New foliar pathogens of *Eucalyptus* from Australia and Indonesia

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Mycosphaerella tasmaniensis is newly described from Mycosphaerella leaf blotch symptoms occurring on *Eucalyptus nitens* in Tasmania, Australia. Single ascospore cultures produced a *Mycovellosiella* anamorph, described here as *M. tasmaniensis*. Both states occurred together, as well as separately on leaf spots. *Phaeophleospora epicoccoides* (= *Kirramyces epicoccoides*) is commonly associated with leaf spots of *Eucalyptus* spp. in Australia. The teleomorph, *Mycosphaerella suttoniae*, previously known only from Indonesia, was also collected on *E. grandis* leaves from Australia. A Cylindrocladium leaf blight disease of young *E. grandis* trees in Indonesia was found to be associated with a new species of *Calonectria*. *Calonectria multiseptata* and its anamorph *Cylindrocladium multiseptatum* is newly described and distinguished from other species based on their larger, multi-septate ascospores and conidia.

Species of *Eucalyptus* L'Hér. provide an important source of fibre to the international paper and pulp industry (Turnbull, 1991). Concurrent with their growing importance, there has been an intensification of management practices including production through vegetative propagation and hybridization. Diseases of eucalypts have also gained considerable importance, particularly where management practices have led to an increase in the genetic uniformity of plantations.

An extraordinary number of fungal species have already been reported from *Eucalyptus* (Sankaran, Sutton & Minter, 1995). Many of these are pathogens, which are specific to certain species or subgenera of *Eucalyptus* (Wall & Keane, 1984; Walker, Sutton & Pascoe, 1992). Although losses incurred by leaf spotting fungi in either plantations or nurseries have been poorly documented, these pathogens have formed the basis of several studies. In recent years Mycosphaerella leaf blotch disease (MLB), associated with several species of *Mycosphaerella* Johanson (Crous & Wingfield, 1996), has received considerable attention. Likewise, Cylindrocladium leaf spot and shoot blight diseases (CLB) are considered to be amongst the most important threats to *Eucalyptus* spp. (Ferreira, 1989; Crous, Phillips & Wingfield, 1991; Sharma & Mohanan, 1991). While several *Mycosphaerella* species are highly host specific on *Eucalyptus* (Crous & Wingfield, 1996), *Cylindrocladium* spp. are much less so, also infecting many other diverse hosts (Crous & Wingfield, 1994).

The present study reports on two newly discovered MLB and CLB diseases, and one new MLB disease record. The new MLB disease was discovered on *E. nitens* (Deane & Maid.) Maid. in Tasmania, while the new disease record was on *E. grandis* Hill ex Maid. in Brisbane, Australia, and the new CLB disease occurred on young *E. grandis* trees in northern Sumatra, Indonesia.

MATERIALS AND METHODS

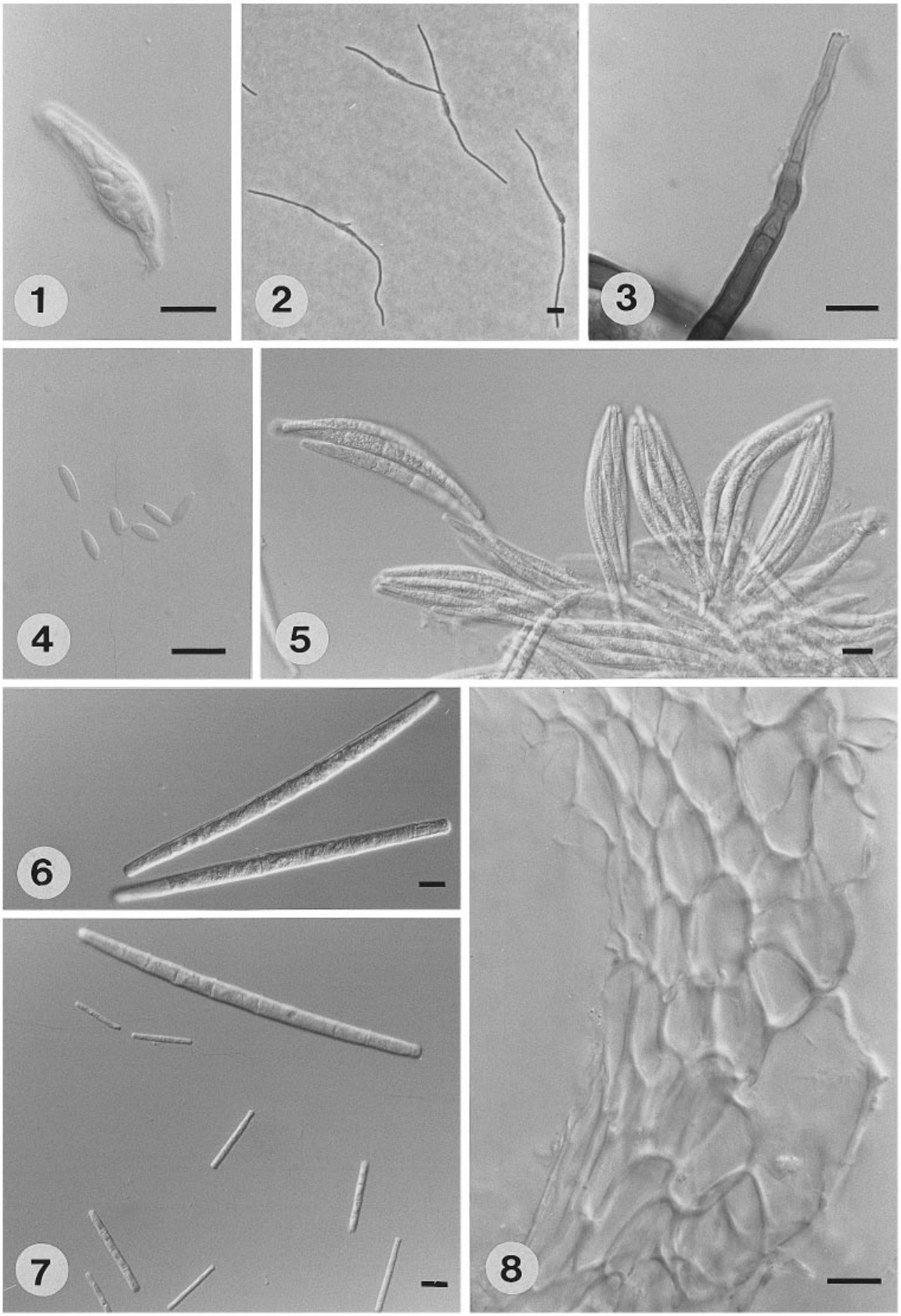
Single-conidial isolates of the *Calonectria* sp. were cultured on 2% malt extract agar (MEA) (Biolab), plated onto carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous, Phillips & Wingfield, 1992), incubated at 25 °C under nuv light, and examined after 7 d. Only material occurring on carnation leaves was examined. Leaves with Mycosphaerella leaf blotch symptoms were collected from several plantations in Tasmania. Lesions were excised from leaves, and single ascospore cultures were established on MEA using the technique described by Crous, Wingfield & Park (1991). Germinating ascospores were examined after 24 h, germination patterns determined, and transferred to MEA. Colonies were sub-cultured onto divided plates with one half containing CLA and the other MEA, incubated at 25° under continuous nuv light. Linear growth on agar for each culture was determined after 6 d for *Calonectria*, and 1 mo for *Mycosphaerella* (Crous & Wingfield, 1996). Colony colours were scored using the colour charts of Rayner (1970). Wherever possible, 30 measurements were made of structures mounted in lactophenol, and the extremes are given in parentheses.

TAXONOMY

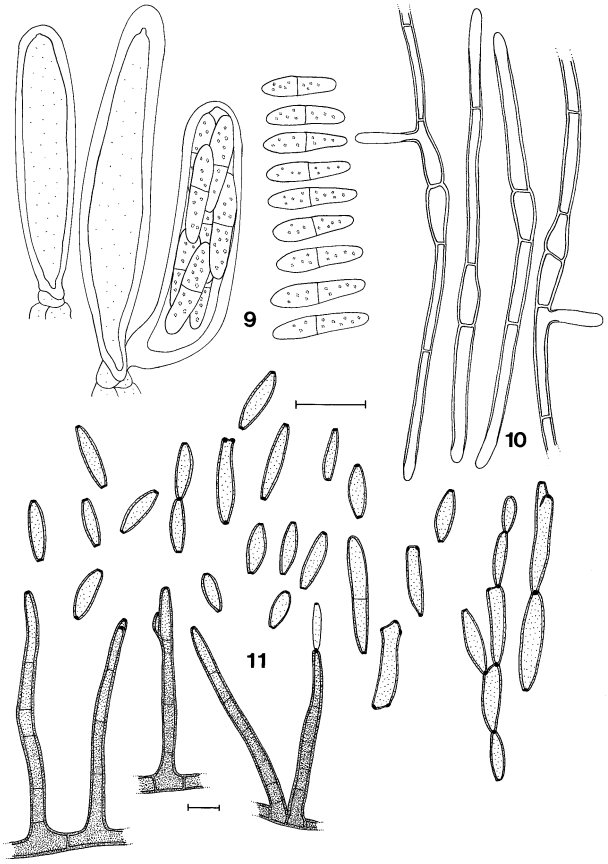
Mycosphaerella tasmaniensis Crous & M. J. Wingf., sp. nov. (Figs 1–4, 9–11)

Anamorph: *Mycovellosiella tasmaniensis* Crous & M. J. Wingf., sp. nov.

Etym.: Named after its country of origin



Figs 1–8. *Mycosphaerella tasmaniensis* and *Calonectria multiseptata*. **Figs 1–4.** *Mycosphaerella tasmaniensis* and its anamorph *Mycovellosiella tasmaniensis*. **Fig. 1.** Obovoid ascus. **Fig. 2.** Ascospores germinating parallel to their long axis on MEA. **Fig. 3.** Conidiophore with thickened, refractive, darkened, terminal, conidial loci. **Fig. 4.** Ellipsoidal, aseptate conidia with thickened, darkened hila. **Figs 5–8.** *Calonectria multiseptata* and its anamorph *Cylindrocladium multiseptatum*. **Fig. 5.** Fusoid ascospores. **Figs 6, 7.** Curved to straight, multiseptate macroconidia, and 1–3-septate microconidia. **Fig. 8.** Vertical section through the side wall of a perithecium showing the various cell layers (bars, 10 μm).



Figs 9–11. *Mycosphaerella tasmaniensis* and its anamorph *Mycovellosiella tasmaniensis*. **Fig. 9.** Ellipsoidal asci and fusoid–ellipsoidal ascospores. **Fig. 10.** Ascospores germinating on MEA. **Fig. 11.** Conidiophores and catenulate conidia with thickened, darkened loci (bars, 10 μm).

Pseudothecia hypophylla, sparse distributa, brunnea vel atra, subepidermalia, erumpentia, globosa vel subglobosa, 80–110 μm diam. *Asci* fasciculati, bitunicati, subcylindracei vel ellipsoidei, recti vel curvati, 30–40 × 7–11 μm. *Ascosporae* tri- vel multiseriatae, imbricatae, hyalinae, guttulatae, parietibus tenuibus, rectae, fusoideo-ellipsoideae, apicibus obtusis, latissimae in medium cellulae apicalis, mediano l-septatae, non constrictae in septum, ad utrosque apices sed base prominentiores, (10–)11–12(–13) × (2·5–)3(–4) μm. *Mycelium* plerumque externum, hyphis septatis, ramosis, laevibus, pallide brunneis, 2–4 μm latis. *Conidiophora* singularia, ex mycelio superficiali enata, medio brunnea, laevia vel subtiliter verruculosa, 3–12-septata, subcylindracea, recta vel parum curvata, 50–150 × 4–6 μm. *Conidiogenae cellulae* terminales, mono- vel polyblasticae, sympodialiter proliferantes, pallide brunneae, laeves, subcylindraceae, ad apices subobtusos contractae, locibus truncatis crassis, atribus et refractivis, 6–15 × 3–4 μm. *Conidia* catenulata, catenis ramosis, olivacea, laevia, eguttulata, subcylindracea, anguste ellipsoidea vel fusiformia, ad apices obtusis, locibus truncatis, atribus refractivis crassis, recta vel parum curvata, 0(–1)-septata, (4–)8–12(–20) × 2–2·5 μm.

Leaf spots amphigenous, subcircular, 2–30 mm diam. grey to light brown, separate, but coalescing with age to form large blotches, surrounded by a raised, medium brown border and a diffuse, red-purple margin. *Pseudothecia* hypophyllous, sparsely distributed, brown to black, subepidermal, becoming erumpent, globose to subglobose, 80–110 μm diam.; ostioles apical, 5–10 μm diam., walls consisting of 2–3 layers of medium brown *textura angularis*, subhymenium layer at base consisting of 1–2 layers of hyaline cells. *Asci* fasciculate, bitunicate, subcylindrical to ellipsoidal, rarely obovoid, straight or cuved, 8-spored, 30–40 × 7–11 μm. *Ascospores* tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid-ellipsoidal with obtuse ends, widest in middle of apical cell, medianly 1-septate, not constricted at septum, tapering toward both apices, but with more prominent taper towards lower end (10–)11–12(–13) × (2·5–)3(–4) μm. *Spermogonia* not observed. *Mycelium* mostly external, consisting of septate, branched, smooth, pale to light brown hyphae, 2–4 μm wide. *Conidiophores* arising singly from superficial mycelium, medium brown, smooth to finely verruculose, 3–12-septate, subcylindrical, straight to slightly curved, mostly unbranched, or branched above, 50–150 × 4–6 μm. *Conidiogenous cells* terminal, mono- to polyblastic, proliferating sympodially, pale brown, smooth, subcylindrical, tapering toward subobtuse apices with flat-tipped loci that are thickened, darkened and refractive, 6–15 × 3–4 μm. *Conidia* catenulate, chains branched, olivaceous, smooth, eguttulate, subcylindrical, narrowly ellipsoidal or fusiform, tapering toward rounded ends with flattened, darkened, refractive, thickened loci, straight to slightly curved, 0(–1)-septate, (4–)8–12(–20) × 2–2·5 μm *in vivo* and *in vitro*.

Ascospore germination on MEA. Germinating from both ends, not darkening or distorting upon germination, becoming constricted at the septum, (3–)4 μm wide, with germ-tubes growing parallel to the long axis of the spore, and occasionally developing lateral branches after 24 h (frequently from original ascospore).

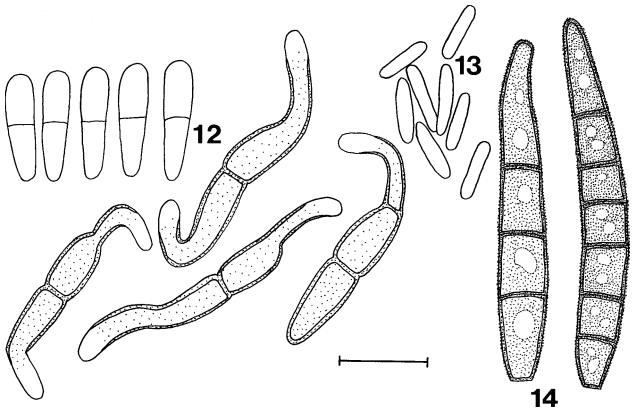
Cultural characteristics. Colonies up to 30 mm diam. on MEA after 1 mo at 25° in the dark, margins smooth, regular, surface not sectored, smooth, aerial mycelium moderate, grey, colonies iron grey, 25′′′′′k (bottom).

Host. *E. nitens*.

Distribution. Tasmania, Australia.

Specimens examined: **Australia**, Tasmania, leaves of *E. nitens*, Nov. 1996, leg. M. J. Wingfield, det. P. W. Crous. (PREM 55339 of *Mycosphaerella tasmaniensis*; PREM 55340 of *Mycovellosiella tasmaniensis*, holotypes, cultures ex-type STE-U 1555-1557); leaves of *E. nitens*, Nov. 1996, leg. M. J. Wingfield, det. P. W. Crous, (PREM 55341 of *Mycovellosiella tasmaniensis*, cultures STE-U 1537-1539).

Mycosphaerella tasmaniensis is morphologically similar to *M. heimii* Crous (anam. *Pseudocercospora heimii* Crous), *M. heimioides* Crous & M. J. Wingf. (anam. *Pseudocercospora heimioides* Crous & M. J. Wingf.), *M. ellipsoidea* Crous & M. J. Wingf. (anam. *Uwebraunia ellipsoidea* Crous & M. J. Wingf.), *M. irregulariramosa* Crous & M. J. Wingf. (anam. *Pseudocercospora irregulariramosa* Crous & M. J. Wingf.) and *M. molleriana* (Thüm.) Lindau (anam. *Colletogloeopsis molleriana* Crous & M. J. Wingf.) (Crous & Wingfield, 1996, 1997*a*, *b*). Other than slight differences in ascospore dimensions, the mode of ascospore germination of *M. tasmaniensis* is similar to that of *M. ellipsoidea* and *M. irregulariramosa*. None of the



Figs 12–14. *Mycosphaerella suttoniae* and its anamorph *Phaeophleospora epicoccoides*. **Fig. 12.** Obovoid ascospores, some germinating. **Fig. 13.** Spermatia. **Fig. 14.** Conidia (bar, 10 μm).

species discussed above has an anamorph resembling *Mycovellosiella tasmaniensis*, and no other species of *Mycovellosiella* has yet been described from *Eucalyptus*.

Carnegie & Keane (1994) reported that *Mycosphaerella grandis* (Carnegie & Keane frequently occurred in association with *M. gregaria* Carnegie & Keane. Similarly *M. grandis* was commonly found on the epiphyllous leaf surfaces of lesions associated with *M. tasmaniensis*. In other collections examined in this study, *M. grandis* was also isolated from typical lesions associated with *M. cryptica* (Cooke) Hansf. and *M. nubilosa* (Cooke) Hansf.

Mycosphaerella suttoniae Crous & M. J. Wingf., *Can. J. Bot.*: **75**: 783 (1997) (Figs 12–14)

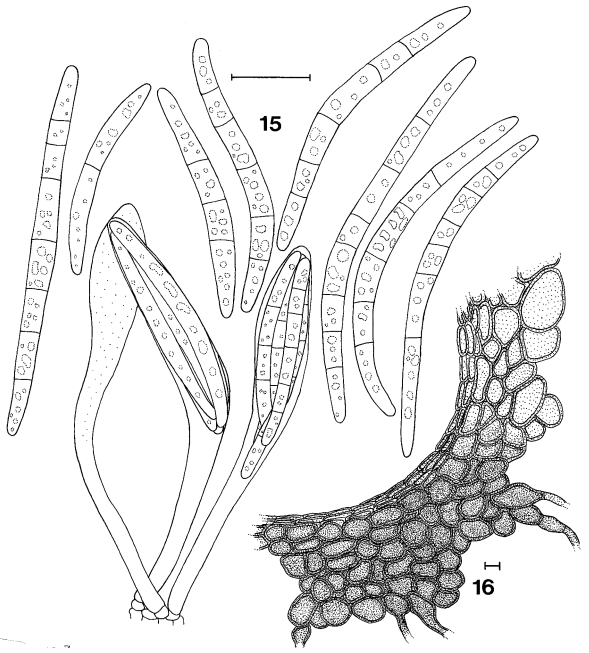
Anamorph: *Phaeophleospora epicoccoides* (Cooke & Massee) Crous, F. A. Ferreira & B. Sutton, *S. Afr. J. Bot.* **63**: 113 (1997).

Cercospora epicoccoides Cooke & Massee apud Cooke, (*Grevillea* **19**: 91 (1891).

Additional synonyms listed in Walker *et al.* (1992).

Leaf spots amphigenous, irregular to angular or subcircular, dark brown to purple with diffuse purple margins, ranging from small specks to larger medium brown spots up to 6 mm diam. Fruiting hypophyllous, with spermogonia and pseudothecia being inconspicuous, intermingled between pycnidia. Ascospores thin-walled, straight to curved, obovoid with obtuse ends, widest near apex, medianly l-septate, not constricted at septum, tapering prominently towards lower end, (10–)11–12(–13) × (2·5–)3–3·5 μm. Spermatia rod-shaped, hyaline, 4–7 × 1–1·5 μm, straight or slightly curved. Conidia exuding from ostiole in long cirri, subcylindrical to narrowly obclavate, apex subobtuse, tapering slightly from the basal septum to a narrowly truncate base, straight or slightly flexuous, thick-walled, medium brown, verruculose, guttulate, 3–7-euseptate, (30–)40–55(–60) × (3–)3·5–5 μm, thus very similar to the description given by Crous & Wingfield (1997*b*).

Ascospore germination on MEA. Germinating from both ends, becoming 3·5–5 μm wide, spore and germ-tubes becoming uniformly olivaceous upon germination, with germ-tubes parallel or perpendicular to the long axis of the spore.



Figs 15, 16. *Calonectria multiseptata*. **Fig. 15.** Asci and ascospores. **Fig. 16.** Vertical section through the basal wall of a perithecium, showing its cell layers (bars, 10 μm).

Cultural characteristics and hosts. As previously described by Crous & Wingfield (1997*b*).

Specimen examined: **Australia**, Brisbane, leaves of *E. grandis*, Nov. 1996, leg. M. J. Wingfield, det. P. W. Crous, PREM 55342, cultures derived from conidia and ascospores STE-U 1549-1551 and STE-U 1579-1581, respectively.

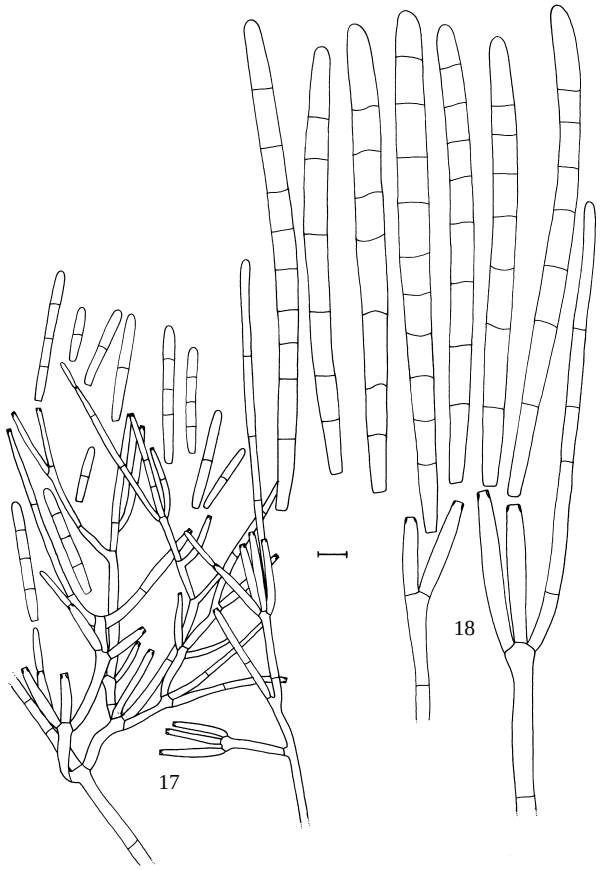
The anamorph, *Phaeophleospora epicoccoides*, occurs on several eucalypts in the subgenus Symphyomyrtus (Nichol, Wingfield & Swart, 1992), and has been recorded from most countries where eucalypts are grown (Sankaran *et al.*, 1995). The teleomorph, *M. suttoniae*, has previously only been reported from Indonesia (Crous & Wingfield, 1997*b*). In the present study, lesions on *E. grandis* leaves colonized by *P. epicoccoides* were soaked in water, and placed over MEA dishes. After 24 h several ascospores were released from pseudothecia. The pseudothecia and spermagonia were inconspicuous, and intermingled with pycnidia of the anamorph. Ascospores germinated in a mode similar to that of the Indonesian type collection, and after 1 mo sporulated on MEA to produce long cirri of conidia.

Because the teleomorph was inconspicuous among pycnidia on infected leaves, leaves infected with *P. epicoccoides* from Brazil and South Africa were treated in a similar fashion to determine if the teleomorph also occurs in these countries. No evidence of a teleomorph could be found, and its role in the genetic structure of populations of *P. epicoccoides* in plantations world-wide remains uncertain.

Calonectria multiseptata Crous & W. J. Wingf. sp. nov. (Figs 5–8, 15–18)

Anamorph: **Cylindrocladium multiseptatum** Crous & M. J. Wingf., sp. nov.

Etym.: Named after its multi-septate ascospores and conidia



Figs 17, 18. *Cylindrocladium multiseptatum*. **Fig. 17.** Microconidiophores and microconidia. **Fig. 18.** Macroconidiophores and macroconidia (bar, 10 μm).

Perithecia crocea deinde croceo-rubra, globosa vel ovoidea, 300–500 μm alta, 250–400 μm lata. Perithecii paries stratorum duorum; strato exteriori texturae globulosae parietibus crassis, ad 40 μm lato, cellulis 20–50 μm altis, 15–35 μm latis; strato interiori texturae angularis ad 20 μm lato, cellulis 15–20 μm altis, 7–11 μm latis. *Asci* unitunicati, clavati vel fusoidei, petiolo longo basali, 80–150 × 12–20 μm. *Ascosporae* in tertium partem superiorem asci, hyalinae, fusoideae, apicibus obtusis, curvatae, raro rectae, 1–9-septatae, in septa non constrictae, guttulatae, (45–)65–75(–110) × (5–)6–7(–8) μm. *Macroconidiophora* stipa, parte elongata sterili et phialidibus paucis. Stripae pars elongata septata, parietibus tenuibus, in vesiculum anguste clavatum terminans; conidiophori primarii rami subcylindracei, recti vel parum curvati, 30–60 × 4–5 μm. *Phialides* stipa vel ramulis primariis exorientes, singulares, vel in gregibus 2–4, cylindraceae, apice truncato, 15–55 × 4–5 μm. *Conidia* cylindracea vel subcylindracea, apice obtusa, base truncata, recta vel parum curvata, in medio latissima (120–)150–170)–200) × 8–9(–10) μm, 6–10-septata. *Microconidiophora* stipa parte elongata et ramulis fertilibus, penicellatis vel subverticillatis. Stipae pars elongata septata, parietibus tenuibus, in vesiculum anguste clavatum terminans. Rami primarii 0(–1)–septati, subcylindracei, recti vel curvati, 30–45 × 2–3 μm; rami secondarii et alteri 0(–1)-septati, 10–30 × 2·5–3 μm, phialibus 1–4, cylindraceis, rectis vel parum curvatis, 15–30 × 2–3 μm terminantes. *Microconidia* cylindracea, pro parte maxima recta, apice obtusa, base truncata, 20–65 × 2·5–3·5 μm, 1–3-septata.

Perithecia on CLA *in vitro*, superficial, solitary or in clusters of 2–6, orange, becoming orange-red with age, globose to ovoid, 300–500 μm high, 250–400 μm wide, turning dark red in 3% KOH; ostiole slightly depressed, concolorous with perithecial body. Perithecial wall consisting of two layers: outside layer of thick-walled *textura globulosa*, up to 40 μm wide, cells 20–50 μm high, 15–35 μm wide; inner layer of *textura angularis*, up to 20 μm wide, cells 15–20 μm high, 7–11 μm wide; perithecial base up to 150 μm wide, consisting of dark red cells; perithecia situated directly on substrate without a tapering foot of pseudoparenchymatal cells. *Asci* unitunicate, 8-spored, clavate to fusoid with long basal stalks, 80–150 × 12–20 μm. *Ascospores* aggregated in upper third of the ascus, hyaline, fusoid with rounded ends, curved, rarely straight, 1–9-septate, not constricted at septa before ascal dehiscence, guttulate, (45–)65–75(–110) × (5–)6–7(–8) μm. *Macroconidiophores* rarely observed, comprising a stipe, a sterile elongation and a few phialides. Stipe elongation rarely formed, septate, thin-walled, terminating in a narrowly clavate vesicle; primary conidiophore branches subcylindrical, straight to slightly curved, 30–60 × 4–5 μm. *Phialides* arising directly from the stipe or from primary branches, occurring singly, or in groups of 2–4, cylindrical, apex truncate, 15–55 × 4–5 μm; periclinal thickening minute. *Conidia* cylindrical to subcylindrical, rounded at apex, flattened at base, straight to slightly curved, widest in middle of conidium, (120–)150–170(–200) × 8–9(–10) μm, 6–10-septate, occurring singly, rarely in small packets. *Microconidiophores* comprising a stipe, a stipe elongation and a penicillate or subverticillate arrangement of fertile branches. Stipe elongation septate, thin-walled, terminating in a narrowly clavate vesicle. Primary branches 0(–1)-septate, subcylindrical, straight to curved, 30–45 × 2–3 μm; secondary and additional branches 0(–1)-septate, 10–30 × 2·5–3 μm, terminating in 1–4 phialides that are cylindrical, straight to slightly curved, 15–30 × 2–3 μm; apex with minute periclinal thickening. *Microconidia* cylindrical, mostly straight, rounded at apex, flattened at base, 20–65 × 2·5–3·5 μm, 1–3-septate, held in packets by colourless slime. Dark brown, thickened chlamydospores are formed throughout the medium in moderate numbers, and aggregate to form microsclerotia.

Cultural characteristics. Colonies 14–18 mm diam. on MEA after 6 d at 25° in the dark, aerial mycelium moderate, colonies umber (13′i; Rayner, 1970) underneath.

Host. *E. grandis*.

Distribution. Northern Sumatra, Indonesia.

Specimens examined: **Indonesia**, northern Sumatra, leaves and stems of *E. grandis*, Jan. 1997, leg. M. J. Wingfield, det. P. W. Crous (PREM 55343 of *Calonectria multiseptata*; PREM 55344 of *Cylindrocladium multiseptatum*, holotypes; cultures ex-type STE-U 1589-1591). **Indonesia**, northern Sumatra, small leaf spots on *E. grandis*, Apr. 1997, leg. M. J. Wingfield, det. P. W. Crous, culture STE-U 1602.

Calonectria multiseptata is easily distinguished from other species by its large (45–)65–75(–110) × (5–)6–7(–8) μm, 1–9-septate ascospores. Ascospores of *Ca. quinqueseptata* Figueirado & Namek. are (1–)3-septate, 54–100 × 4–8 μm, and are most similar, while those of *Ca. indusiata* Seaver [= *Ca. theae* (Petch) Subraman.] and *Ca. reteaudii* (Bugnic.) Booth are only up to 70 μm long. *Cy. multiseptatum* has large

conidia (120–200 × 8–10 μm, 6–10-septate) and clavate stipe extensions, and therefore must be compared with *Cy. heptaseptatum* Sobers, Alfieri & Knauss (conidia 1–8-septate, 96–144 × 6–9 μm) and *Cy. quinqueseptatum* Boedijn & Reitsma (conidia 1–6-septate, 61–101 × 5–7 μm). Although the conidia of *Cy. multiseptatum* are much larger than those of the other two species, they are also peculiar in the sense that they are frequently curved (Figs 6, 18), and that they have their widest points in the middle of conidia, rather than at their bases. Furthermore, this is the only species of *Cylindrocladium* known to have a multi-septate microconidial state. Where known, microconidial states of all other *Cylindrocladium* species have been observed to be l-septate (Crous & Wingfield, 1994). *Cy. multiseptatum* is also peculiar in the sense that it is the teleomorph state that forms predominantly in culture. It appears that although the fungus sporulates occasionally, it forms conidia on phialides which occur separately on unbranched conidiophores, rather than in branched conidiophores with tight, penicillate arrangements of phialides, which is characteristic of the genus.

Although *Ca. multiseptatum* was collected from large, brown leaf blotches and small leaf spots typically associated with Cylindrocladium leaf spot or blight disease, perithecia were common on dying shoots and branches of young trees throughout the plantation. Little is known about the pathogenicity of *Ca. multiseptata*. As no inoculations have yet been done, more attention will have to be given to these aspects of its biology in order to elucidate its role as a *Eucalyptus* pathogen.

The authors thank the South African Foundation for Research Development for financial support.

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(*Accepted 24 June 1997*)