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Species of *Mycosphaerella* and their anamorphs associated with leaf blotch disease of *Eucalyptus* in South Africa

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Abstract: Mycosphaerella leaf blotch is a serious disease of *Eucalyptus nitens* and also occurs on several other *Eucalyptus* spp. in South Africa. The aim of the present study was to characterize the variation observed among South African *Mycosphaerella* strains obtained from eucalypts. Earlier studies had suggested that only one species, *M. molleriana*, occurred on eucalypts in South Africa. Contrary to these reports *M. molleriana* was not found, but five new species of *Mycosphaerella* were collected and are described here. The name *M. juvenis* is proposed for the species commonly associated with leaf spots on juvenile leaves of *E. nitens*. Because its cercosporoid anamorph state could not be suitably accommodated in *Pseudocercospora*, a new genus *Uwebraunia* is introduced for it, and also for the anamorphs of two other new species, *M. ellipsoidea* and *M. lateralis*, occurring on *E. cladocalyx* and *E. grandis* x *saligna*, respectively. *Mycosphaerella crystallina* with a *Pseudocercospora* anamorph is described as a new species from leaves of *E. bicostata*. A *Pseudocercospora* sp. associated with spermogonia of an undescribed *Mycosphaerella* sp., is compared with known species of the genus from eucalypts, and described as *P. epispermogoniana*. *Mycosphaerella marksii*, recently described from eucalypts in Australia, is also reported from South Africa for the first time. A key to the species of *Mycosphaerella* presently known from *Eucalyptus* is also provided.

Key Words: *Pseudocercospora, Uwebraunia*, systematics

INTRODUCTION

Mycosphaerella leaf blotch is a serious disease of *Eucalyptus* species worldwide (Lundquist and Purnell, 1987; Ferreira, 1989; Carnegie et al., 1994; Crous, 1995a, b; Crous et al., 1995a, b, c). In all, 17 species of *Mycosphaerella* Johanson have been described from this host genus, with diseases ranging from serious to insignificant (Crous et al., 1993a; Carnegie and Keane, 1994; Crous, 1995a, b; Crous et al., 1995a, b, c). In South Africa only certain provenances of *E. nitens* (Deane & Maid.) Maid. can be effectively used for commercial forestry because of this disease. Others such as *E. globulus* Labill. have had to be abandoned from commercial plantations due to the severity of Mycosphaerella leaf blotch (Lundquist and Purnell, 1987).

The causal agent of Mycosphaerella leaf blotch in South Africa was first reported by Doidge (1950) to be *M. molleriana* (Thüm.) Lindau. It was later referred to by Lundquist (1985) as *M. nubilosa* (Cooke) Hansf. In a detailed study of *M. nubilosa*, Crous et al. (1991) reported that only one species of *Mycosphaerella* occurred over a wide range of *Eucalyptus* spp. in South Africa, and that these were best referred to as *M. molleriana*. Furthermore, similar symptom expression, ascus and ascospore morphology was seen in the type specimens of *M. molleriana* and *M. nubilosa*. Ascospores of *M. molleriana* are (11–)12–16(–17) × 3–3.5(–4.5) µm, and those of *M. nubilosa* are (11–)13–16 × 3–3.5(–4.5) µm (type specimens lodged at K). *Mycosphaerella nubilosa* was, therefore, reduced to synonymy under the older epithet *M. molleriana*. Crous et al. (1993a, b), following Park and Keane (1982), utilized the characteristics of ascospore germination to distinguish *Mycosphaerella* species. Evidence arising from ascospore germination and cultural studies has recently led to the observation that strains generally accepted as *M. molleriana* represent a complex of species (Crous and Alfenas, 1995). These species could generally be distinguished based on their mode of germination (orientation of the germ tube and morphology of the germinating ascospore), as well as cultural characteristics (growth rate, colony color, mycelial type, etc.). Carnegie and Keane (1994) described several new species of *Mycosphaerella* that are morphologically similar to *M. molleriana* but that have different patterns of ascospore germination. Similarly, *in vitro* colony characteristics of Brazilian and Indonesian strains (Crous et al., 1993a, b; Crous and Alfenas,

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1995), that could be accommodated in *M. molleriana* as interpreted by Crous et al. (1991), suggested a multitude of species. Based on this hypothesis, extensive collections of Mycosphaerella leaf blotch on a wide variety of *Eucalyptus* spp. and from diverse sites in South Africa were studied. The aim of this study, was to compare South African collections of *Mycosphaerella* from *Eucalyptus* with each other, and to contrast them with species known from eucalypts elsewhere in the world.

Symptomatology.—Symptoms induced by *Mycosphaerella* spp. on eucalypts are characteristic for some *Mycosphaerella* species, but variable for others (FIGS. 1–7). Lesions can vary from being angular (*M. martinae* Hansf.) to circular or irregular [*M. cryptica* (Cooke) Hansf.]. Several species also have the ability to form leaf blotches through the coalescence of leaf spots (*M. cryptica*), thus causing a distortion of the leaf lamina. Lesions can vary in color, and can be smooth and amphigenous (*M. molleriana*), or corky and mostly not extending through the lamina (*M. suberosa* Crous et al.). Borders of lesions can be raised, and frequently darker than the centers. Margins surrounding the borders of lesions can be absent (*M. delegatensis* R.F. Park & Keane), or vary from a chlorotic yellow to a red or red-purple color (*M. swartii* R.F. Park & Keane).

Several species are associated with leaves of defined age. For example *M. juvenis* Crous & M.J. Wingf. from South Africa, is mostly found on juvenile foliage, whereas *M. suberosa* is common on older, mature foliage of eucalypts in Brazil (Crous et al., 1993a).

Teleomorphic and cultural characters.—The dimensions of pseudothecia and their distribution in lesions have been shown to be important characteristics of *Mycosphaerella* spp. on eucalypts. Pseudothecia can be amphigenous, but predominantly epi- or hypophyllous. They can also be immersed, becoming erumpent (*M. juvenis*), or superficial, being solitary or arranged in dense clusters [*M. aggregata* Carnegie & Keane; a later homonym of *M. aggregata* (Schwein.) Stevenson (1918), presently being renamed by Carnegie and Keane, pers. comm.], or in concentric rings (*M. suberosa*). Several other features have not been taken into account, because of the considerable overlap that was observed among species. These include the dimensions of the pseudothecial wall cells, and the periphyses present in the ostiolar canals. Asci, which are aparaphysate, bitunicate, subsessile, and formed in a fascicle, vary in shape from obovoid to broadly ellipsoidal (*M. juvenis*, FIGS. 8–10, 11–15; *M. africana* Crous & M.J. Wingf., FIGS. 16–18), or narrowly ellipsoidal to cylindrical (*M. parkii* Crous et al.).

Ascospores are mostly hyaline, or slightly olivaceous in the ascus (*M. suberosa*). They are usually bi- to triseriate in asci of species with large spores, or multiseriate in those with small spores. Ascospores in asci can either be straight, curved, or frequently both curved and straight. They vary from being strongly guttulate (*M. crystallina* Crous & M.J. Wingf.; FIGS. 20–26) to nonguttulate, thin- to thick-walled, and prominently, slightly or not constricted at the septa. Ascospores are bicellular; in most the septum is median but in some species the basal cell is slightly longer than the apical cell (*M. aggregata*). The widest point in the ascospore can either be at the median septum (*M. ellipsoidea* Crous & M.J. Wingf.; FIGS. 27, 28, 35–38), or in the middle of the apical cell (*M. molleriana*), which itself is frequently asymmetrical (*M. marksii* Carnegie & Keane). Ascospores are narrowly ellipsoidal (*M. gracilis* Crous & Alfenas), fusoid-ellipsoidal (*sensu* Snell and Dick, 1971) (*M. ellipsoidea*), or obovoid (*sensu* Hawksworth et al., 1983) (*M. juvenis*). They taper from the middle towards both ends (*M. gracilis*), or more prominently from the tip or middle of the upper cell toward the basal end (*M. molleriana*).

Park & Keane (1982) demonstrated that ascospore germination could be used to distinguish relatively similar species of *Mycosphaerella*. Germ tubes of *M. cryptica* are perpendicular to the long axis of ascospore, whereas germ tubes of *M. molleriana* lie parallel to the long axis of the spore. Subsequent to this work, characteristics of ascospore germination have become a very important feature in species delimitation (Crous et al., 1993a, b; Crous and Alfenas, 1995; Crous and Swart, 1995). Ascospores can germinate from either one (*M. cryptica*) or both cells (*M. africana*), from the polar caps, adjacent to the polar caps, or irregularly along the length of spore. Some species, such as *M. suberosa*, can also germinate via multiple germ tubes. Furthermore, spores of some species are not constricted at the median septum at germination (*M. gracilis*), while others develop a slight constriction (*M. ellipsoidea*), or a severe

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FIGS. 1–7. Leaf symptoms on the adaxial (left) and abaxial (right) surfaces of *Eucalyptus* leaves associated with *Mycosphaerella* spp. 1, 2. *M. juvenis* on *E. nitens* and *E. grandis*, respectively. 3. *M. africana* on *E. viminalis*. 4. *M. crystallina* on *E. bicostata*. 5. *M. ellipsoidea* on *E. cladocalyx*. 6. *M. lateralis* on *E. grandis* × *saligna*. 7. *M. marksii* on *E. grandis*.

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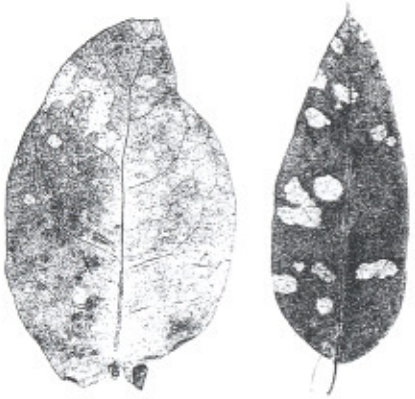

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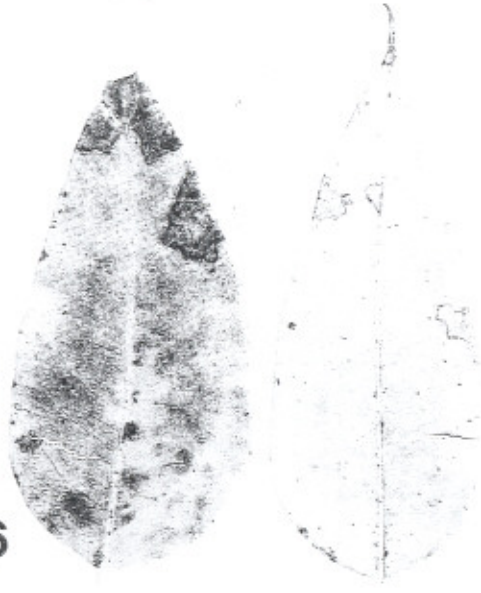

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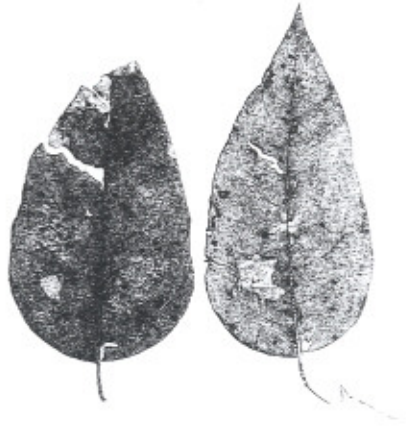

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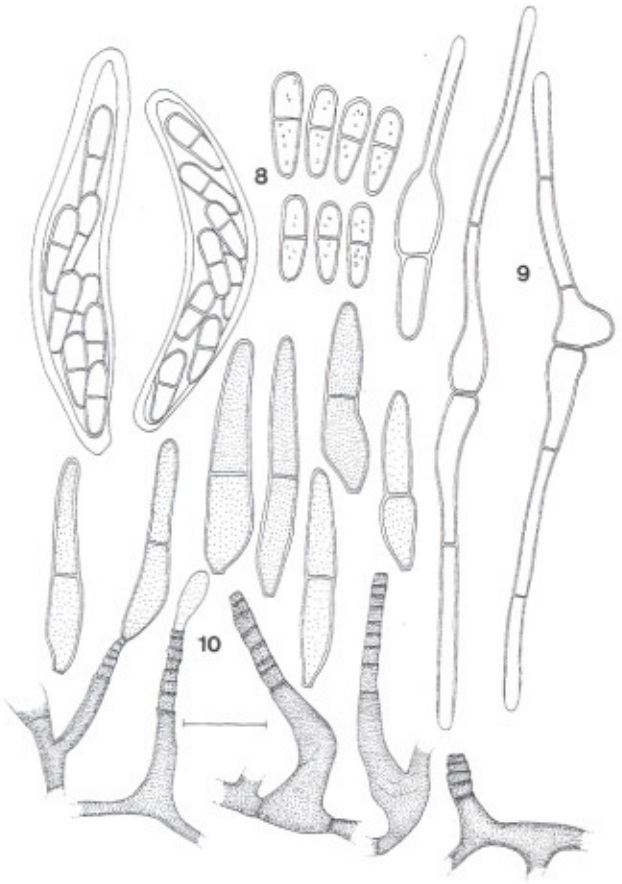
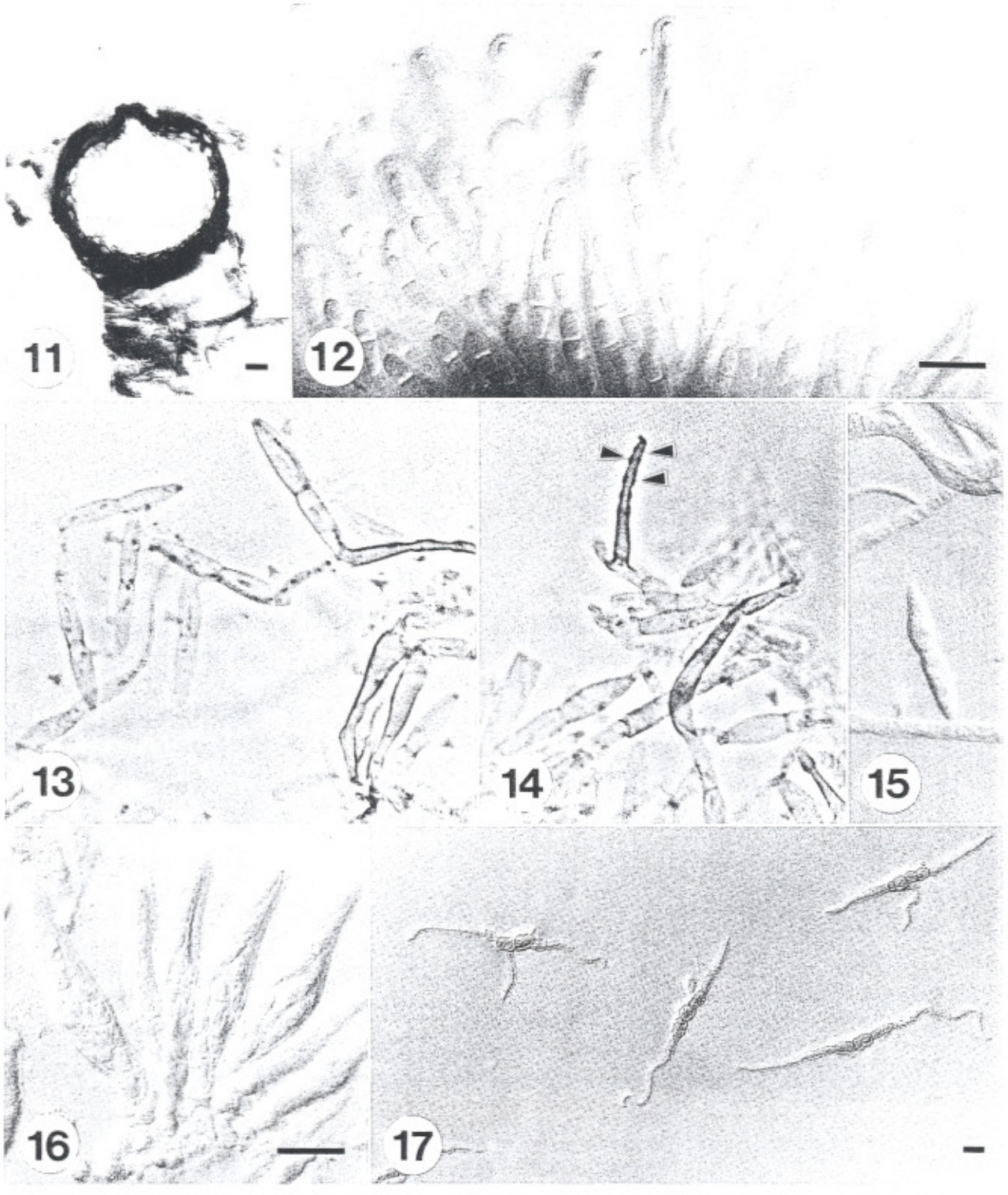


FIGS. 8–10. *Mycosphaerella juvenis* and its anamorph, *Uwebraunia juvenis*. 8. Asci and ascospores. 9. Germinating ascospores after 24 h on MEA. 10. Conidia and conidiophores *in vitro*. Scale bar = 10 μm.

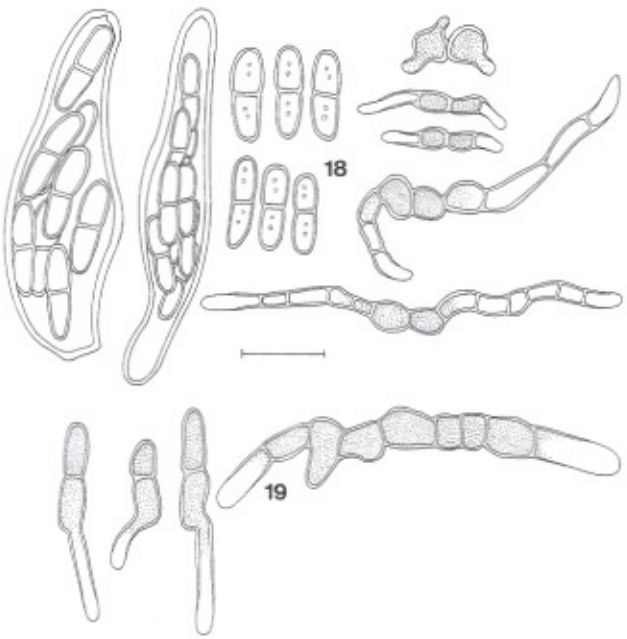
swelling and distortion of the ascospore (*M. juvenis*). Ascospores can darken during germination. The pigmentation can either be evenly distributed throughout the ascospore and germ tubes (*M. grandis* Carnegie & Keane), or be confined to the spore itself (*M. africana*). Germ tubes of some species also form lateral branches 24–48 h after germination (*M. lateralis* Crous & M.J. Wingf.; FIGS. 29, 30, 39–41). The length of the first few germ tube cells is variable and not a useful character, but the shape of the germinated ascospore after 24 h is a valuable feature for identification. For the purpose of this study germinated ascospores were referred to as slighly distorted (appearing slightly swollen), or distorted (prominent swelling of the two ascospore cells). Although the width of the two cells of the germinated ascospores are given for each species, differences in spore volume ensures that a swelling of 2 μm in small-spored species usually indicates gross distortion, while this would not necessarily be the case in large-spored species.

Colony characteristics are generally consistent *in vitro*. However, radial growth rate can frequently be variable, as is cultural color and morphology. For reasons unknown to us, colonies derived from some ascospores obtained from lesions exhibit stunted or retarded growth with poor or no sporulation *in vitro*. Such strains were discarded from further study. In some species ascospores germinate at 25 C, but die soon after (*M. juvenis*). If these germinated spores are incubated at 15 C till colonies become visable to the naked eye, however, colonies will continue to grow further at a wide range of temperatures. This phenomenon was also recently observed in *M. jonkershoekensis* P.S. van Wyk et al. from *Protea*, and it is possible that it occurs much more commonly than earlier expected.

Anamorphs.—Hyphae of *Mycosphaerella* spp. can either be internal in the host tissue, or both internal and external, verrucose (*M. parkii*), or smooth (*M. gracilis*). Anamorphs of *Mycosphaerella* spp. occurring on *Eucalyptus* have been linked to coelomycetes with pycnidia [*Sonderhenia eucalypticola* (A.R. Davis) H.J. Swart & J. Walker and *S. eucalyptorum* (Hansf.) H.J. Swart & J. Walker anamorphs of *M. walkeri* R.F. Park & Keane and *M. swartii*; *Stagonospora delegatensis* R.F. Park & Keane anamorph of *M. delegatensis*], and acervuli (*Colletogloeum nubilosum* Ganap. & Corbin anamorph of *M. cryptica*). Hyphomycete anamorphs have been placed in cercosporoid genera such as *Pseudocercospora* Speg. [*P. gracilis* Crous & Alfenas and *P. heimii* Crous anamorphs of *M. gracilis* and *M. heimii* (Bugnic.) Crous] and *Stenella* Syd. (*S. parkii* Crous & Alfenas anamorph of *M. parkii*). In addition, a new hyphomycete genus is proposed for the cercosporoid anamorphs of three *Mycosphaerella* spp. described in this study. The three anamorph states are characterized by having smooth, olivaceous, obclavate, 1-septate conidia with unthickened hila, produced on light to medium brown conidiogenous cells with several percurrent proliferations. Conidia frequently give rise to secondary conidia by microcyclic conidiation, as is common in cercosporoid fungi (Fernandez et al., 1991). These features suggest a similarity with the genus *Pseudocercospora*. This genus is, however, characterized by scolecospores attached to conidiogenous cells that proliferate sympodially as well as percurrently. Braun (1994) recently introduced the genus *Cercostigmina* U. Braun for species with scolecospores and conidiogenous cells that have percurrent proliferation and unthickened conidiogenous walls. The absence of scolecospores in the three *Mycosphaerella* anamorphs discussed here exclude them from *Cercostigmina*. Furthermore, the characteristic shape of their 1-septate conidia, and separate, single conidiogenous cells with percurrent



FIGS. 11–17. *Mycosphaerella* spp. 11–15. *Mycosphaerella juvenis* and its anamorph, *Uwebraunia juvenis*. 11. Vertical section through a pseudothecium. 12. Asci and ascospores. 13–15. One-septate conidia giving rise to secondary conidia via microcyclic conidiation. Note percurrent proliferations on the germ tubes (arrowed). 16, 17. *M. africana*. 16. Asci with constricted ascospores. 17. Ascospores that darken at germination. Scale bars = 10 μm.



FIGS. 18, 19. *Mycosphaerella africana* and *M. grandis*. 18. Obovoid asci, freshly discharged and germinating ascospores of *M. africana*. 19. Germinating ascospores of *M. grandis*. Scale bar = 10 μm.

proliferations justified their separation into a new genus described below.

Uwebraunia Crous *et* M.J. Wingf, *gen nov.*

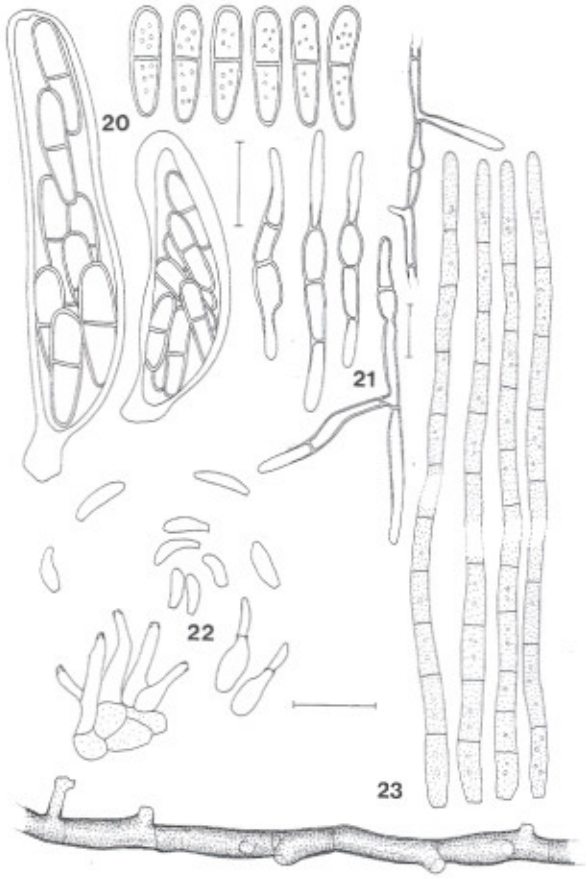
Mycelium internum et externum, constans ex hyphis ramosis septatis, laevibus hyalinis ad olivaceo-brunneis, emittens conidiophora singulare separata. Conidiophora redacta ad cellulis conidiogenis vel cellula sustinenti. Cellulae conidiogenae laeves, pallide ad medio-brunneae, subcylindraceae, vel basale subulatae et superiore subcylindraceae, rectae vel geniculato-sinuatae, monopodiales, hologena proliferationibus 20 percurrentibus, 15–55 × 2–6 μm. Conidia terminalia, solitaria, pallide olivacea, laevia, obclavata, obtusa base obconico-truncata, 1-septata, demum prominenter constricta in septo, recta vel curvata, 16–40 × 3–6 μm; hila non crassa, non atra vel refractiva, 1.5–2 μm lata. Status teleomorphicus *Mycosphaerella*.

Type species. **Uwebraunia juvenis** Crous *et* M.J. Wingf

HOLOTYPE. SOUTH AFRICA. Natal: Pietermaritzburg, leaves of *E. nitens*, Jan. 1995, *M.J. Wingfield* (PREM 51915, cultures ex type STE-U 932–934).

Etymology. Named for Dr. Uwe Braun in recognition of his contribution to the taxonomy of cercosporoid fungi.

Mycelium internal and external, consisting of branched, septate, smooth, hyaline to olivaceous brown hyphae, giving rise to single, separate conidiophores. Conidiophores reduced to conidiogenous

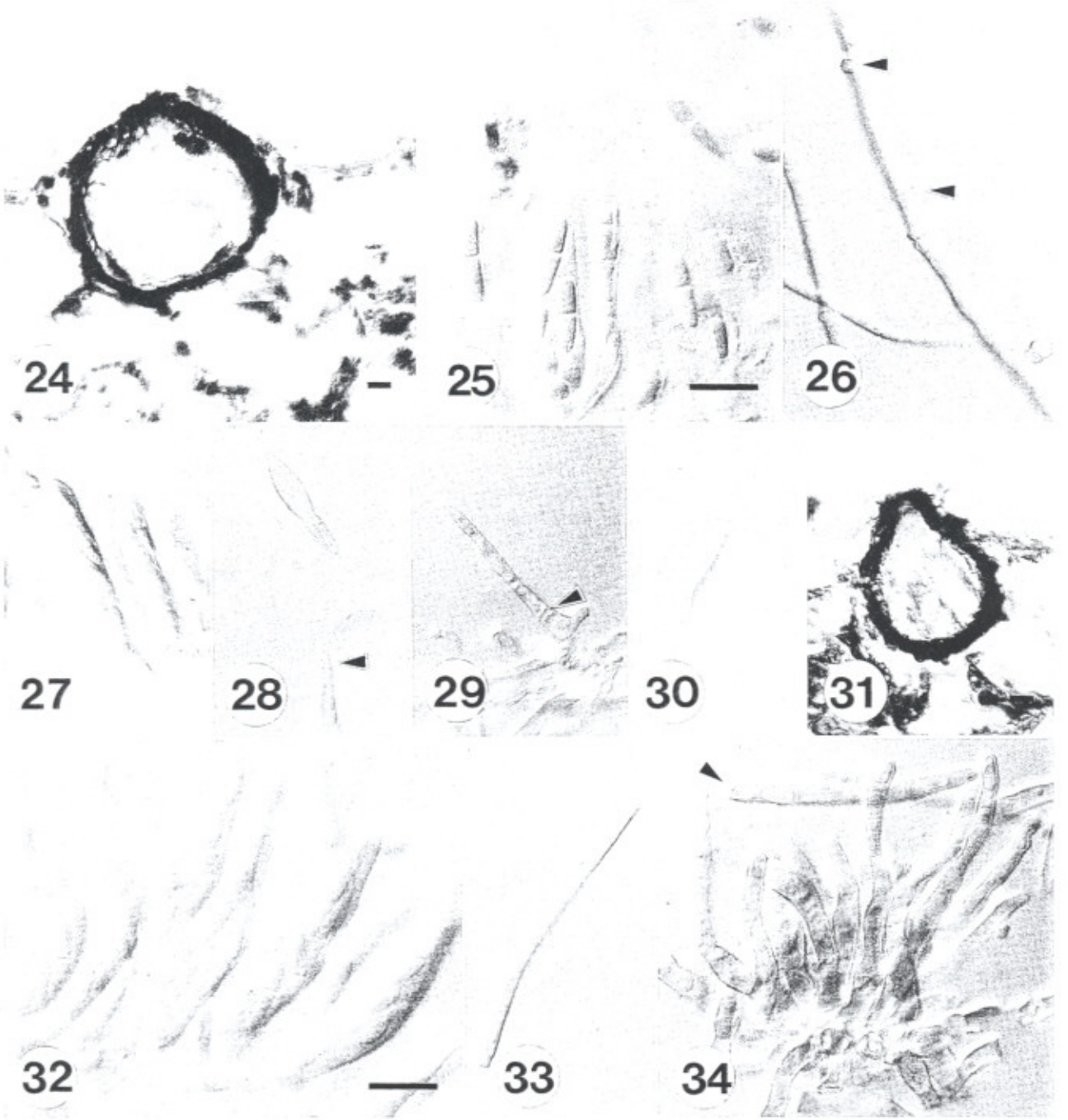


FIGS. 20–23. *Mycosphaerella crystallina* and its anamorph *Pseudocercospora crystallina*. 20. Asci and ascospores. 21. Germinating ascospores after 24 h on MEA. 22. Spermatia and spermatogenous cells. 23. Conidia and conidiogenous cells *in vitro*. Scale bar = 10 μm.

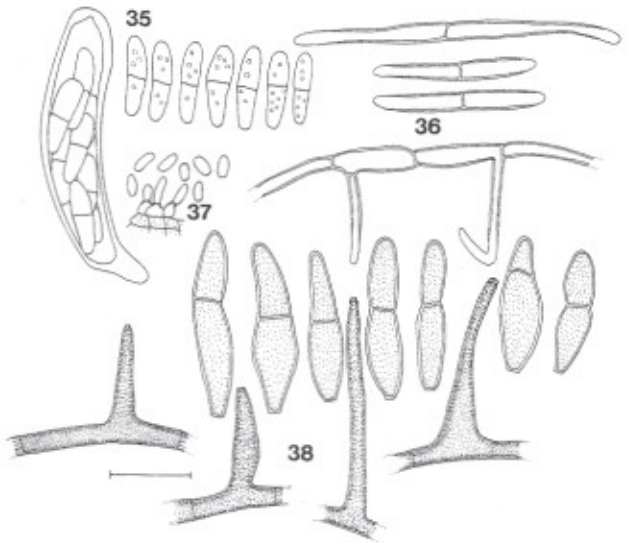
cells or a supporting cell. Conidiogenous cells smooth, light to medium brown, subcylindrical, or with subulate basal parts and subcylindrical upper parts, straight or geniculate-sinuous, monopodial, hologenous with up to 20 percurrent proliferations, 15–55 × 2–6 μm. Conidia terminal, solitary, pale olivaceous, smooth, obclavate with a obtuse apex and obconically truncate base, 1-septate, becoming prominently constricted at septa when mature, straight or curved, 16–40 × 3–6 μm; hila unthickened, not darkened or refractive, 1.5–2 μm wide. Conidia frequently producing secondary conidia via microcyclic conidiation *in vitro*.

Teleomorph. *Mycosphaerella*.

Several *Mycosphaerella* spp. have spermatial synanamorphs that are classified in the genus *Asteromella* Pass. & Thüm. (von Arx, 1983). Spermatia are either rod shaped (*M. cryptica*), or ellipsoidal to cylindrical and allantoid (M. *crystallina*). They are formed on phialides that line the inner wall of spermogonia (pycnidia), which occur freely among pseudothecia,

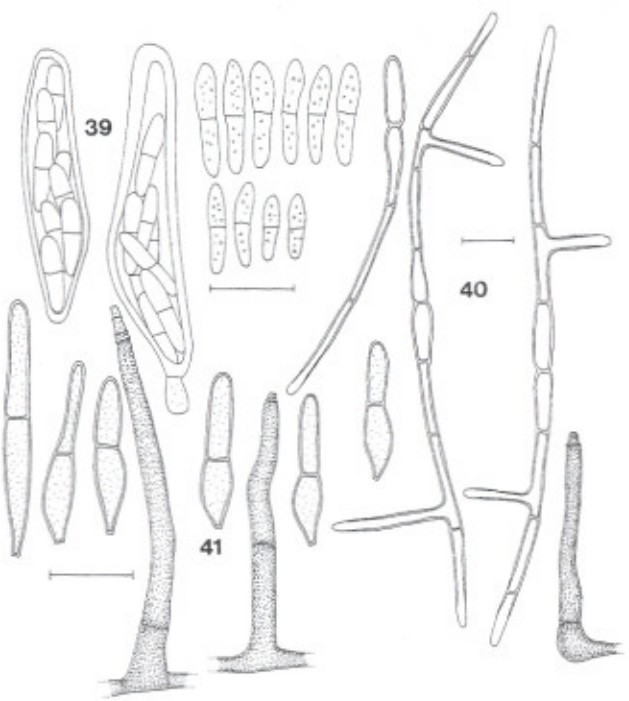


FIGS. 24–34. *Mycosphaerella* and *Pseudocercospora* spp. 24–26. *Mycosphaerella crystallina* and its anamorph, *Pseudocercospora crystallina*. 24. Vertical section through a pseudothecium. 25. Asci and ascospores. 26. Conidiogenous cells on a hypha (arrows). 27, 28. *M. ellipsoidea* and its anamorph, *Uwebraunia ellipsoidea*. 27. Cylindrical ascus with ascospores. 28. Conidium and a conidiogenous cell with a percurrent proliferation (arrow). 29, 30. *Uwebraunia lateralis*, the anamorph of *M. lateralis*. 29. Conidiophore with basal septum. 30. One-septate conidium. 31, 32. *M. marksii*. 31. Vertical section through a pseudothecium. 32. Asci and ascospores. 33. Narrowly obclavate conidium of *Pseudocercospora epispermogoniana*. 34. Fascicle of *P. eucalyptorum* conidiophores, and subcylindrical conidium with a wide subtruncate base (arrow). Scale bars = 10 μm.



FIGS. 35–38. *Mycosphaerella ellipsoidea* and its anamorph, *Uwebraunia ellipsoidea*. 35. Ascus and ascospores. 36. Germinating ascospores after 24 h on MEA. 37. Spermatia and spermatogenous cells. 38. Conidia and conidiogenous cells. Scale bar = 10 μm.

and are also induced to form in culture. Uncertainty remains regarding the possible role of these spermatia/conidial synanamorphs in *Mycosphaerella*, and for the remainder of this study they will be referred to as spermatia.



FIGS. 39–41. *Mycosphaerella lateralis* and its anamorph, *Uwebraunia lateralis*. 39. Asci and ascospores. 40. Germinating ascospores after 24 h on MEA. 41. Conidiophores and conidia. Scale bar = 10 μm.

In a detailed study of *Mycosphaerella* spp. and their anamorphs, von Arx (1983) accepted 23 anamorph genera as having teleomorphs in *Mycosphaerella*. Corlett (1991) listed more than 40 anamorph genera that have been linked to *Mycosphaerella*. Since these publications, however, there have been several generic synonymies, and several additional new anamorph genera linked to *Mycosphaerella*. Presently, we are of the opinion that detailed cultural studies are required to elucidate some of the more obscure anamorph/teleomorph connections in this complex, and that only such research would provide any basis for further phylogenetic research within *Mycosphaerella*. Previous proposals to split the genus *Mycosphaerella* have used features such as ascospore pigmentation, anamorph genera, pseudothecial anatomy, ascus and ascospore morphology, and their parasitic or saprophytic nature. Although the mode of ascospore germination is an important taxonomic feature, it appears to be species specific, and apparently does not indicate distinct groupings within *Mycopshaerella*. However, we hypothesize that *Mycosphaerella* is polyphyletic, thus explaining the correlation with a diversity of (presumably?) monophyletic anamorph genera. More cultural studies on numerous additional species would be required, however, to clearly elucidate genera within *Mycosphaerella*.

MATERIALS AND METHODS

Lesions were excised from leaves, and single ascospore cultures were established on 2 % malt-extract agar (Biolab) (MEA) as described in Crous et al. (1991). Germinating ascospores were examined after 24 h, illustrated, and transferred to MEA. Cultures were incubated for 2 wk at 25 C in the dark, and subcultured onto divided plates with one half containing carnation-leaf agar (CLA) (Crous et al., 1992) and the other MEA, incubated at 25 C under continuous near-ultraviolet light. Single ascospore cultures were examined at weekly intervals for the presence of anamorphs or spermogonia. With the exception of *Pseudocercospora epispermogoniana* Crous & M.J. Wingf., all newly described anamorph states were induced from single ascospore cultures. Linear growth on agar for each culture was determined for three single ascospore colonies on MEA after 1 mo of incubation at 25 C in the dark. Two perpendicular measurements were obtained for each colony, and averages determined. Colony colors (top and bottom) were subsequently rated using the color charts of Rayner (1970). Wherever possible, thirty measurements were made of structures mounted in lactophenol, and the extremes given in parentheses.

KEY TO *MYCOSPHAERELLA* SPECIES OCCURRING ON *EUCALYPTUS*

Two species for which no material could be located are not included in the key. These are *M. didymelloides* Petr. (pseudothecia 80–150 µm diam, asci 38–50 × 7–8 µm, ascospores 7.5–10 × 3–3.5 µm, from *E. globulus* in Spain; Corlett, 1991) and *Sphaerella molleriana* Thüm. var. *megalospora* Sousa da Câmara from *Eucalyptus* in Portugal (asci 50–60 × 18–20 µm, ascospores guttulate, slightly constricted at median septa, 20–25 × 6–8 µm; Saccardo, 1913). The key relies chiefly on symptom expression, ascospore morphology and the availability of fresh field material for ascospore germination studies. In all species except *M. swartii* and *M. walkeri*, the teleomorph is the the most commonly encountered form.

1. Ascospores up to 15 µm in length . . . 2
1. Ascospores longer than 15 µm . . . 12
2. Leaf spots angular, ascospores 11–13 × 2.5–3 µm . . . *M. martinae* (Hansford, 1956)
2. Leaf spots not angular . . . 3
3. Ascospores constricted at septa . . . 4
3. Ascospores not constricted at septa . . . 8
4. Ascospores darkening at germination . . . 5
4. Ascospores not darkening at germination . . . 7
5. Pseudothecia hypophyllous, ascospores 7–10 × 1–3 µm . . . *M. parva* (Park and Keane, 1982)
5. Pseudothecia amphigenous, ascospores wider . . . 6
6. Lesions confined to leaf margins; germination from one cell; ascospores 10.5–14.5 × 3–4.5 µm . . . *M. grandis* (Carnegie and Keane, 1994)
6. Lesions sub-circular, not confined to margins; germination from both cells; ascospores 7–11 × 2–3 µm . . . 1. *M. africana*
7. Pseudothecia in clusters of up to 12, superficial; ascospores 12.5–15 × 2.5–3.5 µm . . . *M. aggregata* (Carnegie and Keane, 1994)
7. Pseudothecia only aggregated against leaf veins, immersed; ascospores 8.5–18 × 4–6 µm . . . *M. cryptica* (Park and Keane, 1982)
8. Ascospores fusoid-ellipsoidal . . . 9
8. Ascospores obovoid . . . 10
9. Colonies olivaceous-green to dark green on MEA; *Pseudocercospora heimii* anamorph . . . *M. heimii* (Crous and Swart, 1995)
9. Colonies white with pink aerial mycelium on MEA; *Uwebraunia ellipsoidea* anamorph . . . 3. *M. ellipsoidea*
10. Germinating with more than one germ tube per cell; hyphae verrucose, anamorph *Stenella parkii*; ascospores 8–14 × 2.5–3 µm . . . *M. parkii* (Crous et al., 1993b)
10. Germinating with one germ tube per cell; hyphae olivaceous to light brown, smooth; anamorph *Pseudocercospora* or *Uwebraunia*; ascospores wider . . . 11
11. Ascospores with prominent distortion 24 h after germination, lateral branches absent; low viability at 25 C; anamorph *Uwebraunia juvenis*. . . . 4. *M. juvenis*
11. Ascospores constricted but not distorted 24 h after germination, lateral branches present; no death of germinated spores at 25 C; red crystals formed in MEA; anamorph *Pseudocercospora crystallina*. . . . 2. *M. crystallina*
12. Ascospores 20 µm or less in length . . . 13
12. Ascospores longer than 20 µm . . . 16
13. Lesions corky; ascospores slightly constricted, darkening at germination, 10–19 × 3–6 µm . . . *M. suberosa* (Crous et al., 1993b)
13. Lesions not corky; ascospores not constricted, not darkening at germination . . . 14
14. Lesions without red margins; ascospores obovoid, 11–17 × 2.5–4.5 µm . . . *M. molleriana* (Crous et al., 1991)
14. Lesions with red margins; ascospores not wider than 3 µm . . . 15
15. Ascospores narrlowly ellipsoidal, not constricted at germination, lateral branches absent, 10–20 × 2–3 µm; anamorph *Pseudocercospora gracilis* . . . *M. gracilis* (Crous and Alfenas, 1995)
15. Ascospores fusoid-ellipsoidal, constricting at germination, lateral branches present, 7–16 × 2–3 µm; anamorph *Uwebraunia lateralis* . . . 5. *M. lateralis*
16. Ascospores 45–50 × 6 µm . . . *M. eucalypti* (Hansford, 1957)
16. Ascospores shorter than above . . . 17
17. Ascospores not constricted at septa; lesions larger; anamorph not *Sonderhenia*. . . . 18
17. Ascospores slightly constricted at septa, 20–27 × 4–6 µm; leaf spots 0.5–2 mm, with red margins . . . 19
18. Lesions with red margins; pseudothecia predominantly epiphyllous; ascospores obovoid with asymmetrical apical cells, 12.5–22.5 × 2.5–5 µm . . . 6. *M. marksii*

18. Lesions without red margins; pseudothecia predominantly hypophyllous; ascospores obovoid with symmetrical apical cells, 16–25 × 3–5 μm ..... *M. delegatensis* (Crous et al., 1995a)
19. Anamorph *Sonderhenia eucalyptorum*; conidia ellipsoidal to cylindrical, 25–49 × 5–10 μm ..... *M. swartii* (Crous et al., 1995c)
19. Anamorph *Sonderhenia eucalypticola*; conidia ellipsoidal to ovoid, 19–31 × 6–12 μm .. *M. walkeri* (Crous et al., 1995c)

DESCRIPTIONS OF NEW SPECIES

1. **Mycosphaerella africana** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 3, 16–18

Laesiones amphigenae, rotundatae, 1–2 mm diam, pallide brunneae, marginibus atrobrunneis, saepe marginibus diffusis, rubropurpureis. Pseudothecia amphigena, solitaria, 4–15 per mm², nigra, subepidermalia, globosa, 50–65 μm lata, 50–70 μm alta; ostiola apicalia papillata 10–15 μm diam; paries consistens in 2–3 stratis *texturae angularis*, mediobrunneae, subhymenium basis consistens in 1–2 stratis cellarum hyalinarum. Asci aparaphysati, fasciculati, bitunicati, subsessiles, obovoidei ad late ellipsoidei, recti vel incurvi, 8-sporis, 28–45 × 8–11 μm. Ascosporae 2–3 seriate, superpositae, hyalinae, guttulatae, parietibus crassis, rectae, fusoideo-ellipsoideae, apicibus obtusis, latissimimae ad mediam cellam superiorem, mediano 1-septatae, non colligatae ad septum, attenuatae ad extrema, sed prominentius attenuatae ad extrema inferioria (7–)8–10(–11) × (2–)2.5–3 μm. Spermogonia ignota. Status anamorphicus ignota.

HOLOTYPE. SOUTH AFRICA. Western Cape: Stellenbosch, Stellenbosch Mountain, leaves of *E. viminalis* Labill., Oct. 1994, *P.W. Crous* (PREM 51917, cultures ex type STE-U 794–796).

Etymology. Named after the continent from which it is described.

Leaf spots amphigenous, round, 1–2 mm diam, light brown, surrounded by dark brown borders, and frequently with diffuse red-purple margins. Pseudothecia amphigenous, single, 4–15 per colonized mm², black, subepidermal, globose, 50–65 μm wide, 50–70 μm high; apical ostioles 10–15 μm diam, 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 obovoid to broadly ellipsoidal, straight or incurved, 8-spored, 28–45 × 8–11 μm. Ascospores bi- to triseriate, overlapping, hyaline, guttulate, thick-walled, straight, fusoid-ellipsoidal with obtuse ends, widest in middle of apical cells, medianly 1-septate, constricted at septa, tapering toward both apices, but with slightly more prominent taper towards lower ends (7–)8–10(–11) × (2–)2.5–3 μm. Spermogonia unknown.

Anamorph. Unknown.

Ascospore germination on MEA after 24 h of incubation. Germination irregular, germinating from both ends, or from different positions in cells, with two or more germ tubes, darkening and distorting, with cells becoming 5–7(–8) μm wide upon germination.

Cultures. Colonies 20–32 mm diam on MEA after 1 mo at 25 C in the dark, even edged, aerial mycelium profuse, olivaceous black, 27""k (bottom), grey olivaceous 23""i (top), colony not-sectored, fluffy, with erect hyphal tufts, and frequently with white-grey aerial mycelium. Most colonies of *M. africana* are black, and produce a diffuse brown pigment on MEA, while some also form masses of brown, thick-walled cells in the agar that can frequently aggregate in clusters.

Cardinal temperature requirements for growth. 5 C min., 20–25 C opt., below 30 C max.

Hosts. E. deanei, E. globulus, E. grandis, E. radiata and *E. viminalis.*

Distribution. Western Cape and Natal Provinces.

Additional collections examined. SOUTH AFRICA. Western Cape: Stellenbosch, Stellenbosch Mountain, leaves of *E. deanei*, Oct. 1994, *P.W. Crous* (PREM 51918, culture STE-U 816); Pampoenvlei, leaves of *E. globulus*, Nov. 1994, *P.W. Crous* (PREM 51919, cultures STE-U 838–840); Pampoenvlei, leaves of *E. grandis*, Nov. 1994, *P.W. Crous* (PREM 51920, cultures STE-U 833–837); Stellenbosch, Rust and Vrede Farm, leaves of *E. radiata*, Nov. 1994, *P.W. Crous* (cultures STE-U 896–898); Natal: Richmond, leaves of *E. smithii*, Nov. 1994, *G. Kemp* (PREM 51921, cultures STE-U 819–821).

Notes. This species is most similar to *M. cryptica* and *M. grandis.* It can, however, be distinguished from *M. cryptica* by its ascospores that darken at germination, smaller ascospores and cultural characteristics. Ascospores of *M. grandis* also darken at germination, they germinate from one cell (adjacent to the polar region), germ tubes subsequently develop parallel to the long axis of spores, and pigmentation in these is uniform (FIG. 19). In *M. africana*, ascospores also germinate adjacent to the polar regions, but germinate from both cells. Furthermore, germinating ascospores become more distorted than those of *M. grandis*, and the original spore is darker brown than the germ tubes. Although *M. africana* forms small, circular lesions on *E. viminalis*, leaf spots up to 10 mm diam were observed on other *Eucalyptus* spp.

2. **Mycosphaerella crystallina** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 4, 20–26

Laesiones amphigenae, subcirculares, 2–10 mm diam, coalescentes, pallide brunneae, marginibus atrobrunneis elevatis, paginis adaxialibus leucobrunneae paginis inferioribus, marginibus elevatis concoloratis paginis abaxialibus. Pseudothecia hypophylla, solitaria, 12–15 per mm², nigra, subepidermalia, demum erumpentia ad superficialia (plus quam *M. juvenis*), globosa, 70–110 µm lata, 70–90 µm alta; ostiola apicalia papillata 10–15 µm diam; paries consistens in 3–4 stratis *texturae angularis*, mediobrunneae, subhymenium basis consistens in 1–2 stratis cellarum hyalinarum. Asci aparaphysati, fasciculati, bitunicati, subsessiles, obovoidei ad late ellipsoidei, recti vel incurvi, 8-sporis, 35–55 × 11–13 µm. Ascosporae 2–3 seriate, superpositae, hyalinae, guttulatae, parietibus tenuibus, rectae rare curvatae, obovoideae, cellulis basalibus et apicalibus obtusis, latissimae apicibus, mediano 1-septatae, non colligatae ad septum, attenuatae ad extremis, sed prominentius attenuatae ad extrema inferioria (11–)12–14(–15) × 3–3.5(–4) µm. Spermatia hyalina, anguste ellipsoidea ad cylindrica et allantoidea, attenuata ad extremis obtusis, basis truncatis, 4.5–8 × 1.5–2.5 µm. Status anamorphicus *Pseudocercospora crystallina* Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Natal: Umvoti, leaves of *E. bicostata*, Oct. 1994, *M.J. Wingfield* (PREM 51922, cultures ex type STE-U 800–802).

Etymology. Name derived from the ability of this fungus to form crystals in culture.

Anamorph. **Pseudocercospora crystallina** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 23, 26

Mycelium internum et externum, consistens in hyphis ramosis, septatis, laevibus, pallide brunneis, 2–4 µm diam. Conidiophorae redactae ad cellulis conidiogenis. Cellulae conidiogenae inconspicuae, in mycelio integratae, mono- ad polyblasticae, sympodiales, cicatricibus, inconspicuis, non crassae, 2–7 × 1.5–2.5 µm. Conidia solitaria, sinuata, laevia, olivacea, anguste obclavata apicibus subobtusis et basibus longis obconicis truncatis, latissima parte mediano cellularum basale, guttulatae, multiseptatae, 50–200 × 2–3 µm. Status teleomorphicus Mycosphaerella crystallina Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Natal: Umvoti, leaves of *E. bicostata*, Oct. 1994, *M.J. Wingfield* (PREM 51923, cultures ex type STE-U 800–802).

Leaf spots amphigenous, subcircular, 2–10 mm diam, coalescing to form larger blotches, light brown, surrounded by raised, dark brown borders on the adaxial surfaces, whitish-brown on the lower surfaces, surrounded by raised, concolorous borders on the abaxial surfaces. Pseudothecia hypophyllous, single, 12–15 per colonized mm², black, subepidermal, becoming erumpent to superficial, globose, 70–110 µm wide, 70–90 µm high; apical ostioles 10–15 µm diam, becoming papillate; walls consisting of 3–4 layers of medium brown *textura angularis*, sub-hymenium layer at base consisting of 1–2 layers of hyaline cells. Asci obovoid to broadly ellipsoidal, straight or incurved, 8-spored, 35–55 × 11–13 µm. Ascospores bi- to triseriate, overlapping, hyaline, guttulate, thin-walled, straight, rarely curved, obovoid, with obtuse basal and bluntly obtuse apical cells, widest near apices, medianly 1-septate, not constricted at septum, tapering toward both ends, but with more prominent taper towards the lower end, (11–)12–14(–15) × 3–3.5(–4) µm. Spermogonia amphigenous, similar to pseudothecia in anatomy. Spermatia hyaline, narrowly ellipsoidal to cylindrical and allantoid, tapering to acutely rounded apices, with truncate bases, 4.5–8 × 1.5–2.5 µm. Mycelium internal and external, consisting of branched, septate, smooth, light brown to olivaceous hyphae, 2–4 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells inconspicuous, integrated on mycelium, mono- to polyblastic, sympodial, scars inconspicuous, unthickened, 2–7 × 1.5–2.5 µm. Conidia solitary, sinuous, smooth, olivaceous, narrowly obclavate with a subobtuse apex and long obconically truncate base, widest in the middle of the basal cell, guttulate, multiseptate, 50–200 × 2–3 µm.

Ascospore germination on MEA after 24 h of incubation. Ascospore germination from both ends, germ tubes parallel to the long axis of the spore, not darkening during germination, distorting slightly, becoming constricted at the septum, ascospore cells becoming 5–5.5(–6) µm wide upon germination, with lateral branches present on germ tubes 24 h after germination.

Cultures. Colonies 37 mm diam on MEA after 1 mo at 25 C in the dark, even edged, but diffuse, aerial mycelium profuse, grey olivaceous, colonies olivaceous black 27""k (bottom), grey olivaceous 23""i (top), not-sectored, fluffy. After 2 wk on MEA at 25 C under continuous near-ultraviolet light, red crystals form in the agar.

Cardinal temperature requirements for growth. 10 C min., 25 C opt., above 35 C max.

Hosts. E. bicostata and *E. grandis* × *camaldulensis*.

Distribution. Natal Province.

Additional collection examined. SOUTH AFRICA. Natal: leaf litter of *E. grandis* × *camaldulensis*, Jun. 1995, *M.J. Wingfield* (PREM 51937, culture STE-U 1178–1180).

Notes. Based on ascospore morphology and leaf symptoms *M. crystallina* is most similar to *M. molleriana* and *M. juvenis*. However, it can be distinguished

from these taxa by its faster growth rate in culture, the formation of red crystals in agar, its more prominently erumpent pseudothecia, as well as its distinct *Pseudocercospora* anamorph. Pseudothecia, spermogonia and the anamorph develop readily on CLA.

3. **Mycosphaerella ellipsoidea** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 5, 27, 28, 35–38

Laesiones amphigenae, subcirculares, 2–10 mm diam, pallide brunneae, marginibus leviter elevatis, adaxiale mediobrunneis, concoloratis paginis inferioribus. Pseudothecia amphigena, solitaria, inconspicua, 5–15 per mm², nigra, subepidermalia, globosa, 40–60 µm lata, 50–70 µm alta; ostiola apicalia papillata 10–15 µm diam; paries consistens in 3–4 stratis *texturae angularis*, mediobrunneae, subhymenium basis consistens in 1–2 stratis cellarum hyalinarum. Asci aparaphysati, fasciculati, bitunicati, cylindracei, recti vel incurvi, 8-spori, 30–45 × 6–8 µm. Ascosporae multiseriatae, imbricatae, hyalinae, prominente guttulatae, parietibus tennibus, rectae vel leviter curvae, fusoideo-ellipsoideae, obtusae, latissimae admodum supra septum, mediano uniseptatae, non constrictae ad septum, attenuatae ad extremis ambobus (8–)10–11 × (2–)2.5–3 µm. Spermogonia in morphologia pseudotheciis similia, et in pagina folii intermixa. Spermatia hyalina, recta, bacilliformia, obtusa, 2–4 × 1–1.5 µm. Status anamorphicus Uwebraunia ellipsoidea Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Western Cape: Pampoenvlei, on leaves of *E. cladocalyx*, Nov. 1994, *P.W. Crous* (PREM 51924, cultures ex type STE-U 849–851).

Etymology. Name refers to the distinct ellipsoidal shape of the ascospores in this species.

Anamorph. **Uwebraunia ellipsoidea** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 28, 38

Mycelium hyphis pallide brunneis, 2–4 µm diam. Cellulae conidiogenae laeves, pallide ad medio-brunneae, subulatae rare lageniformes, proliferationibus usque ad 3 percurrentibus, 15–30 × 2–4 µm. Conidia terminalia, solitaria, pallide olivacea, laevia, obclavata, obtusa base obconico-truncata, inaequaliter 1-septata, (16–)17–21(–22) × (3–)4–5(–6); hila subtruncata, non crassa, non atra vel refractiva, 2 µm lata. Status teleomorphicis Mycosphaerella ellipsoidea Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Western Cape: Pampoenvlei, on leaves of *E. cladocalyx*, Nov. 1994, *P.W. Crous* (PREM 51925, cultures ex type STE-U 849–851).

Leaf spots amphigenous, subcircular, 2–10 mm diam, light brown, surrounded by slightly raised borders, medium brown on the adaxial surfaces, concolorous on the lower surfaces. Pseudothecia amphigenous, single, inconspicuous, 5–15 per colonized mm², black, subepidermal, globose, 40–60 µm wide, 50–70 µm high; apical ostioles 10–15 µm diam, becoming papillate; walls consisting of 3–4 layers of medium brown *textura angularis*, sub-hymenium layer at base consisting of 1–2 layers of hyaline cells. Asci cylindrical, straight or incurved, 8-spored, 30–45 × 6–8 µm. Ascospores multiseriate, overlapping, hyaline, prominently guttulate, thin-walled, straight or slightly curved, fusoid-ellipsoidal with obtuse apices, widest just above the septa, 1-septate, not constricted at median septa, tapering toward both ends, (8–)10–11 × (2–)2.5–3 µm. Spermogonia similar to pseudothecia in morphology, and intermixed on the leaf surface. Spermatia hyaline, straight, rod-shaped with obtuse ends, 2–4 × 1–1.5 µm. Mycelium internal and external, consisting of branched, septate, smooth, hyaline to light brown hyphae, 2–4 µm diam, giving rise to single conidiophores. Conidiophores separate, arising from mycelium, reduced to conidiogenous cells. Conidiogenous cells smooth, light to medium brown, subcylindrical, subulate, rarely lageniform, straight to sinuous, monopodial, hologenous, with up to 3 proliferations at the subtruncate apex, 15–30 × 2–4 µm. Conidia terminal, solitary, pale olivaceous, smooth, obclavate, apex obtuse, base obconically truncate, 1-septate above the middle, (16–)17–21(–22) × (3–)4–5(–6) µm; hilum subtruncate, unthickened, not darkened or refractive, flattened, 2 µm wide.

Ascospore germination on MEA after 24 h of incubation. Ascospores germinating from both ends; germ tubes parallel to the long axis of the spore, not darkening during germination, initially with no constriction at septum, eventually with a slight constriction, cells becoming 3.5–4(–4.5) µm wide, and developing lateral branches 24–48 h after germination.

Cultures. Colonies 24 mm diam on MEA after 1 mo at 25 C in the dark, even or uneven edged; aerial mycelium profuse,white with pink patches; colonies olivaceous black 27""k (bottom), and on the top the margins are olivaceous black, but the rest consists of fluffy aerial mycelium, not-sectored.

Cardinal temperature requirements for growth. Below 5 C min., 25 C opt., below 35 C max.

Host. E. cladocalyx.

Distribution. Western Cape Province.

Notes. In terms of symptom expression, ascospore morphology and germination, there is little difference between *M. ellipsoidea* and *M. heimii*. However, colonies of *M. ellipsoidea* are white on the surface of MEA, and become bright pink under near-ultraviolet light, producing a diffuse brown pigment in the agar with age. In contrast, those of *M. heimii* are olivaceous-green to dark green, and readily produce the anamorph, *Pseudocercospora heimii* Crous, in culture (Crous and Swart, 1995). The anamorph of *M. ellipsoidea* can be distinguished from that of *M. juvenis* by its smaller conidia that are septate above the middle, and few, more tightly aggregated proliferations

on conidiogenous cells that tend to be more subcylindrical in shape.

4. **Mycosphaerella juvenis** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 1, 2, 8–10, 11–15

Laesiones amphigenae, subcirculares, separatae, confluescentes, 2–12 mm diam, aequaliter pallide-brunneae paginis adaxialibus, albo-brunneae paginis inferioribus, marginibus elevatis. Pseudothecia hypophylla, solitaria, aequaliter dispersa, 16–35 per mm², nigra, subepidermalia, demum erumpentia, globosa, 70–90 µm lata, 65–90 µm alta; ostiola apicalia papillata 10–15 µm diam; paries consistens in 3–4 stratis *texturae angularis*, mediobrunneae, subhymenium basis consistens in 1–2 stratis cellarum hyalinarum. Asci aparaphysati, fasciculati, bitunicati, subsessiles, obovoidei ad late ellipsoidei, recti vel incurvi, 8-sporis, 38–55 × 10–13 µm. Ascosporae 2–3 seriate, superpositae, hyalinae, guttulatae, parietibus tennibus, rectae rare curvatae, obovoideae, cellulis basalibus et apicalibus obtusis, latissimae apicibus, mediano 1-septatae, non colligatae ad septum, attenuatae ad extrema, sed prominentius attenuatae ad extrema inferioria (10–)11–13(–15) × 3–3.5(–4) µm. Spermogonia ignota. Status anamorphicus Uwebraunia juvenis Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Natal: Pietermaritzburg, leaves of *E. nitens*, Jan. 1995, *M.J. Wingfield* (PREM 51910, cultures ex type STE-U 932–934).

Etymology. Name depicts the ability of this fungus to infect only juvenile leaves.

Anamorph. **Uwebraunia juvenis** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 10, 13–15

Cellulae conidiogenae laeves, pallide ad medio-brunneae, subcylindraceae, vel basale subulatae et superiore subcylindraceae, rectae vel geniculato-sinuatae, monopodiales, hologena proliferationibus 20 percurrentibus, 20–55 × 4–6 µm. Conidia terminalia, solitaria, pallide olivacea, laevia, obclavata, obtusa base obconico-truncata, 1-septata, demum prominenter constricta in septo, recta vel curvata, (25–)26–30(–40) × (4–)4.5–5.5(–6); hila non crassa, non atra vel refractiva, 1.5–2 µm lata. Status teleomorphicus Mycosphaerella juvenis Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Natal: Pietermaritzburg, leaves of *E. nitens*, Jan. 1995, *M.J. Wingfield* (PREM 51915, cultures ex type STE-U 932–934).

Leaf spots amphigenous, round to irregular, separate, becoming confluent, 2–12 mm diam, evenly light brown on adaxial surface, whitish brown on abaxial surface, surrounded by raised borders, dark brown on the adaxial surfaces, concolorous with the lesion on the abaxial surfaces. Pseudothecia hypophyllous, single, evenly distributed, 16–35 per colonized mm², black, subepidermal, becoming slightly erumpent, globose, 70–90 µm wide, 65–90 µm high; apical ostioles 10–15 µm diam, becoming papillate; walls consisting of 3–4 layers of medium brown *textura angularis*, sub-hymenium layer at base consisting of 1–2 layers of hyaline cells. Asci obovoid to broadly ellipsoidal, straight or incurved, 8-spored, 38–55 × 10–13 µm. Ascospores bi- to tri-seriate, overlapping, hyaline, guttulate, thick-walled, straight, rarely curved in asci, obovoid, with obtuse ends, widest near apex, medianly 1-septate, not constricted at septum, tapering toward both apices, but with more prominent taper towards lower end (10–)11–13(–15) × 3–3.5(–4) µm. Spermogonia not observed. Mycelium internal and external, consisting of branched, septate, smooth, hyaline to olivaceous brown hyphae, 1.5–4 µm diam. Conidiophores separate, arising singly from mycelium, reduced to conidiogenous cells. Conidiogenous cells smooth, light to medium brown, subcylindrical, or with subulate basal half and subcylindrical upper half, straight or geniculate-sinuous, monopodial, hologenous, 20–55 × 4–6 µm with up to 20 widely spaced, percurrent proliferations. Conidia terminal, solitary, pale olivaceous, smooth, obclavate with obtuse apex and obconically truncate base, 1-septate, becoming prominently constricted at septum when mature, straight or curved, (25–)26–30(–40) × (4–)4.5–5.5(–6) µm; hilum not thickened, not darkened or refractive, 1.5–2 µm wide.

Ascospore germination on MEA after 24 h of incubation. Germinating from one or both ends, germ tubes parallel to the long axis of the spore, not darkening during germination, becoming prominently constricted at median septum and distorting, cells becoming (3.5–)5(–7) µm wide upon germination.

Cultures. Colonies 16–29 mm diam on MEA after 1 mo at 25 C in the dark, uneven edged, aerial mycelium sparse, olivaceous black 27""k (bottom), grey olivaceous 23""I (top), colonies frequently sectoring, and developing erect hyphal tufts. A peculiar feature of *M. juvenis* is that ascospores germinate readily at 25 C, but die soon afterwards. To ensure that growth continues after germination, ascospores must be transferred to 15 C. After colonies become visible, cultures can be placed at 25 C, where they will continue to grow. Cultures sporulate well on CLA at 15 C under near-ultraviolet light. Colonies of varying morphology are obtained in culture, and vary from black to olivaceous-black underneath, and grey to grey-olivaceous on the upper surface, frequently with white aerial mycelium. Some colonies can also become brown to black underneath, with a brown to white-brown aerial mycelium and sparse growth. Brown colonies usually sporulate more profusely that the darker colored colonies.

Cardinal temperature requirements for growth. 5–10 C min., 25 C opt., above 30 C max.

Hosts. E. grandis and *E. nitens*.

Distribution: Western Cape, Transvaal and Natal Provinces.

Additional collections examined. SOUTH AFRICA. Transvaal: Piet Retief, Iswepe, leaves of *E. nitens*, Feb. 1995, *M.J. Wingfield* (teleomorph PREM 51911, anamorph PREM 51916, cultures STE-U 929–931); Hazy View, leaves of *E. grandis*, Mar. 1995, *M.J. Wingfield* (PREM 51912, cultures STE-U 1004–1006); Witbank, leaves of *E. grandis*, Mar. 1995, *M.J. Wingfield* (PREM 51913, cultures STE-U 1007–1008). Western Cape: Grabouw, leaves of *E. nitens*, Nov. 1994, *P.W. Crous* (PREM 51914, cultures STE-U 830–832).

Notes. Mycosphaerella juvenis is most similar in ascospore morphology and symptom expression to *M. molleriana*. The two species can, however, easily be distinguished by their mode of ascospore germination, and the *Uwebraunia* anamorph formed by cultures of *M. juvenis*. Ascospores of *M. molleriana* (syn. = *M. nubilosa)* become slightly constricted at their median septa during germination (Park and Keane,1982), but do not become prominently distorted as observed in *M. juvenis*. In subsequent collections obtained from Australia, Crous et al. (1995b) found that ascospores of *M. molleriana* did not necessarily have a constriction at their median septum upon germination. The interpretation of the variation observed in South African herbarium specimens of *M. molleriana* by Crous et al. (1991) was incorrect. Lesions of *M. molleriana* do not have red-purple margins, and ascospores on the type specimen are (11–)12–16(–17) × 3–3.5(–4.5) μm.

5. **Mycosphaerella lateralis** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 6, 29, 30, 39–41

Laesiones amphigenae, subcirculares, 3–12 mm diam, griseo-brunneae, aequaliter medio-brunneae paginis adaxialibus, concoloratis paginis inferioribus, marginibus elevatis. Pseudothecia amphigena, praecipue epiphylla, solitaria, inconspicua, nigra, subepidermalia, demum erumpentia, globosa, 40–60 μm lata, 50–70 μm alta; ostiola apicalia papillata 10–15 μm diam; paries consistens in 3–4 stratis *texturae angularis*, mediobrunneae, subhymenium basis consistens in 1–2 stratis cellarum hyalinarum. Asci aparaphysati, fasciculati, bitunicati, subsessiles, cylindracei, recti vel incurvi, 8-sporis, 30–50 × 6–10 μm. Ascosporae multiseriatae, superpositae, hyalinae, guttulatae, rectae vel leviter incurvae, fusoideo-ellipsoideae, obtusae, latissimae admodum supra septum, mediano uniseptatae, non constrictae ad septum, attenuatae ad extremis ambobus, (7–)8–14(–16) × 2–2.5 (–3) μm. Spermogonia ignota. Status anamorphicus Uwebraunia lateralis Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Transvaal: Tzaneen, Magoebaskloof, on leaves of *E. grandis* × *saligna* hybrid, Oct. 1994, *G. Kemp* (PREM 51926, cultures ex type STE-U 825–826).

Etymology. Named after its ability to form lateral branches 24–48 h after germination.

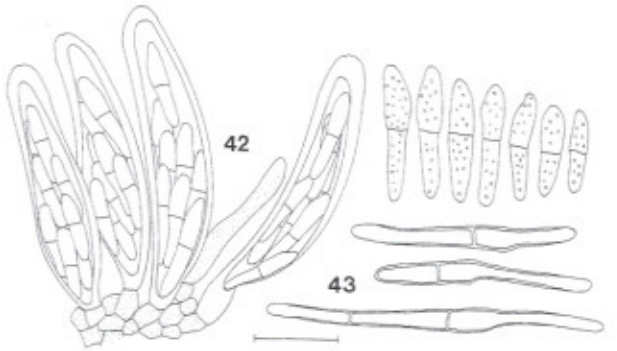
Anamorph. **Uwebraunia lateralis** Crous *et* M.J. Wingf., *sp. nov.* FIGS. 29, 30, 41

Mycelium hyphis pallide brunneis, 1.5–3 μm diam. Conidiophora subcylindracea, laeves, pallide ad medio-brunneae, 0(–2)–septata, 20–70 × 3–5 μm. Cellulae conidiogenae subcylindracea, subulatae rare lageniformes, proliferationibus usque ad 4 percurrentibus, 20–55 × 3–5 μm. Conidia terminalia, solitaria, pallide olivacea, laevia, obclavata, obtusa base obconico-truncata, 1-septata, (15–)17–21(–35) /× (2–)3.5–4(–4.5); hila subtruncata, non crassa, non atra vel refractiva, 1.5–2 μm lata. Status teleomorphicus *Mycosphaerella lateralis* Crous et M.J. Wingf.

HOLOTYPE. SOUTH AFRICA. Transvaal: Tzaneen, Magoebaskloof, on leaves of *E. grandis* × *saligna* hybrid, Oct. 1994, *G. Kemp* (PREM 51929, cultures ex type STE-U 825–826).

Leaf spots amphigenous, subcircular, 3–12 mm diam, grey-brown, surrounded by raised borders, medium brown on the adaxial surfaces concolorous on the lower surfaces. Pseudothecia amphigenous, mainly epiphyllous, single, inconspicuous, sparse on lesions, black, subepidermal becoming erumpent, globose, 40–60 μm wide, 50–70 μm high; apical ostioles 10–15 μm diam; walls consisting of 3–4 layers of medium brown *textura angularis*, sub-hymenium layer at base consisting of 1–2 layers of hyaline cells. Asci cylindrical, straight or incurved, 8-spored, 30–50 × 6–10 μm. Ascospores multiseriate, overlapping, hyaline, guttulate, thin-walled, straight or slightly curved, fusoid-ellipsoidal with an obtuse apex, widest in the middle of the apical cell, medianly 1-septate, not constricted at septum, tapering toward both ends, (7–)8–14(–16) × 2–2.5(–3) μm. Spermogonia unknown. Mycelium internal and external, consisting of branched, septate, smooth, hyaline to light brown hyphae, 1.5–3 μm diam, giving rise to single conidiophores. Conidiophores separate, arising from mycelium, subcylindrical, tapering to a bluntly rounded apex, straight or geniculate-sinuous, smooth, medium brown, 0(–2)–septate, 20–70 × 3–5 μm. Conidiogenous cells smooth, light to medium brown, subcylindrical, subulate, rarely lageniform, straight to sinuous, monopodial, hologenous, with up to 4 proliferations at the subtruncate apex, 20–55 × 3–5 μm. Conidia terminal, solitary, pale olivaceous, smooth, obclavate, apex obtuse, base obconically truncate, medianly 1-septate, (15–)17–21(–35) × (2–)3.5–4 (–4.5) μm; hilum subtruncate, unthickened, not darkened or refractive, flattened, 1.5–2 μm wide.

Ascospore germination on MEA after 24 h of incubation. Ascospores germinating from both ends, germ tubes parallel to the long axis of the spore, not



FIGS. 42, 43. *Mycosphaerella marksii*. 42. Asci and ascospores. 43. Germinating ascospores after 24 h on MEA. Scale bar = 10 μm.

darkening during germination, constricted at septum, developing lateral branches 24–48 h after germination.

Cultures. Colonies 53 mm diam on MEA after 1 mo at 25 C in the dark, even edged; aerial mycelium profuse, colonies grey olivaceous 23""i, on the top reverse.

Cardinal temperature requirements for growth. Below 5 C min., 25 C opt., below 35 C max.

Hosts. *E. grandis* × *saligna*, *E. saligna* and *E. nitens*.

Distribution. Northern Transvaal, Natal Provinces.

Additional collections examined. SOUTH AFRICA. Transvaal: Tzaneen, Magoebaskloof, on leaves of *E. grandis* × *saligna* hybrid, Oct. 1994, *G. Kemp* (teleomorph PREM 51927, anamorph PREM 51930, cultures STE-U 803–805); Tzaneen, Magoebaskloof, on leaves of *E. saligna*, Oct. 1994, *G. Kemp* (teleomorph PREM 51928, anamorph PREM 51931, cultures STE-U 806–808). Natal: Seven Oaks Plantation, on leaves of *E. nitens*, 12 July 1995, *M.J. Wingfield* (cultures STE-U 1188–1190).

Notes. Mycosphaerella lateralis is one of three species described here that forms an *Uwebraunia* anamorph in culture. There are many similarities between *M. lateralis* and *M. ellipsoidea*. The two species can be distiguished by the larger ascospores, mode of ascospore germination and the distinct cultural characteristics of *M. lateralis*. Futhermore, *U. lateralis* has longer, septate conidiophores, and narrower conidia than *U. ellipsoidea*. *Mycosphaerella lateralis* is further distinguished from species such as *M. molleriana* and *M. gracilis* based on symptom expression, distribution of pseudothecia in lesions, ascospore germination and cultural characteristics.

6. *Mycosphaerella marksii* Carnegie & Keane, Mycol. Res. 98: 414. 1994. FIGS. 7, 31, 32, 42, 43

Leaf spots amphigenous, subcircular to irregular, 3–20 mm diam, light brown, surrounded by raised, medium brown borders, and frequently with red-purple margins. Pseudothecia predominantly epiphyllous, single, dispersed, 5–15 per colonized mm^2, black, subepidermal, becoming erumpent and papillate, globose, 50–60 μm wide, 60–70 μm high; apical papillate ostioles 10–20 μm diam; walls consisting of 3–4 layers of medium brown *textura angularis*, subhymenium layer at base consisting of 1–2 layers of hyaline cells. Asci obovoid to broadly ellipsoidal, straight or incurved, 8-spored, 35–50 × 8–10 μm. Ascospores bi- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid-ellipsoidal with an obtuse basal and asymmetrical apical cell, widest in middle of the apical cell, medianly 1-septate, not constricted at septum, tapering toward both ends, but with more prominent taper towards lower end, (11–)12–14(–16) × 2–2.5(–3) μm. Spermogonia observed on leaves, or formed in culture on CLA after 2 wks. Spermatia hyaline, rod shaped with obtuse ends, smooth, 2.5–4 × 1–1.5 μm.

Anamorph. Unknown.

Ascospore germination on MEA after 24 h of incubation. Ascospores germinating from both ends, germ tubes parallel to the long axis of the spore, not darkening or distorting upon germination, cells becoming (3–)3.5–4(–5) μm wide upon germination.

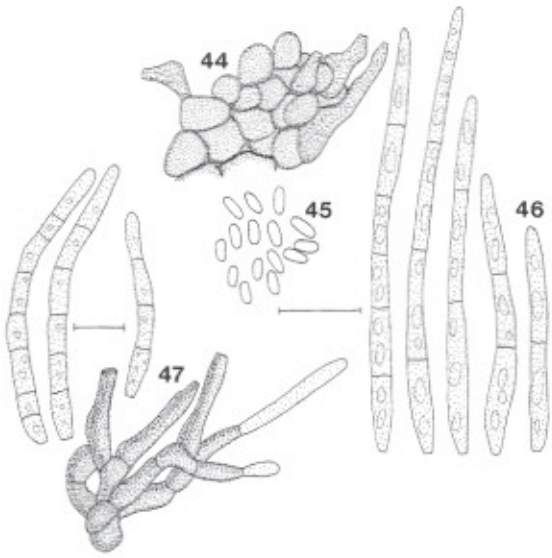
Cultures. Colonies 24–28 mm diam on MEA after 1 mo at 25 C in the dark, even or uneven edged, aerial mycelium sparse, white-grey if present, olivaceous black 27""k (bottom), olivaceous grey 23""I (top), colony often sectored, smooth, spreading, regular or irregular, erect hyphal tufts absent.

Cardinal temperature requirements for growth. Above 5 C min., 25 C opt., below 35 C max.

Hosts. Collected in South Africa from *E. grandis* and *E. nitens*, but reported from numerous hosts in Australia (Crous et al., 1995b).

Distribution. Australia, and the Cape, Natal and Transvaal Provinces of South Africa.

Collections examined. AUSTRALIA. Victoria: Tostaree, on leaves of *E. botroides*, Oct. 1994, *A. Carnegie* (PREM 51932, cultures STE-U 982–984). SOUTH AFRICA. Transvaal: Sabie, on leaves of *E. grandis*, Dec. 1994, *M.J. Wingfield* (PREM 51933, cultures STE-U 893–895); Tzaneen, Magoebaskloof, on leaves of *E. grandis*, Oct. 1994, *G. Kemp* (cultures STE-U 814–815). Western Cape: Grabouw, on leaves of *E. nitens*, Nov. 1994, *P.W. Crous* (cultures STE-U 827–829). Natal: Richmond, on leaves of *E. grandis*, Nov. 1994, *G. Kemp* (PREM 51934, cultures STE-U 843–845); Kwambonambi, on leaves of *E. grandis*, Nov. 1994, *G. Kemp* (PREM 51935, cultures STE-U 797–799, 841–842).



FIGS. 44–47. *Pseudocercospora* spp. 44–46. *Pseudocercospora epispermogoniana*. 44. Conidiogenous cells on the outside of a spermogonium. 45. Spermatia. 46. Narrowly obclavata condia. 47. Subcylindrical conidia of *P. eucalyptorum* with wide, subtruncate hila. Scale bar = 10 μm.

Notes. Mycosphaerella marksii is a very characteristic species in having prominently asymmetrical apical ascospore cells. Cultures sporulate profusely after 2 wk on CLA. Although South African collections have slightly smaller asci and ascospores than reported for the type (Carnegie and Keane, 1994), in an examination of fresh Australian material, ascospores were found to be 11–18 × 2.5–3.5 μm, and thus overlapping with those from South Africa. Furthermore, leaf symptoms, germinating ascospores with cells (3–)3.5–4 μm wide, and cultural characteristics correspond closely with observations from South African collections.

In a collection of *E. grandis* × *saligna* leaves colonized by a species of *Mycosphaerella* similar to *M. marksii*, spermogonia with outer cells giving rise to conidiophores of a *Pseudocercospora* sp. were found. Single ascospore cultures obtained from these lesions were sterile. Hardly any material of the teleomorph, and no cultures of the anamorph were obtained. Because of our inability to clearly prove the link between the *Mycosphaerella* teleomorph and the *Pseudocercospora* anamorph, and the scant material of the teleomorph, only the anamorph is described below.

Pseudocercospora epispermogoniana Crous *et* M.J. Wingf., *sp. nov.* FIGS. 33, 44–46

Mycelium internum et externum consistens in hyphis ramosis septatis laevibus pallide brunneis, 1.5–2.5 μm diam. Conidiophorae redacta ad cellulis conidiogenis vel cellula solitarii, in parte exteriore spermogoniorum maturorum. Cellulae conidiogenae terminales, polyblasticae, sympodiales, rectae ad geniculato-sinuatae, apicalibus subtruncatis, 5–20 × 2.5–4 μm; cicatricibus inconspicuis. Conidia solitaria, anguste obclavata obtusa basibus longe obconicis subtruncatis, recta vel curvata, 28–65 × 2–3 μm, pallide olivacea, laevia, guttulata, 1–7-septata; hila inconspicua, 1–2 μm lata. Status teleomorphicus *Mycosphaerella* sp.

HOLOTYPE. SOUTH AFRICA. Transvaal: Tzaneen, Magoebaskloof, on leaves of *E. grandis* × *saligna* hybrid, Oct. 1994, *G. Kemp* (PREM 51936); cultures of a *Mycosphaerella* sp. possibly related to the anamorph are STE-U 822–824.

Etymology. Named after its conidiogenous cells occurring on spermogonia.

Mycelium internal and external, consisting of branched, septate, smooth, light brown hyphae, 1.5–2.5 μm diam. Conidiophores reduced to conidiogenous cells or one supporting cell, situated on the outside of mature spemogonia. Conidiogenous cells terminal, polyblastic, sympodial, straight to geniculate-sinuous, tapering to a subtruncate apex, 5–20 × 2.5–4 μm; terminal scars inconspicuous. Conidia solitary, narrowly obclavate with a subobtuse apex and long obconically subtruncate base, straight or curved, 28–65 × 2–3 μm, pale olivaceous, smooth, guttulate, 1–7-septate; hilum inconspicuous, 1–2 μm wide.

Teleomorph. *Mycosphaerella* sp., similar to *M. marksii* (presumed).

Host. E. grandis × *saligna* hybrid.

Distribution. Northern Transvaal Province.

Notes. Several cercosporoid fungi are known from *Eucalyptus* spp. (Crous et al., 1989; Crous and Alfenas, 1995; Crous and Swart, 1995; Crous and Braun, 1996). Of these, *P. epispermogoniana* is most similar to *P. eucalyptorum* Crous et al. (FIGS. 34, 47). It can, however, be distinguished from the latter species by its distinct conidial shape, narrower conidia and hila (conidia 25–65 × 2.5–4 μm; hila 2.5–3 μm in *P. eucalyptorum*) and conidiophores that are reduced to conidiogenous cells. Conidia fall into the range of that accepted for *P. crystallina*, but the conidiogenous cells are distinct, and the spermatia are rod-shaped, not ellipsoidal and allantoid as is the case for *M. crystallina*. Although conidiogenous cells are situated on the outside of spermogonia on lesions colonized by *M. marksii*, none of the cultures derived from ascospores produced an anamorph in culture. We have examined numerous *M. marksii*-like collections from Australia, South Africa and Portugal, and are of the opinion that several taxa may be involved in this complex. We are hesitant, therefore, to link *P. epispermogoniana* to any teleomorph before more conclusive evidence or additional collections of the anamorph together with the teleomorph have been obtained.

In this paper we have shown that the genus of *Mycosphaerella* on *Eucalyptus* in South Africa is much more complex and diverse than was ever expected. When taking species reported in this study into consideration, a total of 21 are presently acknowledged to occur on this host genus, with several others yet awaiting description. At this stage many *Mycosphaerella* spp. are not known from within the native range of *Eucalyptus,* and may not even occur there. If this is so, these pathogens have adapted from native plants, probably Myrtaceae, to infect eucalypts. These would then potentially threaten *Eucalyptus* spp. where they are native. Some examples of pathogens with wider host ranges in the Myrtaceae are the guava rust fungus, *Puccinia psidii* G. Winter (Ferreira, 1989), *Harknessia* leaf spot fungi (Sutton and Pascoe, 1989) and the Cryphonectria canker pathogen *Cryphonectria cubensis* (Bruner) Hodges (Hodges et al., 1986). This matter clearly deserves study and is most likely of significant international interest.

ACKNOWLEDGMENTS

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