

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

Mycosphaerella parkii Crous et al. Mycol. Res. 97: 582. 1993. FIGS. 1, 6

ANAMORPH. *Stenella parkii* Crous et Alfenas, sp. nov. FIG. 1

Maculae foliorum ut in *M. parkii*. Mycelium internum, secundarium externum, brunneum, verruculosum ad verrucosum, ramosum, septatum, 1.5–3.5 μm latum. Conidiophora brunnea verruculosa, cum projectionibus lateralibus in mycelio secundario portata, repetite geniculata, 1–3-septata, 15–65 \times 3–5 μm . Conidiogenae cellulae terminales, verruculosae, brunneae, repetite geniculatae, 7–20 \times 3–5 μm ; cicatrices conidiales conspicue incrassatae, fuscatae, refractivae. Conidia olivaceo-brunnea, verruculosa, singularia, obclavata, ad apicem obtusam et basim truncatam contracta, recta vel curvata, indistincte 1–8-septata, 25–200 \times 2–2.5 μm ; hila parum incrassata et fuscata.

STATUS TELEOMORPHICUS. *Mycosphaerella parkii* Crous et al.

HOLOTYPUS. BRAZIL. São Paulo, *Eucalyptus saligna*, Apr. 1993, P.W. Crous (PREM 51713).

Lesions characteristic round to slightly irregular, light brown with raised margins on *E. grandis* and *E. saligna*;

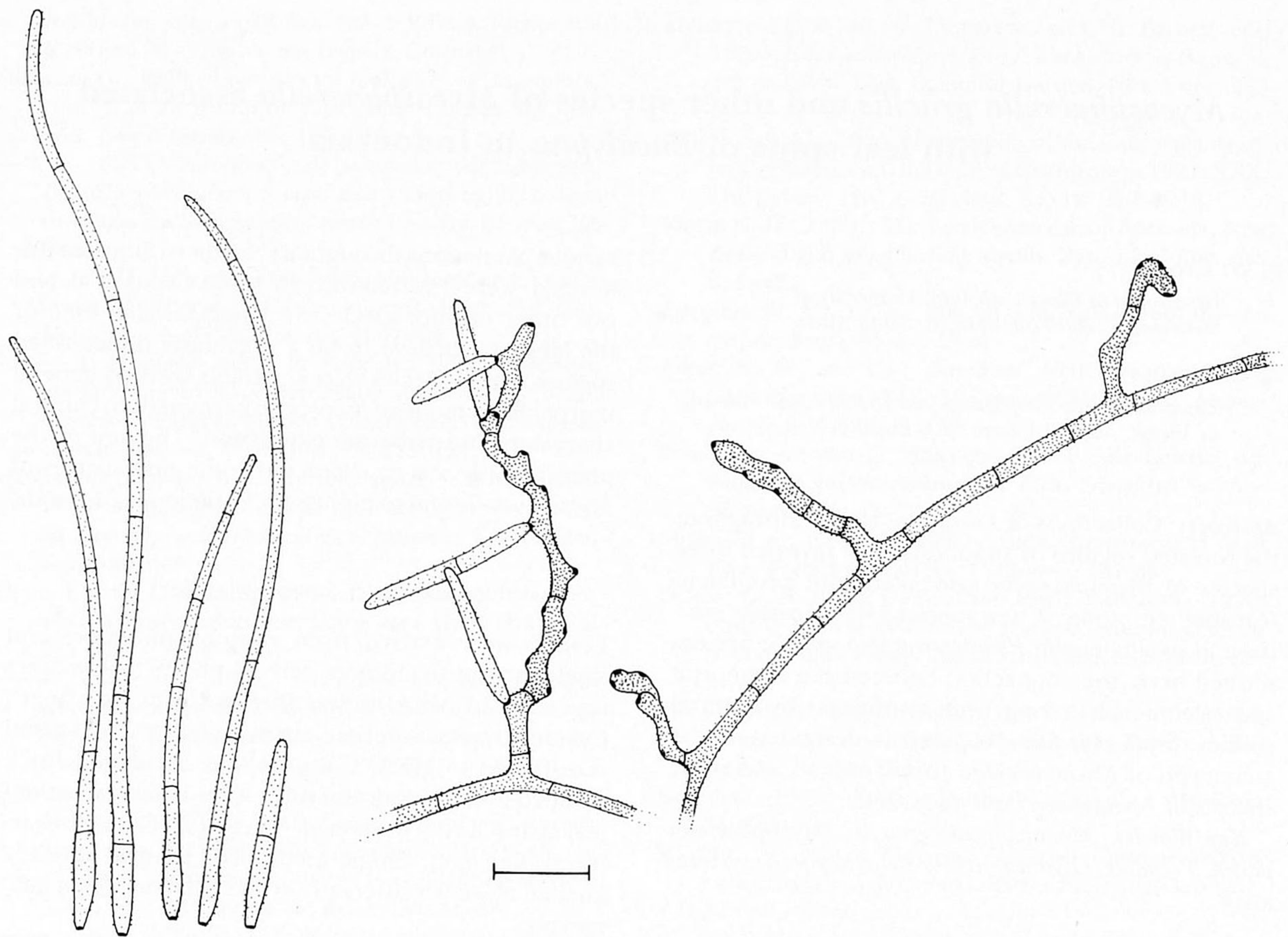


FIG. 1. *Stenella parkii*. Verruculose, olivaceous-brown, obclavate conidia with thickened hila forming on conidiogenous cells with thickened, refractive conidial scars (PREM 51713). Bar = 10 μ m.

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 μ m wide. *Pseudothecia* amphigenous and immersed. *Asci* obclavate to cylindrical, 25–35 \times 6–8 μ m, with overlapping, straight, ellipsoidal ascospores, 8–12 \times 2.5–3 μ m, 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 \times 3–5 μ m. *Conidiogenous cells* terminal, verruculose, brown, repeatedly geniculate, 7–20 \times 3–5 μ m; conidial scars conspicuously thickened, darkened, refractive. *Conidia* olivaceous-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 \times 2–2.5 μ m; hila slightly thickened and darkened. *Cultures* olive-green on MEA, fast grow-

ing, 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).

Specimens examined. BRAZIL. São Paulo, *Eucalyptus saligna*, Apr. 1993, P.W. Crous (PREM 51713, HOLOTYPE). Rio Grande do Sul, *E. globulus*, 7 Jul. 1993, F.A. Ferreira (CPC 651, PREM 51714). INDONESIA. North of Sumatra, *E. grandis*, 22 Nov. 1993, F.A. Alfenas (PREM 51715).

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 *Myc-*

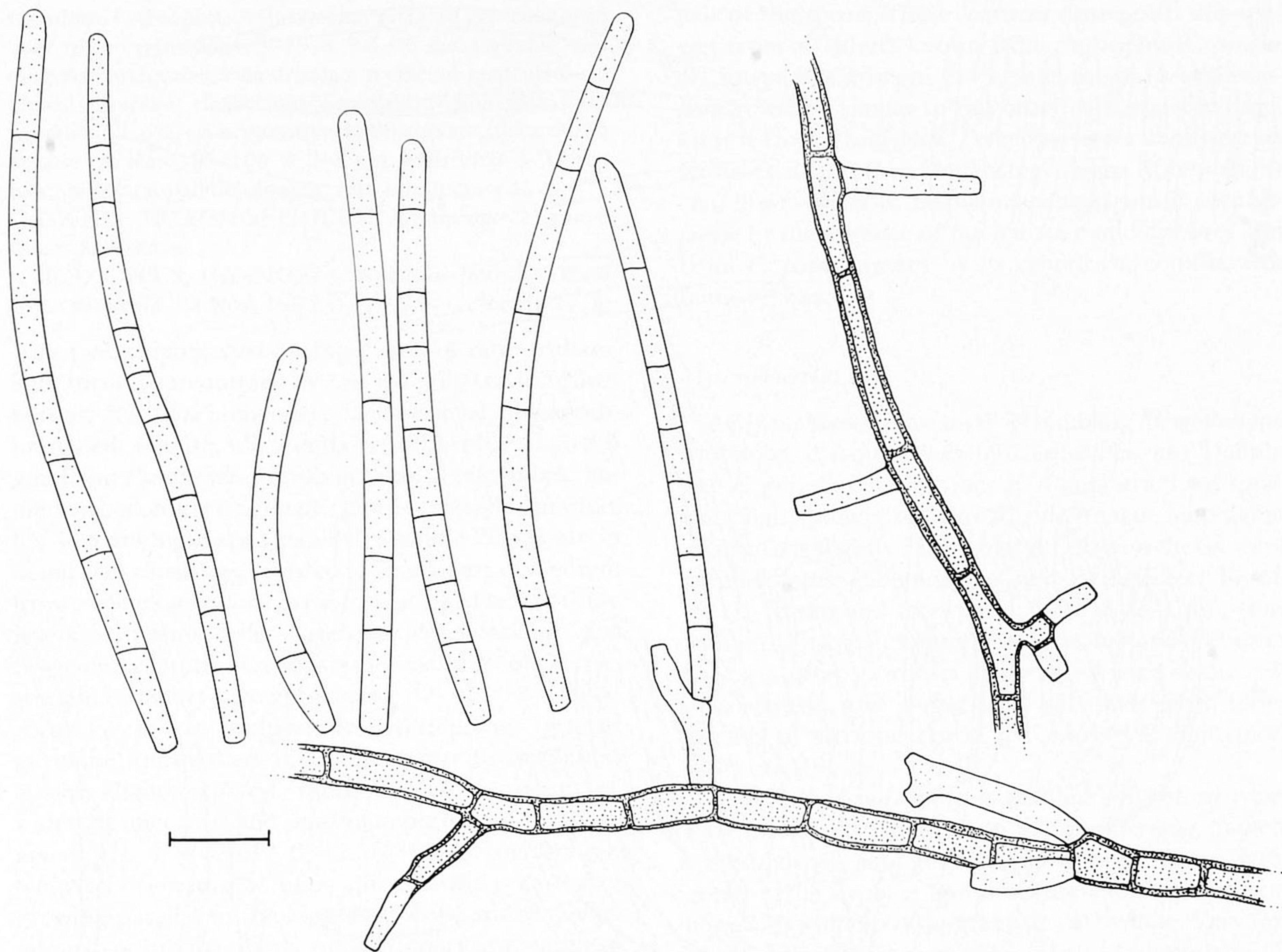


FIG. 2. *Pseudocercospora gracilis*. Cylindrical, olivaceous conidia and conidiogenous cells (PREM 51719). Bar = 10 μ m.

sphaerella species occurring on *Eucalyptus* (Park and Keane, 1982; Crous et al., 1991, 1993a, b; Carnegie and Keane, 1994), it would seem to be more variable in *M. parkii*.

A criterion not mentioned in the original description of *M. parkii* is the verruculose nature of the hyphae. This feature, together with its conspicuous, darkened and refractive conidial scars and verruculose, olivaceous conidia with slightly thickened and darkened hila place this anamorph in *Stenella* H. Sydow. *Stenella* is one of the known anamorphs of *Mycosphaerella* and has been linked to *Mycosphaerella* spp. occurring on hosts such as *Alyxia* (Yip, 1989), *Citrus* (Sivanesan, 1984) and *Maesa* (Crous and Braun, 1994).

***Mycosphaerella gracilis* Crous et Alfenas, sp. nov.**

FIGS. 2–5

Laesiones amphigenae, irregulares, 2–6 mm diam, pallide brunneae, cinctae margine exili elevata rufa ad brunnea. Ascocarpi amphigeni, solitarii, nigri, immersi, ad erumpescentiores, globosi, 60–80 μ m lati, 65–110 μ m alti; ostiolum

apicale papillatum ad 20–35 μ m diam; paries in cellis medio-brunneis consistens, 3–4 stratis textura angularis, basis 2–3 stratis cellularum hyalinarum consistens. Asci aparaphysati, fasciculati, bitunicati, sessilibus, ovoidei ad obclavati, recti vel incurvati, 8-spori, 32–45 \times 8–12 μ m. Ascospores tri- ad multiseriatae, superpositae, hyalinae, guttulatae, parietatibus tenuibus, parum curvatae, rare rectae, anguste ellipsoideae, apicibus obtusis, 1-septatae, latissimae ad septum inconstrictum, ad apices duos attenuatae, apicibus germinantes, tubi ad axem longum paralleli, (10–)16(–20) \times (2–)2.5(–3) μ m.

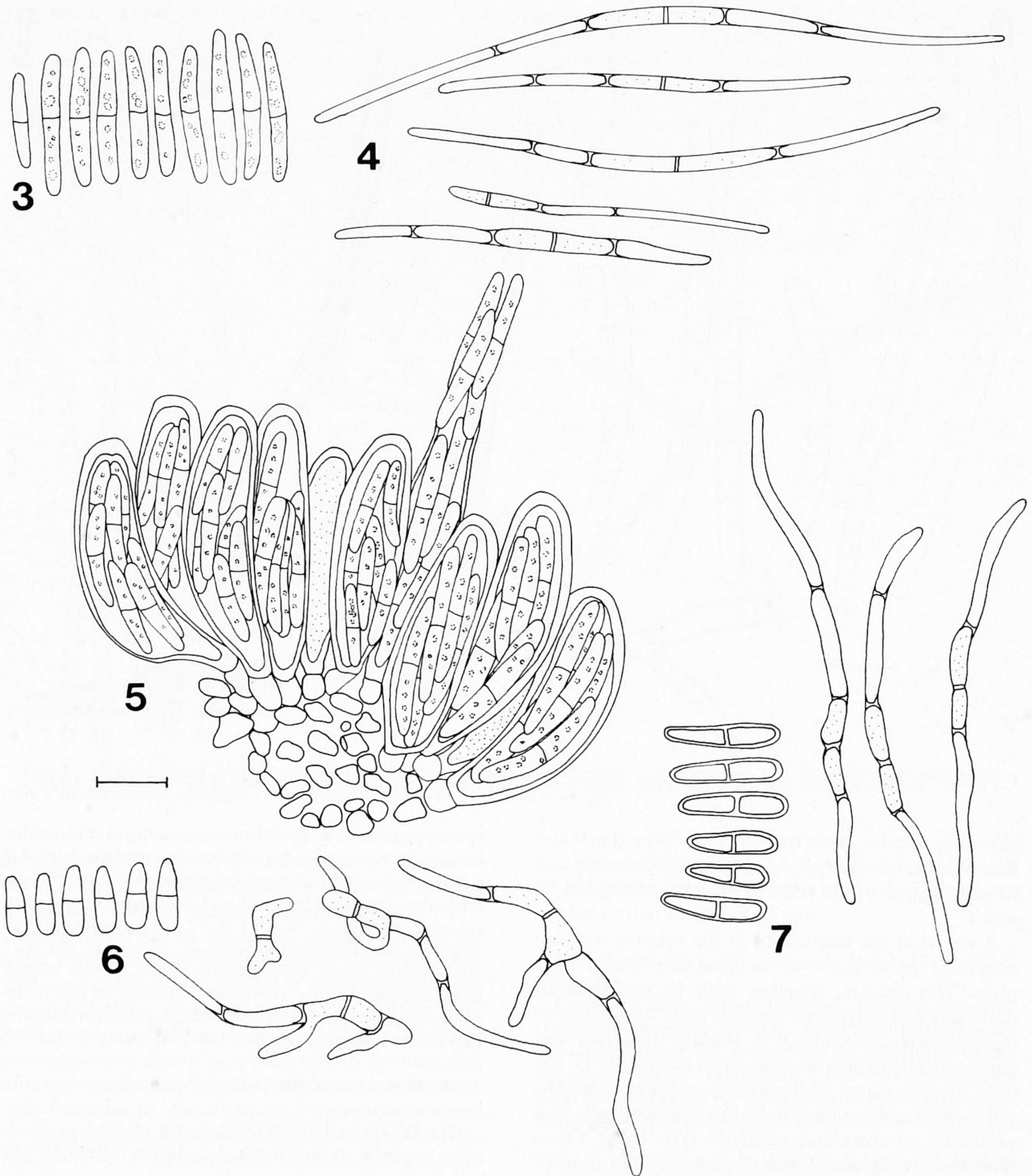
STATUS ANAMORPHICUS. *Pseudocercospora gracilis* Crous et Alfenas.

HOLOTYPUS. INDONESIA. North of Sumatra, *Eucalyptus urophylla*, 22 Nov. 1993, A.C. Alfenas (PREM 51718).

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 μ m diam. Conidiophora singularia in mycelio secundo pro projectionibus lateralibus, simplicia vel



FIGS. 3-7. Asci and ascospores of *Mycosphaerella* spp. 3-5. *M. gracilis* (PREM 51718). 3. Median septate ascospores. 4. Ascospores germinating with germ tubes parallel to the long axis of the spore with no visible distortion. 5. Ovoid to obclavate asci with ascospores. 6. Ellipsoidal ascospores of *M. parkii* germinating with germ tubes parallel and perpendicular to the long axis of the spore (PREM 51714). 7. Ellipsoidal ascospores of a *Mycosphaerella* sp. germinating with germ tubes parallel to the long axis of the spore, with visible signs of distortion. Bar = 10 μ m.

ramulosa, 0–2-septata, cylindracea, recta ad geniculato-sinuata, olivaceo-brunnea, $5\text{--}25 \times 2.5\text{--}3.5 \mu\text{m}$. Cellulae conidiogenae integratae, cylindraceae, rectae ad geniculato-sinuatae, olivaceae, cicatricibus conidialibus non incrassatis. Conidia solitaria, cylindracea, recta vel curvata, apice obtuso et base truncata, $35\text{--}100 \times 2\text{--}3 \mu\text{m}$, indistincte 3–11-septata, guttulata, pallide olivacea, hilo non incrassato.

STATUS TELEOMORPHICUS. *Mycosphaerella gracilis* Crous & Alfenas.

HOLOTYPUS. INDONESIA. North of Sumatra, *Eucalyptus urophylla*, 22 Nov. 1993, 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, globose, 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, subsessile, ovoid to obclavate, straight or incurved, eight-spored, $32\text{--}45 \times 8\text{--}12 \mu\text{m}$. Ascospores tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, narrowly ellipsoid with obtuse apices, slightly curved, rarely straight, one-septate, widest at unconstricted septum, tapering toward both apices, $(10\text{--})16(\text{--}20) \times (2\text{--})2.5(\text{--}3) \mu\text{m}$. 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, straight to geniculate-sinuous, olivaceous brown, $5\text{--}25 \times 2.5\text{--}3.5 \mu\text{m}$. Conidiogenous cells integrated, cylindrical, straight to geniculate-sinuous, olivaceous, monoblastic or polyblastic, $5\text{--}15 \times 2.5\text{--}3.5 \mu\text{m}$, tapering to a truncate apex; conidial scars unthickened. Conidia solitary, cylindrical, straight or curved, with an obtuse apex and truncate base, $35\text{--}100 \times 2\text{--}3 \mu\text{m}$, 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 $(10\text{--})16(\text{--}20) \times (2\text{--})2.5(\text{--}3) \mu\text{m}$, 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 and 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. molleriana* 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 $40\text{--}50 \times 11\text{--}13 \mu\text{m}$ and ascospores $8\text{--}14 \times 2\text{--}4 \mu\text{m}$, thus within the range accepted for *M. molleriana* (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. molleriana*.

Park and Keane (1982) stated that two colony types were associated with cultures of *M. molleriana*, 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. molleriana*, 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. molleriana*, 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. molleriana*-like isolates from Indonesia.

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LITERATURE CITED

- Carnegie, A. J., and P. J. Keane. 1994. Further *Mycosphaerella* species associated with leaf diseases of *Eucalyptus*. *Mycol. Res.* 98: 413–418.
- Corlett, M. 1991. An annotated list of the published names in *Mycosphaerella* and *Sphaerella*. *Mycol. Mem.* 18: 1–328.
- Crous, P. W., and U. Braun. 1994. *Cercospora* species and similar fungi occurring in South Africa. *Sydowia* 46: 204–224.
- , F. A. Ferreira, A. C. Alfenas, and M. J. Wingfield. 1993b. *Mycosphaerella suberosa* associated with corky leaf spots on *Eucalyptus* in Brazil. *Mycologia* 85: 705–710.
- , A. J. L. Phillips, and M. J. Wingfield. 1992. Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. *Mycologia* 84: 497–504.
- , and M. J. Wingfield. 1991. *Eucalyptus* leaf pathogens in South Africa: a national perspective. Pp. 749–759. In: *Proceedings of the IUFRO symposium for intensive forestry. The role of eucalypts*. Ed., A. P. G. Schönau. Southern African Institute of Forestry, Pretoria, South Africa.
- , ———, and R. F. Park. 1991. *Mycosphaerella nubilosa* a synonym of *M. molleriana*. *Mycol. Res.* 95: 628–632.
- , ———, F. A. Ferreira, and A. C. Alfenas. 1993a. *Mycosphaerella parkii* and *Phyllosticta eucalyptorum*, two new species from *Eucalyptus* leaves in Brazil. *Mycol. Res.* 97: 582–584.
- Park, R. F., and P. J. Keane. 1982. Three *Mycosphaerella* species from leaf diseases of *Eucalyptus*. *Trans. Brit. Mycol. Soc.* 79: 95–100.
- Sivanesan, A. 1984. *The bitunicate ascomycetes and their anamorphs*. J. Cramer, Vaduz, Liechtenstein. 701 pp.
- Yip, H.-Y. 1989. *Stenella alyxiae* sp. nov.: the anamorph of *Mycosphaerella alyxiae*. *Mycol. Res.* 92: 373–375.