Phylogenetic reassessment supports accommodation of Phaeophleospora and Colletogloeopsis from eucalypts in Kirramyces

Vera ANDJIC\textsuperscript{a}, Paul A. BARBER\textsuperscript{a}, Angus J. CARNEGIE\textsuperscript{b}, Giles St J. HARDY\textsuperscript{a}, Michael J. WINGFIELD\textsuperscript{c}, Treena I. BURGESS\textsuperscript{a,*}

\textsuperscript{a}School of Biological Sciences and Biotechnology, Murdoch University, Murdoch 6150, Australia
\textsuperscript{b}Forest Resources Research, NSW Department of Primary Industries, PO Box 100, Beecroft, NSW 2119, Australia
\textsuperscript{c}Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

\textbf{ABSTRACT}

Species of Phaeophleospora are anamorphs of Mycosphaerella and they include some of the most serious foliar pathogens of Eucalyptus spp. grown in plantations worldwide. Pathogens assigned to this genus and occurring on Eucalyptus spp. were previously treated in Kirramyces and they are also phylogenetically closely related to other anamorphs of Mycosphaerella residing in the genus Colletogloeopsis. The primary aim of this study was to consider the appropriate taxonomic placement of these species. To achieve this goal, morphological characteristics and DNA sequence data from the ITS and translation EF1-\textit{a} gene regions were used to compare the type species \textit{P. eugeniae}, \textit{Phaeophleospora} spp. and \textit{Colletogloeopsis} spp. occurring on eucalypts, using ex-type cultures and herbarium specimens. Phylogenetic data and morphological comparisons supported the separation of \textit{P. eugeniae} from \textit{Phaeophleospora} species occurring on eucalypts. The name \textit{Phaeophleospora} is retained for \textit{P. eugeniae} and the name \textit{Kirramyces} is resurrected for the species occurring on eucalypts (genera \textit{Eucalyptus}, \textit{Corymbia}, and \textit{Angophora}). Sequence data from the type specimens of two previously described species of \textit{Kirramyces}, \textit{K. lilianiae} and \textit{K. delegatensis}, show they reside in a clade with other \textit{Kirramyces} spp. Morphological and DNA sequence comparisons also showed that there is considerable overlap between species of \textit{Phaeophleospora} and \textit{Colletogloeopsis} from eucalypts. Based on these findings, \textit{Colletogloeopsis} is reduced to synonymy with the older \textit{Kirramyces} and the description of \textit{Kirramyces} is emended to include species with aseptate, as well as multiseptate, conidia produced in acervuli or pycnidia. Two new species of \textit{Kirramyces}, \textit{K. angophorae} and \textit{K. corymbiae}, are also described.

© 2007 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

\textbf{Introduction}

\textit{Phaeophleospora} spp. are anamorphs of \textit{Mycosphaerella} that cause leaf and shoot blight diseases on many plants, including members of the families \textit{Myrtaceae}, \textit{Proteaceae}, \textit{Malvaceae}, \textit{Elaeocarpaceae}, and \textit{Sapotaceae}. The genus \textit{Phaeophleospora} was introduced to accommodate the dark form of \textit{Phleospora} (“\textit{Phleospora}”) by \textit{Rangel} (1916). However, most taxonomists regarded the monotypic genus \textit{Phaeophleospora} as a \textit{nomen dubium} because the mode of conidiogenesis and the form of...
the conidia were not documented in the type description (Sutton 1977). Up until 1997, Phaeophleospora included only two species: P. eugeniae occurring on Eugenia uniflora and P. elaeocarpi occurring on Elaeocarpus spp. (Bond 1947).

Confusion regarding the taxonomic placement of Phaeophleospora first emerged when a suitable name was sought for a Pseudocercospora sp. causing widespread damage on Eucalyptus in South Africa. Type specimens of Cercospora epicipoides and C. eucalypti were examined and it was concluded that neither of these species were correctly placed in the genus Cercospora (Crous et al. 1989). Thus, C. epicipoides was shown to reside in Phaeosporia where it was known as P. eucalypti. Walker et al. (1992) later compared P. eucalypti with the type specimen of the genus Phaeosporia, P. papayae, and noted that P. eucalypti differed from the type specimen in having large, brown, cylindrical, rough-walled, percurrently proliferating conidiogenous cells, that were not present in the type species. P. papayae had also previously been redescribed by Morgan-Jones (1974) and both he and Walker et al. (1992) found large, brown, cylindrical, rough-walled, percurrently proliferating conidiogenous cells were not present.

In a search for a suitable genus for P. eucalypti, several genera were considered (Walker et al. 1992). These included Scoleciasis, Sonderhenia, and Stagonospora, but none were suitable because they were all characterised by smooth-walled conidiogenous cells and distoseptate conidia (Sonderhenia) or smooth-walled, hyaline conidia (Stagonospora). P. eucalypti was therefore removed from Phaeosporia and the new genus Kirramyces was introduced for species with pycnidial conidio mata, brown, euseptate, cylindrical to narrowly obclavate rough-walled conidia and brown roughened annellidic conidiogenous cells (Walker et al. 1992). The genus included three taxa, K. epicipoides, K. lilium, characterised by brown rough-walled conidia, and K. eucalypti with pale yellowish-brown, finely roughened conidia. Walker et al. (1992) recognized the similarity in conidial size and shape between K. eucalypti and the Stagonospora delegatensis anamorph of Mycosphaerella delegatensis, but they noted that S. delegatensis differed from Kirramyces spp. in having paler, slightly less tapered and smooth conidia. These features also indicated that it was poorly accommodated in Stagonospora, but due to the lack of a suitable number of collections, the taxonomic position of this fungus was not resolved. Sankaran et al. (1995) later reduced S. delegatensis to synonymy with K. eucalypti without providing an explanation for their decision.

Crous et al. (1997) redescribed Phaeophleospora eugeniae based on the collection and designation of a neotype. In their study, it was concluded that P. eugeniae resembled species residing in Kirramyces, and Kirramyces was reduced to synonymy under the older name Phaeophleospora. Differences were noted between Phaeophleospora and Kirramyces, particularly in the gradient of pigmentation and number of septa in the conidia, but they did not consider these sufficiently important to justify separation at the generic level Crous et al. (1997). Other species of Kirramyces that had previously been described by Walker et al. (1992), as well as K. proteae, K. hebes, K. phormii and K. destructans, were re-allocated to Phaeophleospora (Crous et al. 1997).

Maxwell et al. (2003) selected Phaeophleospora for the anamorph of M. ambiphylla because the conidia of the fungus were produced in pycnidia. However, these authors noted that asceptate conidia had not previously been described for species residing in Phaeophleospora. Amongst Mycosphaerella anamorphs, asceptate conidia are more typical of Colletogloeopsis spp. with acervular conidiomata; however, the anamorph of M. ambiphylla produced conidia in pycnidia rather than acervuli. More recently, Crous et al. (2004) described the anamorph of M. toledana in the genus Phaeophleospora because the conidiomata were pycnidial rather than acervular. This species, like the anamorph of M. ambiphylla, produces asceptate conidia.

In a taxonomic re-evaluation of Coniothyrium zuluensis, Cortinas et al. (2006a) showed that this pathogen, which produces conidia in pycnidia, always clusters in the same clade as Colletogloeopsis nubilosum and C. molleriana whose conidia are produced in acervuli. Based on phylogenetic data, Cortinas et al. (2006a) emended the description of Colletogloeopsis to accommodate Coniothyrium-like anamorphs residing in Mycosphaerella, and included pycnidial, as well as acervular, conidiomata in this description. The authors could not place this fungus in the genus Phaeophleospora, as the type species P. eugeniae was found to be phylogenetically distant from C. zuluensis (Cortinas et al. 2006a). Likewise, Andjic et al. (2007), who investigated the phylogenetic relationship of Phaeophleospora species from eucalypts, found that these species were phylogenetically distant from the ex-type culture of P. eugeniae but close to C. zuluense and C. nubilosum. As a result, Phaeophleospora was no longer suitable to accommodate Colletogloeopsis-like species with pycnidial conidiomata as had previously been true for the anamorphs of M. ambiphylla (Maxwell et al. 2003) and M. toledana (Crous et al. 2004).

Species of Phaeophleospora s. lat. and Colletogloeopsis are common and important pathogens of eucalypts. New species that might reside in either of these genera are collected regularly. For practical taxonomic reasons and for the establishment of appropriate quarantine regulations, these fungi require appropriate names. This study emerged from the collection of apparently new species in these two genera, and the assignment of these new species to the appropriate genus necessitated a detailed phylogenetic and morphological study of related fungi. These included type specimens representing Phaeophleospora, Kirramyces, and Colletogloeopsis. The correct taxonomic placement of these genera was thus reassessed; suitable synonyms and combinations are proposed in Kirramyces and new species are described. Phaeophleospora remains as the monotypic genus for P. eugeniae.

**Materials and methods**

**Isolates**

Isolates were obtained by collecting conidia exuding from single pycnidia or acervuli using the tip of a sterile needle. These were transferred onto 2 % malt extract agar (MEA) containing streptomycin 150 µg ml⁻¹ (Sigma-Aldrich, Sydney, Australia) in a single spot and allowing it to hydrate for 5 min. Under a dissecting microscope, spores were streaked using a sterile needle and single spores immediately transferred to MEA plates. Cultures were grown at 25 °C for 2 weeks and then...
transferred to fresh MEA plates. Cultures were maintained on 2 % MEA in tubes at 20 °C. Herbarium specimens were obtained for *Phaeophleospora lilianiae* (DAR 3833), *P. delegatensis* (DAR 45718b), species for which an ex-type culture or sequence data do not exist. Herbarium material used for measurements included: *P. destructans* (PREM 59259, PREM 59261), *P. epicocoides* (PREM 59258, PREM 59260, MURU 422, MURU 423), and *P. eucalypti* (MURU 424, MURU 425). The cultures used in this study are maintained in the culture collections of Murdoch University (MUCU), the Forestry and Agricultural Biotechnology Institute, University of Pretoria (CMW), the New South Wales, Plant Pathology Herbarium (DAR), and State Forests of New South Wales (NSWF), Australia.

**DNA extraction and PCR from cultures**

Isolates were grown on 2 % MEA at 20 °C for four weeks and the mycelium harvested and placed in 1.5 ml sterile Eppendorf tubes. Harvested mycelium was frozen in liquid nitrogen, ground to a fine powder, and genomic DNA extracted using a hexadecyl trimethyl ammonium bromide (CTAB) modified protocol of Graham et al. (1994). Six hundred microlitres of extraction buffer (2 % CTAB; 100 mm Tris–HCl (pH 8), 1.4 m NaCl 2 % PVP-40 100 μg ml⁻¹ protease K, 100 μg ml⁻¹ RNase A) was added per 60 mg freeze-dried mycelium and incubated at 55 °C for 20 min. After incubation, the tubes were centrifuged for 2 min at 11 000 × g, and the supernatant transferred to a new tube and extracted with equal volume of chloroform isomyl alcohol 24:1 (IAC), centrifuged for 20 s at 11 000 × g, the upper aqueous phase transferred to a new tube and 0.1 volumes of 7.5 m ammonium acetate and two volumes of 100 % added to precipitate the DNA. The tubes were inverted a few times, incubated at −20 °C for 60 min, centrifuged for 1 min at 11 000 × g, the supernatant discarded and the DNA pellet washed with 1 ml 70 % ice-cold ethanol and re-centrifuged at 1000 × g for 1 min. The ethanol was decanted and the DNA allowed to air dry for 15 min. The DNA was resuspended in 30 μl ultra-pure PCR grade water. ITS1, ITS2 and 5.8S regions of the rDNA operon were amplified using primers ITS-1F (5′ GGT CAT TTA GAA GTA A 3′) and ITS2 (5′GCGTTCCTCACGTCGTTCTTCA 3′) (White et al. 1990) and the translation elongation factor 1-α region was amplified using primers EF1-728F (5′ CAT CGA GAA GGT CGA GAA GG 3′) and EF1-986R (5′ TAC TTG AAG GAA CCC TTA CC 3′) (Carbone & Kohn 1999).

PCR was performed using GeneAmp PCR System 2700 Thermal Sequencer (Applied Biosystems, Foster City, CA). Each 25 μl reaction mixture contained 1 × PCR polymerisation buffer (67 mm Tris–HCl, 16.6 mm ammonium sulphate, 0.45 % Triton X-100, 0.2 mg ml⁻¹ gelatine, 0.2 mM of each dNTPs; Fisher Biotech, Perth, Australia), 25 mM MgCl₂ (Fisher Biotech), 0.6 pmol each primer (GeneWorks, Adelaide, Australia), approximately 5 ng DNA and 1 unit Taq DNA polymerase (Fisher Biotech). During the PCR reaction, the DNA first was denatured at 94 °C for 2 min, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (55 °C for 45 s) and elongation (72 °C for 1 min) and ended with a final elongation step at 72 °C for 5 min. To detect possible contamination in the amplification reaction, a negative control that contained all reaction components except the fungal template DNA, was used with every reaction. The PCR products were visualised on 1 % agarose gel containing ethidium bromide using an uv transilluminator and purified with Ultrabind® DNA purification kit (MO BIO Laboratories, Solana Beach, CA) following the manufacturer’s instructions.

**DNA extraction and amplification from herbarium specimens**

In the cases where ex-type cultures were not available for species required to define genera, DNA extractions were made directly from herbarium specimens representing the types. Several individual pycnidia or acervuli were carefully removed from the herbarium specimens and transferred to a 1.5 ml tube and ground with liquid nitrogen to a fine powder. DNA was extracted with CTAB extraction buffer as described previously (Wittzell 1999).

Initially the DNA from the herbarium specimens was amplified using ITS primers ITS-1F and ITS-4, followed by nested PCR using primers ITS-1 (5′ GTA TCG ATG AAG AAC GCA GC 3′) and ITS2 (GCTCGGTTCCTCACGTCGTTCTTCA 3′) (White et al. 1990) primers with 1:5 dilution of initial PCR product as template. PCR mixtures and running conditions were as described above.

Some isolates showed false-positive amplification when subjected to nested PCR, thus direct PCR was used and the running conditions were changed. The magnesium concentration was increased to 4 mm and 0.8 % bovine serum albumin was added to each reaction. The DNA was denatured at 94 °C for 7 min, followed by 40 cycles of denaturation (94 °C for 2 min), annealing (45 °C for 1 min) and elongation (72 °C for 2 min) and ended with a final elongation step at 72 °C for 10 min.

**Phylogenetic analyses**

In order to compare species of *Phaeophleospora*, sequences in addition to those derived in this study were obtained from GenBank (Table 1). Sequence data were assembled using Sequence Navigator version 1.01 (Perkin Elmer, Melbourne, Australia) 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 listed in Table 1.

Analyses were performed on the combined dataset of complete ITS and EF-1α sequences, after a partition homogeneity test (PHT) had been performed in PAUP version 4.0b10 (Swofford 2003) to determine whether sequence data from the two gene regions were statistically congruent (Farris et al. 1995; Huelsenbeck et al. 1996). Parsimony analysis with heuristic search was performed using PAUP with random stepwise addition in 100 replicates with the tree bisection–reconnection branch-swapping option and the steepest-descent option off. All ambiguous and parsimony-uninformative characters were excluded; gaps were treated as a fifth character. MaxTrees were unlimited, branches of zero length were collapsed, and all multiple equally parsimonious trees saved. Estimated levels of homoplasy and phylogenetic signal; tree length (TL), consistency index (CI) and retention index (RI) were determined (Hillis & Huelsenbeck 1992). Characters were unweighted and
Bayesian analysis was conducted on the same aligned and unordered branch and branch node support was determined using 1 K BS replicates with equal probability (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 that used in the distance analysis. First, MrModeltest v2.2 (Nylander 2004) was used to determine the best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v3.1 (Ronquist & Huelsenbeck 2003) applying a general time reversible (GTR) substitution model with gamma (G) and proportion of invariable site (I) parameters to accommodate variable rates across sites. Two independent runs of MCMC using four chains were run over 10 M generations. Trees were saved each 10 K generations, resulting in 10 001 trees. Burn-in was set at 500 001 generations (i.e. 51 trees), well after the likelihood values converged to stationery, leaving 9950 trees from which the consensus trees and PPs were calculated.

Morphological comparisons

In order to re-assess the taxonomical status of Phaeophleospora spp. associated with eucalypts and to determine the correct generic placement for apparently undescribed species obtained from Corymbia spp. and Angophora floribunda, representative isolates of the unknown species and representative isolates of P. destructans, P. epicocoides and P. eugeniae were compared in vivo and in vitro with type specimens and with previous observations from published literature. The only available isolate of P. eugeniae (CMW5351) was sterile in culture and it could not be used for morphological comparisons. Ex-holotype cultures of P. elaeocarpi,
Results

Phylogenetic analyses

After repeated attempts to amplify the whole ITS region of herbarium specimens of Phaeophleospora delegatensis and P. lilianiae did not exist. The herbarium specimens were examined for P. epicoceoides (DAR 6338), P. eucalypti (Septoria normae) (DAR 65274), P. lilianiae (DAR 3832, DAR 3833), P. delegatensis (DAR 45718b), P. eugeniae (IMI 372655), Colletogloeopsis nibulosum (PDD37677) and C. molleriana (PREM 54395). Where morphological characteristics of species could not be determined from culture or herbarium specimens, data from published literature were included. Available isolates were characterised using cultural characteristics useful for Phaeophleospora species separation, such as conidial pigmentation, number of septa, and conidial size (Crous et al. 1997).

Four replicates of each isolate used in comparisons were prepared using 55 mm diam Petri plates and 2 % MEA. After 30 d, cultures were photographed and squash mounts of fruiting structures were prepared on slides in lactoglycerol and observed under an Olympus BH2 light microscope. Each isolate was assessed for conidial size, shape, pigmentation, and number of septa. Unknown species were also assessed for growth rate after one month growing at 20 °C in the dark. The growth rate was determined by measuring perpendicular colony diameters. Wherever possible, 30 measurements of all taxonomically relevant structures were recorded for each species and the extremes have been presented in parentheses. Colony colour for the unknown species was described using notations in the Munsell Soil Color Charts (Gretag Macbeth, New Windsor, New York, revised 2000). Measurements of conidial size were obtained using the image analysis software Olysia BioReport 3.2 soft imaging system. Data analyses were performed using descriptive statistics in Microsoft Excel. The drawings were prepared using a drawing tube attached to a BH2 Olympus Microscope. These drawings were then scanned on a flatbed scanner at 300 dpi, imported into the software program Macromedia Freehand version 10, and traced into a vector file. This file was then imported into Adobe Photoshop version, airbrushed, and stippled using the Andromeda Series 3 Screen Filter (Barber, unpubl. technique).

Morphological comparisons

Re-examination of the type specimen of Phaeophleospora eugeniae in this study has shown conidia of P. eugeniae to differ in conidial pigmentation, length, width, and number of septa from other Phaeophleospora species. While conidia of P. eugeniae show variation in pigmentation along the conidial length, ranging from light brown cells near the base to subhyaline cells at the apex, the conidia of other Phaeophleospora species are uniformly pigmented. Also, conidia of P. eugeniae are much longer, broader, and have a greater number of septa when compared with conidia of other Phaeophleospora spp. (Table 2). Morphological observations from herbarium specimens agreed well with the published descriptions, although there were minor exceptions (Table 2). Observation of the type specimens of P. eugeniae in the present study showed conidia were slightly shorter (100–115 μm) and had less septa (18–20 septa) than those described previously in the literature (110–120 μm, 16–30 septa). Specimens of P. destructans and P. eucalypti showed high levels of variability in conidial length, depending on the origin of the specimen. For example, specimens of P. destructans from China had shorter conidia (38–47 μm) than those from Indonesia (49–55 μm). The specimen of P. eucalypti from Queensland had slightly longer conidia (42–47 μm) than P. eucalypti from New South Wales (38–46 μm). Specimens of P. destructans had shorter conidia (38–47 μm) than previously recorded (50–65 μm) by Wingfield et al. (1996). Phaeophleospora epicoceoides, P. eucalypti and P. destructans produced shorter conidia in vitro than in vivo.
Based on phylogenetic analyses and morphological observations, it is clear that *Phaeophleospora eugeniae* is not related to other species of *Phaeophleospora* that are found on eucalypts. Although it has conidia that are peripherally similar to other species of *Phaeophleospora*, they are much longer, broader, and have more septa. Re-examination of type specimens in this study has shown that pigmentation is uniform along the length of conidia in all species of *Phaeophleospora* occurring on eucalypts. This is different to *P. eugeniae*, where there is distinct gradation in conidial pigmentation from light brown basal cells to subhyaline apical cells. Moreover, phylogenetic analysis has shown that taxa of *Colletogloeopsis* from eucalypts are congeneric with taxa of *Phaeophleospora* from eucalypts.

A genus is thus needed to accommodate species of *Phaeophleospora* other than *P. eugeniae*. The most appropriate repository for these species is *Kirramyces*, which we resurrect, with an emended description for species of *Phaeophleospora* occurring on eucalypts.

This study has shown that anamorphs of *Mycosphaerella* from eucalypt leaves and stems, currently residing in *Colletogloeopsis*, occur in a single monophyletic assemblage together with species of *Kirramyces*. However, these fungi all have single-celled conidia that are morphologically very different to the multi-septate conidia of *Kirramyces* spp. On the other hand, one of the unknown species emerging from this study, residing in the same phylogenetic group, and for which a name is needed, has either aseptate or up to three septate conidia. This implies that there is an obvious gradation from single-celled to multi-septate conidia in the

**Taxonomy**

Fig 1 – Consensus phylogram of 9500 trees resulting from Bayesian analysis of the combined ITS and EF-1α sequence data for isolates of *Phaeophleospora* and *Colletogloeopsis*. PPs of the branch nodes are indicated in italics and BS values resulting from parsimony analysis are indicated in brackets. The tree is rooted to *Neofusicoccum ribis*.
<table>
<thead>
<tr>
<th>Fungus Specimen number</th>
<th>Pigmentation</th>
<th>Conidial length (in vivo) µm</th>
<th>Conidial length (in vitro) µm</th>
<th>Conidial width (in vivo) µm</th>
<th>Conidial width (in vitro) µm</th>
<th>Number of septa</th>
<th>Schematic drawings of conidia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaeophleospora eugeniae (Crous et al. 1997)</td>
<td>IMI 372655 Sub-hyaline to medium brown</td>
<td>110–120</td>
<td>n/a</td>
<td>7–8</td>
<td>n/a</td>
<td>16–30</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>Present study</td>
<td>IMI 372655 Versicoloured</td>
<td>100–115</td>
<td>n/a</td>
<td>4–5</td>
<td>n/a</td>
<td>18–20</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>P. epicoccoides (Walker et al. 1992) (Crous &amp; Wingfield 1997)</td>
<td>K 39488 Medium brown</td>
<td>32–50.5</td>
<td>n/a</td>
<td>5–6</td>
<td>n/a</td>
<td>1–4</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>Present study</td>
<td>PREM 54963 Medium brown</td>
<td>45–55</td>
<td>40–55</td>
<td>3.5–4</td>
<td>3.5–5</td>
<td>1–7</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>MURU 422 Medium brown</td>
<td>44–50</td>
<td>n/a</td>
<td>3.5–4</td>
<td>n/a</td>
<td>n/a</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>MURU 423 Medium brown</td>
<td>45–48</td>
<td>n/a</td>
<td>3.5–4</td>
<td>n/a</td>
<td>n/a</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>PREM 59260 Medium brown</td>
<td>45–53</td>
<td>36–45</td>
<td>3–4</td>
<td>3–4.5</td>
<td>3–6</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>PREM 59258 Medium brown</td>
<td>41–49</td>
<td>n/a</td>
<td>3–4</td>
<td>n/a</td>
<td>n/a</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>DAR 6338 Medium brown</td>
<td>34–48</td>
<td>n/a</td>
<td>3.5–5</td>
<td>n/a</td>
<td>n/a</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>MURU 422 Medium brown</td>
<td>35–50</td>
<td>n/a</td>
<td>3–4</td>
<td>n/a</td>
<td>n/a</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>PREM 59260 Medium brown</td>
<td>41–49</td>
<td>n/a</td>
<td>3–4</td>
<td>n/a</td>
<td>n/a</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>PREM 59258 Medium brown</td>
<td>49–55</td>
<td>33–40</td>
<td>2–2.5</td>
<td>2–2.5</td>
<td>1–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>DAR 65742 Sub-hyaline</td>
<td>24–57</td>
<td>25–36</td>
<td>2–3</td>
<td>2–3.5</td>
<td>0–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>Septoria normae (Heather 1961)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>Present study</td>
<td>DAR 65742 Pale brown</td>
<td>42–47</td>
<td>25–36</td>
<td>2–3</td>
<td>2–3.5</td>
<td>0–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>MURU 425 Pale brown</td>
<td>38–46</td>
<td>22–28</td>
<td>2–3</td>
<td>2–3</td>
<td>0–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>MURU 424 Sub-hyaline</td>
<td>35–46</td>
<td>n/a</td>
<td>2–3</td>
<td>n/a</td>
<td>1–2</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>PREM 54416 Pale brown</td>
<td>50–65</td>
<td>n/a</td>
<td>2.5–3</td>
<td>n/a</td>
<td>1–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>P. destructans (Wingfield et al. (1996))</td>
<td>PREM 59261 Pale brown</td>
<td>38–47</td>
<td>35–40</td>
<td>2–2.5</td>
<td>2–3</td>
<td>1–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>PREM 59259 Pale brown</td>
<td>49–55</td>
<td>33–40</td>
<td>2–2.5</td>
<td>2–2.5</td>
<td>1–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>Kirramyces corymbiae</td>
<td>DAR 77445 Pale brown</td>
<td>17–23</td>
<td>16.5–22</td>
<td>3.5–5</td>
<td>2.5–3.5</td>
<td>0</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>Present study</td>
<td>DAR 77452 Sub-hyaline to pale brown</td>
<td>9–15</td>
<td>10.5–22.5</td>
<td>2.5–4</td>
<td>3–4.5</td>
<td>0–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>K. angophorae Present study</td>
<td>DAR 3833 Medium brown</td>
<td>35–50</td>
<td>n/a</td>
<td>4–6</td>
<td>n/a</td>
<td>1–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>Present study</td>
<td>DAR 3832 Medium brown</td>
<td>40–48</td>
<td>n/a</td>
<td>5–6</td>
<td>n/a</td>
<td>1–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>DAR 3833 Medium brown</td>
<td>35–43</td>
<td>n/a</td>
<td>5–7</td>
<td>n/a</td>
<td>1–3</td>
<td><img src="image" alt="Schematic drawings" /></td>
<td></td>
</tr>
<tr>
<td>P. delegatensis (Park &amp; Keane 1984)</td>
<td>DAR 45718b Hyaline</td>
<td>21–51</td>
<td>n/a</td>
<td>3–5</td>
<td>n/a</td>
<td>1</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>P. toledana (Crous et al. 2004)</td>
<td>CBS 59896 Medium brown</td>
<td>10–12</td>
<td>n/a</td>
<td>3–3.5</td>
<td>n/a</td>
<td>0</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
<tr>
<td>Colletogloeopsis nubilosum (Crous &amp; Wingfield 1997)</td>
<td>PDD 37677 Medium brown</td>
<td>10–15</td>
<td>n/a</td>
<td>4–5</td>
<td>n/a</td>
<td>0</td>
<td><img src="image" alt="Schematic drawings" /></td>
</tr>
</tbody>
</table>
monophyletic lineage that includes species of Colletogloeopsis and those species of Phaeophleospora known from eucalypts, now shown to be more appropriately accommodated in Kirramyces.

Kirramyces, as emended to include Phaeophleospora and Colletogloeopsis species known from eucalypts, produces fruiting bodies that are pycnidial, acervular, or both, conidiogenous cells that proliferate percurrently and/or sympodially, and conidia that are rough-walled, or in the case of Colletogloeopsis smooth-walled, pigmented, subhyaline to medium brown, with none or up to seven septa (Table 3). Thus, the generic description of Kirramyces is emended to accommodate additional species with black, erumpent acervuli, cylindrical to subcylindrical, subhyaline conidiogenous cells and aseptate, fusoid and ellipsoidal, smooth conidia. Phaeophleospora is distinguished from Kirramyces by the patterns of pigmentation, length of the conidia, and number of septa.

An emended description for Kirramyces is as follows:


**Mycelium** immersed.

**Conidiomata** pycnidoid to acervuloid, immersed to erumpent, brown to black, solitary, unilocular; wall 2–5 cells thick, of brown textura angularis or textura epi-dermoidea; ostiole central, circular, not papillate.

**Conidiogenous cells** discrete or produced on superficial hyphae (when cultivated), ampulliform, doliiform to lageniform or short cylindrical to sub-cylindrical, subhyaline to brown, verruculose, with 1-several percurrent or sympodial proliferations, formed from the inner cells of the pycnidial wall.

**Conidia** holoblastic, pigmented, aseptate or euseptate, fusoid to cylindrical to long obclavate, ellipsoidal tapered to obtuse apices, bases truncate to subtruncate with a marginal frill, smooth to verruculose.


The following additional species are thus accepted in Kirramyces:

**Kirramyces delegatensis** (R.F. Park & Keane) Andjic, *comb. nov.*

MycoBank no.: MB511196

Table 3 – Comparison of morphological characters defining *Phaeophleospora*, *Kirramyces* and *Colletogloeopsis*

<table>
<thead>
<tr>
<th>Morphological Characters</th>
<th><em>Phaeophleospora</em> (Crous et al. 1997)</th>
<th><em>Colletogloeopsis</em> (Crous &amp; Wingfield 1997) (Cortinas et al. 2006a)</th>
<th><em>Kirramyces</em> (present study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conidiomata</td>
<td>Pycnidial</td>
<td>Pycnidial, acervular</td>
<td>Pycnidial, acervular</td>
</tr>
<tr>
<td>Conidiogenous cell</td>
<td>Pigmented, cylindrical to ampulliform, proliferation percurrent</td>
<td>Sub-hyaline to pigmented, doliiform to subcylindrical or somewhat irregular, proliferation percurrent and sympodial</td>
<td>Sub-hyaline to pigmented, ampulliform, doliiform to lageniform, or short cylindrical to subcyllindrical, somewhat irregular proliferation percurrent and sympodial</td>
</tr>
<tr>
<td>Conidia</td>
<td>Pigmented, basal cell light-brown, apical cell pale brown, euseptate, verriform, long, subcylindrical to obclavate, smooth to rough walled</td>
<td>Pigmented, aseptate rarely 1-septate, subcylindrical, fusoid to ellipsoidal, smooth to verruculose</td>
<td>Pigmented, 0–7, cylindrical-subcylindrical, fusoid to ellipsoidal, rough to smooth walled</td>
</tr>
</tbody>
</table>


Teleomorph: not seen but presumed to be a *Mycosphaerella* sp. based on phylogenetic analysis.


Teleomorph: not seen but presumed to be a *Mycosphaerella* sp. based on phylogenetic analysis.


Teleomorph: not seen but presumed to be a *Mycosphaerella* sp. based on phylogenetic analysis.

*Kirramyces toledana* (Crous & G. Bills), Andjic, *comb. nov.* Mycobank no. MB511197


Teleomorph: *Mycosphaerella toledana* Crous & Bills

*Colletogloeopsis* spp. from eucalypts are synonymised with *Kirramyces* spp. and new combinations are proposed as follows:


Mycobank no. MB571199


Teleomorph: not seen but presumed to be a *Mycosphaerella* sp. based on phylogenetic analysis.

**Kirramyces molleriana** (Crous & M.J. Wingf.), Andjic, & M.J. Wingf. *comb. nov.*

Mycobank no. MB11199


**Kirramyces nubilosum** (Ga, nap. & Corbin), Andjic, *comb. nov.*

Mycobank no. MB511200


**Kirramyces stellenbochiana** (Crous) Andjic, *comb. nov.*

Mycobank no. MB511201


**Kirramyces sp.**


**Kirramyces zuluensis** (M.J. Wingf., Crous & T.A. Cout.) Andjic & M.J. Wingf., *comb. nov.*

Mycobank no. MB511202


Teleomorph: Not seen but presumed to be a Mycosphaerella sp. based on phylogenetic analysis.

Based on phylogenetic analysis and morphological observations it is clear that the new species isolated from Angophora and Corymbia should reside in the genus Kirramyces and they are described below.

**Kirramyces corymbiae** Carnegie, Andjic & P.A. Barber, sp. nov.

Figs 2, 3A–C

MycoBank no.: MB510110

Etym.: Named after the host on which this fungus is found.

Conidiomata pycnidialia amphigena, sub-epidermal, single to occasionally aggregated, black, globose, unilocular, to 90 μm diam; wall of 2-3 layers of textura angularis. Conidiogenous cells discrete, subhyaline to light brown, doliiform, 6–13 μm. Conidia holoblastic, fusiform, straight to mostly curved, occasionally sigmoidal, apex sub-obtuse, base truncate, not prominently guttulate, pale brown, aseptate, (14–)17–23(–24) × 3.5–5 μm.

Conidial germination on MEA after 24 h: Conidia becoming 1–2-septate, germ tubes growing at an acute angle from both ends of the conidia, each germ tube less than 10 μm long at 24 h.

Cultures: Colonies slow-growing, 9–14 mm diameter on MEA after one month at 25°C in the dark, margin white 5Y 8/1, top dark grey 5Y 3/1, bottom light pink 5YR 8/3, colony sectored. Conidia: fusiform, pale brown, straight to mostly curved, aseptate (8.5–)10.5–22.5(–25) × (1.5–)3–4.5(–5) μm (mean = 16.5 × 3.5 μm).

Hosts: *C. variegata*, *C. maculata*, and *C. henryi*.

Geographical distribution: Native forests and plantations in NSW Australia, very common and occasionally damaging.


**Kirramyces angophorae** Andjic, Carnegie & P.A. Barber, sp. nov.

Figs 2D–F, 4

MycoBank no.: MB510110

Etym.: Named after the host on which this species was found.
Teleomorph: not seen but presumed to be a *Mycosphaerella* sp. based on phylogenetic analysis.

Conidiomata pycnidialia amphigena, plerumque, hypophyllous, solitaria, atrobrunnea ad atra, uniloculata, ad 92 μm diam. paryetibus 3 – stratis texturae angularis. Cellulae conidiogenae cellulis superis stromatum orientes, doliformes ad subcylindraceae vel ampuliformes, aseptatae ad 1-septatae, 6.5–12 × 2.5–4 μm, paryetibus crassis, subhyalinae ad pallide brunneae, verruculosae, enteroblasticae prolificantes, 1-3 percurrenter, raro sympodialiter. Conidia solitaria, aseptata ad 1-3 septata, subhyalina et pallide brunnea, verrucolosa, fusiformia, subcylindraceae ad ellipsoidalae, recta ad parvum curvata, basis truncata, fimbriata, margine imbricato, apice subobtuso ad obtuso, (4.5–)9–15(–19) × (1.5–)2.5–4(–4.5) (mean = 12 × 3.5 μm).

**Typus:** Australia: NSW, Greenwich, Lane Cove Bushland, on leaves of *Angophora floribunda*, 27 Feb. 2005, A.J. Carnegie MURU 426 (DAR 77452 - holotypus; culture ex-type DAR 77452).

Leaf spots amphigenous, circular to irregular, 2–8 mm diam., single to confluent, red-brown with prominent purple border. Conidiomata pycnidial, amphigenous, predominantly hypophyllous, solitaria, atrobrunnea ad atra, uniloculata, up to 92 μm diam; wall of three layers of textura angularis. Conidiogenous cells arising from upper cells of the stroma, doliform to subcylindrical or ampulliform, aseptate to 1-septate, 6.5–12 × 2.5–4 μm, thick-walled, subhyaline to pale brown, verruculose, proliferating enteroblastically, 1-3 times percurrently, occasionally sympodially. Conidia single, aseptate to 1-3 euseptate, subhyaline to pale brown, verruculose, fusoid, subcylindrical to ellipsoidal, straight to slightly curved; base truncate with a marginal frill, apex sub-obtuse to obtuse, (4.5–)9–15(–19) × (1.5–)2.5–4(–4.5) (mean = 12 × 3.5 μm).

Cultures: Colonies 28–22 mm after one month at 25 °C in the dark, produce red pigmentation (10R 5/6) in agar, upper surface of culture olive SY 5/3, reverse dark olive SY 3/2. Conidiogenous cells 5.5–1.5 × 2.5–6 μm. Conidia (8.5–)10.5–22.5 (–25.5) (mean = 16.5 μm) × (1.78–)3–4.5(5–) (mean = 3.5 μm) 0–3 euseptate, lateral branches present as secondary conidia, mycelium in culture producing a synanamorph resembling...
Key to Kirramyces species occurring on eucalypts

1 Conidia versicoloured, apex and basal cells lighter than the rest of conidial body, on average >100 μm long................................................................. Phaeophleospora
Conidia uniformly pigmented, on average <70 μm long................................................................................................................ Kirramyces

2(1) Conidia aseptate to rarely 1-septate ................................................................................................................................. 3
Conidia 1 to multi-septate, rarely aseptate........................................................................................................................... 10

3(2) Conidia aseptate, fusiform, straight to mostly curved, occasionally sigmoidal, apex sub-obtuse, base truncate, on average >17 μm in length .............................................................. Corymbia
Conidia on average <15 μm in length ................................................................................................................................. 4

4(3) Conidia on average <6 μm in length ............................................................................................................................. 5
Conidia on average >6 μm in length ................................................................................................................................. 6

5(4) Conidia broadly ellipsoidal, finely verruculose, apex obtuse to sub-obtuse, base sub-truncate to bluntly rounded, 5–6 × 2.5 μm .................................................................................................................................................. Gauchensia
Conidia fusoid to subcylindrical to ellipsoidal, smooth to verruculose, apex obtuse, base, subtruncate, 4.5–5 × 2–2.5 μm ............................................................................................................................ Zulius

6(4) Conidia on average <10 μm in length ................................................................................................................................. Stellenboschiana
Conidia on average >10 μm in length .................................................................................................................................. 7

7(6) Conidia fusoid ..................................................................................................................................................................... 8
Conidia subcylindrical to ellipsoidal .......................................................................................................................................... 9

8(7) Conidia, finely verruculose, 12–14 × 4 μm; teleomorph Mycosphaerella pseudocryptica ........................................................................... Kirramyces sp.
Conidia, verruculose, apex acutely rounded, base truncate with a minute marginal frill, 10–12 × 3–3.5 μm; teleomorph Mycosphaerella toledana ................................................................................................................................... Toledana

9(7) Conidia, aseptate rarely becoming 1-septate in culture, 9–12 × 3–3.5 μm; teleomorph Mycosphaerella molleriana ................................................ Molleriana
Conidia, aseptate, 10–15 × 4–5 μm; teleomorph Mycosphaerella cryptica ................................................................................. Nubilosum

10(2) Conidia on average <30 μm in length ................................................................................................................................. Angophorae
Conidia on average >30 μm in length ............................................................................................................................................. 11

11(10) Conidia medium brown, typically 3–5 septate ....................................................................................................................... 12
Conidia hyaline to sub-hyaline to pale brown, typically 1–3 septate .............................................................................................. 13

12(11) Conidia typically 3–5-septate, occasionally with up to 7 septa, subcylindrical to narrowly obclavate, apex sub-obtuse 45–55 × 3.5–4 μm; teleomorph Mycosphaerella suttoniae ....................................................................................................................... Epicoccoides
Conidia typically 3-septate, cylindrical, apex obtuse, 40–48 × 5–6 μm, no known teleomorph ................................................... Lilianiae

13(11) Conidia 1-septate, hyaline, cylindrical, straight or curved, smooth, thin walled, apex obtuse, base truncate 21–51 × 3–5 μm; teleomorph Mycosphaerella delegatensis ...................................................... Delegatensis
Conidia subhyaline to pale brown ............................................................................................................................................. 14

14(13) Conidia typically 1–2-septate, less typically 0–3-septate, subcylindrical to narrowly obclavate, thick walled, finely verruculose, apex sub-obtuse, inconspicuous marginal frill present 35–50 × 3–4 μm, no known teleomorph .... Eucalypti
Conidia 1-3-septate, cylindrical, verruculose, apex obtuse, marginal frill mostly absent 50–65 × 2.5 μm, no known teleomorph ............................................................... Destructans

**Discussion**

Mycosphaerella spp. and their anamorphs include some of the most important pathogens of eucalypts. Many of them have also been moved around the world through the establishment of plantations of these trees. In recent years, numerous new species of these fungi have been described (Crous et al. 2004, 2006), and based on the large number of eucalypt species, it seems likely that many more will be discovered in the future. Many of these fungi are morphologically similar or difficult to distinguish based on morphology and their contemporary taxonomy relies heavily on DNA sequence comparisons. Results of this study, using phylogenetic inference and morphological characteristics, have led to the discovery of two new species of these fungi. In order to accommodate these species, the need to modify the boundaries of the genera to which they belong became evident.

The present phylogenetic and morphological study has shown the type specimen of the genus Phaeophleospora, *P. eugeniae*, is well separated from *Phaeophleospora* spp. occurring on eucalypts. This phylogenetic separation logically led to the resurrection of the previous generic name, *Kirramyces*, for these species. Furthermore, phylogenetic analysis combined with the overlapping morphological characters of *Kirramyces* spp. and Colletogloeopsis spp. occurring on eucalypts, supporting the synonymy of these genera. Thus, anamorphs of *Mycosphaerella* residing in *Phaeophleospora* occurring on eucalypts, as well as species of *Colletogloeopsis*, have been transferred to the newly resurrected genus *Kirramyces*. Re-examination in the present study of the type specimens of both *Phaeophleospora* and *Kirramyces* has shown that variation in pigmentation of conidia is a useful morphological feature in distinguishing between these two genera.

The phylogenetic relationship between the genera *Phaeophleospora* (now *Kirramyces*) and Colletogloeopsis has been shown in previous studies (Andjic et al. 2007; Cortinas et al. 2006b; Crous et al. 2001, 2006; Hunter et al. 2006). The two genera have not previously been combined, mainly because *Phaeophleospora* produced pycnidial conidiomata and 0-multiseptate conidia, whereas *Colletogloeopsis* produced acervular conidiomata and asceptate or rarely 1-septate conidia. The emendment of the description of *Colletogloeopsis* to accommodate species with pycnidia (Cortinas et al. 2006a) resulted in conidial size and septation being the only morphological characters separating the two genera. However, discovery of new species such as *K. angophorae*, with asceptate as well as multiseptate conidia, precludes retaining *Colletogloeopsis* for asceptate species in this group. Furthermore, these differences are not phylogenetically supported between species within the genus.

In the present study, it was possible to obtain the sequences for the ITS1 region from the type specimens of *K. lilianiae* and *K. delegatensis*. Walker et al. (1992) described *K. lilianiae* as morphologically very similar to *K. epicoccoides*. However, due to the lack of a suitable number of collections of *K. lilianiae*, comparison between the two species was not possible. *Stagonospora delegatensis*, first described by Park & Keane (1984) was later considered by Swart (1988) to be congeneric with *Septoria pulcherrima*. Walker et al. (1992) noted that *S. delegatensis* was similar to *K. eucalypti* and thus was a possible candidate to transfer to *Kirramyces*, but required further collections. It was later reduced to synonymy with *K. eucalypti* (Sankaran et al. 1995). Subsequently, Crous (1998) re-examined the type specimen of *S. delegatensis* and supported the placement of this fungus in the genus *Kirramyces*. However, based on conidial shape, they chose to retain the species as separate from *K. eucalypti* and transferred it to *Phaeophleospora* as *P. delegatensis*. Results of the present study based on ITS1 sequence data have shown that *K. delegatensis* and *K. lilianiae* cluster together with other *Kirramyces* species occurring on eucalypts, therefore confirming its placement in *Kirramyces*.

*Kirramyces* was originally described for three species: *K. epicoccoides*, *K. eucalypti* and *K. lilianiae*. Based on the results of the present and previous studies, the genus now includes 14 species. These all reside in a well-resolved, monophyletic clade based on DNA sequence comparisons. They also have conidia ranging from those that are aseptate to multisepate.

*Mycosphaerella* is a heterogenous genus that is linked closely to a large number of anamorphs that lack known teleomorphs (Crous & Braun 2003). Previous authors have debated whether anamorphic states should be used to separate genera, subgenera, or sections within *Mycosphaerella*. Sutton & Hennebert (1994) held the view that different conidiogenous events and conidial modes in anamorphs linked to *Mycosphaerella* may prove useful in grouping species at some subgeneric level. Based on phylogeny, this has not proved to be true, as many anamorphs of *Mycosphaerella* spp. are currently polyphyletic (Crous et al. 2006).

Hunter et al. (2006) suggested that anamorph relationships based on phylogenetic position within *Mycosphaerella* cannot be predicted. However, results of the present study have shown that all *Kirramyces* spp. from eucalypts (including several as yet undescribed species) reside in the same strongly supported clade. This is also true for species of *Pseudocerospora* (Crous & Braun 2003) and *Readeriella* (Crous et al. 2004, 2006; Hunter et al. 2006). Morphologically, *Readeriella* spp. are somewhat similar to species in the genus *Kirramyces*, but are distinctly different as *Readeriella* spp. have obvious phialidic conidiogenesis. This was a key feature used by Sutton (1980) to differentiate between the genera *Microsphaeropsis* and *Coniothyrium* occurring on eucalypts. A number of species collected and identified as *Microsphaeropsis* or *Kirramyces*, based on morphological characters, have subsequently been compared based on DNA sequence data and these have resolved taxonomic conflicts between *Readeriella* and *Kirramyces* (Andjic, unpubl. data). In the case of *Readeriella* and *Kirramyces*, the mode of conidiogenesis appears to be phylogenetically significant. We suspect that new collections and subsequent DNA sequence comparisons for previously described *Microsphaeropsis* spp. from eucalypts, including *M. conielloides*, *M. callista*, *M. globulosa*, and *M. olivaceae* will show that these fungi reside in the genus *Readeriella*.

Data emerging from this study provide clear evidence that at least some groups of anamorphs of *Mycosphaerella* spp.
Taxonomic revision of Phaeophleospora spp. from eucalypts

reside in strongly monophyletic lineages. These are generally also consistent with their morphological features. These are interesting and important observations that most likely reflect ecological adaptation and evolutionary events. How these relate to a possible subdivision of Mycosphaerella based on phylogenetic inference is difficult to predict. Clearly, many anamorph genera are emerging in discrete clades that are very different to the one that accommodates M. punctiformis, the type species of the genus. There is good evidence that Mycosphaerella is polyphyletic and its subdivision into more natural subdivisions will emerge in time. We believe the anamorphs of this important genus encompass valuable ecological inference and should not be lost from future phylogenetic treatments of the group.

This study has clarified the generic placement of a large number of Mycosphaerella spp. or their anamorphs occurring on leaves, shoots, and stems of eucalypts. These also include some of the most important pathogens of Eucalyptus residing in Mycosphaerella. For example, the stem pathogens, K. zulenis and K. gauchensis cause the disease known as Coniothyrium canker, which is one of the most important diseases of Eucalyptus spp. grown in plantations (van Zyl 1999; Wingfield; Crous & Coutinho 1997). Likewise, K. destructans, K. eucalypti, K. epipodites, and K. rubebubum (anamorph of M. cryptica) represent four of the most important leaf pathogens of Eucalyptus spp. (Park et al. 2000; Barber 2004; Burgess et al. 2006; Carnegie 2007). Of these, K. destructans is particularly damaging because it infects both leaves and shoots of trees and it has caused substantial damage to plantations in southeast Asia. The majority of the most important Mycosphaerella spp. that infect eucalypts reside in the phylogenetic clade accommodating species of Kiramycetes. The evolutionary significance of this relationship deserves further study.

Acknowledgements

This work was funded in part by the Australian Research Council DP0343600, ‘Population genetics of fungal pathogens that threaten the biosecurity of Australia’s eucalypts’. V.A. is a recipient of a Murdoch University Doctoral Research Scholarship. Pedro W. Crous is thanked for useful discussion on the correct generic name for this group. We acknowledge funding from various grants to the University of Pretoria linked to tree protection research and a collaborative research agreement linking the University of Pretoria and Murdoch University. Finally we thank Alex George for editing the Latin descriptions.

References


