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Diversimorbus metrosiderotis gen. et sp. nov. and three new species of *Holocryphia* (*Cryphonectriaceae*) associated with cankers on native *Metrosideros angustifolia* trees in South Africa

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ABSTRACT

The *Cryphonectriaceae* includes important tree pathogens, especially on the *Myrtales*. During a routine disease survey in the Western Cape Province of South Africa, a fungus resembling the *Eucalyptus* pathogen *Holocryphia eucalypti* was observed on native *Metrosideros angustifolia* (*Myrtales*). The aims of this study were to identify the fungus and to expand surveys for fungi in the *Cryphonectriaceae* on *M. angustifolia*. Fungi were identified based on DNA sequence comparisons and morphological features, and their pathogenicity was tested on *M. angustifolia* under field conditions. Based on morphology and multigene phylogenetic analyses of DNA sequence data from six gene regions, we describe a new genus including a single species and three new species of *Holocryphia* (*Cryphonectriaceae*) from *M. angustifolia*. These fungi are provided with the names *Diversimorbus metrosiderotis* gen. et sp. nov., *Holocryphia capensis* sp. nov., *Holocryphia gleniana* sp. nov., and *Holocryphia mzansi* sp. nov. We also revise *H. eucalypti*, the type of the genus, to include only isolates from *Eucalyptus* in South Africa. Research results indicated that *H. mzansi* may undergo host shifts between different tree genera in the *Myrtaceae*. Inoculation tests showed that isolates of all the newly described species can cause lesions on the branches of *M. angustifolia*, indicating that they are all pathogens of this tree.

Introduction

The family *Cryphonectriaceae* was established in 2006 (Gryzenhout *et al.* 2006a) and includes many important pathogens of trees, the best known of which is the chestnut blight pathogen, *Cryphonectria parasitica* (Anagnostakis 1987, 1992). Other tree pathogens in the family of 15 genera (Cheewangkoon *et al.* 2009; Begoude *et al.* 2010; Gryzenhout *et al.* 2010a; Vermeulen *et al.* 2011; Chen *et al.* 2012) include the *Eucalyptus* (*Myrtaceae*, *Myrtales*) pathogens *Chrysoporthe austroafricana*, *Chrysoporthe cubensis*, and *Chrysoporthe deuterocubensis* (Wingfield 2003; Gryzenhout *et al.* 2009; Van der Merwe *et al.* 2010), the pin oak pathogen *Endothia gyrosa* (Stipes & Phipps 1971) and a recently described aggressive

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pathogen of native *Rapanea melanophloeos* (*Myrsinaceae*, *Ericales*), *Immersiporthe knoxdaviesiana*, in South Africa (Chen *et al.* 2012).

The 15 genera of *Cryphonectriaceae* have been reported from 11 orders of trees (Gryzenhout *et al.* 2009, 2010a; Begoude *et al.* 2010; Vermeulen *et al.* 2011; Chen *et al.* 2012). Some of the species in the *Cryphonectriaceae* have been shown to have undergone host shifts between trees of distinctly different genera, although restricted to the same order. Species of *Chrysoporthe*, for example, have spread from native *Myrtales* in their areas of origin to cause serious diseases of non-native *Eucalyptus* spp. in Africa, South America, and Southeast Asia (Wingfield 2003; Heath *et al.* 2006; Wingfield *et al.* 2008; Gryzenhout *et al.* 2009; Van der Merwe *et al.* 2010). This illustrates the potential of these fungi to cause dramatic losses to forests and forestry in the future.

On the African continent, six genera of *Cryphonectriaceae* have been reported to be associated with canker diseases of trees. These include *Aurifilum* (Begoude *et al.* 2010), *Celoporthe* (Nakabonge *et al.* 2006), *Chrysoporthe* (Gryzenhout *et al.* 2004, 2009), *Holocryphia* (Gryzenhout *et al.* 2006c), *Immersiporthe* (Chen *et al.* 2012), and *Latruncellus* (Vermeulen *et al.* 2011). Four of these, *Celoporthe*, *Chrysoporthe*, *Holocryphia*, and *Immersiporthe*, have been recorded in South Africa (Gryzenhout *et al.* 2004, 2006c, 2009; Nakabonge *et al.* 2006; Chen *et al.* 2012). Of these, *Chr. austroafricana* is an important pathogen of plantation-grown *Eucalyptus* species (Gryzenhout *et al.* 2009) and *I. knoxdaviesiana* was recently described as a devastating pathogen of native *R. melanophloeos* in the country (Chen *et al.* 2012). Others, such as *Holocryphia eucalypti*, although not killing trees, result in stem cankers (Van der Westhuizen *et al.* 1993; Venter *et al.* 2002; Gryzenhout *et al.* 2009).

Chrysoporthe austroafricana was first recognised as a serious pathogen of non-native plantation-grown *Eucalyptus* in South Africa approximately 25 y ago when it was thought to represent *Cryphonectria cubensis* (Wingfield *et al.* 1989; Conradie *et al.* 1990). This fungus was subsequently discovered on stem and branch cankers on native *Syzygium* species (Heath *et al.* 2006). This discovery and subsequent research has supported the notion that *Chr. austroafricana* is native to Africa and that it has undergone a host shift from native trees to non-native *Eucalyptus* (Slippers *et al.* 2005; Heath *et al.* 2006).

Closely related plants are assumed to be more likely to be susceptible to the same plant pathogen (Gilbert & Webb 2007; Vienne *et al.* 2009). In South Africa, the closest relatives of *Eucalyptus* species are species of *Syzygium*, *Metrosideros*, and *Eugenia* (Coates Palgrave 2002). Of these, the only naturally occurring, native plant in the *Myrtales* occurring in the Western Cape Province of the country is *Metrosideros angustifolia* (*Myrtaceae*). *Metrosideros angustifolia* is a shrub or small tree up to 4 m in height, occasionally reaching 7 m. It grows naturally only in relatively small areas along streams and rivers where it can become locally common (Coates Palgrave 2002).

During recent surveys for pathogens of native trees (http://www.fabinet.up.ac.za/research/cthb), especially species in the *Myrtales*, fruiting structures resembling species in the *Cryphonectriaceae* were observed on stem and branch cankers on native *M. angustifolia* trees in several areas of the Western Cape Province (Roux *et al.* 2011). The aims of this study were to identify these fungi based on phylogenetic analyses and morphological studies, expand surveys to better understand their distribution, and to investigate their possible role as tree pathogens.

Materials and methods

Disease symptoms, samples, and isolation of fungi

Metrosideros angustifolia trees in the Western Cape Province were surveyed along rivers in several regions stretching from Clanwilliam in the north to Kleinmond in the south of the province (Fig 1). Specific areas surveyed included those of Algeria, Bainskloof, Citrusdal, Du Toitskloof, Kleinmond, Porterville, and Stellenbosch (Fig 1). Surveys continued until a total of at least ten trees having symptoms and signs of possible *Cryphonectriaceae* infection were identified in each area. Typical symptoms of stem and branch cankers, branch and shoot dieback and dead/dying trees were selected for investigation. These included trees damaged by fire, wind or other unknown physical factors. Symptoms were scrutinized for the presence of yellow, orange or black fruiting structures typical of fungi in the *Cryphonectriaceae* (Gryzenhout *et al.* 2009). Where these were observed, pieces of infected bark, or sections of infected branches bearing fruiting structures were removed from the trees and taken to the laboratory in brown paper bags.

In the laboratory, samples were studied using a binocular microscope and single spore-tendrils were lifted from fruiting structures using a sterile needle and transferred to 2 % Malt Extract Agar (MEA; 20 g Biolab Malt Extract, 20 g Biolab Agar, 1 L water) and incubated at 25 °C. In some instances the material was first incubated in moist chambers to induce spore production. Where no fresh spore-tendrils were visible, stromata were exposed using a sharp scalpel blade to cut open fruiting bodies, and spore masses extracted and transferred to MEA. To obtain pure cultures, single hyphal tips were transferred to 2 % MEA and incubated at 25 °C. Resulting cultures are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative isolates were also deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands (Table 1). The original bark specimens were deposited in the National Collection of Fungi (PREM), Pretoria, South Africa.

DNA extraction, polymerase chain reaction (PCR), and sequencing

Representative isolates from different *Metrosideros angustifolia* trees in each of the surveyed areas of the Western Cape Province were selected for DNA sequence comparisons (Table 1). Prior to DNA extraction, selected isolates were grown on 2 % MEA at 25 °C for 14 d. For each isolate, actively growing mycelium was transferred to 1.5 mL Eppendorf tubes by scraping them from the surfaces of cultures on MEA. DNA was extracted following the method described by Myburg *et al.* (1999). To degrade RNA, samples were treated with RNase (3 uL, 1 mg mL^{-1}) and left 12 h at 25 °C. The success of DNA extraction was evaluated on 1 % agarose gels after staining with

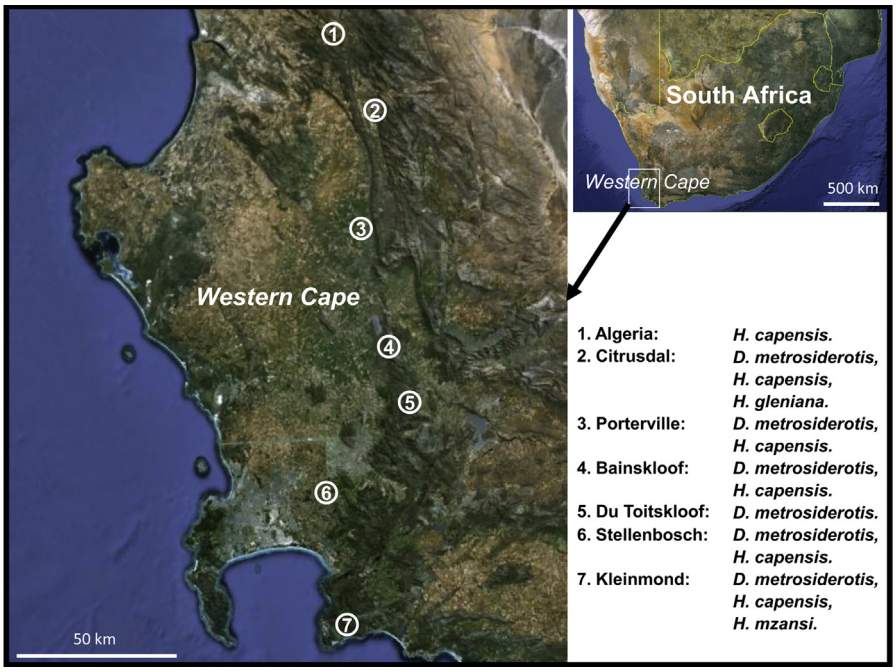

Fig 1 – Map of South Africa indicating seven areas where *Metrosideros augustifolia* trees were sampled in the Western Cape Province, and the species of *Cryphonectriaceae* obtained from each area. The seven surveyed areas were indicated as numbers '1' to '7', and the species of *Cryphonectriaceae* identified in relative areas were indicated.

GelRed (Biotium, Hayward, California, USA; 3 μL DNA extraction product with 2 μL GelRed) and visualisation under ultraviolet (UV) light.

Six regions from five genes were amplified with the PCR. These included the actin (ACT), β-tubulin 1 and 2 (BT1, BT2), calmodulin (CAL), internal transcribed spacer (ITS) regions including the 5.8S gene of the ribosomal DNA operon, the conserved nuclear large subunit (LSU) ribosomal DNA, and the translation elongation factor 1-alpha (TEF-1α) gene regions. The BT1, BT2, ITS, LSU, and TEF-1α regions were amplified using the primers and method previously presented by Chen *et al.* (2011). The ACT gene region was amplified using primers ACT-512F and ACT-783R (Carbone & Kohn 1999) and CAL gene region using primers CAL-228F and CAL-737R (Carbone & Kohn 1999). The PCR products were sequenced following the method described by Chen *et al.* (2011). Nucleotide sequences were edited with MEGA4 (Tamura *et al.* 2007). All the sequences obtained in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

To determine the generic placement of the isolates collected from *Metrosideros angustifolia* trees in the Western Cape Province, sequences of the LSU gene region, as well as the 5.8S rDNA and the exon regions of the BT1 and BT2 (including partial exon 4, exon 5, partial exon 6, and partial exon 7) genes of previously described genera/species in the *Cryphonectriaceae* (Gryzenhout *et al.* 2009, 2010a; Begoude *et al.* 2010; Chen *et al.* 2011, 2012; Vermeulen *et al.* 2011) as well as isolates collected for the current study, were analysed. To determine whether conflict existed between the 5.8S rDNA and the exon regions of the BT gene (BT1/BT2), a partition homogeneity test (PHT) was performed in PAUP*4.0b10 (Swofford 2003) before conducting phylogenetic analyses. The datasets of Chen *et al.* (2012) were used as templates for the phylogenetic analyses. *Togninia minima*, *Togninia fraxinopennsylvanica*, and *Phaeoacremonium aleophilum* were used as outgroup taxa for analyses using sequence data from the LSU gene region. *Diaporthe ambigua* was used as the outgroup taxon for the 5.8S rDNA and the exon regions of the BT gene region analyses.

After the generic identities of the isolates from *M. angustifolia* had been determined, *M. angustifolia* isolates and previously described phylogenetically related species were analysed using sequences obtained for the ACT, BT (BT1 and BT2), CAL, ITS, and TEF-1α genes, separately and in combination. A PHT was also conducted to determine whether conflict existed among the datasets for the six regions from five genes.

Sequences for all the datasets were aligned with the iterative refinement method (FFT-NS-i settings) of the online version of MAFFT 5.667 (Katoh *et al.* 2002) and the alignments were edited manually in MEGA4 (Tamura *et al.* 2007). All the sequence alignments for each of the datasets were deposited in TreeBASE (http://www.treeBASE.org). Two different phylogenetic analyses were performed for each of the datasets. Maximum parsimony (MP) analyses were conducted with PAUP*4.0b10 (Swofford 2003) and Bayesian inference was performed using the Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Both of the phylogenetic analyses for each of the datasets were determined using the methods previously outlined by Chen *et al.* (2011).

To investigate the shared alleles for the *Holocryphia* isolates, allele networks were analysed using the software

Table 1 – Isolates sequenced and used for phylogenetic studies and pathogenicity tests.

Identity	Isolate no.[a,b]	Host	Location	Collector[c]	GenBank accession no.[d] ACT/BT1/BT2/CAL/ITS/LSU/TEF-1α
Celoporthe eucalypti	CMW26900 = CBS 127191	*Eucalyptus* clone	GuangDong, China	XDZ & SFC	**JQ862992**/HQ730816/HQ730826/**JQ863029**/HQ730836/HQ730862/HQ730849
C. eucalypti	CMW26908 = CBS 127190	*Eucalyptus* clone	GuangDong, China	XDZ & SFC	**JQ862993**/HQ730817/HQ730827/**JQ863030**/HQ730837/HQ730863/HQ730850
Diversimorbus metrosiderotis	CMW37320[b]	*Metrosideros angustifolia*	Bainskloof, Western Cape, South Africa	JR	NA[e]/**JQ862910**/**JQ862951**/NA/**JQ862869**/**JQ862826**/NA
D. metrosiderotis	CMW37321[b] = CBS 132865	*M. angustifolia*	Du Toitskloof, Western Cape, South Africa	JR	NA/**JQ862911**/**JQ862952**/NA/**JQ862870**/**JQ862827**/NA
D. metrosiderotis	CMW37322[b] = CBS 132866	*M. angustifolia*	Citrusdal, Western Cape, South Africa	JR & SFC	NA/**JQ862912**/**JQ862953**/NA/**JQ862871**/**JQ862828**/NA
D. metrosiderotis	CMW37323[b] = CBS 132866	*M. angustifolia*	Porterville, Western Cape, South Africa	JR & SFC	NA/**JQ862913**/**JQ862954**/NA/**JQ862872**/**JQ862829**/NA
D. metrosiderotis	CMW37324[b]	*M. angustifolia*	Stellenbosch, Western Cape, South Africa	JR, SFC, & FR	NA/**JQ862914**/**JQ862955**/NA/**JQ862873**/**JQ862830**/NA
D. metrosiderotis	CMW37325[b]	*M. angustifolia*	Kleinmond, Western Cape, South Africa	JR & SFC	NA/**JQ862915**/**JQ862956**/NA/**JQ862874**/**JQ862831**/NA
Holocryphia gleniana	CMW37334[b] = CBS 132871	*M. angustifolia*	Citrusdal, Western Cape, South Africa	JR & SFC	**JQ862957**/**JQ862875**/**JQ862916**/**JQ862994**/**JQ862834**/**JQ862791**/**JQ863031**
H. gleniana	CMW37335[b] = CBS 132872	*M. angustifolia*	Citrusdal, Western Cape, South Africa	JR & SFC	**JQ862958**/**JQ862876**/**JQ862917**/**JQ862995**/**JQ862835**/**JQ862792**/**JQ863032**
H. gleniana	CMW37336[b] = CBS 132873	*M. angustifolia*	Citrusdal, Western Cape, South Africa	JR & SFC	**JQ862959**/**JQ862877**/**JQ862918**/**JQ862996**/**JQ862836**/**JQ862793**/**JQ863033**
H. eucalypti	CMW7033	*Eucalyptus grandis*	KwaZulu/Natal, South Africa	MV	**JQ862960**/**JQ862878**/**JQ862919**/**JQ862997**/**JQ862837**/**JQ862794**/**JQ863034**
H. eucalypti	CMW7035	*Eucalyptus saligna*	KwaZulu/Natal, South Africa	MV	**JQ862961**/**JQ862879**/**JQ862920**/**JQ862998**/**JQ862838**/**JQ862795**/**JQ863035**
H. eucalypti	CMW7036	*Eucalyptus* sp.	Mpumalanga, South Africa	IVDW	**JQ862962**/**JQ862880**/**JQ862921**/**JQ862999**/**JQ862839**/**JQ862796**/**JQ863036**
H. eucalypti	CMW14545	*Eucalyptus* sp.	South Africa	IVDW	**JQ862963**/**JQ862881**/**JQ862922**/**JQ863000**/**JQ862840**/**JQ862797**/**JQ863037**
H. mzansi	CMW37337[b] = CBS 132874	*M. angustifolia*	Kleinmond, Western Cape, South Africa	JR & SFC	**JQ862964**/**JQ862882**/**JQ862923**/**JQ863001**/**JQ862841**/**JQ862798**/**JQ863038**
H. mzansi	CMW37338[b] = CBS 132875	*M. angustifolia*	Kleinmond, Western Cape, South Africa	JR & SFC	**JQ862965**/**JQ862883**/**JQ862924**/**JQ863002**/**JQ862842**/**JQ862799**/**JQ863039**
H. mzansi	CMW11689	*E. grandis* clone	Swaziland	JR	**JQ862966**/**JQ862884**/**JQ862925**/**JQ863003**/**JQ862843**/**JQ862800**/**JQ863040**
H. mzansi	CMW11690	*E. grandis* clone	Swaziland	JR	**JQ862967**/**JQ862885**/**JQ862926**/**JQ863004**/**JQ862844**/**JQ862801**/**JQ863041**
Holocryphia sp.	CMW6246	*Tibouchina urvilleana*	Melbourne, Victoria, Australia	MJW	**JQ862968**/**JQ862886**/**JQ862927**/**JQ863005**/**JQ862845**/**JQ862802**/**JQ863042**
Holocryphia sp.	CMW6249	*T. urvilleana*	Melbourne, Victoria, Australia	MJW	**JQ862969**/**JQ862887**/**JQ862928**/**JQ863006**/**JQ862846**/**JQ862803**/**JQ863043**
Holocryphia sp.	CMW10010	*Eucalyptus fastigata*	Castleridge, Wellington, New Zealand	BJR	**JQ862970**/**JQ862888**/**JQ862929**/**JQ863007**/**JQ862847**/**JQ862804**/**JQ863044**
Holocryphia sp.	CMW10011	*Eucalyptus* sp.	Central Park, Wellington, New Zealand	BJR	**JQ862971**/**JQ862889**/**JQ862930**/**JQ863008**/**JQ862848**/**JQ862805**/**JQ863045**
Holocryphia sp.	CMW10015	*E. fastigata*	Bay of Plenty, New Zealand	RJVB	**JQ862972**/**JQ862890**/**JQ862931**/**JQ863009**/**JQ862849**/**JQ862806**/**JQ863046**
Holocryphia sp.	CMW10016	*Eucalyptus nitens*	Omatoroa, Bay of Plenty, New Zealand	MRT	**JQ862973**/**JQ862891**/**JQ862932**/**JQ863010**/**JQ862850**/**JQ862807**/**JQ863047**
Holocryphia sp.	CMW10017	*Eucalyptus ficifolia*	Lambton Quay, Wellington, New Zealand	LR	**JQ862974**/**JQ862892**/**JQ862933**/**JQ863011**/**JQ862851**/**JQ862808**/**JQ863048**
Holocryphia sp.	CMW10021	*Eucalyptus globulus*	Westport, Buller, New Zealand	PMB	**JQ862975**/**JQ862893**/**JQ862934**/**JQ863012**/**JQ862852**/**JQ862809**/**JQ863049**
Holocryphia sp.	CMW10729	*T. urvilleana*	Coffs Harbour, New South Wales, Australia	MJW	**JQ862976**/**JQ862894**/**JQ862935**/**JQ863013**/**JQ862853**/**JQ862810**/**JQ863050**

Table 1 – (*continued*)

Identity	Isolate no.[a,b]	Host	Location	Collector[c]	GenBank accession no.[d] ACT/BT1/BT2/CAL/ITS/LSU/TEF-1α
H. capensis	CMW37887[b] = CBS 132870	*M. angustifolia*	Stellenbosch, Western Cape, South Africa	JR, SFC, & FR	**JQ862977/JQ862895/JQ862936/JQ863014/JQ862854/JQ862811/JQ863051**
H. capensis	CMW37888	*M. angustifolia*	Stellenbosch, Western Cape, South Africa	JR, SFC, & FR	**JQ862978/JQ862896/JQ862937/JQ863015/JQ862855/JQ862812/JQ863052**
H. capensis	CMW37326	*M. angustifolia*	Algeria, Western Cape, South Africa	JR	**JQ862979/JQ862897/JQ862938/JQ863016/JQ862856/JQ862813/JQ863053**
H. capensis	CMW37327	*M. angustifolia*	Bainskloof, Western Cape, South Africa	JR	**JQ862980/JQ862898/JQ862939/JQ863017/JQ862857/JQ862814/JQ863054**
H. capensis	CMW37328[b] = CBS 132868	*M. angustifolia*	Citrusdal, Western Cape, South Africa	JR & SFC	**JQ862981/JQ862899/JQ862940/JQ863018/JQ862858/JQ862815/JQ863055**
H. capensis	CMW37329	*M. angustifolia*	Porterville, Western Cape, South Africa	JR & SFC	**JQ862982/JQ862900/JQ862941/JQ863019/JQ862859/JQ862816/JQ863056**
H. capensis	CMW37331[b] = CBS 132869	*M. angustifolia*	Kleinmond, Western Cape, South Africa	JR & SFC	**JQ862983/JQ862901/JQ862942/JQ863020/JQ862860/JQ862817/JQ863057**
H. capensis	CMW37332[b]	*M. angustifolia*	Kleinmond, Western Cape, South Africa	JR & SFC	**JQ862984/JQ862902/JQ862943/JQ863021/JQ862861/JQ862818/JQ863058**
H. capensis	CMW37333[b]	*M. angustifolia*	Kleinmond, Western Cape, South Africa	JR & SFC	**JQ862985/JQ862903/JQ862944/JQ863022/JQ862862/JQ862819/JQ863059**
H. capensis	CMW37339[b]	*M. angustifolia*	Citrusdal, Western Cape, South Africa	JR & SFC	**JQ862986/JQ862904/JQ862945/JQ863023/JQ862863/JQ862820/JQ863060**
H. capensis	CMW37340[b]	*M. angustifolia*	Citrusdal, Western Cape, South Africa	JR & SFC	**JQ862987/JQ862905/JQ862946/JQ863024/JQ862864/JQ862821/JQ863061**
H. capensis	CMW37341[b]	*M. angustifolia*	Porterville, Western Cape, South Africa	JR & SFC	**JQ862988/JQ862906/JQ862947/JQ863025/JQ862865/JQ862822/JQ863062**
H. capensis	CMW37342	*M. angustifolia*	Porterville, Western Cape, South Africa	JR & SFC	**JQ862989/JQ862907/JQ862948/JQ863026/JQ862866/JQ862823/JQ863063**

a Designation of isolates and culture collections: CMW = Tree Protection Co-operative Program, FABI, University of Pretoria, South Africa; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
b Isolates used in field pathogenicity trails.
c XDZ = X.D. Zhou, SFC = S.F. Chen, JR = J. Roux, FR = F. Roets, MV = M. Venter, IVDW = I. Van der Westhuizen, MJW = M.J. Wingfield, BJR = B.J. Rogan, RJVB = R.J. van Boven, MRT = M.R. Twaddle, LR = L. Renney, PMB = P.M. Bradbury.
d GenBank numbers in boldface were sequenced in this study.
e NA: GenBank No. not available in this study.

programme TCS (Clement *et al.* 2000). Fixed alleles were sought for the ACT, BT1/BT2, CAL, ITS, and TEF-1α gene regions for all the *Holocryphia* isolates.

Morphology

Morphological features of the fungi obtained from *Metrosideros angustifolia* were determined using a Zeiss Axioplan microscope, Zeiss HRc Axiocam digital camera with Zeiss Axiovision 3.1 software (Carl Zeiss Ltd., München, Germany). Characteristics studied included those of the stromata, asci, ascospores, conidiophores, conidiogenous cells, and conidia. The sizes of the various taxonomically important features were recorded as (min–) (mean – st. dev.) – (mean + st. dev.) (–max). The characteristics of specimens were compared with those of previously described species in the *Cryphonectriaceae* (Gryzenhout *et al.* 2009, 2010a; Begoude *et al.* 2010; Chen *et al.* 2011, 2012; Vermeulen *et al.* 2011). For previously unknown species, a total of 50 measurements were made for each character. The specimens with structures of anamorph and teleomorph were connected by sequence data.

To study culture characteristics, 16 representative isolates (CMW37320–CMW37326, CMW37328, CMW37329, CMW37331, CMW37334–CMW37338, CMW37887, CMW37888) from different *M. angustifolia* trees in different areas, representing different species as determined using the phylogenetic studies, were used. The optimal growth conditions for each culture were obtained using the method presented by Chen *et al.* (2011) and the entire experiment was repeated once. For the descriptions of the fruiting structures and cultures, colour designations were determined using the colour charts of Rayner (1970).

Pathogenicity tests

Nineteen isolates (CMW37320–CMW37325, CMW37328, CMW37331–CMW37341, CMW37887) were selected for field inoculations on *Metrosideros angustifolia* to test their potential to cause disease on these trees. The selected isolates were grown on 2 % MEA at 25 °C for 10 d before inoculation. Isolates were inoculated into young, actively growing branches of *M. angustifolia* trees. The branches selected for inoculation were approximately 1 cm in diameter. Using a cork borer (7 mm diam.), wounds were made on the branches, by removing the bark to expose the cambium. Agar discs of the same size were removed from the actively growing margins of

cultures and placed into the wounds with the mycelium facing the exposed cambium. Sterile MEA discs were used for the negative controls. Wounds with the inoculated mycelium or sterile MEA were covered with masking tape to prevent contamination and desiccation.

Ten trees, each tree with seven branches suitable for inoculation, were selected for the study. Each branch on each tree was inoculated with one of six fungal isolates (CMW37320–CMW37325) or sterile MEA. An additional ten trees, each tree with 14 branches suitable for inoculation, were selected to inoculate 13 isolates (CMW37328, CMW37331–CMW37341, CMW37887) and sterile MEA.

The inoculations were conducted on 9 January 2012, and results were collected after 6 wk by measuring the lesion lengths in the cambium. Small pieces of wood from the edges of lesions were removed aseptically and transferred to 2 % MEA at 25 °C to confirm that the lesions had been caused by the inoculated fungi. All branches inoculated as controls and four randomly selected branches per isolate were used for the re-isolations.

The results were analysed in EXCEL (2003). To determine the effects of fungal strain/sterile MEA on lesion/wound length, single factor analysis of variance (ANOVA) was used. To test significance among means, F values with $P < 0.05$ were considered significantly different. For each fungal strain, the standard errors of means of lesion/wound length were calculated.

Results

Disease symptoms, samples, and isolation of fungi

Fungi resembling the *Cryphonectriaceae* were obtained from *Metrosideros angustifolia* trees in all of the regions (Fig 1) surveyed in the Western Cape Province. It was possible to find at least ten infected trees in each region, within a distance of less than 1 km along the banks of the selected streams or rivers relatively easily, suggesting that the *Cryphonectriaceae* are common. Typical symptoms associated with the *Cryphonectriaceae* included dead and dying branches and cankers on these stems or branches. In all cases the level of damage to trees was reasonably restricted, requiring careful observation to detect the fungi and there was no sign of large scale mortality. On most trees, only a single branch, canker or patch of bark bearing fruiting bodies was observed.

Fruiting structures, with the typical morphological characteristics of fungi in the *Cryphonectriaceae* were observed on the surfaces of cankers. In some cases the fruiting bodies were imbedded in the plant material, with infection being visible only as a result of spore-tendrils exuding from the bark. Microscopic examination revealed the presence of mostly asexual fruiting structures, while sexual structures were observed only occasionally. Isolates on MEA were white and fluffy when young, but turned yellow white to chestnut after 2–3 wk, typical of species in the *Cryphonectriaceae* (Gryzenhout *et al.* 2009).

DNA extraction, PCR, and phylogenetic analyses

DNA was successfully extracted from 26 cultures and PCR and sequence products obtained from each of them (Table 1). Results of the phylogenetic analyses for the different gene regions, as well as for the combined datasets, including the numbers of base pairs, parsimony informative characters, and other parameters for the best suitable substitution models are provided in Table 2. Selected phylogenetic trees, based on MP analyses, are presented in Figs 2–4.

For the LSU dataset, the aligned sequences included 98 taxa and 627 characters (TreeBASE No.: 12586). For the MP and Bayesian analyses, the position of the genera in the *Cryphonectriaceae*, and related families, in relation to each other was different, but all the genera could be clearly distinguished from each other (Fig 2). Isolates collected from *M. augustifolia* in this study clearly resided in the family *Cryphonectriaceae*, forming two distinct lineages (Fig 2). The first phylogenetic lineage, including isolates CMW37320–CMW37325, did not group with any known genus (Fig 2). The second lineage for isolates (CMW37326–CMW37329, CMW37331–CMW37342, CMW37887, CMW37888) from *Metrosideros angustifolia* grouped within the genus *Holocryphia*, a genus of *Eucalyptus*-infecting fungi (Gryzenhout *et al.* 2006a) (Fig 2).

For the datasets of the 5.8S rRNA gene and exons of the BT (BT1 and BT2) gene region, the PHT gave a value of $P = 0.992$, suggesting that the two datasets have no significant conflict and could be combined for further phylogenetic analyses. The aligned sequences of the combined dataset included 94 taxa and 760 characters (TreeBASE No.: 12586) (Table 2). For the MP and Bayesian analyses, the internal arrangement of genera relative to each other was slightly different, but all the genera could be consistently distinguished from each other (Fig 3). Consistent with the LSU analyses, the isolates collected from *M. angustifolia* trees grouped in two different phylogenetic lineages comprising the same isolates and representing species of *Holocryphia* and an unknown genus (Fig 3).

To identify possible differences between isolates within genera, the sequence data for five different genes, ACT, BT1/BT2, ACT, ITS, and TEF-1α, were used. Apart from the *Holocryphia* isolates collected from *M. angustifolia* in this study, isolates previously identified as *Holocryphia eucalypti* from various geographic regions were included. These included the type species of the genus, *H. eucalypti* (CMW7033 and CMW7035) (Gryzenhout *et al.* 2006b), as well as isolates of *Holocryphia* from Australia, New Zealand, and Swaziland (Heath *et al.* 2007; Gryzenhout *et al.* 2010b; Vermeulen *et al.* 2011). The PHT test for the five genes gave a value of $P = 0.597$, suggesting that the five datasets could be combined for further phylogenetic analyses. This was also evident from the similar topologies of the trees obtained for the individual datasets and the combined dataset (Fig 4).

Phylogenetic analyses of the five genes as well as the combined dataset (TreeBASE No.: 12586) consistently showed that the *Holocryphia* isolates resided in five distinct phylogenetic groups within the monotypic genus *Holocryphia* (Fig 4A–F). Phylogenetic Group One included isolates only from *M. angustifolia* (CMW37326–CMW37329, CMW37331–CMW37333, CMW37339–CMW37342, CMW37887, CMW37888) and formed a single phylogenetic group based on the ACT, BT, and TEF-1α gene regions (Fig 4A,B,E). In analyses of the CAL and ITS gene regions, two and three separate subclades emerged respectively (Fig 4C,D) in phylogenetic Group One. Group Two also included only *Metrosideros* isolates (CMW37334,

Table 2 – Statistics resulting from phylogenetic analyses.

Dataset	No. of taxa	No. of bp[a]	MP					
			PIC[b]	No. of trees	Tree length	CI[c]	RI[d]	HI[e]
LSU	98	627	125	100	281	0.544	0.845	0.46
5.8S rRNA/exons of BT12	94	760	114 (5.8S: 6; BT: 108)	1	255	0.545	0.883	0.455
ACT	35	359	91	1	95	0.989	0.994	0.011
BT2/1	35	1080	142	1	167	0.934	0.979	0.066
CAL	35	473	73	1	84	0.964	0.987	0.036
ITS	35	644	90	9	108	0.907	0.964	0.093
TEF-1α	35	370	73	2	87	0.931	0.979	0.069
ACT/BT2&1/CAL/ITS/TEF-1α	35	2926	469	2	552	0.924	0.972	0.076

Dataset	MrBayes				
	Subst.[f] model	Prset statefreqpr	NST[g]	Rates	Burn-in
LSU	GTR + I + G	Dirichlet (1,1,1,1)	6	Invgamma	100 000
5.8S rRNA/exons of BT12	GTR + I + G	Dirichlet (1,1,1,1)	6	Invgamma	100 000
ACT	HKY	Dirichlet (1,1,1,1)	2	Equal	50 000
BT2/1	GTR + I	Dirichlet (1,1,1,1)	6	Propinv	100 000
CAL	HKY + G	Dirichlet (1,1,1,1)	2	Gamma	50 000
ITS	SYM + I	Fixed (equal)	6	Propinv	100 000
TEF-1α	HKY + G	Dirichlet (1,1,1,1)	2	Gamma	50 000
ACT/BT2&1/CAL/ITS/TEF-1α	GTR + G	Dirichlet (1,1,1,1)	6	Gamma	100 000

a bp = base pairs.
b PIC = number of parsimony informative characters.
c CI = consistency index.
d RI = retention index.
e HI = homoplasy index.
f Subst. model = best fit substitution model.
g NST = number of substitution rate categories.

CMW37335, CMW37336) (Fig 4A–F). Phylogenetic Group Three included *Metrosideros* isolates (CMW37337, CMW37338) and *Eucalyptus* isolates (CMW11689, CMW11690) from Swaziland (Fig 4A–F). *Eucalyptus* isolates CMW7033, CMW7035, CMW7036, CMW14545 from South Africa all resided in Group Four (Fig 4). Phylogenetic Group Five included *Holocryphia* isolates (CMW6246, CMW6249, CMW10010, CMW10011, CMW10015–CMW10017, CMW10021, CMW10729) from *Tibouchina* and *Eucalyptus* trees in Australia and New Zealand (Fig 4A–F), forming a number of subgroups for each of the five genes (Fig 4A–E), and the combined dataset of the five genes (Fig 4F). The fungi in Phylogenetic Group Five, from Australia and New Zealand, were not treated further here since the focus of this study was on those collections from *Metrosideros* and *Eucalyptus* in Africa.

Single nucleotide polymorphism (SNP) analyses of the sequence results confirmed that the *Holocryphia* isolates from *M. angustifolia* represent three unique groups, which we have referred to as phylogenetic Group One, Two, and Three (Fig 4A–F). The isolates from *Eucalyptus* in South Africa (phylogenetic Group Four, Fig 4A–F) represent the fourth group. For these four Groups, comparisons of sequences for five gene regions showed clearly that each group separated from the others by 1–26 unique SNPs for each gene region (Table 3). For all five gene regions combined, the total number of differences between the four groups varied between 17 and 64 unique SNPs (Table 3).

The nucleotide difference analyses showed that in Group One, two different phylogenetic subgroups were recognised for the CAL gene analyses (Fig 4C), with four base pairs differing between the two subgroups. Three phylogenetic subgroups were found in the ITS gene analyses (Fig 4D), with three to eight base pairs different between the three phylogenetic subgroups in Group One. No nucleotide differences were found for isolates in Group one when comparing sequences of the ACT, BT2/1, and TEF-1α gene regions. For the isolates within groups Two, Three, and Four, no nucleotide differences existed in the five gene regions.

The allele network drawn for the combined genes of the *Holocryphia* isolates provided strong support for the phylogenetic analyses (Fig 5). In this analysis, the *Holocryphia* isolates collected from *M. angustifolia* grouped in three networks (Network 1, 2, 3) respectively, and the isolates from *Eucalyptus* in South Africa were grouped in Network 4. These four networks grouped the same isolates as those emerging from the phylogenetic analyses. Two networks existed for the isolates from *Tibouchina* and *Eucalyptus* in Australia and New Zealand.

Morphology

Consistent with the phylogenetic analyses, the morphology of the fungi from *Metrosideros angustifolia* shared the typical characteristics of fungi within the *Cryphonectriaceae*. Fungi obtained from *M. angustifolia* turned yellow in lactic acid and purple in 3 % KOH (Castlebury *et al.* 2002; Gryzenhout *et al.* 2009). Furthermore, the isolates from *M. angustifolia* could be separated into two distinct groups based on morphological characteristics of the fruiting structures, which is consistent with the two phylogenetic lineages representing the different genera recognised in this study.

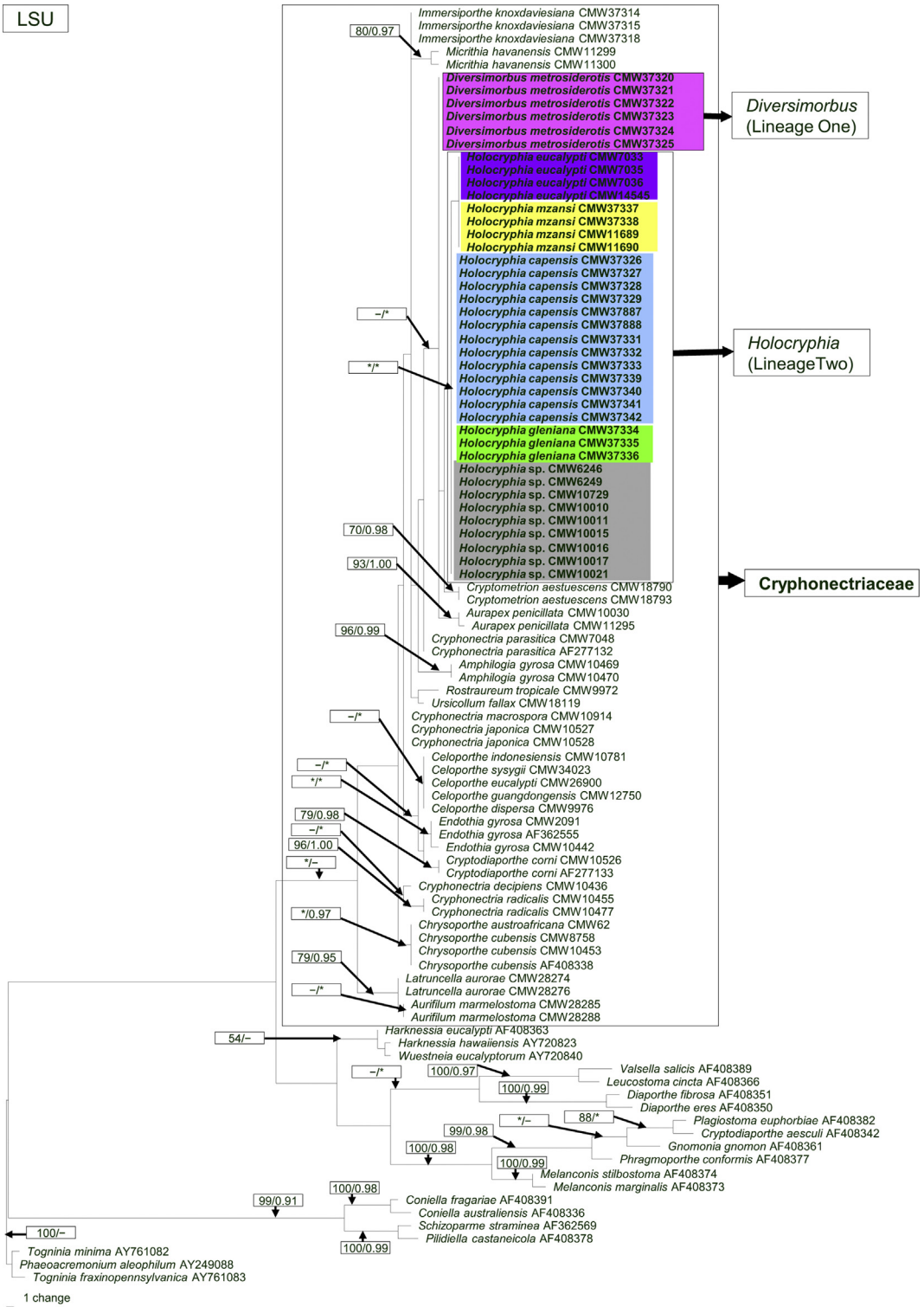

Fig 2 – Phylogenetic tree based on MP analysis of LSU DNA sequences for various genera in the *Diaporthales*. Isolates in boldface were sequenced in this study. Sequences with AF and AY numbers originated from Zhang & Blackwell (2001) and Castlebury *et al.* (2002), while others were obtained from Gryzenhout *et al.* (2006a, 2009) and Chen *et al.* (2012). Bootstrap values >70 % for MP, and posterior probabilities >95 % obtained from Bayesian analyses are presented above branches as follows: MP/Bayesian. Bootstrap values lower than 70 % and posterior probabilities lower than 95 % are marked with *. Absent analysis values of bootstrap and posterior probabilities are marked with –. *Togninia minima* (AY761082), *T. fraxinopennsylvanica* (AY761083), and *Phaeoacremonium aleophilum* (AY249088) in the family *Togniniaceae* represent the outgroups.

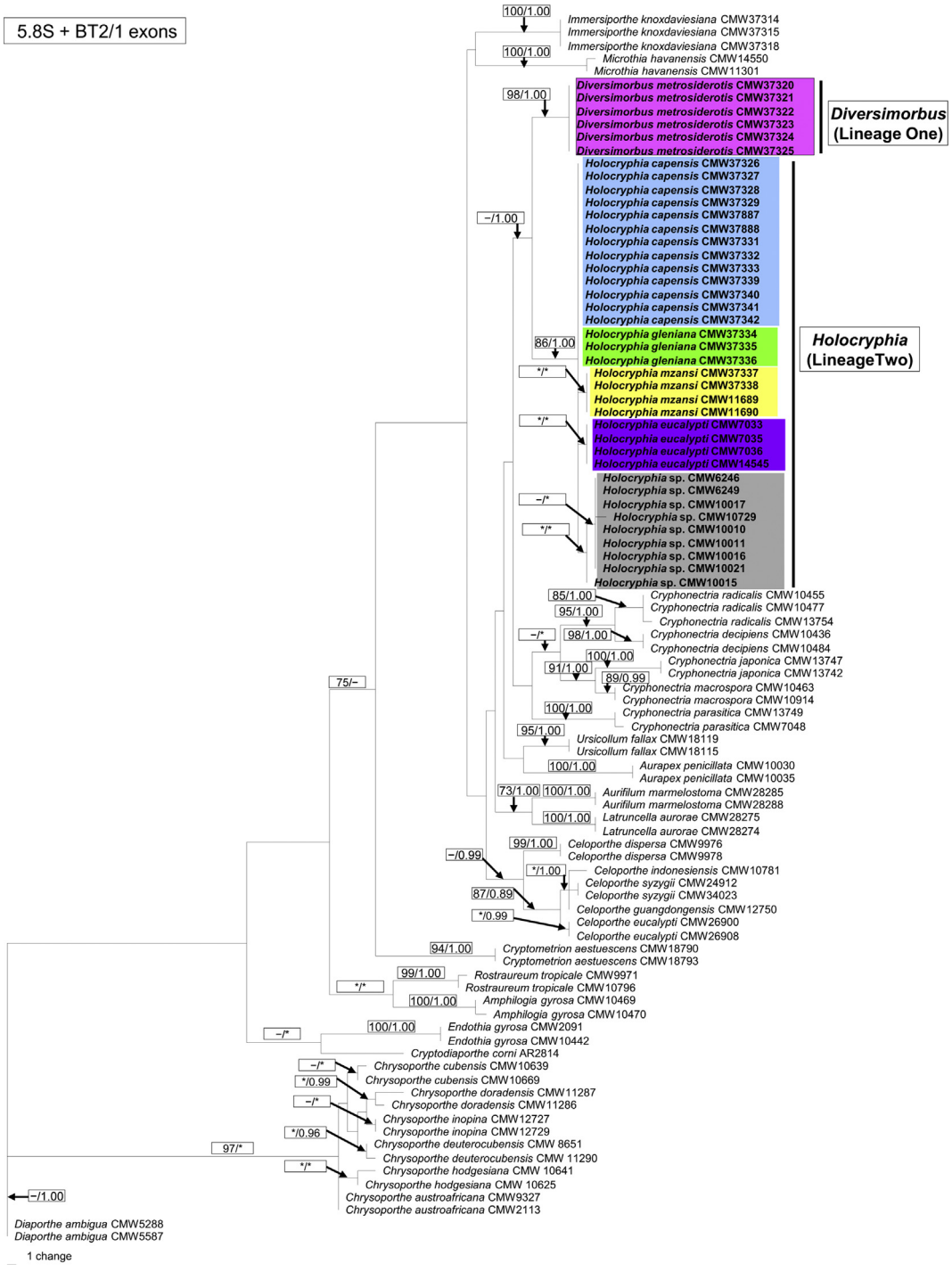

Fig 3 – Phylogenetic tree based on MP analysis of a combined DNA sequence dataset of gene regions of the partial exon 4, exon 5, partial exon 6, and partial exon 7 of the BT genes, and the 5.8S rRNA gene region for various genera in the *Cryphonectriaceae*. Isolates in boldface were sequenced in this study. Bootstrap values >70 % for MP and posterior probabilities >95 % obtained from Bayesian analyses are presented above branches as follows: MP/Bayesian. Bootstrap values lower than 70 % and posterior probabilities lower than 95 % are marked with *, while absent values are marked with –. Two isolates of *Diaporthe ambigua* (CMW5587, CMW5288) represent the outgroups.

Isolates of the purportedly new genus had black conidiomata, which distinguishes it from the majority of genera in the *Cryphonectriaceae* that have orange conidiomata (Gryzenhout *et al.* 2009, 2010a; Begoude *et al.* 2010; Chen *et al.* 2011, 2012; Vermeulen *et al.* 2011). Genera in the *Cryphonectriaceae* with black conidiomata include only *Aurapex*, *Celoporthe*, and *Chrysoporthe* (Gryzenhout *et al.* 2006c, 2009; Nakabonge *et al.* 2006) (Table 4). Based on the morphology of the

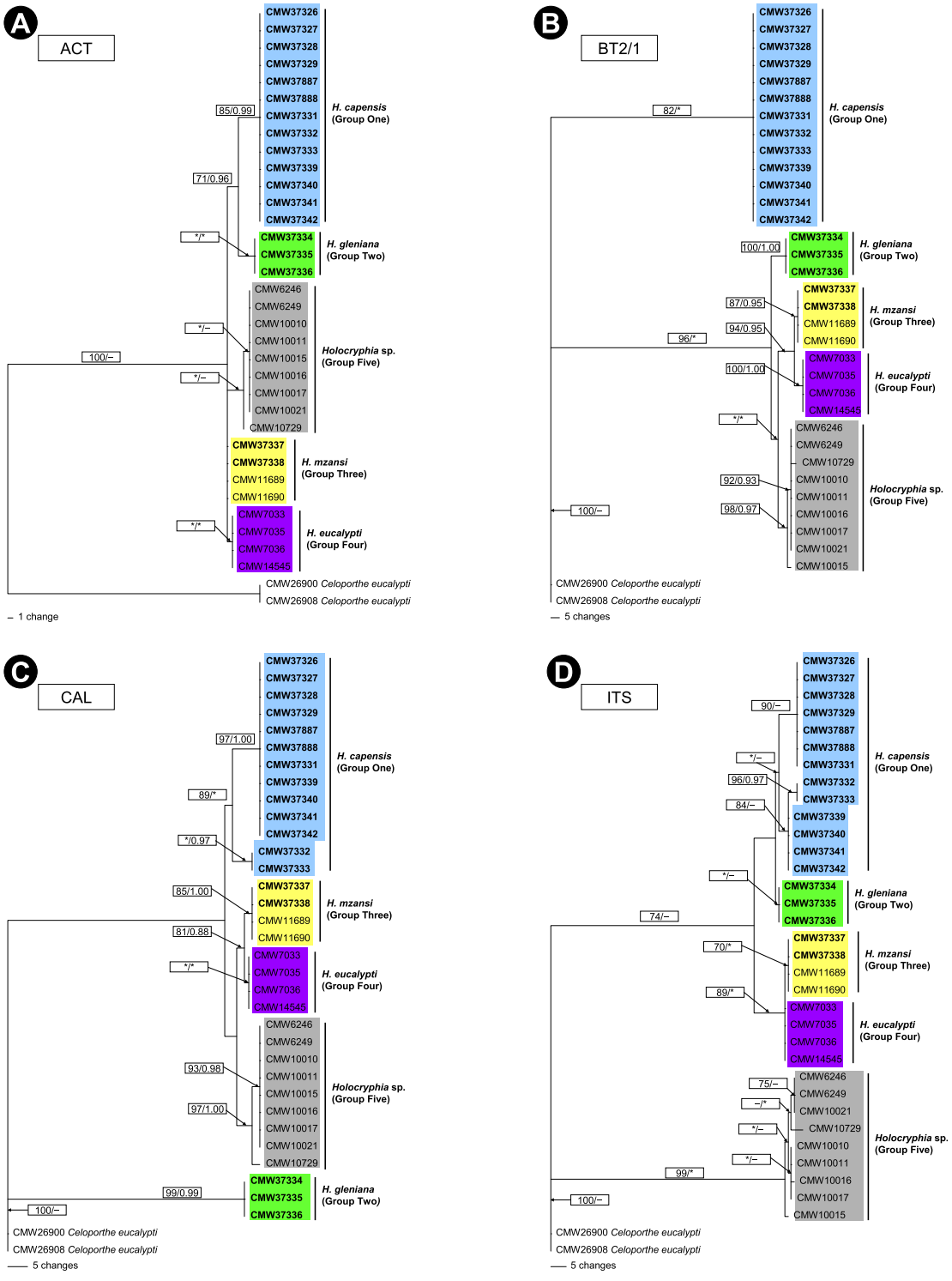

Fig 4 – Phylogenetic trees based on MP analysis for various species of *Holocryphia*. (A) ACT gene region. (B) Two regions of the BT (BT2/1). (C) CAL gene region. (D) ITS nuclear ribosomal DNA (ITS). (E) TEF-1α gene region. (F) Combination of ACT, BT2/1, CAL, ITS, TEF-1α regions. Isolates in boldface were isolated in this study. Bootstrap values >70 % for MP and posterior probabilities >95 % obtained from Bayesian analyses are presented above branches as follows: MP/Bayesian. Bootstrap values lower than 70 % and posterior probabilities lower than 95 % are marked with *, while absent values are marked with –. Two isolates of *Celoporthe eucalypti* (CMW26900, CMW26908) represent the outgroups.

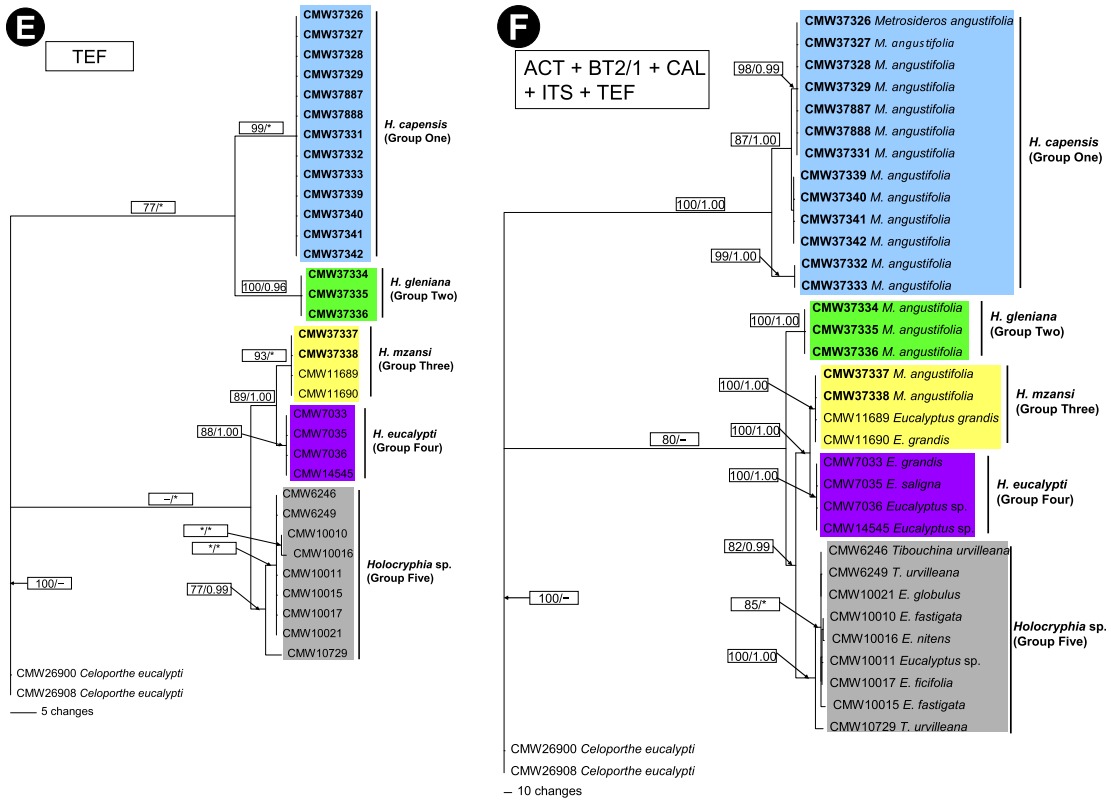

Fig. 4 – (*continued*).

conidiomata, paraphyses, and perithecia, the *Metrosideros* lineage (conidiomata without necks, base tissue pseudoparenchymatous, paraphyses present, perithecial necks black) could, however, be distinguished from *Aurapex* (conidiomata with long orange necks, base tissue textura globulosa, paraphyses absent, perithecial necks colour unknown) (Gryzenhout *et al.* 2006c), *Celoporthe* (conidiomata occasionally with necks, base tissue prosenchymatous, paraphyses present, perithecial necks orange to umber) (Nakabonge *et al.* 2006), and *Chrysoporthe* (conidiomata with attenuated necks, base tissue textura globulosa, paraphyses absent, perithecial necks black) (Gryzenhout *et al.* 2009) (Table 4).

Isolates from *M. angustifolia* that grouped in the genus *Holocryphia* based on sequence analyses displayed typical morphological features of that genus. These characteristics include pulvinate stromata that are semi-immersed within the bark, orange conidiomata without necks, paraphyses, and aseptate ascospores. Furthermore, isolates from *M. angustifolia* could be distinguished from the types of *Holocryphia eucalypti* based on the sizes of their asci and conidia. The asci (longer than 40 μm) and conidia (longer than 5.0 μm) of the fungi from *M. angustifolia* are larger than those of *H. eucalypti* (asci shorter than 35 μm, conidia no more than 5 μm). Also, the fungi from *M. angustifolia* grew more slowly (covering a 90 mm diam. plate after 17–18 d at their optimal temperature 25–30 °C) than those of *H. eucalypti* (covering 90 mm diam. plate in 9 d at the optimal temperature 25–30 °C).

Isolates of *Holocryphia* from *M. angustifolia* could also be distinguished from each other based on several morphological features, especially the sizes of ascospores, asci, and conidia. Sizes for isolates in phylogenetic Group One (asci up to 41 μm, ascospores up to 12 μm, conidia up to 5.1 μm), phylogenetic Group Two (asci up to 44 μm, ascospores up to 14 μm, conidia up to 6.2 μm), and phylogenetic Group Three (asci up to 46 μm, ascospores up to 16 μm, conidia up to 6.2 μm) were consistently different from each other. They could also be distinguished from each other based on optimal growth temperature, with those in phylogenetic Group One growing best at 20–25 °C, while those of isolates in phylogenetic Group Two and Three grew optimally at 25–30 °C and 25 °C, respectively.

Table 3 – Number of unique alleles between *Holocryphia capensis*, *H. gleniana*, *H. mzansi*, and *H. eucalypti*.

ACT/BT2&1/CAL/ITS/TEF-1α[a]	*H. gleniana* (Group Two)	*H. mzansi* (Group Three)	*H. eucalypti* (Group Four)
H. capensis (Group One)	50(3/21/9/2/15)	60(5/23/10/5/17)	63(6/26/9/4/18)
H. gleniana (Group Two)		63(5/23/9/3/23)	64(6/26/8/2/22)
H. mzansi (Group Three)			17(1/7/3/1/5)

a The order of the five genes: ACT, BT2&1, CAL, ITS, and TEF-1α.

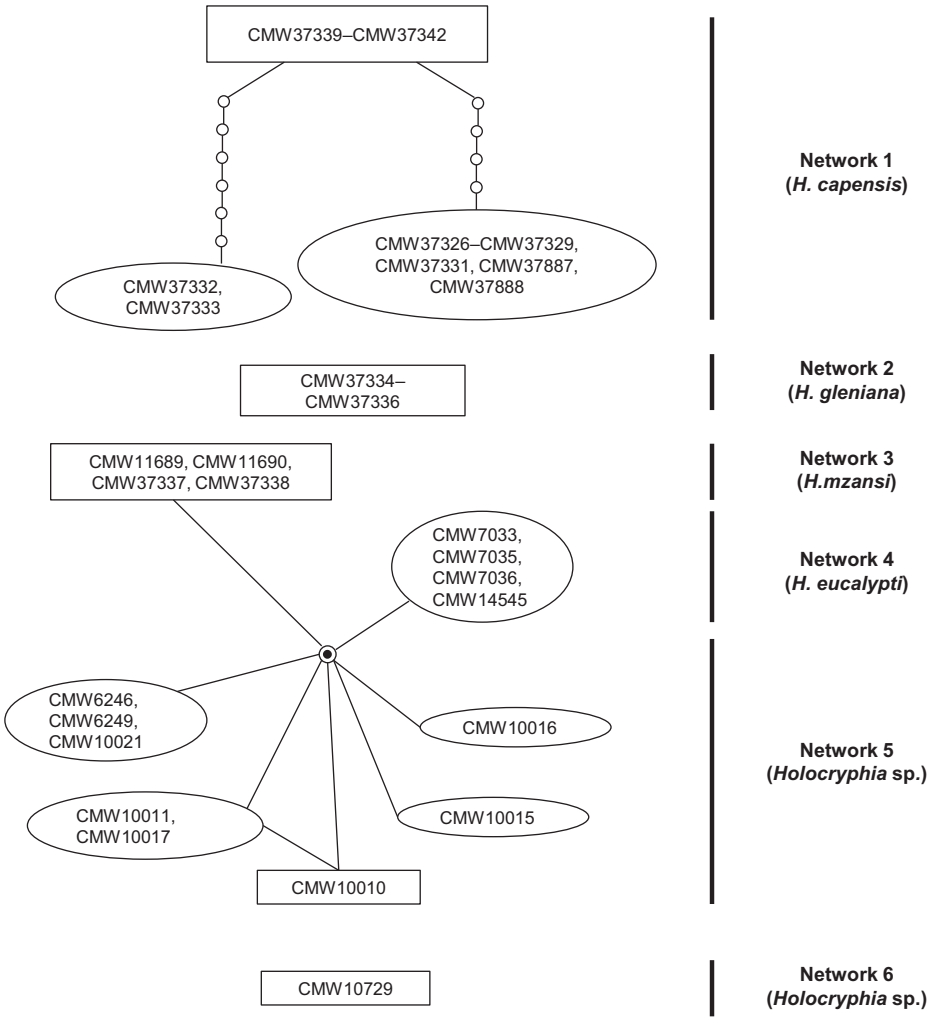

Fig 5 – Allele networks for isolates of *Holocryphia*. The ACT, BT1/BT2, CAL, ITS, and TEF-1α gene regions were combined and six allele networks were obtained.

Taxonomy

Based on phylogenetic analyses, combined with morphological characteristics, the isolates from *M. augustifolia* in the Western Cape Province represent two distinct genera in the *Cryphonectriaceae*. Isolates residing in Phylogenetic Lineage One clearly represent a previously undescribed genus and species, which is named as *Diversimorbus metrosiderotis* gen. et sp. nov., while those in Phylogenetic Lineage Two represent species in the genus *Holocryphia*. Furthermore, isolates in the genus *Holocryphia* represent three previously unknown species, *Holocryphia capensis* sp. nov., *Holocryphia gleniana* sp. nov., and *Holocryphia mzansi* sp. nov. The unknown genus and species are described as follows:

Diversimorbus S.F. Chen & Jol. Roux, **gen. nov.**
MycoBank No.: MB564805

Etymology: *Diversis*, Latin for different, diverse, describing the different colours of the conidiomata and ascostromata of this fungus, and *morbus*, Latin for disease, describing the pathogenic potential of the fungus to its host.

Type species: *D. metrosiderotis* S.F. Chen & Jol. Roux

Ascostromata on bark gregarious or single, superficial to slightly immersed, orange to umber, pulvinate; ascostromatic tissue pseudoparenchymatous, covering the tops of the perithecial bases. ***Perithecia*** valsoid, fuscous black, embedded beneath surface of bark at base of stromata, bases globose to subglobose; perithecial necks black, emerging at stromatic surface as black ostioles with orange stromatic tissue of *textura porrecta*, no neck extending above stromatic surface. ***Asci*** fusoid to ellipsoidal, biseriate, unitunicate, released from inner wall of perithecia when mature, nonstipitate, eight-spored. ***Ascospores*** hyaline, one-septate with septum variously placed in the spore but usually central, ascospores constricted at septum, fusoid to ellipsoidal, ends round to slightly tapered.

Conidiomata part of ascomata as conidial locules or as separate structures, black, pulvinate, globose to conical, superficial to slightly immersed, without necks, uni to multilocular structures, with locules often convoluted, ostioles covered with orange tissue; stromatic tissue pseudoparenchymatous. ***Conidiophores*** nonseptate, cylindrical, occasionally with separating septa and branching, hyaline. ***Conidiogenous cells***

Table 4 – Morphological characteristics of genera in the *Cryphonectriaceae* having black conidiomata compared with those of *Diversimorbus*.

Morphological characteristics	*Aurapex*	*Celoporthe*	*Chrysoporthe*	*Diversimorbus*
Teleomorph				
(1). Structure of ascostromata	Not known	Pulvinate, semiimmersed	Beneath or erumpent through the bark surface	Pulvinate, superficial to slight immersed
(2). Perithecial necks extending above stromatic surface	Not known	Short or absent	Long	Absent
(3). Colour of perithecia and perithecial necks	Not known	Black	Black	Black
(4). Colour of ascostromatal tissue	Not known	Orange to umber	Orange	Orange
(5). Colour of perithecial necks covered tissue	Not known	Umber	Umber	Orange
(6). Ascospore shape	Not known	Hyaline, oblong to ellipsoidal	Hyaline, fusoid to oval	Hyaline, fusoid to ellipsoidal
(7). Ascospore septation	Not known	One median septate	One-septate, usually central	One-septate, usually central
Anamorph				
(8). Structure of conidiomata	Globose to pyriform, superficial to slight immersed	Pulvinate to conical, superficial	Pyriform to pulvinate, superficial	Pulvinate, globose to conical, superficial to slightly immersed
(9). Colour of conidiomata when mature	Black with orange necks	Uniformly black	Uniformly black	Uniformly black
(10). Conidiomatal necks	Present	Absent or occasionally with short necks	With attenuated necks	Absent
(11). Conidiomatal stromatic tissue	*Textura globulosa* and prosenchymatous	Prosenchymatous	*Textura globulosa*	Pseudoparenchymatous
(12). Paraphyses	Absent	Present	Absent	Present
(13). Conidia	Hyaline, oval to oblong, aseptate	Hyaline, oblong to cylindrical to ovoid, occasionally allantoid, aseptate	Hyaline, oblong, aseptate	Hyaline, cylindrical to fusoid, aseptate

cylindrical or flask-shaped with attenuated apices. Cylindrical ***paraphyses*** occur among conidiogenous cells, slightly tapered towards apex, branching occasionally. ***Conidia*** hyaline, cylindrical to fusoid, aseptate.

Diversimorbus metrosiderotis S.F. Chen & Jol. Roux, **sp. nov.**
(Fig 6)
MycoBank No.: MB564862

Etymology: Name reflects the host genus *Metrosideros* on which the fungus was first found.

Ascostromata on bark gregarious or single, superficial to slightly immersed, orange to umber, pulvinate; ascostromatic tissue pseudoparenchymatous, covering the tops of the perithecial bases; ascostromata extending 120–420 μm high above the bark, 300–1200 μm diam. ***Perithecia*** valsoid, up to 13 per stroma, fuscous black, embedded beneath surface of bark at base of stromata; perithecial bases globose to subglobose, 100–230 μm diam.; perithecial necks black, emerging at the stromatic surface as black ostioles with orange stromatic tissue of *textura porrecta*, necks 20–90 μm wide, up to 410 μm long, no neck extending above stromatic surface. ***Asci*** fusoid to ellipsoidal, biseriate, unitunicate, released from inner wall of perithecia when mature, nonstipitate, eight-spored, (40.5–)46.5–53.5(–55) × (7.5–)9.5–12.0(–14.5) μm (av. 50 × 11 μm). ***Ascospores*** hyaline, one-septate with septum variously placed in the spore but usually central, ascospores slightly constricted at septum, fusoid to ellipsoidal, ends round to slightly tapered, (7.5–)8.5–10.5(–12.5) × (2.5–)3.0–4.0(–4.5) μm (av. 10 × 3.5 μm).

Conidiomata part of ascomata as conidial locules or as separate structures, black, pulvinate, globose to conical, superficial to slightly immersed, without necks, uni to multilocular structures, with locules often convoluted, ostioles covered with orange tissue; stromatic tissue pseudoparenchymatous; conidiomatal bases 160–500 μm high above the bark surface, 300–1400 μm diam.; locus 60–620 μm diam. ***Conidiophores*** nonseptate, cylindrical, (7–)13.5–21(–32) μm long, occasionally with separating septa and branching, hyaline. ***Conidiogenous cells*** 1–2 μm wide, cylindrical or flask-shaped with attenuated apices. Cylindrical ***paraphyses*** occurring among conidiogenous cells, up to 220 μm long, 1–2.5 μm wide, slightly tapered towards apex, branching occasionally. ***Conidia*** hyaline, cylindrical to fusoid, aseptate, (3.0–)3.5–4(4.5) × (1.0–)1–1.5 μm (av. 3.8 × 1 μm).

Culture characteristics: On MEA *D. metrosiderotis* fluffy with an uneven margin, white when young, turning ochraceous to umber with umber/chestnut patches after 14 d. Colony reverse umber to chestnut. Optimal growth temperature 25 °C, no growth at 35 °C. In 15 d, colonies at 5 °C intervals from 5 °C to 30 °C reached 10.0 mm (5 °C), 30.0 mm (10 °C), 45.0 mm (15 °C), 65.0 mm (20 °C), 85 mm (25 °C), and 10.0 mm (30 °C), respectively. Asexual fruiting structures occasionally formed in primary isolations of the fungus.

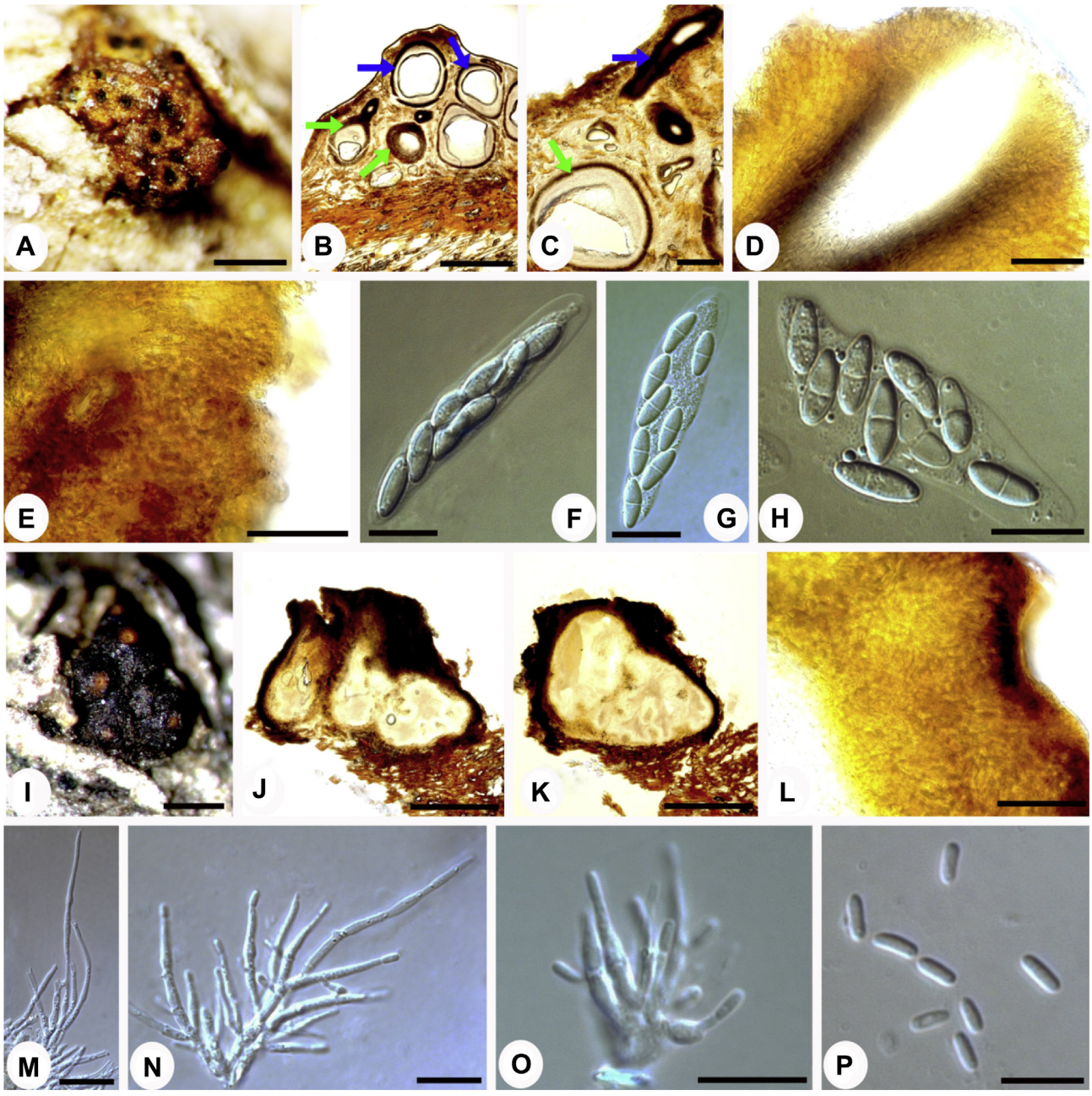

Fig 6 – Fruiting structures of *Diversimorbus metrosiderotis*. (A) Ascostroma on bark. (B) Longitudinal section through ascostroma showing orange, superficial (blue arrows indicate) to slightly immersed (green arrows indicate) ascostromata. (C) Perithecia, neck is indicated with blue arrow, perithecial base is indicated with green arrow. (D) Perithecial neck tissue of textura porrecta. (E) Pseudoparenchymatous stromatic tissue of the ascostroma. (F, G) Ascus with ascospores. (H) Fusoid ascospores. (I) Conidiomata on the bark. (J, K) Longitudinal section through conidioma showing black conidiomata. (L) Pseudoparenchymatous stromatic tissue of conidioma. (M) Paraphyses. (N, O) Conidiophores and conidiogenous cells. (P) Fusoid conidia. Scale bars: A, I = 300 μm; B, J, K = 200 μm; C = 50 μm; D, E, L = 20 μm; F–H, M–P = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Substrate: Bark of *Metrosideros angustifolia*.
Distribution: Western Cape Province, South Africa.
Specimens examined: South Africa, from bark of *M. angustifolia*. Western Cape Province, Citrusdal area, 8 March 2011, Jolanda Roux & ShuaiFei Chen, HOLOTYPE PREM 60741 (branches with mature ascostromata and conidiomata), extype culture CMW37322 = CBS 132866 (isolated from ascostromata); Western Cape Province, Du Toitskloof area, 2009, Jolanda Roux, PARATYPE PREM 60742 (branches with mature ascostromata and conidiomata), living culture CMW37321 = CBS 132865 (isolated from ascostromata); Western Cape Province, Porterville area, 9 March 2011, Jolanda Roux & ShuaiFei Chen, PARATYPE PREM

60743 (branches with mature conidiomata), living culture CMW37323 = CBS 132867; Western Cape Province, Bainskloof area, 2009, Jolanda Roux, living culture CMW37320; Western Cape Province, Stellenbosch area, 9 March 2011, Jolanda Roux, ShuaiFei Chen & Francois Roets, living culture CMW37324; Western Cape Province, Kleinmond area, 10 March 2011, Jolanda Roux & ShuaiFei Chen, living culture CMW37325.

Holocryphia capensis S.F. Chen & Jol. Roux, **sp. nov.**
(Fig 7)
MycoBank No.: MB564863

Etymology: Name refers to the fact that the species was isolated in the Western Cape Province of South Africa.

Ascostromata on bark gregarious or single, orange, pulvinate, semiimmersed in the bark; ascostromatic tissue pseudoparenchymatous, covering the tops of the perithecial bases; ascostromata extending 50–200 μm high above the bark, 100–700 μm diam. ***Perithecia*** valsoid, up to nine per stroma, fuscous black, embedded beneath surface of bark at base of stromata; perithecial bases globose to subglobose, 80–380 μm diam.; perithecial necks black, emerging at stromatal surface as black ostioles with orange stromatic tissue of *textura porrecta*, necks 40–130 μm wide, up to 520 μm long, no neck extending above stromatic surface. ***Asci*** fusoid to ellipsoidal, biseriate, unitunicate, released from inner wall of perithecia when mature, nonstipitate, eight-spored, (24.0–)27.0–31.0(–41.0) × (5.0–)5.5–7.0(–10.0) μm (av. 29.0 × 6.0 μm). ***Ascospores*** hyaline, aseptate, cylindrical to fusiform, occasionally allantoid, ends round to slightly tapered, (5.0–)6.0–9.0(–12.0) × (1.2–)1.5–2 μm (av. 7.0 × 1.5 μm).

Conidiomata part of ascomata as conidial locules or as separate structures, orange, pulvinate, semiimmersed, without necks, uni to multilocular structures, with locules often convoluted, ostioles covered with orange tissue; stromatic tissue pseudoparenchymatous; conidiomatal bases 60–300 μm high above the bark surface, 280–800 μm diam.; locus 40–420 μm diam. ***Conidiophores*** nonseptate, cylindrical, (6–)9–20(–28) μm long, occasionally with separating septa and branching, hyaline. ***Conidiogenous cells*** 1–2 μm wide, cylindrical or flask-shaped

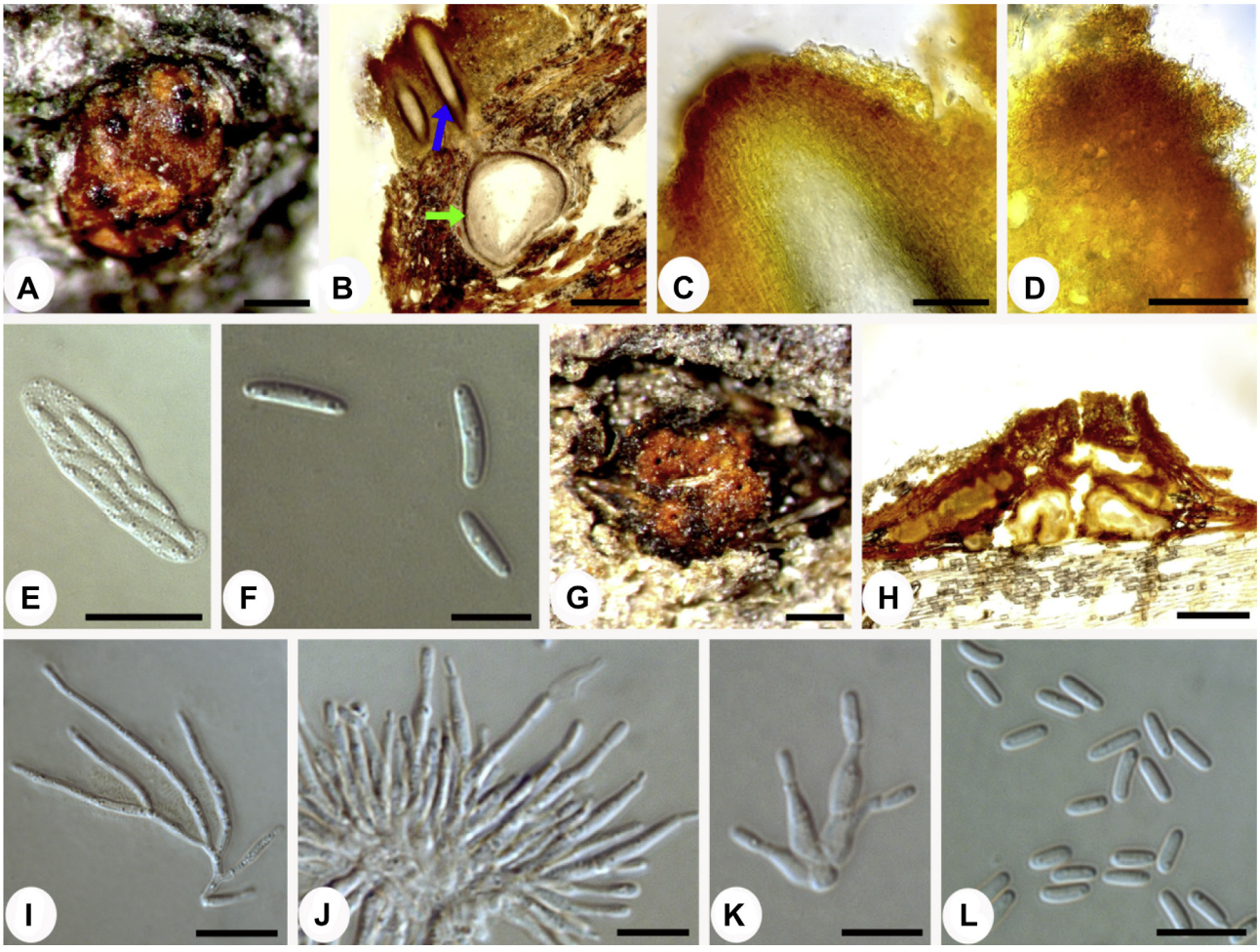

Fig 7 – Fruiting structures of *Holocryphia capensis*. (A) Ascostroma on bark. (B) Longitudinal section through ascostroma showing orange, semi-immersed ascostromata, perithecial neck is indicated with blue arrow, perithecial base is indicated with green arrow. (C) Perithecial neck tissue of textura porrecta. (D) Pseudoparenchymatous stromatic tissue of the ascostroma. (E). Ascus with ascospores. (F) Fusiform ascospores. (G) Conidiomata on the bark. (H) Longitudinal section through conidioma showing orange conidiomata. (I) Paraphyses. (J, K) Conidiophores and conidiogenous cells. (L) Fusoid conidia. Scale bars: A, G = 200 μm; B, H = 100 μm; C, D = 20 μm; E, I, J = 10 μm; F, K, L = 5 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with attenuated apices. Cylindrical ***paraphyses*** occur among conidiogenous cells, up to 70 μm long, 1–3 μm wide, slightly tapered towards apex, branching occasionally. ***Conidia*** hyaline, cylindrical to fusoid, occasionally allantoid, aseptate, (3–)3.5–4.5(–5.1) × 1–1.5 μm (av. 4.0 × 1.1 μm).

Culture characteristics: On MEA *H. capensis* fluffy with an uneven margin, white when young, turning yellow–white with yellow–white to yellow patches after 14 d. Colony reverse yellow–white. Optimal growth temperature 20–25 °C, no growth at 35 °C. In 15 d, colonies at 5 °C intervals from 5 °C to 30 °C reaching 10.0 mm (5 °C), 20.0 mm (10 °C), 55.0 mm (15 °C), 80 mm (20–25 °C), and 20.0 mm (30 °C), respectively.

Substrate: Bark of *M. angustifolia*.

Distribution: Western Cape Province, South Africa.

Specimens examined: South Africa, from bark of *M. angustifolia*. Western Cape Province, Stellenbosch area, 9 March 2011, Jolanda Roux, ShuaiFei Chen & Francois Roets, HOLOTYPE PREM M60744 (branches with mature ascostromata and conidiomata), extype culture CMW37887 = CBS 132870 (isolated from ascostromata); Western Cape Province, Kleinmond area, 10 March 2011, Jolanda Roux & ShuaiFei Chen, PARATYPE PREM 60745 (branches with mature ascostromata and conidiomata), living culture CMW37331 = CBS 132869 (isolated from ascostromata); Western Cape Province, Citrusdal area, 8 March 2011, Jolanda Roux & ShuaiFei Chen, PARATYPE PREM 60746 (branches with mature conidiomata), living culture CMW37328 = CBS 132868; Western Cape Province, Stellenbosch area, 9 March 2011, Jolanda Roux, ShuaiFei Chen, & Francois Roets, living culture CMW37888; Western Cape Province, Algeria area, Clanwilliam, 2009, Jolanda Roux, living culture CMW37326; Western Cape Province, Bainskloof area, 2009, Jolanda Roux, living culture CMW37327; Western Cape Province, Porterville area, 9 March 2011, Jolanda Roux & ShuaiFei Chen, living culture CMW37329.

Holocryphia gleniana S.F. Chen & Jol. Roux, **sp. nov.**
(Fig 8)
MycoBank No.: MB564864

Etymology: This species is named for Dr Hugh Glen recognising his passion for biology and the support that he has given to mycologists in South Africa and especially at FABI.

Ascostromata on bark gregarious or single, orange, pulvinate, semiimmersed in the bark; ascostromatic tissue

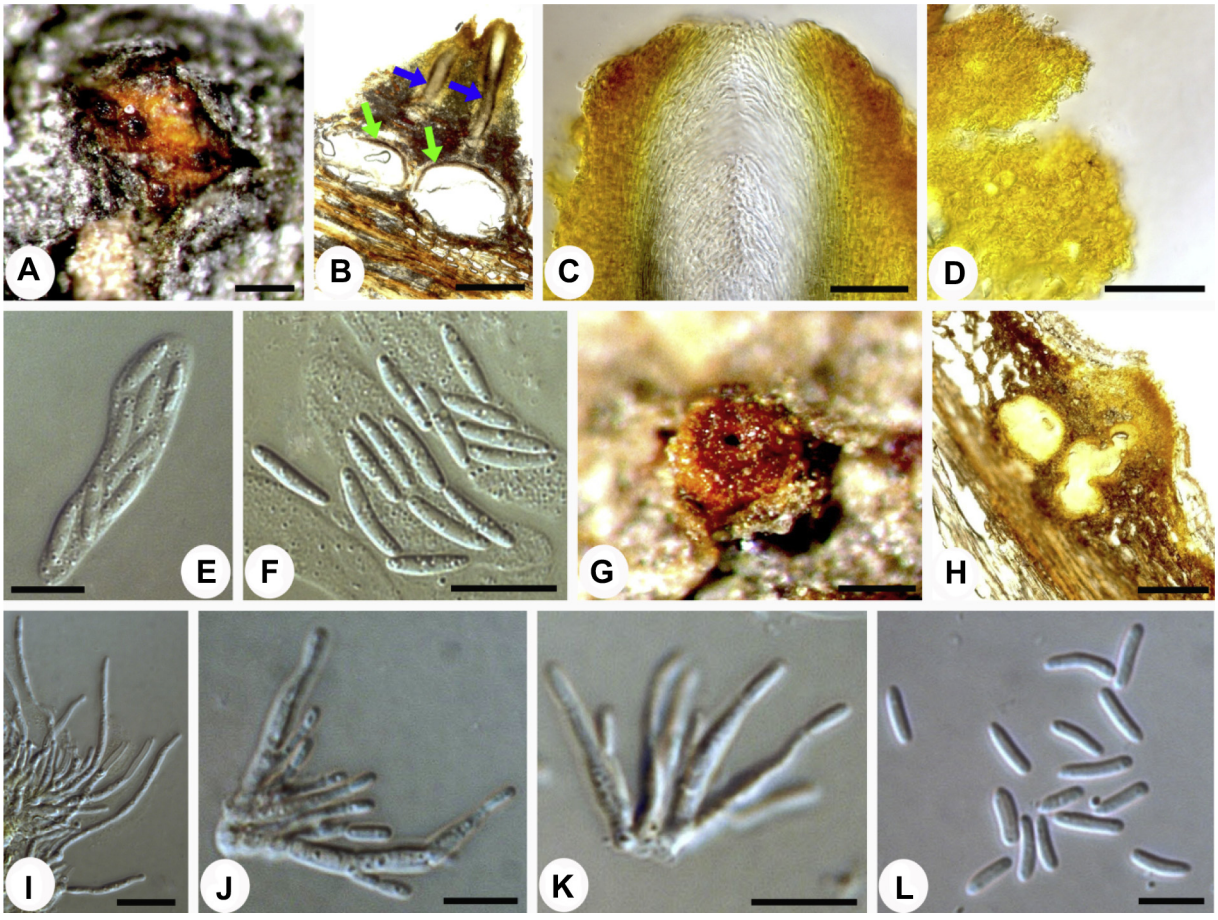

Fig 8 – Fruiting structures of *Holocryphia gleniana*. (A) Ascostroma on bark. (B) Longitudinal section through ascostroma showing orange, semi-immersed ascostromata, perithecial necks are indicated with blue arrow, perithecial bases are indicated with green arrow. (C) Perithecial neck tissue of textura porrecta. (D) Pseudoparenchymatous stromatic tissue of the ascostroma. (E) Ascus with ascospores. (F) Fusiform ascospores. (G) Conidiomata on the bark. (H) Longitudinal section through conidioma showing orange conidiomata. (I) Paraphyses. (J, K) Conidiophores and conidiogenous cells. (L) Fusoid conidia. Scale bars: A, G = 200 μm; B, H = 100 μm; C, D = 20 μm; E, F, I, J, K = 10 μm; L = 5 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pseudoparenchymatous, covering the tops of the perithecial bases; ascostromata extending 40–220 μm high above the bark, 120–600 μm diam. ***Perithecia*** valsoid, fuscous black, embedded beneath surface of bark at base of stromata; perthecial bases globose to subglobose, 70–320 μm diam.; perithecial necks black, emerging at stromatic surface as black ostioles with orange stromatic tissue of *textura porrecta*, necks 50–110 μm wide, up to 440 μm long, no necks extending above stromatic surface. ***Asci*** fusoid to ellipsoidal, biseriate, unitunicate, released from inner wall of perithecia when mature, nonstipitate, eight-spored, (24.0–)29.0–36.0(–44.0) × (5.0–)5.5–8.0(–10.0) μm (av. 33.0 × 7.0 μm). ***Ascospores*** hyaline, aseptate, cylindrical to fusiform, occasionally allantoid, ends round to slightly tapered, (6.5–)7.0–11.0(–14.0) × (1.2–)1.6–2.0(–2.3) μm (av. 9.0 × 1.8 μm). ***Conidiomata*** part of ascomata as conidial locules or as separate structures, orange, pulvinate, semiimmersed, without neck, uni to multilocular structures, with locules often convoluted, ostioles covered with orange tissue; stromatic tissue pseudoparenchymatous; conidiomatal bases 50–250 μm high above the bark surface, 240–750 μm diam.; locus 30–380 μm diam. ***Conidiophores*** nonseptate, cylindrical, (4–)12–25(–34) μm long, occasionally with separating septa and branching, hyaline. ***Conidiogenous cells*** 1–2 μm wide, cylindrical or flask-shaped with attenuated apices. Cylindrical **paraphyses** occur among conidiogenous cells, up to 85 μm long, 1–2.5 μm wide, slightly tapered towards apex, branching occasionally. ***Conidia*** hyaline, cylindrical to fusoid, occasionally allantoid, aseptate, (3.5–)4.5–5(–6.2) × (0.9–)1.1–1.4(–1.5) μm (av. 4.7 × 1.2 μm).

Culture characteristics: On MEA *H. gleniana* fluffy with an uneven margin, white when young, turning yellow–white to pale luteous with pale luteous patches after 14 d. Colony reverse luteous. Optimal growth temperature 25–30 °C, no growth at 35 °C. In 15 d, colonies at 5 °C intervals from 5 °C to 20 °C reached 8.0 mm (5 °C), 25.0 mm (10 °C), 50.0 mm (15 °C), 80.0 mm (20 °C), and 85 mm (25–30 °C), respectively.

Substrate: Bark of *M. angustifolia*.

Distribution: Western Cape Province, South Africa.

Specimens examined: South Africa, Western Cape Province, Citrusdal area, from bark of *M. angustifolia*. 8 March 2011, Jolanda Roux & ShuaiFei Chen, HOLOTYPE PREM 60747 (branches with mature ascostromata and conidiomata), extype culture CMW37334 = CBS 132871 (isolated from ascostromata); 8 March 2011, Jolanda Roux & ShuaiFei Chen, PARATYPE PREM 60748 (branches with mature conidiomata), living culture CMW37335 = CBS 132872; 8 March 2011, Jolanda Roux & ShuaiFei Chen, PARATYPE PREM 60749 (branches with mature conidiomata), living culture CMW37336 = CBS 132873.

Holocryphia mzansi S.F. Chen & Jol. Roux, **sp. nov.**
(Fig 9)
MycoBank No.: MB564865

Etymology: Mzanzi (isiXhosa language) is the word for south and often used to refer to South Africa. The name is thus indicative of the region South Africa and Swaziland where the fungus was first found.

Ascostromata on bark gregarious or single, orange, pulvinate, semiimmersed in the bark; ascostromatic tissue pseudoparenchymatous; covering the tops of the perithecial bases; ascostromata extending 60–260 μm high above the bark, 150–900 μm diam. ***Perithecia*** valsoid, fuscous black, embedded beneath surface of bark at base of stromata; perithecial bases globose to subglobose, 90–390 μm diam.; perithecial necks black, emerging at stromatic surface as black ostioles with orange stromatic tissue of *textura porrecta*, necks 54–120 μm wide, up to 540 μm long, no neck extending above stromatal surface. ***Asci*** cylindrical to fusoid, biseriate, unitunicate, released from inner wall of perithecia when mature, non-stipitate, eight-spored, (26.0–)32.0–39.0(–46.0) × (7.0–)7.5–9.5(–11.0) μm (av. 36.0 × 8.5 μm). ***Ascospores*** hyaline, aseptate, cylindrical to fusiform, occasionally allantoid, ends round to slightly tapered, (8.0–)9.0–12.0(–16.0) × (1.4–)1.7–2.1(–2.4) μm (av. 10.5 × 1.9 μm). ***Conidiomata*** part of ascomata as conidial locules or as separate structures, orange, pulvinate, semiimmersed, without necks, uni to multilocular structures, with locules often convoluted, ostioles covered with orange tissue; stromatic tissue pseudoparenchymatous; conidiomatal bases 40–340 μm high above the bark surface, 300–850 μm diam.; locus 50–500 μm diam. ***Conidiophores*** nonseptate, cylindrical, (4–)13–24(–38) μm long, occasionally with separating septa and branching, hyaline. ***Conidiogenous cells*** 1–2 μm wide, cylindrical or flask-shaped with attenuated apices. Cylindrical ***paraphyses*** occur among conidiogenous cells, up to 60 μm long, 1–2.5 μm wide, slightly tapered towards apex, branching occasionally. ***Conidia*** hyaline, cylindrical to fusoid, occasionally allantoid, aseptate, (3.5–)4–5(–6.2) × 1.0–1.5 μm (av. 4.5 × 1.2 μm).

Culture characteristics: On MEA *H. mzansi* is fluffy with an uneven margin, white when young, turning luteous with luteous/sienna patches after 14 d. Colony reverse luteous to umber. Optimal growth temperature 25 °C, no growth at 35 °C. In 15 d, colonies at 5 °C intervals from 5 °C to 30 °C reaching an average diameter of 8.0 mm (5 °C), 15.0 mm (10 °C), 45.0 mm (15 °C), 80.0 mm (20 °C), 85 mm (25 °C), and 65.0 mm (30 °C), respectively.

Substrate: Bark of *M. angustifolia* and *Eucalyptus grandis*.

Distribution: Western Cape Province, South Africa and Swaziland.

Specimens examined: South Africa, Western Cape Province, Kleinmond area, from bark of *M. angustifolia*. 10 March 2011, Jolanda Roux & ShuaiFei Chen, HOLOTYPE PREM 60750 (branches with mature ascostromata and conidiomata), extype culture CMW37337 = CBS 132874 (isolated from ascostromata); 10 March 2011, Jolanda Roux & ShuaiFei Chen, PARATYPE PREM 60751 (branches with mature conidiomata), living culture CMW37338 = CBS 132875.

Dichotomous key to genera of Cryphonectriaceae

This key is adapted from Vermeulen *et al.* (2011), and based on both anamorph and teleomorph characteristics.

1. Orange conidiomata.....**2**
Conidiomata uniformly black to black with orange necks.....**12**
2(1). Conidiomata pulvinate, without a neck; ascospores septate or aseptate.....**3**
Conidiomata conical or rostrate or pyriform or convex, with or without a neck; ascospores septate.....**8**
3(2). Paraphyses absent.....**4**
Paraphyses present.....**6**
4(3). Ascospores septate; stromata semiimmersed; conidiomata uni to multilocular or unilocular.....**5**
Ascospores aseptate; stromata mostly superficial; conidiomata multilocular..... ***Endothia***

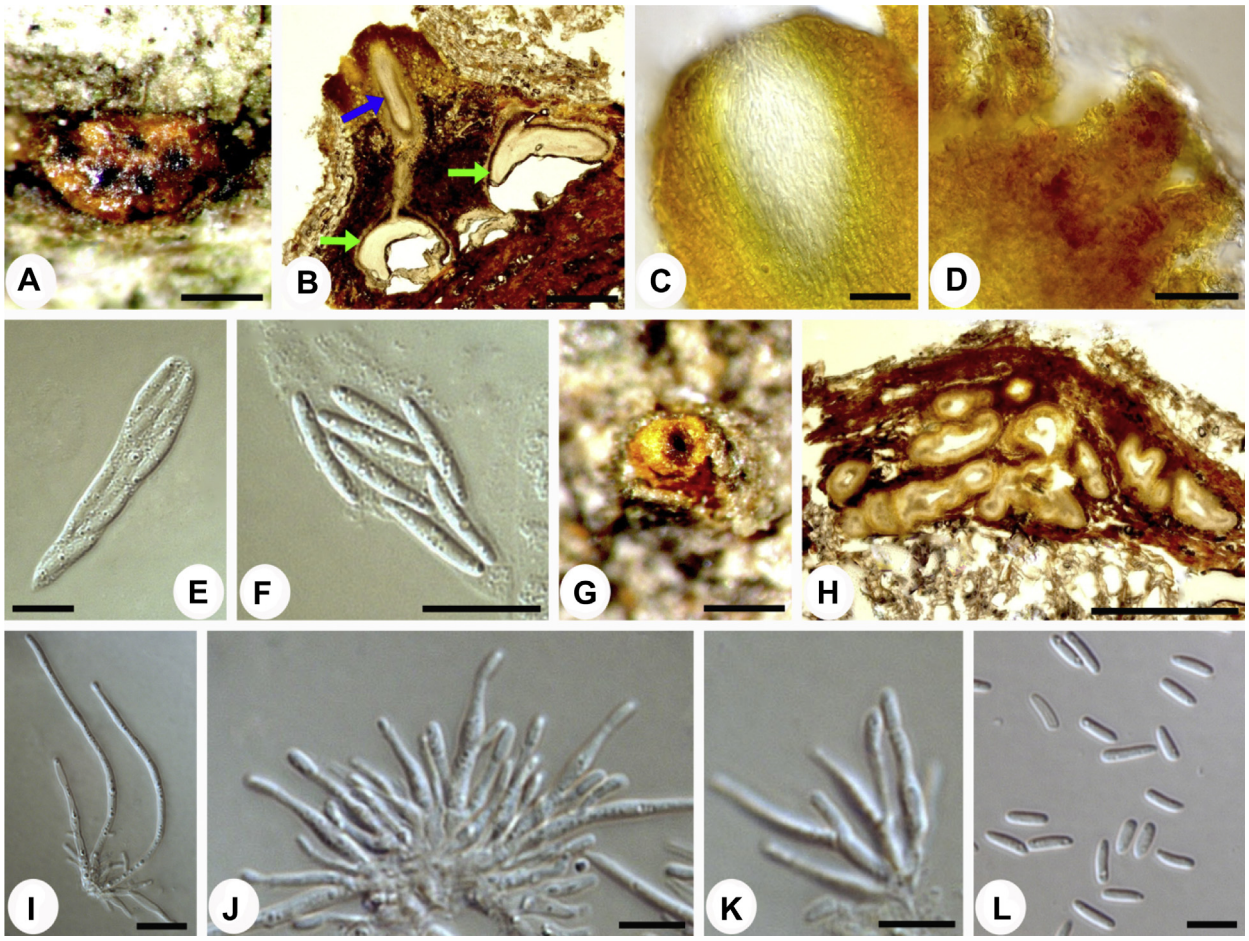

Fig 9 – Fruiting structures of *Holocryphia mzansi*. (A) Ascostroma on bark. (B) Longitudinal section through ascostroma showing orange, semiimmersed ascostromata, perithecial neck is indicated with blue arrow, perithecial bases are indicated with green arrow. (C) Perithecial neck tissue of textura porrecta. (D) Pseudoparenchymatous stromatic tissue of the ascostroma. (E) Ascus with ascospores. (F) Fusiform ascospores. (G) Conidiomata on the bark. (H) Longitudinal section through conidioma showing orange conidiomata. (I) Paraphyses. (J, K) Conidiophores and conidiogenous cells. (L) Fusoid conidia. Scale bars: A, G = 200 μm; B, H = 100 μm; C, D = 20 μm; E, F, I, J, K = 10 μm; L = 5 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5(4). Conidiomata usually larger than 350 μm diam., uni to multilocular; ascospores with median septum..... ***Cryphonectria***

Conidiomata usually smaller than 350 μm diam., unilocular; ascospores with median to submedian septum..... ***Cryptometrion***

6(3). Stromata usually smaller than 900 μm, stromata superficial to semiimmersed; ascospores septate or aseptate..... **7**

Stromata usually larger than 1000 μm, stromata immersed; teleomorph still unknown..... ***Immersiporthe***

7(6). Ascospores septate; stromata semiimmersed to superficial..... ***Microthia***

Ascospores aseptate; stromata semiimmersed..... ***Holocryphia***

8(2). Conidiomata with necks; ascospores single septate..... **9**

Conidiomata without necks; ascospores single to multiple septate..... **11**

9(8). Conidiomata with prominent, delimited necks; perithecial necks covered with orange tissue when teleomorph known..... **10**

Conidiomata with neck continuous with base, rostrate; white sheath of tissue surrounding perithecial necks when sectioned longitudinal..... ***Rostraureum***

10(9). Conidiomata rostrate to pyriform with large base, necks attenuated or not; teleomorph still unknown..... ***Ursicollum***

Conidiomata conical with constricted, fattened necks, shape like a chess pawn; teleomorph known..... ***Latruncellus***

11(8). Conidiomata conical, orange, nonostiolate; ascospores one to three-septate..... ***Amphilogia***

Conidiomata convex, orange, with blackened ostiolar openings; ascospores one-septate..... ***Aurifilum***

12(1). Conidiomata uniformly black when mature; teleomorph known..... **13**

Conidiomata black with orange necks; teleomorph still unknown..... ***Aurapex***

13(12). Conidiomata without or occasionally with short necks; paraphyses present; perithecial necks above the stromatic surface short or absent, perithecial necks black, covered with the same colour tissue as ascostromata..... **14**

Conidiomata with attenuated necks; paraphyses absent; perithecial necks above the stromatic surface long, perithecial necks black, covered with umber tissue, different from the orange tissue of ascostromata..... ***Chrysoporthe***

14(13). Conidiomata occasionally with short necks; conidiomata base tissue prosenchymatous; perithecia above the stromatic surface with short necks, perithecial necks black,

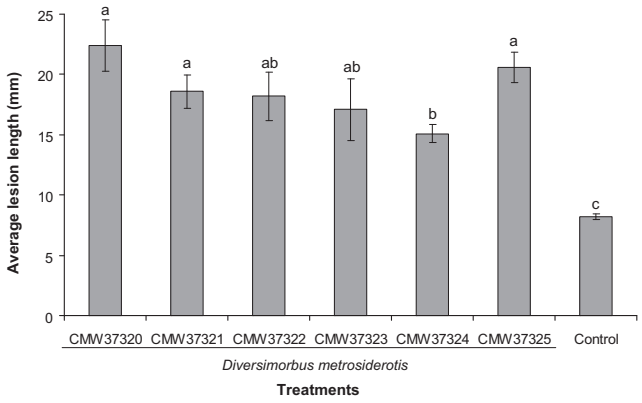

Fig 10 – Column chart indicating the average lesion lengths (mm) resulting from inoculation trials with *D. metrosiderotis* onto stems/branches of *M. angustifolia*. Vertical bars represent standard error of means. Different letters above the bars indicate treatments that were significantly different ($P = 0.05$).

covered with the same colour (orange to umber) tissue as ascostromata.....***Celoporthe***

Conidiomata without necks; conidiomata base tissue pseudoparenchyma; perithecia above the stromatic surface absent, perithecial necks black, covered with the same colour (orange) tissue as ascostromata.....***Diversimorbus***

Dichotomous key to Holocryphia *species*

The key is based on characteristics of asci, ascospores, and conidia:

1. Asci longer than 40 μm; conidia cylindrical to fusiform, occasionally allantoid, conidia longer than 5 μm.....**2**

Asci shorter than 35 μm; conidia cylindrical, occasionally allantoid, conidia no longer than 5 μm.....***Holocryphia eucalypti***

2(1). Asci fusoid to ellipsoidal, asci shorter than 45 μm; ascospores shorter than 15 μm.....**3**

Asci cylindrical to fusiform, asci longer than 45 μm; ascospores longer than 15 μm.....***Holocryphia mzansi***

3(2). Ascospores shorter than 13 μm; conidia shorter than 5.5 μm.....***Holocryphia capensis***

Ascospores longer than 13 μm; conidia longer than 6.0 μm.....***Holocryphia gleniana***

Pathogenicity tests

The six isolates of *Diversimorbus metrosiderotis* (CMW37320–37325) inoculated on the branches of *M. augustifolia* in the natural environment in which this fungus occurs produced lesions within 6 wk, while wounds associated with the control inoculations were covered by callus in the same period (Fig 10). The mean comparison tests showed that the lesions caused by isolates of *D. metrosiderotis* were all significantly longer ($P < 0.001$) than the wounds caused by the controls (Fig 10). *Diversimorbus metrosiderotis* was successfully reisolated from all the lesions resulting from the inoculations but never from the control inoculations.

The lesions produced by the 13 isolates of *Holocryphia*, representing *Holocryphia capensis* (CMW37328, CMW37331–CMW37333, CMW339–CMW37341, CMW37887), *Holocryphia gleniana* (CMW37334–CMW37336), and *Holocryphia mzansi* (CMW37337, CMW37338) were all significantly longer ($P < 0.001$) than the wounds associated with control inoculations (Fig 11). All species had similar levels of aggressiveness on *Metrosideros angustifolia* and significant differences were observed between isolates of the same species (Fig 11). *Holocryphia capensis*, *H. gleniana*, and *H. mzansi* were successfully reisolated

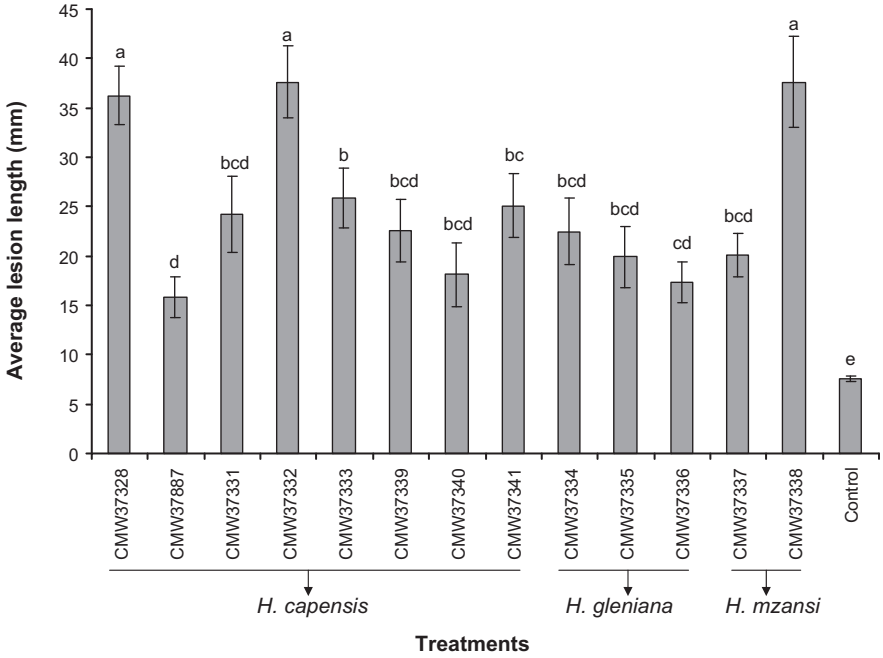

Fig 11 – Column chart indicating the average lesion lengths (mm) resulting from inoculation trials with *H. capensis*, *H. gleniana*, and *H. mzansi* onto stems/branches of *M. angustifolia*. Vertical bars represent standard error of means. Different letters above the bars indicate treatments that were significantly different ($P = 0.05$).

from the lesions, but not from the wounds made for the control inoculations.

Discussion

Very little is known regarding the diseases of the megadiverse woody-plant flora of southern Africa. In this study, we considered the presence of cankers on the native Myrtaceous tree *Metrosideros angustifolia* that is restricted to the Western Cape Province. The motivation for choosing this tree for investigation was the fact that native *Myrtaceae* have previously (Heath *et al.* 2006; Gryzenhout *et al.* 2009; Vermeulen *et al.* 2011) been shown to harbour important plant pathogens residing in the *Cryphonectriaceae*. The study has led to the discovery of a new genus and species as well as three new species of *Holocryphia* residing in this fungal family. Based on previous findings (Wingfield 2003; Gryzenhout *et al.* 2009), these fungi, all shown to be pathogens, have the potential to undergo host shifts to infect other species of *Myrtaceae* including commercially important crop plants and trees that are valuable components of natural ecosystems, both in southern Africa and elsewhere.

Diversimorbus represents the fifth genus in the *Cryphonectriaceae* to be discovered in South Africa and is the 16th genus to be added to this family that includes many important tree pathogens (Gryzenhout *et al.* 2009; Begoude *et al.* 2010; Vermeulen *et al.* 2011; Chen *et al.* 2012). It can be distinguished from all other genera in the family based on morphology and DNA sequence data. It has a wide geographic distribution on *M. angustifolia* in the Western Cape Province, being found from Citrusdal in the north of the survey area to Kleinmond in the south; a distance of more than 200 km. The fungus was shown to be pathogenic on its host, although not highly virulent and it is most likely a native fungus in the area.

Three new species of *Holocryphia* were found from *M. angustifolia* trees in this study. *Holocryphia capensis* was widely distributed in most of the areas surveyed and it was the most commonly encountered *Holocryphia* sp. in the study. In contrast, *Holocryphia gleniana* and *Holocryphia mzansi* were each found only in a single area. Where all three species (*H. gleniana*, *H. capensis*, and *H. mzansi*) were present in the same area, they could all be found together on a single *M. angustifolia* tree.

In this study, it was possible to reevaluate the taxonomy of *Holocryphia*, a genus for which the taxonomy has been somewhat confused in the past. Previously, the genus included only the single species *Holocryphia eucalypti*, a relatively mild pathogen of *Eucalyptus* trees in South Africa and one that was believed to have been introduced into the country from Australia (Gryzenhout *et al.* 2006b; Nakabonge *et al.* 2008). All previous studies deal with *Holocryphia* treated isolates collected from Australia, New Zealand, South Africa, Swaziland, and Uganda and these were all considered to represent the single species, *H. eucalypti* (Venter *et al.* 2002; Gryzenhout *et al.* 2006b, 2010b; Heath *et al.* 2007; Roux & Nakabonge 2010; Vermeulen *et al.* 2011). A study by Heath *et al.* (2007), recognised the presence of subclades in analyses of DNA sequence data for this fungus, but a lack of useful herbarium material, including morphological structures, precluded studies that might have led to the description of new species. The addition of a large collection of isolates from *M. angustifolia* emerging from this study and the inclusion of gene regions not previously considered allowed for a more robust analysis of the genus. This has shown clearly that the type species, *H. eucalypti* should be restricted to isolates from *Eucalyptus* trees in South Africa. These are clearly distinct from isolates on the same host in other parts of the world and they are also distinct from any of those collected on *M. angustifolia* in this study. Although evidence suggests that isolates from *Tibouchina* spp. and *Eucalyptus* spp. in Australia and New Zealand represent distinct taxa, we have chosen not to describe them as this step requires the collection of additional material and it would also detract from the focus on isolates from *M. angustifolia*.

Results in this study have shown that species of *Holocryphia* may undergo host shifts between different tree genera in the *Myrtaceae*. The phylogenetic analyses have provided good evidence that *Holocryphia* isolates from *Eucalyptus* trees in Swaziland are very closely related to those of *H. mzansi* from *M. angustifolia* in South Africa. This is an intriguing discovery since there is a considerable distance (more than 1500 km) between the Western Cape Province, where *H. mzanzi* was collected, and Swaziland. Furthermore, *M. angustifolia* is a native tree restricted to the Western Cape Province while *Eucalyptus* spp. are non-natives in South Africa. Given its distribution and the lack of serious disease symptoms, *H. mzansi* is most likely native to the southern part of South Africa and this would imply that it represents another example of a host shift in this group of pathogens (Slippers *et al.* 2005; Heath *et al.* 2006; Van der Merwe *et al.* 2010) in the region. While the areas of occurrence of *M. mzansi* are separated by a considerable distance, a relatively uninterrupted continuum of *Eucalyptus* spp. is found along the eastern sea-board of South Africa and the pathogen could easily have moved in this area along with extensive *Eucalyptus* forestry operations. Further studies, including population genetic analyses will contribute to a better understanding of this potentially important host shifts.

Pathogenicity tests with all four new fungal species collected from *M. angustifolia* showed that they are able to cause disease on their apparently native host trees. All four fungi produced lesions within 6 wk of inoculation whereas the controls resulted in callusing and wound healing. The fungi are clearly canker pathogens on *M. angustifolia* but they did not show signs of causing serious disease problems. This supports the view that they are likely native pathogens and have the potential to be able to spread to new hosts and areas (Heath *et al.* 2006; Nakabonge *et al.* 2008; Gryzenhout *et al.* 2009).

Results of this study emphasise the fact that there are many pathogens of woody plants, including some iconic trees, which remain to be discovered in South Africa. The country has a megadiverse flora; for example the area where this study was undertaken is part of the Cape Fynbos Biome, which is the smallest yet most diverse of all the Floral Kingdoms on earth (Myers *et al.* 2000; Born *et al.* 2006). Very little is known regarding the fungal diversity in this fragile ecosystem (Crous *et al.* 2006; Lee *et al.* 2008) and importantly the threat of pathogens to it (Knox-Davies 1981; Roets *et al.* 2009; Chen *et al.* 2012). Studies such as this one are required to avert dramatic damage to such native woody

ecosystems, as has occurred for example with Chestnut Blight, Dutch Elm disease, and other diseases elsewhere in the world.

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REFERENCES

Anagnostakis SL, 1987. Chestnut blight: the classical problem of an introduced pathogen. *Mycologia* **79**: 23–37.

Anagnostakis SL, 1992 Annual Report of the Northern Nut Growers Association. *Chestnuts and the Introduction of Chestnut Blight* **83**: 39–42.

Begoude BAD, Gryzenhout M, Wingfield MJ, Roux J, 2010. *Aurifilum*, a new fungal genus in the *Cryphonectriaceae* from *Terminalia* species in Cameroon. *Antonie van Leeuwenhoek* **98**: 263–278.

Born J, Linder HP, Desmet P, 2006. The greater cap floristic region. *Journal of Biogeography* **34**: 147–162.

Carbone I, Kohn LM, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN, 2002. A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.

Cheewangkoon R, Groenewald JZ, Summerell BA, Hyde KD, Toanun C, Crous PW, 2009. *Myrtaceae*, a cache of fungal biodiversity. *Persoonia* **23**: 55–85.

Chen SF, Gryzenhout M, Roux J, Xie YJ, Wingfield MJ, Zhou XD, 2011. Novel species of *Celoporthe* from *Eucalyptus* and *Syzygium* trees in China and Indonesia. *Mycologia* **103**: 1384–1410.

Chen SF, Wingfield MJ, Roets F, Roux J, 2012. A serious canker disease caused by *Immersiporthe knoxdaviesiana* gen. et sp. nov. (*Cryphonectriaceae*) on native *Rapanea melanophloeos* in South Africa. *Plant Pathology*. http://dx.doi.org/10.1111/j.1365-3059.2012.02671.x.

Clement M, Posada D, Crandall K, 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1660.

Coates Palgrave K, 2002. *Trees of Southern Africa*, 3rd edn. Struik, Cape Town, South Africa.

Conradie E, Swart WJ, Wingfield MJ, 1990. Cryphonectria canker of *Eucalyptus*, an important disease in plantation forestry in South Africa. *South African Forestry Journal* **152**: 43–49.

Crous PW, Rong IH, Wood A, Lee S, Glen HF, Botha W, Slippers B, De Beer ZW, Wingfield MJ, Hawksworth DL, 2006. How many species of fungi are there at the tip of Africa? *Studies in Mycology* **55**: 13–33.

Gilbert GS, Webb CO, 2007. Phylogenetic signal in plant pathogen–host range. *Processing of the National Academy of Sciences of the USA* **104**: 4979–4983.

Gryzenhout M, Myburg H, Van der Merwe NA, Wingfield BD, Wingfield MJ, 2004. *Chrysoporthe*, a new genus to accommodate *Cryphonectria cubensis*. *Studies in Mycology* **50**: 119–142.

Gryzenhout M, Myburg H, Wingfield BD, Wingfield MJ, 2006a. *Cryphonectriaceae* (*Diaporthales*), a new family including *Cryphonectria*, *Chrysoporthe*, *Endothia* and allied genera. *Mycologia* **98**: 239–249.

Gryzenhout M, Myburg H, Hodges CS, Wingfield BD, Wingfield MJ, 2006b. *Microthia*, *Holocryphia* and *Ursicollum*, three new genera on *Eucalyptus* and *Coccoloba* for fungi previously known as *Cryphonectria*. *Studies in Mycology* **55**: 35–52.

Gryzenhout M, Myburg H, Rodas CA, Wingfield BD, Wingfield MJ, 2006c. *Aurapex penicillata* gen. sp. nov. from native *Miconia theaezans* and *Tibouchina* spp. in Colombia. *Mycologia* **98**: 105–115.

Gryzenhout M, Wingfield BD, Wingfield MJ, 2009. *Taxonomy, Phylogeny, and Ecology of Bark-inhabiting and Tree Pathogenic Fungi in the* Cryphonectriaceae. APS Press, St. Paul, MN, USA.

Gryzenhout M, Tarigan M, Clegg PA, Wingfield MJ, 2010a. *Cryptometrion aestuescens* gen. sp. nov. (*Cryphonectriaceae*) pathogenic to *Eucalyptus* in Indonesia. *Australasian Plant Pathology* **39**: 161–169.

Gryzenhout M, Vermeulen M, Dick M, Wingfield MJ, 2010b. The *Eucalyptus* canker pathogen *Holocryphia eucalypti* on *Eucalyptus* in New Zealand. *Australasian Plant Disease Notes* **5**: 5–8.

Heath RN, Gryzenhout M, Roux J, Wingfield MJ, 2006. Discovery of the canker pathogen *Chrysoporthe austroafricana* on native *Syzygium* spp. in South Africa. *Plant Disease* **90**: 433–438.

Heath RN, Roux J, Gryzenhout M, Carnegie AJ, Smith IW, Wingfield MJ, 2007. *Holocryphia eucalypti* on *Tibouchina urvilleana* in Australia. *Australasian Plant Pathology* **36**: 560–564.

Katoh K, Misawa K, Kuma K, Miyata T, 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.

Knox-Davies PS, 1981. Comments on fungal diseases of plants indigenous to the south western Cape. *Veld and Flora* **67**: 88–91.

Lee S, Crous PW, Groenewald E, Wingfield MJ, 2008. *Fungi on the Proteaceae*. *CBS Fungal Biodiversity Series No. 7*. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Myburg H, Wingfield BD, Wingfield MJ, 1999. Phylogeny of *Cryphonectria cubensis* and allied species inferred from DNA analysis. *Mycologia* **91**: 243–250.

Myers N, Mittelmeier RA, Mittelmeier CG, da Fonseca GAB, Kent J, 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.

Nakabonge G, Gryzenhout M, Roux J, Wingfield BD, Wingfield MJ, 2006. *Celoporthe dispersa* gen. et sp. nov. from native *Myrtales* in South Africa. *Studies in Mycology* **55**: 255–267.

Nakabonge G, Burgess T, Gryzenhou M, Wingfield BD, Wingfield MJ, Roux J, 2008. Population structure of the fungal pathogen *Holocryphia eucalypti* in Australia and South Africa. *Australasian Plant Pathology* **37**: 154–161.

Rayner RW, 1970. *A Mycological Colour Chart*. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey, UK.

Roets F, Wingfield MJ, Crous PW, Dreyer LL, 2009. Fungal radiation in the Cape Floristic Region: an analysis based on *Gondwanamyces* and *Ophiostoma*. *Molecular Phylogenetics and Evolution* **51**: 111–119.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Roux J, Nakabonge G, 2010. First report of *Holocryphia eucalypti* on *Eucalyptus grandis* in Uganda. *Plant Pathology* **59**: 409.

Roux J, Chen SF, Gryzenhout M, Wingfield MJ, 2011. The *Cryphonectriaceae*: a threat to native *Metrosideros angustifolia* in the Western Cape? In: The 47th Congress of the Southern African

Society for Plant Pathology, Berg en Dal, Kruger National Park, South Africa, 23–26 January 2011.

Slippers B, Stenlid J, Wingfield MJ, 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology and Evolution* **20**: 420–421.

Stipes RJ, Phipps PM, 1971 Plant Disease Report. *A Species of* Endothia *Associated with a Canker Disease of Pin Oak (*Quercus palustris*) in Virginia*, vol. 55467–469.

Swofford DL, 2003. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10*. Sinauer Associates, Sunderland, MA, USA.

Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biological and Evolution* **24**: 1596–1599.

Van der Merwe NA, Gryzenhout M, Steenkamp ET, Wingfield BD, Wingfield MJ, 2010. Multigene phylogenetic and population differentiation data confirm the existence of a cryptic species within *Chrysoporthe cubensis*. *Fungal Biology* **114**: 966–979.

Van der Westhuizen IP, Wingfield MJ, Kemp GHJ, Swart WJ, 1993. First report of the canker pathogen *Endothia gyrosa* on *Eucalyptus* in South Africa. *Plant Pathology* **42**: 661–663.

Venter M, Myburg H, Wingfield BD, Coutinho TA, Wingfield MJ, 2002. A new species of *Cryphonectria* from South Africa and Australia, pathogenic to *Eucalyptus*. *Sydowia* **54**: 98–117.

Vermeulen M, Gryzenhout M, Wingfield MJ, Roux J, 2011. New records of *Cryphonectriaceae* from southern Africa including *Latruncellus aurorae* gen. sp. nov. *Mycologia* **103**: 554–569.

Vienne DM, Hood ME, Giraud T, 2009. Phylogenetic determinants of potential host shifts in fungal pathogens. *Journal of Evolutionary Biology* **22**: 2532–2541.

Wingfield MJ, Swart WJ, Abear BJ, 1989. First record of *Cryphonectria* canker of *Eucalyptus* in South Africa. *Phytophylactica* **21**: 311–313.

Wingfield MJ, 2003. Increasing threat of diseases to exotic plantation forests in the Southern Hemisphere: lessons from Cryphonectria canker. *Australasian Plant Pathology* **32**: 133–139.

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J, 2008. Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests* **70**: 139–144.

Zhang N, Blackwell M, 2001. Molecular phylogeny of dogwood anthracnose fungus (*Discula destructiva*) and the Diaporthales. *Mycologia* **93**: 355–365.