Scientific paper

Foliicolous Fungi of *Eucalyptus* spp. from Eastern Madagascar: Implications for South Africa

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SYNOPSIS

A collection of *Eucalyptus* leaves from Madagascar revealed several foliicolous fungi to be present, most being new records for the country. Known foliar pathogens such as *Aulographina eucalypti, Cylindrocladium quinqueseptatum, Kirramyces epicoccoides, Pseudocercospora eucalyptorum, Harknessia hawaiiensis* and *Mycosphaerella heimii* were found associated with leaf spots. *Codinea eucalypti, C. septata, Arnaudiella eucalyptorum, Mycotribulus mirabilis, Clypeophysalospora latitans, Glomerella cingulata, Cylindrocladium candelabrum* and *Propolis emarginata* were isolated from *Eucalyptus* leaf litter. Previous studies have shown all these fungi to be present in South Africa, except *C. quinqueseptatum,* which is not known from Africa, and *M. heimii* which is known only from Madagascar. *M. heimii* is distinguished from similar *Mycosphaerella* spp. occurring on *Eucalyptus* leaves by its symptom expression, ascospore morphology and cultural characteristics. A Latin diagnosis is provided for *M. heimii* to validate the name, and its anamorph described as *Pseudocercospora heimii* sp. nov.

INTRODUCTION

Commercial plantations of exotic pine and eucalypt species in South Africa are under constant threat of new pests and diseases. In recent years, a number of new tree pathogens have been reported from South Africa, especially on eucalypts. Because most of these pathogens have caused extensive losses to eucalypt crops in other countries, it is assumed that they are of exotic origin (Crous *et al.,* 1989a; Wingfield *et al.,* 1989). The introduction of these pathogens into South Africa is therefore of obvious concern to the forestry industry. Needless to say, the increasing mobility of people and plant products around the world will inevitably result in new and damaging pathogens appearing in countries where they were previously unknown.

The cultivation of plants outside their native range can also result in the appearance of new diseases caused by pathogens which originate on indigenous hosts, and eventually develop the capacity to infect closely related exotics. A good example of this phenomenon is eucalypt rust caused by *Puccinia psidii* G. Winter which is native to Central and South America, and has adapted from *Psidium* to *Eucalyptus* in the Myrtaceae. If introduced to South Africa, this pathogen could pose a substantial threat to the future cultivation of eucalypts locally.

Clonal plantations have resulted in tremendous gains in productivity and relatively few losses due to disease (Wingfield, 1990). Intensive clonal propagation and hybridisation of eucalypts can however lead to the development of new susceptible genotypes. It is therefore not surprising that recently reported diseases such as *Sporothrix* twig and leaf blight, and *Coniothyrium* and *Cryphonectria* canker (Kemp and Wingfield, 1992; Wingfield *et al.,* 1989, 1993) have been observed to be more severe on certain hybrids or genotypes than others.

Eucalyptus pathogens occurring in countries where eucalypts are grown as exotics could eventually have substantial implications for the South African forestry industry should they be introduced into the country. For this reason, surveys in these countries are a valuable strategy for keeping abreast of diseases occurring on eucalypts. Such a preliminary survey was conducted when the authors visited Madagascar during April 1994. By systematically collecting eucalypt foliage at 30 km intervals along a 400 km section of road between Antananarivo and Tamatave on the east coast, a good general impression was obtained of foliicolous eucalypt fungi occurring in this area. This report lists the most prevalent fungi collected, and provides a brief description of the pathogenic status of each fungus.

RESULTS AND DISCUSSION

Kirramyces epicoccoides (Cooke & Massee) J. Walker, B. Sutton & Pascoe (= *Phaeoseptoria eucalypti* Hansf.)

Kirramyces epicoccoides has been reported from countries such as Brazil, Chile, India, Italy, Malawi, Portugal, South Africa and Taiwan (Park *et al.,*

1994). The pathogenicity, host range and infection process of this fungus has been well studied in Australia and South Africa (Heather, 1965; Crous *et al.*, 1989a; Nichol *et al.*, 1992). It has furthermore been associated with a serious leaf disease of *Eucalyptus* spp. in the subgenus Symphyomyrtus leading to a defoliation of plantation trees, and seedling mortality. Although *K. epicoccoides* is unlikely to cause the death of trees in plantations, it has the potential to cause substantial losses in nurseries due to defoliation of seedlings (Chipompa, 1987). Severe defoliation of older trees, however, can affect photosynthesis and reduce eventual timber yield (Nichol *et al.*, 1992)

Of all the leaf spot diseases occurring on *Eucalyptus* spp. in Madagascar, *K. epicoccoides* is the most common. It occurred very prominently in and around Antananarivo, but became less obvious toward Tamatave on the east coast. The latter may be either because the *Eucalyptus* sp. on the east coast is different from the one in Antananarivo, or because the climate becomes more humid towards the east.

Harknessia hawaiiensis F. Stevens & E. Young

Harknessia hawaiiensis has been recorded from Brazil, Hawaii and Florida, USA, Zambia and South Africa (Sutton, 1980; Crous *et al.*, 1993a). In South Africa, it has been found to cause an important leaf spot disease of *E. grandis,* and several other *Eucalyptus* spp. In Madagascar it was found in association with *K. epicoccoides* and *Myscosphaerella heimii* Bouriquet in Andranokobaka and Moramanga (east of Antananarivo).

Aulographina eucalypti (Cooke & Massee) Arx & E. Müll. [anamorph: *Thyrinula eucalypti* (Cooke & Massee) H.J. Swart]

Aulographina eucalypti is known as a common leaf spot of *Eucalyptus* spp. world-wide, and is mainly associated with *Eucalyptus* spp. in the subgenus Monocalyptus (Park *et al.*, 1994). Although it is commonly found in association with pathogens such as *Mycosphaerella* and *K. epicoccoides* in South Africa, this was not the case in Madagascar, where it occurred on its own, causing serious foliage and petiole necrosis of *Eucalyptus* spp. in Antananarivo, as well as further east towards the coast.

Pseudocercospora eucalyptorum Crous, M.J. Wingf., Marasas & B. Sutton

Pseudocercospora eucalyptorum is a common leaf spot of *Eucalyptus* spp. in South Africa, Brazil, Australia, Italy and New Zealand. In Italy, it has been associated with leaf spots on 45 different *Eucalyptus* spp. (Magnani, 1965). In South Africa, it is most serious on *E. nitens,* on which it causes an angular leaf spot (Crous and Wingfield, 1991). In Madagascar it was found once only, occurring on a *Eucalyptus* sp. in Antananarivo.

Cylindrocladium quinqueseptatum Boedijn & Reitsma (teleomorph: *Calonectria quinqueseptata* Figueiredo & Namek.)

Cylindrocladium quinqueseptatum has been recorded from *Eucalyptus* spp. in Australia, Brazil, Indonesia, Malaysia, Mauritius, India, Sri Lanka and Hong Kong (Crous and Wingfield, 1994). It is interesting to note that this important *Eucalyptus* pathogen has not yet been recorded from Africa. However, it does occur in Indonesia, and taking the strong Indonesian influence in Madagascar into consideration, it is possible that *C. quinqueseptatum* was introduced into Madagascar from the east.

Cylindrocladium quinqueseptatum was reported as one of the most common and destructive diseases of *Eucalyptus* spp. in India (Anahosur *et al.*, 1976; Seghal, 1983; Rattan and Dhanda, 1984), and as a nursery disease in Brazil (Figueiredo and Namaketa, 1967). Research conducted by Anahosur *et al.* (1976) suggested that there may be a toxin involved, while Sharma and Mohanan (1991) found different physiological strains of the fungus to be present in India. In Madagascar this pathogen was associated with a serious leaf spotting disease of coppice regrowth of a *Eucalyptus* sp. in Tamatave on the east coast.

Mycosphaerella heimii Bouriquet *ex* Crous sp. nov. *Figure 1*

Mycospaerella heimii Bouriquet, *Encycl. Mycol.* 12: 418 (1946), *nom non rite publ.*

Laesiones amphigenae, pallide brunneae, elongatae, irregulares, 5-15 mm diam. *Ascocarpi* amphigena, solitaria, nigra, immersa, globosa, glabra, usque ad 90 µm lata, 100 µm alta; paries consistens in cellis medio-brunneis, 3-4 stratis texturae angularis, basis consistens in 2-3 stratis cellarum hyalinarum; *Asci* in fascia una, bitunicati, aparaphysati, subsessiles, octospori, ellipsoidei vel obclavati vel cylindrici, recti vel incurvi, numerosi, 25-40 x 6-9 µm. *Ascosporae* 2-3 seriatae, obliquae, superpositae, recte ellipsoidales, obtusae ad extremum utrumque, hyalinae, laevae, uniseptatae, guttulatae, non colligatae ad septum, latissimae ad mediam cellam superiorem, prominentius attenuatae ad extremum hoc quam ad illud, (8-)10(-12) x (2-)2,5(-3) µm.

Lesions discrete, amphigenous, medium brown, elongated, irregular, 5-15 mm diam., surrounded by a prominently-raised, brown margin; also associated with tip blight symptoms. *Pseudothecia* amphigenous, single, black, immersed, globose, glabrous, up to 90 µm wide, 100 µm high; wall consisting of medium brown cells, 3-4 layers of *textura angularis;* base consisting of 2-3 layers of hyaline cells. *Asci* fasciculate, bitunicate, aparaphysate, subsessile, 8-spored, ellipsoid to obclavate or cylindrical, straight or curved, numerous, 25-40 x 6-9 µm. *Ascospores* 2-3 seriate, oblique, overlapping, straight ellipsoidal, obtuse at each end, hyaline, smooth, 1-septate, prominently guttulate, not constricted at the median septum, widest in the middle of upper cell, tapering

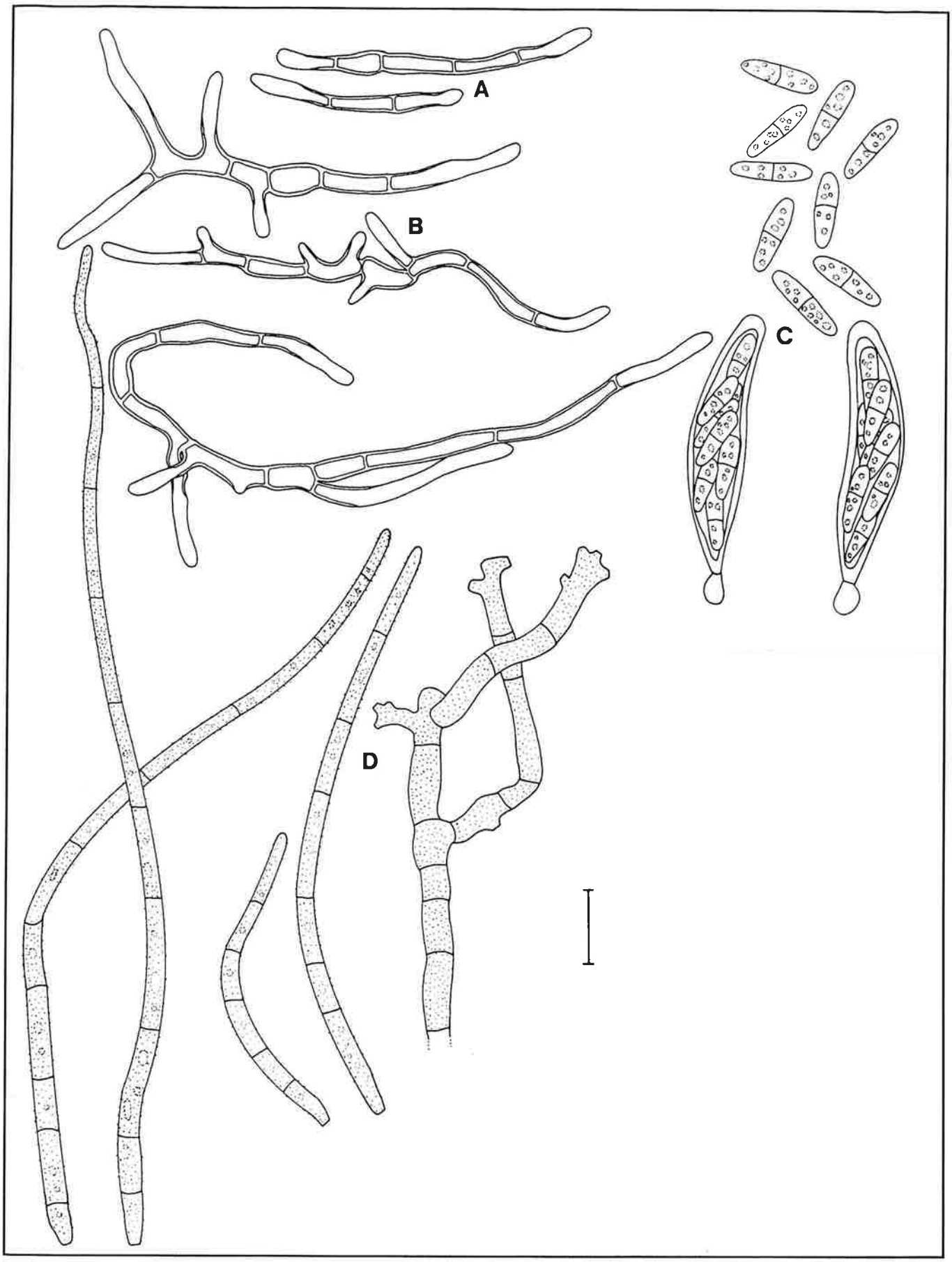

FIGURE 1. Mycosphaerella heimii *and its anamorph* Pseudocercospora heimii. *A, B. Germinating ascospores on MEA after 12 and 24 h, respectively. C. Asci and ascospores* in vivo. *D. Conidiophores and conidia* in vitro (bar = 10 μm).

more prominently toward the lower end (8-)10(-12) x (2-)2,5(-3) μm.

Specimens examined: MADAGASCAR. Moramanga, *Eucalyptus* sp., Apr. 1994, P.W. Crous, PREM 51749 (holotype); Tamatave, *Eucalyptus* sp., Apr. 1994, P.W. Crous & W.J. Swart, PREM 51750.

Pseudocercospora heimii Crous sp. nov. *Figure 1*

Mycelium glabrum, ramulosum, septatum, olivaceo-brunneum, 2-4 μm diam. *Conidiophora* fasciculata vel singularia in mycelio secondario pro parte ectionibus lateralibus, simplicia vel ramulosa, 0-3-septata, cylindracea, recta ad geniculato-sinuata, olivaceo-brunnea, 15-50 x 2-3 μm. *Conidiogenae cellulae* integratae, cylindraceae, rectae ad geniculato-sinuatae, olivaceae-brunneae, polyblasticae, 5-20 x 2,5-4 μm, ad apice truncato contractae; cicatricibus conidialibus non incrassatis. *Conidia* solitaria, obclavata, recta vel curvata, apice subacuto et base obconico truncata ad truncata, 55-300 x 2,5-3 μm, multiseptata.

Produced in culture from single ascospores of *M. heimii*. *Colonies* on 2 % malt extract agar (MEA) (Biolab), black (bottom), olivaceous-green to dark green (surface), with fluffy aerial mycelium, resembling that of *M. parkii*. *Mycelium* olivaceous brown, smooth, branched, septate, 2-4 μm diam. *Conidiophores* fasciculate, or occurring singly on secondary mycelium as lateral projections, simple or branched, 0-3 septate, cylindrical, straight to geniculate-sinuous, olivaceous brown, 15-50 x 2-3 μm. *Conidiogenous cells* integrated, cylindrical, straight to geniculate-sinuous, olivaceous brown, polyblastic, 5-20 x 2,5-4 μm, terminating in a clavate, truncate, apex with unthickened conidial scars. *Conidia* solitary, irregularly curved, guttulate, olivaceous brown, narrowly obclavate with a subacute apex and obconically truncate to truncate base and unthickened hilum, 55-300 x 2,5-3 μm, multiseptate.

Specimens examined: MADAGASCAR. Moramanga, *Eucalyptus* sp., Apr. 1994, P.W. Crous, derived from single-ascospores of *M. heimii*, PREM 51748 (holotype); Tamatave, *Eucalyptus* sp., Apr. 1994, P.W. Crous & W.J. Swart, derived from single-ascospores of *M. heimii*, lodged at PREM.

Mycosphaerella heimii was described from leaf spots of a *Eucalyptus* sp. in Mananjary on the east coast of Madagascar (Bouriquet, 1946). However, as no Latin diagnosis was provided this name was never validly published (art. 36.1 of the International Code of Botanical Nomenclature). Furthermore, no type specimen could be located by Park and Keane (1984), who stated that further material is needed to adequately redescribe this species. In the present study, two collections of *M. heimii* were obtained, namely from Moramanga, and from Tamatave on the east coast.

In the original description Bouriquet (1946) gave accurate illustrations of a pseudothecium, ascus and ascospores of *M. heimii*. Pseudothecia were described as being 74 μm high and 64 μm wide, asci 42 x 9 μm, and ascospores 10 x 2,5 μm. Of the small-spored *Mycosphaerella* species known from *Eucalyptus* (Crous *et al.* 1993b, 1993c; Carnegie and Keane, 1994), *M. heimii* is similar to *M. didymelloides* Petr., *M. martinae* Hansf., *M. parkii* Crous, M.J. Wingf., F.A. Ferreira & Alfenas, and *M. parva* R.F. Park & Keane. Based on its narrower ascospore dimensions, *M. heimii* can be distinguished from *M. didymelloides* (7,5-10 x 3-3,5 μm; Corlett, 1991). *M. martinae* Hansf. has slightly longer ascospores (11-13 x 2,5-3 μm; Hansford, 1956), and is associated with angular leaf spots, whereas *M. parva* has smaller ascospores (7-10 x 1-3 μm; Park and Keane, 1982), that darken with germination. Of all the species discussed above, *M. heimii* is most similar to *M. parkii*.

M. parkii has ascospores that are 8-14 x 2,5-3 μm in size, eguttulate, and not constricted at the median septum. It is furthermore associated with round to slightly irregular, light-brown leaf spots with raised margins (Crous *et al.*, 1993b). Ascospores have been observed to germinate with germ tubes parallel, as well as perpendicular to the long axis of the spore, producing green to grey, fast-growing colonies on MEA. In additional collections recently obtained from Brazil and Indonesia, hyphae were observed to be verrucose, and to produce a *Stenella* anamorph in culture (Crous and Alfenas, in preparation).

In the original description Bouriquet illustrated the ascospores of *M. heimii* to be unconstricted at the median septum, to have big guttules, and to have a prominent taper towards the one end. Other than the ascospores of *M. heimii* tending to be slightly narrower than those of *M. parkii*, the taper in the wider, apical cell is also distinct. The widest point in ascospores of *M. parkii* is slightly higher than in *M. heimii*, thus leading to a more abrupt taper of the apical cell. Furthermore, in contrast to *M. parkii*, both collections obtained in the present study have ascospores with big guttules as illustrated by Bouriquet (1946), suggesting that this is characteristic of *M. heimii*. Bouriquet (1946) described lesions as starting at the leaf margin, and slowly spreading over the whole leaf. In the two collections obtained in the present study, *M. heimii* was found on irregular, elongated lesions (Moramanga), and on a necrotic leaf tip (Tamatave). The latter symptoms are distinct from that of *M. parkii* (Crous *et al.*, 1993b).

Using the technique as explained in Crous *et al.* (1991), ascospores of *M. heimii* were observed to germinate with germ tubes parallel to the long axis of the spore after 12 h on MEA, but to develop multiple germ tubes after 24 h. Colonies were olivaceous-green to dark green (surface) on MEA, being similar to those of *M. parkii*, but with less aerial mycelium.

M. heimii can therefore be distinguished from *M. parkii* on leaf symptoms, ascospore germination after 24 h, its smooth as opposed to verrucose hyphae, and its ascospore morphology. Ascospores are slightly smaller, prominently guttulate, and have a more gradual apical taper in the upper cell.

The anamorph *Pseudocercospora heimii* was read-

ily formed in culture from single-ascospore colonies of *M. heimii.* Two cercosporoid fungi have been described from *Eucalyptus,* namely *P. eucalyptorum* and *Cercospora paraguayensis* Kobayashi. An additional two species have recently been collected from *Eucalyptus* leaves in Indonesia, namely a species of *Pseudocercospora,* and the *Stenella* anamorph of *M. parkii* (Crous and Alfenas, in prep.). *P. heimii* has smooth hyphae and conidia with unthickened hila, which distinguishes it from the *Stenella* anamorph of *M. parkii.* It can be distinguished in culture from *P. eucalyptorum* and the Indonesian *Pseudocercospora* species by having olivaceous-green to dark green colonies on MEA, in contrast to the grey colonies of the latter species. Furthermore, its conidia are distinct from *P. eucalyptorum, C. paraguayensis* and the Indonesian *Pseudocercospora* sp. by being darker olivaceous brown, narrower, and longer (Kobayashi, 1984; Crous *et al.,* 1989b; Crous and Alfenas, in prep.).

Other leaf fungi isolated from *Eucalyptus* leaf litter that also occur in South Africa include *Clypeophysalospora latitans* (Sacc.) H.J. Swart, *Mycotribulus mirabilis* Nag Raj & W.B. Kendr. and its anamorph *Xenogliocladiopsis eucalyptorum* Crous & W.B. Kendr., Glomerella cingulata (Stoneman) Spauld. & Schenk and its anamorph *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Cylindrocladium candelabrum* Viégas, *Codinea septata* B. Sutton & Hodges, and *Codinea eucalypti* B. Sutton & Hodges, which was till now unreported from South Africa (PREM 51439) (Crous *et al.*, 1990; Crous, 1993; Crous and Van der Linde, 1993; Crous and Kendrick, 1994; Crous and Wingfield, 1994). Voucher specimens of the respective fungi discussed in this paper have been lodged at the National Collection of Fungi, Pretoria (PREM), and reference strains of several are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch.

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