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# *Kirramyces viscidus* sp. nov., a new eucalypt pathogen from tropical Australia closely related to the serious leaf pathogen, *Kirramyces destructans*

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**Abstract.** *Kirramyces destructans* is a serious pathogen causing a leaf, bud and shoot blight disease of *Eucalyptus* plantations in the subtropics and tropics of South-East Asia. During surveillance of eucalypt taxa trials in northern Queensland, symptoms resembling those of *K. destructans* were observed on *Eucalyptus grandis* and *E. grandis* × *E. camaldulensis*. Phylogenetic and morphological studies revealed that the *Kirramyces* sp. associated with these symptoms represents a new taxon described here as *K. viscidus* sp. nov., which is closely related to *K. destructans*. Plantation assessments revealed that while *E. grandis* from the Copperload provenance, collected in northern Queensland, recovered from disease, *E. grandis* × *E. camaldulensis* hybrids from South America were highly susceptible to infection by *K. viscidus* and are not recommended for planting in northern Queensland. Preliminary results suggest the fungus probably originates from Australia. *K. viscidus* is closely related to *K. destructans* and causes a disease with similar symptoms, suggesting that it could seriously damage Australian eucalypt plantations, especially those planted off-site.

**Additional keywords:** phylogenetics.

## Introduction

The eucalypt plantation industry in Australia is relatively new, with major expansion from ~200 000 to 600 000 ha occurring only in the last 10 years (National Forestry Inventory 2004). These plantations are predominantly of *Eucalyptus globulus*, situated in southern Australia and the major end-use is paper and pulp. A comparatively small eucalypt plantation industry for both timber and pulpwood is developing in the subtropical regions of northern New South Wales (NSW) and southern Queensland (Qld) and expanding into the tropical regions of northern Qld (Dickinson *et al*. 2004; Carnegie *et al*. 2005). The majority of these plantations are established on previous agricultural land and do not involve the clearing of native vegetation.

Eucalypt species trials have been established throughout Australia to test the suitability of different environments for growing eucalypts. Some of these trials have been planted in the east coast tropics in a region referred to as Far North Queensland (FNQ) (Dickinson *et al*. 2004; Lee 2007), where the climate is similar to parts of South-East Asia. The eucalypt species being tested in these trials include *E. grandis*, *E. camaldulensis* and hybrids between these two species, which are commonly used in tropical plantation forestry worldwide (Turnbull 2000).

Several eucalypt diseases have emerged in South-East Asia that are unknown in Australia (Wingfield *et al*. 1996*b*; Barber 2004; Burgess *et al*. 2006). These diseases threaten the biosecurity of Australia's eucalypts. Under an existing project, eucalypt taxa trials in northern Australia are currently being monitored for incursions of pathogens from Australia's northern neighbours such as Indonesia and Papua New Guinea (T. I. Burgess, pers. comm.).

*Kirramyces* J. Walker *et al*. species are anamorphs of *Mycosphaerella* Johanson. Species such as *K. zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Andjic & M.J. Wingf., *K. destructans* (M.J. Wingf. & Crous), *K. eucalypti* (Cooke & Massee) J. Walker, B. Sutton & Pascoe and *K. epicoccoides* (Cooke & Massee) J. Walker, B. Sutton & Pascoe, are common and important pathogens of eucalypts (Wingfield *et al*. 1996*a*; Park *et al*. 2000; Carnegie 2007*b*). *K. zuluensis* and *K. destructans* are unknown in Australia. *K. zuluensis* causes a serious stem canker disease on *Eucalyptus* species in subtropical areas of South Africa (Wingfield *et al*. 1996*a*), Hawaii (Cortinas *et al*. 2004), Ethiopia and Uganda (Gezahgne *et al*. 2005),

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Argentina and Vietnam (Gezahgne *et al*. 2003), China (Cortinas *et al*. 2006) and Uruguay (M. J. Wingfield, unpubl. data). *K. destructans* is an aggressive, destructive pathogen causing distortion of infected leaves and blight of young leaves, buds and shoots (Wingfield *et al*. 1996*b*). This pathogen was first discovered in Indonesia in 1996 and has subsequently been detected in Thailand, China, Vietnam and Timor (Wingfield *et al*. 1996*b*; Old *et al*. 2003*a*, 2003*b*; Burgess *et al*. 2006). *K. eucalypti* and *K. epicoccoides* are endemic to Australia and outbreaks have been noted in off-site plantations in the subtropics. For example, in northern NSW, *K. eucalypti* can cause severe damage to *E. nitens* plantations and *K. epicoccoides* can cause severe, repeated defoliation in *E. grandis* and *E. grandis* × *E. camaldulensis* plantations (Carnegie 2007*b*). *K. eucalypti* has also been introduced into New Zealand where it has resulted in the complete defoliation of juvenile leaves of *E. nitens* in New Zealand (Dick 1982). *K. epicoccoides* is known from many countries (Sankaran *et al*. 1995; Park *et al*. 2000) and generally causes disease on older leaves, although leaf blights have been observed in the tropics (B. Dell, pers. comm.).

During monitoring of eucalypt species trials in FNQ in July 2005, we observed leaves exhibiting symptoms similar to those observed for *K. destructans*. Samples were collected across several sites and preliminary examination revealed a fungus with a conidial morphology similar to that of *K. destructans*. This study describes the fungus as a new *Kirramyces* species from northern Qld and discusses its relationship to *K. destructions*. The origin of these species and the necessity for conducting surveillance and risk assessment are also considered.

## Materials and methods

### Collection

*Eucalyptus* leaves with symptoms resembling those of *K. destructans* were collected from a eucalypt species trial site situated 3 km north-west of Mareeba, near Cairns in FNQ. The trial was established in August 2004 to evaluate hardwood species grown using effluent irrigation in the dry tropics. Species planted in the trial included *Corymbia* spp., *E. pellita*, *E. grandis* and hybrids of *E. grandis* × *E. camaldulensis* from South America. Collected leaves were wrapped in paper tissues, placed in paper bags, packed in tight-fitting plastic bags from which the air had been displaced to form a vacuum and stored in a fridge before the isolation of fungi.

### Fungal isolates

Leaves were examined under a dissecting microscope, and conidia observed oozing from single pycnidia, were collected at the tip of a sterile needle. The conidia were transferred to malt extract (20 g/L) agar (MEA) containing streptomycin at 150 µg/mL (Sigma-Aldrich, Sydney, Australia) in a single spot and allowed to hydrate for 2–4 h. Under a dissecting microscope, conidia were then streaked across the surface of the medium using a sterile needle and single conidia were picked off the agar and transferred to new MEA plates. Cultures were grown at 20°C in the dark for 2 weeks and then transferred to fresh MEA plates. Cultures were maintained on 2% MEA in tubes at 20°C. Morphological characteristics of the isolates from this study were compared with those of other closely related species (Table 1). All isolates are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa or at Murdoch University, Perth, Western Australia (MUCC). Ex-type cultures have been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands. The holotype has been lodged in the herbarium of the Department of Primary Industries, Brisbane, Qld, Australia (BRIP). Isotype and paratype material is available from the Murdoch University herbarium (MURU).

### DNA extraction and PCR amplification

The isolates were grown on 2% MEA at 20°C for 4 weeks and the mycelium was harvested and placed in a 1.5-mL sterile Eppendorf tube. Harvested mycelium was frozen in liquid nitrogen, ground to a fine powder and genomic DNA was extracted as described previously (Andjic *et al*. 2007*a*). The region spanning the second internal transcribed spacer (ITS) and part of 5.8 S region of the rDNA was amplified using the primers ITS-3 (5′GTA TCG ATG AAG AAC GCA GC 3′) and ITS-4 (5′TCC TCC GCT TAT TGA TAT GC 3′) White *et al*. (1990). Part of the β-tubulin (βt) gene region was amplified with the primers βt2a (5′GGT AAC CAA ATC GGT GCT GCT TTC 3′) and βt2b (5′ACC CTC AGT GTA GTG ACC CTT GGC 3′) Glass and Donaldson (1995), part of translation elongation factor 1α (EF-1α) gene with the primers EF1–728 (5′CAT CGA GAA GTT CGA GAA GG 3′) and EF1–986R (5′ TAC TTG AAG GAA CCC TTA CC 3′) Carbone and Kohn (1999). The PCR reaction mixture, PCR conditions, the clean-up of products and sequencing were as described previously (Andjic *et al*. 2007*a*).

### Phylogenetic analysis

In order to compare *Kirramyces* isolates used in this study with other closely related species, additional sequences were obtained from GenBank (Table 1). Sequence data were assembled using Sequence Navigator version 1.01 (Perkin Elmer) and aligned in Clustal X (Thompson *et al*. 1997). Manual adjustments were made visually by inserting gaps where necessary. All sequences derived in this study were deposited in GenBank and accession numbers are shown in Table 1.

Analyses were performed on individual datasets in phylogenetic analysis using parsimony (PAUP) version 4.0b10 (Swofford 2003). The most parsimonious trees were obtained using heuristic searches with random stepwise addition 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 parsimonious trees were saved. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indices) were determined (Hillis and Huelsenbeck 1992). Branch and branch node support was determined using 1000 bootstrap replicates (Felsenstein 1985). Trees were rooted to *Neofusicoccum ribis*, which was treated as the outgroup taxon.

Bayesian analysis was conducted on the same aligned and combined dataset as the one used in the distance analysis. First, MrModelltest v. 3.5 (Nylander 2004) was used to determine the

**Table 1.** ***Kirramyces*** **species and isolates considered in the phylogenetic study**

| Culture no.[A] | Teleomorph | Anamorph | Host | Location | Collector | GenBank accession number[B] | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Internal transcribed spacer | β-tubulin | Elongation factor 1α |
| MUCC 452, CBS 121156 | – | *Kirramyces viscidus* | *Eucalyptus grandis* | Mareeba, Australia | T. I. Burgess | **EF031471** | **EF031483** | **EF031495** |
| MUCC 453, CBS 121157 | – | *K. viscidus* | *E. grandis* | Mareeba, Australia | T. I. Burgess | **EF031472** | **EF031484** | **EF031496** |
| MUCC 454 | – | *K. viscidus* | *E. grandis* | Mareeba, Australia | T. I. Burgess | **EF031473** | **EF031485** | **EF031497** |
| MUCC 45 | – | *K. viscidus* | *E. grandis* | Mareeba, Australia | T. I. Burgess | **EF031474** | **EF031486** | **EF031498** |
| MUCC 456, CBS 121155 | – | *K. viscidus* | *E. grandis* | Mareeba, Australia | T. I. Burgess | **EF031475** | **EF031487** | **EF031499** |
| MUCC 468 | – | *K. viscidus* | *E. grandis* × *E. camaldulensis* | Mareeba, Australia | T. I. Burgess | **EF527436** | **EF527430** | **EF527433** |
| MUCC 469 | – | *K. viscidus* | *E. grandis* × *E. camaldulensis* | Mareeba, Australia | T. I. Burgess | **EF527438** | **EF527432** | **EF527435** |
| MUCC 467 | – | *K. viscidus* | *E. grandis* × *E. camaldulensis* | Mareeba, Australia | T. I. Burgess | **EF527437** | **EF527431** | **EF527434** |
| CMW 22553 | – | *K. destructans* | *E. grandis* | Sumatra, Indonesia | P. A. Barber | DQ632667 | DQ632625 | DQ632732 |
| CMW 17918 | – | *K. destructans* | *E. grandis* | Sumatra, Indonesia | P. A. Barber | DQ632666 | DQ632624 | DQ632731 |
| CMW 19832 | – | *K. destructans* | *E. grandis* | Sumatra, Indonesia | P. A. Barber | DQ632665 | DQ632623 | DQ632730 |
| CMW 17919 | – | *K. destructans* | *E. urophylla* | Guangzhou, China | T. I. Burgess | DQ632701 | DQ632622 | DQ632729 |
| CMW 19909 | – | *K. destructans* | *E. urophylla* | Guangzhou, China | T. I. Burgess | EF031464 | **EF031476** | **EF031488** |
| CMW 15089 | – | *K. destructans* | *E. camaldulensis* | Vietnam | T. I. Burgess | EF031465 | **EF031477** | **EF031489** |
| CMW 15090 | – | *K. destructans* | *E. camaldulensis* | Vietnam | T. I. Burgess | EF031466 | **EF031478** | **EF031490** |
| CMW 15092 | – | *K. destructans* | *E. camaldulensis* | Vietnam | T. I. Burgess | EF031467 | **EF031479** | **EF031491** |
| CMW 16123 | – | *K. destructans* | *E. camaldulensis* | Thailand | M. J. Wingfield | EF031468 | **EF031480** | **EF031492** |
| CMW 13337 | – | *K. destructans* | *E. camaldulensis* | Thailand | M. J. Wingfield | EF031469 | **EF031481** | **EF031493** |
| CMW 16120 | – | *K. destructans* | *E. camaldulensis* | Thailand | M. J. Wingfield | EF031470 | **EF031482** | **EF031494** |
| CMW 17915 | – | *K. eucalypti* | *E. nitens* | Victoria, Australia | P. A. Barber | DQ632664 | DQ632626 | DQ632727 |
| CMW 17917 | – | *K. eucalypti* | *E. grandis* × *E. tereticornis* | New South Wales, Australia | A. J. Carnegie | DQ632711 | DQ632630 | DQ632725 |
| CMW 17916 | – | *K. eucalypti* | *E. grandis* × *E. camaldulensis* | Queensland, Australia | A. J. Carnegie | DQ632659 | DQ632628 | DQ632722 |
| CMW 11687 | – | *K. eucalypti* | *E. nitens* | New Zealand | M. Dick | DQ240001 | DS890168 | DQ235115 |
| MUCC 538 | *M. suttonii* | *K. epicoccoides* | *E. globulus* | Western Australia | S. Jackson | DQ632702 | DQ632619 | DQ632716 |
| MUCC 425 | *M. suttonii* | *K. epicoccoides* | *E. grandis* | New South Wales, Australia | T. I. Burgess | DQ632655 | DQ632613 | DQ632713 |
| CMW 22484 | *M. suttonii* | *K. epicoccoides* | *E. urophylla* | China | T. I. Burgess | DQ632705 | DQ632616 | DQ632714 |
| SA12 | *M. suttonii* | *K. epicoccoides* | *E. fragrata* | South Africa | M. N. Cortinas | DQ632657 | DQ632614 | DQ632718 |
| CBS 113313, CMW 14457 | *M. toledana* | *K. toledana* | *E. globulus* | Spain | P. W. Crous | AY725581 | DQ658235 | DQ235120 |
| CMW 11560 | *M. nubilosa* | | *E. globulus* | Tasmania, Australia | A. Milgate | DQ658232 | DQ658236 | DQ240176 |
| CMW 3279 | *M. cryptica* | *K. nubilosum* | *E. globulus* | Australia | A. J. Carnegie | AY309623 | DQ658234 | DQ235119 |
| CBS 117262, CMW 7449 | – | *K. zuluensis* | *E. grandis* | South Africa | L. Van Zyl | DQ240021 | DQ240102 | DQ240155 |
| CBS 113399, CMW 13328 | – | *K. zuluensis* | *E. grandis* | South Africa | L. Van Zyl | DQ240018 | DQ658233 | DQ240172 |
| CBS 110499, CMW 13704 | *M. molleriana* | *K. molleriana* | *E. globulus* | Western Australia | A. Maxwell | AY150675 | DQ240116 | DQ240169 |
| CMW 4940 | *M. molleriana* | *K. molleriana* | *Eucalyptus* sp. | Portugal | M. J. Wingfield | DQ239969 | DQ240115 | DQ240168 |
| CMW 11588 | *M. molleriana* | *K. molleriana* | *E. globulus* | Tasmania, Australia | A. Milgate | DQ239968 | DQ240114 | DQ240167 |
| CMW 7773 | *Neofusicoccum ribis* | – | *Ribes* sp. | New York, USA | B. Slippers | AY236936 | AY808170 | AY236878 |

[A]Designation of isolates and culture collections: CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; MUCC = Murdoch University culture collection, Australia.

[B]Sequences in bold were obtained during this study.

best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v. 3.1 (Ronquist and Heuelsenbeck 2003) applying a general time reversible substitution model with gamma and proportion of invariable site parameters to accommodate variable rates across sites. Two independent runs of Markov Chain Monte Carlo using 4 chains were run over 1 000 000 generations. Trees were saved every 1000 generations, resulting in 10 001 trees. Burn-in was set at 50 001 generations (i.e. 51 trees), well after the likelihood values converged to the stationary, leaving 9950 trees from which the consensus trees and posterior probabilities were calculated.

### *Morphological characterisation*

In order to determine the taxonomic position of the *Kirramyces* species considered in this study, plugs (2-mm diameter) were cut from actively growing cultures and placed at the centres of Petri dishes (55 mm) containing one of three different nutrient media. Three replicates of each isolate (five isolates in total) were grown on 2% MEA, oatmeal agar (OMA) and sterilised eucalypt leaves placed on the surface of tap water agar (TWA) at 20 and 28°C in the dark. After 30 days, cultures were assessed for growth and photographed. Squash mounts of fruiting structures were prepared on slides in lacto-glycerol (1 : 1 : 1 volume of lactic acid, glycerol and water) and observed at ×1000 magnification with an Olympus BH2 light microscope. The growth of cultures was determined by taking two measurements of colony diameter perpendicular to each other. Measurements of relevant taxonomic features used to distinguish between currently described *Kirramyces* spp. were made. Thus, each isolate was assessed for conidial size, shape, pigmentation and number of septa. Wherever possible, 30 measurements (×1000 magnification) of all taxonomically relevant structures were recorded for each species and the extremes are presented in parentheses. Colony colour was described using notations in the Munsell Soil Colour Charts (Gretag Macbeth, New Windsor, NY, revised 2000). Measurements of conidial size were obtained using the image analysis software Olysia BioReport 3.2 software imaging system. Data analyses were performed using descriptive statistics in Microsoft Excel.

## Results

### *Distribution, impact and symptoms of* Kirramyces *sp.*

The disease was first detected in August 2005 causing leaf blight to *E. grandis* and *E. grandis* × *E. camaldulensis* hybrids at a single site in Mareeba, north Qld. Infection was limited to the lower canopy and no defoliation was observed. The site at Mareeba was revisited in August 2006 and defoliation levels of 95% and greater were noted on the *E. grandis* × *E. camaldulensis* hybrids (Fig. 1). New shoots were infected with both *K. epicoccoides* and the *Kirramyces* sp. considered in this study. *K. epicoccoides* was also detected on the leaves of *E. grandis* and *E. pellita*. While the hybrids were heavily infected with the *Kirramyces* sp., the damage on *E. grandis* of the Copperload provenance was limited to the lower canopy with less then 15% of foliage affected. Several other taxa trials in the region, which included *E. grandis* and *E. grandis* × *E. camaldulensis* hybrids, were surveyed but the *Kirramyces* sp. found at Mareeba was not detected.

**Fig. 1.** Defoliated 15-month-old *Eucalyptus grandis* × *E. camaldulensis* at Mareeba, Atherton Tableland, Queensland, Australia. A year earlier the trees had full foliage and early symptoms of infection with *Kirramyces viscidus*.

Symptoms on leaves of *E. grandis* and *E. grandis* × *E. camaldulensis* were very similar to those caused by *K. destructans* (Fig. 2*a*, *d*). Necrotic spots appeared on both sides of the leaves and were circular to irregular, 3–20 mm in diameter, single to confluent, medium brown to light brown with red brown borders on the adaxial surface, and a light brown colour on the abaxial surface. In some cases, young leaves were severely distorted. Conidia accumulated on the lower surface of the lesions giving rise to crusty black masses (Fig. 2*c*, *f*).

### *DNA sequence comparisons*

The ITS2 dataset consisted of 203 characters, of which 51 were parsimony-informative and were used in the analysis. These data contained significant phylogenetic signal [$P < 0.01$; phylogenetic signal (gl) = −0.88]. Heuristic searches of unweighted characters in PAUP resulted in a single most parsimonious tree of 106 steps [consistency index (CI) = 0.65, retention index (RI) = 0.88]. Bayesian analysis resulted in a tree with the same topology and clades as those revealed in the parsimony tree [Fig. 3*a*, TreeBASE (http://www.treebase.org/treebase/) = SN3318]. The EF-1α dataset consisted of 337 characters, of which 156 were parsimony informative and were used in the analysis.

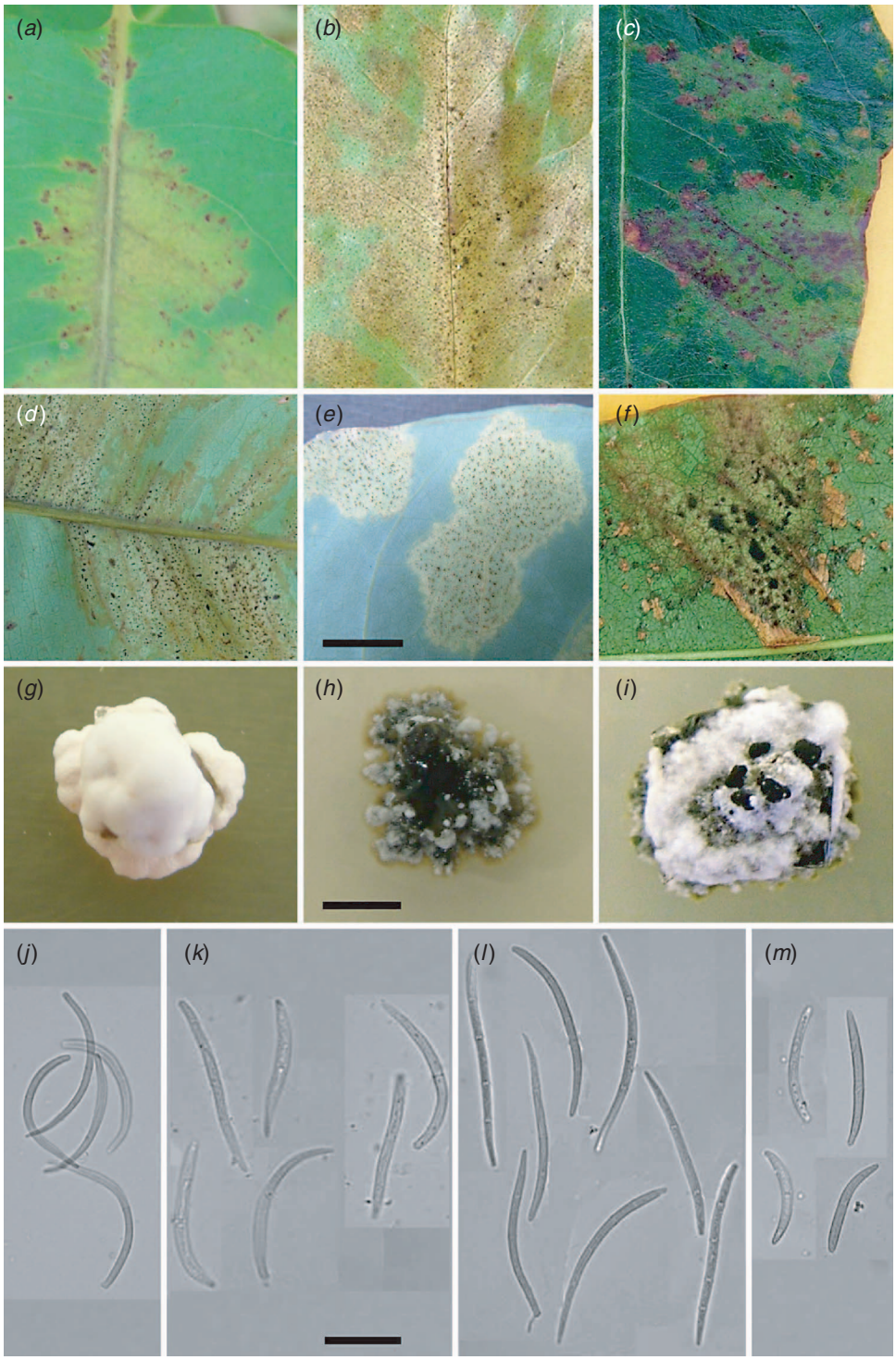


**Fig. 2.** A comparison between the foliar disease symptoms and cultural morphology of three *Kirramyces* spp. Symptoms of (*a*, *d*) *K. destructans* on leaves of *Eucalyptus urophylla* hybrids from Guangdong Province, China, (*b*, *e*) *K. eucalypti* on leaves of *E. grandis*, from Queensland, Australia and (*c*,*f*) *K. viscidus* sp. nov. on leaves of *E. grandis* from Mareeba, Queensland. Upper photo, adaxial surface; lower photo, abaxial surface. Bar = 10 mm. Cultures on malt extract agar of (*g*) *K. destructans* isolate CMW 17918 (CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa), (*h*) *K. eucalypti* isolate MUCC 384 (MUCC = Murdoch University culture collection, Perth, Western Australia) and (*i*) *K. viscidus* isolate CBS 121156 (CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands). Bar = 5 mm. Conidia of (*j*) *K. destructans* specimen PREM (PREM = South African National Mycological Herbarium) 59261, (*k*) *K. eucalypti* specimen MURU 425 (MURU = Murdoch University herbarium) and (*l*) *K. viscidus* specimen BRIP 49804 (BRIP = herbarium of the Department of Primary Industries, Brisbane, Queensland, Australia) and (*m*) *K. viscidus* isolate CBS 121156. Bar = 15 µm.

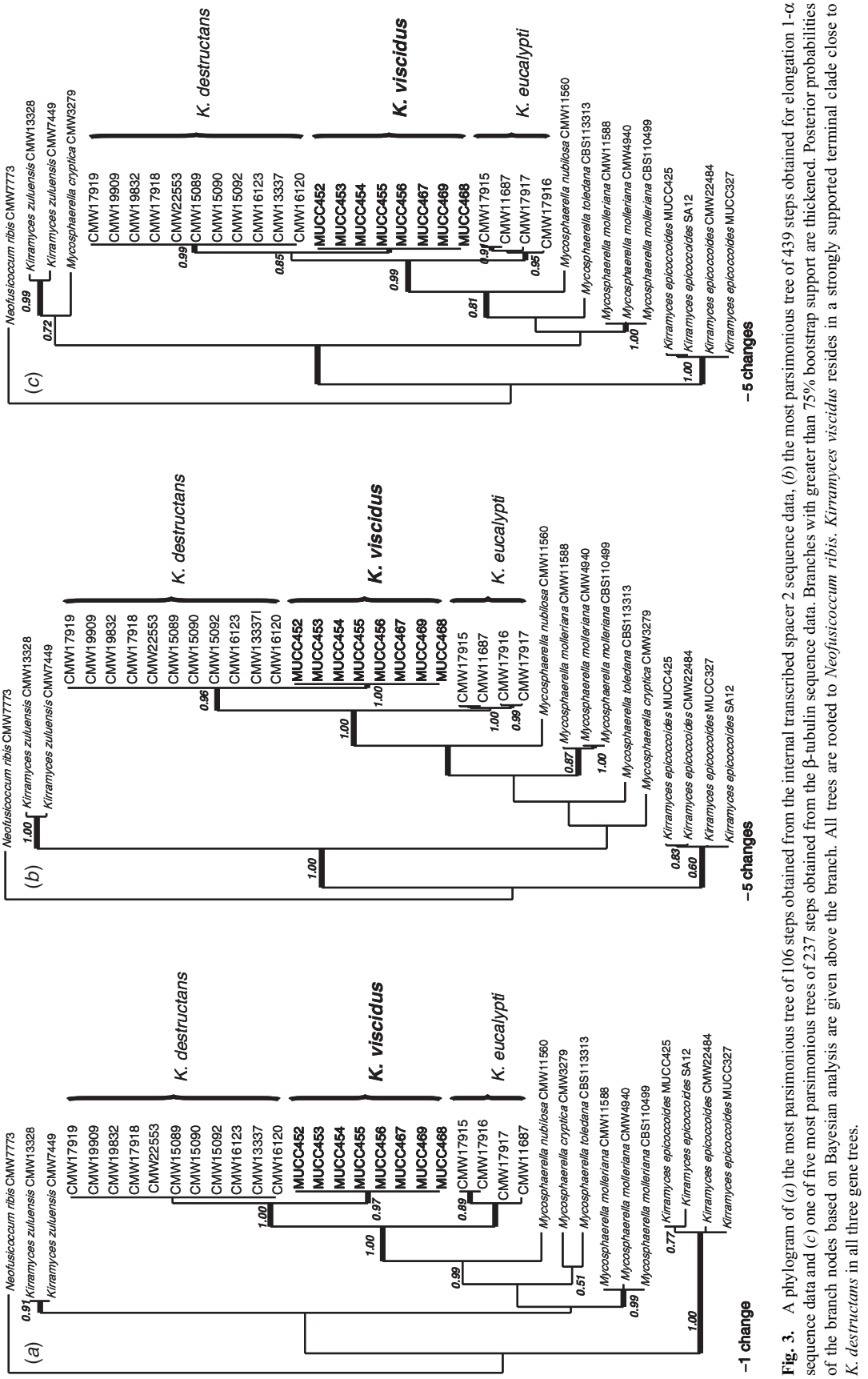


**Fig. 3.** A phylogram of (*a*) the most parsimonious tree of 106 steps obtained from the internal transcribed spacer 2 sequence data, (*b*) the most parsimonious tree of 439 steps obtained for elongation 1-α sequence data and (*c*) one of five most parsimonious trees of 237 steps obtained from the β-tubulin sequence data. Branches with greater than 75% bootstrap support are thickened. Posterior probabilities of the branch nodes based on Bayesian analysis are given above the branch. All trees are rooted to *Neofusicoccum ribis*. *Kirramyces viscidus* resides in a strongly supported terminal clade close to *K. destructans* in all three gene trees.

These data contained significant phylogenetic signal ($P < 0.01$; gl = −0.93). Heuristic searches of unweighted characters in PAUP resulted in a single most parsimonious trees of 106 steps (CI = 0.72, RI = 0.89). Bayesian analysis resulted in a tree with the same topology and clades as the parsimony tree (Fig. 3*b*, TreeBASE = SN3318). The βt dataset consisted of 384 characters, of which 90 were parsimony informative and were used in the analysis. These data contained significant phylogenetic signal ($P < 0.01$; gl = −0.80). Heuristic searches of unweighted characters in PAUP resulted in a single most parsimonious tree of 106 steps (CI = 0.72, RI = 0.88). Bayesian analysis resulted in a tree with the same topology and clades as the parsimony tree (Fig. 3*c*, TreeBASE = SN3318).

In all three analyses, the *Kirramyces* sp. from FNQ was closely related to, but phylogenetically distinct from *K. destructans* (Fig. 3). Isolates of both *K. destructans* and the *Kirramyces* sp. were monomorphic with no sequence variation observed for all isolates tested. Across the three gene regions sequenced, there were 12 fixed polymorphisms distinguishing *K. destructans* from the *Kirramyces* sp. (Table 2).

### Morphological characterisation

At first appearance, the conidia of the *Kirramyces* sp. were very similar to those of *K. destructans*. The first difference noted between the *Kirramyces* sp. and *K. destructans* was the extremely hydrophobic and sticky spores and these required 2–4 h of rehydration before the conidial masses could be teased apart. This is in contrast to *K. destructans* and *K. eucalypti*, where conidia are easily separated after rehydration for 1–5 min. The conidia of the *Kirramyces* sp. were very similar to those of *K. destructans* and *K. eucalypti* in pigmentation, shape, size and number of septa (Fig. 2*j–m*).

Conidia of the new *Kirramyces* sp. were subhyaline to pale brown, 0–3-septate and longer (54 µm on average, range 47–60 µm) than those of *K. eucalypti* (mean 43 µm, range 35–50 µm, which are also 0–3-septate) (Heather 1961; Walker *et al*. 1992; Andjic *et al*. 2007*b*). The conidia of the *Kirramyces* sp. were also longer than the conidia of specimens of *K. destructans* collected from China and Indonesia (mean 47 µm, range 38–55µm, 1–3-septate) that were given by Andjic *et al*. (2007*b*) but slightly shorter (50–65 µm, 1–3-septate) than conidia of specimen of *K. destructans* collected from Indonesia that were given by Wingfield *et al*. (1996*b*).

Pycnidia on leaf material collected at Mareeba were over-mature and it was not possible to obtain measurements of conidiogenous cells from these specimens. New collections at the same location were made, but all trees were more then 95% defoliated and the remaining leaves were covered with *K. epicoccoides* mixed with over-mature pycnidia of the *Kirramyces* sp. Thus, the only description and measurements obtained from leaf specimens were those for the conidia. Consequently, pycnidia produced in culture were used to characterise the morphology of conidiogenous cells.

After 30 days of growth at 28°C in the dark on MEA, colonies of the *Kirramyces* sp. were 20–30 mm in diameter, the upper surface was white to pinkish white with sectors of light greenish grey and they were covered with pycnidia, the lower surface was pinkish grey to reddish brown with irregular margins (Fig. 2*i*). These characteristics are typical of both *K. destructans* and *K. eucalypti* (Crous 1998) (Fig. 2*g–h*). On OMA, colonies were

**Table 2. Polymorphic nucleotides from sequence data of internal transcribed spacer (ITS), EF-1α and β-tubulin gene regions showing the variation between isolates of *Kirramyces destructans* and *Kirramyces* sp. nov.**

| Culture no.[A] | ITS | | | EF-1α | | | | β-tubulin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 155 | 194 | 226 | 211 | 228 | 295 | 296 | 131 | 134 | 222 | 224 | 270 |
| | | | | | *K. destructans* | | | | | | | |
| CMW17919 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW19909 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW17918 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW22553 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW19832 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW15089 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW15090 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW15092 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW16123 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW13337 | G | C | C | C | C | – | – | A | G | C | G | C |
| CMW16210 | G | C | C | C | C | – | – | A | G | C | G | C |
| | | | | | *Kirramyces* sp. nov. | | | | | | | |
| MUCC452 | T | T | T | T | A | T | G | G | A | T | A | T |
| MUCC453 | T | T | T | T | A | T | G | G | A | T | A | T |
| MUCC454 | T | T | T | T | A | T | G | G | A | T | A | T |
| MUCC455 | T | T | T | T | A | T | G | G | A | T | A | T |
| MUCC456 | T | T | T | T | A | T | G | G | A | T | A | T |
| MUCC468 | T | T | T | T | A | T | G | G | A | T | A | T |
| MUCC469 | T | T | T | T | A | T | G | G | A | T | A | T |
| MUCC470 | T | T | T | T | A | T | G | G | A | T | A | T |

[A]Designation of isolates and culture collections: CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; MUCC = Murdoch University culture collection, Australia.

20–29 mm in diameter, pinkish white on the upper surface, and pink to dark olive grey with a regular margin on the reverse side. On TWA, colonies grew 18–28 mm in diameter, white to pink on the upper surface with sectors of reddish brown, and olive grey below with a regular margin. Some colonies produced pycnidia on their surfaces. Growth was slower at 20°C on all three media, though the colour and shape on each was similar. Conidia from culture were shorter (33–45 µm) than conidia measured from leaf material (47–60 µm). This is also true for *K. eucalypti* where conidia produced in culture were shorter (24–32 µm) than conidia produced on leaf material (35–50 µm) (Andjic *et al*. 2007*b*). Of the five isolates used in this study, only one produced pycnidia and conidiogenous cells in culture, on MEA at 28°C.

### *Taxonomy*

Phylogenetic inference, and to a lesser extent morphological characteristics, have provided robust evidence that the *Kirramyces* sp. causing a serious leaf disease on hybrids of *E. grandis* × *E. camaldulensis* at Mareeba represents a unique taxon. The fungus is described here as a new species:

***Kirramyces viscidus*** Andjic, P.A. Barber, T.I. Burgess sp. nov. (Fig. 4)

Mycobank no. MB 510859.
*Teleomorph*: ***Mycosphaerella*** sp. (based on phylogenetic inferences, but not seen).
*Etymology*: Name refers to the hydrophobic and exceptionally sticky conidia of the fungus.

*Conidiomata* pycnidialia hypophylla, solitaria, atrobrunnea ad atra. *Conidia* solitaria, 0–3-septata, subhyalina et pallide brunnea, parum verruculosa, cylindracea, recta ad diverse curvata, parietibus crassis, ad basim truncata, margine interdum fimbriato, apice obtuso, (30.5–)47–60(–78.5) × (2–)2.5–3.5(4.0–) µm.

*Leaf spots*: circular to irregular, 3–20 mm in diameter, single to confluent, medium brown to light brown with red brown border on the top surface, light brown at the bottom. *Conidiomata*: pycnidial, hypophyllous, single, dark brown to black. Conidiophores reduced to conidiogenous cells. *Conidia*: solitary, 0–3-septate, subhyaline to pale brown, slightly verruculose, cylindrical, straight to variously curved, thick-walled, base truncate sometimes with marginal frill, apex obtuse, (30.5–)47–60(–78.5) × (2–)2.5–3.5(4.0–) (mean = 54 × 3 µm).

*Cultures*: Colonies 29 mm in diameter after 1 month at 28°C in the dark on MEA, white 5Yellow-Red (YR) 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*: doliiform to subcylindrical, smooth to slightly verruculose, aseptate to 1-septate, (5.5–)8–10.5 (–12.5) × (3–)4.5–8(–9.0) (mean = 6.4 × 9.5) µm, subhyaline to pale brown, proliferating enteroblastically, 1–2 times percurrently. *Conidia*: solitary, 0–1-septate, subhyaline to pale brown, smooth to slightly verruculose, cylindrical, straight to variously curved (29–)35–40(–47.5) × (2.0–)2.5–3.5(–4.0) (mean = 37 × 3.0 µm), lateral branches present as secondary conidia, mycelium in culture produce a synanamorph resembling chlamydospores (12.5 × 11 µm) that are dark brown and thick walled.

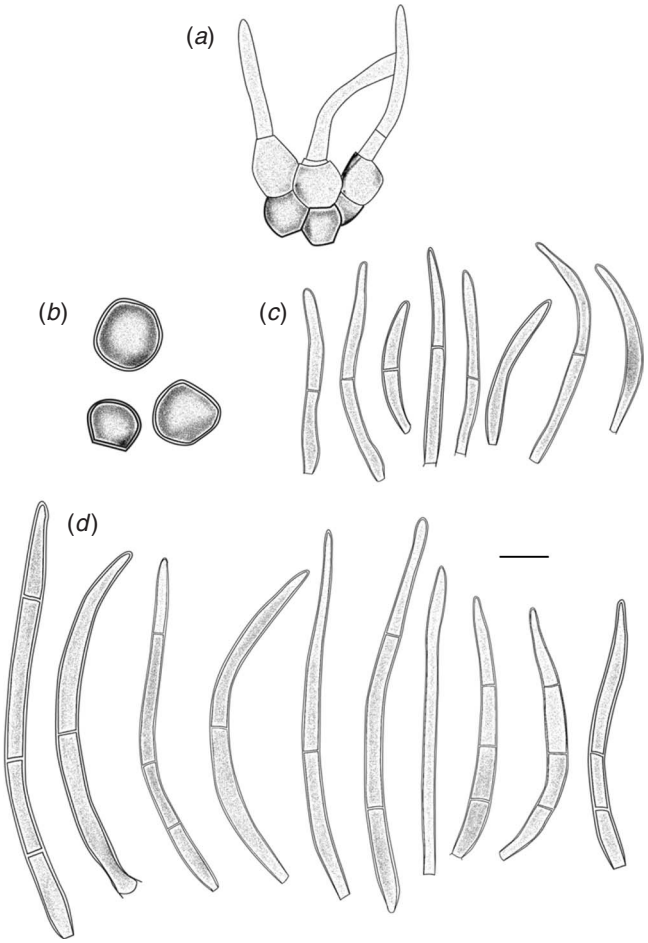


**Fig. 4.** *Kirramyces viscidus* (*a*) conidiogenous cells, (*b*) mycelium in culture producing chlamydospore-like synanamorph, (*c*) conidia produced on malt extract agar and (*d*) conidia produced *in vivo*. Scale bar = 10 µm.

*Holotype*: on leaves of *E. grandis* Hill ex Maiden, Mareeba, Qld, Australia, T.I. Burgess, G.E.St.J Hardy, A.J. Carnegie, G. Pegg, August 2005, (HOLOTYPE BRIP 49804; culture ex-type CBS 121157).

*Hosts: E. grandis*, *E. grandis* × *E. camaldulensis*.

*Geographic distribution:* Mareeba, north Qld.

*Additional specimens examined: K. viscidus* on *E. grandis*, Mareeba, Qld, Australia, T.I. Burgess, G.E.St.J Hardy, A.J. Carnegie, G. Pegg, August 2005, (MURU427; culture ex-isotypes, CBS 121155, CBS 121156, MUCC 454, MUCC 455) and *E. grandis* × *E. camaldulensis*, (MURU431; culture ex-paratypes, MUCC 467, MUCC 468, MUCC 469).

*Notes: K. viscidus* can be distinguished from *K. destructans* and *K. eucalypti* by the production of highly hydrophobic and viscous spore masses. *In vivo*, *K. viscidus* produces longer conidia (47–60 µm) than those of *K. eucalypti* (35–50 µm). In contrast to *K. destructans*, whose conidia are 1–3-septate, the conidia of *K. viscidus* are 0–3-septate. Unlike *K. destructans*, *K. viscidus* produces a synanamorph with chlamydospore-like structures in culture. The conidia of *K. viscidus* produced *in vitro*

were shorter 35–40, (mean = 37 × 2.8 µm) than those produced *in vivo* 47–60, (mean = 54 × 3 µm) (Fig. 2*m*).

## Discussion

Comparisons of DNA sequence data revealed that the *Kirramyces* sp. collected from leaves of *E. grandis* and the hybrid of *E. grandis* × *E. camaldulensis* from Mareeba represents a new taxon. This fungus has been named *K. viscidus*, a name emphasising the stickiness of its conidia. Based on phylogenetic analyses of sequence data obtained for the ITS, EF-1α and βt gene regions, *K. viscidus* has 12 polymorphic sites, which distinguish it from the closely related *K. destructans*. Based on morphological characteristics, *K. viscidus* can be distinguished from *K. destructans* by its remarkably sticky and hydrophobic conidia and the number of septa in the conidia. This report represents the first record of a new *Kirramyces* species very closely related to the destructive leaf blight and shoot pathogen, *K. destructans*. The latter fungus is considered a serious threat to the biosecurity of native eucalypts (Burgess *et al*. 2006) but has not been detected in Australia. The presence of a very similar but different fungus in the country will necessitate very careful comparisons when new records of similar *Kirramyces* spp. are made.

Sequence comparisons for all the isolates of *K. viscidus* examined in this study were identical. Conidial measurements for specimens on leaves also revealed no obvious differences between different collections. *Kirramyces* spp. from eucalypts are morphologically similar and thus difficult to distinguish from each other. Their current taxonomy consequently relies more heavily on DNA sequence comparisons than on morphology (Andjic *et al*. 2007*a*, 2007*b*).

The eucalypt taxa trial where *K. viscidus* was discovered was established in an ex-pasture area near remnant vegetation. In this trial, the *E. grandis* × *camaldulensis* hybrids from South America are highly susceptible to infection by *K. viscidus*, while endemic *Eucalyptus* spp. are less susceptible. These hybrids, a range of clones initially tested in the late 1990s, have also shown high susceptibility to *K. epicoccoides* in NSW (Carnegie 2007*a*, 2007*b*) and to *K. eucalypti* in southern Qld (G. S. Pegg, pers. obs.). Therefore, they would not be suitable for planting in FNQ. This is not surprising, as these hybrids were specifically selected for planting in South America; an area free of most Australian native pests and diseases to which they may be susceptible. *E. grandis*, selected from seed derived from the Copperload provenance near Cairns in north Qld, was less susceptible to infection by *K. viscidus*, with only 15% of the foliage affected. *K. viscidus* was not detected on *E. pellita* and *Corymbia* spp., which was also planted in the trial. Based on these findings, *K. viscidus* is most probably endemic to Australia and would be unlikely to cause serious diseases on endemic *Eucalyptus* spp. The most likely source of the pathogen is the remnant vegetation close to the trial site.

The close relativity of *K. viscidus* to the aggressive pathogen *K. destructans* and the similarity in disease symptoms, suggests that the pathogen could seriously damage Australian eucalypt plantations in the future. There is an urgent need to study the biology and ecology of *K. viscidus* and to determine its potential impact to the eucalypt industry. Thus, surveys to find possible hosts in both native forests and plantations need to be conducted in tropical regions, where non-endemic eucalypt plantations are being established. Here, priority should be given to native stands of *E. grandis* to target potential resistance for breeding programs using Australian derived material.

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