The *Eucalyptus* canker pathogen *Holocryphia eucalypti* on *Eucalyptus* in New Zealand

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Abstract

Holocryphia eucalypti is an opportunistic canker pathogen of *Eucalyptus* and *Corymbia* spp. (Myrtaceae, Myrtales) in Australia and South Africa. It is also known in Australia on *Tibouchina* trees (Melastomataceae, Myrtales). Using DNA sequence comparisons and morphological characterisation, we show for the first time that *H. eucalypti* is present in New Zealand on *Eucalyptus* spp.

Article

Holocryphia eucalypti (=*Cryphonectria eucalypti*) causes die-back, and stem and branch cankers of several *Eucalyptus* and *Corymbia* spp. in natural forests and commercial plantations in Australia and South Africa (Walker *et al*. 1985; Van der Westhuizen *et al*. 1993; Carnegie 2007). The pathogen has also recently been reported on *Tibouchina urvilleana* (Lassiandra) planted as ornamentals in Australia (Heath *et al*. 2007). The canker disease caused by *H. eucalypti* is closely associated with stress conditions such as drought and cankers can be relatively mild or lethal where host trees are susceptible and stress is severe (Old *et al*. 1986; Wardlaw 1999; Gryzenhout *et al*. 2003). Because predisposition plays such an important role in disease development, *H. eucalypti* is not considered as severe (Yuan and Mohammed 2000) as other closely related *Eucalyptus* pathogens such as species of *Chrysoporthe* (Gryzenhout *et al*. 2009).

Canker caused by *H. eucalypti* is common on many *Eucalyptus* and *Corymbia* species in forests and plantations of eastern Australia including Tasmania, and it also occurs in Western Australia (Old *et al*. 1986; Davison and Coates 1991; Wardlaw 1999; Yuan and Mohammed 2000; Carnegie 2007). In South Africa, it occurs on commercially propagated, non-native *Eucalyptus* spp. in plantations (Van der Westhuizen *et al*. 1993; Gryzenhout *et al*. 2003). Population genetic studies employing microsatellite data have shown that *H. eucalypti* was most likely introduced into South Africa, although evidence for its origin in Western Australia was less convincing (Nakabonge *et al*. 2008).

During the course of the past 24 years, isolates of a fungus in the Cryphonectriaceae have been collected from various *Eucalyptus* species in New Zealand. Most locations were in the southern half of the North Island with one from the north of the South Island (Table 1). Based on cultural characteristics and the morphology of conidia produced in culture, the isolates were identified as

Endothiella-like, which represent the anamorph state of some species in the Cryphonectriaceae (Gryzenhout *et al*. 2009). However, in the absence of sexual fruiting bodies or well-defined fruiting bodies on host tissue and in culture, a definitive identification could not be made. The aim of this study was thus to characterise the isolates from *Eucalyptus* stems in New Zealand based on DNA sequence comparisons for the β-Tubulin gene region.

Table 1. Isolates and specimens used in this study.

Species identity	Isolate[A]	Host	Origin	Collector	Date	GenBank accession[B]
Holocryphia eucalypti	CMW7036/CBS119478	*Eucalyptus* sp.	Mpumalanga South Africa	I van der Westhuizen	n/a	AF 368341, AF 368340
	CMW7037/CBS119477	*Eucalyptus delegatensis*	NSW, Australia	KM Old	n/a	AF 368343, AF 368342
	CMW7038/CBS119475	*Eucalyptus globulus*	WA, Australia	MJ Wingfield	September 1997	AF 368345, AF 368344
	CMW14545/CBS115852	*Eucalyptus* sp.	South Africa	I van der Westhuizen	n/a	DQ 368730, DQ 368731
	CMW8541/CBS115720	*Eucalyptus* sp.	Nseleni, KwaZulu-Natal, South Africa	J Roux	July 2007	GQ 465234
	CMW7033/CBS115842	*Eucalyptus grandis*	South Africa	M Venter		DQ 368728, DQ 368729
	CMW6246	*Tibouchina urvilleana*	Melbourne, Australia	MJ Wingfield	September 2000	EF 127995, EF 128000
	CMW6249	*T. urvilleana*	Melbourne, Australia	MJ Wingfield	September 2000	EF 127996, EF 128001
	CMW6244	*T. urvilleana*	Melbourne, Australia	MJ Wingfield	September 2000	EF 127997, EF 128002
	CMW10729	*T. urvilleana*	Brisbane, Australia	MJ Wingfield	2000	EF 127998, EF 128003
	CMW10010/NZFS79/E	*Eucalyptus fastigata*	Castleridge, Wellington, New Zealand	BJ Rogan	1997	AY 214236, AY 214272
	CMW10011/NZFS79/F	*Eucalyptus* sp.	Central Park, Wellington, New Zealand	BJ Rogan	1998	AY 214237, AY 214273
	CMW10015/NZFS79.15	*Eucalyptus fastigata*	Jackson Road, Genmarie Blk., Bay of Plenty., New Zealand	RJ van Boven	1999	GQ 427071
	CMW10016/NZFS79.17	*Eucalyptus nitens*	Omatoroa, Bay of Plenty, New Zealand	MR Twaddle	1999	GQ 427068
	CMW10017/NZFS372	*Eucalyptus ficifolia*	Lambton Quay, Wellington, New Zealand	L Renney	2000	GQ 427069
	CMW10021/NZFS833	*E. globulus*	Westport, Buller, New Zealand	PM Bradbury	2001	GQ 427072
	CMW10797/NZFS79	*Eucalyptus regnans*	Wanganui, New Zealand	MA Dick	1985	AY 214238, AY 214274
	CMW12723[C]	*E. globulus*	Auckland, New Zealand	MJ Wingfield	February 2003	GQ 427070
Cryphonectria parasitica	CMW7048/ATCC48198	*Quercus virginiana*	Virginia, USA	FF Lombard	May 1960	AF 273076, AF 273470
Endothia gyrosa	CMW2091/ATCC48192	*Quercus palustris*	Virginia, USA	RJ Stipes	n/a	AF 368337, AF 368336
Microthia havanensis	CMW14550/CBS115855	*Eucalyptus saligna*	Tabasco State, Mexico	CS Hodges	February 1998	DQ 368741, DQ 368742

[A]CMW = Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; NZFS = National Forestry Culture Collection, Scion, New Zealand, Forest Research Institute Ltd.
[B]Accession numbers refer to the combined sequence data of the β-tubulin 1 and 2 regions.
[C]Original bark material deposited as PREM 60237 in the National Collection of Fungi (PREM), Pretoria, South Africa.

Original isolates curated in NZFS (National Forestry Culture Collection) were obtained from discoloured yellowish wood, from branch cankers and die-back. These isolates are maintained at 4°C in the culture collection (NZFS) of Scion, New Zealand Forest Research Institute, Rotorua, New Zealand (Table 1). Duplicates of the cultures, as well as an isolate (CMW12723) obtained from asexual fruiting bodies from a later collection, are housed in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (Table 1). The representative specimen of the original bark material from which the culture CMW12723 was made has been deposited in the National Collection of Fungi (PREM), Pretoria, South Africa (Table 1).

For DNA sequence comparisons, isolates were grown in 2% Malt Extract broth (100 µL in 1.5 µL Eppendorf tubes), the mycelium harvested and the DNA extracted following Gryzenhout *et al*. (2006). The DNA was used in polymerase chain reactions (PCR) of the β-Tubulin gene regions 1 and 2 and sequenced using the same protocols as those described by Gryzenhout *et al*. (2006). The sequences were included in a data matrix containing sequences of *H. eucalypti* isolates previously published (Gryzenhout *et al*. 2006; Heath *et al*. 2007). Sequences of isolates CMW10010 and CMW10011 were obtained from the study of Myburg (2003). Species of other genera in the Cryphonectriaceae (*Endothia gyrosa*, *Cryphonectria parasitica*, *Microthia havanensis*) were defined as outgroups. The dataset consisted of 20 taxa and the sequences were aligned with the CLUSTAL function of the program MEGA ver. 4 (Tamura *et al*. 2007) and verified manually.

Phylogenetic analyses were run with PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002). Phylogenetic trees were obtained with maximum parsimony (uninformative sites excluded, heuristic search with 100 random sequence additions and tree-bisection-reconnection branch swapping, MULTREES off, base pairs re-weighted according to the consistency index). The strength of branches was tested with a 70% bootstrap analysis (1 000 replicates).

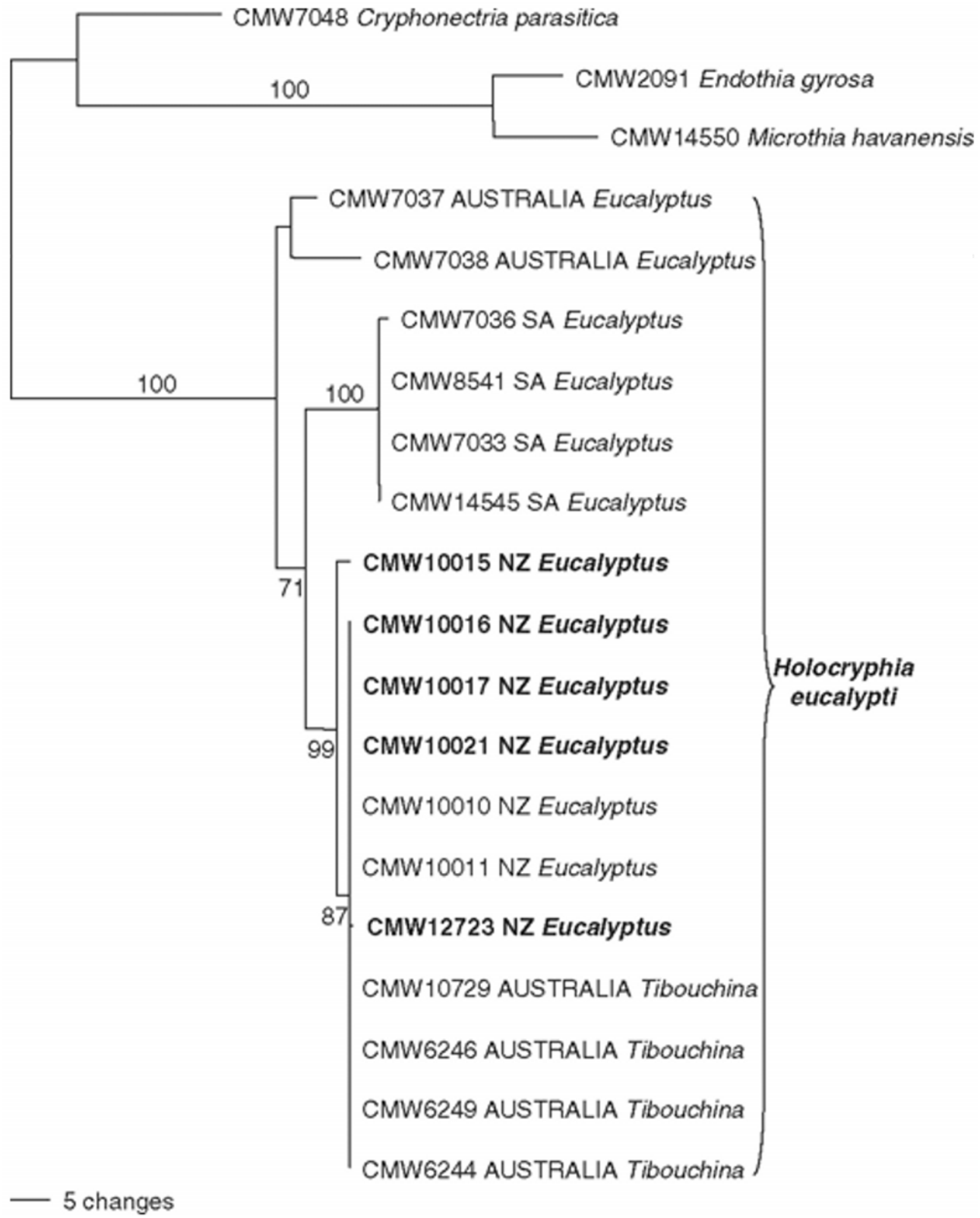

Fig. 1. Most parsimonious tree based on DNA sequences of the β-tubulin gene. Confidence levels based on a 70% bootstrap analysis are indicated on the branches, with isolates sequenced in this study in bold typeface. *Cryphonectria parasitica*, *Endothia gyrosa* and *Microthia havanensis* were defined as outgroups.

Of the 928 characters in the DNA matrix, 165 were uninformative, 645 were constant and 118 were informative. Two trees that were identical topologically but differed slightly based on length of some branches (Tree Length = 170.3, Consistency Index = 0.882, Retention Index = 0.895, g1 value = –2.62), were obtained in the analysis. The phylogenetic trees (Fig. 1) clearly showed that the

isolates from New Zealand grouped with those of *H. eucalypti* from South Africa and Australia (bootstrap support 100%). The isolates from New Zealand formed a group with those from *T. urvilleana* in Australia (bootstrap support 87%). Two other groups, including South African and Australian isolates, were also evident (bootstrap support 100% and below 50%, respectively).

This study reports the presence of the *Eucalyptus* pathogen *H. eucalypti* in New Zealand for the first time. This report further contributes to the databases and checklists of pathogens and fungi in New Zealand (http://nzfungi.landcareresearch.co.nz/html/mycology.asp database; Dingley 1969; Pennycook 1989; McKenzie *et al*. 2000; Pennycook and Galloway 2004). Due to the opportunistic nature of *H. eucalypti* on *Eucalyptus* and *Tibouchina*, the occurrence of the fungus in New Zealand is most likely not of economic significance, although its presence deserves to be monitored.

Relatively little is known regarding the Cryphonectriaceae in New Zealand. Although there are reports of *Endothia gyrosa*, a previous name used for *H. eucalypti* (Gryzenhout *et al*. 2006), in the country (Gryzenhout *et al*. 2009), these reports are from *Nothofagus* (McKenzie *et al*. 2000; Pennycook and Galloway 2004), *Myrsine salicina* and dead wood of an unknown host (http://nzfungi.landcareresearch.co.nz/html/mycology.asp database) and need to be verified based on recent taxonomic changes to the group (Gryzenhout *et al*. 2009). Reports of other Cryphonectriaceae that could represent incorrectly identified *H. eucalypti* specimens in New Zealand, include those of *Rostraureum longirostris* on *Nothofagus* (Nothofagaceae, Fagales), *Amphilogia gyrosa* and *Amphilogia major* on *Elaeocarpus* spp. (Elaeocarpaceae, Oxalidales), an *Endothiella* sp. and *Cryphonectria radicalis* (http://nzfungi.landcareresearch.co.nz/html/mycology.asp database; Dingley 1969; Pennycook 1989; McKenzie *et al*. 2000; Pennycook and Galloway 2004; Gryzenhout *et al*. 2005). In the NZFS collection, cultures of an *Endothiella* sp. identified only on the basis of culture characteristics, originate from *Myrsine chathamica* and *Leptospermum scoparium* as well as various species of eucalypts. A member of the Cryphonectriaceae has also been found on a *Coriaria* sp. (Coriariaceae, Cucurbitales) in New Zealand (PDD28477, Waiomu, Thames, Auckland, J.M. Dingley, August 1958) but the identity of this collection relies only on a herbarium specimen and cannot be confirmed (M. Gryzenhout, unpubl. data). Thus, only the identities of *A. gyrosa* and *A. major* have been verified based on recent phylogenies (Gryzenhout *et al*. 2005).

The relative proximity of New Zealand to Australia, as well as the common occurrence of *H. eucalypti* in the eastern part of Australia indicates a possibility that the fungus originated in the latter country. However, it is also possible that *H. eucalypti* occurs on native Myrtaceae in New Zealand. It may also occur on other non-native shrubs such as *Tibouchina* spp. (Melastomataceae, Myrtales), which are hosts of this fungus (Heath *et al*. 2007) and many other members of the Cryphonectriaceae (Gryzenhout *et al*. 2009). Answers to these intriguing questions will require further surveys and population genetic studies.

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