doi:10.5598/imafungus.2016.07.02.05

IMA FUNGUS · 7(2): 253–263 (2016)

ARTICLE

New cryptic species of *Teratosphaeria* on *Eucalyptus* in Australia

Vera Andjic[1], Aaron Maxwell[1], Giles E. StJ. Hardy[1], and Treena I. Burgess[1,2]

[1]School of Veterinary and Life Sciences, Murdoch University, South St, Murdoch, 6150, Australia; corresponding author e-mail: v.andjic@murdoch.edu.au

[2]Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

Abstract: *Teratosphaeria destructans* and *T. viscida* are serious pathogens causing leaf, bud and shoot blight diseases of *Eucalyptus* plantations in the subtropics and tropics of South-East Asia (*T. destructans*) and North Queensland, Australia (*T. viscida*). During disease surveys in northern Western Australia and the Northern Territory of Australia, symptoms resembling those of *T. destructans* were observed on young and adult leaves of native and plantation *Eucalyptus* spp. and its hybrids. Phylogenetic studies revealed *Teratosphaeria* species associated with these symptoms are new taxonomic novelties described here as *T. novaehollandiae* and *T. tiwiana* spp. nov. Isolates from previous records of *T. destructans* recorded in Australia were re-examined and based upon the phylogenetic evidence are reassigned to these new taxa. We conclude that *T. destructans* is absent from Australia.

Key words:
biosecurity
DNA phylogeny
Kirramyces

Article info: Submitted: 25 February 2016; Accepted: 6 October 2016; Published: 20 October 2016.

INTRODUCTION

Teratosphaeria leaf diseases (TLD; Crous *et al*. 2006, Hunter *et al*. 2006, Crous 2009, Crous *et al*. 2009a, b) have emerged as significant foliar diseases impacting on the eucalypt plantation industry in subtropical and tropical areas of Australia (Carnegie *et al*. 2007a, b, c). *Teratosphaeria* species with kirramyces-like asexual morphs have emerged as the most significant foliar pathogens of this genus; namely *T. destructans, T. eucalypti*, *T. pseudoeucalypti, T. suttonii,* and *T. viscida* (Wingfield *et al*. 1996, Park *et al*. 2000, Carnegie 2007a, b, Andjic *et al*. 2010a). These five species cause a serious leaf blight disease, leading to premature defoliation and in some instances tree mortality (Andjic *et al*. 2007b, 2010a, Carnegie 2007a, b, c). Symptoms are similar and include brown to purple spots on leaves with diffuse border and red brown margin, necrotic lesions delimited by veins and presence of spore masses and conidia (Dick 1982, Walker *et al*. 1992, Wingfield *et al*. 1996, Burgess *et al*. 2006, Andjic *et al.* 2007a, b, c, Andjic *et al*. 2010a). Conidia of these species are all long, variously curved, subhyaline to pale brown, smooth to verruculose and are virtually indistinguishable by morphology (excluding *T. suttonii*), thus making diagnostics based on morphology impossible (Andjic *et al.* 2010b, Hunter *et al*. 2011).

Teratosphaeria destructans is an aggressive pathogen causing a leaf, bud and shoot blight disease (Wingfield *et al*. 1996). This pathogen was first discovered in Indonesia in 1996 and has since been detected in Thailand, China, Vietnam, and most recently South Africa (Burgess *et al*. 2006, Old *et al*. 2003a, b, Wingfield *et al.* 1996, Greyling *et al*. 2016). In Australia, *T. destructans* is only reported from Tiwi Island in the Northern Territory (on introduced plantation *Eucalyptus* hybrids) and Derby in Western Australia (on amenity *Eucalyptus* sp.) (Burgess *et al*. 2007). *Teratosphaeria eucalypti* is a leaf parasite of endemic *Eucalyptus* species (eastern Australia) but under favourable conditions can cause a serious leaf blight disease mostly infecting juvenile leaves of some *Eucalyptus* species in plantations (Carnegie 2007b). *Teratosphaeria eucalypti* is known to have been introduced with plantings of *E. nitens* from Australia into New Zealand, where it has resulted in complete defoliation of juvenile leaves of *E. nitens* (Dick 1982, Miller *et al*. 1992). *Teratosphaeria pseudoeucalypti* was first discovered on an unidentified *Eucalyptus* sp. and hybrids of *E. grandis* × *camaldulensis* in Central Queensland, where it caused severe outbreaks and damage (Andjic *et al*. 2010a). Since then the pathogen has been detected in Argentina (Ramos & Perez 2015), Brazil (de Souza *et al*. 2014), and Uruguay (Soria *et al*. 2014). *Teratosphaeria suttonii* is known from many countries (Park *et al*. 2000, Sankaran *et al*. 1995, Taole *et al.* 2015) and can cause severe damage in eucalypt plantations (Carnegie 2007b). *Teratosphaeria viscida* was first detected in 2005 causing leaf and shoot blight in *E. grandis* and complete defoliation of *E. grandis* × *camaldulensis* hybrids in Mareeba, North Queensland (Andjic *et al*. 2007b).

Whilst *T. eucalypti, T. pseudoeucalypti, T. suttonii,* and *T. viscida* are all native to Australia, the origin of *T. destructans* is still unclear (Andjic *et al*. 2011). Based on DNA sequence variation of Australian isolates, it was thought that *T. destructans* originated from Australia (Burgess *et al*. 2007). *Teratosphaeria destructans* was a high risk pathogen for Australia and was on the Northern Australia Quarantine Strategy (NAQS) biosecurity target list (as *Kirramyces*

destructans), but later removed after being reported in northern Australia by Burgess *et al*. (2007) (Jane Ray pers. comm.).

During surveys of native and plantation eucalypt species in northern Western Australia and the north-western Northern Territory over the years 2006–12, we observed leaves exhibiting symptoms similar to those of *T. destructans*. Samples were collected across several sites and preliminary examination revealed a fungus with a conidial morphology similar to that of *T. destructans*. This study describes two new species of *Teratosphaeria* with long conidia found in northern Western Australia and the Northern Territory that are distinct from *T. destructans*.

MATERIALS AND METHODS

Collection and isolation

Eucalypt leaves with symptoms resembling those of *Teratosphaeria destructans* were collected from several different locations in Australia: (a) adult mature trees of a *Eucalyptus* sp. in Derby, Western Australia; (b) juvenile leaves from *Eucalyptus* hybrids in plantations on Tiwi Island, Northern Territory; and (c) juvenile and adult foliage from eucalypt woodlands at several locations in northern, Western Australia and north-western parts of Northern Territory (Table 1, Fig. 1C). Isolations were made as described previously (Andjic *et al*. 2007 a). Isolates are maintained in culture collections at Murdoch University, Perth, Western Australia (MUCC) and the Department of Agriculture and Water Resources (AQISWA), Perth, Western Australia. Ex-type cultures and leaf material have been deposited in the fungal collection of Queensland Plant Pathology Herbarium (BRIP), Brisbane, Queensland, Australia, and the KNAW-CBS Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands.

Morphological identification and characterisation

Preliminary identification of the *Teratosphaeria* isolates was by microscopic examination and culturing. Plugs (2 mm diam) were cut from actively growing cultures and placed at the centres of Petri dishes (55 mm diam) containing 2 % Malt Extract Agar (MEA). After 30 d, cultures were assessed for growth-rate, by taking two measurements of colony diameter perpendicular to each other. Colony colour was described using notations in the Munsell® Soil Colour Charts (Gretag Macbeth, New Windsor, NY, revised 2000).

Squash mounts of sporing structures were prepared, from hand sections of lesions and from culture, on slides in lacto-glycerol (1:1:1 v lactic acid: glycerol: water) and observed at 1000× magnification with Leica DM5000 light microscope. Morphological characters used in this study to distinguish *Teratosphaeria* species producing kirramyces-like long conidia included: conidial size, shape, pigmentation and number of septa. Wherever possible, 30 measurements of all potentially taxonomically relevant structures were recorded for each species and the extremes are presented in parentheses. Measurements of conidial size were obtained using image analysis software Leica Image Application Suite (LAS) and adjusted to the nearest 0.5 μm. Conidium lengths were recorded as straight-line (linear) length following the method of previous studies of *Teratosphaeria* (Wingfield *et al*. 1996). Data analyses were performed using descriptive statistics in Microsoft Excel.

DNA Extraction, PCR amplification and sequencing

Isolates were grown on 2 % MEA at 20 °C for 4 wk and the mycelium was harvested and placed in a 1.5 mL sterile Eppendorf® tube. Harvested mycelium was ground to a fine powder using cordless motor pellet pestle (Sigma-Aldrich) and genomic DNA was extracted using a DNeasy® Plant Mini Kit (Qiagen) following the manufacturer's instructions. ITS2 and part of the 5.8S region of the rDNA (ITS2), and two partial protein-coding genes, β-tubulin (*tub2*) and translation elongation factor (*tef1*), were sequenced for all isolates as described previously (Andjic *et al*. 2007a).

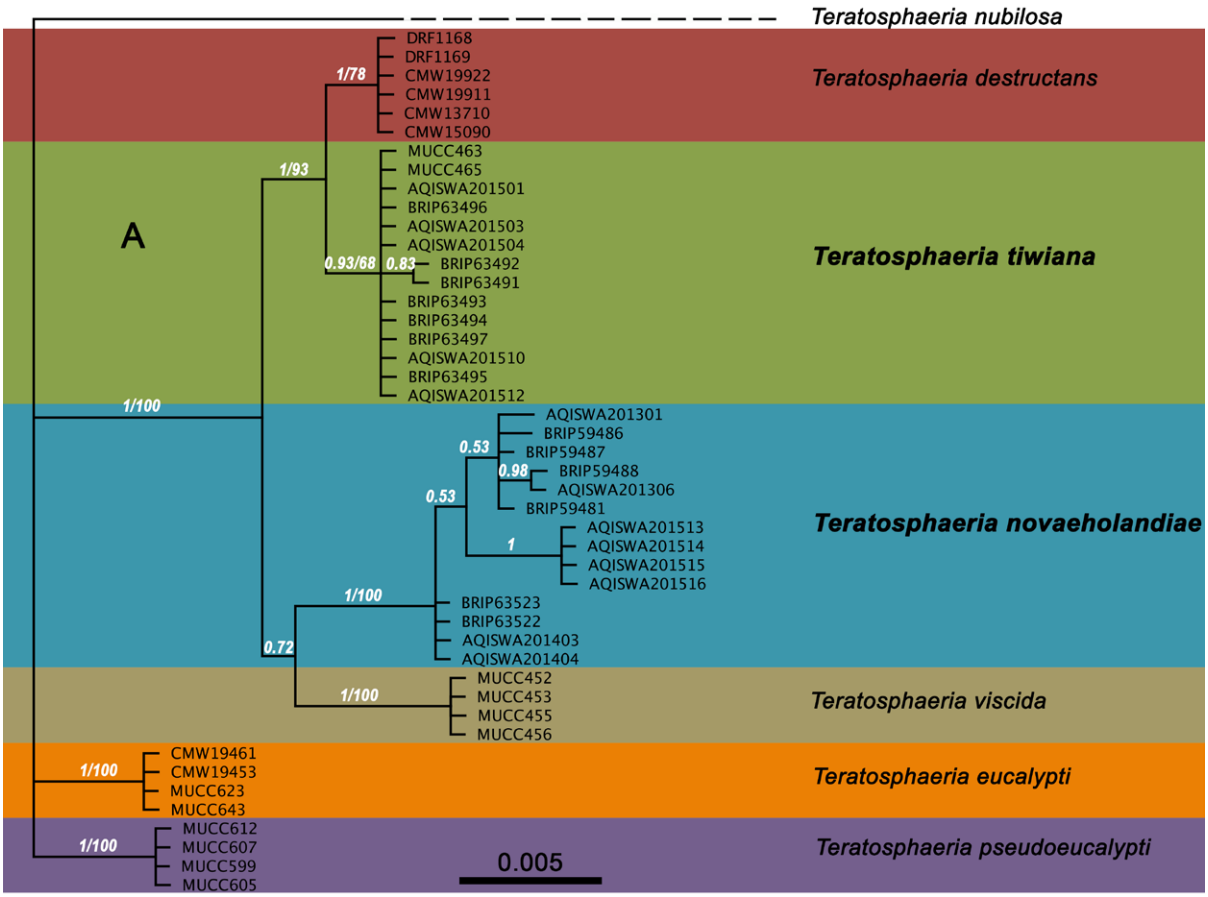
Phylogenetic analysis

The phylogeny of the new *Teratosphaeria* isolates was estimated using parsimony and maximum likelihood methods. In order to compare *Teratosphaeria* species used in this study with other closely related species, additional ITS2, *tub2* and *tef1* sequences were obtained from GenBank (Table 1). Sequence data were assembled and aligned using the CLUSTALW algorithm implemented in Geneious R7 v. 7.0.4 (Biomatters). Adjustments to the alignments were made manually by inserting gaps where necessary.

Maximum parsimony analyses were performed on individual (data not shown) and combined data sets in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) after a partition homogeneity test (PHT) of the combined ITS2, *tub2* and *tef1* alignments was conducted in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) to test pairwise congruence between the sequence data sets.

The equally most parsimonious trees were obtained using heuristic searches with random stepwise taxon additions in 100 replicates, with the tree bisection-reconnection branch-swapping option on and the steepest-descent option off. Maxtrees were unlimited, branches of zero length were collapsed and all multiple equally most parsimonious trees were saved. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indices) were determined (Hillis & Heuelsenbeck 1992). Branch and branch node support was determined using 1000 bootstrap replicates (Felsenstein 1985). Trees were rooted to *Teratosphaeria nubilosa* (CBS 116005).

The same aligned datasets were used for the Bayesian analysis, which was performed with MrBayes v. 3.2.6 (Ronquist & Heuelsenbeck 2003) as implemented as Geneious plug-in after MrModeltest v. 3.5 (Nylander 2004) was used to determine the best nucleotide substitution model per gene region. For all gene regions the Hasegawa, Kishino and Yano (HKY85) nucleotide substitution model with gamma (G) and proportion of invariable site (I) parameters was the best model. Two independent runs of Markov Chain Monte Carlo (MCMC) were run over 1 100 000 generations. The heating parameter was set at 0.2 and trees were saved each 1 000 generations, resulting in 1 100 trees. Burn-in was set



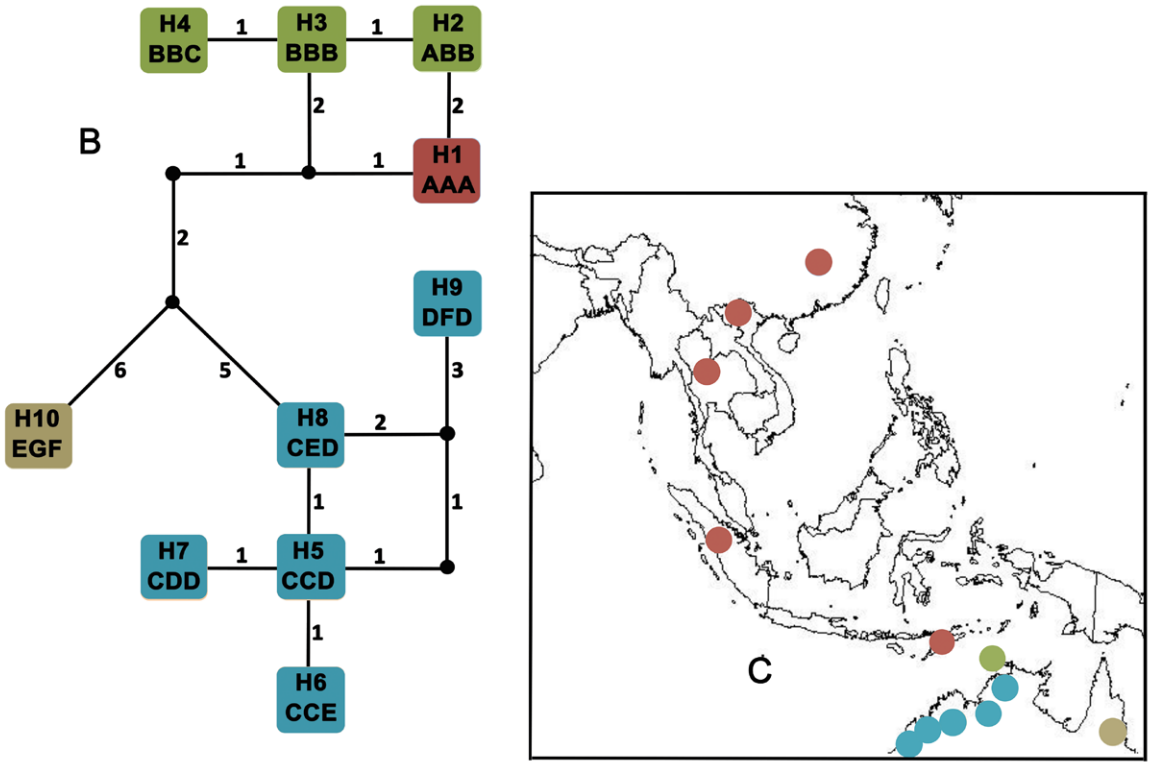


Fig. 1. A. Bayesian phylogram obtained from the combined ITS2, translation elongation factor 1-α and the β-tubulin sequence alignment. Bootstrap support based on parsimony analysis and posterior probabilities of the branch nodes based on Bayesian analysis (italics) are given at the nodes. All trees are rooted to *Teratosphaeria nubilosa*. **B.** Haplotype network based on sequence data from ITS2, *tub2* and *tef1* gene regions. Colours following the blocks in Fig. 1. A indicate the isolates and the localities in Fig. 1. C. Red=*T. destructans*; Green=*T. tiwiana*; Orange=*T. novaehollandiae*; Brown=*T. viscida* **C.** Map showing collection localities of species described with colour coding aligned to the phylogram (Fig. 1A) and haplotype network (Fig. 1 B).

Table 1. Host and locations of *Teratosphaeria* isolates considered in this study and the GenBank accession numbers for the three nuclear gene regions, ITS2, *tub2*, *tef1*.

Fungus	Culture no.[a]	Host	Location	Collector	GenBank accession no.b,c		
					tef1	*tub2*	ITS2
Teratosphaeria destructans	**DRF1168**	*E. grandis*	Aek Nauli, Sumatra, Indonesia	MJ Wingfield	KT972342	KT972310	KT972278
	DRF1169	*E. grandis*	Sumatra, Indonesia	MJ Wingfield	KT972343	KT972311	KT972279
	CMW19922	*E. urophylla*	Goungdong, China	TI Burgess	KT972344	KT972312	KT972280
	CMW19911	*E. urophylla*	Goungdong, China	TI Burgess	EF686474	EU046365	EU046369
	CMW13710	*E. camaldulensis*	Tatoom, Thailand	MJ Wingfield	EF686464	EF686274	EF686505
	CMW15090	*E. camaldulensis*	MinhDuc, S-E Vietnam	TI Burgess	EF031490	EF031478	EF031466
T. eucalypti	CMW19461	*E. nitens*	Sun Valley, New Zealand	M Dick	EU101583	EU101527	FJ793232
	CMW19453	*E. nitens*	Settlement Rd, New Zealand	M Dick	EU101585	EU101529	FJ793234
	MUCC 623	*E. nitens*	Dorrigo,HAN-NSW, Australia	AJ Carnegie	EU101623	EU101566	FJ793255
	MUCC 643	*E. nitens*	Roses Tier, TAS, Australia	T Wardlaw	EU101635	EU101578	EU101656
T. novaehollandiae	AQISWA 201513	*Eucalyptus* sp.	Derby, WA, Australia	MJ Wingfield	KT972355	KT972323	KT972291
	AQISWA 201514	*Eucalyptus* sp.	Derby, WA, Australia	MJ Wingfield	KT972356	KT972324	KT972292
	AQISWA 201515	*Eucalyptus* sp.	Derby, WA, Australia	MJ Wingfield	KT972357	KT972325	KT972293
	AQISWA 201516	*Eucalyptus* sp.	Derby, WA, Australia	MJ Wingfield	KT972358	KT972326	KT972294
	BRIP59486	*E. camaldulensis*	Kununurra, WA, Australia	A Maxwell	KT972345	KT972313	KT972281
	AQISWA 201301	*E. camaldulensis*	Kununurra, WA, Australia	A Maxwell	KU567981	KU567980	KU547982
	BRIP59487	*E. camaldulensis*	Kununurra, WA, Australia	A Maxwell	KT972346	KT972314	KT972282
	BRIP59488,CBS 141552	*E. camaldulensis*	NT, Australia	V Andjic	KT972347	KT972315	KT972283
	BRIP59490	*E. camaldulensis*	NT, Australia	V Andjic	KT972349	KT972317	KT972285
	BRIP59481	*E. camaldulensis*	NT, Australia	A Maxwell	KT972350	KT972318	KT972286
	BRIP63522	*E. victrix*	Pilbara, WA, Australia	G Hardy	KT972352	KT972320	KT972288
	BRIP63523,CBS 141554	*E. victrix*	Pilbara, WA, Australia	G Hardy	KT972351	KT972319	KT972287
	AQISWA 201403	*E. victrix*	Pilbara, WA, Australia	G Hardy	KT972353	KT972321	KT972289
	AQISWA 201404	*E. victrix*	Pilbara, WA, Australia	G Hardy	KT972354	KT972322	KT972290
	MUCC 599	*E. grandis* x *camaldulensis*	Harrisville, S-QLD, Australia	AJ Carnegie	EU101593	EU101537	FJ793216
	MUCC 605	*E. grandis* x *camaldulensis*	Harrisville, S-QLD, Australia	AJ Carnegie	EU101616	EU101559	FJ793225
	MUCC 607	*E. grandis* x *camaldulensis*	Miriam Vale, C-QLD,Australia	G. Pegg	EU101598	EU101542	FJ793220
	MUCC 612	*E. grandis* x *camaldulensis*	Miriam Vale, C-QLD,Australia	G. Pegg	EU101601	EU101545	FJ793223
T. tiwiana	MUCC463	*E. grandis* x *camaldulensis*	Tiwi Island, Australia	TI Burgess	EU009640	EU009649	EU009631
	MUCC465	*E. grandis* x *camaldulensis*	Tiwi Island, Australia	TI Burgess	EU009641	EU009650	EU009632
	AQISWA 201501,CBS 141547	*E. grandis* x *urophylla*	Tiwi Island, Australia	TI Burgess	KT972361	KT972329	KT972298
	BRIP63496, CBS 141549	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972362	KT972330	KT972298

Table 1. (Continued).

Fungus	Culture no.[a]	Host	Location	Collector	GenBank accession no.b,c		
	AQISWA 201503	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972363	KT972331	KT972299
	AQISWA 201504	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972364	KT972332	KT972300
	BRIP63492,CBS 141553	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972365	KT972333	KT972301
	BRIP63491,CBS 141551	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972366	KT972334	KT972302
	BRIP63493	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972368	KT972336	KT972304
	BRIP63494	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972367	KT972335	KT972303
	BRIP63497,CBS 141550	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972369	KT972376	KT972305
	BRIP63495,CBS 141548	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972370	KT972377	KT972306
	BRIP63524	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972371	KT972378	KT972307
	AQISWA 201512	*E. urophylla* hybrids	Tiwi Island, Australia	TI Burgess	KT972372	KT972379	KT972308
T. viscida	MUCC 452, CBS 121156	*E. grandis*	Mareeba, Australia	TI Burgess	EF031495	EF031483	EF031471
	MUCC 453, CBS 121157	*E. grandis*	Mareeba, Australia	TI Burgess	EF031496	EF031484	EF031472
	MUCC 456	*E. grandis*	Mareeba, Australia	TI Burgess	EF031497	EF031485	EF031473
	MUCC 455	*E. grandis*	Mareeba, Australia	TI Burgess	EF031498	EF031486	EF031474

[a]Designation of isolates and culture collections: AQISWA = Biosecurity Australia Fungal Culture Collection, Perth, Western Australia, Australia; CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; BRIP = Biosecurity Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa. MUCC= Murdoch University Culture Collection, Perth, Western Australia, Australia. Specimens in **bold** are type specimens.

at 100 000 generations (i.e. 100 trees), well after the likelihood values converged to the stationery, leaving 1 000 trees from which the consensus trees and posterior probabilities were calculated.

Sequences obtained from this study have been deposited in GenBank and accession numbers are shown in Table 1. The sequence alignment was lodged in TreeBASE (www.treebase.org/) and taxonomic novelties in MycoBank (www.MycoBank.org).

Haplotype network estimation

Haplotype networks were used to compare isolates in order to infer which isolates were most closely related to one another. Haplotype networks were generated using the statistical parsimony method in the TCS v. 1.21 software programme (Clement *et al*. 2000). The program collapses DNA sequences into haplotypes and calculates the frequencies of haplotypes in the sample, which are used to estimate haplotype out-group probabilities that correlate with haplotype age (Donelly & Tavare 1986, Castelloe & Tempelton 1994). It then calculates an absolute distance matrix from which it estimates phylogenetic networks using a probability of parsimony, until the probability exceeds 0.95 (Templeton *et al*. 1992). The analysis was performed on the combined dataset of ITS2, *tub1* and *tef1* DNA sequences.

RESULTS

Morphological identification

The fungal isolates obtained in this study were characterised as slow-growing cultures on MEA. Morphological characteristics of the conidia of the *Teratosphaeria* isolates were similar in pigmentation, length, size, shape, and septa number (Table 2). Conidia were hyaline, subhyaline to pale brown, straight to variously curved, with 0–3 septa, and ranging from 30–50 × 2–3.5 µm (*in vivo*). These characteristics are typical for all *Teratosphaeria* species with kirramyces-like long conidia isolated from *Eucalyptus*. Morphological features of *Teratosphaeria* asexual morphs with long and short conidia are variable and not reliable for species separation, therefore the identification of those species relies on DNA sequencing (Andjic *et al.* 2007c, 2010b).

Molecular identification and phylogenetic analysis

A BLASTn search was conducted in GenBank to compare the ITS2 sequences of the *Teratosphaeria* isolates being examined in this study with those already there. The returned sequences were most similar to *T. destructans* (for isolates from Tiwi Island) and *T. viscida* (for isolates from Western Australia, WA, and Northern Territories, NT) and these and other less related species (*T. eucalypti* and *T.*

Table 2. Morphological features of conidia of *Teratosphaeria destructans, T. viscida, T. novaehollandiae,* and *T. tiwiana* species from eucalypts. *In vivo* = herbarium specimens, *in vitro* = isolates from culture, n/a=not applicable (the isolates did not produce conidia in culture or were not available.)

Fungus	Specimen number	Pigmentation	Conidial length (*in vivo*) µm	Conidial length (*in vitro*) µm	Conidial width (*in vivo*) µm	Conidial width (*in vitro*) µm	Number of septa
T. destructans							
Wingfield *et al*. 1992	PREM54416	Pale brown	50–65	n/a	2.5–3	n/a	1–3
Andjic *et al.* 2007	PREM59261	Pale brown	38–47	35–40	2–2.5	2–3	1–3
Andjic *et al*. 2007	PREM59259	Pale brown	49–55	33–40	2–2.5	2–2.5	1–3
T. viscida							
Andjic *et al.* 2007	BRIP 49804	Subhyaline to pale brown	47–60	35–40	2.5–3.5	2.5–3.5	0–3
T. novaehollandiae							
Present study	BRIP59486 Kununurra	Hyaline to subhyaline	35–40	30–35	2.5–3	2.0–2.8	1–3
Present study	BRIP59488 NT	Hyaline to subhyaline	30–35	25–30	2.0–3	1.5–2.0	1–3
Present study	BRIP63523 Pilbara	Hyaline to pale brown	25–30	26–30	3–3.5	2.3–2.8	1–2
Present study	AQISWA201513	Subhyaline to pale brown	45–50	n/a	2.5–3	n/a	1–3
T. tiwiana							
Present study	BRIP63496	Pale brown	35–40	35–40	2.5–3	2.5–3	1–2

pseudoeucalypti) were used in the phylogenetic analyses. The aligned combined data set of ITS2, *tub2,* and *tef1* consisted of 870 characters, of which 72 were parsimony-informative and were used in the analysis. The partition homogeneity test showed no significant difference (P > 0.01; P = 0.32) between data from different gene regions, and so these data were combined. These data contained significant phylogenetic signal (P< 0.01; gl = - 0.95). Heuristic searches of unweighted characters in PAUP resulted in 18 equally most parsimonious trees of 91 steps (CI=0.89, RI=0.98; TreeBASE S18826; Fig. 1). The Bayesian analysis resulted in a tree with the same topology and clades as those revealed in the parsimony analysis and presented as Fig. 1A (TreeBASE S18826; Fig. 2). In the Bayesian analysis, the *tub2*, *tef1* and ITS2 regions consisted of 47, 55 and 31 unique site patterns respectively.

The phylogeny generated from the combined alignment (Fig. 1A) resulted in three major clades: the first major clade comprising *T. destructans* and isolates from Tiwi Island with 93 % bootstrap support and a Bayesian posterior probability of 1.0; the second comprising isolates from WA and the NT with 100 % bootstrap support and a Bayesian posterior probability of 1.0; and the third containing isolates of *T. viscida* with 100 % bootstrap support and a Bayesian posterior probability of 1.0. Furthermore, the first major clade was subdivided in two sub-clades, one containing isolates of *T. destructans* and the second containing *Teratosphaeria* isolates from Tiwi Island. The *T. destructans* sub-clade was well supported with 78 % bootstrap support and a Bayesian posterior probability of 1.0. The Tiwi Island isolates subclade was supported with 68 % bootstrap support and a strong Bayesian posterior probability of 0.93. Although the bootstrap support for isolates from Tiwi Island was relatively low, posterior probability was strong for that node and the tree topology was consistent in all 18 equally most parsimonious trees. *Teratosphaeria* isolates from Tiwi Island were monophyletic in all 18 equally most parsimonious trees and consistently separated from *T. destructans* (data not shown).

The second clade containing isolates from WA and NT was well supported with a 100 % bootstrap value and a posterior probability of 1.0. The unnamed *Teratosphaeria* from this clade was closely related to, but phylogenetically distinct from *T. viscida* (Fig 1A). Isolates of both *T. viscida* and the undescribed *Teratosphaeria* were monophyletic with some sequence variation observed amongst new *Teratosphaeria* isolates. There were 12 fixed polymorphic sites distinguishing *T. viscida* from the new *Teratosphaeria* across the three gene regions indicating that isolates from this clade represent a new taxon (Table 3).

Haplotype network

Haplotype networks constructed in TCS software resulted in ten haplotypes (H-1–H-10) amongst the isolates used in this study (Fig. 1B): *Teratosphaeria destructans* was represented by one haplotype, H-1 (six isolates from Asia, AAA); *T. tiwiana* from Tiwi Islands was represented by three haplotypes (H-2,two isolates, ABB; H-3, six isolates, BBB; and H-4, six isolates BBC); the new *Teratosphaeria* isolates were represented by five haplotypes: H-5 (two isolates from the Kimberly region in WA and one from the NT, CCD); H-6 (one isolate from the NT, CCE); H-7 (two isolates from the NT, CDD); H-8 (four isolates from the Pilbara, WA, CED);

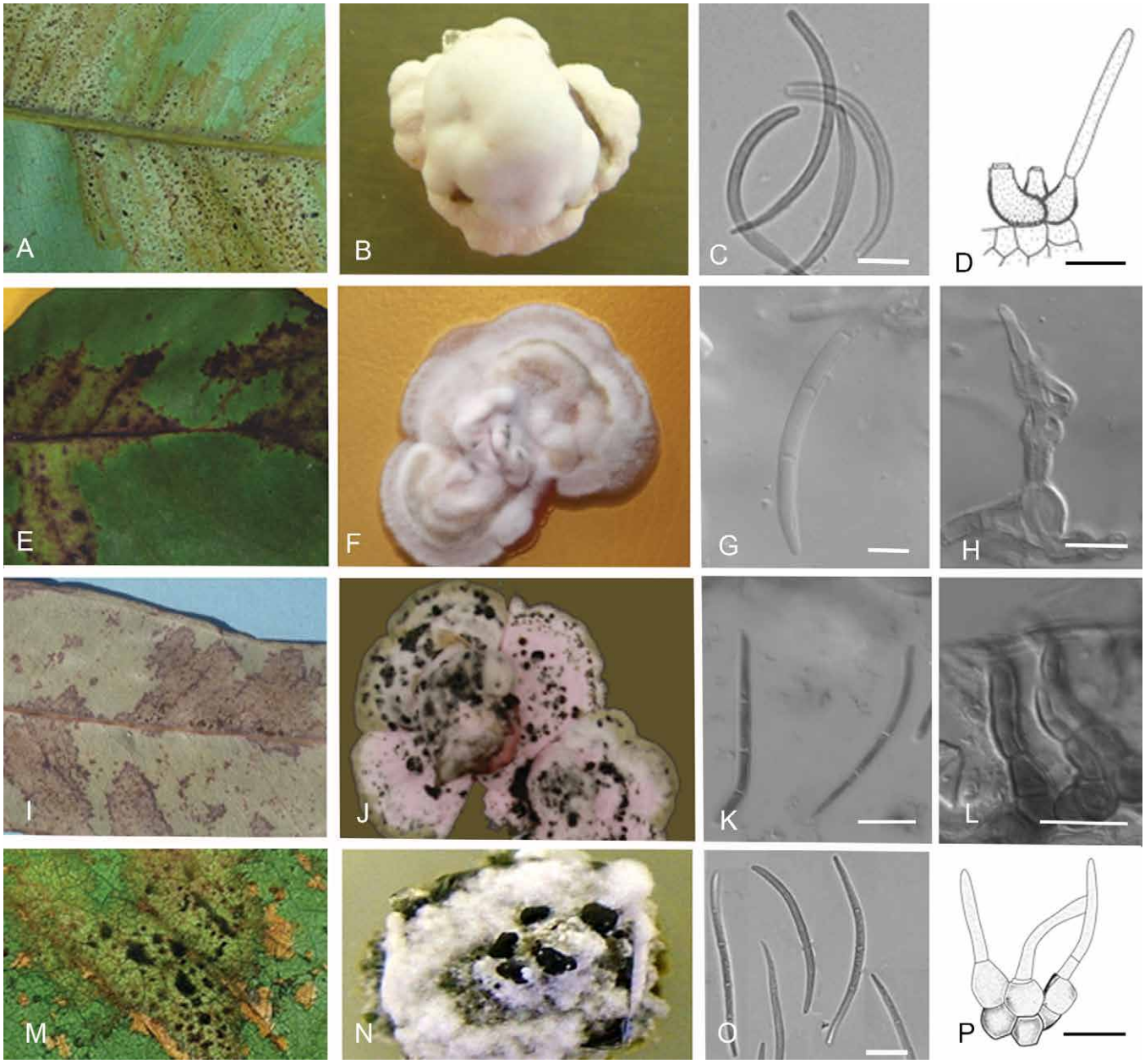


Fig. 2. Morphological features of *Teratosphaeria destructans, T. tiwiana, T. novaehollandiae,* and *T. viscida* from eucalypts. **A–D**. *T. destructans* specimen PREM 59261(CMW 17919). **A.** Leaf symptoms. **B.** Culture morphology on MEA. **C.** Conidia morphology. **D.** Conidiogenous cells and conidiogenesis. **E–H**. *T. tiwiana* holotype specimen and ex type culture BRIP 63496 (CBS 141549). **E.** Leaf symptoms. **F.** Culture morphology on MEA. **G.** Conidia morphology. **H.** Conidiogenous cells and conidiogenesis. **I–L.** *T. novaehollandiae* holotypespecimen and ex type culture BRIP 59486. **I.** Leaf symptoms. **J.** Culture morphology on MEA. **K.** Conidia morphology. **L.** Conidiogenous cells and conidiogenesis. **M–P.** *T. viscida* specimen BRIP 49804 CBS 121156). **M.** Leaf symptoms. **N.** Culture morphology on MEA. **O.** Conidia morphology. **P.** Conidiogenous cells and conidiogenesis. Bars = 10 µm.

and H-9 (four isolates from Derby, WA, DFD); and *T. viscida* was represented by one haplotype, H-10 (four isolates from Queensland, EGF).

Three different haplotypes (H-2, H-3, H-4) were observed in the population from Tiwi Island, but none of them were shared with the phylogenetically closely related *T. destructans* (H-1).

Five haplotypes were detected in the population from WA and NT. Only one haplotype was shared among isolates from WA and the NT (H-5), and none of the haplotypes were shared with the closely related *T. viscida* (H-10).

Morphological examination did not show any major differences between the *Teratosphaeria* isolates obtained in this study. This situation is common in species lacking a known sexual morph. However, the combination of phylogenetic inference and haplotype analysis provides robust evidence that isolates from Tiwi Island, NT and WA are distinct from both *T. destructans* and *T. viscida.* They are therefore described as new species here.

Table 3. Polymorphic nucleotides from sequence data of ITS2, *tef1* and *tub2* gene regions showing the variation between isolates of *Teratosphaeria viscida* and *T. novaehollandiae*. Ex-type cultures are indicated in bold face.

	tub2				*tef1*						*ITS2*	
	96	99	105	229	158	166	169	173	209	211	181	183
Teratosphaeria viscida												
CBS 121156	G	A	G	T	T	G	A	A	G	G	T	G
CBS 121157	G	A	G	T	T	G	A	A	G	G	T	G
MUCC 456	G	A	G	T	T	G	A	A	G	G	T	G
MUCC 455	G	A	G	T	T	G	A	A	G	G	T	G
Teratosphaeria novaehollandiae												
BRIP59486	A	G	T	C	C	A	C	T	A	A	C	C
BRIP59488	A	G	T	C	C	A	C	T	A	A	C	C
BRIP63523	A	G	T	C	C	A	C	T	A	A	C	C
BRIP63523	A	G	T	C	C	A	C	T	A	A	C	C
AQISWA201513	A	G	T	C	C	A	C	T	A	A	C	C

TAXONOMY

Teratosphaeria novaehollandiae V. Andjic, T.I. Burgess, A. Maxwell, **sp. nov.**
MycoBank MB815681
(Fig. 2I–L)

Etymology: Name refers to original Dutch name for the geographic western half of Australia, where the fungus was collected.

Diagnosis: Distinguished from *T. viscida* (cfr. Figs. 2 I–L and 2 M–P) in not producing highly hydrophobic and viscous spore masses. *In vivo*, *T. novaehollandiae* produces shorter conidia (33–40 µm) than those of *T. viscida* (47–60 µm). *In vitro*, *T. novaehollandiae* produce shorter conidia (27–31 µm) than *T. viscida* (35–40 µm). Unlike *T. viscida, T. novaehollandiae* does not produce a synasexual morph with chlamydospore-like structures in culture. Based on phylogenetic analyses of sequence data obtained for the ITS2, *tef1* and *tub2* gene regions, *T. novaehollandiae* has 12 fixed polymorphic sites across three gene regions which distinguish it from the closely related *T. viscida* (Table 3).

Type: **Australia**: *Western Australia*: Kununurra, isolated from leaves of *Eucalyptus camaldulensis*, Apr. 2012, *A. Maxwell & V. Andjic* (BRIP 59486 – holotype).

Description: *Leaf spots* circular to irregular, 2.5–35 mm diam, single to confluent, pale to medium brown with a red brown border on the top surface, and light brown from below. *Conidiomata* pycnidial, hypophyllous, single, dark brown to black. *Conidiophores* reduced to conidiogenous cells. *Conidia* solitary, 1–3-septate, hyaline to pale brown, slightly verruculose, cylindrical, straight to slightly curved, thick-walled, base truncate sometimes with marginal frill, apex obtuse, (20–)33–40(–62) × (1.5–)2–3.5(–4.0) (mean = 38 × 2.5 µm).

Culture characteristics: *Colonies* 35 × 25 mm after 1 mo at 25 °C in the dark on MEA, white 5YR 8/1 to pink 5YR 8/4 on the upper surface, olive grey 5YR 7/1 on reverse. *Mycelium* subhyaline to pale brown, septate, branched. *Conidiomata* pycnidial, single, dark brown to black, globose to subglobose, unilocular: wall of *textura angularis*. *Conidiogenous cells* not seen in culture. *Conidia* solitary, 1–3-septate but mostly 3-septate, hyaline to subhyaline, slightly verruculose, cylindrical, straight to slightly curved (22–)27–30(–41) × (1–)2–2.5(–4) (mean = 30 × 2.5 µm).

Additional material examined: **Australia***: Western Australia and Northern Territory*: Kununurra and Bachelor, isolated from *E. camaldulensis*, Apr. 2012, *A. Maxwell & V. Andjic* (BRIP 59487, BRIP 59488 = CBS 141552, BRIP 59490, and BRIP 59481); *Western Australia*: Pilbara isolated from *Eucalyptus victrix*, Aug. 2013, *G. Hardy* (BRIP 63522, BRIP 63523 = CBS 141554, AQISWA 201403, AQISWA 201404); Derby, isolated from leaves of *Eucalyptus* sp., July 2006, *T.I Burgess & M.J. Wingfield* (BRIP 64754, culture not viable).

Teratosphaeria tiwiana V. Andjic, T.I. Burgess, A. Maxwell, **sp. nov.**
MycoBank MB815680
(Fig. 2 E–H)

Etymology: Named after Tiwi Island, the type locality.

Diagnosis: Distinguished from *T. destructans* (cfr.Fig. 2 E–H v. A–D) by producing slightly shorter conidia and in septa number. *In vivo*, *T. tiwiana* produces shorter and less curved conidia (35–40 µm) than those of *T. destructans* (38–65 µm). In contrast to *T. destructans,* whose conidia is 1–3-septate, the conidia of *T. tiwiana* are 1–2-septate (Fig. 2C, G). Based on multi-gene phylogeny *T. tiwiana* can be distinguished from *T. destructans* with 6 bp differences across three gene regions.

Type: **Australia**: *Northern Territory*: Tiwi Island, isolated from leaves of *Eucalyptus* hybrids *E. grandis* × *E. urophylla,* Aug. 2007, *T.I. Burgess* (BRIP 63496– holotype; BRIP 63496 = CBS 141549– ex-type cultures).

Description: *Leaf spots* circular to irregular, 3–20 mm diam, single to confluent, pale to medium brown with red brown border on the top surface, light brown below. *Conidiomata* pycnidial, hypophyllous, single, dark brown. *Conidiophores* reduced to conidiogenous cells. *Conidia* solitary, 1–2-septate, predominantly with 1-septum, pale brown, slightly verruculose, cylindrical, straight to variously curved, thick-walled, base truncate, sometimes with marginal frill, apex obtuse, (26.5–)35–40(–44.5) × (2–)2.5–3.0(–3.5) (mean = 35 × 2.8 µm).

Culture characteristics: *Colonies* 25 × 25 mm after 1 mo at 25 °C in the dark on MEA, white 5YR 8/1 to pink 5YR 8/4 on the upper surface, olive grey 5YR 7/1 on reverse. *Mycelium* subhyaline to pale brown, septate, branched. *Conidiomata,* if present, pycnidial, single, dark brown to black, globose to subglobose, unilocular: wall of *textura angularis*. *Conidiogenous cells* not seen in culture. *Conidia* solitary, 0–1-septate, subhyaline to pale brown, slightly verruculose, cylindrical, straight to variously curved (25–)35–40(–56.5) × (2–)2.5–3(–3.5) (mean = 38 × 3.0 µm), lateral branches occasionally present as secondary conidia.

Additional specimens examined: **Australia***: Northern Territory*: Tiwi Island, isolated from *E. grandis* and *E. urophylla* hybrids, Aug. 2007, *T.I. Burgess* (BRIP 63492 = CBS 141553, BRIP 63491 = CBS 141551, BRIP 63493, BRIP 63494, BRIP 63497 = CBS 141550, BRIP 63495 = CBS 141548, BRIP 63524).

DISCUSSION

We describe two new cryptic *Teratosphaeria* species isolated from *Eucalyptus* in northern Australia: *T. tiwiana* and *T. novaehollandiae*. Australian isolates previously described as *T. destructans* were re-examined and are here assigned to these two new taxa. *Teratosphaeria destructans* s. str., therefore, has not been correctly recorded in Australia, and remains restricted to South-East Asia and Africa.

The two new *Teratosphaeria* species could not be morphologically distinguished, thus the description was based on data inferred from multi-gene phylogeny: applying the Genealogical Concordance for Phylogenetic Species Recognition (GCPSR; Taylor *et al.* 2000) criteria, and noting the haplotype analysis of combined sequence data for the ITS2, *tub2* and *tef1* gene regions. The GCPSR concept uses the phylogenetic concordance of multiple unlinked genes to indicate a lack of genetic exchange and thus evolutionary independence of lineages (Geiser *et al*. 1998, Taylor *et al*. 2000, Starkey *et al.* 2007, Cai *et al*. 2011). It is a useful criterion for the discrimination of species when other species recognition criteria (morphological, physiological, reproduction, host specificity) fail (Cai *et al*. 2011). GCPSR has already proved to be a valuable tool for recognising cryptic species in *Colletotrichum*, *Diaporthe*, *Phyllosticta*, and *Fusarium* species complexes (Glienke *et al.* 2011, Damm *et al.* 2012, Shivas & Cai 2012, Gomes *et* al. 2013, Hansen & Olariaga 2015).

Teratosphaeria tiwiana, a cryptic species similar to *T. destructans,* was isolated from non-endemic juvenile eucalypt leaves from a clonal taxa trial from Tiwi Island, NT, Australia. Previously, based on symptoms, conidial morphology and multilocus sequence data, the isolates from Tiwi Island had been identified as *T. destructans* although they grouped separately from *T. destructans* from Asia (Burgess *et al.* 2007). As a consequence, *T. destructans* was removed from the NAQS target list for exotic invasive plant pathogens. This study included more isolates from Tiwi Island than the initial study, and re-evaluated the relationship between Australian and Asian isolates using multi-locus sequence data and haplotype analysis. The DNA sequence analysis obtained in this study was in agreement with the findings in Burgess *et al.* (2007); there were 6 bp differences amongst isolates across three gene regions. In that previous study, it was thought that 6 bp difference was within the normal limits of infraspecies variation and the isolates from Australia were identified as *T. destructans* However, multigene phylogeny and haplotype analysis obtained in the present study provided sufficient evidence to support the separation of Australian isolates as a separate species described here as *T. tiwiana*. According to the Genealogical Concordance Concept of Dettman *et al.* (2003), a clade is recognised as an independent evolutionary lineage if the clade was present in the majority of the single locus genealogies and the clade is identified from the majority rule consensus tree regardless of its bootstrap or posterior probability support. In this study, the *T. tiwiana* clade was recovered in all three single strict consensus trees satisfying the criterion of genealogical concordance (data not shown); the clade was monophyletic in the phylogenetic trees inferred from single gene regions and from the combined dataset; and the lineage was supported by Bayesian analyses. Furthermore, *T. tiwiana* isolates showed sequence variation and were split in three haplotypes, while the sequences of *T. destructans* isolates were identical and contained only one haplotype. The haplotypes were not shared between these two species. This suggests that *T. tiwiana* is an endemic Australian cryptic species.

Teratosphaeria novaehollandiae was found on amenity plantings of an unidentified native *Eucalyptus* species in Derby, endemic *E. camaldulensis* woodlands in northern WA and the NT, and on *E. victrix* in the Pilbara, WA. These isolates were collected from adult and juvenile eucalypt foliage across an extensive area of northern Australia and where present caused minor to moderate levels of damage to *Eucalyptus* leaves. The sequences of *T. novaehollandiae* were variable and split into five haplotypes. Two haplotypes contained isolates from northern WA, one haplotype contained isolates from NT, one contained isolates from Pilbara and one contained isolates from Derby, WA. Despite having unique haplotypes, isolates from across northern WA and the NT could not be consistently split into separate phylogenetic species.

In a previous study, based on conidial morphology and phylogenetic analysis, isolates from Derby (WA) were assigned to *T. destructans* (Burgess *et al.* 2007). We have now re-evaluated the taxonomic position of the Derby isolates using multigene sequence data including haplotype analysis. The results obtained in this study have demonstrated that all isolates from Derby grouped together and were separated from Asian and Australian isolates previously named as *T. destructans* (i.e. *T. tiwiana*). The Derby isolates clustered

within the *T. novaehollandiae* clade, which was well supported (1.00 Posterior probabilities) and was distinct from both *T. destructans* and *T. viscida*. The Derby isolates were not consistently separated from other isolates within *T. novaehollandiae* and are therefore here recognised as the new cryptic species *T. novaehollandiae*.

Sequence data and ex-type cultures are now available for for eight *Teratosphaeria* species described from eucalypts and for which a kirramyces-like asexual morph is known. Conidia range in size from the shortest *T. novaehollandiae* (33–40 µm) to the longest, *T. destructans* (50–65 µm), pigmentation and septation also varies, but generally conidia are septate. These species produce lesions on leaves with various symptoms. All except *T. destructans* have been reported in Australia. The expansion of eucalypt plantation forestry into the subtropics of Australia has led to the discovery of many new *Teratopshaeria* species, and it appears to be the dominant fungal genus on subtropical eucalypt leaves. Currently, the two species newly described in this study are not causing any significant damage to Australian eucalypt plantations, but the threat they may pose to the forestry industry is unknown.

ACKNOWLEDGEMENTS

This work was funded in part by the Australian Research Council DPO343600 “Population genetics of fungal pathogens that threaten the biosecurity of Australia’s eucalypts”. We also thank Dianne White for technical assistance.

REFERENCES

Andjic V, Barber PA, Pegg GS, Carnegie AJ, Hardy GEStJ *et al.* (2007b) *Kirramyces viscidus* sp. nov, a new eucalypt pathogen from tropical Australia is closely related to the serious leaf pathogen, *Kirramyces destructans*. *Australasian Plant Pathology* **36**: 478–487.

Andjic V, Barber PA, Carnegie AJ, Hardy GEStJ, Wingfield MJ *et al.* (2007c) A morphological and phylogenetic reassessment of the genus *Phaeophleospora* and the resurrection of the genus *Kirramyces*. *Mycological Research* **111**: 1184–1198.

Andjic V, Cortinas MN, Hardy GEStJ, Burgess TI, Wingfield MJ (2007a) Multiple gene genealogies reveal important relationships between *Phaeophleospora* spp. infecting *Eucalyptus* leaves. *FEMS Microbiology Letters* **268**: 22–33.

Andjic V, Dell B, Barber P, Hardy GEStJ, Wingfield MJ, *et al.* (2011) Plants for planting; indirect evidence for the movement of a serious forest pathogen, *Teratosphaeria destructans*, in Asia. *European Journal of Plant Pathology* **131**: 49–58.

Andjic V, Pegg GS, Carnegie AG, Callister A, Hardy GEStJ *et al.* (2010a) *Teratosphaeria pseudoeucalypti*, new cryptic species responsible for leaf blight of *Eucalyptus* in subtropical and tropical Australia. *Plant Pathology* **59**: 900–912.

Andjic V, Whyte G, Hardy GEStJ, Burgess TI (2010b) New *Teratosphaeria* species occurring on eucalypts in Australia. *Fungal Diversity* **43**: 27–38.

Burgess TI, Andjic V, Hardy GEStJ, Dell B, Xu D (2006) First report of *Phaeophleospora destructans* in China. *Journal of Tropical Forest Science* **18**: 144–146.

Burgess TI, Andjic V, Wingfield MJ, Hardy GEStJ (2007) The eucalypt leaf blight pathogen *Kirramyces destructans* discovered in Australia. *Australasian Plant Disease Notes* **2**: 141–144.

Carnegie AJ (2007a) Forest health condition in New South Wales, Australia, 1996-2005. I Fungi recorded from eucalypt plantations during forest health surveys. *Australasian Plant Pathology* **36**: 213–224.

Carnegie AJ (2007b) Forest health condition in New South Wales, Australia, 1996-2005. II Fungal damage recorded in eucalypt plantations during forest health surveys and their management. *Australasian Plant Pathology* **36**: 225–239.

Carnegie AJ, Burgess TI, Beilharz V, Wingfield MJ (2007c) New species of *Mycosphaerella* in plantations and native forests in Eastern Australia. *Mycologia* **99**: 461–474.

Cai L, Giraud T, Zhang N, Begerow D, Cai G *et al*. (2011) The evolution of species concepts and species recognition criteria in plant pathogenic fungi. *Fungal Diversity* **50**: 121–133.

Castelloe J, Templeton AR (1994) Root probabilities for intraspecific gene trees under neutral coalescent theory. *Molecular Phylogenetics and Evolution* **3**: 102–113.

Clement M, Posada D, Crandall KA (2000) TCS: A computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1659.

Crous PW (2009) Taxonomy and phylogeny of the genus *Mycosphaerella* and its anamorphs. *Fungal Diversity* **38**: 1–24.

Crous PW, Wingfield MJ, Mansilla JP, Alfenas AC, Groenewald JZ (2006) Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus* II. *Studies in Mycology* **55**: 99–131.

Crous PW, Groenewald JZ, Summerell BA, Wingfield BD, Wingfield MJ (2009a) Co-occurring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* **22**: 38–48.

Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC *et al.* (2009b) Unravelling *Mycosphaerella*: do you believe in genera? *Persoonia* **23**: 99–118.

Damm U, Cannon PF, Woudenberg JHC, Crous PW (2012) The *Colletotrichum acutatum* species complex. *Studies in Mycology* **73**: 37-113.

de Souza TC, de Silva AC, de Silva LMG, Ferraz HGM, Junior NB *et al.* (2014) *Teratosphaeria pseudoeucalypti* on eucalyptus in Brazil. *Tropical Plant Pathology* **39**: 407–412.

Dettman JR, Jacobson DJ, Taylor JW (2003) A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution* **57**: 2703–2720.

Dick M (1982) Leaf-inhabiting fungi of eucalypts in New Zealand. *New Zealand Journal of Forestry Science* **12**: 525–537.

Felsenstein J (1985) Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* **39**: 783–791.

Geiser DM, Pitt JI, Taylor JW (1998) Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*. *Proceedings of the National Academy of Sciences, USA* **95**: 388–393.

Glienke C, Pereira OL, Stringari D, Fabris, J., Kava-Cordeiro *et al.* (2011) Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. *Persoonia* **26**: 47–56.

Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia* **31**:1–41.

Greyling I, Wingfield MJ, Coetzee Martin PA, Marincowitz Seonju, Roux J (2016) The *Eucalyptus* shoot and leaf pathogen

Teratosphaeria destructans recorded in South Africa. *Southern Forests: a Journal of Forest Science* **78**: 123–129.

Hansen K, Olariaga I (2015) Species limits and relationships within *Otidea* inferred from multiple gene phylogenies. *Persoonia* **35**: 148–165.

Hillis DM, Huelsenbeck JP (1992) Signal noise and reliability in molecular phylogenetic analysis. *Journal of Heredity* **83**: 189–195.

Hunter GC, Wingfield BD, Crous PW, Wingfield MJ (2006) A multigene phylogeny for species of *Mycosphaerella* occurring on *Eucalyptus* leaves. *Studies in Mycology* **55**: 147–161.

Hunter GC, Crous PW, Carnegie JA, Burgess IT, Wingfield MJ (2011) Mycosphaerella and Teratosphaeria diseases of *Eucalyptus*: easily confused and with serious consequences. *Fungal Diversity* **50**: 145–166.

Miller JT, Cannon PG, Ecroyd CE (1992) Introduced forest trees in New Zealand: recognition, role, and seed source part 11. *Eucalyptus nitens* (Deane et Maiden) Maiden. *New Zealand Ministry of Forestry, Forest Research Bulletin* **124**: 27–28.

Nylander JAA (2004) *MrModeltest v2*. Program distributed by the author. Uppsala: Evolutionary Biology Centre.

Old KM, Pongpanich K, Thu PQ, Wingfield MJ, Yuan ZQ (2003a) *Phaeophleospora destructans* causing leaf blight epidemics in South East Asia. In: *8th International Congress of Plant Pathology, Feb.* Vol. 2 – *Offered papers* ICPP: 165, Christchurch, New Zealand.

Old KM, Wingfield MJ, Yuan ZQ (2003b) *A Manual of Diseases of Eucalypts in South-East Asia*. Bogor: Centre for International Forestry Research.

Park RF, Keane PJ, Wingfield MJ, Crous PW (2000) Diseases and pathogens of eucalypts. In: *Fungal Diseases of Eucalypt Foliage* (Keane PJ, Kile GA, Podger FD, Brown BN, eds): 153–240. Melbourne: CSIRO Publishing.

Ramos SO, Perez CA (2015) First report of *Teratosphaeria pseudoeucalypti* on *Eucalyptus* hybrids in Argentina. *Plant Disease* **99**: 554.

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

Sankaran KC, Sutton BC, Minter DW (1995) A checklist of fungi recorded on eucalypts. *Mycological Papers* **170**: 1–375.

Shivas RG, Cai L (2012) Cryptic fungal species unmasked. *Microbiology Australia* **1**: 36–37.

Soria S, Alonso R, Bettucci L, Lupo S (2014) First report of *Teratosphaeria pseudoeucalypti* in Uruguay. *Australasian Plant Disease Note* **9**: 146.

Starkey DE, Ward TJ, Aoki T, Gale LR, Kistler HC, *et al.* (2007) Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. *Fungal Genetics and Biology* **44**: 1191–1204.

Swofford DL (2003) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4.0b10. Sunderland, MA: Sinauer Associates.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM *et al.* (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**: 21–32.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data III. Cladogram estimation. *Genetics* **132**: 619–633.

Taole M, Bihon W, Wingfield B, Wingfield MJ, Burgess TI (2015) Multiple introductions from multiple sources: Invasion patterns for an important *Eucalyptus* leaf pathogen. *Ecology and Evolution* **5**: 1–11.

Walker J, Sutton BC, Pascoe IG (1992) *Phaeoseptoria eucalypti* and similar fungi on *Eucalyptus* with description of *Kirramyces* gen. nov. (*Coelomycetes*). *Mycological Research* **96**: 911–924.

Wingfield MJ, Crous PW, Boden D (1996) *Kirramyces destructans* sp. nov., a serious leaf pathogen of *Eucalyptus* in Indonesia. *South African Journal of Botany* **62**: 325–327.