**Mycosphaerella gracilis** and other species of *Mycosphaerella* associated with leaf spots of *Eucalyptus* in Indonesia

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**Abstract:** Collections of *Eucalyptus* leaves throughout the forested regions of Indonesia have revealed three species of *Mycosphaerella* associated with prominent leaf spot symptoms. A new species *Mycosphaerella gracilis* and its anamorph *Pseudocercospora gracilis* are described here, the connection between the anamorph and teleomorph having been confirmed by cultural studies. Similarly, *Stenella parkii* is described as the anamorph of *Mycosphaerella parkii*, and an additional species of *Mycosphaerella* is recorded.

**Key Words:** *Mycosphaerella gracilis*, *Mycosphaerella parkii*, *Pseudocercospora gracilis*, *Stenella parkii*, systematics

**INTRODUCTION**

Species of *Mycosphaerella* Johanson are regarded as among the most important leaf pathogens of *Eucalyptus* worldwide (Crous and Wingfield, 1991). Although several species have been recorded from this host (Corlett, 1991), recent studies have led to the description of a further two species from Brazil (Crous et al., 1993a, b), and three from Australia (Carnegie and Keane, 1994). Several workers (Park and Keane, 1982; Crous et al., 1991; Carnegie and Keane, 1994) have shown the importance of ascospore germination and cultural characteristics as additional criteria in distinguishing among the *Mycosphaerella* species occurring on *Eucalyptus*. Although leaf symptoms have been found to vary for *M. molleriana* (Thüm.) Lindau on different *Eucalyptus* species and on leaves of different ages in South Africa (Crous et al., 1991), Carnegie and Keane (1994) have shown that symptom expression is still valuable as a criterion in distinguishing the various species.

Collections of symptomatic *Eucalyptus* leaves from various plantations throughout Northern Sumatra (Indonesia) were infected with *M. parkii* Crous et al. and two other distinct species of *Mycosphaerella*. One of the latter we believe to be a previously undescribed species. These species were separable by their general morphology, mode of ascospore germination, cultural characters and symptom expression. The aim of the present study was to characterize the new species of *Mycosphaerella* and to discuss the variation in *M. parkii*.

**MATERIALS AND METHODS**

Lesions were excised from symptomatic leaves and single ascospore cultures derived on 2% malt-extract agar (Biolab) (MEA) as described by Crous et al. (1991). Germinating ascospores were examined after 24 h and transferred to MEA. Cultures were incubated for 2 wk at 25°C in the dark and subcultured onto carnation-leaf agar (CLA) (Crous et al., 1992) at 25°C under near-ultraviolet light. Single ascospore cultures were examined at weekly intervals for the presence of an anamorph.

**RESULTS AND DISCUSSION**

**Figs. 1, 6**

**ANAMORPH. Stenella parkii** Crous and Alfenas, sp. nov.  
**Fig. 1**


**STATUS TELEOMORPHICUS. Mycosphaerella parkii** Crous et al.


Lesions characteristic round to slightly irregular, light brown with raised margins on *E. grandis* and *E. saligna*;
on *E. globulus*, however, these margins can occasionally also be dark brown. *Mycelium* immersed in substrate, secondary mycelium superficial, brown, verruculose to verrucose, branched, septate, 1.5–3.5 μm wide. *Pseudothecia* amphigenous and immersed. Asci obclavate to cylindrical, 25–35 × 6–8 μm, with overlapping, straight, ellipsoidal ascospores, 8–12 × 2.5–3 μm, not constricted at the median septum. *Ascospore germination* parallel to perpendicular to the long axis of the spore. Anamorph occurring in close association with teleomorph. *Conidiophores* brown, verruculose, borne as lateral projections on curved, repeatedly geniculate, one to three-septate, 15–65 × 3–5 μm. *Conidiogenous cells* terminal, verruculose, brown, repeatedly geniculate, 7–20 × 3–5 μm; conidial scars conspicuously thickened, darkened, refractive. *Conidia* oliveaceous-brown, verruculose, single, obclavate, tapering to an obtuse apex and a long, obconically truncate base, straight or curved, indistinctly one to eight-septate, 25–200 × 2–2.5 μm; hila slightly thickened and darkened. *Cultures* olive-green on MEA, fast growing, sporulating well on CLA 3–4 wk after inoculation of the plates; mycelium and conidia in culture are similar to that observed in vivo.

*Hosts*. *E. globulus* Labill., *E. grandis* Hill ex Maid. and *E. saligna* Sm.

*Known distribution*. Brazil, Indonesia (northern Sumatra).


Since the description of *M. parkii* from *E. grandis* leaves in Brazil, it has also been associated with severe leaf spotting of other species such as *E. saligna* and *E. globulus*. Germinating ascospores from these Brazilian collections showed that germ tubes could also occur at right angles to the long axis of the spore, thus resembling *M. cryptica* (Park and Keane, 1982). Although particular modes of ascospore germination have been reported as characteristic of several *Mycro-
*Myosphaerella gracilis* Crous et Alfenas, sp. nov.

Figs. 2–5

*Laesiones amphigenae, irregulares, 2–6 mm diam, pallide brunneaee, cinctae margine exili elevata rufa ad brunnea. Ascocarpi amphigeni, solitarii, nigri, immersi, ad erumpentescentiores, globosi, 60–80 μm lati, 65–110 μm alti; ostiolum apicale papillatum ad 20–35 μm diam; paries in cellis medio-brunneis consistens, 3–4 stratis textura angularis, basis 2–3 stratis cellularum hyalinarum consistens. Asci aparaphysati, fasciculati, bitunicati, subsessiles, ovoidei ad obclavati, recti vel incurvati, 8-sporei, 32–45 × 8–12 μm. Ascosporae tri-ad multiseriatae, superpositae, hyalinae, guttatae, parietatis tenuibus, parum curvatae, rare rectae, anguste ellipsoidae, apicibus obtusis, 1-septatae, latissimae ad septum inconstrictum, ad apices duos attenuatae, apicibus germinantes, tubi ad axem longum paralleli, (10–)16–(20) × (2–)2.5–(3) μm.

STATUS ANAMORPHICUS. *Pseudocercospora gracilis* Crous et Alfenas.


ANAMORPH. *Pseudocercospora gracilis* Crous et Alfenas, sp. nov.

Fig. 2

Maculae foliorum ut in *M. gracilis*. Mycelium internum et externum, glabrum, ramulosum, septatum, olivaceo-brunneum, 1.5–3.5 μm diam. Conidiophora singularea in mycelio secondario pro projectionibus lateralibus, simplicia vel
ramulosa, 0–2-septata, cylindracea, recta ad geniculato-sinuata, olivaceo-brunnea, 5–25 × 2.5–3.5 μm. Cellulae conidiogenae integrae, cylindraceae, rectae ad geniculato-sinuatae, olivaceae, cicastricus conidia libus non incrassati. Conidia solitaria, cylindracea, recta vel curvata, apice obtusae et base truncata, 35–100 × 2–3 μm, indistincte 3–11-septata, guttulata, pallide olivacea, hilo non incrassato.

STATUS TELEMORPHICUS. Mycosphaerella gracilis Crous & Alfenas.

HOLOTYPUS. INDONESIA. North of Sumatra, Eucalyptus urophylla, 22 Nov. 1995, A.C. Alfenas (PREM 51719).

Lesions amphigenous, irregular, 2–6 mm in diam., light brown, surrounded by a raised, thin red to brown border. Mycelium immersed and superficial, of smooth, branched, septate, olivaceous brown hyphae, 1.5–3.5 μm diam. Pseudothecia amphigenous, single, black, immersed becoming erumpent, globoso, 60–80 μm wide, 65–110 μm high; apex papillate, ostiole 20–35 μm in diam.; wall consisting of three to four layers of medium brown textura angularis, base consisting of two to three layers of hyaline cells. Pseudoparaphyses lacking. Asci fasciculate, bitunicate, sessilissime, ovato obclavate, straight or incurvate, eight-spored, 32–45 × 8–12 μm. Ascospores tri- to multisierate, overlapping, hyaline, guttulate, thin-walled, narrowly ellipsoid with obtuse apices, slightly curved, rarely straight, one-septate, widest at unconstricted septum, tapering toward both apices, (10−)16−20 × (2−)2.5−3 μm. Ascospore germination occurring at both apices, with germ tubes growing parallel to the long axis of the spore. Conidiophores occurring singly on secondary mycelium as lateral projections, unbranched or branched, 0− to 2-septate, cylindrical to geniculate-sinuous, olivaceous brown, 5–25 × 2.5–3.5 μm. Conidiogenous cells integrated, cylindrical, straight to geniculate-sinuous, olivaceous, monoblastic or polyblastic, 5–15 × 2.5–3.5 μm, tapering to a truncate apex; conidial scars unthickened. Conidia solitary, cylindrical, straight or curved, with an obtuse apex and truncate base, 35–100 × 2–3 μm, indistinctly 3− to 11-septate, guttulate, pale olivaceous, hilum unthickened. Cultures grey on MEA, reaching 25–40 mm in diam. after 8 wk at 25 C under near-ultraviolet light. Colonies were grey with a dark grey to black submerged mycelium, and fluffy grey-white aerial hyphae. After 4 wk a Pseudocercospora anamorph developed on MEA, identical to that observed in vitro.

Host. E. urophylla S.T. Blake.

Known distribution. Indonesia (northern Sumatra), known only from type collection.

Ascospores of M. gracilis are (10−)16−20 × (2−)2.5−3 μm, thin-walled, narrowly ellipsoid with obtuse ends, widest in the middle, germinate from both apices, and have germ tubes growing parallel to the long axis of the spore. These features distinguish this species from all others known from Eucalyptus (Crous et al., 1993a, b; Carnegie & Keane, 1994). Pseudocercospora gracilis is similar to two other cercosporoid fungi known from Eucalyptus, Pseudocercospora eucalyptorum Crous et al. and Cercospora paraguayensis Kobayashi. It can, however, easily be distinguished from P. eucalyptorum by the absence of fasciculate conidiophores and from C. paraguayensis by its cylindrical conidia with truncate bases.

Mycosphaerella sp.

A Mycosphaerella sp. closely resembling M. moleriana was isolated from leaves of E. camaldulensis Dehnh. and E. grandis collected north of Sumatra. Leaf spots were light brown, circular to subcircular, amphigenous, with a slightly raised margin. Pseudothecia were amphigenous, subepidermal and yielded asci 40–50 × 11–13 μm and ascospores 8–14 × 2–4 μm, thus within the range accepted for M. moleriana (Crous et al., 1991). Ascospores were not constricted at the median septum, and germinated with their germ tubes parallel to the long axis of the spore (Fig. 7), typical of M. moleriana.

Park and Keane (1982) stated that two colony types were associated with cultures of M. moleriana, namely a black, dense, submerged mycelium with dense dark-green aerial hyphae, and a diffuse submerged mycelium with white to olive-green aerial hyphae. Very few of the germinated ascospores of the Indonesian collections of this species continued to grow in culture. Generally, colonies were black with a diffuse black submerged mycelium and black aerial mycelium. Colonies grew slower than observed for South African collections of M. moleriana, and leaf lesions were frequently indistinct. Furthermore, asci and ascospores were distinctly thick-walled. Ascospores also appeared slightly olivaceous (as observed for M. suberosa Crous et al.), and gave rise to verruculose hyphae. Although these collections share several characters with M. moleriana, we are of the opinion that the Indonesian material might represent yet another species. Additional material and cultural studies are required, however, to suitably characterize M. moleriana-like isolates from Indonesia.

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LITERATURE CITED


