New species of *Mycosphaerella* occurring on *Eucalyptus* leaves in Indonesia and Africa

P.W. Crous and M.J. Wingfield

Abstract: Although Africa and Indonesia have not been particularly well surveyed for *Mycosphaerella* leaf spot fungi, several species are known to occur on *Eucalyptus* leaves in these areas. Three new species of *Mycosphaerella* with anamorph states are described from herbarium specimens and cultures in the present study. *Mycosphaerella suttoniae* and *Mycosphaerella heimioides* are described from *Eucalyptus* leaves from Indonesia. The former species is of particular interest, because its anamorph *Phaeophleospora epicoccoides* is the first species of *Phaeophleospora* linked to *Mycosphaerella*. *Mycosphaerella irregulariramosa* is described from *Eucalyptus saligna* growing in the Northern Province of South Africa. Both *M. irregulariramosa* and *M. heimioides* have *Pseudocercospora* anamorphs, and these are described as *Pseudocercospora irregulariramosa* and *Pseudocercospora heimioides*. Notes are also provided on the host range and geographic distribution of previously described species of *Mycosphaerella* on *Eucalyptus* leaves in other parts of Africa and in Indonesia.

Key words: *Eucalyptus*, *Kirramyces*, *Mycosphaerella*, *Phaeophleospora*, *Pseudocercospora*, systematics.

Résumé : Bien que l'Afrique et l'Indonésie aient été particulièrement bien explorées pour déceler les *Mycosphaerella*, champignons responsables de taches foliaires, on connait plusieurs espèces qui poussent sur les feuilles d'*Eucalyptus* dans ces régions. Dans cette étude, les auteurs décrivent trois nouvelles espèces de *Mycosphaerella* avec leurs stades anamorphes provenant de spécimens d'herbiers et de cultures. Ils décrivent le *Mycosphaerella suttoniae* et le *Mycosphaerella heimioides* provenant de feuilles d'*Eucalyptus* originaires d'Indonésie. La première espèce présente un intérêt particulier, parce que son anamorphe, le *Phaeophleospora epicoccoides* est la première espèce de *Phaeophleospora* liées au *Mycosphaerella*. Ils décrivent le *Mycosphaerella irregulariramosa* provenant de l'*E. saligne* poussant dans la province du Nord-Ouest de l'Afrique du Sud. Le *M. irregulariramosa* ainsi que le *M. heimioides* possèdent des anamorphes de type *Pseudocercospora* qui sont alors décrits comme *Pseudocercospora irregulariramosa* et *Pseudocercospora heimioides*. Les auteurs présentent également des notes sur l'amplitude des hôtes et la distribution géographique d'espèces de *Mycosphaerella* déjà décrites sur feuilles d'Eucalyptus, dans d'autres parties de l'Afrique et de l'Indonésie.

Mots clés : *Eucalyptus*, *Kirramyces*, *Mycosphaerella*, *Phaeophleospora*, *Pseudocercospora*, systématique.
[Traduit par la rédaction]

Introduction

Eucalyptus L'Hér. plantations cover more than eight million hectares internationally, and thus represent a biomass resource of international importance (Turnbull 1991). In their centres of origin (primarily Australia), there are more than 600 species that form a major component of a unique ecosystem. *Eucalyptus* spp. have also been planted as exotics in plantations in various parts of the tropics and southern hemisphere.

Diseases pose a great threat to *Eucalyptus* spp., both in natural ecosystems and in plantations. Pathogens introduced into native eucalypt forests have the potential to cause epidemic disease situations. Dieback of Jarrah (*Eucalyptus marginata* Donn ex Sm.) in Western Australia, caused by *Phytophthora cinnamomi* Rands, appears to represent such a situation (Zentmeyer 1980). Many pathogens have been recorded on exotic *Eucalyptus* spp., of which several have caused serious disease problems (Sankaran et al. 1995) enhanced by the clonal nature of the plantations.

Species of *Mycosphaerella* Johanson are well known as important pathogens of *Eucalyptus* spp. The so-called Mycosphaerella leaf blotch (MLB) disease is one of the important constraints to *Eucalyptus* propagation in various parts of the world (Lundquist and Purnell 1987; Carnegie et al. 1994). More than 22 species of *Mycosphaerella* have been associated with MLB (Carnegie and Keane 1994; Crous and Wingfield 1996), although very little is known about the relative importance of most of these. Detailed studies have also shown that more than one species of *Mycosphaerella* is commonly associated with diseases previously thought to be caused by *Mycosphaerella molleriana* (Thüm.) Lindau (Crous et al. 1991; Crous and Wingfield 1996).

In recent years, new characters have been defined that have made it possible to recognise distinct taxa among *Mycosphaerella* spp. associated with MLB. Because teleomorph structures on leaves are the dominant signs associated with *Mycosphaerella* infections, morphological characteristics associated with these structures have been the primary basis

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for the circumscription of these fungi. Many of these fungi also have unique anamorphs, although these can usually only be detected in axenic culture. Characteristics associated with ascospore germination and colony growth in culture are also useful and reliable taxonomic tools (Crous and Wingfield 1996).

Recent surveys of plantation-grown *Eucalyptus* spp. in Indonesia and South Africa have led to the description of several new species of *Mycosphaerella* (Crous and Alfenas 1995; Crous and Wingfield 1996). These studies have now been intensified to survey additional sites in the two countries. A number of new taxa were collected and are described here. Furthermore, data pertaining to the host range and geographic distribution of previously described species are also updated.

Materials and methods

Eucalyptus leaves with MLB symptoms were collected from plantations in Indonesia (Lake Toba area, northern Sumatra) and Africa (Kenya, South Africa, Tanzania, and Zambia). Lesions were excised from leaves, and single ascospore cultures were established on 2% malt extract agar (Biolab) (MEA) using the technique described by Crous et al. (1991). Germinating ascospores were examined after 24 h, their germination patterns were determined, and then they were transferred to MEA. Cultures were incubated for 2 weeks at 25°C in the dark and subcultured onto divided plates with one half containing carnation leaf agar (CLA) (Fisher et al. 1982; Crous et al. 1992) and the other MEA, incubated at 25°C under continuous near-ultraviolet light. Linear growth on agar for each culture was determined after 1 month (Crous and Wingfield 1996). Colony colors (top and bottom) were scored using the color charts of Rayner (1970). Wherever possible, 30 measurements were made of structures mounted in lactophenol, and the extremes are given in parentheses. Herbarium specimens were lodged at the National Collection of Fungi, Pretoria (PREM).

Results

Material of MLB disease collected from *Eucalyptus* spp. from Africa and Indonesia included three undescribed *Mycosphaerella* spp., which are dealt with in the taxonomy section below. In addition, materials collected from Kenya, Tanzania, and Zambia were colonized by several species known to occur in South Africa. In Kenya and Zambia, leaves of *Eucalyptus globulus* Labill. were colonized by *Mycosphaerella juvenis* Crous & M.J. Wingf. (PREM 54972, PREM 54973), with the same species also occurring on *Eucalyptus maidenii* F. Muell. in Tanzania (PREM 54971). Furthermore, Tanzanian collections of *E. maidenii* leaves were also frequently colonized by *Mycosphaerella marksii* Carnegie & Keane (PREM 54971). Other than *M. juvenis*, leaves of *E. globulus* from Zambia were also colonized by *Mycosphaerella africana* Crous & M.J. Wingf. and *Mycosphaerella lateralis* Crous & M.J. Wingf. (PREM 54973). The common species associated with the most serious leaf spotting on *E. globulus* in these countries was *M. juvenis*. This species is also the most common and serious pathogen of *Eucalyptus nitens* (Deane et Maid.) Maid. in South Africa. An examination of older herbarium specimens lodged at PREM led us to conclude that this species made *E. globulus* unsuitable for afforestation in South Africa and made it possible to plant only certain provenances of *E. nitens* (Lundquist and Purnell 1987).

In their study of *Mycosphaerella* spp. occurring on eucalypts in Indonesia, Crous and Alfenas (1995) described *Mycosphaerella gracilis* Crous & Alfenas (anam. *Pseudocercospora gracilis* Crous & Alfenas) from leaf spots on *Eucalyptus urophylla* S.T. Blake. They also recorded *Mycosphaerella parkii* Crous et al. from leaf spots on *Eucalyptus grandis* Hill ex Maid. The latter species is well known from *Eucalyptus saligna* Sm. and *E. globulus* in Brazil. On fresh material obtained in the present study, several collections of MLB from *E. urophylla* were associated with *M. gracilis* (PREM 54977), while collections from *E. grandis* were commonly associated with *M. parkii* (PREM 54968). Ascospore morphology and germination of *M. gracilis* were the same as those observed for the type collection. Agar colonies were grey with a dark grey to black submerged mycelium, smooth and even edged, with fluffy grey-white aerial mycelium, and readily produced the anamorph *P. gracilis* (Crous et al. 1995*a*). Colonies of *M. parkii* were generally fast growing and olive-green with abundant aerial mycelium (Crous et al. 1995*b*), consistent with the type collection from Brazil. Most single-ascospore colonies produced the anamorph *Stenella parkii* Crous & Alfenas in culture. Although conidia were within the range described for the type collection, several of these also occurred in branched chains, a feature not observed in the type material. Furthermore, although pseudothecia of *M. parkii* are know to be amphigenous on leaf spots, several collections had pseudothecia that were either more prominently epiphyllous or hypophyllous.

Another well-known species from *Eucalyptus dunnii* Maid. and *E. grandis* in Brazil is *Mycosphaerella suberosa* Crous et al., which was also collected from a *Eucalyptus* sp. (PREM 54970) in Indonesia during this study. This is the first record of *M. suberosa* from outside South America.

Mycosphaerella marksii was recently described from Australia, where it occurs on several *Eucalyptus* spp. (Carnegie and Keane 1994). This species was subsequently recorded from leaves of *E. grandis* and *E. nitens* in South Africa (Crous and Wingfield 1996). It is characterized by epiphyllous pseudothecia occurring on light brown, irregular to subcircular lesions with red-purple margins. Ascospores are in the range $11-18 \times 2.5-3.5$ μm, have asymmetrical apical cells, and germinate with germ tubes parallel to the long axis of the spore. In the present study, isolates corresponding to *M. marksii* were collected from *E. globulus* in Indonesia (PREM 54976).

Mycosphaerella heimii Crous has previously been known only from *Eucalyptus* in Madagascar. In the present study, *M. heimii* was commonly collected on *E. urophylla* leaves in Indonesia. Leaf spots were either irregular or subcircular, 2–20 mm in diameter, light brown, becoming dark brown towards the raised border, with red-brown to purple margins. Ascospores were ellipsoidal, not constricted at their septa, guttulate, and widest in the middle of the apical cell, $(9-)10-11(-13) \times 2-2.5(-3)$ μm. Single-ascospore colonies readily formed *Pseudocercospora heimii* Crous in culture. Conidia were within the range observed for the type

collection, being 55–200 × 2–3 μm, multiseptate, variously curved, with sub-obtuse apices and narrow obconically truncate bases with hila 1–1.5 μm in diameter.

Taxonomy

Mycosphaerella suttoniae Crous et M.J. Wingf. sp.nov. Figs. 1–5

ANAMORPH: ***Phaeophleospora epicoccoides*** (Cooke & Massee) Crous, F.A. Ferreira & B. Sutton, S. Afr. J. Bot. 63. In press. 1997 Fig. 4

syn. *Cercospora epicoccoides* Cooke & Massee apud Cooke, Grevillea **19**: 91. 1891. Additional synonyms listed in Walker et al. (1992).

SYNANAMORPH: ***Cercostigmina*** sp. Fig. 5

ETYMOLOGY: named for Dr. B.C. Sutton, who contributed greatly to our knowledge of the coelomycetes.

Laesiones amphigenae, irregulares ad suborbiculares, 5–25 mm diam., pallide brunneae. Pseudothecia hypophylla, nigra, subepidermalia, globosa, 70–90 μm lata, 60–90 μm alta. Asci fasciculati, bitunicati, obovoidei ad late ellipsoidei, recti vel parum incurvati, 8-sporis, 35–45 × 10–12 μm. Ascosporae multiseriatae, imbricatae, hyalinae, guttulatae, parietibus tenuibus, rectae ad parum curvatae, obovoideae, base obtusa et apice obtuso, latissimae prope apicem, mediano 1-septatae, ad septum non constrictae, (10–)11–12(–13) × (2.5–)3–3.5 μm.

Leaf spots amphigenous, subcircular to irregular, 5–25 mm in diameter, light brown, surrounded by a diffuse, red-purple margin on the upper surface, which is concolorous with the leaf surface on the lower surface. Pseudothecia hypophyllous, single, up to 20 per colonized mm^2, black, subepidermal, globose, 70–90 μm wide, 60–90 μm high; ostioles apical, 5–10 μm diameter, becoming papillate; walls consisting of 2–3 layers of medium brown textura angularis, sub-hymenium layer at base consisting of 1–2 layers of hyaline cells. Asci fasciculate, bitunicate, obovoid to broadly ellipsoidal, straight or curved, 8-spored, 35–45 × 10–12 μm. Ascospores multiseriate, overlapping, hyaline, guttulate, thin walled, straight to curved, obovoid with obtuse ends, widest near apex, medianly 1-septate, not constricted at septum, tapering prominently towards lower end, (10–)11–12(–13) × (2.5–)3–3.5 μm. Spermogonia intermixed with pseudothecia, up to 100 μm wide and 80 μm high. Spermatia rod shaped, hyaline, 5–7 × 1 μm, straight or slightly curved. Mycelium mostly internal on leaves, consisting of brown, verruculose, septate, branched hyphae, 2–4 μm diam. *Phaeophleospora* conidiomata pycnidial, amphigenous, substomatal, scattered, black, globose to subglobose, unilocular, up to 100 μm high and 130 μm in diameter; wall of textura angularis in surface view, consisting of 2–3 layers of brown cells, becoming light brown towards the inner layer. Ostiole single, central. Conidiophores absent. Conidiogenous cells discrete, pale brown, ampulliform or doliiform to subcylindrical, pale brown, verruculose, with 1–15 percurrent proliferations, 4–15 × 4.5–7 μm. Conidia holoblastic, solitary, exuding from ostiole in long cirri, subcylindrical to narrowly obclavate, apex subobtuse, tapering slightly from the basal septum to a narrowly truncate base, straight or slightly flexuous, thick walled, medium brown, verruculose, guttulate, 3–5-euseptate, (30–)45–55(–65) × (3–)3.5–4(–5) μm in vitro, (35–)45–55(–60) × (3–)3.5–4(–5) μm in vivo; lateral branches frequently present in vitro; hila thickened and sometimes refractive in vitro with a marginal frill. *Cercostigmina* synanamorph: mycelium in some cultures giving rise to dark brown or back stromata on MEA, up to 150 μm wide and 100 μm high, composed of dark pseudoparenchymatous cells. Conidiomata sporodochial, brown, up to 250 μm wide and 170 μm high (including the conidiophores). Conidiophores irregular, subcylindrical, frequently branched below, straight or geniculate–sinuous, 1–6-septate, medium brown, verruculose, arising from the upper cells of the stroma, 20–60 × 5–7 μm. Conidiogenous cells terminal, subcylindrical or slightly doliform, medium to light brown, verruculose, with up to 5 enteroblastic percurrent proliferations, 7–15 × 5–6 μm. Conidia holoblastic, apical, solitary, medium brown, (15–)30–35(–55) × 4–5 μm, verruculose, 0–5 transversely euseptate, guttulate, straight to variously curved, obclavate to subcylindrical, apex rounded to obtuse, base truncate to obconically truncate with a marginal frill; primary conidia frequently forming lateral branches or secondary conidia via microcyclic conidiation.

ASCOSPORE GERMINATION ON MEA: germinating from both ends, becoming up to 6 μm wide, spore and germ tubes becoming uniformly olivaceous upon germination, with germ tubes parallel or almost perpendicular to the long axis of the spore, becoming coiled and distorted.

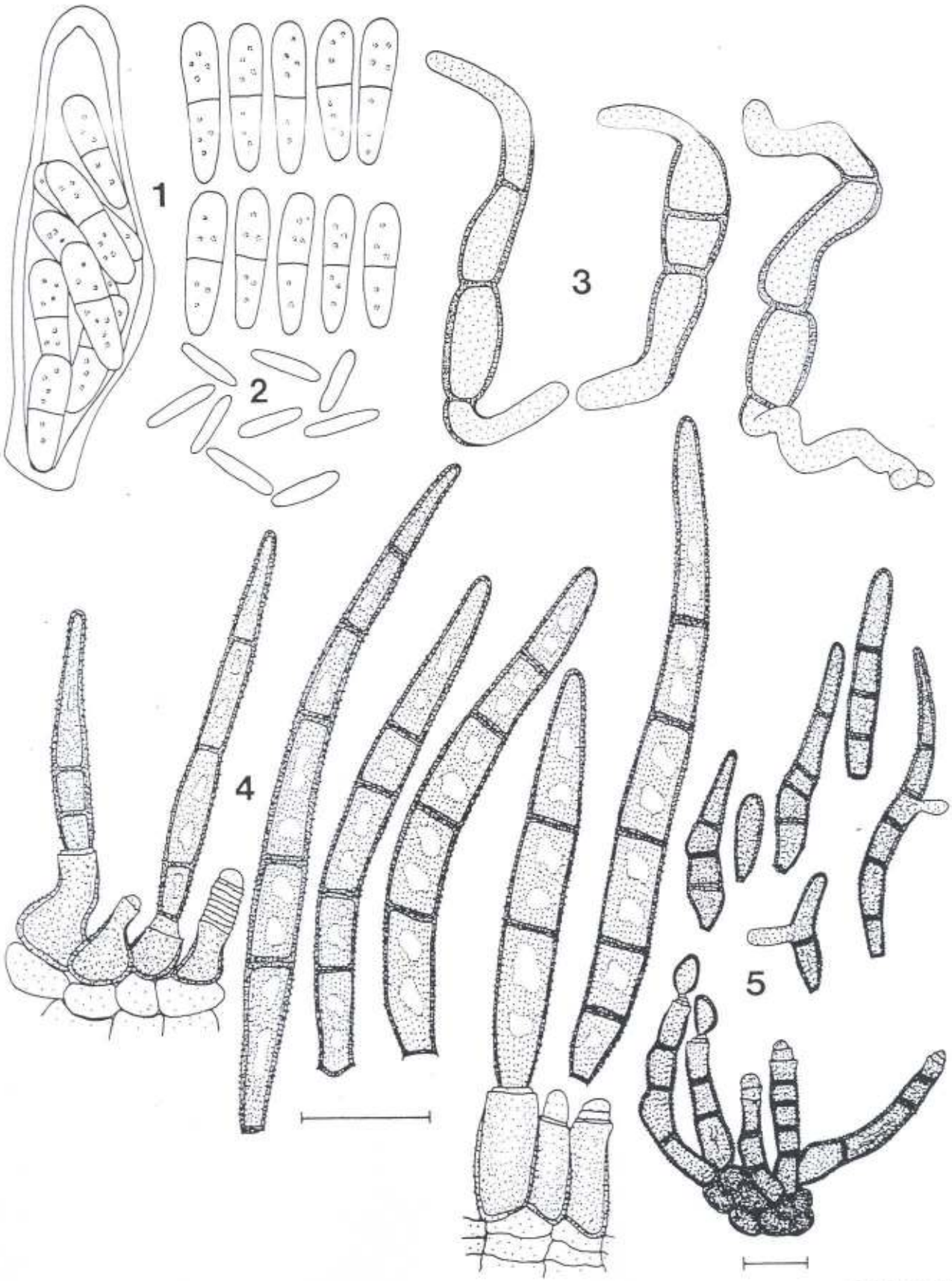
CULTURES: colonies 10–12 mm in diameter on MEA after 1 month at 25°C in the dark, margins smooth, regular, aerial mycelium sparse, white-grey when present, outer colony olivaceous grey, 23""'i (top), with numerous spore cirri in central part; greenish black 33""'k (bottom).

CARDINAL TEMPERATURES FOR GROWTH: above 5°C min., 20–25°C optimum (opt.), below 35°C max.

HOSTS: *Eucalyptus amplifolia* Naudin, *Eucalyptus camaldulensis* Dehnh., *Eucalyptus citriodora* Hook., *Eucalyptus cladocalyx* F. Muell., *Eucalyptus crebra* F. Muell., *Eucalyptus dealbata* A. Cunn. ex Schau., *Eucalyptus delegatensis* R.T. Bak., *Eucalyptus drepanophylla* F. Muell. ex Benth., *E. dunnii*, *Eucalyptus exserta* F. Muell., *E. globulus*, *E. globulus* ssp. *bicostata* (Maid. et al.) Kirkp., *E. globulus* ssp. *maidenii* (F. Muell.) Kirkp., *E. grandis*, *Eucalyptus longifolia* Link et Otto, *Eucalyptus macarthurii* Deane et Maid., *Eucalyptus maculata* Hook., *Eucalyptus major* (Maid.) Blakely, *Eucalyptus microcorys* F. Muell., *E. nitens*, *Eucalyptus nova-anglica* Deane et Maid., *Eucalyptus pellita* F. Muell., *Eucalyptus platypus* Hook., *Eucalyptus punctata* DC., *Eucalyptus quadrangulata* Deane et Maid., *Eucalyptus radiata* ssp. *robertsonii* (Blakely) L. Johnson et D. Blaxell, *Eucalyptus resinifera* Sm., *Eucalyptus robusta* Sm., *Eucalyptus rostrata* Schlecht., *Eucalyptus saligna*, *Eucalyptus sideroxylon* A. Cunn. ex Woolls, *Eucalyptus tereticornis* Sm., *E. urophylla*, *Eucalyptus viminalis* Labill., and *Eucalyptus* sp. (Sankaran et al. 1995).

DISTRIBUTION: Argentina, Australia, Bhutan, Brazil, Ethiopia, Hong Kong, India, Indonesia, Madagascar, Malawi, Myanmar, New Zealand, Philippines, South Africa, Taiwan, Tanzania, United States (Hawaii), and Zambia (Crous and Swart 1995; Sankaran et al. 1995).

Figs. 1–5. *Mycosphaerella suttoniae* and its anamorph *Phaeophleospora epicoccoides* (PREM 54963). Fig. 1. Asci and ascospores. Fig. 2. Rod-shaped spermatia. Fig. 3. Germinating ascospores on MEA. Fig. 4. Conidiogenous cells and conidia of *Phaeophleospora epicoccoides* in vitro (left) and in vivo (right). Fig. 5. *Cercostigmina* synanamorph. Scale bars = 10 μm.



HOLOTYPE: INDONESIA: northern Sumatra, Lake Toba area, leaves of a *Eucalyptus* sp., Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54963, cultures ex type STE-U 1345–1347).

NOTES: based on ascospore dimensions *M. suttoniae* (10–)11–12(–13) × (2.5–)3–3.5 μm is most similar to *M. parkii* (8–)9–13(–15) × (2–)2.5–3(–3.5) μm, *M. juvenis* (10–)11–13(–15) × 3–3.5(–4) μm, and *Mycosphaerella crystallina* Crous & M.J. Wingf. (11–)12–14(–15) × 3–3.5(–4) μm. However, the latter three species have different modes of ascospore germination, and their anamorphs are accommodated in *Stenella* Syd., *Uwebraunia* Crous & M.J. Wingf., and *Pseudocercospora* Speg., respectively. Several species of *Phaeophleospora* Rangel (= *Kirramyces* J. Walker et al.) are known from eucalypts (Walker et al. 1992; Crous et al. 1997). Of these, *Phaeophleospora epicoccoides* has the widest distribution, and occurs in most areas where eucalypts are grown (Sankaran et al. 1995). Conidia of the type specimen of *Phaephleospora epicoccoides* lodged at (K) are verruculose, medium brown, 1–4-euseptate, 32–50.5 × 5–6 μm, and have verruculose, pale brown conidiogenous cells, 6.5–11 × 3–5 μm, with 1–2 distinctly roughened annellations (Walker et al. 1992). The present collection is somewhat different by having larger, light brown, subcircular leaf spots, and slightly narrower, obclavate to subcylindrical conidia. However, given the variation we have observed in different collections of *Phaeophleospora epicoccoides*, it is best to accept this species as morphologically variable until more information can be obtained using more objective molecular techniques. In culture, colonies also produced a *Cercostigmina* synanamorph. No *Cercostigmina* state has yet been reported among the *Mycosphaerella* anamorphs from *Eucalyptus*.

Braun (1993) transferred several *Stigmina*-like anamorphs of *Mycosphaerella* (*Stigmina concentrica* (Cooke & Ellis) Deighton, *Stigmina dictamni* (Fuckel) U. Braun) to a new genus, *Cercostigmina* U. Braun. This was based on the fact that they were not congeneric with *S. platani*, but more *Pseudocercospora*-like with narrow, obclavate, thin-walled, smooth, transversely septate conidia, and conidiogenous cells with smooth percurrent proliferations. Sutton and Pascoe (1989*b*) suggested that *Stigmina* Sacc. should be restricted to species that are foliicolous, always associated with stomata, and with superficial and immersed mycelium. Species of *Stigmina* possess conidiogenous cells that are rough, irregular, and flaring, with percurrent proliferations, and conidia that are usually transversely and occasionally longitudinally distoseptate, brown, ellipsoidal to cylindrical. However, the type species of *Stigmina*, *Stigmina platani* (Fuckel) Sacc., is the subject of considerable controversy. Although Barr (1972), Sivanesan (1984), and Farr et al. (1989) accepted this species as the anamorph of *Mycosphaerella stigmina-platani* F.A. Wolf, this connection was refuted by von Arx (1983), who considered *Stigmina platani* to be congeneric with *Sporocadus lichenicola* Corda, the anamorph of *Discostroma corticola* (Fuckel) Brockmann. Shoemaker and Müller (1964) and Sutton (1980) listed *Sporocadus lichenicola* as a synonym of *Seimatosporium lichenicola* (Corda) Shoemaker & E. Müll. Although *Stigmina platani* appears to be distinct from *Seimatosporium lichenicola* (Shoemaker and Müller 1964; Sivanesan 1984), uncertainty remains regarding its connection to *Mycosphaerella* (Smith and Smith 1941).

Crous and Braun (1996) described many intermediate morphological forms in the *Cercostigmina*–*Stigmina* complex. This suggests that the present generic circumscription of *Cercostigmina* is tentative. Sutton and Crous (1997) provisionally accepted *Cercostigmina* for species with brown sporodochial conidiomata, and integrated conidiogenous cells that proliferate percurrently rather than sympodially and have euseptate, verrucose conidia. Based on these features, the synanamorph of *M. suttoniae* is accommodated in *Cercostigmina*.

In a recent comparison of the presently described species of *Phaeophleospora* (as *Kirramyces*), Palm (1996) speculated that, although no teleomorph had yet been linked to *Phaeophleospora* spp., it would probably be a bitunicate ascomycete in the Dothideales. The description of *M. suttoniae* as teleomorph of *Phaeophleospora epicoccoides* confirms this hypothesis. Furthermore, the *Stagonospora* (Sacc.) Sacc. anamorph of *Mycosphaerella delegatensis* R.F. Park & Keane could possibly be congeneric to the pale-spored species of *Phaeophleospora*, as speculated by Walker et al. (1992).

Mycosphaerella irregulariramosa Crous et M.J. Wingf. sp.nov. Figs. 6–8

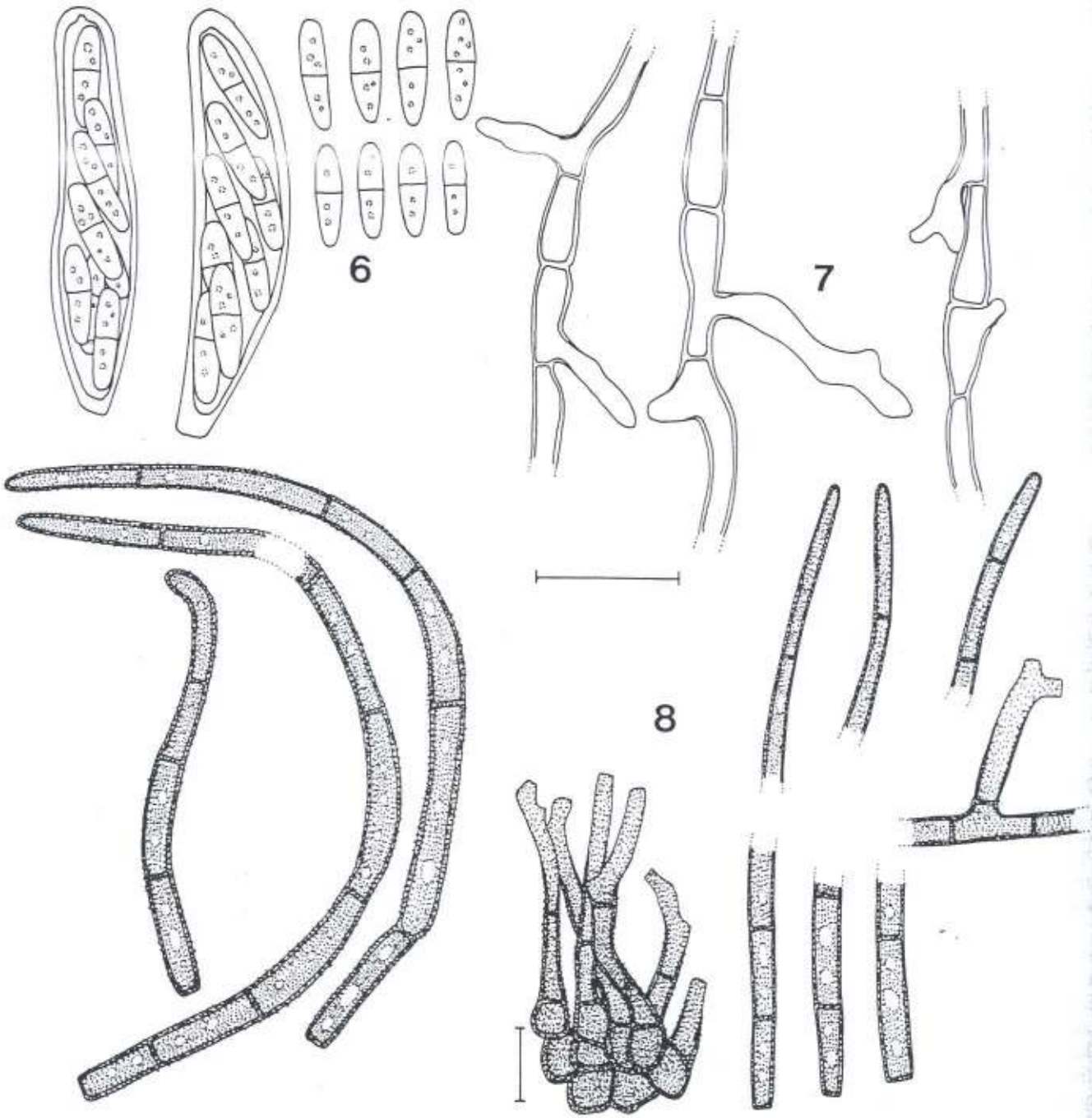
ANAMORPH: ***Pseudocercospora irregulariramosa*** Crous et M.J. Wingf. sp.nov. Fig. 8.

ETYMOLOGY: named for the irregular swellings on lateral branches that develop after ascospore germination.

Laesiones amphigenae, suborbiculares, 3–15 mm diam., griseae ad pallide brunneae. Pseudothecia amphigena, nigra, subepidermalia, subglobosa ad globosa, 50–90 μm lata, 60–90 μm alta. Asci fasciculati, bitunicati, obovoidei ad anguste ellipsoidei, recti vel parum incurvati, 8-sporis, 25–35 × 7–8 μm. Ascosporae multiseriatae, imbricatae, hyalinae, guttulatae, parietibus tenuibus, rectae ad parum curvatae, fusoideo–ellipsoideae, base obtusa et apice obtuso, latissimae supra mediam cellae apicalis, mediano 1-septatae, ad septum non constrictae, (7–)8–10 × (1.5–)2–2.5 μm. Spermogonia inter fasciculos anamorphae mixta. Spermatia hyalina, bacilliformia, 2–3 × 1 μm. Mycelium internum et externum, pallide brunneum, hyphis 2–3.5 μm diam. Caespituli brunnei, amphigeni. Conidiophora fasciculata, verruculosa, pallide brunnea, 1–4-septata, subcylindracea, recta ad geniculato–sinuosa, simplicia vel ad basim ramosa, 15–45 × 3.5–5.5 μm. Conidiogenae cellulae terminales, pallide brunneae, verruculosae, subcylindraceae, ad apicem truncatae, sympodialiter proliferantes, 7–17 × 2.5–3.5 μm. Conidia solitaria, pallide ad medio brunnea, verruculosa, parietibus crassis, guttulata, recta vel curvata, subcylindracea, base truncata, apice subobtusa, multi-septata, (35–)45–75(–85) × 2.5–3 μm in vivo.

Leaf spots amphigenous, subcircular, 3–15 mm in diameter, grey to light brown, surrounded by a slightly raised border and dark brown margin. Pseudothecia amphigenous, single, 10–15 per colonized mm^2, black, subepidermal, becoming erumpent, globose to subglobose, 50–90 μm wide, 60–90 μm high; ostioles apical, 5–10 μm in diameter, walls consisting of 2–3 layers of medium brown *textura angularis*, sub-hymenium layer at base consisting of 1–2 layers of hyaline cells. Asci fasciculate, bitunicate, obovoid to narrowly ellipsoidal, straight or curved, 8-spored, 25–35 × 7–8 μm. Ascospores multiseriate, overlapping,

Figs. 6–8. *Mycosphaerella irregulariramosa* and its anamorph *Pseudocercospora irregulariramosa* (PREM 54964, 54965). Fig. 6. Asci and ascospores. Fig. 7. Germinating ascospores on MEA. Fig. 8. Conidia and conidiophores in vivo (left) and in vitro (right). Scale bars = 10 μm.



hyaline, guttulate, thin walled, straight, fusoid–ellipsoidal with obtuse ends, widest in middle of apical cells, medianly 1-septate, not constricted at septum, tapering toward both apices, but with slightly more prominent taper towards lower end (7–)8–10 × (1.5–)2–2.5 μm. Spermogonia intermixed between caespituli and pseudothecia. Spermatia hyaline, rod shaped, 2–3 × 1 μm. Mycelium internal and external, consisting of septate, branched, smooth, olivaceous to light brown hyphae, 2–3.5 μm wide. Caespituli brown, amphigenous. Conidiophores fasciculate, arising from the upper cells of a well-developed brown stroma, up to 100 μm wide and 60 μm high; conidiophores subcylindrical, verruculose, light brown, 1–4-septate, straight to geniculate–sinuous, rarely branched below, 15–45 × 3.5–5.5 μm. Conidiogenous cells terminal, monoblastic to polyblastic, proliferating sympodially, light brown, verruculose, subcylindrical, terminating in truncate loci, 7–17 × 2.5–3.5 μm. Conidia solitary, light to medium brown, verruculose, guttulate, thick walled, subcylindrical with a subobtuse apex and truncate base, multiseptate, irregular in width along

its length on host material (not in culture), irregularly curved, (35–)45–75(–85) × 2.5–3 μm in vivo, 70–200 × 1.5–2 μm in vitro.

ASCOSPORE GERMINATION ON MEA: germinating from both ends, not darkening upon germination, becoming constricted at the septum, 3–3.5 μm wide, distorting slightly, with germ tubes growing parallel to the long axis of the spore, and lateral branches appearing after 24 h (frequently from original ascospore); lateral branches are irregular in width, geniculate and branched.

CULTURES: colonies 43–47 mm in diameter on MEA after 1 month at 25°C in the dark, margins irregular, feathery, surface not sectored, aerial mycelium sparse, colonies iron grey, 25""k (surface), greenish black 33""k (bottom).

CARDINAL TEMPERATURES FOR GROWTH: above 5°C min., 20–25°C opt., below 35°C max.

HOST: *Eucalyptus saligna*.

DISTRIBUTION: South Africa.

HOLOTYPE: SOUTH AFRICA: Northern Province, Tzaneen, leaves of *E. saligna*, Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54964 of *M. irregulariramosa*; PREM 54965 of *P. irregulariramosa*; cultures ex type STE-U 1360–1362).

NOTES: ascospores of *M. irregulariramosa* (7–)8–10 × (1.5–)2–2.5 μm are most similar to *M. heimii* (8–)9–11(–12) × 2–2.5(–3) μm (anam. *Pseudocercospora heimii*) and *M. ellipsoidea* Crous & M.J. Wingf. (8–)10–11 × (2–)2.5–3 μm (anam. *Uwebraunia ellipsoidea* Crous & M.J. Wingf.). *Mycosphaerella irregulariramosa* can be distinguished from these species by its smaller ascospores, subcircular, grey leaf spots, and irregular swellings of its lateral branches at ascospore germination.

Several species of *Pseudocercospora* are presently known from *Eucalyptus* (Crous and Braun 1996; Crous and Wingfield 1996). Of these, only one species has truncate conidial bases, namely *Pseudocercospora eucalyptorum* Crous et al. *Pseudocercospora irregulariramosa* can be distinguished from the latter species based on its larger conidia (35–)45–75(–85) × 2.5–3 μm, and the prominent apical taper, which is absent in the more cylindrical conidia of *Pseudocerospora eucalyptorum* (25–65 × 2.5–4 μm).

Mycosphaerella heimioides Crous et M.J. Wingf. sp.nov. Figs. 9–11

ANAMORPH: ***Pseudocercospora heimioides*** Crous et M.J. Wingf. sp.nov. Fig. 11

ETYMOLOGY: morphologically similar to *M. heimii* Crous.

Laesiones absentes. Pseudothecia amphigena, nigra, subepidermalia, globosa, 60–80 μm diam. Asci fasciculati, bitunicati, obovoidei ad late ellipsoidei, recti vel parum incurvati, 8-sporis, 20–45 × 7–9 μm. Ascosporae multiseriatae, imbricatae, hyalinae, guttulatae, parietibus tenuibus, rectae ad parum curvatae, fusoideo–ellipsoideae, base obtusa et apice obtuso, latissimae supra mediam cellae apicalis, mediano 1-septatae, ad septum non constrictae, (7.5–)8–10(–11) × (2–)2.5–3 μm. Spermogonia ignota. Mycelium internum et externum, pallide brunneum, hyphis 1.5–4 μm diam. Conidiogenae cellulae inconspicuae, in mycelio integratae, monoblasticae ad polyblasticae, sympodiales, laeves, pallide brunneae, 3–10 × 2–3.5 μm. Conidia solitaria, olivacea ad pallide brunnea, subtiliter verruculosa, guttulata, anguste obclavata, apicibus subobtusis et basibus longis obconicis truncatis, 4-multiseptata, latissima parte mediano cellularum basale, (25–)40–90(–150) × (2–)2.5–3(–3.5) μm *in vitro*.

Leaf spots absent. Pseudothecia amphigenous, single, black, subepidermal, globose, 60–80 μm wide and high; ostioles apical, 5–15 μm in diameter, walls consisting of 2–3 layers of medium brown *textura angularis*, subhymenium layer at base consisting of 1–2 layers of hyaline cells. Asci fasciculate, bitunicate, obovoid to broadly ellipsoidal, straight or curved, 8-spored, 20–45 × 7–9 μm. Ascospores multiseriate, overlapping, hyaline, guttulate, thin walled, straight to slightly curved, fusoid–ellipsoidal with obtuse ends, widest in middle of apical cells, medianly 1-septate, not constricted at septum, tapering toward both apices, but with slightly more prominent taper towards lower end, (7.5–)8–10(–11) × (2–)2.5–3 μm. Spermogonia unknown. Mycelium internal and external, consisting of septate, branched, smooth olivaceous hyphae, 1.5–4 μm wide. Conidiophores reduced to conidiogenous cells. Conidiogenous cells inconspicuous, integrated on mycelium, 3–10 × 2–3.5 μm, monoblastic to polyblastic, proliferating sympodially, light brown, smooth, subcylindrical, terminating in truncate loci. Conidia solitary, terminal, olivaceous to light brown, finely verruculose, guttulate, narrowly obclavate with a rounded to subobtuse apex and long obconically truncate to truncate base, 4-multiseptate, widest near the first basal septum or in the middle of the basal cell, (25–)40–90(–150) × (2–)2.5–3(–3.5) μm in vitro.

ASCOSPORE GERMINATION ON MEA: germinating from both ends, not darkening upon germination, becoming slightly constricted at the septum, and up to 3.5 μm wide, with germ tubes growing perpendicular to the long axis of the spore.

CULTURES: colonies 39–41 mm in diameter on MEA after 1 month at 25°C in the dark, growing in concentric circles at different elevations; inner four circles olivaceous grey, 23""i (surface), outer two circles submerged in agar, dark mouse grey, 15""k, with red crystals visible in agar; bottom dark mouse grey, margin even, smooth, clearly delimited. Aerial mycelium moderate, olivaceous grey, present on zones between different circles; colonies flat, spreading.

CARDINAL TEMPERATURES FOR GROWTH: above 5°C min., 20–25°C opt., below 35°C max.

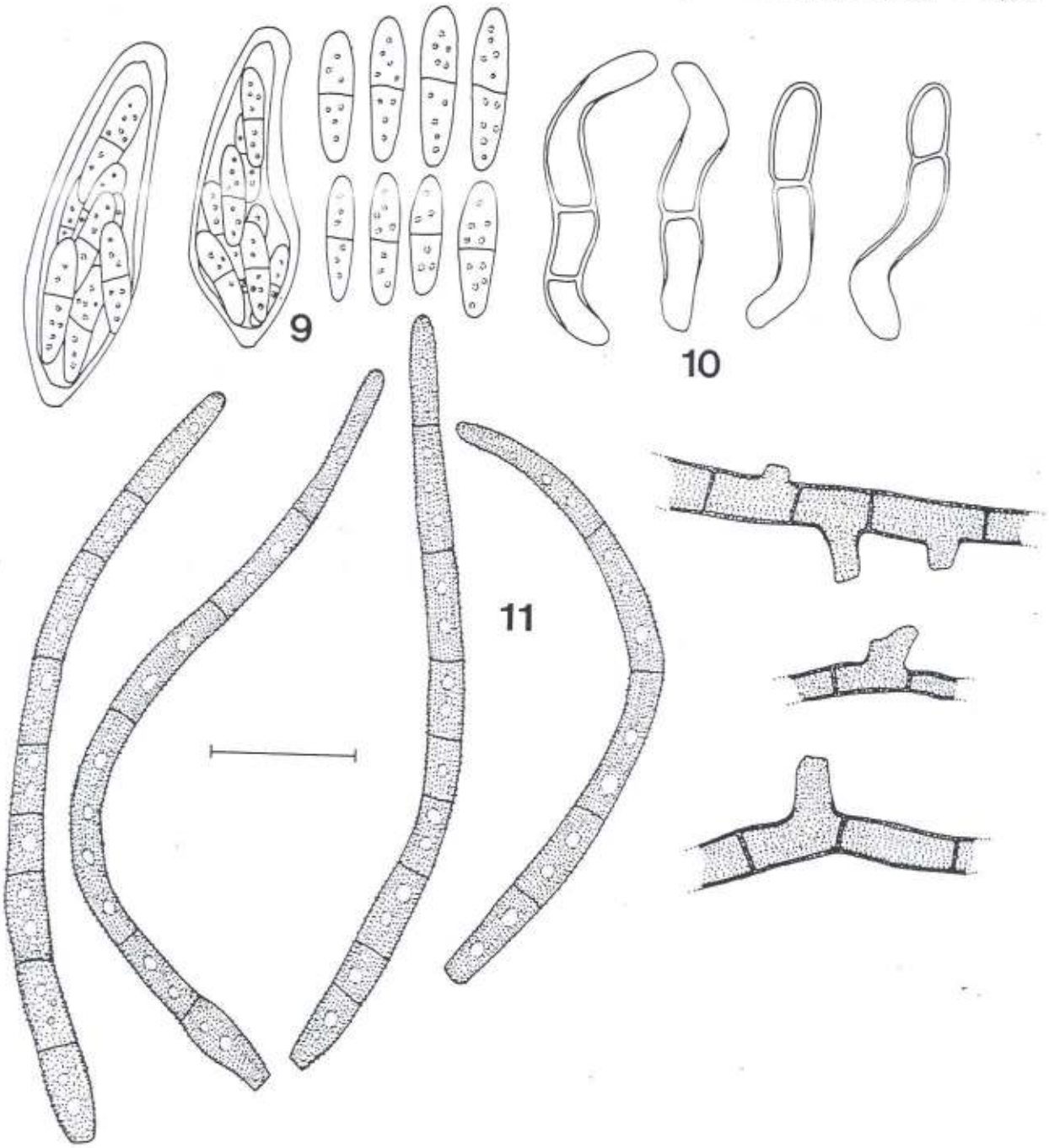
HOST: *Eucalyptus* sp.

DISTRIBUTION: Indonesia.

HOLOTYPE: INDONESIA: northern Sumatra, Lake Toba area, leaves of a *Eucalyptus* sp., Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54966 of *M. heimioides*; PREM 54967 of *Pseudocercospora heimioides*; cultures ex type STE-U 1311–1312).

NOTES: Ascospore shape and dimensions of *M. heimioides* (7.5–)8–10(–11) × (2–)2.5–3 μm are very similar to those of *M. heimii* ((8–)9–11(–12) × 2–2.5(–3) μm (PREM 51750, type), and (9–)10–11(–13) × 2–2.5(–3) μm (PREM 54969, 54975) for Indonesian collections). However, there are some clear differences between these species. The type and Indonesian collections of *M. heimii* have ascospores that germinate with germ tubes parallel to the long axis of the spore, whereas ascospores of *M. heimioides* produce germ tubes that arise perpendicular to the long axis. Furthermore, colonies of *M. heimioides* also

Figs. 9–11. *Mycosphaerella heimioides* and its anamorph *Pseudocercospora heimioides* (PREM 54966, 54967). Fig. 9. Asci and ascospores. Fig. 10. Germinating ascospores on MEA. Fig. 11. Conidia and conidiogenous cells in vitro. Scale bar = 10 μm.



produce red crystals in MEA, have different cultural features to *M. heimii*, and have a *Pseudocercospora* anamorph with shorter conidia than those of *Pseudocercospora heimii*.

ADDITIONAL SPECIMENS EXAMINED: INDONESIA: *Mycosphaerella suberosa* on leaves of an *Eucalyptus* sp., Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54970); *M. parkii* on leaves of *E. grandis*, Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54968, cultures STE-U 1299–1301); *M. gracilis* on leaves of *E. urophylla*, Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54977, cultures STE-U 1313–1315); *M. marksii* (cultures STE-U 1196–1198) and *M. gracilis* on leaves of *E. globulus*, Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54976); *M. heimii* on leaves of *E. urophylla*, Mar. 1996, leg. M.J. Wingfield, det. P.W. Crous (PREM 54975, 54969, cultures STE-U 1302–1304, 1319–1320). PORTUGAL: *Mycosphaerella africana* on leaves of *E. globulus*, June 1995, leg. S. McRae, det. P.W. Crous (PREM 54974, cultures STE-U 1196–1198). ZAMBIA: *Mycosphaerella africana* (cultures STE-U 1229–1231), *M. lateralis* (cultures STE-U 1232–1234), and *M. juvenis* on leaves of *E. globulus*, Aug. 1995, leg. T. Coutinho, det. P.W. Crous (PREM

54973). KENYA: *Mycosphaerella juvenis* on leaves of *E. globulus*, May 1995, leg. T. Coutinho, det. P.W. Crous (PREM 54972, cultures STE-U 1078–1080). TANZANIA: *Mycosphaerella marksii* (cultures STE-U 1072–1074) and *M. juvenis* (cultures STE-U 1098–1100) on leaves of a *Eucalyptus* sp., May 1995, leg.T. Coutinho, det. P.W. Crous (PREM 54971). COLOMBIA: Sinai, *Mycosphaerella* spp. and *M. africana* on leaves of *E. grandis*, 1995, leg. M.J. Wingfield, det. P.W. Crous (PREM 54978).

Discussion

The surveys that have given rise to the present study have led to the description of three additional species of *Mycosphaerella*. It is thus apparent that intensified surveys, particularly in areas where *Eucalyptus* leaf fungi have only been considered superficially, will lead to the discovery of more species. The large number of species of *Mycosphaerella* that have been described on this single host genus might be considered surprising. In this regard, various factors should be considered. The genus *Eucalyptus* is large and diverse, and this would presumably lead to abundant speciation events in fungi occurring on this host. Evidence also exists that some fungi on other Myrtaceous genera have become adapted to infect *Eucalyptus* spp. (Ferreira 1989; Sutton and Pascoe 1989*a*). Furthermore, the distinct anamorph form genera associated with *Mycosphaerella* spp. occurring on *Eucalyptus* suggest that *Mycosphaerella* could also represent a number of discrete generic entities.

Until recently, MLB disease of eucalypts in South Africa was attributed either to *M. molleriana* or *Mycosphaerella nubilosa* (Cooke) Hansf. (Doidge 1950; Lundquist and Purnell 1987; Crous et al. 1991). Other records from Africa referred to the disease as being caused by a *Mycosphaerella* sp. (Shakacite 1991). In a study of the species associated with MLB disease in South Africa, Crous and Wingfield (1996) found no material of either *M. molleriana* or *M. nubilosa*, but recorded six different species, five of which were new to science. The findings of the present study further suggest that most of the South African species associated with MLB also occur elsewhere on the continent. Some of these species, such as *M. marksii*, may prove to be heterogeneous (Crous and Wingfield 1996). Ascospores of the Indonesian collection of *M. marksii* showed some distortion upon germination which was not found in material obtained from Australia or South Africa.

Both *M. parkii* and *M. suberosa*, which were previously only known from Brazil, are now also known to occur in Asia. The fact that they occur on the latter continent, which has some native eucalypts and is geographically close to Australia, suggests that they may also occur on *Eucalyptus* spp. in Australia.

Eucalyptus leaves from Indonesia associated with *M. heimii* showed extensive leaf spotting, thus suggesting that it is an important leaf pathogen, as initially reported by Bouriquet (1946). Crous and Swart (1995) speculated that *Calonectria quinqueseptatum* Figueiredo & Namek., which is presently not known from Africa (Crous and Wingfield 1994), probably reached Madagascar via *Eucalyptus* material introduced from Indonesia. Similarily, *M. heimii*, which has hitherto been known only from Madagascar and is now also known from Indonesia, might have originated from the latter country.

In this study, we found evidence that many *Mycosphaerella* spp. pathogenic to *Eucalyptus* spp. have apparently moved between continents. Although it is difficult to determine areas of origin, distribution patterns are beginning to emerge from these surveys. *Mycosphaerella cryptica*, which is known from Australia and New Zealand, has recently been observed in Chile (Wingfield et al. 1995). *Mycosphaerella africana*, known only from Africa (Crous and Wingfield 1996), has recently been identified from *E. globulus* leaves from Portugal (PREM 54974) and *E. grandis* leaves from Colombia (PREM 54978). *Mycosphaerella marksii*, recently described from Australia (Carnegie and Keane 1994), also occurs in Africa and Portugal (Crous and Wingfield 1996). In the present study, it was also isolated from *E. globulus* leaves from Indonesia (PREM 54976).

Mycosphaerella spp. are commonly isolated as endophytes from *Eucalyptus* leaves (P. Crous, unpublished data), and it is possible that species could also be distributed in asymptomatic, apparently healthy plant material. Patterns pertaining to the distribution of these fungi on different continents are beginning to arise. Evidence for species having moved between continents is also emerging, although the basis for this movement, or the time at which it occurred is unknown. Additional surveys in the many areas where eucalypts are grown, but where leaf-infecting fungi have not been studied, are required. A more complete perspective of the distribution of these fungi will contribute substantially to the taxonomy of *Mycosphaerella* and its anamorphs, as well as to our knowledge of these fungi as pathogens of *Eucalyptus*.

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References

Barr, M.E. 1972. Preliminary studies on the Dothideales in temperate North America. Contrib. Univ. Mich. Herb. **9**: 523–638.

Bouriquet, G. 1946. Les maladies des plantes cultivées á Madagascar. Encycl. Mycol. **12**: 44–45.

Braun, U. 1993. New genera of phytopathogenic Deuteromycetes. Cryptogam. Bot. **4**: 107–114.

Carnegie, A.J., and Keane, P.J. 1994. Further *Mycosphaerella* species associated with leaf diseases of *Eucalyptus*. Mycol. Res. **98**: 413–418.

Carnegie, A.J., Keane, P.J., Ades, P.K., and Smith, I.W. 1994. Variation in susceptibility of *Eucalyptus globulus* provenances to *Mycosphaerella* leaf disease. Can. J. For. Res. **24**: 1751–1757.

Crous, P.W., and Alfenas, A.C. 1995. *Mycosphaerella gracilis* sp.nov. and other species of *Mycosphaerella* associated with leaf spots of *Eucalyptus* in Indonesia. Mycologia, **87**: 121–126.

Crous, P.W., and Braun, U. 1996. Cercosporoid fungi from South Africa. Mycotaxon, **57**: 233–321.

Crous, P.W., and Swart, W.J. 1995. Foliicolous fungi of

Eucalyptus spp. from eastern Madagascar: implications for South Africa. S. Afr. For. J. **172**: 1–5.

Crous, P.W., and Wingfield, M.J. 1994. A monograph of *Cylindrocladium*, including anamorphs of *Calonectria*. Mycotaxon, **51**: 341–435.

Crous, P.W., and Wingfield, M.J. 1996. Species of *Mycosphaerella* and their anamorphs associated with leaf blotch disease of *Eucalyptus* in South Africa. Mycologia, **88**: 441–458.

Crous, P.W., Wingfield, M.J., and Park, R.F. 1991. *Mycosphaerella nubilosa*, a synonym of *M. molleriana*. Mycol. Res. **95**: 628–632.

Crous, P.W., Phillips, A.J.L., and Wingfield, M.J. 1992. Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. Mycologia, **84**: 497–504.

Crous, P.W., Carnegie, A.J., and Keane, P.J. 1995*a*. IMI descriptions of fungi and bacteria, set 121. *Mycosphaerella gracilis*. Mycopathologia, **130**: 49–50.

Crous, P.W., Carnegie, A.J., and Keane, P.J. 1995*b*. IMI descriptions of fungi and bacteria, set 121. *Mycosphaerella parkii*. Mycopathologia, **130**: 57–58.

Crous, P.W., Ferreira, F.A., and Sutton, B.C. 1997. A comparison of the fungal genera *Phaeophleospora* and *Kirramyces* (coelomycetes). S. Afr. J. Bot. **63**: In press.

Doidge, E.M. 1950. The South African fungi and lichens to the end of 1945. Bothalia, **5**: 1–1094.

Farr, D.F., Bills, G.F., Chamuris, G.P., and Rossman, A.Y. 1989. Fungi on plants and plant products in the United States. APS Press, St. Paul, Minn.

Ferreira, F.A. 1989. Patologia florestal. Principais doenças florestais no Brasil. Sociedade de Investigaçoes Florestais, Viçosa, Brazil.

Fisher, N. L., Burgess, L. W., Toussoun, T. A., and Nelson, P. E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology, **72**: 151–153.

Lundquist, J.E., and Purnell, R.C. 1987. Effects of *Mycosphaerella* leaf spot on growth of *Eucalyptus nitens*. Plant Dis. **71**: 1025–1029.

Palm, M. 1996. *Kirramyces phormii* comb.nov. from leaves of *Phormium*. Mycol. Res. **100**: 373–376.

Rayner, R.W. 1970. A mycological colour chart. CMI and British Mycological Society, Kew, Surrey, England.

Sankaran, K.V., Sutton, B.C., and Minter, D.W. 1995. A checklist of fungi recorded on *Eucalyptus*. Mycol. Pap. **170**: 1–375.

Shakacite, O. 1991. A review on pathology of eucalypts in Zambia. *In* Intensive Forestry: the role of eucalypts, Proceedings of the IUFRO Symposium, Durban, South Africa, 2–6 Sept. 1991. *Edited by* A.P.G. Schönau. Southern African Institute of Forestry, Pretoria, South Africa. pp. 790–799.

Shoemaker, R.A., and Müller, E. 1964. Generic correlations and concepts: *Clathridium* (= *Griphosphaeria*) and *Seimatosporium* (= *Sporocadus*). Can. J. Bot. **42**: 403–410.

Sivanesan, A. 1984. The bitunicate ascomycetes and their anamorphs. J. Cramer, Germany.

Smith, D.J., and Smith, C.O. 1941. Species of *Stigmina* and *Stigmella* occurring on *Platanus*. Hilgardia, **14**: 205–231.

Sutton, B.C. 1980. The coelomycetes. Fungi imperfecti with acervuli and stromata. Commonwealth Mycological Institute, Kew, Surrey, England.

Sutton, B.C., and Crous, P.W. 1997. *Lecanostictopsis* gen.nov., and related leaf-spotting fungi on *Syzygium* species. Mycol. Res. **101**: 215–225.

Sutton, B.C., and Pascoe, I.G. 1989*a*. Addenda to *Harknessia* (Coelomycetes). Mycol. Res. **92**: 431–439.

Sutton, B.C., and Pascoe, I.G. 1989*b*. Reassessment of *Peltosoma*, *Stigmina* and *Batcheloromyces* and description of *Hyphothyrium* gen.nov. Mycol. Res. **92**: 210–222.

Turnbull, J.W. 1991. Future use of *Eucalyptus*: opportunities and problems. *In* Intensive forestry: the role of eucalypts. Proceedings of the IUFRO Symposium, Durban, South Africa, 2–6 Sept. 1991. *Edited by* A.P.G. Schönau. Southern African Institute of Forestry, Pretoria, South Africa. pp. 1–27.

von Arx, J.A. 1983. Mycosphaerella and its anamorphs. Proc. K. Ned. Akad. Wet. Ser. C Biol. Med. Sci. **86**: 15–54.

Walker, J., Sutton, B.C., and Pascoe, I. 1992. *Phaeoseptoria eucalypti* and similar fungi on *Eucalyptus*, with description of *Kirramyces* gen.nov. (Coelomycetes). Mycol. Res. **96**: 911–924.

Wingfield, M.J., Crous, P.W., and Peredo, H.L. 1995. Foliar pathogens of *Eucalyptus* spp. in Chile. S. Afr. For. J. **173**: 53–57.

Zentmeyer, G.A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Monograph 10. APS Press, St. Paul, Minn.