(continued)

Table 1 (continued)

Identity	Isolate no.[a]	Host	Location	Collector	GenBank accession no.[b]				Reference
					LSU	ITS	*BT1*	*BT2*	
Foliocryphia eucalypti	CBS124779	*Eucalyptus coccifera*	Australia	C. Mohammed	GQ303307	GQ303276	N/A	N/A	Cheewangkoon *et al.* (2009)
Holocryphia capensis	CMW37887	*M. angustifolia*	South Africa	J. Roux, S. F. Chen & F. Roets	JQ862811	JQ862854	JQ862895	JQ862936	Chen *et al.* (2013b)
	CMW37329	*M. angustifolia*	South Africa	J. Roux & S. F. Chen	JQ862816	JQ862859	JQ862900	JQ862941	Chen *et al.* (2013b)
Holocryphia eucalypti	CMW7033	*E. grandis*	South Africa	M. Venter	JQ862794	JQ862837	JQ862878	JQ862919	Chen *et al.* (2013b)
	CMW7035	*E. saligna*	South Africa	M. Venter	JQ862795	JQ862838	JQ862879	JQ862920	Chen *et al.* (2013b)
Holocryphia gleniana	CMW37334	*M. angustifolia*	South Africa	J. Roux & S. F. Chen	JQ862791	JQ862834	JQ862875	JQ862916	Chen *et al.* (2013b)
	CMW37335	*M. angustifolia*	South Africa	J. Roux & S. F. Chen	JQ862792	JQ862835	JQ862876	JQ862917	Chen *et al.* (2013b)
Holocryphia mzansi	CMW37337	*M. angustifolia*	South Africa	J. Roux & S. F. Chen	JQ862798	JQ862841	JQ862882	JQ862923	Chen *et al.* (2013b)
	CMW37338	*M. angustifolia*	South Africa	J. Roux & S. F. Chen	JQ862799	JQ862842	JQ862883	JQ862924	Chen *et al.* (2013b)
Holocryphia sp.	CMW6246	*T. granulosa*	Australia	M. J. Wingfield	JQ862802	JQ862845	JQ862886	JQ862927	Chen *et al.* (2013b)
Holocryphia sp.	CMW10015	*Eucalyptus fastigata*	New Zealand	R. J. van Boven	JQ862806	JQ862849	JQ862890	JQ862931	Chen *et al.* (2013b)
Immersiporthe knoxdaviesiana	CMW37314	*Rapanea melanophloeos*	South Africa	M. J. Wingfield & J. Roux	JQ862755	JQ862765	JQ862785	JQ862775	Chen *et al.* (2013a)
	CMW37315	*R. melanophloeos*	South Africa	M. J. Wingfield & J. Roux	JQ862756	JQ862766	JQ862786	JQ862776	Chen *et al.* (2013a)
Latruncellus aurorae	CMW28274	*Galpinia transvaalica*	Swaziland	J. Roux	HQ171213	GU726946	GU726958	GU726958	Vermeulen *et al.* (2011)
	CMW28276	*G. transvaalica*	Swaziland	J. Roux	HQ730872	GU726947	GU726959	GU726959	Chen *et al.* (2011); Vermeulen *et al.* (2011)
	CMW28275	*G. transvaalica*	Swaziland	J. Roux	HQ171214	HQ171209	HQ171207	HQ171207	Vermeulen *et al.* (2011)
Luteocirrhus shearii	CBS130775	*Banksia baxteri*	Australia	C. Crane	KC197018	KC197024	KC197015	KC197009	Crane & Burgess (2013)
	CBS130776	*B. baxteri*	Australia	C. Crane	KC197019	KC197021	KC197012	KC197006	Crane & Burgess (2013)
Micrithia havanensis	CMW11299	*Myrica faya*	Madeira	N/A	AY194087	N/A	N/A	N/A	Gryzenhout *et al.* (2009)
	CMW11300	*M. faya*	Madeira	N/A	AY194088	N/A	N/A	N/A	Gryzenhout *et al.* (2009)
	CMW11301	*M. faya*	Azores	C. S. Hodges & D. E. Gardner	N/A	AY214323	AY214251	AY214287	Gryzenhout *et al.* (2006d)
	CMW14550	*E. saligna*	Mexico	C. S. Hodges	N/A	DQ368735	DQ368741	DQ368742	Gryzenhout *et al.* (2006d)

(continued)

Table 1 (continued)

Identity	Isolate no.[a]	Host	Location	Collector	GenBank accession no.[b]				Reference
					LSU	ITS	*BT1*	*BT2*	
Rostraureum tropicale	CMW9972	*T. ivorensis*	Ecuador	M. J. Wingfield	AY194092	AY167436	AY167426	AY167431	Gryzenhout *et al.* (2005c, 2006a)
	CMW10796	*T. ivorensis*	Ecuador	M. J. Wingfield	N/A	AY167438	AY167428	AY167433	Gryzenhout *et al.* (2005c)
	CMW9971	*T. ivorensis*	Ecuador	M. J. Wingfield	N/A	AY167435	AY167425	AY167430	Gryzenhout *et al.* (2005c)
Ursicollum fallax	CMW18119	*Coccoloba uvifera*	USA	C. S. Hodges	EF392860	DQ368755	DQ368758	DQ368759	Gryzenhout *et al.* (2006d, 2009)
	CMW18115	*C. uvifera*	USA	C. S. Hodges	N/A	DQ368756	DQ368760	DQ368761	Gryzenhout *et al.* (2006d)

[a]Designation of isolates and culture collections: CMW, Tree Protection Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; MES, CTS represent isolates in Beier *et al.* (2015); ATCC, American Type Culture Collection, Manassas, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CERC, China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), Zhanjiang, GuangDong, China.

[b]GenBank numbers in boldface were sequenced in this study.

[c]N/A, not available.

[d]Isolates used in pathogenicity tests.

saplings were inoculated with sterile MEA plugs to serve as negative controls. The inoculated plants were arranged in a randomized design in the same shade house.

After 4 weeks, the inoculated plants were evaluated by measuring the lengths of the lesions in the cambium. Reisolations were made from the inoculated branches/saplings by cutting small pieces of discoloured tissue from the lesion edges and transferring them onto 2% MEA at 25 °C. Reisolations were made from four randomly selected branches/saplings per isolate and all branches/saplings that served as negative controls.

Lesion length results were analysed in EXCEL. Single-factor analysis of variance (ANOVA) was used to define the effects of isolates/negative control on lesion length, and to test the significance among means of lesion lengths. *F*-values with $P < 0.05$ were considered significantly different. The standard errors of means of lesion lengths for each isolate and control were calculated.

Results

Sample collection and isolations

Seven *L. speciosa* trees with fruiting bodies typical of fungi in the Cryphonectriaceae (Fig. 1) were identified. These included a single tree in a park in the HaiNan Province and six trees in a park in the ZhanJiang region of GuangDong Province. Seventeen isolates were obtained from these trees: four from the tree in HaiNan and the remainder from the trees in GuangDong. Only asexual fruiting structures (conidiomata) with orange stromatic tissue were observed on the surfaces of *L. speciosa* stems and branches (Fig. 1b,c,e,f). The isolates on MEA were white in colour when young and became yellow to orange with age (Fig. 2g–i), with the typical morphological characteristics of fungi in the Cryphonectriaceae. All cultures displayed the same morphology.

Phylogenetic analysis

Ten representative isolates, four from the tree in HaiNan Province and six from the six trees in GuangDong Province, were selected for DNA sequencing and phylogenetic analysis (Table 1). The aligned *LSU* sequence dataset consisted of 83 taxa and 626 characters (TreeBASE no. 20882; http://www.treebase.org). Statistical values of both ML and MP analyses are provided in Table 2. Other than *Cryphonectria*, each genus in the Cryphonectriaceae formed a unique phylogenetic clade in both the MP and ML analyses (Fig. 3), although the inferred phylogenetic relationships among genera differed between ML and MP analyses. Isolates collected from *L. speciosa* grouped together in a distinct clade in the Cryphonectriaceae, separate from all other genera and supported by high bootstrap values (ML = 98%, MP = 100%). This supported the view that they represent a novel genus in the family (Fig. 3).

The partition homogeneity test for the 5.8S rRNA gene and exons of the *BT1* and *BT2* gene region datasets indicated that the two datasets were congruent

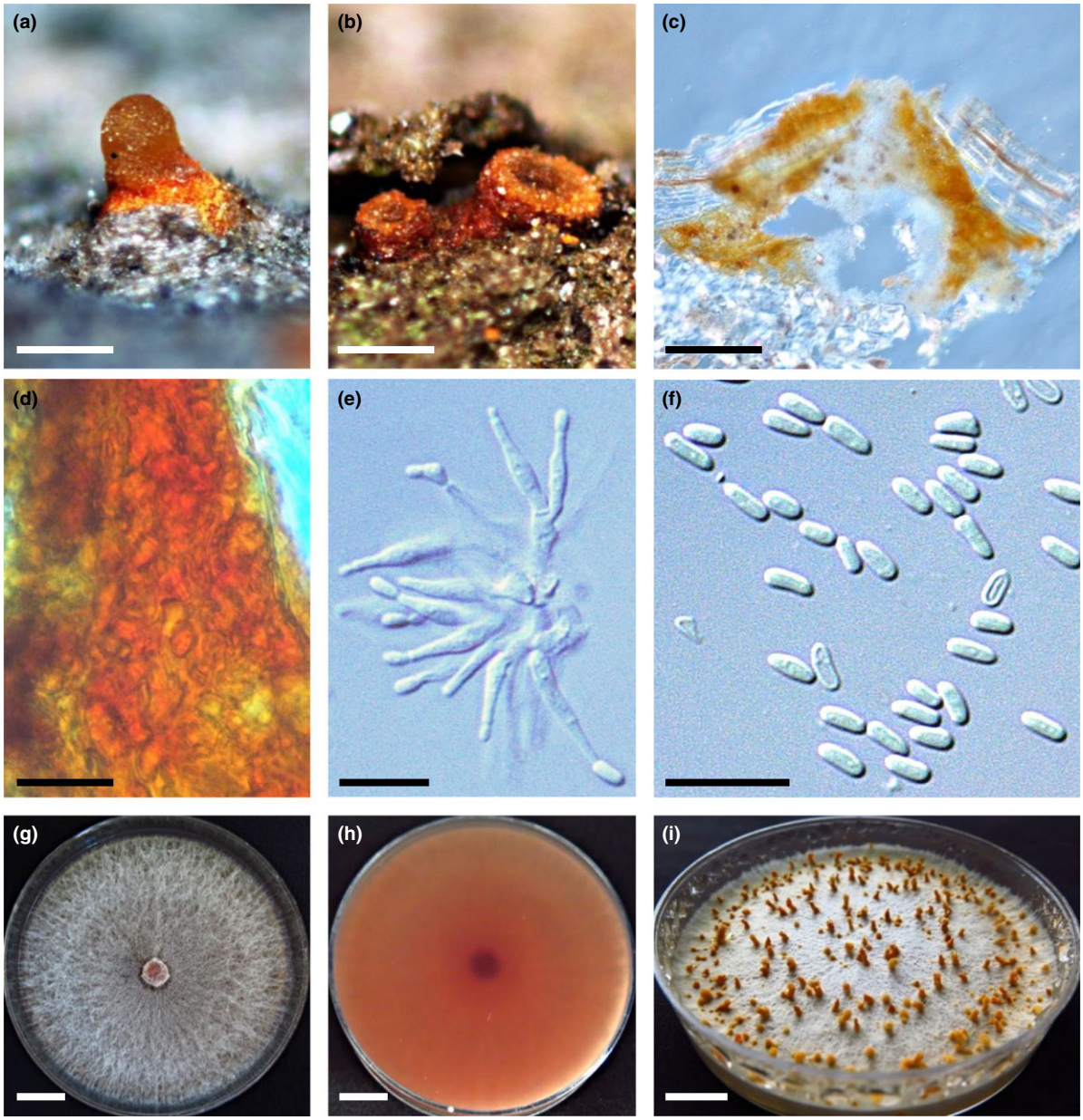

Figure 2 Asexual fruiting structures of *Chrysomorbus lagerstroemiae*. (a) Conidiomata on the bark, orange and globose; (b) orange and convex conidiomata; (c) longitudinal section through conidioma showing orange and unilocular stroma; (d) *textura globulosa* stromatic tissue of the conidioma; (e) non-septate, cylindrical conidiophores and cylindrical conidigenous cells; (f) fusoid to oval, aseptate conidia; cultures grown on malt extract agar at 25 °C after 7 days (g, h) and 90 days (i), showing the culture colour and development of conidiomata. Scale bars: a, b = 100 μm; c, d = 50 μm; e, f = 10 μm; g–i = 10 mm.

(P = 0.906) and they were consequently combined for further analyses. The alignment of the combined dataset consisted of 85 taxa and 760 characters (TreeBASE no. 20882; Table 2). With the exception of *Cryphonectria*, all the genera in the Cryphonectriaceae formed independent phylogenetic clades with high bootstrap values (>80%) in both MP and ML analyses (Fig. 4). The topology of the MP and ML analyses was similar, although the position of genera relative to each other were slightly different in the two analyses. Based on the phylogenetic analyses of the combined sequences of the 5.8S rRNA and *BT* exons, the isolates from *L. speciosa* in China formed a strongly defined phylogenetic clade, distinct from other Cryphonectriaceae and supported by high bootstrap values (MP = 100%, ML = 100%; Fig. 3). All analyses showed that the isolates from *L. speciosa* in southern China represented a single novel genus and species of Cryphonectriaceae.

Table 2 Statistics resulting from phylogenetic analyses

Analysis	Dataset	
	LSU	5.8S rRNA with exons of *BT1* & *BT2*
No. of taxa	83	85
No. of characters (bp)	626	760
Maximum parsimony[a]		
Parsimony informative characters	123	126
Tree length	340	317
Consistency index	0.532	0.577
Retention index	0.815	0.881
Homoplasy index	0.468	0.423
Maximum likelihood[b]		
Rate matrix	1.6671	0.3765
	6.5433	1.8248
	3.7482	0.2614
	0.8893	0.9832
	21.1859	5.7962

[a]100 trees.

[b]Best fit substitution model GTR+I+G, using six substitution rate categories with gamma rates.

Morphology

The characteristics of the asexual structures on *L. speciosa* were typical of fungi in the Cryphonectriaceae. These included orange stromatic tissue. Stromatic tissue and mycelium stained purple in 3% KOH (Castlebury *et al.*, 2002; Gryzenhout *et al.*, 2006a, 2009). The fungus from *L. speciosa* had superficial to slightly immersed conidiomata, conidiomatal tissues of *textura globulosa*, orange conidiomata that were convex to globose in shape, non-ostiolate, without necks and no paraphyses. These characters distinguished the fungus from other genera with orange conidiomata, including *Amphilogia* (Gryzenhout *et al.*, 2005a), *Aurantioporthe* (Beier *et al.*, 2015), *Aurantiosacculus* (Crous *et al.*, 2012a), *Aurifilum* (Begoude *et al.*, 2010), *Cryphonectria* (Gryzenhout *et al.*, 2009), *Cryptometrion* (Gryzenhout *et al.*, 2010), *Endothia* (Gryzenhout *et al.*, 2006a), *Filiocryphia* (Cheewangkoon *et al.*, 2009), *Holocryphia* (Gryzenhout *et al.*, 2006d), *Immersiporthe* (Chen *et al.*, 2013a), *Latruncellus* (Vermeulen *et al.*, 2011), *Microthia* (Gryzenhout *et al.*, 2006d), *Rostraureum* (Gryzenhout *et al.*, 2005c) and *Ursicollum* (Gryzenhout *et al.*, 2006d) in the Cryphonectriaceae.

Taxonomy

Based on the phylogenetic analyses of *LSU* and combination of 5.8S rRNA and *BT1* and *BT2* exon gene regions, as well as morphological characteristics, the fungus from cankers on *L. speciosa* clearly represents a species in the Cryphonectriaceae. It is also different to any previously described genus and species in this family. A novel genus and species is consequently described to accommodate the fungus.

Chrysomorbus S. F. Chen gen. nov.

MycoBank MB 821021

Etymology: Greek, *chrysous*, golden, referring to the orange stromatic tissue, and *morbus*, disease, describing that the fungus occurs on dead and dying bark and stems/branches of living trees, and occurs on cankers.

Type species: *Chrysomorbus lagerstroemiae* S. F. Chen & Q. L. Liu

Conidiomata orange, convex to globose, superficial to semi-immersed, without necks, uni- to multilocular structures, with locules often convoluted, non-ostiolate; stromatic tissue *textura globulosa*. *Conidiophores* aseptate, cylindrical, occasionally with separating septa and branching, hyaline. *Conidiogenous cells* cylindrical or flask-shaped with attenuated apices. *Paraphyses* absent. *Conidia* hyaline, fusoid to oval, aseptate.

Chrysomorbus lagerstroemiae S. F. Chen & Q. L. Liu, sp. nov. (Fig. 2)

MycoBank MB 821022

Etymology: Refers to the host genus *Lagerstroemia* from which the fungus was isolated.

Conidiomata on bark, yellow when young, orange when mature, convex to globose, superficial to semi-immersed, without necks, uni- to multilocular structures, with locules often convoluted, non-ostiolate; stromatic tissue *textura globulosa*; conidiomatal bases 74–506 μm high above bark surface (av. 50 conidiomata 224 μm), 138–652 μm diameter (av. 50 conidiomata 368 μm); locules 82–212 μm diameter (av. 50 locules 146 μm). *Conidiophores* non-septate, cylindrical, (4.8–)5.3–14.7(–16.5) μm long (av. 50 conidiophores 10.0 μm), occasionally with separating septa and branching, hyaline. *Conidiogenous cells* (0.6–)1.0–1.4(–1.8) μm wide (av. 50 conidiogenous cells 1.2 μm), cylindrical or flask-shaped with attenuated apices. *Paraphyses* absent. *Conidia* hyaline, fusoid to oval, aseptate, (2.6–)3.1–3.7(–4.9) × (1.0–)1.3–1.5(–1.8) μm (av. 100 conidia 3.4 × 1.4 μm), exuded as orange droplets.

Culture characteristics: On MEA mycelium fluffy with uneven margin, colony white when young, occasionally with orange patches, turning orange after 14 days. Colony reverse white yellow, orange to sienna. Optimal growth temperature 25 °C, no growth at 5 °C. In 7 days, colony diameters at 5 °C intervals from 10 to 35 °C reached averages of 8.5 mm (10 °C), 18.5 mm (15 °C), 47.5 mm (20 °C), 75.5 mm (25 °C), 29.0 mm (30 °C) and 6 mm (35 °C). Conidiomata produced on MEA after 60 days, mature after 90 days producing conidial spore masses.

Substrate: Bark of *Lagerstroemia speciosa*.

Distribution: GuangDong and HaiNan Provinces, China.

Specimens examined: China, GuangDong Province, ZhanJiang region (21°14′44.5″N, 110°24′23.2″E), *L. speciosa*, 14 February 2016, ShuaiFei Chen, HOLOTYPE CSFF2016 (branches with mature conidiomata), ex-type culture CERC8810 = CMW49289 = CBS 142594; China, HaiNan Province, HaiKou region (20°0′54.8″N, 110°19′2.4″E), *L. speciosa*, 2 November

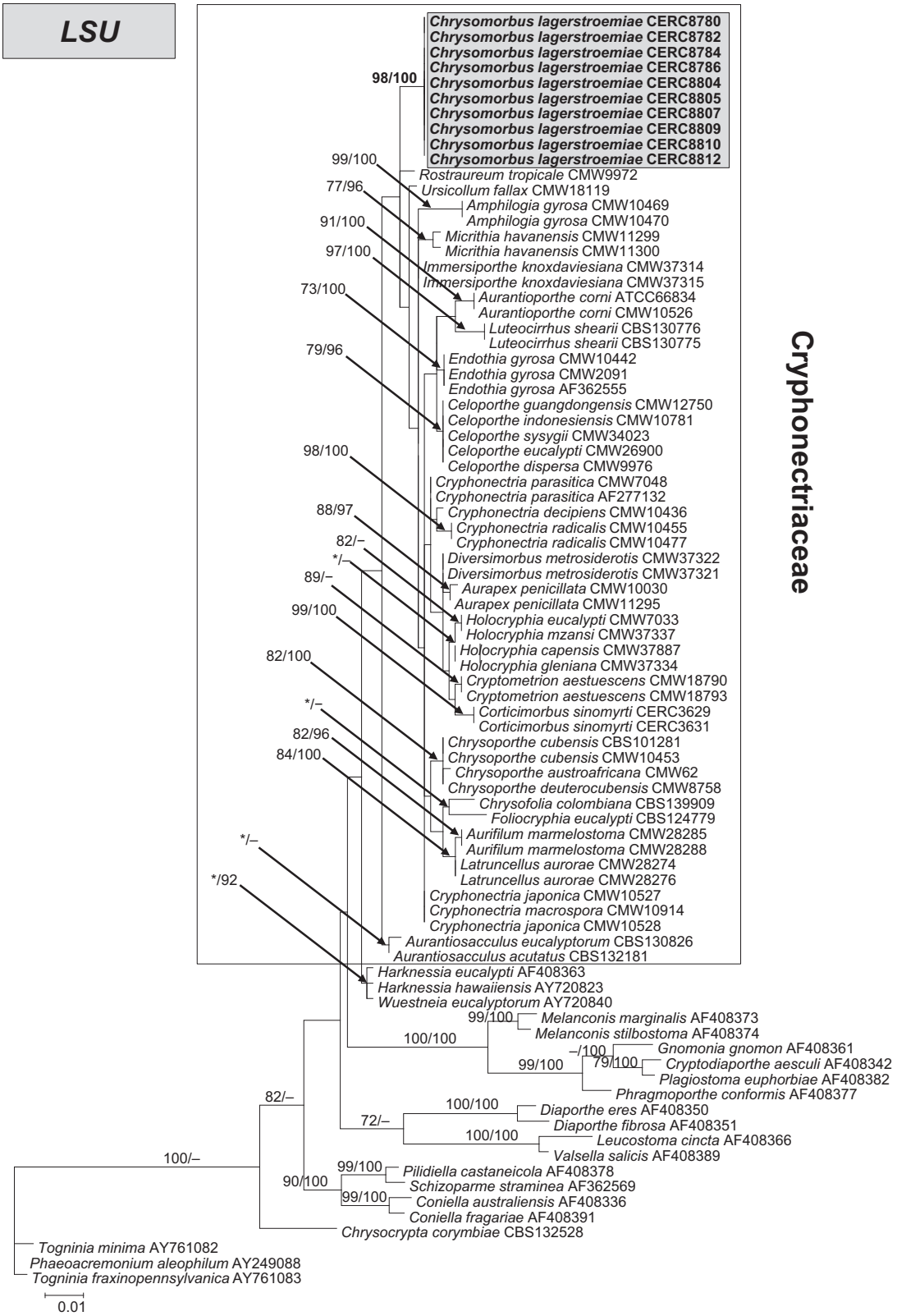

Figure 3 Phylogenetic tree based on maximum likelihood (ML) analysis of *LSU* DNA sequences for genera in the Diaporthales. Bootstrap values >70% for ML/MP (maximum parsimony) analyses are presented at branches. Bootstrap values lower than 70% are marked with *, and absent values are marked with –. Isolates from *Lagerstroemia speciosa* are in boldface and highlighted.

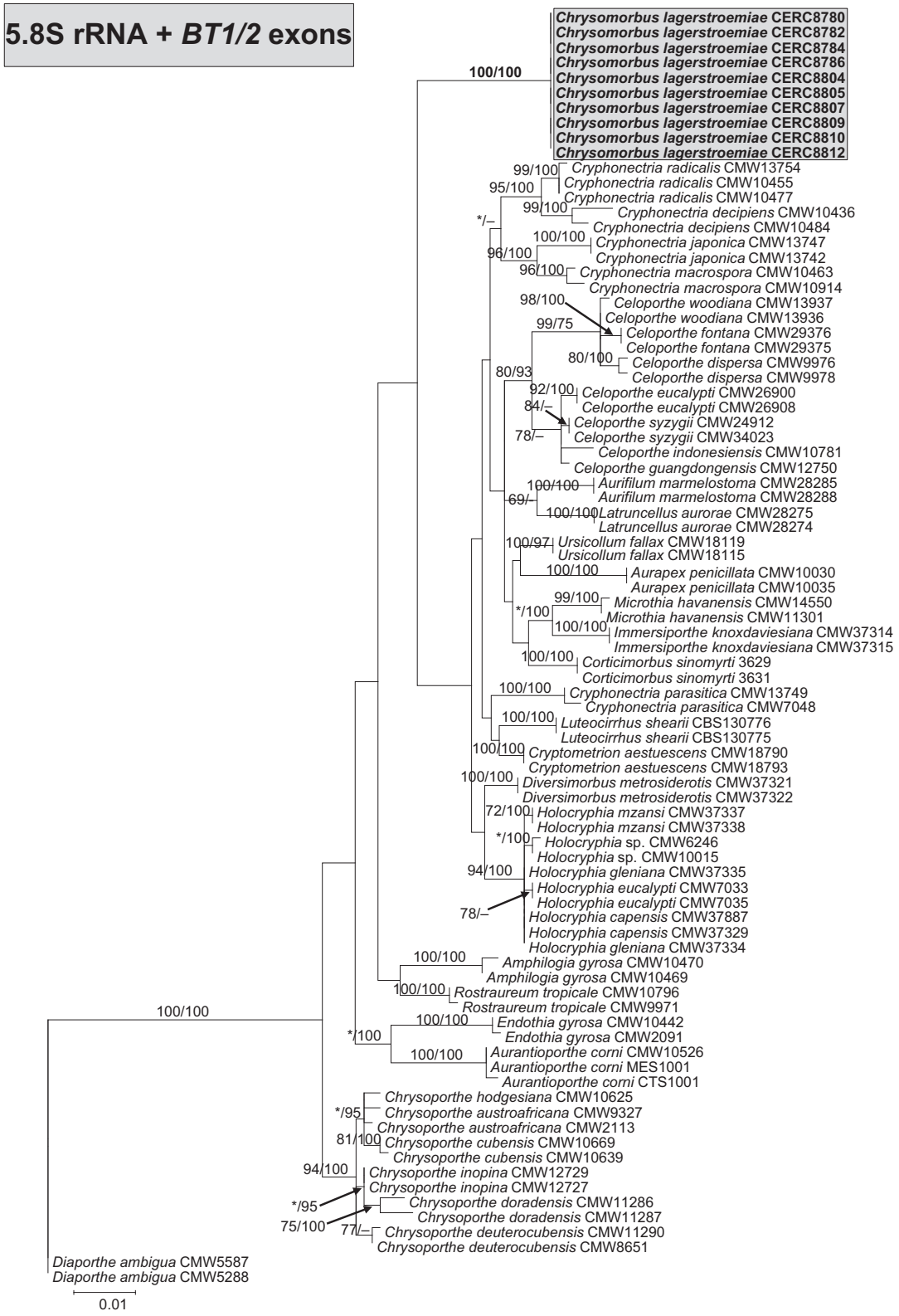

Figure 4 Phylogenetic tree based on maximum likelihood (ML) analysis of a combined DNA sequence dataset of regions of the 5.8S rRNA gene, and partial exon 4, exon 5, partial exon 6 and partial exon 7 of the *BT1* and *BT2* genes. Bootstrap values >70% for ML/MP (maximum parsimony) analyses are presented at branches. Bootstrap values <70% are marked with *, and absent analyses values are marked with –. Isolates from *Lagerstroemia speciosa* are in boldface and highlighted.

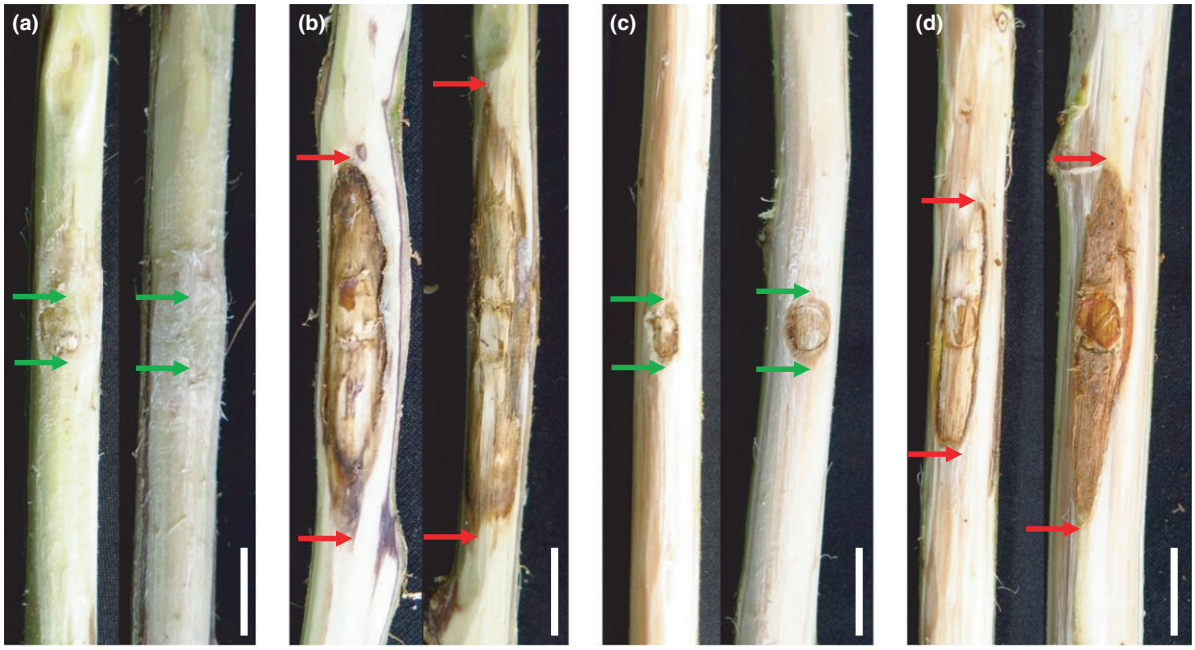

Figure 5 Lesions resulting from inoculation of *Chrysomorbus lagerstroemiae* onto *Lagerstroemia speciosa* branches (a, b) and *Eucalyptus* saplings (c, d), and wound response on the negative controls. (a, c) Negative controls showing absence of lesion development; (b) lesion associated with isolate CERC8805; (d) lesion associated with isolate CERC8782. Arrows indicate the terminal ends of wound response. Scale bars: 10 mm.

2015, Jolanda Roux & ShuaiFei Chen, PARATYPE CSFF2017 (trunk with mature conidiomata), living culture CERC8780 = CMW49281 = CBS142592; China, GuangDong Province, ZhanJiang region (21°14′44.5″N, 110°24′23.2″E), *L. speciosa*, 14 February 2016, ShuaiFei Chen, CSFF2018 (branches with mature conidiomata), living culture CERC8805 = CMW49286 = CBS142593.

Notes. The morphology of *C. lagerstroemiae* is most similar to species of *Amphilogia*, *Aurantiosacculus* and *Aurifilum*. Conidiomata of the four genera are orange, not pulvinate, and without necks. The sigmoid conidia distinguish *Aurantiosacculus* from *Amphilogia* (having cylindrical to allantoid conidia), *Aurifilum* (having oblong conidia) and *Chrysomorbus* (having fusoid to oval conidia). The convex to globose conidiomata and lack of ostioles distinguish *Chrysomorbus* from *Amphilogia* (having conical conidiomata, ostiole absent) and *Aurifilum* (having convex conidiomata, and ostiole present with black opening). *Celoporthe eucalypti*, *Celoporthe guangdongensis*, *Celoporthe syzygii*, *Chrysoporthe deuterocubensis* and *Corticimorbus sinomyrti* have previously been isolated on Myrtales in China, but the conidiomata of these species are black, which is different to *C. lagerstroemiae* in which the conidiomata are orange.

Pathogenicity tests

Four weeks after inoculation, all six *C. lagerstroemiae* isolates produced lesions on *L. speciosa* branches, while none were produced by the negative controls (Fig. 5a,b). The mean comparison tests indicated that average lesion length caused by the six *C. lagerstroemiae* isolates were all significantly longer ($P < 0.05$) than the wounds caused by the controls (Fig. 6a). Isolates CERC8805, CERC8807 and CERC8810 were more aggressive than the other isolates (Fig. 6a). *Chrysomorbus lagerstroemiae* was successfully reisolated from the lesions, but not from the controls.

The six isolates also produced lesions on *Eucalyptus* clone CEPT-10 within 4 weeks; no lesions developed in the control inoculations (Fig. 5c,d). The lesions caused by the six isolates were all significantly longer than the wounds caused by the controls (Fig. 6b). *Chrysomorbus lagerstroemiae* was successfully reisolated from the lesions on inoculated *Eucalyptus* seedlings but not from the controls.

Discussion

In this study, a previously unknown genus and species of fungus in the Cryphonectriaceae was discovered on *L. speciosa* trees in two parks in the GuangDong and HaiNan Provinces of South China. Justification for describing the fungus as a new taxon was based on the results of phylogenetic analyses of sequence data for multiple gene regions as well as morphological characteristics. The newly described fungus typifies the new genus *Chrysomorbus* and has been provided with the name *C. lagerstroemiae*. Inoculation tests showed that

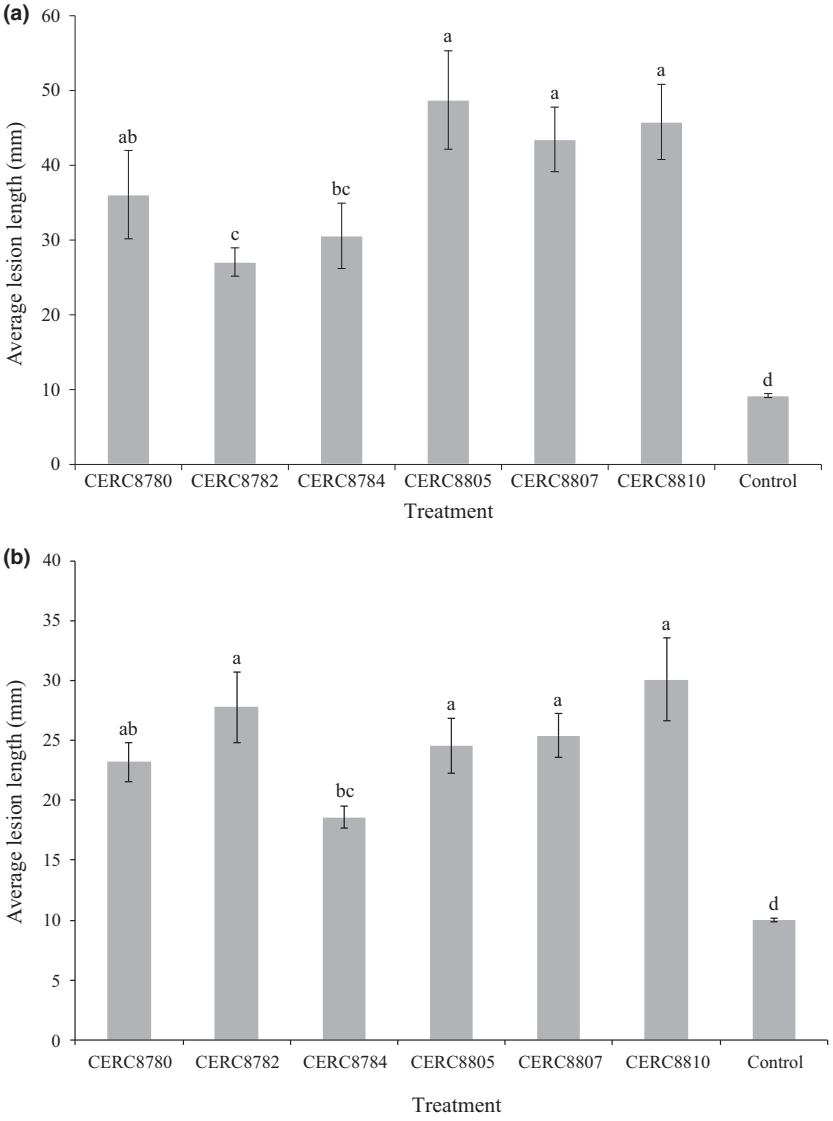

Figure 6 Histogram showing the average lesion lengths resulting from inoculation of *Lagerstroemia speciosa* branches (a) or saplings of *Eucalyptus* clone CEPT-10 (b) with six isolates of *Chrysomorbus lagerstroemiae* and a control. Vertical bars represent standard error of means. Different letters above the error bars indicate treatments that were significantly different ($\alpha = 0.05$).

C. lagerstroemiae is pathogenic to *L. speciosa* as well as to a *Eucalyptus* clone commonly planted in China.

This is the first report of a species of Cryphonectriaceae on Lythraceae in China. Unlike species of Cryphonectriaceae that have been widely reported from Myrtaceae trees (Gryzenhout *et al.*, 2009), only two species of Cryphonectriaceae have previously been recorded on the Lythraceae. These include *C. cubensis* collected on the non-native tree *L. indica* in Cuba (Gryzenhout *et al.*, 2006b) and *L. aurorae* from native *G. transvaalica* trees in Swaziland (Vermeulen *et al.*, 2011). The results of the present study suggest that the Cryphonectriaceae have wider host and geographical ranges on Lythraceae than those currently known for these trees, both in China and in other regions of the world.

Isolates of *C. lagerstroemiae* were obtained from stems of dead trees, as well as from cankered stems and cracked bark on living trees. The inoculation tests undertaken in this study showed that the fungus can produce lesions on branches of *L. speciosa*, and thus confirm that it was most likely responsible for the cankers of trees in the areas sampled. This might also suggest that *C. lagerstroemiae* poses a threat to *L. speciosa* in areas where the tree is planted as a non-native ornamental and possibly also where the tree is native.

The origin of *C. lagerstroemiae* is unknown. It is possible that it occurs on *Lagerstroemia* species where these trees are native and that it has been introduced into China. Alternatively, it might be a fungus native to China that has undergone a host shift (Slippers *et al.*, 2005) to infect *L. speciosa*. This would be similar to situations where other members of the Cryphonectriaceae on native Myrtales have adapted to infect *Eucalyptus* (Myrtaceae) in various parts of the world (Heath *et al.*, 2006; Vermeulen *et al.*, 2011, 2013a; van der Merwe *et al.*, 2013). In this regard, these fungi appear to be

common as natives on the Myrtales and to have the capacity to easily adapt to infect hosts in this plant order (Burgess & Wingfield, 2017).

Preliminary inoculation tests showed that *C. lagerstroemiae* is able to infect the stems of a *Eucalyptus* clone. This is similar to the situation in Africa and South America where *C. austroafricana* and *C. cubensis*, from native *Syzygium* species or *Tibouchina* species respectively, have adapted to become a serious pathogen of plantation-grown *Eucalyptus* (Rodas *et al.*, 2005; Heath *et al.*, 2006; Gryzenhout *et al.*, 2009). Regardless as to whether *C. lagerstroemiae* is native to China or not, it clearly poses a threat to *Eucalyptus* propagated as non-natives in that country or elsewhere in the world. More importantly, it could easily be introduced into new areas such as Australia where Myrtaceae are native and where very serious damage to the natural environment could result. This would be similar to the situation that has arisen with the myrtle rust pathogen *Puccinia psidii* in Australia (Pegg *et al.*, 2014; Burgess & Wingfield, 2017) and it should be actively avoided.

Other than *Cryphonectria nitschkei* and *C. parasitica* that occur on the Fagaceae, species of Cryphonectriaceae occurring in China have all been isolated from Myrtaceae (Myrtales) trees. These trees include the native *R. tomentosa* and the non-native *S. cumini*, *Eucalyptus* species, and in this study *L. speciosa*, which are all found in South China. There are large numbers of native Myrtales in China including at least 19 genera (Chen, 1984). Consequently, surveys of these trees in China are likely to reveal additional members of the Cryphonectriaceae in this region of the world. The aggressive nature of many of these fungi as tree pathogens suggest that such surveys would be justified as part of a global strategy (Wingfield *et al.*, 2015) to reduce the impact of tree diseases globally.

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