New species of *Mycosphaerella* from Myrtaceae in plantations and native forests in eastern Australia

Angus J. Carnegie[1]

Forest Resources Research, NSW Department of Primary Industries, PO Box 100, Beecroft, New South Wales 2119, Australia

Treena I. Burgess

School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA 6150, Australia

Vyrna Beilharz

Primary Industries Research Victoria, Department of Primary Industries, Knoxfield, Victoria 3180, Australia

Michael J. Wingfield

Forestry and Agricultural Biotechnology Institute, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa

Abstract: The majority of *Mycosphaerella* species from eucalypts (*Eucalyptus, Corymbia* and *Angophora*) in Australia have been recorded only from trees growing in plantations. This illustrates a bias in research in the past two decades toward commercial enterprise, and it emphasises a lack of understanding of the occurrence of these important fungi under natural conditions. Surveys of foliar fungi in native forests in eastern Australia, as well as adjacent plantations, thus have been initiated in recent years. In this study we describe four new species of *Mycosphaerella* from *Eucalyptus* spp. as well as other Myrtaceae. *Mycosphaerella tumulosa* sp. nov. (anamorph: *Pseudocercospora* sp.) was found on more than seven species of *Eucalyptus* and *Corymbia* in native forests and plantations in northeastern New South Wales and southeastern Queensland and appears to be relatively common, although not damaging to these trees. *Mycosphaerella multiseptata* sp. nov. was recorded from several locations on species of *Angophora* in native forests and amenity plantings. *Mycosphaerella pseudovespa* sp. nov. was found in one location in native forest on *E. biturbinata*. The first species of *Mycosphaerella* to be described from *Syncarpia, M. syncarpiae* sp. nov., was found in native forests in numerous locations from Sydney through to northeastern New South Wales and appears to be relatively common.

Key words: *Angophora, Corymbia, Eucalyptus,* ITS, *Mycosphaerella, Pseudocercospora, Syncarpia,* taxonomy

Accepted for publication 2 February 2007.

[1] Corresponding author. E-mail: angusc@sf.nsw.gov.au

INTRODUCTION

The family Myrtaceae incorporates a large number of tree genera, including *Eucalyptus, Corymbia, Angophora* and *Syncarpia*. More than 700 species are accommodated in these four genera, the majority endemic to Australia (Pryor and Johnson 1971, Boland et al 1992), and they are the dominant tree species over most of the Australian landscape. Many species of *Eucalyptus* and *Corymbia* are grown widely in Australia and elsewhere for commercial purposes and as amenity trees, while *Syncarpia* is commercially important in eastern Australia.

More than 500 species of leaf-infecting fungi have been recorded from eucalypts (including the genera *Eucalyptus* and *Corymbia*) (Sankaran et al 1995). *Aulographina eucalypti* (Cooke & Massee) Arx & E. Müll. and species of *Cylindrocladium* Morgan, *Mycosphaerella* Johansen and its associated anamorphs are considered to be the most common and numerous (Park et al 2000). Some of these fungi are serious pathogens, particularly of plantation-grown *Eucalyptus* spp. In this regard these fungi threaten the economic viability of paper and pulp industries based on fiber from these trees.

More than 60 species of *Mycosphaerella* have been described from eucalypts in Australia and elsewhere (Park and Keane 1982, Carnegie and Keane 1994, 1998, Crous 1998, Maxwell et al 2003, Crous et al 2004, 2006, 2007, Hunter et al 2004, Burgess et al 2006). Most of these have been described from plantations but have not been found in native forests. This is not surprising because most studies on *Mycosphaerella* in Australia in the past two decades have concentrated on eucalypt plantations (e.g. Park 1984, Carnegie 1991, 2000, Maxwell 2004, Barber 2005, Milgate 2005). Only three species are commonly found in native forests in Australia: *M. cryptica*, which occurs on many hosts, the anamorph of *M. swartii* (*Sonderhenia eucalyptorum* [Hansf.] H.J. Swart & J. Walker), which occurs on several hosts, and *M. nubilosa* on *E. globulus* (Park et al 2000, Carnegie pers obs).

More than 20 species of *Pseudocercospora* Deighton also have been described from *Eucalyptus* spp. (Crous et al 1989, Crous 1998, Yuan et al 2000, Braun and

Dick 2002, Crous et al 2004, 2007, Hunter et al 2006), the majority of which are known only from countries other than Australia. Many of these have been linked to *Mycosphaerella* teleomorphs (e.g. Crous 1998, Burgess et al 2007) and others are known to be linked to this genus based on phylogenetic inference (Crous et al 2001, 2004). A number of other species of *Pseudocercospora* currently are being described as a result of detailed studies of this genus in Australia (V. Beilharz unpubl). Of the numerous species of *Pseudocercospora* detected on *Eucalyptus* in countries outside Australia, only two species, found in New Zealand, are known to occur in Australia: *P. subulata* Z.Q. Yuan, D. de Little & C. Mohammed (as *P. pseudobasitruncata* U. Braun & M. Dick) and *P. crousii* U. Braun & M. Dick. *Pseudocercospora subulata* occurs on *E. nitens* in both countries, while *P. crousii*, found on a number of *Eucalyptus* spp. including *E. regnans* in New Zealand, has been identified on *E. regnans* in eastern Australia. Only *P. eucalyptorum* however is considered a significant pathogen of eucalypts. It occurs mostly in *Eucalyptus* plantations (Crous et al 1989, Park et al 2000) and is not known in Australia.

Twelve foliar fungi have been identified from *Syncarpia*, most from single collections, and lodged in Australian herbaria (www.planthealthaustralia.com.au). Few species of foliar fungi have been described from *Angophora* (Park et al 2000) and only 10 are lodged in Australian herbaria (www.planthealthaustralia.com.au). In a detailed study of the then seven species of *Mycosphaerella* and related anamorphs from Myrtaceae, excluding *Eucalyptus*, Crous (1999) did not report any species from *Syncarpia* and only one species, *M. angophorae* Hansf., was described from *Angophora*. One other species has been reported from Myrtaceae, namely *M. melaleucoides* Sivan. & R.G. Shivas, from native *Melaleuca* in Queensland (Sivanesan and Shivas 2002).

Because of the bias of surveys toward *Eucalyptus* spp. in plantations, and thus the large number of identified species from this artificially established environment, the first author has made a purposeful effort to survey the species of *Mycosphaerella* in native forests of eastern Australia. This has revealed several undescribed species of *Mycosphaerella* and their anamorphs that are found in both native forests and plantations. The aim of this study is to provide names for these fungi and to discuss their relative significance.

MATERIALS AND METHODS

Sample collection.—Samples of diseased leaves were collected from various eucalypt plantations and native forests throughout eastern Australia over several years. These were placed in paper bags, pressed and kept cool during transportation back to the laboratory, where they were stored at 4 C.

Examination of samples and isolation from samples.—Lesion characteristics, including size and color, were recorded for each fungal species on each host. Pseudothecia were removed from diseased leaves and mounted in ammoniacal Congo red, lactic acid or acid fuchsin, squashed under a cover slip and examined with a compound microscope. Thin sections through pseudothecia were examined in a similar manner. Ascospore germination patterns were observed by excising mature pseudothecia from lesions on fresh leaves and attaching these to the undersides of 90 mm Petri dish lids with double-sided tape. The lesions were moistened for up to 2 h, blotted dry, then inverted over 2% malt-extract agar (MEA) in the Petri dish base and placed on a laboratory bench at room temperature for 24 h. Slabs of agar were cut from the dishes after 24 h, placed on a microscope slide, stained with ammoniacal Congo red, covered with a cover slip and examined with a compound microscope. Ascospore germination Type (A–N) was described following the definitions of Crous (1998). Further agar slabs were observed after 12 h and 36 h so that variation in germination patterns could be observed over time.

Germinating ascospores were removed from MEA plates with a sterile needle, transferred to fresh Petri dishes containing 2% MEA and incubated in the dark at 25 C (single-spore cultures). Plates were examined at weekly intervals and linear growth determined after 1 mo. Culture color was rated with the aid of color charts (Kornerup and Wanscher 1983). Mycelium was removed with a sterile needle from the edges of single spore cultures, replated on carnation leaf agar (CLA) and incubated under continuous near-ultraviolet light at room temperature. Plates were examined for the presence of anamorphs at weekly intervals or until 3 mo. Specimens and cultures used in this study are kept at herbaria DAR (Orange Agricultural Institute, Orange, NSW, Australia), VPRI (Victorian Department of Primary Industries, Knoxfield, Australia) and NSWF (State Forests of NSW, Sydney, Australia), with ex-type cultures also lodged at Centraalbureau voor Schimmelcultures (CBS).

Molecular phylogenetic characterization.—For each isolate approximately 50 mg of fungal mycelium was scraped from the surface of 21 d old cultures, ground with a glass rod, suspended in 200 µL of DNA extraction buffer (200 mM Tris-HCL pH 8.0, 150 mM NaCl, 25 mM EDTA, 0.5% SDS) and incubated 1 h at 70 C. DNA was purified with the Ultrabind® DNA purification kit according to the manufacture's instructions (MO BIO Laboratories, Solana Beach, California). A part of the internal transcribed spacer (ITS) region of the ribosomal DNA operon was amplified with the primers ITS-1F (5′CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns 1993) and ITS4 (5′TCCTCCGCTTATTGATATGC) (White et al 1990). The PCR reaction mixture (25 µL), PCR conditions and visualization of products were as described by Burgess (et al 2001) except that 1 U of Taq

TABLE I. Isolates considered in the study

Culture no.	teleomorph	anamorph	Host	Location	Collector	GenBank accession No.
DAR 77433	*M. syncarpiae*		*Syncarpia glomulifera*	New South Wales	AJ Carnegie	DQ530219
NSWF 005320	*M. syncarpiae*		*S. glomulifera*	New South Wales	AJ Carnegie	DQ530220
DAR 77439	*M. multiseptata*		*Angophora subvelutina*	New South Wales	AJ Carnegie	DQ530223
DAR 77438	*M. multiseptata*		*A. subvelutina*	New South Wales	AJ Carnegie	DQ530224
DAR 77440	*M. multiseptata*		*A. costata*	New South Wales	AJ Carnegie	DQ530225
DAR 77432	*M. pseudovespa*		*E. biturbinata*	New South Wales	AJ Carnegie	DQ530216
DAR 77424	*M. tumulosa*	*P. tumulosa*	*E. moluccana*	Queensland	AJ Carnegie	DQ530217
NSWF 005313	*M. tumulosa*	*P. tumulosa*	*E. moluccana*	Queensland	AJ Carnegie	DQ530218

polymerase (Biotech International, Needville, Texas) was used in each reaction. PCR products were cleaned with Ultrabind® DNA purification kit (MO BIO Laboratories). Products were sequenced with the BigDye terminator cycle sequencing kit (PE Applied Biosystems, Foster City, California) with the same primers that were used in the initial amplification. The products were separated with an ABI 3730·48 capillary sequencer (Applied Biosystems) using a BioRad Biofocus 2000 capillary gel electrophoresis system.

To compare *Mycosphaerella* isolates in this study with other *Mycosphaerella* spp., ITS rDNA sequences obtained from GenBank, including isolates used in recent studies (Burgess et al 2006, Cortinas et al 2006, Crous et al 2006, Crous et al 2004, Crous et al 2001, Hunter et al 2004, Maxwell et al 2003), were used in the phylogenetic analysis (culture number, identity and GenBank number are available at TreeBASE SN3238). ITS trees were rooted with *Neofusicoccum ribis* as outgroup taxon.

Sequence data were analysed with Sequence Navigator version 1.0.1™ (Perkin Elmer Corp. Foster City, California) and manually aligned by inserting gaps. Gaps were treated as a fifth character; all ambiguous characters and parsimony uninformative characters were excluded before analysis. The most parsimonious trees were obtained with heuristic searches with random stepwise addition in 100 replicates, with the tree bisection-reconnection branch-swapping option on and the steepest-descent option off. MAXTREES were unlimited, branches of zero length were collapsed and all multiple equally parsimonious trees were saved. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indices) were determined (Hillis and Huelsenbeck 1992). Branch and branch node supports were determined with 1000 bootstrap replicates (Felsenstein 1985).

RESULTS

Phylogenetic analyses.—A BLAST search was first conducted on GenBank to compare the ITS sequences for the *Mycosphaerella* spp. collected in this study (TABLE I) with those for existing species that have been lodged in GenBank. The four purported new species were closely related to but different from currently described *Mycosphaerella* spp. The sequence for these species then was compared with other *Mycosphaerella* spp. isolated from eucalypts. Due to the large number of species, two separate analyses were conducted, both containing representative isolates from the different phylogenetic lineages in *Mycosphaerella* (Burgess et al 2006, Crous et al 2006). The first aligned dataset, concentrating on the 'nubilosa clade', consisted of 572 characters of which 191 were parsimony informative. The dataset contained significant phylogenetic signal compared to 1000 random trees ($P < 0.01$, g1 = −1.33). Heuristic searches in PAUP resulted in one most parsimonious tree of 582 steps (CI = 0.59, RI = 0.79) (FIG. 1, TreeBASE SN3238-13881). Two of the new *Mycosphaerella* spp. treated in this study were found in strongly supported (100% bootstrap) terminal clades and appeared to be distinct from all known related *Mycosphaerella* spp. *Mycosphaerella* sp. from *Syncarpia* and *Mycosphaerella* sp. from *Angophora* are most similar primarily to each other, although bootstrap analysis does not support subgroupings within the 'nubilosa clade' (FIG. 1)

The second aligned dataset, concentrating on species with *Pseudocercospora* anamorphs, consisted of 586 characters of which 172 were parsimony informative. The dataset contained significant phylogenetic signal compared to 1000 random trees ($P < 0.01$, g1 = −0.57). Initial heuristic searches of unweighted characters in PAUP resulted in 1000 most parsimonious trees of 676 steps (CI = 0.51, RI = 0.73). Characters were weighted according to the CI (32 characters had a weight of 1, 140 a weight of <1) and subsequent heuristic searches resulted in 30 most parsimonious trees of 348 steps (CI = 0.60, RI = 0.78). (FIG. 2, TreeBASE SN3238-13882). *Mycosphaerella* sp. from *E. moluccana* resided in a strongly supported (100% bootstrap) terminal clade. Compared to other *Pseudocercospora* anamorphs, which are closely related to each other, this species differed by

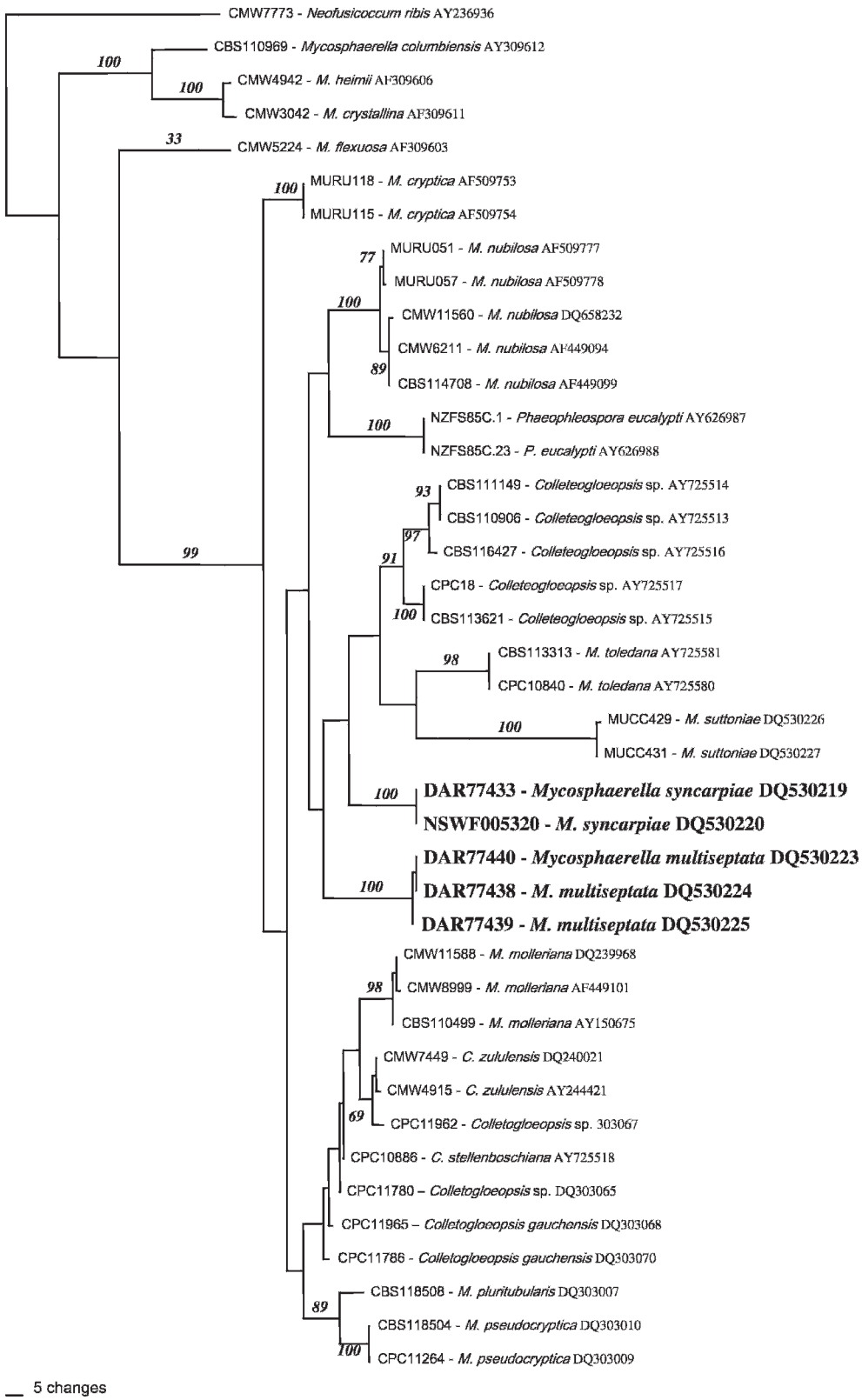


FIG. 1. A phylogram of the most parsimonious tree of 582 steps obtained from ITS sequence data, indicating the placement of two new *Mycosphaerella* spp. in bold. Bootstrap support is given above the branches. The trees were rooted to *Neofusicoccum ribis*.

at least 12 steps (FIG. 2). *Mycosphaerella* spp. associated with wasp galls on *E. biturbinata* was related to but different from *M. citri*, which is not known to be a pathogen of *Eucalyptus, M. obscures*, which was isolated from eucalypts in southeastern Asia, and an undescribed *Mycosphaerella* sp. (FIG. 2).

Based on DNA sequence comparisons the four *Mycosphaerella* spp. found in this study clearly represent discrete taxa. These fungi also could be discriminated from species to which they are most closely related with morphological characteristics. We therefore describe them as new in the following taxonomic treatments.

TAXONOMY

Mycosphaerella tumulosa Carnegie & Beilharz sp. nov. FIGS. 3–8

MycoBank MB500742

Anamorph: Pseudocercospora sp.

Etymology. Referring to the mounded shape of the culture (*tumulosus*, mounded).

Mycosphaerella marksii subsimilis, differt ascosporis grandioribus 15–19 × 4.5–5 μm (*M. marksii* 11–16 × 2–3 μm) et cum cellvla apicali asymmetrica rariore, etcoloniis in agaro tardioribus crescentibus (*M. tumulosa* 9–12 mm, *M. marksii* 24–29 mm, post mensam unam).

Pseudocercosporae crousii subsimilis sed conidiis minus obclavatis, conidiophoris simplicibus plerumque irregularibus et brevioribus (conidiophora in *P. tumulosa* 26–60 μm longa, in *P. crousii* 5–100 μm longa) differt.

Leaf spots amphigenous, subcircular to irregular, 2–15 mm diam, single to confluent, beginning as irregular chlorotic blotches, turning carmine-red before necrosis, slightly raised and corky, yellow-brown to red-brown on the abaxial surface, medium brown to gray-brown on the adaxial surface, becoming gray-brown to gray with age, especially in center, maturation of leaf spot often not even, with irregular margins and red-brown border 1 mm wide, often with diffuse chlorotic and red-brown to purple halo surrounding leaf spot. Pseudothecia amphigenous, predominantly epigenous, single, dispersed to occasionally aggregated, subepidermal becoming erumpent, black, globose, 95–120 μm. Asci fasciculate, bitunicate, ovoid to narrowly ellipsoidal, straight or curved, 8-spored, (40–)45–65(–81) × (10–)11–15(–17) μm. Ascospores 2–3-seriate, overlapping, narrowly clavate to fusoid-ellipsoidal, straight to slightly curved, hyaline, basal end obtuse, apical end prominently tapered, apical cell occasionally asymmetrical, widest at midpoint of apical cell, medianly 1-septate, not constricted at septum or slightly so, 15–19 × 4.5–5.5 μm.

Sporulation amphigenous, mostly hypogenous, seen as inconspicuous light brown fascicles and external mycelium on the abaxial surface, and occasionally as dense erumpent sporodochia on the adaxial surface. Primary mycelium internal; secondary mycelium external, superficial. Immersed hyphae intercellular, pale olivaceous, smooth, septate, branched, irregular in diameter, 2–4.5 μm wide. Superficial hyphae pale to medium olivaceous brown, septate, branched, smooth, developing from the bases of caespituli, 2–3 μm diam and bearing erect lateral conidiogenous cells 42–70 μm long × 5.5 μm wide. Conidiophores solitary or up to 12, caespitose, initially arising from a few olivaceous hyphae in the substomatal cavity but occasionally seen borne on cells of the outer cell layer of ascomata of the teleomorph, which have developed in the substomatal cavities, mildly divergent or erect, pale to medium olivaceous brown, pale to hyaline at the growing apex, ±cylindrical or of irregular diameter, often slightly swollen near the apex, straight, curved or occasionally bent, sometimes 1-branched, smooth throughout or somewhat roughened toward the apex, anastomosing, 0–8-septate, (26–)40–60 μm long × 4.5–6 μm wide. Conidiogenous cells terminal, integrated, pale olivaceous brown, smooth to slightly rough, straight to mildly geniculate or sinuous, often slightly swollen toward the apex before attenuating markedly toward the conidiogenous locus, (8–)19–32 μm long × 4–5 μm wide. Conidiogenous loci 1–3, left on short pegs after enteroblastic sympodial proliferation of the conidiogenous cell, not darkened, not thickened, nonrefractive, 1–2 μm diam. Conidia solitary, pale olivaceous to subhyaline, smooth to faintly verruculose, straight or slightly curved, narrowly obclavate or subulate to ±cylindrical, often variable in diameter, gradually attenuating to a subobtuse or subacute apex and more abruptly to an obconically or narrowly obclavate, subtruncate or truncate base, anastomosing, 3–7-septate, (31–)50–80(–95) μm long × 3.5–4.5 μm wide. Conidiogenous loci and hila not darkened, not thickened, nonrefractive, 1–2(–2.5) μm diam.

HOLOTYPES: *Mycosphaerella tumulosa* and *Pseudocercospora* sp.: AUSTRALIA: QUEENSLAND: Kingaroy, Coolabunia Plantation, on living leaves of *Eucalyptus moluccana*, 14 Feb 2004, *A.J. Carnegie* (DAR 77424, culture ex-type DAR 77424 = CBS 121158; isotype NSWF 005313, culture ex-isotype NSWF 005313).

Ascospore germination on MEA after 24 h. Germ tubes grow from both ends, initially parallel to the long axis of the spore, often becoming sigmoidal, occasionally with a third or fourth germ tube emanating obliquely from the basal or apical cell or from either germ tube; ascospores do not darken or become distorted but become prominently constricted; length of ascospore plus both germ tubes

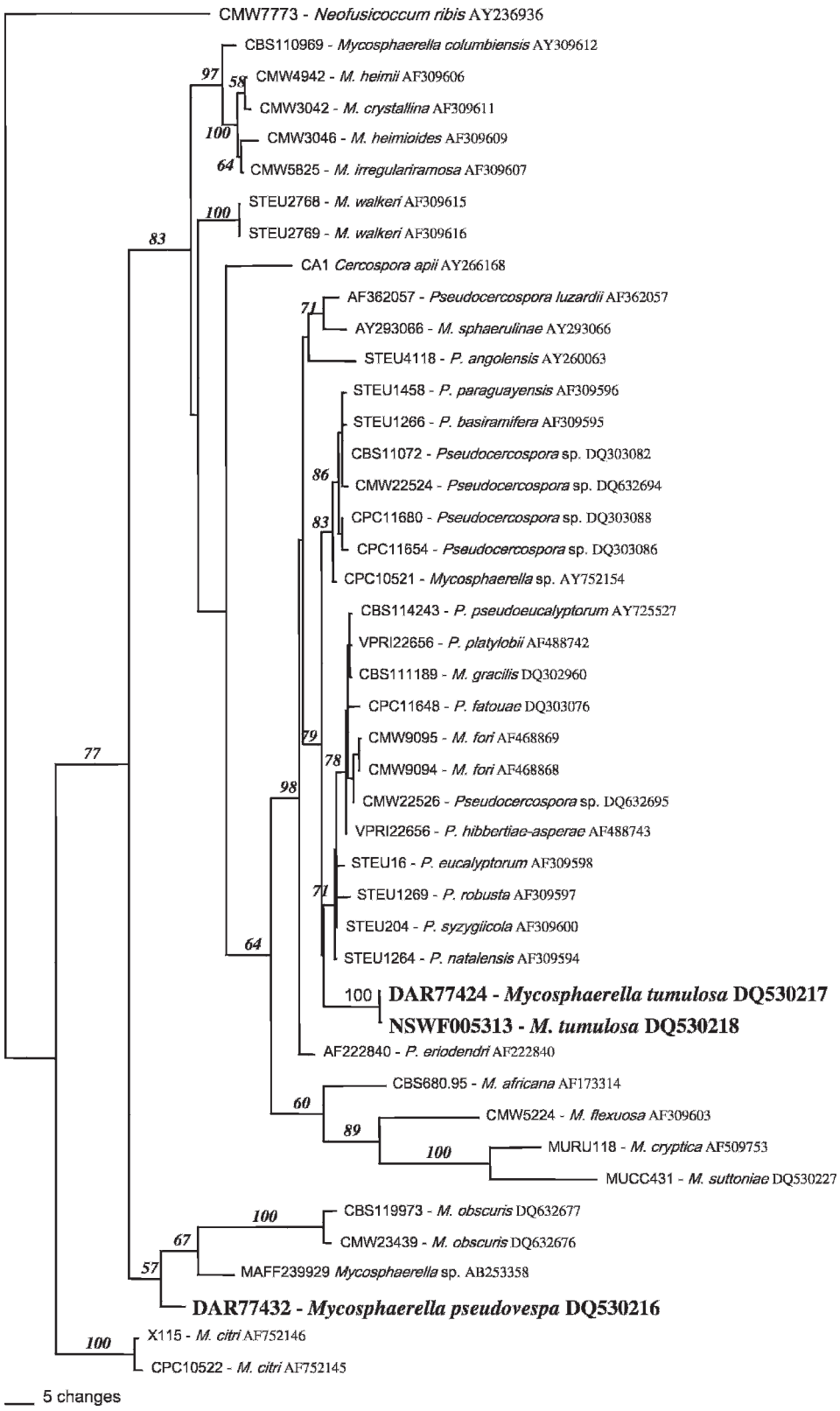
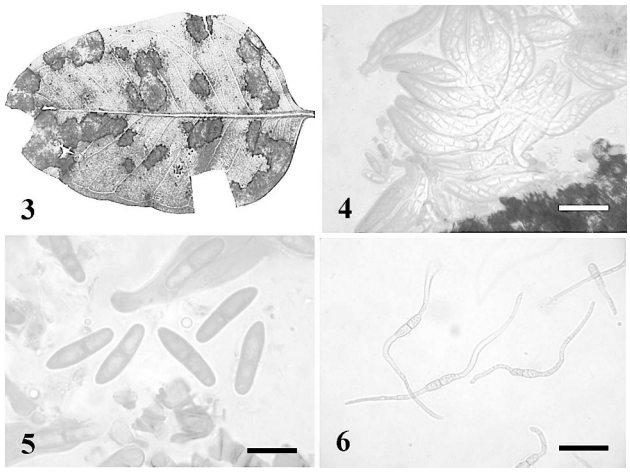


FIG. 2. A phylogram of one of the 30 most parsimonious tree of 348 steps obtained from ITS sequence data, indicating the placement of two new *Mycosphaerella* spp. in bold. Bootstrap support is given above the branches. The trees were rooted to *Neofusicoccum ribis*.



FIGS. 3–6. *Mycosphaerella tumulosa.* 3. Leaf of *E. moluccana* showing symptoms. 4. Asci (bar = 30 μm). 5. Ascospores (bar = 10 μm). 6. Ascospore germination after 24 h on 2% MEA (bar = 20 μm).

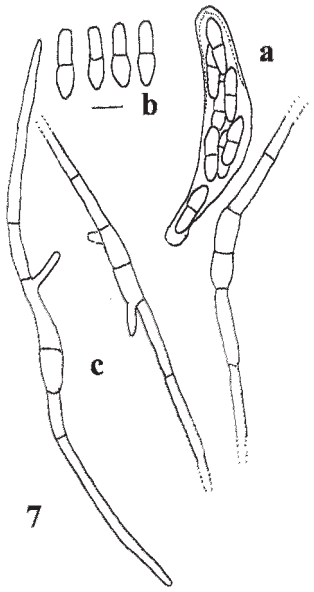


FIG. 7. *Mycosphaerella tumulosa.* a. Ascus (bar = 20 μm). b. Ascospores (bar = 10 μm). c. Ascospore germination after 24 h on 2% MEA (bar = 20 μm).

after 24 h (96–)130–150(–165) μm. This germination pattern most closely resembles Type C and Type D of Crous (1998).

Variation in germination patterns of *M. tumulosa* was observed between 12 h, 24 h and 36 h periods. At 12 h germ tubes grew parallel to the long axis of the spore, often becoming sigmoidal, with no secondary germ tubes; the length of spore plus two germ tubes was (51–)64–102(–124) μm. At 24 h most germ tubes were obviously sigmoidal and the length of spore plus germ tubes was (96–)130–150(–165) μm. A small proportion of ascospores also had started to produce up to two secondary germ tubes, which emanated obliquely from the basal or apical cell or from either germ tube. At 36 h most ascospores had produced two or three secondary germ tubes and were distorted, and the length of the ascospore plus original germ tubes was mostly greater than 178 μm.

Cultures. Single ascospore colonies grew slowly on MEA, reaching 9–12 mm diam after 1 mo, with a mound of light gray (2D1) aerial hyphae and an outer edge (1–2 mm wide) of olive-gray (2F2) aerial hyphae; submerged hyphae dark gray to black; readily producing conidiophores of the *Pseudocercospora* anamorph in culture after 1 mo on CLA. Single conidia formed in single ascospore cultures, and single conidia produced in *Pseudocercospora* colonies growing in situ in close association with the teleomorph on the leaf surface, were germinated and grown on PDA 3 wk at 25 C in 12 h dark/12 h fluorescent light. The cultures were compared based on DNA sequences with the methods of Beilharz and Cunnington (2003) and were found to be of the same fungus, thus confirming the anamorph-teleomorph connection.

Hosts. On living leaves of *E. moluccana, E. tereticornis, E. amplifolia, Eucalyptus* sp., *Corymbia variegata, E. acmenoides, E. seeana.*

Known distribution. Australia, eucalypt plantations and native forests in New South Wales and southeastern Queensland; relatively common but not damaging.

Notes. Mycosphaerella tumulosa was found on several *Eucalyptus* and *Corymbia* species in plantations as well as on species of *Eucalyptus* in native forests in New South Wales and southeastern Queensland. It is not considered a significant pathogen in plantations or native forests, although individual trees can be heavily infected. Although distinguishable from other *Mycosphaerella* species on eucalypts it most closely resembles *M. marksii* Carnegie & Keane in having an apical cell that is occasionally asymmetrical (Carnegie and Keane 1994). However ascospores of *M. marksii* are smaller (11–16 × 2–3 μm) than those of *M. tumulosa* (15–19 × 4.5–5.5 μm), and cultures of *M. marksii* are faster growing (24–29 mm diam) than those of *M. tumulosa* (9–12 mm). Also *M. marksii* more commonly has ascospores with an asymmetrical apical cell while in *M. tumulosa* this is less common.

There are several species of *Mycosphaerella* from *Eucalyptus* with *Pseudocercospora* anamorphs (Crous 1998, Park et al 2000, Burgess et al 2006), including *M. colombiensis* Crous & M.J. Wingf., *M. crystallina* Crous & M.J. Wingf., *M. gracilis* Crous & Alfenas, *M. heimii* Crous, *M. irregulariramosa* Crous & M.J. Wingf. and *M. obscuris* Barber & T.I. Burgess. These can be distinguished from *M. tumulosa* based on differences in morphology of the teleomorph and anamorph, as well as the ascospore germination patterns. Several

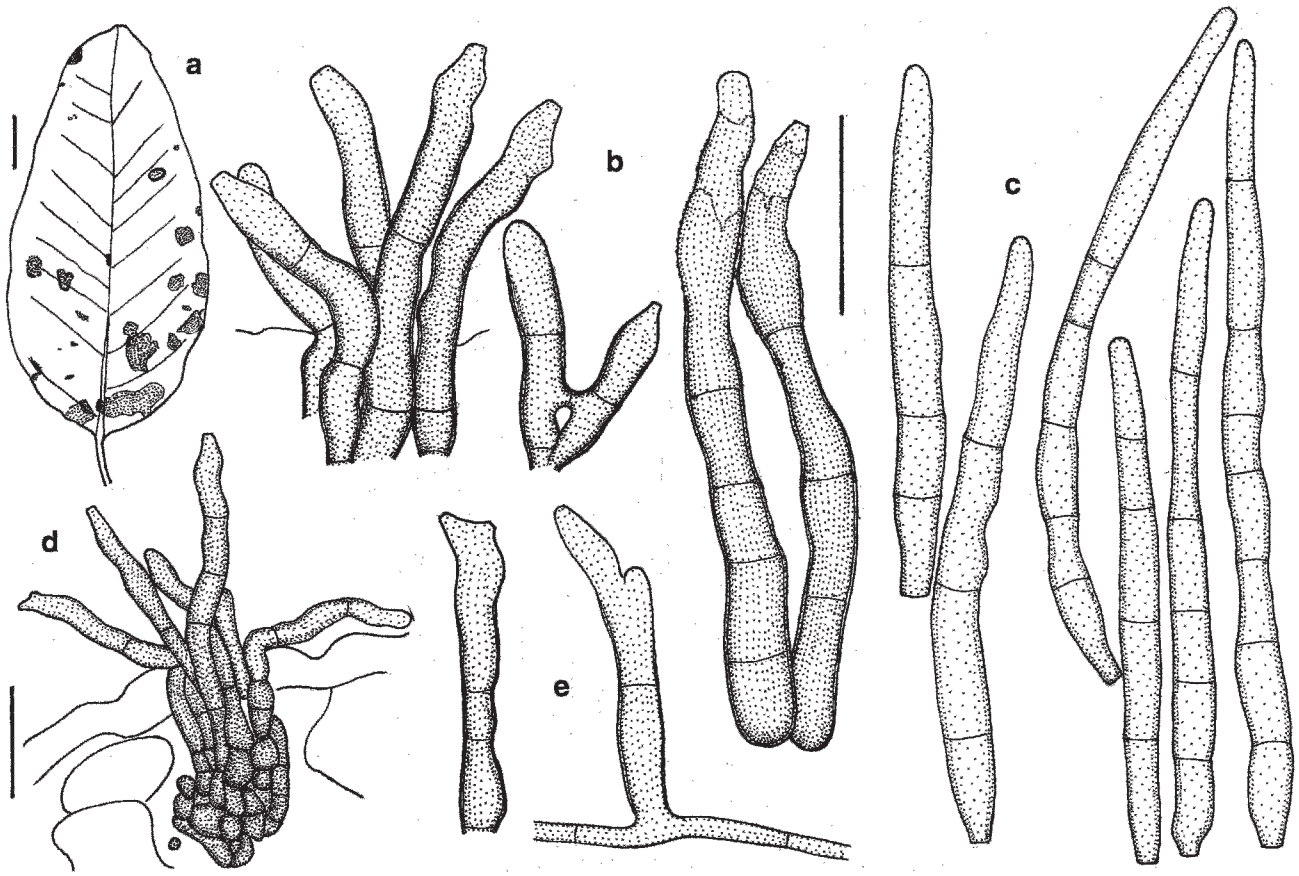


FIG. 8. *Pseudocercospora* sp. anamorph of *M. tumulosa* on *E. moluccana*. a. Leaf symptoms. b. Conidiophores (fascicle and anastomosing conidiophores VPRI 24973, longer conidiophores VPRI 24949). c. Conidia (two on left VPRI 24949, others VPRI 24973). d. Conidioma (VPRI 24973. e. External conidiophores (VPRI 24949). Bars = 1 cm (a), 20 µm (b–e).

species of *Pseudocercospora* from *Eucalyptus* spp. do not have a known teleomorph (Crous 1998, Braun and Dick 2002, Hunter et al 2006), including *P. basiramifera* Crous, *P. basitruncata* Crous, *P. irregularis* Crous, *P. eucalyptorum* Crous, M.J. Wingf., Marasas & B. Sutton, *P acerosa* U. Braun & M. Dick, *P crousii* U. Braun & M. Dick and *P. flavomarginata* G.C. Hunter, Crous & M.J. Wingf. These can be distinguished from the *Pseudocercospora* anamorph of *M. tumulosa* based on conidial morphology and the association with a sexual state.

Additional specimens examined. *Mycosphaerella tumulosa* and *Pseudocercospora* sp.: AUSTRALIA: NEW SOUTH WALES: Richmond, University of Western Sydney, Koala Food Plantation, on living leaves of *E. moluccana*, 15 Feb 2002, *A.J. Carnegie* (DAR 77425; NSWF 005010); Richmond, University of Western Sydney, Koala Food Plantation, on living leaves of *E. tereticornis*, 15 Feb 2002, *A.J. Carnegie* (DAR 77426); Whiporie, Whiporie State Forest, native forest, on living leaves of *Eucalyptus* sp., 21 Jan 2005, *A.J. Carnegie* (DAR 77429); Bungawalbin, Robinson Plantation, on living leaves of *C. variegata*, 23 Jan 2005, *A.J. Carnegie* (DAR 77427); Lawrence, Maunders Plantation, native forest regeneration within plantation boundary, on living leaves of *E. amplifolia*, 21 Jan 2005, *A.J. Carnegie* (DAR 77428); Ewingar, roadside native regeneration, on living leaves of *E. seeana*, 15 Apr 2005, *A.J. Carnegie* (DAR 77430); Urbenville, Smith Plantation, on living leaves of *E. acmenoides*, 9 Feb 2006, *A.J. Carnegie* (DAR 77431); Richmond, University of Western Sydney, Koala Food Plantation, on living leaves of *E. moluccana*, 7 May 2002, *A.J. Carnegie* (VPRI 24973).

Mycosphaerella pseudovespa Carnegie sp. nov.
FIGS. 9–13

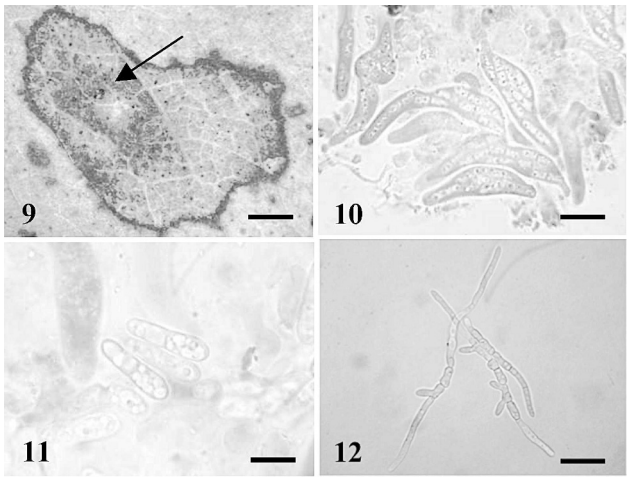
MycoBank MB500743

Anamorph: Not seen

Etymology. Morphologically similar to *M. vespa* Carnegie & Keane.

Mycosphaerellae vespa subsimilis sed tubis germinationibus duobus ascosporae extremis orentibus et cum tubis germinationibus secondariis usque ad quatuor (in *M. vespa* tubis germinationibus ascosporis duobus, rarior tribus) differt.

Leaf spots amphigenous, subcircular, 3–7 mm diam, single, yellow-brown with thin red-brown to purple margin, associated with wasp gall, often with raised center and empty gall, wasp pupae rarely



FIGS. 9–12. *Mycosphaerella pseudovespa*. 9. Leaf spot on *E. biturbinata* (adaxial surface) showing wasp exit hole (arrowed) (bar = 1 mm). 10. Asci (bar = 30 µm). 11. Ascospores (bar = 10 µm). 12. Ascospore germination after 24 h on 2% MEA (bar = 20 µm).

evident but exit hole often observed on abaxial surface of lesion. Pseudothecia amphigenous, more prominent on abaxial surface, single to aggregated, often more numerous around center of lesion, immersed to occasionally erumpent, black, globose, 75–125 µm diam. Asci bitunicate, fasciculate, subsessile, obclavate to fusoid-ellipsoidal, 8-spored, (40–)48–60 × 12.5–15 µm. Ascospores 2–3-seriate, fusoid with obtuse ends, slightly tapered to basal end, slightly constricted or not so, widest in middle of apical cell, straight, (10.5–)11.5–13.5(–14.5) × 3–4 µm.

HOLOTYPE: AUSTRALIA: NEW SOUTH WALES: Urbenville, Reid Plantation, native regeneration within plantation boundary, on living leaves of *E. biturbinata*, 14 Apr 2005, *A.J. Carnegie* (DAR 77432, culture ex-type DAR 77432 = CBS 121159).

Ascospore germination on MEA after 24 h. Germi-Germinates with two germ tubes from the apices roughly parallel to the long axis of the spore, mostly with secondary germ tubes (up to four) from the spore or primary germ tubes, which are mostly shorter than the primary germ tubes; spore becomes prominently constricted and slightly distorted, especially if secondary germ tube emanates from the spore, spore not darkening; length of ascospore and primary germ tubes at 24 h: 60–150 µm. This germination pattern is a combination of Type D and Type I described by Crous (1998).

Cultures. Cultures grow 25–30 mm diam in 1 mo, with olive-brown (4D8) to yellowish-brown (5E8) submerged hyphae, and olive-brown (4D8) to yellow-brown (5E5) aerial hyphae, the center slightly raised.

Host. *E. biturbinata*.

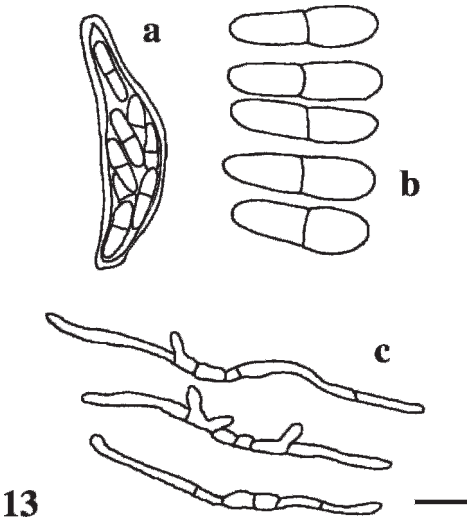


FIG. 13. *Mycosphaerella pseudovespa*. a. Ascus (bar = 20 µm). b. Ascospores (bar = 10 µm). c. Ascospore germination after 24 h on 2% MEA (bar = 20 µm).

Known distribution. Native forest in NSW, Australia; rare.

Notes. *Mycosphaerella pseudovespa* is morphologically similar to *M. vespa* (Carnegie and Keane 1998). Both are associated with wasp galls and small leaf spots with red-brown margins. The distribution of pseudothecia on the surface of lesions is also similar in these two species. Although the ascospores of *M. vespa* are more commonly constricted than those of *M. pseudovespa*, the sizes of these two species are similar and there is overlap of ascospore morphology. The main difference is in ascospore germination patterns: *M. pseudovespa* commonly produces secondary germ tubes, with up to four germ tubes common, whereas *M. vespa* commonly produces two germ tubes and less commonly produces a third germ tube. These two species also reside in separate phylogenetic clades. Recent research has shown that *M. vespa* and *M. molleriana* represent the same taxon and *M. vespa* has been synonimised with *M. molleriana* (Hunter et al 2006).

Mycosphaerella syncarpiae Carnegie & M.J. Wingf. sp. nov. FIGS. 14–18

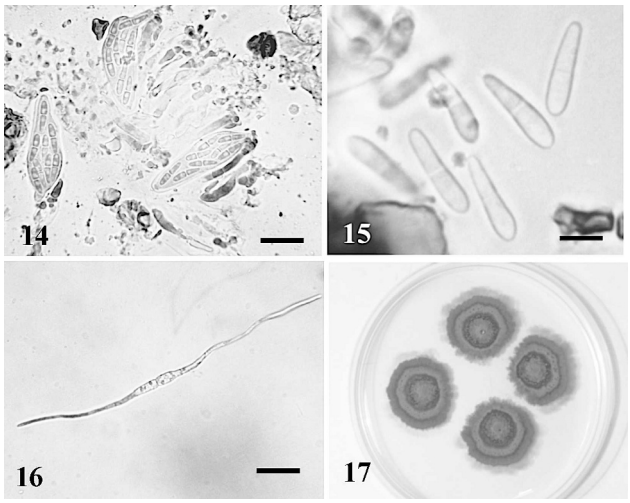
MycoBank MB500746

Anamorph: Not seen.

Etymology. Named after host.

Mycosphaerellae nubilosae, M. mollerianae, M. ohnowae et *M. communis* (omnes in *Eucalypto* parasiticae) in morphologia ascosporarum subsimilis sed in *Syncarpia* parasitica, pseudothesiis praecipue epiphyllis et coloniis in agaro cum annulis concentricis diverse colaratis post mensam unam differt.

Leaf spots amphigenous, circular to irregular with



FIGS. 14–17. *Mycosphaerella syncarpiae*. 14. Asci (bar = 30 µm). 15. Ascospores (bar = 10 µm). 16. Ascospore germination after 24 h on 2% MEA (bar = 20 µm). 17. Four single-spore cultures after 1 mo on 2% MEA.

an irregular margin, 4–12 mm diam, red-brown on the adaxial and yellow-brown on the abaxial surface, often associated with leaf distortion and often cracking. Pseudothecia amphigenous, predominantly epiphyllous, immersed to erumpent, scattered, black, globose, 55–88 µm. Asci bitunicate, fasciculate, ovoid to ellipsoidal, straight or curved, 8-spored, 48–59 × 10–13 µm. Ascospores bi- to multiseriate, overlapping, narrowly clavate, with obtuse ends, tapering toward basal end, not constricted at median septum or only slightly so, widest in middle of apical cell, hyaline, 15–18 × 3.5–5 µm.

HOLOTYPE: AUSTRALIA: NEW SOUTH WALES: Nana Glen, Orara State Forest, native forest, on living leaves of *Syncarpia glomulifera*, 23 Aug 2003, *A.J. Carnegie & M.J. Wingfield* (DAR 77433, culture ex-type DAR 77433, = CBS 121160; isotype NSWF 005320, culture ex-isotype NSWF 005320).

Ascospore germination on MEA after 24 hr. Germ tubes grow from both ends parallel to the long axis of the spore, spore not darkening or distorting but becoming prominently constricted; length of ascospore plus both germ tubes after 24 h (120–)165–216(–239) µm. Germination Type C.

Cultures. Colonies 20–25 mm after 1 mo, in concentric rings of color: submerged hyphae olive-gray (2F2) in center to olive (2F8) with outermost ring(s) sparse and olive (2F8) to olive-yellow (2D8); aerial hyphae in center brownish-gray (6C2) and raised, with several thin rings of gray (6B1) out from center; reverse homogenous dark gray to black.

Host. Syncarpia glomulifera.

Known distribution. New South Wales, Australia; common.

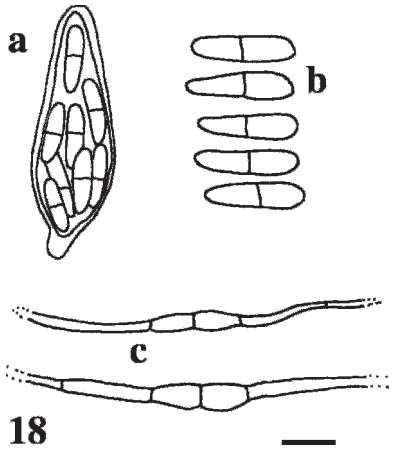
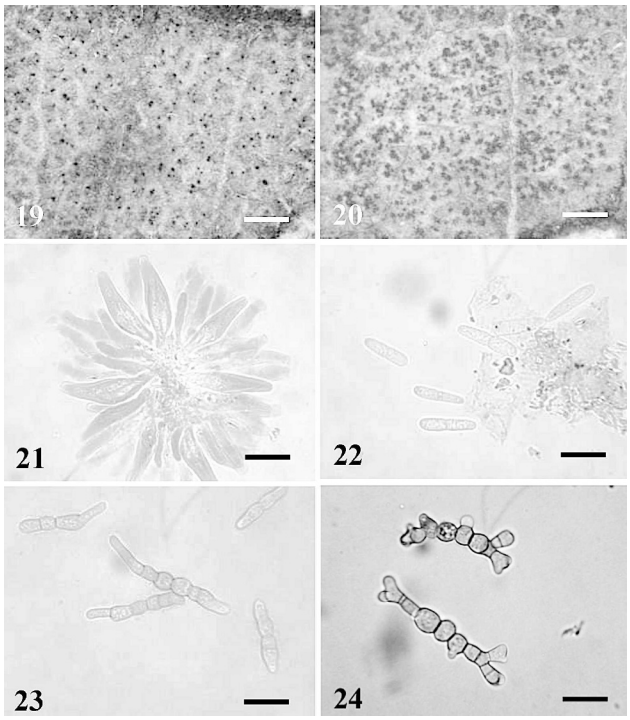


FIG. 18. *Mycosphaerella syncarpiae*. a. Ascus (bar = 20 µm). b. Ascospores (bar = 10 µm). c. Ascospore germination after 24 h on 2% MEA (bar = 15 µm).

Notes. Mycosphaerella syncarpiae was found at numerous locations in native forests of *Syncarpia glomulifera* in northeastern NSW and also from specimens in herbarium DAR collected mostly from the Sydney region. These latter specimens had been examined by R.F. Park in 1983, who noted that they represented a distinct, undescribed species. Like our observations R.F. Park (in 1983) and J. Walker (in 1962) noted that leaf spots were similar to *M. cryptica* (especially in pseudothecial distribution) and ascospores were similar to *M. nubilosa*. During this study *M. marksii* was observed commonly on *S. glomulifera* throughout eastern NSW; however the asymmetrical apical cell of *M. marksii* ascospores (Carnegie and Keane 1994) clearly distinguishes this species from *M. syncarpiae*. No other species of *Mycosphaerella* have been reported from *Syncarpia*. Based on ascospore morphology and germination *M. syncarpiae* resembles several species of *Mycosphaerella* from *Eucalyptus*, including *M. nubilosa, M. molleriana, M. crystallina, M. communis* and *M. ohnowa* (Park and Keane 1982, Crous 1998, Crous et al 2004). However *M. syncarpiae* can be distinguished from these latter species by its different pseudothecial distribution and distinctive cultural characteristics.

Crous (1999) reviewed the species of *Mycosphaerella* from Myrtaceae (other than *Eucalyptus*) and reported seven species. Only one of these, *M. angophorae*, has been recorded in Australia where it occurs on *Angophora bakeri* in NSW. *Mycosphaerella angophorae* has slightly corky lesions, predominantly hypogenous pseudothecia and small, broadly ellipsoidal ascospores (Hansford 1957, Crous 1999), features that distinguish it from *M. syncarpiae*. Based on lesion type, pseudothecial distribution and ascospore morphology, *M. syncarpiae* is also distinct from the other



FIGS. 19–24. *Mycosphaerella multiseptata*. 19. Pseudothecial distribution on abaxial surface of leaf spot on *A. subvelutina* (bar = 1 mm). 20. Pseudothecial distribution on abaxial surface of leaf spot on *A. costata* (bar = 1 mm). 21. Asci (bar = 30 μm). 22. Ascospores (bar = 20 μm). 23–24. Ascospore germination after 24 h on 2% MEA (bar = 15 μm).

species dealt with by Crous (1999). In their examination of herbarium specimens from Queensland, Sivanesan and Shivas (2002) described 12 new species of *Mycosphaerella* from a range of hosts, including one from Myrtaceae, given the name *M. melaleucoides* from *Melaleuca quinquenervia*. Although ascospores of *M. melaleucoides* and *M. syncarpiae* are similar, the epigenous leaf spots and ascomata of *M. melaleucoides* help distinguish it from *M. syncarpiae*.

Additional specimens examined. AUSTRALIA: NEW SOUTH WALES: Urunga, Newry State Forest, native forest, on living leaves of *S. glomulifera*, 20 Feb 2004, *A.J. Carnegie* (DAR 77434); Morrisett, Olney State Forest, native forest, on living leaves of *S. glomulifera*, 21 Nov 1996, *J.A. Simpson & A.J. Carnegie* (DAR 77436; NSWF 005525); Nana Glen, Wedding Bells State Forest, native forest, on living leaves of *S. glomulifera*, 11 Apr 2005, *A.J. Carnegie* (DAR 77437); as *Mycosphaerella* sp., Baulkham Hills, on *S. glomulifera*, Jun 1957, *J. Walker* (DAR 5200); Pittwater, on *S. glomulifera*, Mar 1948, *L. Fraser* (DAR 4787); Mountain Lagoon, on *S. glomulifera*, 28 Mar 1982, *M. Priest* (DAR 45633); as *M. nubilosa*, Baulkham Hills, on *S. glomulifera*, 1 Jan 1957, *J. Walker* (DAR 4986); Kurrajong Heights, on *S. glomulifera*, 27 Apr 1949, *L. Fraser* (DAR 3870); Palm Beach, on *S. glomulifera*, Apr 1949, *L. Fraser* (DAR 3869).

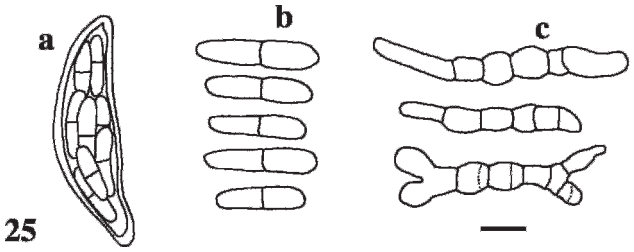


FIG. 25. *Mycosphaerella multiseptata*. a. Ascus (bar = 20 μm). b. Ascospores (bar = 8 μm). c. Ascospore germination after 24 h on 2% MEA (bar = 8 μm).

Mycosphaerella multiseptata Carnegie sp. nov.
FIGS. 19–25

MycoBank MB500744

Anamorph: not seen.

Etymology. Forming multiple septa in the spore body and germ tubes after 24 h germination.

Mycosphaerellae mexicanae in *Eucalypto* ascosporarum morphologia et modo germinationis subsimilis sed *Angophora* parasitica, coloniis in agaro lente crescentibus 5–6 mm post mensam unam et septis transverses numerosis in tubis germinationibus post 24 h.

Leaf spots on *A. subvelutina*: amphigenous, circular to irregular, single to confluent, 2–7 mm diam, yellow-brown becoming gray-brown on adaxial surface, yellow-brown to red-brown on abaxial surface, with prominent red-brown border; on *A. costata*: amphigenous, subcircular with irregular margins, bordered by veins, single to confluent, 3–12 mm diam, yellow-brown becoming gray-brown on adaxial surface, yellow-brown to red-brown on abaxial surface, with prominent red-brown border, often with anthocyanin pigmentation surrounding border. Pseudothecia on *A. subvelutina*: hypophyllous, scattered singly, immersed, black, globose, 75–100 μm; on *A. costata*: hypophyllous, in numerous clusters, immersed becoming erumpent, black, globose, 85–110 μm. Asci aparaphystae, fasiculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to slightly incurved, 8-spored, 43–56 × 10–15 μm. Ascospores 2–3-seriate, overlapping, hyaline, straight, rarely curved, narrowly obovoid to fusiform with obtuse ends, medianly or unequally 1-septate, widest in middle of apical cell, mostly not constricted at septum, tapering to basal end, (12.5–)13.5–17(–19) × (3–)3.5–4.5 (–5) μm.

HOLOTYPE: AUSTRALIA: NEW SOUTH WALES: Whiporie, Pintexan Property, native forest, on living leaves of *Angophora subvelutina*, 18 Jan 2005, *A. J. Carnegie* (DAR 77438; culture ex-type DAR 77438 = CBS 121312).

Ascospore germination on MEA after 24 h. Ascospores germinating from both ends, with germ tubes

parallel to the long axis of the spore, spore body becoming 2–3-septate, spore darkening and becoming distorted with a prominent constriction at the ascospore septum; even though relatively short, germ tubes have many septa, occasionally producing secondary germ tubes; length of ascospore and germ tubes after 24 h (38–)58–71(–81) μm. This germination pattern most closely resembles Type E and Type H.

Cultures. Slow growing, reaching 5–6 mm diam in 1 mo; submerged hyphae olive (1F4), aerial hyphae olive-brown (4E5) with white patches.

Hosts. A. subvelutina, A. costata

Known distribution. Native forests and amenity plantings in NSW, Australia; rare.

Notes. Based on symptoms and pseudothecia, this species on the two different host species initially was thought to represent two different taxa. On *A. subvelutina* leaf spots are mostly circular, while on *A. costata* they are subcircular to irregular. Pseudothecia on *A. subvelutina* are mostly scattered and immersed, and on *A. costata* they often are clustered and immersed to erumpent. However on both hosts ascospore morphology and germination patterns are the same and DNA sequence comparisons have shown that they are the same species of *Mycosphaerella*.

Mycosphaerella multiseptata differs from other species of *Mycosphaerella* from *Eucalyptus* based on a combination of host (*Angophora*), symptoms, ascospore morphology and ascospore germination pattern. It is most similar in ascospore morphology to *M. mexicana*; however ascospores of *M. multiseptata* become more constricted on germination and produce multiple germ tubes within the spore and germ tubes. *Mycosphaerella marksii* has been found on *Angophora*, as well as other Myrtaceae, but has different ascospore morphology and germination patterns (Carnegie and Keane 1994) to *M. multiseptata*. The only other species of *Mycosphaerella* found from *Angophora, M. angophorae* Hansf., can be distinguished from *M. multiseptata* by having smaller ascospores.

Additional specimens examined. AUSTRALIA: NEW SOUTH WALES: Baryugil, Yuligibar Property, native forest within plantation boundary, on living leaves of *A. subvelutina*, 20 Jan 2005, *A. J. Carnegie* (DAR 77439, culture DAR 77439 = CBS 121161); Sydney, Greenwich, Frenchs Road, amenity planting, on living leaves of *A. costata*, 22 Dec 2004, *A.J. & G. F. Carnegie* (DAR 77440, culture DAR 77440).

DISCUSSION

We have described four new species of *Mycosphaerella* from Myrtaceous trees growing in native forests as well as in plantations. *Mycosphaerella tumulosa* was found from many locations in both native forests and plantations in northern NSW and southeastern Queensland and on at least eight hosts in *Eucalyptus* and *Corymbia*. Although individual trees can be heavily infected this pathogen did not cause significant damage at a stand or plantation level. *Mycosphaerella syncarpiae* was identified from many localities in native forests and amenity plantings only from *Syncarpia glomulifera*. It was observed causing minor damage, mostly on individual trees in a stand. *Mycosphaerella multiseptata* was observed causing minor damage on species of *Angophora* in several native stands and an amenity planting. *Mycosphaerella pseudovespa* was recorded from only one locality and was not associated with significant damage.

A specific focus of this study was to survey for *Mycosphaerella* spp. on Myrtaceae in native forest situations or on trees in plantations adjacent to native forests, where the plantations might have acted as ''sinks'' for these fungi. Ultimately such collections might assist us in understanding why only six of the more than 40 species of *Mycosphaerella* first described from outside Australia have ever been found in this country. These include *M. suberosa* Crous, F.A. Ferreira, Alfenas & M.J. Wingf. (Carnegie et al 1997), *M. lateralis* Crous & M.J. Wingf. (Maxwell et al 2000), *M. mexicana* Crous (Maxwell et al 2003), *M. fori* Hunter et al (Jackson et al 2005), *M. heimii* Crous (Whyte et al 2005) and *M. ohnowa* Crous & M.J. Wingf. (Crous et al 2007). It is most likely that these species originated in Australia but were detected overseas first. This is because these species cause little damage in native ecosystems in Australia but, when introduced to susceptible, even aged exotic plantations, their impact is greater, disease symptoms are obvious and they are collected, isolated and described.

Recent surveys in native forests in NSW, including the current work, have resulted in the discovery of numerous new species of *Mycosphaerella* and other foliar fungi from eucalypts (Summerell et al 2006, Crous et al 2007). It seems likely that future surveys will reveal additional species of these fungi, including species currently known only from other countries. Although it might seem unusual that there are so many species of *Mycosphaerella* on *Eucalyptus*, we support the view of Crous et al (2006) that there could be at least as many species of *Mycosphaerella* on these trees as the number of species of the trees themselves. This would imply that ultimately there will more than 700 species of *Mycosphaerella* on *Eucalyptus* and that the majority of species have not yet been discovered.

ACKNOWLEDGMENTS

We thank John Walker for assistance with the Latin and for reviewing the manuscript, Dianne White for technical support associated with the DNA comparisons presented in this study and Pedro Crous for reviewing the manuscript.

LITERATURE CITED

Barber PA. 2005. A study of foliar diseases of eucalypts in plantations and native forests of south-eastern Australia [Doctoral dissertation]. Australia: La Trobe University. p 1–200.

Boland DJ, Brooker MIH, Chippendale GM, Hall N, Hyland BPM, Johnston RD, Kleinig DA, Turner JD. 1992. Forest trees of Australia. Melbourne: Australian Government Publishing Service. 687 p.

Braun U, Dick MA. 2002. Leaf spot diseases of eucalypts in New Zealand caused by *Pseudocercospora* species. NZ J For Sci 32:221–234.

Burgess T, Wingfield MJ, Wingfield BD. 2001. Simple sequence repeat (SSR) markers distinguish between morphotypes of *Sphaeropsis sapinea*. Appl Environ Microbiol 67:354–362.

———, Barber PA, Sufaati S, Xu D, Hardy GESJ, Dell B. 2006. *Mycosphaerella* spp. on eucalypts in Asia; new species, new hosts and new records. Fungal Divers 24: 135–157.

Carnegie AJ. 1991. The susceptibility of certain *Eucalyptus* species and provenances to infection by *Mycosphaerella* spp. and other leaf parasites (Honors dissertation). Australia: La Trobe University. p 1–160.

———. 2000. A study of the species of *Mycosphaerella* on eucalypts in Australia and the impact of *Mycosphaerella* leaf diseases on *Eucalyptus globulus* Labill. [Doctoral dissertation]. Australia: University of Melbourne. p 1–203.

———, Keane PJ. 1994. Further *Mycosphaerella* species associated with leaf diseases of *Eucalyptus*. Mycol Res 98:413–418.

———, ———. 1998. *Mycosphaerella vespa* sp. nov. from diseased *Eucalyptus* leaves in Australia. Mycol Res 102: 1274–1276.

———, ———, Podger FD. 1997. The impact of three species of *Mycosphaerella* newly recorded on *Eucalyptus* in Western Australia. Aust Plant Path 26:71–77.

Cortinas M.-N, Burgess TI, Dell B, Xu D, Wingfield MJ, Wingfield BD. 2006. First record of *Colletogloeopsis zuluense* comb. nov., causing a stem canker of *Eucalyptus* spp. in China. Mycol Res 110:229–236.

Crous PW. 1998. *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. Mycol Mem 21:1–170.

———. 1999. Species of *Mycosphaerella* and related anamorphs occurring on Myrtaceae (excluding *Eucalyptus*). Mycol Res 103:607–621.

———, Groenewald JZ, Mansilla JP, Hunter GC, Wingfield MW. 2004. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. Stud Mycol 50:195–214.

———, Hong L, Wingfield BD, Wingfield MJ. 2001. ITS rDNA phylogeny of selected *Mycosphaerella* species and their anamorphs occurring on *Myrtaceae*. Mycol Res 105:425–431.

———, Knox-Davies PS, Wingfield MJ. 1989. A summary of fungal leaf pathogens of *Eucalyptus* and the diseases they cause in South Africa. SA For J 149:9–16.

———, Summerell BA, Carnegie AJ, Mohammed C, Himaman W, Groenewald JZ. 2007. Foliicolous *Mycosphaerella* spp. and their anamorphs on *Corymbia* and *Eucalyptus*. Fungal Divers 26. (In press).

———, Wingfield MJ, Mansilla JP, Alfenas AC, Groenewald JZ. 2006. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. II. Stud Mycol 55:99–131.

Dick MA, Dobbie K. 2001. *Mycosphaerella suberosa* and *M. intermedia* sp. nov. on *Eucalyptus* in New Zealand. NZ J Bot 39:269–279.

Felsenstein J. 1985. Confidence intervals on phylogenetics: an approach using bootstrap. Evolution 39:783–791.

Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhiza and rusts. Mol Ecol 2:113–118.

Hansford CG. 1957. Australian fungi IV. New species and revisions (continued). Proc Linn Soc NSW 82:209–229.

Hillis DM, Huelsenbeck JP. 1992. Signal, noise and reliability in molecular phylogenetic analysis. J Hered 83: 189–195.

Hunter GC, Roux J, Wingfield BD, Crous PW, Wingfield MJ. 2004. *Mycosphaerella* species causing leaf disease in South African *Eucalyptus* plantations. Mycol Res 108: 672–681.

———, Wingfield BD, Crous PW, Wingfield MJ. 2006a. A multi-gene phylogeny for species of *Mycosphaerella* occurring on *Eucalyptus* leaves. Stud Mycol 55:147–161.

———, Crous PW, Wingfield BD, Pongpanich K, Wingfield MJ. 2006b. *Pseudocercospora flavomarginata* sp. nov., from *Eucalyptus* leaves in Thailand. Fungal Divers 22: 71–90.

Jackson SL, Maxwell A, Collins S, Dell B, Hardy GEStJ. 2005. *Mycosphaerella* species present on *Eucalyptus globulus* in Western Australia, new records for WA and Australia. In: 15th Biennial Australasian Plant Pathology Society Conference Handbook. 185 p.

Kornerup A, Wanscher JH. 1978. Methuen Handbook of Colour. London: Eyre Methuen. p 1–252.

Maxwell A. 2004. The taxonomy, phylogeny and impact of *Mycosphaerella* species on eucalypts in south-western Australia [Doctoral dissertation]. Western Australia: Murdoch University. p 1–225.

———, Dell B, Neumeister-Kemp HG, Hardy GEStJ. 2003. *Mycosphaerella* species associated with *Eucalyptus* in south-western Australia: new species, new records and a key. Mycol Res 107:351–359.

———, Hardy GEStJ, Wingfield MJ, Dell B. 2000. First record of *Mycosphaerella latreralis* on *Eucalyptus* in Australia. Aust Plant Path 29:279.

Milgate A. 2005. *Mycosphaerella* species of Tasmania and their interactions with *Eucalyptus* plantations [Doctoral

dissertation]. Tasmania: University of Tasmania. p 1–199.

Park RF. 1984. The taxonomy, pathology and epidemiology of *Mycosphaerella* species associated with leaf diseases of *Eucalyptus* in Australia [Doctoral dissertation]. Australia: La Trobe University. p 1–205.

———, Keane PJ. 1982. Three *Mycosphaerella* species from leaf diseases of *Eucalyptus*. Trans Brit Mycol Soc 79:95–100.

———, ———, Wingfield MJ, Crous PW. 2000. Fungal diseases of *Eucalyptus* foliage. In: Keane PJ, Kile GA, Podger FD, Brown BN, eds. Diseases and pathogens of eucalypts. Melborne: CSIRO. p 153–240.

Pryor LD, Johnson LAS. 1971. A classification of the eucalypts. Canberra: Australian National University. p 1–102.

Sankaran KV, Sutton BC, Minter D. 1995. A checklist of fungi recorded on *Eucalyptus*. United Kingdom: CAB International. p 1–376.

Sivanesan A, Shivas RG. 2002. Studies on *Mycosphaerella* species in Queensland, Australia. Myco Res 106:355–364.

Slippers B, Stenlid J, Wingfield MJ. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends Ecol Evol 20:420–421.

Summerell BA, Groenewald JZ, Carnegie AJ, Summerbell RC, Crous PW. 2006. *Eucalyptus* microfungi known from culture 2. *Alysidiella, Fusculina* and *Phlogicylindrium* genera nova, with notes on some other poorly known taxa. Fungal Divers 23:323–350.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Snisky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.

Whyte G, Burgess TI, Barber PA, Hardy GEStJ. 2005. First record of *Mycosphaerella heimii* in Australia. Aust Plant Path 34:605–606.

Yuan Z.-Q, de Little DAR, Mohammed C. 2000. A new species of *Pseudocercospora* (Hyphomycetes) described on *Eucalyptus nitens* from Tasmania, Australia. Nov Hedwig 71:415–419.