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ORIGINAL ARTICLE

Novel Cryphonectriaceae from La Réunion and South Africa, and their pathogenicity on *Eucalyptus*

Daniel B. Ali[1] · Seonju Marincowitz[1] · Michael J. Wingfield[1] · Jolanda Roux[2] · Pedro W. Crous[1] · Alistair R. McTaggart[1]

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Abstract

Fungi in the Cryphonectriaceae are important canker pathogens of plants in the Melastomataceae and Myrtaceae (Myrtales). These fungi are known to undergo host jumps or shifts. In this study, fruiting structures resembling those of Cryphonectriaceae were collected and isolated from dying branches of *Syzygium cordatum* and root collars of *Heteropyxis natalensis* in South Africa, and from cankers on the bark of *Tibouchina grandifolia* in La Réunion. A phylogenetic species concept was used to identify the fungi using partial sequences of the large subunit and internal transcribed spacer regions of the nuclear ribosomal DNA, and two regions of the β-tubulin gene. The results revealed a new genus and species in the Cryphonectriaceae from South Africa that is provided with the name *Myrtonectria myrtacearum* gen. et sp. nov. Two new species of *Celoporthe* (*Cel.*) were recognised from La Réunion and these are described as *Cel. borbonica* sp. nov. and *Cel. tibouchinae* sp. nov. The new taxa were mildly pathogenic in pathogenicity tests on a clone of *Eucalyptus grandis*. Similar to other related taxa in the Cryphonectriaceae, they appear to be endophytes and latent pathogens that could threaten *Eucalyptus* forestry in the future.

Keywords Diaporthales · Die-back · Myrtales · New taxa · Stem canker · Tree disease · Taxonomic novelties: *Myrtonectria myrtacearum* gen. et sp. nov., *Celoporthe borbonica* sp. nov., *Celoporthe tibouchinae* sp. nov.

Introduction

The Cryphonectriaceae (Diaporthales, Ascomycota) accommodate fungi previously classified in the *Cryphonectria-Endothia* complex (Castlebury et al. 2002; Gryzenhout et al. 2006b). They include 22 genera and 74 species of facultative parasites, endophytes and saprobes (Chen et al. 2016, 2017; Gryzenhout et al. 2005a, b, 2010). Seven of these genera have been reported from Africa including *Aurifilum* (Begoude et al. 2010), *Celoporthe* (Nakabonge et al. 2006a), *Chrysoporthe* (Gryzenhout et al. 2004), *Diversimorbus* (Chen et al. 2013a), *Holocryphia* (Gryzenhout et al. 2006a), *Immersiporthe* (Chen et al. 2013b) and *Latruncellus* (Vermeulen et al. 2011).

In the Southern Hemisphere, various species of Cryphonectriaceae are regarded as high-risk pathogens because they cause canker and die-back diseases and have undergone host switches between native and cultivated trees, particularly in the Myrtales (Burgess and Wingfield 2017; Slippers et al. 2005; Van der Merwe et al. 2013; Wingfield et al. 2015). For example, *Chrysoporthe (Chr.) austroafricana* has undergone a host shift from species of native South African Myrtaceae to infect introduced species of *Eucalyptus* (Conradie et al. 1990; Heath et al. 2006; Myburg et al. 2002a; Nakabonge et al. 2006b; Vermeulen et al. 2011). Two other important species in this group, *Chr. cubensis* and *Chr. deuterocubensis* from South America and Southeast Asia respectively, have switched hosts between *Eucalyptus* and species of Melastomataceae (Myrtales) (Rodas et al. 2005; Seixas et al. 2004; Van der Merwe et al. 2013).

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✉ Seonju Marincowitz
Seonju.Marincowitz@fabi.up.ac.za

[1] Department of Biochemistry, Genetics and Microbiology, Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences (NAS), University of Pretoria, Private Bag X20, Pretoria 0028, South Africa

[2] Department of Plant and Soil Sciences, Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences (NAS), University of Pretoria, Private Bag X20, Pretoria 0028, South Africa

In Africa, species of Cryphonectriaceae infect genera of Myrtales including *Eucalyptus*, *Heteropyxis*, *Metrosideros*, *Syzygium* (Myrtaceae) (Chen et al. 2013a; Heath et al. 2006; Nakabonge et al. 2006a), *Terminalia* (Combretaceae) (Begoude et al. 2010) and *Tibouchina* (Melastomataceae) (Myburg et al. 2002a). These hosts are either non-native species, such as *Eucalyptus* and *Tibouchina*, or are native to South Africa including species of *Heteropyxis*, *Metrosideros*, *Syzygium* and *Terminalia*. Another genus of Cryphonectriaceae, *Diversimorbus*, is also known in South Africa where it causes a serious canker disease on *Rapanea* (Primulaceae, Ericales) (Chen et al. 2013a).

Several fungi with orange fruiting structures resembling species of Cryphonectriaceae that caused girdling cankers on species of Melastomataceae and Myrtaceae were found during disease surveys in La Réunion and South Africa. The aim of this study was to (i) characterise these fungi in the Cryphonectriaceae based on morphology and a phylogenetic species concept and (ii) determine their pathogenicity to *Eucalyptus*.

Materials and methods

Taxon sampling

Isolates used in this study arose from disease surveys of *Tibouchina grandifolia* in La Réunion and native Myrtales in Tzaneen (Limpopo Province), South Africa. These surveys targeted disease symptoms such as stem cankers and die-back and where orange or yellow fruiting structures were obvious on the bark associated with the cankers. Bark tissue was collected from infected trees and transported in brown paper bags to the laboratory in order to make isolations.

Asexual and sexual fruiting structures were observed on the sampled bark tissues. Using a dissecting microscope, these fruiting structures were dissected using a scalpel blade to open and reveal spore masses, which were transferred with a sterile needle to 2% malt extract agar (MEA) containing 100 mg/L streptomycin. Plates were incubated at 25 °C and pure cultures were obtained by transferring single hyphal tips to clean MEA plates. Cultures were deposited in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative cultures were deposited in the live culture collection (PPRI) of the South African National Collection of Fungi, Roodeplaat, Pretoria, South Africa (Table 1). Original bark specimens bearing fruiting structures associated with representative isolates were deposited in the dried herbarium collection (PREM) of the South African National Collection of Fungi, Roodeplaat, Pretoria, South Africa.

DNA extraction, PCR and sequencing

DNA was extracted from the mycelium following the methods described by Myburg et al. (1999). Concentrations and purity of the extracted DNA were determined with a NanoDrop 3.1.0 ND-1000u*v*/*V*is spectrophotometer (NanoDrop Technologies, Wilmington, Delaware).

Polymerase chain reactions (PCRs) were performed following the method described by Glass and Donaldson (1995). The nuclear large subunit (LSU) and the internal transcribed spacer (ITS) regions of ribosomal DNA were amplified using primer pairs LR0R/LR5 (Vilgalys and Hester 1990) and ITS1/ITS4 (White et al. 1990). The β-tubulin gene region (BT) was amplified using primer pairs BT1a/1b and BT2a/2b (Myburg et al. 1999, 2002b). The PCR products were sequenced in both directions with the Big Dye Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI PRISM™ 3100 automated DNA sequencer (Applied Biosystems, Foster City, California) at the Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria. Gene sequences were viewed and edited with CLC Main Workbench, CLC BIO 5.5 (CLC bio A/S, Science Park Aarhus, Finlandsgade 10–12, 8200 Aarhus N, Denmark).

Phylogenetic analyses

A phylogenetic species concept was used to identify the isolates. Isolates from this study were compared to published sequences of type species of the Cryphonectriaceae (Table 1). The generic relationships in the Cryphonectriaceae were analysed with a concatenated dataset of LSU, ITS and BT (including partial exon 4 and 5, partial exons 6 and 7) sequences with taxa selected from ex-types of described genera in the Cryphonectriaceae (Begoude et al. 2010; Chen et al. 2013a, 2016, 2017; Crane and Burgess 2013). The relationships between species were revealed in analyses of concatenated ITS and BT genes. Each gene region was analysed separately to determine whether they were concordant (Taylor et al. 2000). *Diaporthe ambigua* (CMW 5288) was used as an out-group taxon for phylogenetic analyses of genera in the Cryphonectriaceae, while *Aurapex penicillata* (CMW 10030) was used as an out-group for species of *Celoporthe*.

Sequences were aligned using the iterative refinement method (FFT-NS-I settings) of MAFFT 5.667 (Katoh et al. 2009). The alignments were concatenated and deposited in TreeBASE (www. treebase.org accession 21,995, Reviewer access: http://purl.org/phylo/treebase/phylows/study/TB2:S21995?x-access-code=9ed7741049c56af410fda93ec32200b3&format=html). Maximum likelihood searches for the best scoring tree were conducted with RAxML v8.2.X using the fixed General Time Reversal (GTR) model with non-parametric bootstrapping of 1000 replicates (command–f a) (Stamatakis 2014). Bayesian analyses were performed using Mr Bayes v3.2 (Ronquist and

Table 1 Isolates of Cryphonectriaceae sequenced and used in phylogenetic analyses and pathogenicity test in this study

Identity	Isolate no.	Host	Location	GenBank accession no.				References
				LSU	ITS	BT1	BT2	
Amphilogia gyrosa	CMW10469	*Elaeocarpus dentata*	New Zealand	AY194107	AF452111	AF525707	AF525714	Gryzenhout et al. (2005a, 2006b)
Aurantioporthe corni	MES1001	*Cornus alternifolia*	USA	N/A	KF495039	KF495069	N/A	Beier et al. (2015)
Aurapex penicillata	CMW10030	*Microthia theaezens*	Colombia	AY194103	AY214311	AY214239	AY214275	Gryzenhout et al. (2006c, 2009)
Aurifilum marmelostroma	CMW28285	*Terminalia mantaly*	Cameroon	HQ171215	FJ882855	FJ900585	FJ900590	Begoude et al. (2010)
Myrtonectria myrtacearum	CMW46433	*Heteropyxis natalensis*	South Africa	MG585750	MG585736	MG585720	MG585734	This study
Myrtonectria myrtacearum	CMW46435	*Syzygium cordatum*	South Africa	MG585751	MG585737	MG585721	MG585735	This study
Celoporthe dispersa	CMW9976	*Syzygium cordatum*	South Africa	HQ730853	DQ267130	DQ267136	DQ267142	Nakabonge et al. (2006a)
Cel. dispersa	CMW9978	*S. cordatum*	South Africa	HQ730852	DQ267136	DQ267142	AY214316	Nakabonge et al. (2006a, b)
Cel. eucalypti	CMW26900	*Eucalyptus* clone EC48	China	HQ730862	DQ267136	HQ730816	HQ730826	Chen et al. (2011)
Cel. eucalypti	CMW26908	*Eucalyptus* clone EC48	China	HQ730863	HQ730837	HQ730817	HQ730827	Chen et al. (2011)
Cel. fontana	CMW29376	*Syzygium guineense*	Zambia	NA	GU726941	GU726953	GU726953	Chen et al. (2011)
Cel. fontana	CMW29375	*Syzygium guineense*	Zambia	N/A	GU726940	GU726952	GU726952	Vermeulen et al. (2013)
Cel. guangdongensis	CMW12750	*Eucalyptus* sp.	China	HQ730856	HQ730830	HQ730810	HQ730820	Chen et al. (2011)
Cel. indonesiensis	CMW10781	*Syzygium aromaticum*	Indonesia	HQ730855	AY084009	AY084033	AY084021	Chen et al. (2011)
Cel. borbonica	CMW44121	*Tibouchina grandiflora*	La Réunion	NA	MG585738	NA	NA	This study
Cel. borbonica	CMW44123	*T. grandiflora*	La Réunion	NA	MG585739	MG585723	NA	This study
Cel. borbonica	CMW44125	*T. grandiflora*	La Réunion	NA	MG585740	MG585724	NA	This study
Cel. borbonica	CMW44128	*T. grandiflora*	La Réunion	NA	MG585741	MG585725	NA	This study
Cel. borbonica	CMW44139	*T. grandiflora*	La Réunion	NA	MG585742	MG585726	NA	This study
Cel. borbonica	CMW44143	*T. grandiflora*	La Réunion	NA	MG585743	MG585727	NA	This study
Cel. borbonica	CMW44144	*T. grandiflora*	La Réunion	NA	MG585744	MG585728	NA	This study
Cel. borbonica	CMW44146	*T. grandiflora*	La Réunion	NA	MG585745	MG585729	NA	This study
Cel. borbonica	CMW44150	*T. grandiflora*	La Réunion	NA	MG585746	MG585730	NA	This study
Cel. syzygii	CMW24912	*Syzygium cumini*	China	HQ730859	HQ730833	HQ730813	HQ730823	Chen et al. (2011)
Cel. syzygii	CMW34023	*Syzygium cumini*	China	HQ730857	HQ730831	HQ730811	HQ730821	Chen et al. (2011)
Cel. tibouchineae	CMW44126	*T. grandiflora*	La Réunion	NA	MG585747	MG585731	NA	This study
Cel. tibouchineae	CMW44127	*T. grandiflora*	La Réunion	NA	MG585748	MG585732	NA	This study
Cel. tibouchineae	CMW44147	*T. grandiflora*	La Réunion	NA	MG585749	MG585733	NA	This study
Cel. woodiana	CMW13936	*Tibouchina granulosa*	South Africa	NA	DQ267131	DQ267137	DQ267143	Vermeulen et al. (2013)
Cel. woodiana	CMW13937	*T. granulosa*	South Africa	NA	DQ267132	DQ267138	DQ267144	Vermeulen et al. (2013)
Corticimorbus sinomyrti	CERC 3055	*Rhodomyrtus tomentosa*	China	KT167172	KT167162	KT167182	KT167182	Chen et al. (2016)

Table 1 (continued)

Identity	Isolate no.	Host	Location	GenBank accession no.				References
				LSU	ITS	BT1	BT2	
Cryphonectria nitschkei	CMW13742	*Quercus grosseserrata*	Japan	NA	AY697936	AY697961	AY697962	Myburg et al. (2004)
Cryphonectria decipiens	CMW10436	*Quercus suber*	Portugal	JQ862750	AF452117	AF525703	AF525710	Myburg et al. (2004)
Cryphonectria macrospora	CMW10463	*Castanea cuspidata*	Japan	NA	AF368331	AF368351	AF368350	Gryzenhout et al. (2006c)
Cryphonectria parasitica	CMW7048	*Quercus virginiana*	USA	AY194100	AF368330	AF273076	AF273470	Gryzenhout et al. (2006a), Venter et al. (2001)
Cryphonectria radicalis	CMW10477	*Quercus suber*	Italy	AY194102	AF368328	AF368347	AF368347	
Cryptometrion aestuescens	CMW18790	*Eucalyptus grandis*	Indonesia	HQ171211	GQ369458	GQ369455	GQ369455	Gryzenhout et al. (2010)
Diversimorbus metrosiderotis	CMW37321	*Metrosideros angustifolia*	South Africa	JQ862827	JQ862870	JQ862911	JQ862952	Gryzenhout et al. (2010)
Holocryphia capensis	CMW37329	*Metrosideros angustifolia*	South Africa	JQ862816	JQ862859	JQ862900	JQ862941	Chen et al. (2013a)
Holocryphia eucalypti	CMW7033	*Eucalyptus grandis*	South Africa	JQ862794	JQ862837	JQ862878	JQ862919	Chen et al. (2013b)
Immersiporthe knoxdaviesiana	CMW37314	*Rapanea melanophiloeos*	South Africa	JQ862755	JQ862765	JQ862785	JQ862775	Chen et al. (2013a, b)
Latruncellus aurorae	CMW28274	*Galpinia transvaalica*	Swaziland	HQ171213	GU726946	GU726958	GU726958	Vermeulen et al. (2011)
Luteocirrhus shearii	CBS 130775	*Bankesia baxteri*	Australia	KC197018	KC197024	KC197015	KC197009	Crane & Burgess (2013)
Microthia havanensis	CMW11301	*Myrica faya*	Azores	N/A	AY214323	AY214251	AY214287	Gryzenhout et al. (2006a)
Rostraureum tropicale	CMW10796	*Terminalia ivorensis*	Ecuador	N/A	AY167438	AY167428	AY167433	Gryzenhout et al. (2005b)
Ursicollum fallax	CMW18115	*Coccoloba uvifera*	USA	N/A	DQ368756	DQ36860	DQ368761	Gryzenhout et al. (2006a)

Huelsenbeck 2003). An MCMC analysis was run for 10 million generations with four runs each consisting of four chains heated at the default temperature. Trees were sampled every 1000 generations and a 25% burn-in was used to summarise a consensus from 30,000 trees.

Microscopy and growth study

In order to observe the configuration of fruiting structures and morphology of stromatic tissues in the substrate, pieces of bark bearing fungal fruiting structures were dissected under a dissecting microscope. These specimens were boiled in water for 1 min to hydrate the specimens and mounted with Leica Tissue Freezing Medium® on a disc. Frozen tissue was sectioned (12–16 μm thick) using a Leica CM1100 cryostat (Leica, Wetzlar, Germany) set at − 20 °C. Fungal structures were mounted on microscope slides in water that was later replaced with 85% lactic acid in which measurements were made and photographic images captured. For the holotype specimens of putative new taxa, up to 50 measurements were made for each of the structures whenever possible, while characters for the remaining specimens were measured 25 times. Nikon cameras (DS-Ri2, SMZ18) with NIS Elements software (Nikon, Tokyo, Japan) were used to capture images and to determine dimensions of the structures. Characteristics of specimens were compared with those published for closely related species in the Cryphonectriaceae (Begoude et al. 2010; Chen et al. 2016, 2017; Gryzenhout et al. 2009; Vermeulen et al. 2011).

Culture characteristics were studied for the putative new taxa using two or three representative isolates from different areas and hosts. Growth in culture was assessed by transferring 5-mm-diameter discs of mycelium from 7-day-old cultures to the centres of 90-mm plates containing 2% MEA. The cultures were grown in the dark and incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Five replicate plates were used for each isolate at each temperature. Growth rate of cultures was measured by the diameter of two points at right angles to each other. Measurements were taken daily until the colonies reached the edges of the plates and average growth rates were calculated. The entire experiment was repeated once and colour designations were obtained for the descriptions of cultures and fruiting bodies using the colour charts of Rayner (1970).

Two South African isolates (CMW 46433 and CMW 46435) were grown on media that contained bark and wood tissue taken from branches of *Syzygium cordatum* to stimulate development of fruiting structures. The *Syzygium cordatum* branch sections (1.5–2-cm diameter × 5-cm length) were collected from trees in the Limpopo Province. These were autoclaved at 121 °C for 20 min, placed on the surfaces of 2% water agar in Petri dishes, inoculated with fungi and incubated at 25 °C for 6 weeks until fruiting structures appeared.

Pathogenicity tests

Pathogenicity tests were conducted using two isolates, each of the putative new taxa. These included four isolates from La Réunion (CMW 44126–44128, CMW 44139) and two from South Africa (CMW 46433, CMW 46435). The isolates were inoculated onto 10 trees each of a *Eucalyptus grandis* clone (TAG 5) in a temperature-controlled greenhouse. *Eucalyptus grandis* clone TAG 5 was used because it is moderately susceptible to species of *Cryphonectriaceae*. *Chrysoporthe austroafricana* (CMW 2113), known to be highly pathogenic to *Eucalyptus*, was used as a positive control, and sterile plugs of MEA were used as negative controls.

Trees for inoculation were acclimatised in a greenhouse environment at 25 °C with 14 h of daylight for approximately 1 month prior to inoculations. Cultures of the six test isolates, and the positive control isolate, were grown at 25 °C under continuous fluorescent light for 6 days before making the inoculations. The inoculated trees were 2 years old and 2 m tall with main stems having diameters between 10 and 15 mm.

Agar discs (3-mm diameter) were cut from the margins of actively growing fungal cultures and placed, mycelial surface facing the cambium, into wounds of the same size made on the stems of trees with a cork borer. The wounds were covered with a strip of Parafilm (Bemis, Wisconsin) to prevent desiccation or cross contamination. All six test isolates as well as the positive and negative controls were inoculated onto the stems of 10 trees each. The experiment thus included a total of 80 inoculated trees, which were arranged in a fully randomised block design in a greenhouse maintained at approximately 25 °C and with natural daylight.

After 6 weeks, lesions that had developed in the cambium were measured after the bark had been removed from the sites of inoculation. Pieces of necrotic tissue were taken from lesions on four trees representing each source of inoculum and the controls. The pieces were placed onto the surface of 2% MEA and incubated at 25 °C for re-isolation of the inoculated fungi. The pathogenicity trial was repeated once under the same conditions.

To assess the variation in lesion length associated with the inoculations, means were analysed in SAS 8 with PROC GLM (general linear model) (SAS Institute Inc. 1999). Analysis of variance (ANOVA) was conducted to determine the effects of the fungal strains on lesion length. Before ANOVA, homogeneity of variance across treatments was confirmed. Fisher's protected test was used to determine the significance among means and $P < 0.05$ for the F value taken as significant in difference.

Results

Taxon sampling

In total, 14 samples bearing structures resembling species in the Cryphonectriaceae, with characteristic orange/yellow fruiting structures, were collected from bark on the stems and branches of diseased plants. Of these, 12 samples were collected from *T. grandiflora* in La Réunion, while two samples were collected from native *Heteropyxis natalensis* and *S. cordatum* in Limpopo, South Africa. All isolates were obtained from asexual (conidiomata) fruiting structures except for CMW 44128 from La Réunion for which cultures were isolated from sexual structure.

DNA sequencing and phylogenetic analyses

Phylogenetic analyses using Bayesian inference and maximum likelihood of the combined datasets of LSU, ITS and BT genes identified a putative new genus for the two isolates collected from *H. natalensis* and *S. cordatum* in South Africa (Fig. 1). The isolates were not congeneric with any described genera in the Cryphonectriaceae. A new genus and species are thus described to accommodate them.

Two putative new species of *Celoporthe* were recognised based on analyses of sequences for the ITS and BT gene regions for isolates from *T. grandifolia* in La Réunion. The 12 isolates collected from this host were not conspecific with any of the described species of *Celoporthe* (Fig. 2). Species names are consequently provided for them.

Taxonomic part

Myrtonectria Marinc., D. B. Ali, & J. Roux, *gen. nov.*—MycoBank MB824022; Fig. 3

Etymology—Name refers to the fact that the fungus can potentially kill trees belonging to the Myrtaceae.

Sexual state not observed. *Conidiomata* semi-immersed or superficial, single or gregarious, irregular shape or globose to pyriform, with or without protruding necks, excreting orange pigment in lactic acid and purple in 2% KOH; *necks* cylindrical, tapering towards apex, ostioles brown to dark brown. *Stromatic tissue* varies from *textura intricata*, *globulosa* to *angularis* depending on location of layers. *Periphyses* present near ostiole. *Conidiophores* branched at base, less along length, septate, occasionally reduced to conidiogenous cells. *Conidiogenous cells* blastic, discrete, lateral or terminal, lageniform and abruptly tapering to apex, with very narrow aperture. *Conidia* hyaline, aseptate, oblong with pointed base.

Myrtonectria myrtacearum Marinc., D. B. Ali & J. Roux. *sp. nov.*—MycoBank MB824023; Fig. 3

Etymology—Name refers to the occurrence of this fungus on species of Myrtaceae.

Conidiomata semi-immersed to superficial, dark greyish brown, glossy, uniloculate, convoluted, with or without protruding necks with spore droplets at apex, 345–1340 μm long, 240–660 μm wide; *necks* cylindrical, tapering towards apex, 240–535 μm long, 115–260 μm wide, excreting orange pigment in lactic acid and purple pigment in 2% KOH. *Stromata* eustromatic except for base (pseudostromatic); s*tromatic tissue*s in middle layers *textura intricata*, in inner and outer layers *textura globulosa* to *textura angularis*; innermost walls composed of a few layers of compressed thin-walled cells, outermost walls composed of a few layers of thick-walled cells. *Periphyses* present near ostiole. *Paraphyses* not observed. *Conidiophores* borne in a single layer along locule, branched at base, less along length, septate, occasionally reduced to conidiogenous cells. *Conidiogenous cells* blastic, discrete, lateral or terminal, lageniform and abruptly tapering to apex, with very narrow aperture, 5.5–12.5 μm long, 1.5–3 μm wide near base. *Conidia* hyaline, aseptate, oblong with pointed base, 3–5.5 × 1.5–2 μm (avg. 3.9 × 1.7 μm).

Culture characteristics—On 2% MEA colonies optimum growth at 25 °C covering the entire 90 mm plate in 7 days, but no growth at 10 and 35 °C, mycelium flat and smooth, white when young, becoming pale to moderate yellow with orange tint at the centre.

Substrate—Bark of *H. natalensis* and *S. cordatum*

Distribution—South Africa (Limpopo Province, Tzaneen)

Specimens examined. South Africa, Limpopo Province, Tzaneen, New Agatha plantation (23° 53′ 18.89″ S, 30° 05′ 07.29″ E), on bark of *Syzygium cordatum* Hochst. ex C. Krauss., 29 June 2015, B. D. Ali & J. Roux, holotype PREM 62179, ex-holotype PPRI 25128 = CMW 46433; ibid. on bark of *Heteropyxis natalensis* Harvey, 29 June 2015, B. D. Ali & J. Roux, PREM 62180, culture PPRI 25129 = CMW 46435.

Notes—*Myrtonectria* can be distinguished from other genera in the Cryphonectriaceae by its shiny dark grey and globose to pyriform conidiomata, orange stromatic tissue and the presence of periphyses. Besides *Myrtonectria*, *Aurapex* is the only genus which produces periphyses in conidiomatal neck in the family based on published data.

Celoporthe tibouchinae Marinc., D. B. Ali & M. J. Wingf. *sp. nov.*—MycoBank MB824024; Fig. 4

Etymology—Name refers to the genus *Tibouchina*, the shrub from which the fungus was isolated.

Conidiomata immersed, erumpent, dark brown to black, single, scattered or gregarious, hemispherical or conical, 125–395 μm long, 90–400 μm wide, with an elevated ostiole (or short neck), uni- or multiloculate, convoluted. *Stromatic tissues* in middle often scanty but filled with reflective granules, in innermost and outermost walls

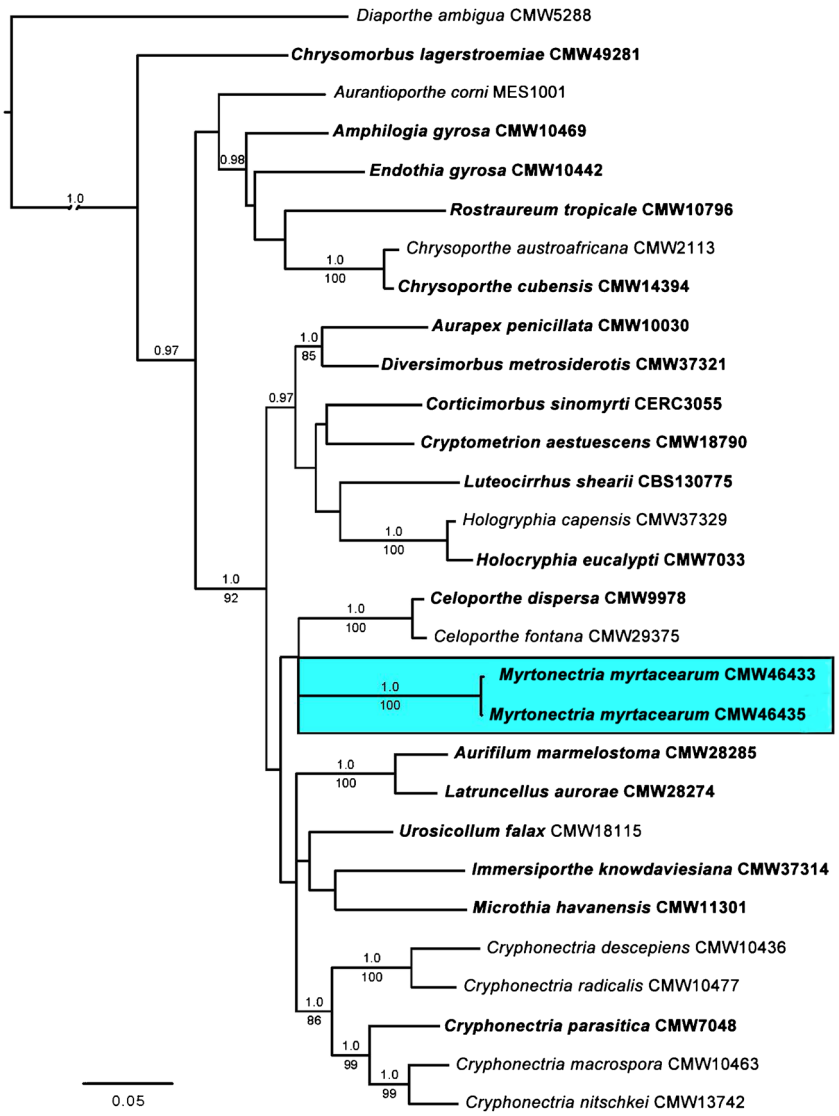


Fig. 1 A maximum likelihood (ML) phylogram from combined data sets of the LSU and ITS regions of rDNA and partial exon 4 and exon 5, and partial exon 6 and 7 of the BT1 and BT2 genes. Statistical bootstrap values > 70% for ML analysis are shown above nodes, and posterior probabilities > 0.95 from Bayesian Inference are shown below nodes. Isolates of the new genus are in highlighted. The type species of the genera are in bold

composed of compressed cells of *textura angularis*, near apex *textura globulosa*. *Periphyses* not observed. *Paraphyses* present, hyaline, simple, cylindrical, septate, 16–43 µm long, 1–2 µm wide. *Conidiophores* borne along the locular walls, branched at the base. *Conidiogenous cells* blastic, discrete, hyaline, lageniform, 5.5–10 µm long, 1–3 µm wide. *Conidia* hyaline, aseptate, oblong to ellipsoidal, with pointed base, 2.5–4.5 × 1–1.5 µm (avg. 3.1 × 1.2 µm).

Culture characteristics—On 2% MEA colonies optimum growth at 30 °C covering entire 90 mm plate in 8 days, orange yellow with white margins and darker centres in reverse, mycelium flat.

Substrate—Bark of *Tibouchina grandifolia*

Distribution—La Réunion (St. Joseph)

Specimens examined. La Réunion (French territory), St. Joseph region (20° 54′ 38.09″ S, 55° 36′ 04.73″ E), on bark of *Tibouchina grandifolia* Cogn., March 2015, M. J. Wingfield, holotype PREM 62178, culture ex-holotype PPRI 25130 = CMW 44126); other cultures CMW 44127 = PPRI 25131, CMW 44147 = PPRI 25132.

Note—The two undescribed species of *Celoporthe* from La Réunion were closely related to each other and formed sister taxa to *Cel. guangdongensis* and *Cel. indonesiensis*. These species are morphologically alike, but the presence of reflective granules in the middle of stromatic tissue is unique to *Cel. tibouchineae*, and has not been reported in other species. The optimal growth temperature of *Cel. tibouchineae* was also similar to that of *Cel. guangdongensis* and *Cel. indonesiensis* at 30 °C, whereas *Cel. borbonica* grew best at 25 °C.

Celoporthe borbonica Marinc., D. B. Ali & M. J. Wingf. sp. nov—MycoBank MB824025; Fig. 5

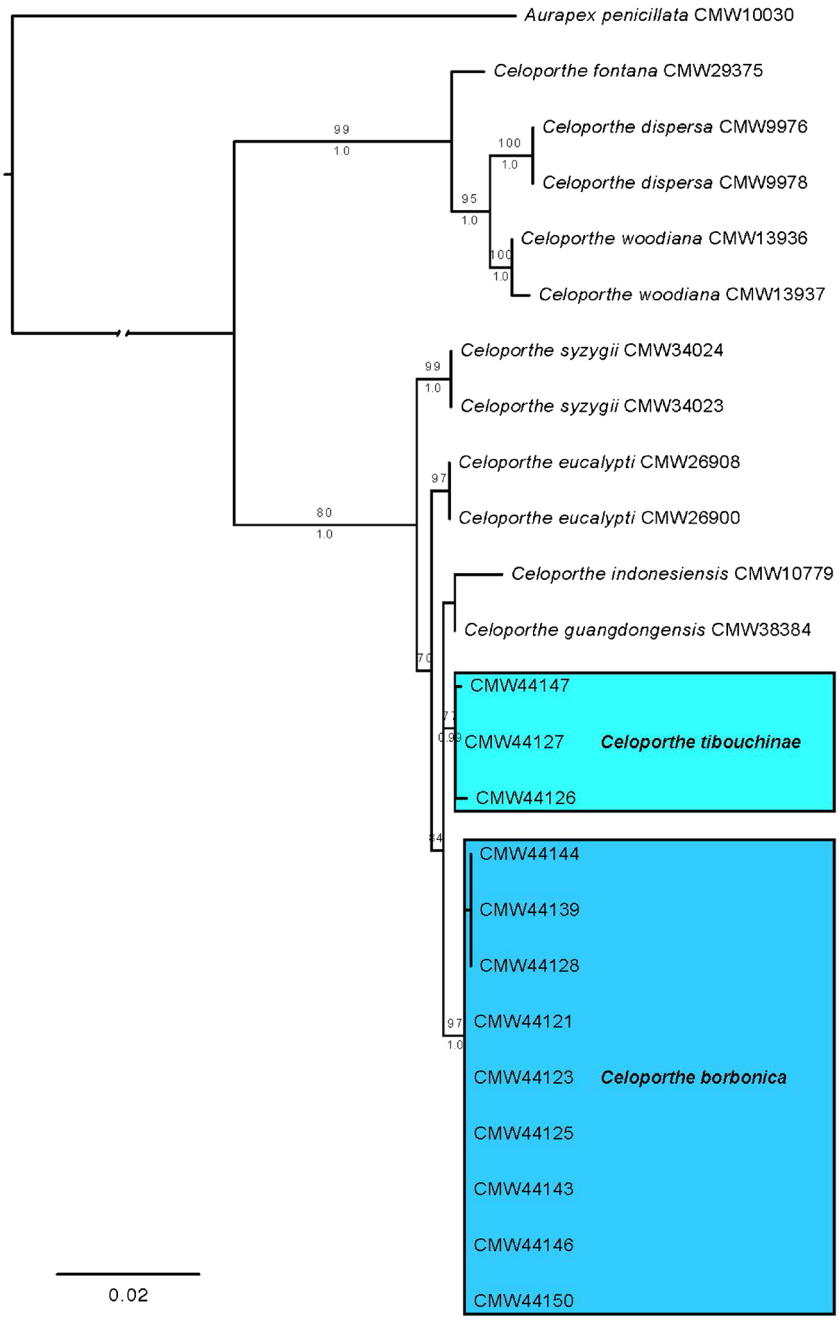
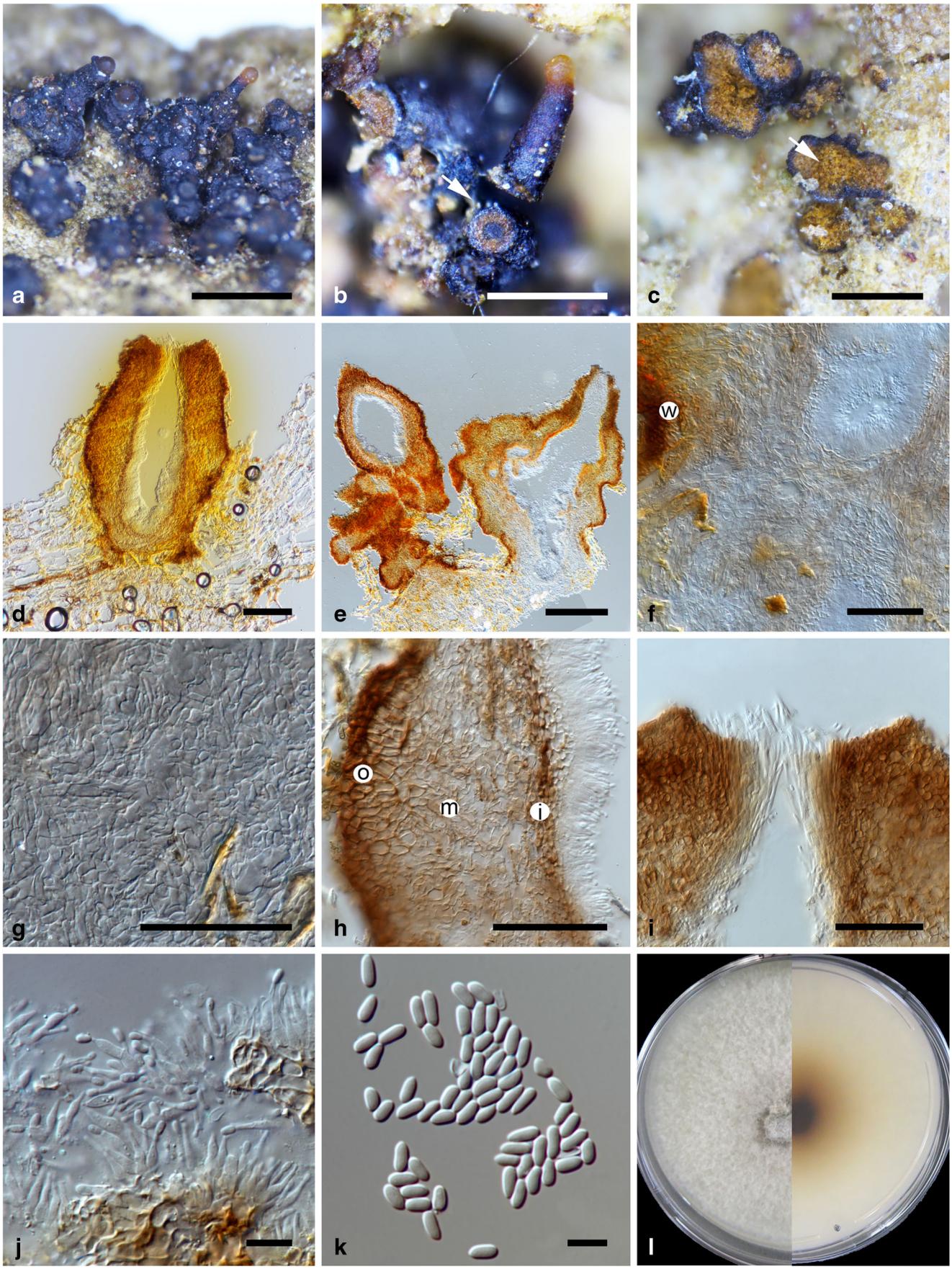


Fig. 2 A maximum likelihood (ML) phylogram from combined data sets of the ITS region of rDNA and partial exon 4 and exon 5, and partial exon 6 and 7 of the BT1 and BT2 genes. Statistical bootstrap values > 70% for ML are shown on nodes and posterior probabilities > 0.95 from Bayesian Inference are shown below nodes. Isolates of the new species are in bold face and highlighted

Etymology—Name refers to Bourbon, the former name of La Réunion, from where the fungus was collected.

Ascostromata semi-immersed, erumpent, off-white to creamy, with necks, single or gregarious. *Stromatic tissues* prosenchymatous at sides and base, *textura angularis* at the base of neck. *Perithecia* sub-globose to ellipsoidal, valsoid, necks convergent, erumpent separately, periphyses present along length, peridial walls pseudoparenchymatous, dark olivaceous brown, outer wall composed of a few layers of compressed, brown, thick-walled cells, inner wall composed of hyaline, thin-walled cells; *necks* cylindrical, 230–395 μm long, 45–70 μm wide, *Asci* clavate to

Fig. 3 Micrographs illustrating *Myrtonectria myrtacearum* (holotype ▶ PREM 62179, ex-holotype CMW 46433 = PPRI 25128). **a** Conidiomata on the substrate; **b**, **c** broken neck and conidioma showing orange stromatic structure (arrows) and dark-coloured outer wall; **d**, **e** vertical section of conidiomata mounted in 85% lactic acid (**d** showing exuding yellow to orange pigment); **f**, **g** pseudostromatic structure at the base of conidioma (w = conidiomatal wall); **h** close-up of conidiomatal wall and stromatic structure; in the middle *textura intricata* (m), and in the outermost (o) and the innermost wall (i) *textura globulosa* to *textura angularis*; **i** periphyses near the ostiole; **j** conidiophores and conidiogenous cells; **k** conidia; **l** culture grown at 25 °C in the dark for 7 days on 2% MEA (right, below; left, above). Scale bars: **a**–**c**, **e** = 250 μm; **d** = 100 μm; **f**–**i** = 50 μm; **j** = 10 μm; **k** = 5 μm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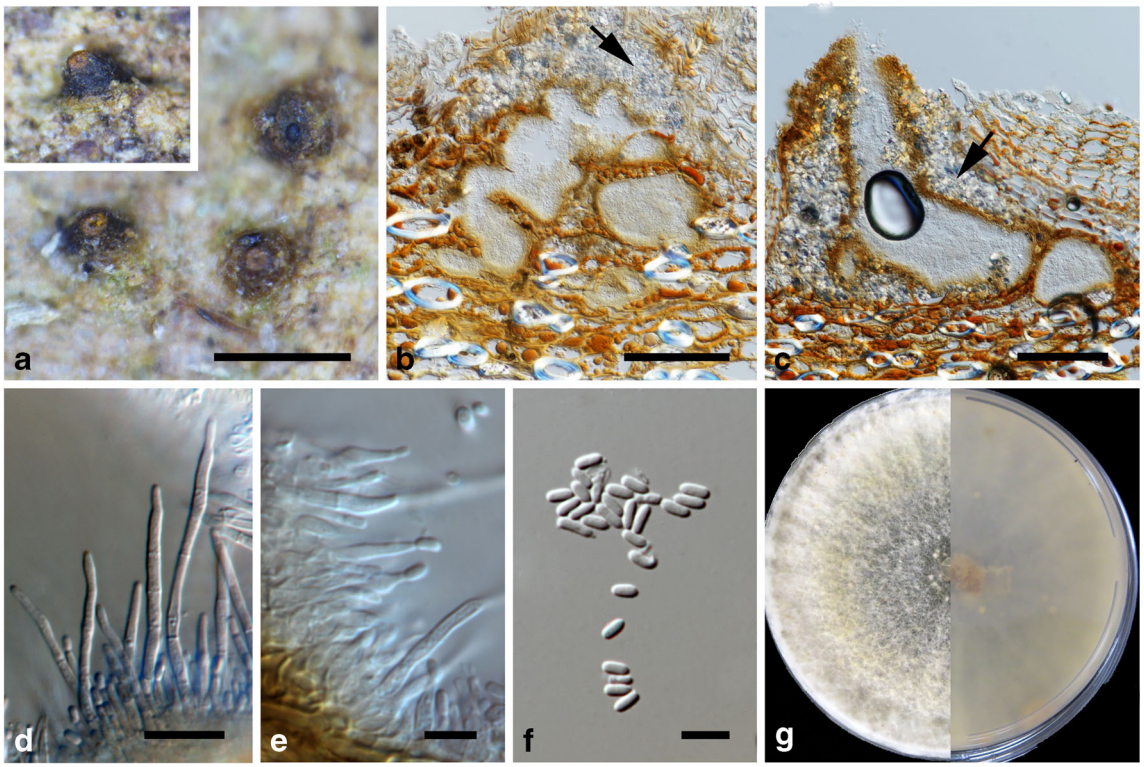


Fig. 4 Micrographs illustrating *Celoporthe tibouchinae* (holotype PREM 62178, ex-holotype CMW 44126 = PPRI 25130). **a** Conidiomata in the substrate; **b**, **c** vertical section of conidiomata showing shiny granules (arrows) in the middle layer of stroma; **d** paraphyses; **e** conidiogenous cells; **f** conidia; **g** culture grown at 30 °C in the dark for 8 days on 2% MEA (left, above; right, below). Scale bars: **a** = 250 μm; **b**, **c** = 100 μm; **d** = 10 μm; **e**, **f** = 5 μm

cylindrical, with non-amyloid refractive ring in apex, with deliquescent base, and lying free in ascoma cavity, 30–42 μm long, 4.5–7 μm wide. *Ascospores* hyaline, ellipsoidal, 2-celled, septum mostly median, straight or slightly curved, 6–10 × 2–3.5 μm (avg. 8.1 × 2.5 μm). *Conidiomata* immersed, erumpent, single, brown to black, scattered or gregarious, hemispherical or conical, uni- or multiloculate, convoluted, 280–415 μm long, 385–550 μm wide, with an ostiole: *ostioles* bright coloured. *Stromatic tissues* pale brown. *Periphyses* not observed. *Paraphyses* hyaline, cylindrical, septate, occasionally branched, 6.5–13.5 μm long, 3–7 μm wide. *Conidiophores* borne along locule, branched at base or reduced to conidiogenous cells. *Conidiogenous cells* hyaline, lageniform, 5–9.5 × 1–2 μm (avg. 7 × 1.7 μm). *Conidia* hyaline, aseptate, oblong to allantoid 2.5–4.5 × 1–1.5 μm (avg. 3.3 × 1.3 μm).

Culture characteristics—On 2% MEA colony optimum growth at 25 °C in dark for 7 days, limited growth at 10 and 35 °C. Mycelium buff to honey, being cinnamon at the centre, flat and smooth with even margin. Colonies white when young, turns dark with age. Colony colour the same on the reverse.

Substrate—Bark of *Tibouchina grandifolia*

Distribution—La Réunion (St. Joseph)

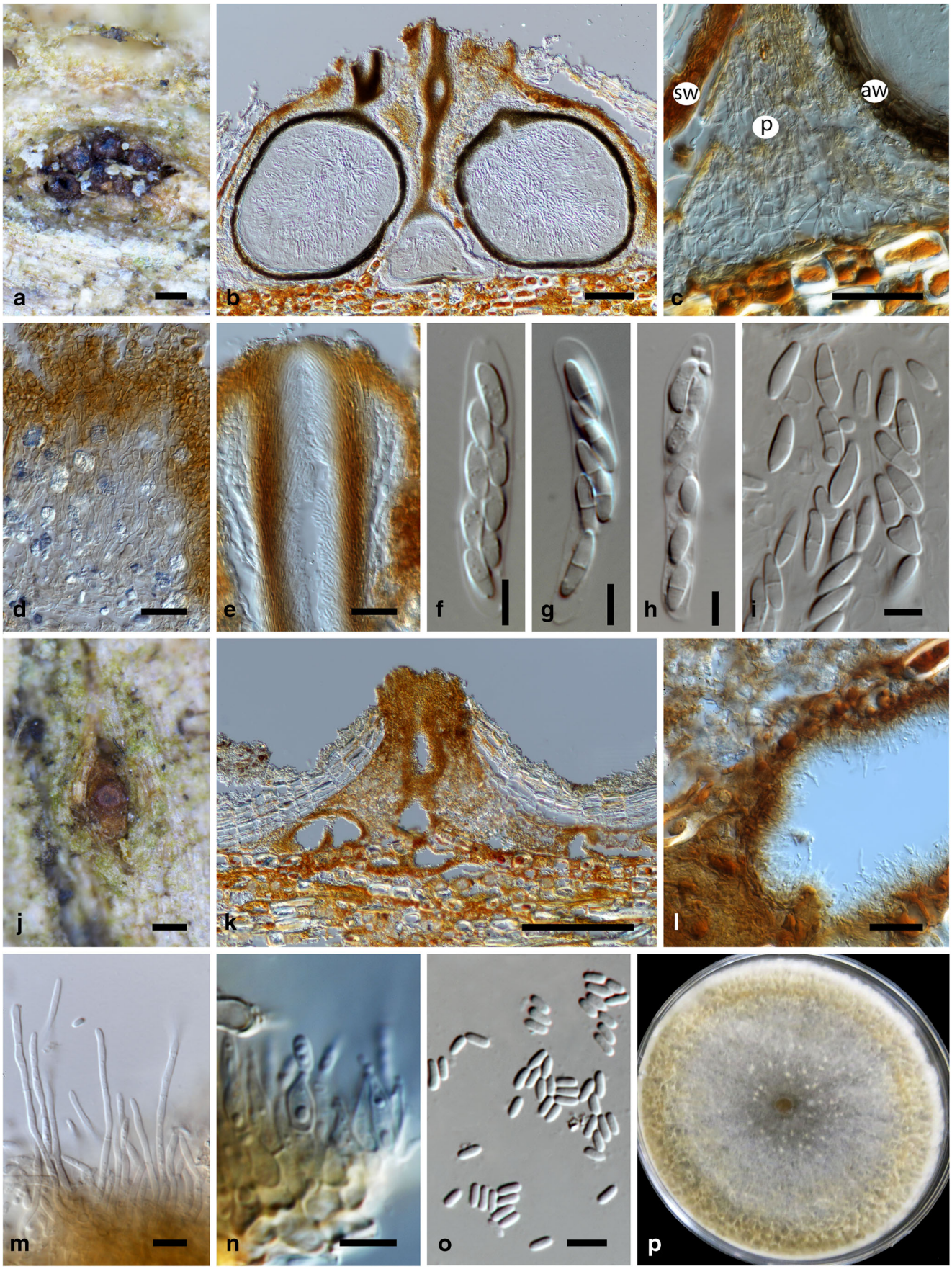
Specimens examined. La Réunion (French territory), St. Joseph region (20°54′38.09″S, 55°36′04.73″E), on bark of *Tibouchina grandifolia*, March 2015, M. J. Wingfield, holotype PREM 62177, culture ex-holotype PPRI 25133 = CMW 44128); CMW 44139 = PPRI 25134, CMW 44144 = PPRI 25135.

Note—The asexual morph of *Cel. borbonica* is very similar to that of *Cel. tibouchinae*. *Celoporthe borbonica* has a lower optimal growth temperature (25 °C) than *Cel. tibouchinae* (30 °C). In comparison with other species of *Celoporthe* that produce a sexual morph, *Cel. borbonica* has slightly larger ascospores (6–10 × 2–3.5 μm) than *Cel. dispersa* (4.5–8 × 2–3.5 μm) and *Cel. syzygii* (5–8.5 × 2.5–3.5 μm).

Pathogenicity to Eucalyptus

All the isolates used in the pathogenicity trials gave rise to lesions on the stems of inoculated *Eucalyptus* clone TAG 5.

Fig. 5 Micrographs illustrating *Celoporthe borbonica* (holotype PREM 62177, ex-holotype CMW 44128 = PPRI 25133). **a** Ascostroma in the substrate; **b** vertical section of ascostroma; **c** close-up of ascostroma (sw = stromatal wall, p = prosenchymatous tissue, aw = ascomatal wall); **d** stromatic tissue in the middle; **e** vertical section of ascomatal neck; **f**–**h** ascus; **i** ascospores; **j** conidioma in the substrate; **k** vertical section of conidioma; **l** stromatic tissue of conidioma; **m** paraphyses; **n** conidiogenous cells; **o** conidia; **p** culture grown at 25 °C in the dark for 7 days on 2% MEA.—Scale bars: **k** = 250 μm; **a**, **b**, **j** = 100 μm, **c** = 50 μm; **d**, **e**, **l** = 25 μm; **f**–**i**, **m**–**o** = 5 μm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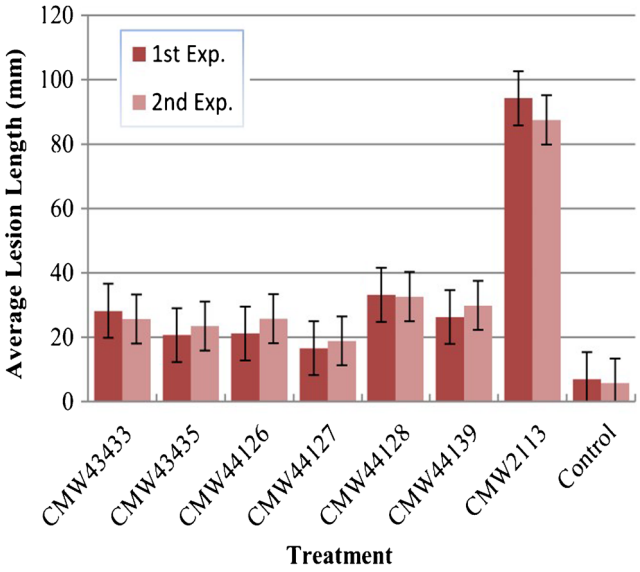

Fig. 6 Histogram showing the average lesion lengths resulting from inoculation trials for experiment 1 and 2 on stems of *Eucalyptus* clone TAG 5. The treatment includes two isolates of *Myrtonectria myrtacearum* (CMW 46433, CMW 46435), two isolates of *Celoporthe tibouchinae* (CMW 44126, CMW 44127), two isolates of *Celoporthe borbonica* (CMW 44128, CMW 44139) and a positive (CMW 2113) and negative control. Vertical bars represent standard error of means

The negative controls formed callus tissue around the inoculation wounds and no lesions developed. The mean comparison tests showed that the average lesion lengths caused by the two isolates of *Myrtonectria myrtacearum*, two isolates of *Cel. tibouchinae*, two of *Cel. borbonica*, and one isolate of *Chrysoporthe austroafricana* used as a positive control, were all significantly longer ($p < 0.001$) than the negative control. The results of the repeat experiment were the same. Isolates CMW 46433, CMW 44128 and CMW 44126 which represented *Myr. myrtacearum*, *Cel. borbonica* and *Cel. tibouchinae*, respectively, were more aggressive than the remaining isolates of those species. However, these isolates were all less aggressive than the positive control (*Chr. austroafricana)*, which killed some plants during the course of the experiment (Figs. 6, and 7).

Discussion

In this study, a combination of morphological features and phylogenetic inference based on DNA sequence data was used to identify a new genus and two new species of Cryphonectriaceae from cankers on trees and shrubs in the Myrtales. The new genus was identified from two species of Myrtaceae in South Africa and was named *Myrtonectria* to accommodate the new species *Myr. myrtacearum*. Two new species of *Celoporthe* were found associated with cankers on *Tibouchina grandifolia* in La Réunion, and these were provided with the names *Cel. borbonica* and *Cel. tibouchinae*. Pathogenicity tests on *E. grandis* saplings showed that none of the new taxa had high levels of aggressiveness.

Myrtonectria myrtacearum described here is the 23rd genus recognised in the Cryphonectriaceae as defined by Gryzenhout et al. (2006b). Based on phylogenetic inference, this fungus belongs to a group of genera that are closely related to the important canker pathogens of trees and shrubs in the Myrtales and mostly the Myrtaceae. The occurrence of *Myr. myrtacearum* associated with cankers on *H. natalensis* and *S. cordatum* is consistent with the niche on which other genera and species of Cryphonectriaceae have been found in southern hemisphere in the past. These species of Cryphonectriaceae are related to important canker pathogens on non-myrtaceae hosts in the northern hemisphere. Those species include *Cryphonectria parasitica*, the causal agent of chestnut blight, which has devastated *Castanea* spp. (Fagaceae, Fagaceales) in Europe and North America, and the more recently discovered *Aurantioporthe corni* which causes a canker disease on *Cornus alternifolia* (Cornaceae, Cornales) in North America (Beier et al. 2015).

The two new species of *Celoporthe* found on *T. grandifolia* in La Réunion are the first fungi in this genus and family to have been recorded on that island. Their descriptions bring the number of species described in the genus to 11. The genus *Celoporthe*, typified by *Cel. dispersa*, was first described on species of Melastomataceae and Myrtaceae in South Africa (Nakabonge et al. 2006a). The type species is restricted to South Africa and has been found on native *Heteropyxis*

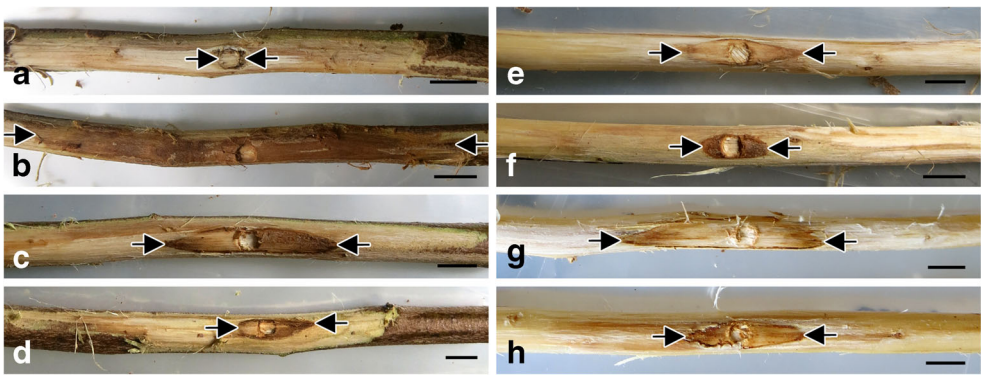

Fig. 7 Symptoms after inoculations on *Eucalyptus grandis* (TAG 5) stems. **a** Negative control (clean agar); **b** positive control (CMW 2113); **c**, **d** *Myrtonectria myrtacearum* (CMW 46433, CMW 46435); **e**, **f** *Celoporthe tibouchinae* (CMW 44126, CMW 44127); **g**, **h** *Celoporthe borbonica* (CMW 44128, CMW 44139). Arrows indicating the margin of lesions. Scale bars = 5 mm

canescens, *Syzygium cordatum* and non-native *Tibouchina granulosa* (Nakabonge et al. 2006a). The other species have previously been found on species of Myrtales in Asia and Africa. Vermeulen et al. (2013) described *Cel. fontana* and *Cel. woodiana*, which occurred on the same hosts as *Cel. dispersa*, in South Africa and Zambia. Chen et al. (2011) described *Cel. eucalypti*, *Cel. guangdongensis* and *Cel. syzygii* from *Eucalyptus* and *Syzygium aromaticum* in China, and *Cel. indonesiensis* from *S. aromaticum* in Indonesia. *Celoporthe* spp. are often associated with canker and die-back of branches and stems of Myrtales. More specifically, these canker pathogens are common on trees or shrubs in the Myrtaceae and Melastomataceae. The former family includes important plantation trees such as *Eucalyptus*, and the Melastomataceae accommodates many flowering trees and shrubs such as *Tibouchina* widely planted as ornamentals in parks and gardens.

Pathogenicity tests in this study showed that *Myr. myrtacearum* and the two new species of *Celoporthe* can result in lesions on a clone of *Eucalyptus grandis*. These were significantly larger than those for the negative control inoculations. They were, however, relatively small compared with the lesions caused by an isolate of *Chrysoporthe austroafricana*, a related fungus that is found on *Eucalyptus* and *Tibouchina* in South Africa (Heath et al. 2006). Although the original hosts of the three new taxa were not tested, the overall results suggest a low level of pathogenicity.

Myrtonectria myrtacearum was found on native trees in South Africa (*H. natalensis* and *S. cordatum*) and it is most likely a native pathogen on these trees. In contrast, the two new species of *Celoporthe* were from *T. grandifolia*, which is an alien plant in La Réunion. These fungi could be native on trees such as those in the *Myrtacaeae* in La Réunion, having undergone a host shift to *T. grandifolia*, as has been observed for other taxa in the Cryphonectriaceae (Burgess et al. 2016; Burgess and Wingfield 2017; Slippers et al. 2005; Van der Merwe et al. 2013). Alternatively, they could have been introduced to the island with their host that is commonly planted in hedges and gardens. This is a probable hypothesis because species of *Tibouchina* are easily propagated from cuttings, and movement of planting stock could have brought asymptomatic fungal endophytes to a new environment.

Various species of the Cryphonectriaceae occurring on the Myrtales have been shown to exist as endophytes in healthy host tissue (Mausse-Sitoe et al. 2016). These include important pathogens such as *Chrysoporthe cubensis* and *Chr. austroafricana* (Heath et al. 2006; Rodas et al. 2005). The three new taxa are also hypothesised to be endophytes on the host plants from which they were isolated. This is despite the fact that they were collected from cankers and are apparently able to also cause disease. The ability of species of the Cryphonectriaceae to infect and live in healthy plant tissue in the absence of symptoms implies that they can easily be introduced into new environments without detection using commonly applied quarantine procedures (Burgess and Wingfield 2017; Mausse-Sitoe et al. 2016). This pathway of movement has not been particularly well considered in the past and it may have contributed to the global spread of many important pathogens including for example the chestnut blight pathogen, *Cryphonectria cubensis*. These fungi deserve far greater attention as threats to natural forest ecosystems as well as to commercial tree crops.

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