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Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*

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Abstract: Species of *Eucalyptus*, mostly native to Australia, are widely planted as exotics in the tropics and Southern Hemisphere. These plantations represent an important source of fuel-wood, structural timber and pulp. *Eucalyptus* plantations are, however, vulnerable to infection by pathogens, including *Mycosphaerella* spp. and their anamorphs, which have caused substantial damage, in many parts of the world. More than 30 species of *Mycosphaerella*, and close to 30 anamorph species for which the *Mycosphaerella* state remains unknown, are associated with leaf and shoot disease on *Eucalyptus* spp., worldwide. Although several studies using DNA sequence data have been applied to resolve the phylogenetic relationships between *Mycosphaerella* spp. on *Eucalyptus*, the number of species treated has been incomplete. In the present study, isolates of 44 *Mycosphaerella* species or their anamorphs associated with lesions on *Eucalyptus* leaves were compared based on DNA sequence data for the internal transcribed spacer region (ITS1 & ITS2) and the 5.8S gene. In addition, DNA sequence data from the elongation factor 1-α and the β-tubulin gene regions were used to resolve species in the *M. nubilosa* species complex. As a result of these comparisons, 11 new species are described. *Mycosphaerella juvenis* is reduced to synonymy with *M. nubilosa* and an epitype specimen and ex-epitype culture are designated for the latter. *Mycosphaerella nubilosa* is recorded as a serious agent of Mycosphaerella leaf blotch on *E. globulus* in Spain. This is also the first definitive record of this pathogen occurring on *Eucalyptus* in Europe.

Taxonomic novelties: *Mycosphaerella madeirae* Crous & Denman sp. nov., *M. toledana* Crous & G. Bills sp. nov. (anamorph *Phaeophleospora toledana* Crous & G. Bills sp. nov.), *M. readeriellophora* Crous & J.P. Mansilla sp. nov. (anamorph *Readeriella readeriellophora* Crous & J.P. Mansilla sp. nov.), *M. communis* Crous & J.P. Mansilla sp. nov. (anamorph *Dissoconium commune* Crous & J.P. Mansilla sp. nov.), *M. ohnowa* Crous & M.J. Wingf. sp. nov., *Passalora zambiae* Crous & T. Coutinho sp. nov., *Pseudocercospora pseudoeucalyptorum* Crous sp. nov., *Readeriella novaezelandiae* Crous sp. nov.
Key words: *Ascomycetes*, *Dissoconium*, DNA sequence comparisons, *Mycosphaerella*, *Passalora*, *Phaeophleospora*, *Pseudocercospora*, *Readeriella*, systematics.

INTRODUCTION

Species of *Eucalyptus* L'Hérit., primarily native to Australia, are widely planted as exotics in the tropics, Mediterranean region and Southern Hemisphere. These plantations that cover more than 8 million hectares, sustain major industries producing timber products and pulp. They also represent important sources of income and fuel wood for resource-poor farmers. *Eucalyptus* spp. planted as exotics are well-known for their exceptional growth, probably due to the separation of these trees from their natural enemies (Wingfield 2001). However, diseases have had a serious negative impact on plantations in some parts of the world, and this is a situation that appears to be worsening. Mycosphaerella leaf blotch (MLB) was one of the first diseases to seriously damage plantations of *Eucalyptus* outside their native range (Crous 1998). For example, early plantations of *Eucalyptus globulus* Labill. in South Africa were devastated by MLB, and the disease resulted in the abandonment of this species for plantation development (Purnell & Lundquist 1986).

Several species of *Mycosphaerella* Johanson, such as *M. cryptica* (Cooke) Hansf. and *M. nubilosa* (Cooke) Hansf., cause severe defoliation and leaf blotch symptoms, particularly of *E. globulus* and *E. nitens* Maiden in Australia, South Africa, and elsewhere (Carnegie *et al*. 1994, Crous & Wingfield 1996, Dungey *et al*. 1997). In New Zealand, *M cryptica* is documented to have caused an epidemic in over 1000 ha of *E. delegatensis* R.T. Bak. (Cheah 1977). More recently, an asexual state of *Mycosphaerella*, *Phaeophloespora destructans* (M.J. Wingf. & Crous) Crous, F.A. Ferreira & B. Sutton, has begun to cause devastating leaf and shoot blight of *E. grandis* W. Hill ex Maiden, *E. camaldulensis* Dehnh. and hybrids of these and other species in South-East Asia (Wingfield *et al*.

1996). These fungi are clearly amongst the most important and most threatening pathogens of *Eucalyptus* spp., and they are likely to become increasingly important in the future.

Mycosphaerella is one of the largest genera of *Ascomycetes*, for which more than 2000 species names have been proposed (Corlett 1991). It also has several thousand anamorph species that lack known teleomorphs (Crous & Braun 2003). In a recent taxonomic treatment, Crous (1998) included 55 species that were known from *Eucalyptus*, although subsequent studies have shown that many more species are present on this host (Carnegie & Keane 1998, Braun & Dick 2002, Maxwell *et al*. 2003, Hunter *et al*. 2004).

Species identification in *Mycosphaerella* is extremely difficult. This is particularly because 4–5 different species frequently inhabit the same lesion, and these often also overlap in morphological characteristics. Ascospore germination patterns, characteristics of the fungi in culture and anamorph morphology, have made it possible to distinguish some of these taxa (Crous 1998). The more recent incorporation of DNA sequence data has allowed for more accurate species delimitation and has elucidated phylogenetic relationships in these fungi (Crous *et al*. 2000, 2001a, b). DNA sequence comparisons have, however, also shown that there can be several phylogenetic species encompassed in what have been perceived to represent well-defined morphological taxa (Crous *et al*. 2000, 2001a, b).

The aim of this study was to compare the largest possible number of *Mycosphaerella* species from *Eucalyptus*, based on DNA, cultural characteristics and morphology. In this way we wished to test the reliability of the morphological species defined by Crous (1998). All isolates used in the study were compared based on the sequences of their internal transcribed spacer region (ITS-1 & ITS-2) and the 5.8S gene. Furthermore, isolates of *M. juvenis* Crous & M.J. Wingf., a species that is morphologically similar to the important pathogen, *M. nubilosa*, were compared using sequences for the elongation factor 1-α and the β-tubulin gene regions.

MATERIALS AND METHODS

Isolates

Leaves showing symptoms of MLB or leaf and shoot blight associated with *Mycosphaerella* spp. and their anamorphs, were chosen for isolations. Excised lesions were placed in water for approximately 2 h, after which they were placed on double-sided tape and fastened to the insides of Petri dish lids, suspended over 2 % malt extract agar (MEA) (2 g/L) (Biolab, Midrand, South Africa). Germination patterns of ascospores were examined after 24 h, and single-ascospore and conidial cultures established as explained by Crous (1998). Colonies were sub-cultured onto carnation leaf agar (CLA) [1 % water agar (1 g/L) (Biolab) with autoclaved carnation leaves placed onto the medium] and incubated at 25 °C under continuous near-ultraviolet light, to promote sporulation.
To resolve the ascospore germination patterns of *M. juvenis* and *M. nubilosa*, original material, slides and cultures used by Crous (1998) were re-examined. Fresh material was also studied from South Africa (Hunter *et al*. 2004), as well as Australia, New Zealand and Spain.

DNA phylogeny

The protocol of Lee & Taylor (1990) was used to isolate genomic DNA from fungal mycelium, grown on MEA in Petri dishes. The primers ITS1 and ITS4 (White *et al*. 1990) were used to amplify part of the nuclear rRNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The PCR reaction mixture consisted of 0.75 units Biotaq (Bioline, London, U.K.), 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 µM of each dNTP, 5 pmol of each primer, approximately 10 to 30 ng of fungal genomic DNA and was made up to a total volume of 25 µL with sterile water. Reactions were performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) and the cycling conditions consisted of denaturation for 5 min at 96 °C, followed by 30 cycles at 96 °C (30 s), 55 °C (30 s), 72 °C (90 s) and a final 7 min extension step at 72 °C to complete the reaction.

For isolates of *M. juvenis* and *M. nubilosa* part of the elongation factor 1-alpha (EF-1α) gene was amplified with primers EF1-728F and EF1-986R (Carbone & Kohn 1999) and part of the β-tubulin gene was amplified with primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995). PCR conditions for EF-1α and β-tubulin genes were the same as those for ITS, except for the $MgCl_2$ concentration, which was increased to 2.0 mM for β-tubulin. PCR products were separated by electrophoresis at 80 V for 1 h in a 0.8 % (w/v) agarose gel in 0.5× TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, U.K.) following ethidium bromide staining. The amplification products were purified according to the manufacturer's instructions using a commercial kit (GFX PCR DNA and Gel Band Purification Kit, Amersham Pharmacia Biotech Europe GmbH, Germany). Sequencing reactions were carried out using the PCR primers in ABI PRISM Big Dye Terminator Cycle v 3.0 Sequencing Ready Reaction Kit (Applied Biosystems) following the manufacturer's recommendations. The reactions were analysed on an ABI Prism 3100 Genetic Analyser (Applied Biosystems).

Table 1. *Mycosphaerella* isolates included in this study for sequence analysis and morphological comparison.

Teleomorph	Anamorph	Accession no.[1]	Substrate	Country	Collector	GenBank accession[3]		
						ITS	EF	TUB
Unknown	*"Coniothyrium ovatum"*	CBS 110906; CPC 40	*E. cladocalyx*	South Africa	P.W. Crous	AY725513		
		CBS 111149; CPC 23	*E. cladocalyx*	South Africa	P.W. Crous	AY725514		
		CBS 113621; CPC 42	*E. cladocalyx*	South Africa	P.W. Crous	AY725515		
		CBS 116427; CPC 10941	*Eucalyptus sp.*	South Africa	P.W. Crous	AY725516		
		CPC 18	*E. cladocalyx*	South Africa	P.W. Crous	AY725517		
Unknown	*"Coniothyrium"* sp.	CBS 116428; CPC 10886	*Eucalyptus* sp.	South Africa	P.W. Crous	AY725518		
Unknown	*Dissoconium aciculare*	CBS 201.89	*Brassica* sp.	Netherlands	T. Hijwegen	AY725519		
		CBS 204.89	*Astragalus* sp.	Germany	T. Hijwegen	AY725520		
		CBS 116429; CPC 10805	*Amorpha fruticosa*	Korea	H.D. Shin	AY725521		
Unknown	*Passalora zambiae*	CBS 112970[2]; CPC 1228	*E. globulus*	Zambia	T. Coutinho	AY725522		
		CBS 112971[2]; CPC 1227	*E. globulus*	Zambia	T. Coutinho	AY725523		
Unknown	*Pseudocercospora "eucalyptorum"*	CBS 116291; CPC 10503	*Eucalyptus* sp.	China	A. Aptroot	AY725525		
Unknown	*Ps. pseudoeucalyptorum*	CPC 10390[2]; CBS 114242	*E. globulus*	Spain	J.P.M. Vazquez	AY725526		
		CPC 10500; CBS 114243	*E. nitens*	New Zealand	W. Gams	AY725527		
		CBS 116371; CPC 10507	*E. nitens*	New Zealand	W. Gams	AY725528		
Unknown	*Readeriella mirabilis*	CBS 116293; CPC 10506	*E. fastigata*	New Zealand	W. Gams	AY725529		
M. ambiphylla	*Phaeophleospora* sp.	CBS 110499[2]	*E. globules*	Australia	A. Maxwell	AY725530		
		CPC 4577	*Eucalyptus* sp.	Australia	A. Maxwell	AY725524		
M. aurantia	Unknown	CBS 110500[2]	*E. globulus*	Australia	A. Maxwell	AY725531		
M. colombiensis	*Ps. colombiensis*	CBS 110967[2]; CPC 1104	*E. urophylla*	Colombia	M.J. Wingfield	AY725532		
		CBS 110968[2]; CPC 1105	*E. urophylla*	Colombia	M.J. Wingfield	AY725533		
		CBS 110969[2]; CPC 1106	*E. urophylla*	Colombia	M.J. Wingfield	AY725534		
M. communis	*Dissoconium commune*	CBS 110747; CPC 831	*E. nitens*	South Africa	P.W. Crous	AY725535		
		CBS 110809; CPC 830	*E. nitens*	South Africa	P.W. Crous	AY725536		
		CBS 110976; CPC 849	*E. cladocalyx*	South Africa	P.W. Crous	AY725537		
		CBS 111270; CPC 1190	*E. nitens*	South Africa	M.J. Wingfield	AY725538		
		CBS 112889; CPC 3359	*Protea magnifica*	Australia	P.W. Crous	AY725539		
		CBS 112890; CPC 1189	*E. nitens*	South Africa	M.J. Wingfield	AY725540		
		CPC 10440[2]; CBS 114238	*E. globulus*	Spain	J.P.M. Vazquez	AY725541		
		CPC 10492; CBS 114239	*E. globulus*	New Zealand	W. Gams	AY725542		
		CBS 116284; CPC 10510	*E. globulus*	New Zealand	W. Gams	AY725543		
		CBS 116286; CPC 10515	*E. globulus*	New Zealand	W. Gams	AY725544		
M. ellipsoidea	*Uwebraunia ellipsoidea*	CBS 110843[2]; CPC 850	*E. cladocalyx*	South Africa	P.W. Crous	AY725545		
M. intermedia	Unknown	CPC 10902; CBS 114356	*E. saligna*	New Zealand	M. Dick	AY725546		
		CPC 10922; CBS 114415	*E. saligna*	New Zealand	M. Dick	AY725547		
M. juvenis	*Uwebraunia juvenis*	CPC 933[2]; CBS 115669	*E. nitens*	South Africa	M.J. Wingfield	AY725548	AY725582	AY725597
		CBS 116292; CPC 934[2]	*E. nitens*	South Africa	M.J. Wingfield	AY725549	AY725583	AY725598
M. lateralis	*Uwebraunia dekkeri*	CBS 111169; CPC 1232	*E. globulus*	Zambia	T. Coutinho	AY725550		
		CBS 111272; CPC 1188	*E. nitens*	South Africa	M.J. Wingfield	AY725551		
		CBS 111282; CPC 1233	*E. globulus*	Zambia	T. Coutinho	AY725552		

M. madeirae	Unknown	CBS 112895; CPC 3745	*Eucalyptus* sp.	Madeira	S. Denman	AY725553		
M. marksii	Unknown	CBS 116290; CPC 10873	*E. botryoides*	New Zealand	M. Dick	AY725554		
		CBS 116285; CPC 10876	*E. botryoides*	New Zealand	M. Dick	AY725555		
		CBS 116288; CPC 10892	*E. botryoides*	New Zealand	M. Dick	AY725556		
		CBS 116287; CPC 10359	*E. globulus*	Spain	J.P.M. Vazquez	AY725557		
M. mexicana	Unknown	CBS 110502	*E. globulus*	Australia	A. Maxwell	AY725558		
M. molleriana	*Colletogloeum molleriana*	CBS 116368; CPC 10391	*E. globulus*	Spain	J.P.M. Vazquez	AY725559		
		CBS 116369; CPC 10394	*E. globulus*	Spain	J.P.M. Vazquez	AY725560		
		CBS 116370; CPC 10397	*E. globulus*	Spain	J.P.M. Vazquez	AY725561		
M. nubilosa	*Uwebraunia juvenis*	CBS 111968; CPC 1079	*E. globulus*	Kenya	M.J. Wingfield	AY725562	AY725584	AY725599
		CBS 111969; CPC 1078	*E. globulus*	Kenya	M.J. Wingfield	AY725563	AY725585	AY725600
		CBS 112972; CPC 1007	*E. nitens*	South Africa	M.J. Wingfield	AY725564	AY725586	AY725601
		CBS 113064; CPC 4665	*E. globulus*	Spain	J.P.M. Vazquez	AY725565	AY725587	AY725602
		CPC 10360; CBS 114241	*E. globulus*	Spain	J.P.M. Vazquez	AY725566	AY725588	AY725603
		CPC 1099	*Eucalyptus* sp.	Tanzania	M.J. Wingfield	AY725567	AY725589	AY725604
		CPC 3722	*E. globulus*	Spain	J.P.M. Vazquez	AY725568	AY725590	AY725605
		CBS 111445; CPC 4659	*E. globulus*	Spain	J.P.M. Vazquez	AY725569	AY725591	AY725606
		CPC 4661	*E. globulus*	Spain	J.P.M. Vazquez	AY725570	AY725592	AY725607
		CPC 4663	*E. globulus*	Spain	J.P.M. Vazquez	AY725571	AY725593	AY725608
		CBS 116005[2]; CPC 937	*E. globulus*	Australia	A. Carnegie	AY725572	AY725594	AY725609
M. "nubilosa"	Unknown	CBS 116283; CPC 10495	*E. globulus*	New Zealand	W. Gams	AY725573	AY725595	AY725610
		CPC 10497; CBS 114419	*E. globulus*	New Zealand	W. Gams	AY725574	AY725596	AY725611
M. ohnowa	Unknown	CBS 110949[2]; CPC 1006	*E. grandis*	South Africa	M.J. Wingfield	AY725575		
M. parva	Unknown	CBS 116289; CPC 10935	*Eucalyptus* sp.	South Africa	P.W. Crous	AY725576		
M. readeriellophora	*R. readeriellophora*	CPC 10375[2]; CBS 114240	*E. globulus*	Spain	J.P.M. Vazquez	AY725577		
Mycosphaerella sp.	*R. novaezelandiae*	CPC 10895[2]; CBS 114357	*E. botryoides*	New Zealand	M. Dick	AY725578		
M. suberosa	Unknown	CPC 515[2]	*E. dunnii*	Brazil	M.J. Wingfield	AY725579		
M. toledana	*Ph. toledana*	CBS 113313[2]	*Eucalyptus* sp.	Spain	P.W. Crous	AY725580		
		CPC 10840; CBS 115513	*Eucalyptus* sp.	Spain	P.W. Crous	AY725581		

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS. [2]Ex-type cultures. [3]GenBank accession numbers for sequence data.

The ITS nucleotide sequences generated in this study were added to other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) and the alignment was assembled using Sequence Alignment Editor v 2.0a11 (Rambaut 2002) with manual adjustments for visual improvement where necessary.

Phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2000). Phylogenetic analysis of the complete ITS alignment consisted of neighbour-joining analysis with the uncorrected ("p"), the Jukes-Cantor and the Kimura 2-parameter substitution model in PAUP. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. When they were encountered, ties were broken randomly.

For parsimony analysis of *M. juvenis* and *M. nubilosa* isolates, alignment gaps were treated as both a fifth character state and as missing and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option with 100 random taxon additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Measures calculated for parsimony included tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC, respectively). The robustness of the resulting phylogenetic trees was evaluated by 1000 bootstrap replications (Hillis & Bull 1993) and the trees were printed with TreeView v. 1.6.6 (Page 1996). A partition homogeneity test (Farris *et al.* 1994) was conducted in PAUP to consider the feasibility of combining the various sequence data sets used for the *M. juvenis* and *M. nubilosa* isolates. Sequence data were deposited in GenBank (Table 1) and the alignments in TreeBASE (accession number S1157).

Taxonomy

Wherever possible, thirty measurements (× 1000 magnification) were made of structures mounted in lactic acid, and the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 1 mo on MEA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands (Table 1).

RESULTS

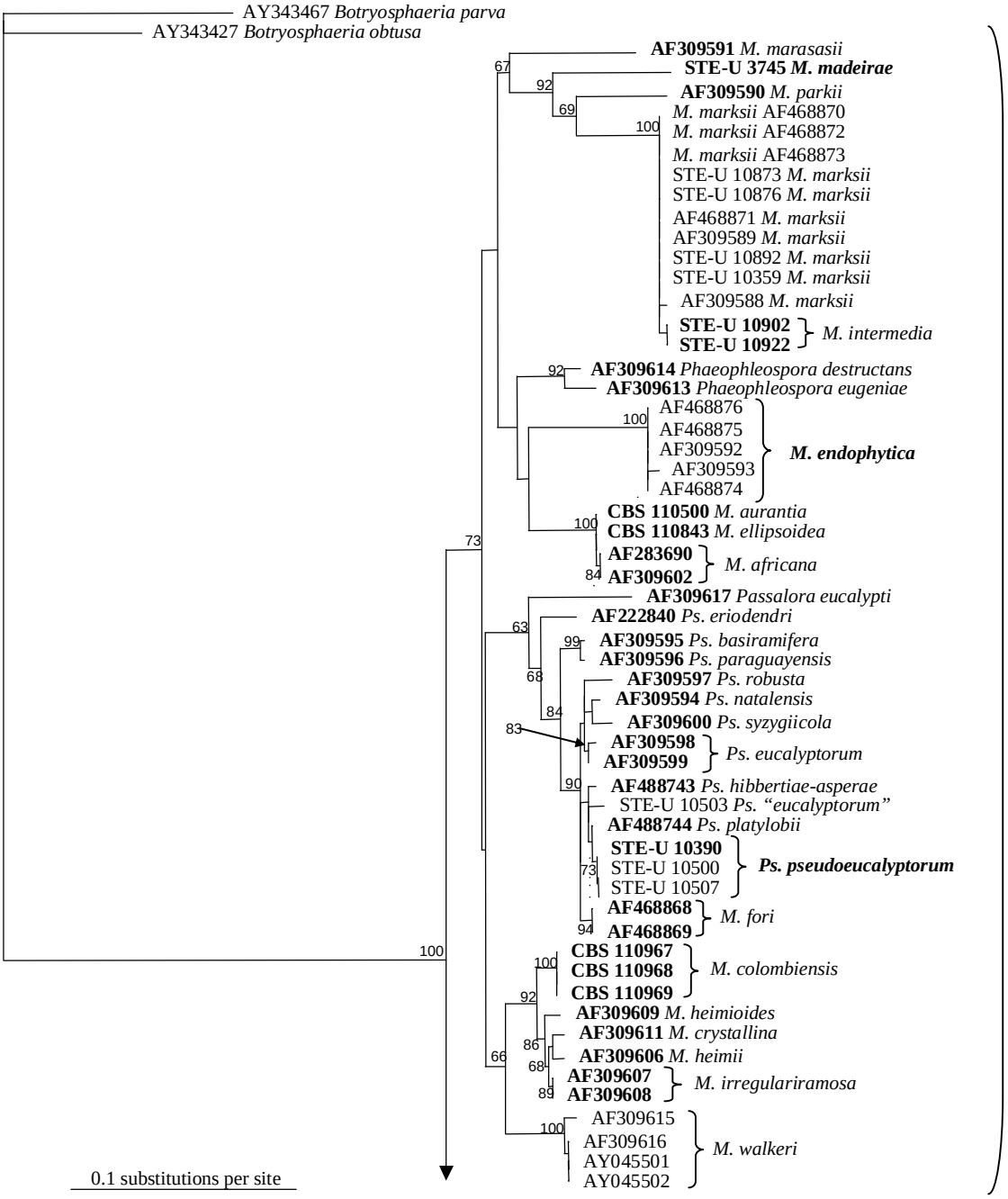
DNA Phylogeny

For the ITS region, approximately 500 to 560 bases were determined for all isolates (Table 1). The manually adjusted alignment of the ITS nucleotide sequences contained 134 taxa (including the two outgroups) and 572 characters including alignment gaps (TreeBASE accession number S1157). Neighbour-joining analysis using the three substitution models, yielded trees with similar topology and bootstrap values. The topology of the trees generated with the Jukes-Cantor and Kimura-2-parameter models were identical, whereas the uncorrected "p" model yielded a tree that differed from the other two models mainly in the higher hierarchy (data not shown). The distance tree obtained using the Kimura 2-parameter substitution model is shown in Fig. 1.

Four well-supported major clades, each containing several sub-clades, were delimited in the tree (Fig. 1). The major clade (clade 1) (73 % bootstrap support), contained a sub-clade (100 % bootstrap support) with isolates of *M. marksii* Carnegie & Keane and *M. intermedia* M. Dick & K. Dobbie. Clade 1 also included two *Phaeophleospora* Rangel species (92 % bootstrap support), a sub-clade of five isolates of *M. endophytica* Crous & H. Smith (100 % bootstrap support) and a sub-clade (100 % bootstrap support) containing *M. ellipsoidea* Crous & M.J. Wingf., *M. aurantia* A. Maxwell and two *M. africana* Crous & M.J. Wingf. isolates. Clade 1 also included a sub-clade with *Pseudocercospora* Speg. species as well as *Mycovellosiella eucalypti* Crous & Alfenas and two isolates of *M. fori* G.C. Hunter, Crous & M.J. Wingf. (63 % bootstrap support). Another sub-clade (66 % bootstrap support) included *M. colombiensis* Crous & M.J. Wingf., *M. irregulariramosa* Crous & M.J. Wingf. and *M. walkeri* R.F. Park & Keane, as well as single isolates of *M. heimioides* Crous & M.J. Wingf., *M. crystallina* Crous & M.J. Wingf. and *M. heimii* Crous.

The second major clade (clade 2) in the phylogenetic tree (Fig. 1) (73 % bootstrap support) contained isolates and sub-clades that are basal to each other. Single isolates that did not form clear groupings with significant bootstrap support were those of *M. mexicana* Crous, *M. tasmaniensis* Crous & M.J. Wingf. and *M. suttonii* Crous & M.J. Wingf. Isolates of *Readeriella* Syd. and *M. readeriellophora* sp. nov. clustered together (100 % bootstrap support), as did those of *M. suberosa* Crous, F.A. Ferreira, Alfenas & M.J. Wingf. (100 % bootstrap support) and *Passalora zambiae* sp. nov. (100 % bootstrap support). However, these isolates did not form well-supported associations with other isolates in the tree. Clade 2 (Fig. 1) contained *M. flexuosa* Crous & M.J. Wingf. and sequences of *M. ohnowa*. A sequence of *M. parva* R.F. Park & Keane and *M.* "*grandis*" Carnegie & Keane grouped with a 100 % bootstrap support in this clade.

The third major clade (clade 3) (86 % bootstrap support) in the phylogenetic tree contained a well-supported sub-clade grouping *M. nubilosa* (Cooke) Hansf. and *M. juvenis* Crous & M.J. Wingf. isolates (98 % bootstrap support) that clustered together (100 % bootstrap support) with four other isolates that had tentatively been assigned to *M. "nubilosa"* (96 % bootstrap support).


AY343467 Botryosphaeria parva
AY343427 Botryosphaeria obtusa
AF309591 M. marasasii
STE-U 3745 M. madeirae
AF309590 M. parkii
M. marksii AF468870
M. marksii AF468872
M. marksii AF468873
STE-U 10873 M. marksii
STE-U 10876 M. marksii
AF468871 M. marksii
AF309589 M. marksii
STE-U 10892 M. marksii
STE-U 10359 M. marksii
AF309588 M. marksii
STE-U 10902
STE-U 10922
M. intermedia
AF309614 Phaeophleospora destructans
AF309613 Phaeophleospora eugeniae
AF468876
AF468875
AF309592
AF309593
AF468874
M. endophytica
CBS 110500 M. aurantia
CBS 110843 M. ellipsoidea
AF283690
AF309602
M. africana
AF309617 Passalora eucalypti
AF222840 Ps. eriodendri
AF309595 Ps. basiramifera
AF309596 Ps. paraguayensis
AF309597 Ps. robusta
AF309594 Ps. natalensis
AF309600 Ps. syzygiicola
AF309598
AF309599
Ps. eucalyptorum
AF488743 Ps. hibbertiae-asperae
STE-U 10503 Ps. "eucalyptorum"
AF488744 Ps. platylobii
STE-U 10390
STE-U 10500
STE-U 10507
Ps. pseudoeucalyptorum
AF468868
AF468869
M. fori
CBS 110967
CBS 110968
CBS 110969
M. colombiensis
AF309609 M. heimioides
AF309611 M. crystallina
AF309606 M. heimii
AF309607
AF309608
M. irregulariramosa
AF309615
AF309616
AY045501
AY045502
M. walkeri
0.1 substitutions per site
Clade 1

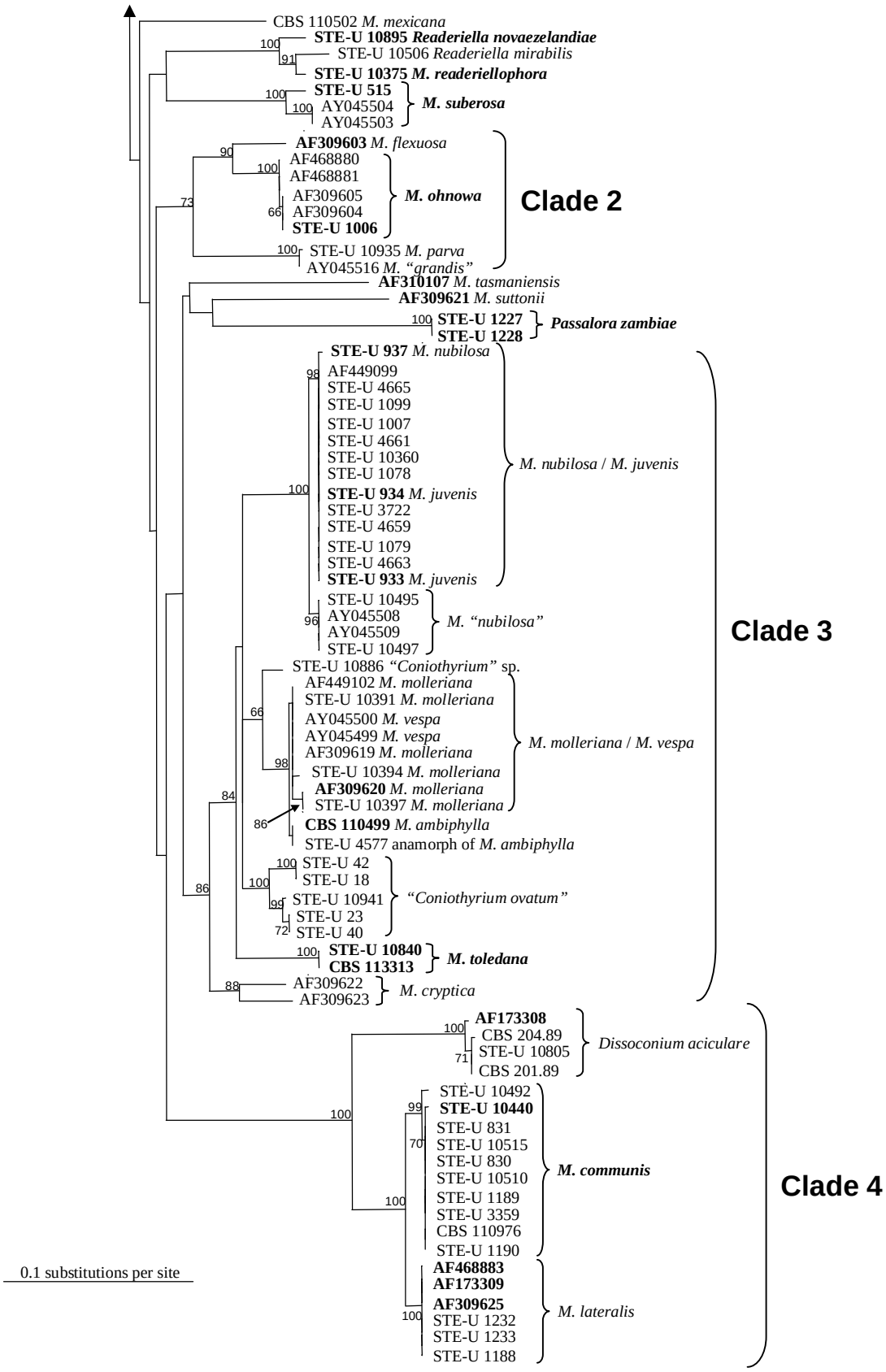


Fig. 1. Neighbour-joining tree obtained from a distance analysis using the Kimura-2-parameter substitution model on ITS sequence data. The scale bar shows 0.1 substitutions per site and bootstrap replicate values from 1000 replicates are shown at the nodes (only values higher than 64 %). Ex-type strains are shown in bold print. The tree was rooted to two *Botryosphaeria* species.

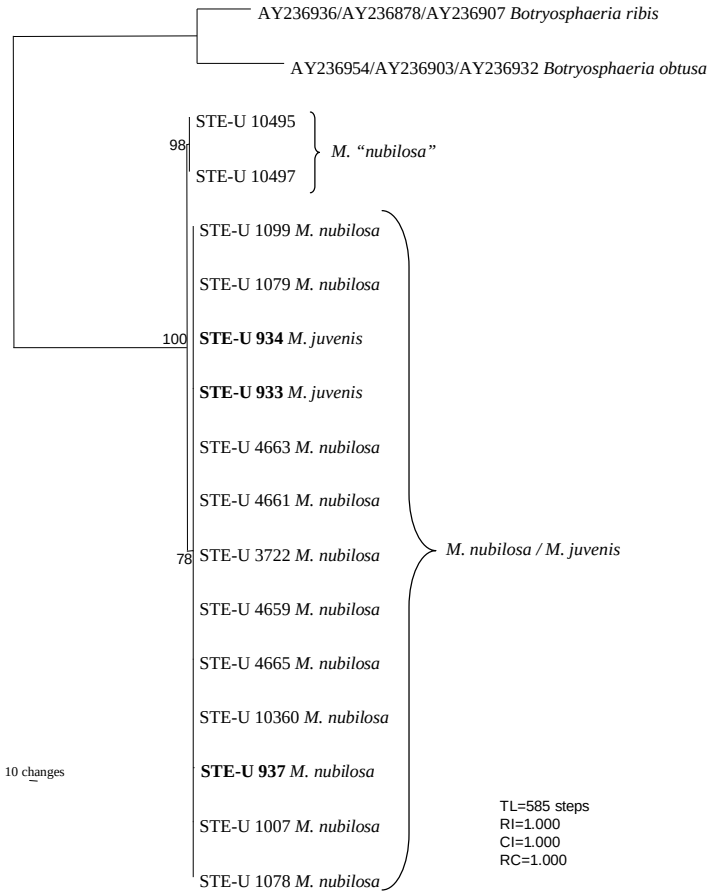


Fig. 2. Single most parsimonious tree obtained from a heuristic search with 100 random taxon additions of a combined ITS, elongation factor 1-alpha and β-tubulin sequence alignment. The scale bar shows 10 changes and bootstrap replicate values from 1000 replicates are shown at the nodes. The tree was rooted to two *Botryosphaeria* species.

Clade 3 also included a well-supported sub-clade (98 % bootstrap support) containing *M. vespa* Carnegie & Keane and *M. molleriana* (Thüm.) Lindau isolates as well as isolates of *M. ambiphylla* A. Maxwell and its *Phaeophleospora* anamorph. This sub-clade also contained five isolates tentatively assigned to "*Coniothyrium ovatum*" H.J. Swart (100 % bootstrap support), a single isolate of a "*Coniothyrium*" sp., two isolates of *M. toledana* sp. nov. (100 % bootstrap support) and two isolates of *M. cryptica* (88 % bootstrap support).

Clade 4 (100 % bootstrap support) consisted of *Mycosphaerella* isolates with *Dissoconium* de Hoog, Oorschot & Hijwegen anamorphs and included four isolates of *Dissoconium aciculare* de Hoog, Oorschot & Hijwegen and two separate sub-clades, one with a bootstrap support value of 99 % containing *M. commune* sp. nov. isolates, and the other with a 100 % bootstrap support containing *M. lateralis* Crous & M.J. Wingf. isolates.

Approximately 500 bases of the β-tubulin gene and 300 bases of the EF-1α were determined for isolates of *M. juvenis* and *M. nubilosa* and these were added to the alignment (TreeBASE accession number S1157). The manually adjusted alignment of the combined ITS, EF-1α and β-tubulin nucleotide sequences contained seventeen isolates (including the two outgroups) and 1184 characters (489, 268 and 427 bases, respectively) including alignment gaps. Of the aligned nucleotide sites for the data set, 348 characters were parsimony-informative, 163 variable characters were parsimony-uninformative and 673 were constant. The results of the pairwise and combined partition homogeneity tests did not reject the null hypothesis of congruence (P = 1.000 for all tests) and indicated that the ITS, β-tubulin and EF-1α data sets could be combined. A single most parsimonious tree (Fig. 2) was obtained for the combined data and in this tree the two New Zealand isolates (bootstrap support value of 98 % for the group) grouped separately from the rest of the isolates, which formed a strongly supported clade (bootstrap = 78 %).

Taxonomy

Results from the phylogenetic analysis have revealed five new species of *Mycosphaerella*, three of which have undescribed anamorphs, and a further three species that are known only from their anamorph states. Furthermore, these data also revealed that two species occurring on *Eucalyptus* should be reduced to synonymy. These species are described below.

Mycosphaerella communis Crous & J.P. Mansilla, **sp. nov.** MycoBank MB500050. Figs 3–10.
Anamorph: *Dissoconium commune* Crous & J.P. Mansilla, sp. nov.

Etymology: Referring to the common occurrence of this species.

Mycosphaerellae nubilosa similis, sed coloniis avellaneis distinguenda.

Leaf spots amphigenous, sub-circular to circular, 4–12 mm diam, medium brown, surrounded by a thin, raised, concolorous border. *Ascomata* pseudothecial, hypophyllous, single, black, immersed becoming erumpent, globose, up to 120 µm diam; apical ostiole 10–15 µm diam; wall of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight or slightly incurved, 8-spored, 35–50 × 10–14 µm. *Ascospores* 2–3-seriate, overlapping, hyaline, guttulate, thick-walled, straight to slightly curved, obovoid with subobtuse ends, medianly or unequally 1-septate, widest in middle of apical cell, or close to the apex of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (12–)13–15(–17) × (3.5–)4–4.5 µm *in vivo*.

Dissoconium commune Crous & J.P. Mansilla, **sp. nov.** MycoBank MB500051.

Dissoconio dekkeri simile, sed coloniis avellaneis distinguendum.

Mycelium internal and external, consisting of smooth, branched, septate, pale brown to olivaceous, 1.5–3 µm wide hyphae. *Conidiophores* arising from mycelium, single, 0–1-septate, smooth, medium brown, base subulate, upper part subcylindrical, simple or branched, 15–30 × 4–6 µm. *Conidiogenous cells* smooth, pale brown, subcylindrical, tapering to a truncate apex with 1–2 loci, straight to curved, 15–20 × 3–4 µm. *Conidia* terminal, pale olivaceous, smooth, obclavate with obtuse apex and obconical-truncate base, 0–1-septate, constricted at the septum, straight or curved, 20–30 × 4–5 µm (avg. 25 × 4.5 µm); hila inconspicuous. *Secondary conidia* developing from loci at the same level as the primary conidia, hyaline to pale olivaceous, aseptate, pyriform with a truncate base, 4–5 × 3–4 µm; hila inconspicuous.

Holotypes: **Spain**, Pontevedra, Lourizán, Areeiro, on leaves of *E. globulus*, Dec. 2002, J.P. Mansilla, herb. CBS 9900, **holotype** of *M. communis* and *D. commune*; culture ex-type CBS 114238 = CPC 10440.

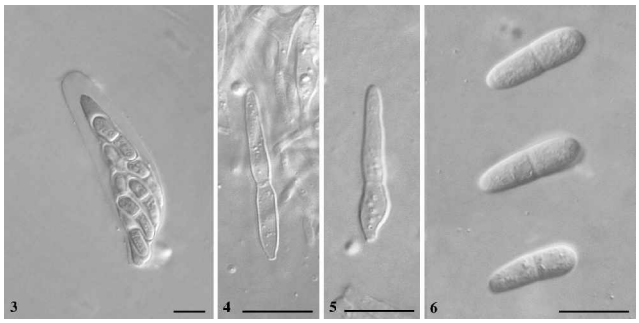
Ascospore germination on MEA after 24 h: Type F. Ascospores not darkening on MEA, and germinating from both ends, with germ tubes parallel to the long axis of the spore, and distorting prominently upon germination, becoming 7–9 µm diam.

Cultures: Colonies irregular, erumpent, uneven, folded, aerial mycelium moderate to sparse, 19’”i, hazel (surface), 27””m, olivaceous-black (reverse). Colonies reaching 20–35 mm diam on MEA after 1 mo at 25 °C in the dark; readily producing conidiophores of *D. commune* in culture after 14 d.

Hosts: *E. globulus*, *Protea* sp.

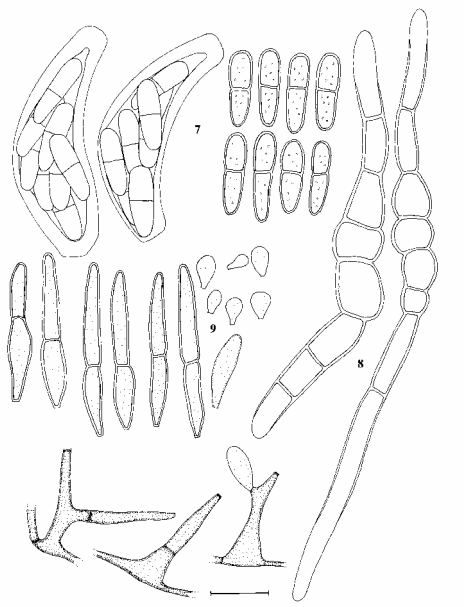
Distribution: Australia, New Zealand, South Africa, Spain.

Notes: *Mycosphaerella communis* is relatively common, and appears to have a wide host range beyond *Eucalyptus*, as well as a wide geographic distribution. In the past, isolates representing this species were erroneously treated as either *M. lateralis* or *M. juvenis* (= *M. nubilosa*). Although *M. lateralis* has a similar *Dissoconium* anamorph, its ascospores are fusoid–ellipsoidal, and are thus distinct from the obovoid ascospores of *M. communis*. Ascospore morphology of *M. communis* is similar to that of *M. nubilosa*, *M. ohnowa* and *M. readeriellophora*. In culture, colonies of *M. communis* are hazel in colour, while those of these other, morphologically similar species are pale olivaceous-grey (*M. nubilosa*), greenish black (*M. ohnowa*) or olivaceous (*M. readeriellophora*).



Figs 3–6. *Mycosphaerella communis* and its anamorph *Dissoconium commune* (CBS 114238). 3. Ascus. 4, 5. Conidia. 6. Ascospores. Scale bars = 10 µm.

Additional specimens and cultures examined: **Australia**, NSW, Mount Tomah Botanic Gardens, on leaves of *Protea magnifica*, Aug. 1999, P.W. Crous & B. Summerell, CPC 3359 = CBS 112889. **New Zealand**, on leaves of *E. globulus*, Feb. 2003, W. Gams, CPC 10510, 10515, 10492 = CBS 114239. **South Africa**, Western Cape province, Grabouw, on leaves of *E. nitens*, Nov. 1994, P.W. Crous (PREM 51914, cultures CPC 830–832, 831 = CBS 110747; KwaZulu-Natal, Seven Oaks Plantation, on leaves of *E. nitens*, 12 Jul. 1995, M.J. Wingfield (CPC 1188–1190 = CBS 111272, 112890, 111270).



Figs 7–10. *Mycosphaerella communis* and its anamorph *Dissoconium commune* (CBS 114238). 7. Asci and ascospores. 8. Germinating ascospores. 9. Primary and secondary conidia. 10. Conidiophores. Scale bar = 10 µm.

Mycosphaerella madeirae Crous & Denman, **sp. nov.** MycoBank MB500052. Figs 11–13.
Anamorph: *Pseudocercospora* sp. (unconfirmed).

Etymology: Named after the location from which it was collected.

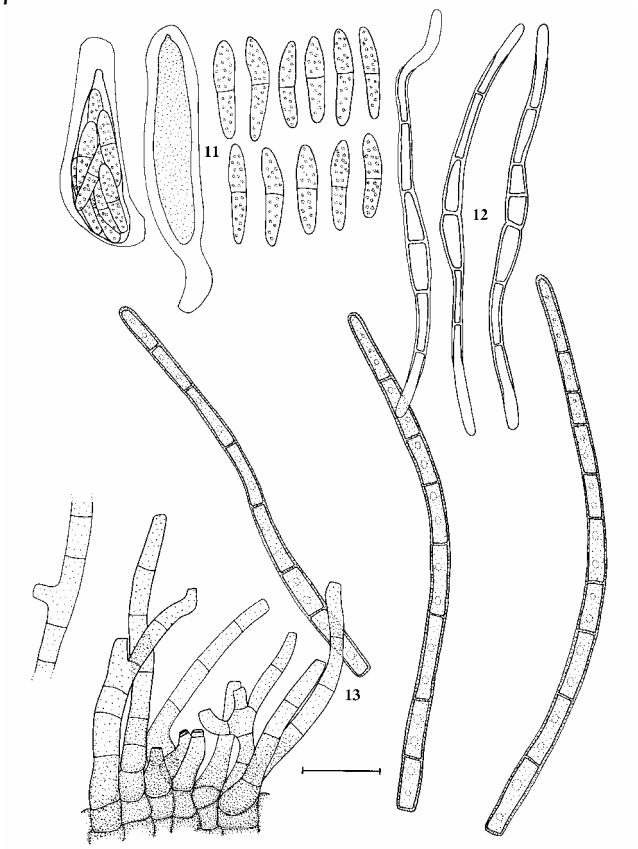
Mycosphaerellae heimioide similis, sed ascosporis germinantibus ad septum non constrictis distinguenda.

Leaf spots amphigenous, subcircular, 2–15 mm diam, medium brown, surrounded by a slightly raised, red-purple border. *Ascomata* pseudothecial, predominantly epiphyllous, single, black, immersed, becoming erumpent, globose, up to 120 µm diam; apical ostiole 10–15 µm diam; wall of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to narrowly ellipsoid, straight or slightly incurved, 8-spored, 30–50 × 8–12 µm. *Ascospores* 3- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid–ellipsoid with subobtuse ends, apex frequently acutely rounded, medianly 1-septate, widest in the middle of the apical cell, not constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (9–)10–13(–15) × 2.5–3(–3.5) µm *in vivo*.

Mycelium internal and external, consisting of smooth, branched, septate, pale to medium brown, 3–6 µm wide hyphae; external mycelium extensive on abaxial leaf surface. *Conidiomata* fasciculate, hypophyllous, medium brown, up to 90 µm wide and 150 µm high. *Conidiophores* arising from superficial mycelium, or aggregated in loose fascicles arising from the upper cells of a brown stroma up to 80 µm wide and 90 µm high; conidiophores pale to medium brown, smooth, unbranched or branched, 1–5-septate, subcylindrical, straight to variously curved, 15–45 × 2.5–4 µm. *Conidiogenous cells* terminal or lateral, unbranched, subcylindrical, pale brown, smooth, proliferating sympodially, or 1–4 times percurrently near apex, 7–15 × 2.5–3 µm; conidial scars inconspicuous. *Conidia* solitary, pale brown, smooth, subcylindrical, but tapering from a subtruncate base towards a subobtuse apex, 3–6- or multiseptate, 35–85 × 2.5–4 µm; hila inconspicuous.

Specimen examined: **Madeira**, Party Farm, on leaves of *E. globulus*, Apr. 2000, S. Denman, herb. CBS 9898 **holotype**, cultures ex-type CPC 3745 = CBS 112895, CPC 3747 = CBS 112301.

Ascospore germination on MEA after 24 h: Type C. Ascospores not darkening on MEA, and germinating from both ends, with germ tubes parallel to the long axis of the spore, and with no or slight constriction at the ascospore septum, with ascospores becoming 3–4 µm diam.



Figs 11–13. *Mycosphaerella madeirae* and its presumed *Pseudocercospora* anamorph (holotype). 11. Asci and ascospores. 12. Germinating ascospores. 13. Conidia and conidiogenous cells. Scale bar = 10 µm.

Cultures: Colonies olivaceous-grey (21”””1) on the surface, iron-grey (23”””k) in reverse; erumpent, folded, with sparse aerial mycelium, and a smooth, catenulate margin. Colonies 20–30 mm diam on MEA after 1 mo at 25 °C in the dark; teleomorph but no conidia formed in culture.

Host: *E. globulus.*

Distribution: Madeira.

Notes: *Mycosphaerella madeirae* is most similar to *M. heimioides* Crous & M.J. Wingf. (Crous 1998), but can be distinguished by its ascospore germination pattern as well as on its cultural characteristics. A *Pseudocercospora* occurred in close proximity to *M. madeirae*, but the connection between these states could not be established in culture and remains unconfirmed. The *Pseudocercospora* species resembled *P. robusta* in conidium shape, but was distinct in having paler conidia. As no cultures could be obtained of the *Pseudocercospora* species to facilitate a more detailed comparison, this fungus will not be treated further here.

Mycosphaerella nubilosa (Cooke) Hansf., Proc. Linn. Soc. N.S.W. 81: 36. 1965. Figs 14–19.

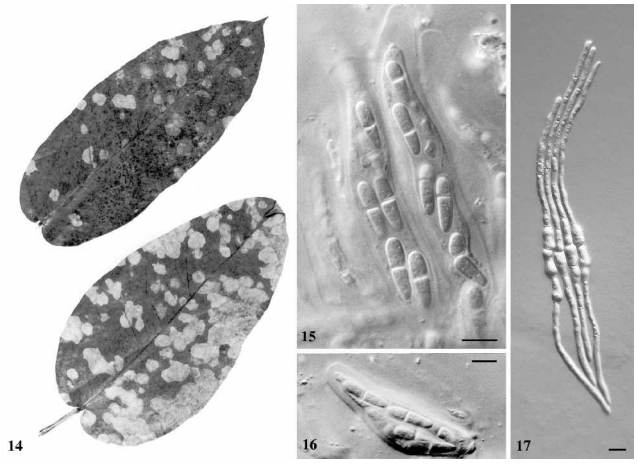
≡ *Sphaerella nubilosa* Cooke, Grevillea 19: 61. 1892.

= *Mycosphaerella juvenis* Crous & M.J. Wingf., Mycologia 88: 453. 1996.

Anamorph: *Uwebraunia juvenis* Crous & M.J. Wingf., Mycologia 88: 453. 1996.

Leaf spots amphigenous, varying from pin spots or flecks to small, round or irregular spots, frequently circular to irregular, up to 15 mm diam, becoming confluent to form larger blotches up to 3 cm diam on older leaves, pale brown, surrounded by a raised dark brown border, and a thin red-purple diffuse margin. *Ascomata* pseudothecial, amphigenous, predominantly hypophyllous, single, black, immersed, becoming erumpent, globose, up to 150 µm diam; apical ostiole 10–15 µm diam; wall of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to ellipsoid, straight or incurved, 8-spored, 30–50(–68) × 9–14(–18) µm. *Ascospores* bi- to triseriate, overlapping, hyaline, non-guttulate, thin-walled, but the septum appearing thicker than the side walls, straight to slightly curved, obovoid with obtuse ends, medianly or unequally 1-septate, not or slightly constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)13–14(–16) × (2.5–)3–4(–4.5) µm *in vivo*; apical cell (4–)5–6 µm, basal cell (4–)7–9 µm long.

Types: **Australia**, Victoria, Melbourne, on leaves of *Eucalyptus* sp., Martin 584 (K), **holotype**. **Australia**, Victoria, Briagalong, on leaves of *E. globulus*, 16 Sep. 1994, A. Carnegie, herb. CBS 9902 **epitype designated here**, ex-epitype culture CPC 937 = CBS 116005.



Figs 14–17. *Mycosphaerella nubilosa* (CBS 113064). 14. Leaf symptoms on upper and lower leaf surfaces of *Eucalyptus globulus* leaves. 15, 16. Asci. 17. Germinating ascospores. Scale bars = 10 µm.

Ascospore germination on MEA after 24 h: Type F, not type C as reported in Crous (1998). Ascospores not darkening on MEA, germinating from both ends, with germ tubes parallel to long axis of spore, with a gross distortion of the original spore; ascospores becoming 6–8 µm diam.

Cultures: Margins irregular but not feathery; surface folded; aerial mycelium moderate to sparse, more whitish in the centre, becoming pale olivaceous-grey, 23’’’’b, towards margins (surface), olivaceous-grey, 23’’’’i (reverse). Colonies 10–30 mm diam on MEA after 1 mo at 25 °C in the dark; conidiophores of *U. juvenis* rarely formed in culture.

Hosts: *E. bridgesiana*, *E. cypellocarpa*, *E. globulus*, *E. nitens* and *E. quadrangulata* (Crous 1998). Records on *E. grandis* and *E. botryoides* are doubtful.

Distribution: Australia, Kenya, New Zealand, South Africa, Spain, Tanzania, Zambia.

Notes: Confusion regarding the ascospore germination pattern for *M. nubilosa* (Park & Keane 1982, Crous & Wingfield 1996, Crous 1998), and the presence or absence of an anamorph, led Crous & Wingfield (1996) to describe *M. juvenis* as a distinct species, and also led Crous (1998) to conclude that *M. juvenis* was the major pathogen causing MLB on *E. globulus* and *E. nitens* in South Africa. Hunter *et al*. (2004) have, however, recently shown that *M. nubilosa* is the major pathogen on *E. nitens* in South Africa. Results of the present study show that *M. juvenis* should be treated as a synonym of this species.

Original slides of germinating ascospores in MEA were re-examined in this study, along with fresh collections obtained from Australia, South Africa, New Zealand and Spain. Germinating ascospores of *M. nubilosa* were seen to have the same germination pattern as that described for *M. juvenis* (type F), with

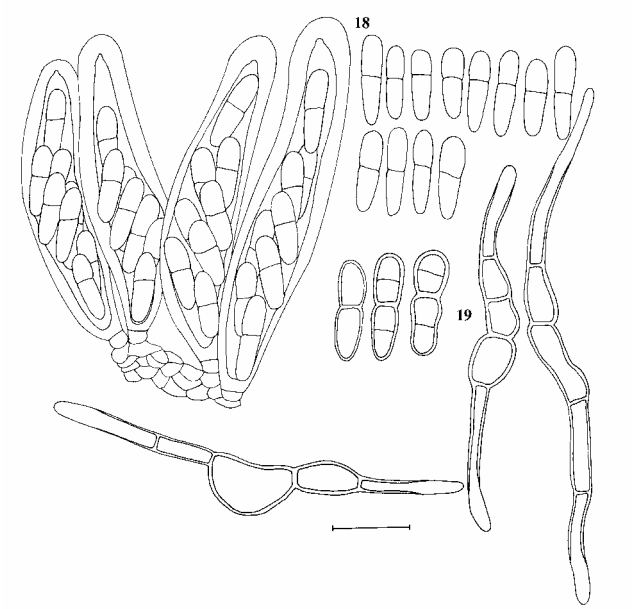
massive distortion within 24 h after germination. A re-examination of the original slide with germinating ascospores described by Crous (1998), received from A. Carnegie, showed that only 3 of the spores present had germinated. Hence the process had been terminated before 24 h had passed, and the germination pattern was described as type C. The same is presumably true for the illustrations provided by Park & Keane (1982). Fresh material studied from several plantations in South Africa, Spain, as well as a few randomly collected specimens from Australia and New Zealand, have shown that spores germinate, then become constricted (type C), and after 24 h become distorted (type F), similar to those observed for *M. juvenis* (Crous 1998).

Additional specimens and cultures examined: **Australia**, On leaves of *E. globulus*, Sep. 2000, M.J. Wingfield (CMW 6210 = CBS 114706, 6211 = CBS 114707). **Kenya**, on leaves of *E. globulus*, May 1995, T. Coutinho (PREM 54972, cultures CPC 1078 = CBS 111969, CPC 1079 = CBS 111968). **New Zealand**, on leaves of *E. globulus*, Feb. 2003, W. Gams (CPC 10495, 10497 = CBS 114419). **Spain**, Reboreda, on leaves of *E. globulus*, Dec. 2001, J.P. Mansilla (cultures CPC 4666, 4665 = CBS 113064); Lago, on leaves of *E. globulus*, Dec. 2001, J.P. Mansilla (cultures CPC 4659, 4660 = CBS 111445); Ponteareas, on leaves of *E. globulus*, Dec. 2001, J.P. Mansilla (cultures CPC 4661, 4662 = CBS 114513); Castrove, on leaves of *E. globulus*, Dec. 2001, J.P. Mansilla (cultures CPC 4663, 4664 = CBS 114244); Pontevedra, Lourizán, Areeiro, on leaves of *E. globulus*, 2003, J.P. Mansilla (CPC 10360 = CBS 114241); on leaves of *E. globulus*, 2001, J.P. Mansilla, CPC 3722. **South Africa**, KwaZulu-Natal, Pietermaritzburg, on leaves of *E. nitens*, Jan. 1995, M.J. Wingfield (PREM 51910 teleomorph, PREM 51915 anamorph, cultures CPC 932–934, ex-type of *M. juvenis* and *U. juvenis*); KwaZulu-Natal, Pietermaritzburg, Bulwer, on leaves of *E. nitens*, June 2000, G.C. Hunter (CMW 9000); Mpumalanga, Witbank, on leaves of *E. grandis*, Mar. 1995, M.J. Wingfield (PREM 51913, cultures CPC 1007 = CBS 112972); KwaZulu-Natal, Pietermaritzburg, Bulwer, on leaves of *E. nitens*, May 2000, G.C. Hunter (CMW 9002); KwaZulu-Natal, Pietermaritzburg, Bulwer, on leaves of *E. nitens*, May 2000, G.C. Hunter (CMW 9003 = CBS 114708). **Tanzania**, on leaves of *Eucalyptus* sp., May 1995, T. Coutinho (PREM 54971, culture CPC 1099).

Mycosphaerella ohnowa Crous & M.J. Wingf., **sp. nov.** MycoBank MB500053. Figs 20–23.

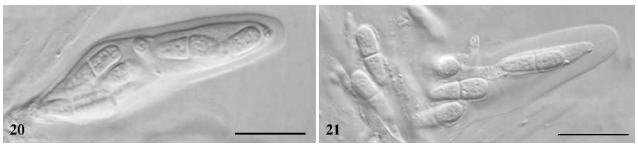
Etymology: Exclamation upon finding this morphologically nondescript, but genetically and culturally distinct taxon.

Mycosphaerellae nubilosae similis, sed coloniis mucidis viridi-atris distinguenda.



Figs 18, 19. *Mycosphaerella nubilosa* (CBS 113064). 18. Asci and ascospores. 19. Germinating ascospores. Scale bar = 10 µm.

Leaf spots amphigenous, irregular to subcircular, 2–10 mm diam, medium brown, with a raised border which is red-brown on the adaxial surface, and medium brown on the abaxial surface. *Ascomata* pseudothecial, amphigenous, single, black, immersed, becoming erumpent, globose, up to 100 µm diam; apical ostiole 5–10 µm diam; wall of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight or slightly incurved, 8-spored, 40–60 × 8–11 µm. *Ascospores* 2–3-seriate, overlapping, hyaline, guttulate, thick-walled, straight, obovoid with obtuse ends, medianly to unequally 1-septate, widest near the apex of the apical cell, not constricted at the septum, tapering towards both ends, but more prominently towards lower end, (10–)12–14(–15) × (3–)3–4 µm *in vivo*.



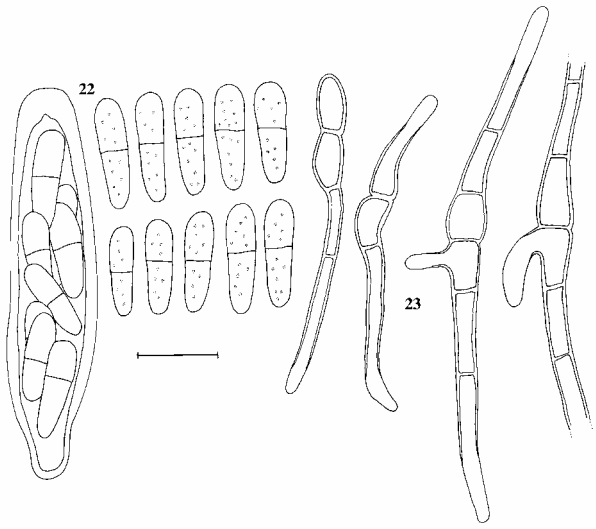
Figs 20, 21. *Mycosphaerella ohnowa* (PREM 51912). 20. Ascus. 21. Broken ascus with ascospores. Scale bar = 10 µm.

Holotype: **South Africa**, Mpumalanga, Hazy View, on leaves of *E. grandis*, 27 Mar. 1995, M.J. Wingfield, PREM 51912 **holotype**; cultures ex-type CPC 1004 = CBS 112896, CPC 1005 = CBS 112973, CPC 1006 = CBS 110949.

Ascospore germination on MEA after 24 h: Type C. Ascospores not darkening on MEA, germinating predominantly from one end, but also from both ends, with germ tubes parallel to the long axis of the

spore, and with a constriction at the ascospore septum; ascospores becoming 3.5–5 µm diam.

Cultures: Colonies smooth, with extensive aerial mycelium that collapses with age, giving a flat, slimy surface, 33””’k, greenish black (surface and reverse); margins smooth. Colonies reaching 40–50 mm diam on MEA after 1 mo at 25 °C in the dark; cultures remaining sterile on a variety of media.



Figs 22, 23. *Mycosphaerella ohnowa* (PREM 51912). 22. Ascus with ascospores. 23 Germinating ascospores. Scale bar = 10 µm.

Hosts: *E. grandis, E. smithii*.

Distribution: South Africa.

Notes: *Mycosphaerella ohnowa* is morphologically similar to *M. nubilosa*, and is also associated with similar leaf spots, and hypophyllous fruiting. It can be distinguished, from the latter species by its colonies that become slimy, greenish black, whereas those of *M. nubilosa* are pale olivaceous-grey.

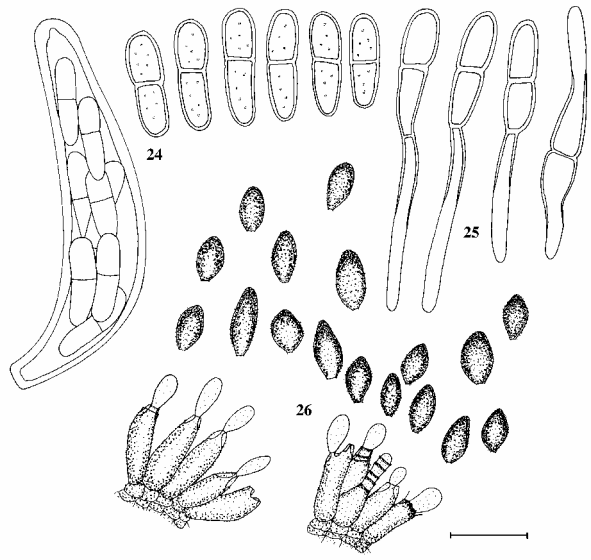
Additional specimens and cultures examined: **South Africa**, Eastern Cape Province, Umtata, on leaves of *E. grandis*, 2001, G.C. Hunter (CMW 9101 = CBS 113289); KwaZulu-Natal, Richmond, on leaves of *E. smithii*, Jun. 2000, G.C. Hunter (CMW 9102 = CBS 113290); KwaZulu-Natal, Richmond, on leaves of *E. smithii*, Jun. 2000, G.C. Hunter (CMW 9103 = CBS 113291).

Mycosphaerella readeriellophora Crous & J.P. Mansilla, **sp. nov.** MycoBank MB500054. Figs 24–26.

Anamorph: *Readeriella readeriellophora* Crous & J.P. Mansilla, sp. nov.

Etymology: Named after the anamorph genus *Readeriella*.

Mycosphaerellae nubilosae similis, sed coloniis olivaceis distinguenda.



Figs 24–26. *Mycosphaerella readeriellophora* and its anamorph *Readeriella readeriellophora* (CBS 114240). 24. Ascus and ascospores. 25. Germinating ascospores. 26. Conidiogenous cells and conidia. Scale bar = 10 µm.

Leaf spots amphigenous, subcircular, 4–6 mm diam, grey to medium brown, with a raised, red-brown border. *Ascomata* pseudothecial, amphigenous, single, black, immersed becoming erumpent, globose, up to 90 µm diam; apical ostiole 5–10 µm diam; wall of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight or slightly incurved, 8-spored, 35–60 × 8–11 µm. *Ascospores* 2–3-seriate, overlapping, hyaline, guttulate, thick-walled, straight, obovoid with obtuse ends, unequally 1-septate, widest in the middle of the apical cell, not to slightly constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (11–)13–14(–16) × (3.5–)4–4.5 µm *in vivo*.

Readeriella readeriellophora Crous & J.P. Mansilla, **sp. nov.** MycoBank MB500055.

Readereillae mirabili similis, sed conidiis minoribus, (5–)6–7(–9) × (3–)4(–4.5) µm, distinguenda.

Mycelium internal, consisting of branched, septate, medium brown, smooth, 2.5–3.5 µm wide hyphae. *Conidiomata in vitro* pycnidial, globose to subglobose, up to 130 µm diam; wall of 2–4 layers of dark brown *textura angularis*. *Conidiogenous cells* discrete, doliiform to subcylindrical, hyaline, smooth, monophialidic, rarely polyphialidic, with prominent periclinal thickening, but also percurrent, becoming pale yellow-brown, finely verruculose, later verruculose, green-brown, with irregular, percurrent proliferation and flared collarettes, 8–15 × 3–4 µm. *Conidia* holoblastic, solitary, ellipsoidal to limoniform, tapering towards a bluntly rounded, subobtuse, thickened apex, base subtruncate, initially hyaline, becoming yellow- to green-brown, and finally dark

brown, aseptate, finely verruculose, (5–)6–7(–9) × (3–)4(–4.5) µm; inconspicuous marginal frill present.

Holotypes: **Spain**, Pontevedra, Lourizán, Areeiro, on leaves of *E. globulus*, 2003, J.P. Mansilla, herb. CBS 9901, **holotype** of *M. readeriellophora* and *R. readeriellophora*; culture ex-type for both morphs CBS 114240 = CPC 10375.

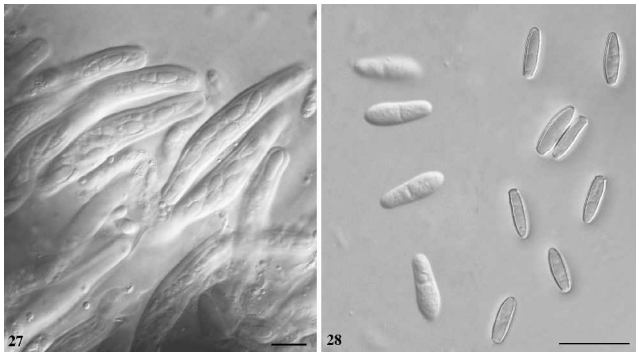
Ascospore germination on MEA after 24 h: Type C. Ascospores not darkening on MEA, germinating predominantly from one end, but also from both ends, with germ tubes parallel to the long axis of the spore, and with a constriction at the ascospore septum; ascospores becoming 4–5 µm diam.

Cultures: Colonies with extensive, pale brown aerial mycelium, surface becoming slimy, with green-brown masses of exuding conidia becoming visible in older cultures; colonies 21”k, olivaceous (surface), 27””m, olivaceous-black (reverse); reaching 50 mm diam on MEA after 1 mo at 25 °C in the dark; readily forming conidiomata of *R. readeriellophora* in culture.

Host: *E. globulus.*

Distribution: Spain.

Notes: *Mycosphaerella readeriellophora* is morphologically similar to *M. nubilosa*, and is also associated with similar leaf spots, and hypophyllous fruiting on *E. globulus*. It can be distinguished from the latter species by its colonies that are olivaceous, producing a *Readeriella* anamorph, while those of *M. nubilosa* are pale olivaceous-grey, sterile, or produce an *Uwebraunia* anamorph. Furthermore, ascospores of *M. readeriellophora* do not distort on MEA (type C), while those of *M. nubilosa* and *M. ohnowa* do (type F).



Figs 27, 28. *Mycosphaerella toledana* and its anamorph *Phaeophleospora toledana* (CBS 113313). 27. Asci. 28. Ascospores (left) and conidia (right). Scale bars = 10 µm.

Mycosphaerella toledana Crous & G. Bills, **sp. nov.** MycoBank MB500056. Figs 27–31.

Anamorph: *Phaeophleospora toledana* Crous & G. Bills, sp. nov.

Etymology: Named after the location from which it was collected.

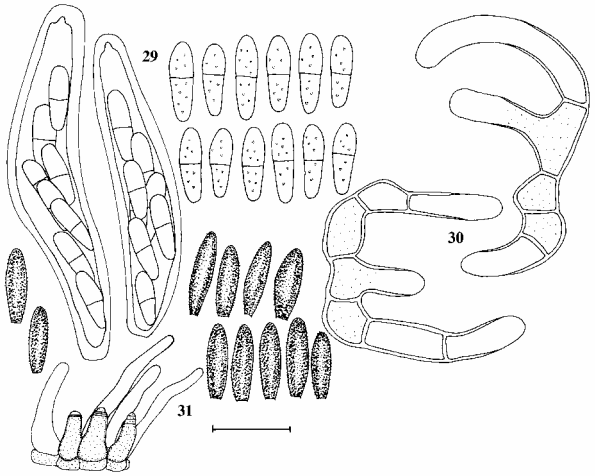
Mycosphaerellae ellipsoideae similis, sed ascosporis fuscescentibus multis hyphis germinantibus distinguenda.

Leaf spots amphigenous, irregular to subcircular or angular, frequently confined by leaf veins, 3–6 mm diam, medium brown, with or without a red-purple border, spots aggregating with age, forming irregular blotches. *Ascomata* pseudothecial, amphigenous, single, black, immersed becoming erumpent, globose, up to 80 µm diam; apical ostiole 10–20 µm diam; wall of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, subsessile, narrowly ellipsoid, straight or slightly incurved, 8-spored, 35–50 × 8–10 µm. *Ascospores* 2–3-seriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid–ellipsoid with subobtuse ends, medianly 1-septate, widest in the middle of the apical cell, not constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (7–)8–10(–11) × 3(–3.5) µm.

Phaeophleospora toledana Crous & G. Bills, **sp. nov.** MycoBank MB500057.

Colletogloeopsi nubilosae (Ganap. & Corbin) Crous & M.J. Wingf. similis sed pycnidiis clausis et conidiis minoribus, (8–)10–12(–14) × (2.5–)3–3.5(–4) µm, distinguenda.

Mycelium internal, consisting of smooth, branched, septate, medium brown, 3–4 µm wide hyphae. *Conidiomata* amphigenous, pycnidial, substomatal; wall consisting of 3–4 layers of *textura angularis*. *Conidiophores* reduced to conidiogenous cells.



Figs 29–31. *Mycosphaerella toledana* and its anamorph *Phaeophleospora toledana* (CBS 113313). 29. Asci and ascospores. 30. Germinating ascospores. 31. Conidiogenous cells, paraphyses and conidia. Scale bar = 10 µm.

Conidiogenous cells ampulliform to subcylindrical, pale brown, smooth to finely verruculose, proliferating 1–3 times percurrently near apex, 6–10 × 3–4 µm; occurring intermixed between hyaline, smooth, subcylindrical, aseptate paraphyses, 10–20 × 2–2.5 µm. *Conidia* fusoid with acutely rounded apices and truncate bases, medium brown, verruculose, aseptate, (8–)10–12(–14) × (2.5–)3–3.5(–4) µm; base with minute marginal frill.

Holotypes: **Spain**, Toledo, on leaves of *Eucalyptus* sp., May 2003, P.W. Crous & G. Bills, herb. CBS 9896, **holotype** of *M. toledana* and *P. toledana*; culture ex-type of both morphs CBS 113313.

Ascospore germination on MEA after 24 h: Type E. Ascospores becoming pale brown on MEA, germinating from both ends, with multiple germ tubes growing irregular to the long axis of spore, with prominent distortion; ascospores becoming 5–6 µm diam.

Cultures: Colonies smooth, irregular, with moderate aerial mycelium, grey in the centre, white towards the margin, 21””b, grey-olivaceous (surface), 15””i, greyish sepia (reverse). Colonies reaching 55 mm diam on MEA after 1 mo at 25 °C in the dark; cultures sterile.

Host: *Eucalyptus* sp.

Distribution: Spain.

Notes: The only other *Mycosphaerella* species known from *Eucalyptus* that has a type E germination pattern is *M. suberosa* (Crous 1998). *Mycosphaerella toledana* can easily be distinguished from *M. suberosa* by its smaller, fusoid–ellipsoid ascospores, and a *Phaeophleospora* anamorph.

Additional specimen examined: **Spain**, Ribera del Alberche, on leaves of *Eucalyptus* sp., May 2003, P.W. Crous & G. Bills, herb. CBS: 9897, culture CPC 10840 = CBS 115513.

Passalora zambiae Crous & T. Coutinho, **sp. nov.** MycoBank MB500058. Figs 32, 33.

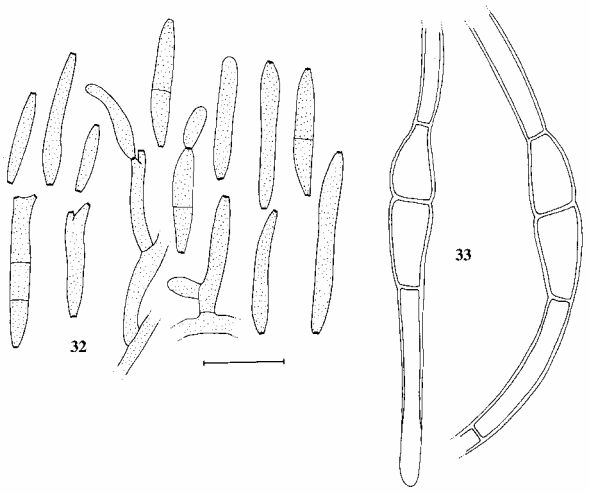
Etymology: Named after the country from which it was collected.

Passalorae morrisii similis, sed conidiis minoribus, 0(–2)-septatis, anguste ellipsoideis, 10–20 × 2–3 µm, distinguenda.

Leaf spots amphigenous, subcircular, 3–10 mm diam, medium brown, surrounded by a raised, brown border. *Mycelium* consisting of smooth to rough, irregularly branched, septate, brown, 2–7 µm wide hyphae; frequently with hyphal swellings that develop into thick-walled, dark brown chlamydospore-like structures, up to 15 µm diam. *Conidiophores* arising from the mycelium, medium brown, smooth, branched or unbranched, 0–2-septate, subcylindrical, straight to variously curved, 10–30 × 2–4 µm. *Conidiogenous cells* terminal and intercalary, subcylindrical, tapering to truncate apices, pale to medium brown, smooth, proliferating sympodially, 10–30 × 2–4 µm; conidial scars conspicuous, darkened, refractive. *Conidia* catenulate, chains simple or branched, medium brown, smooth, narrowly ellipsoidal, tapering to subtruncate, with flattened ends, straight or slightly curved, 0(–2)-septate, 10–20 × 2–3 µm *in vitro*.

Holotype: **Zambia**, on leaves of *E. globulus*, 21 Aug. 1995, T. Coutinho, herb. CBS 9895 **holotype**; cultures ex-type CPC 1227 = CBS 112971, CPC 1228 = CBS 112970).

Ascospore germination on MEA after 24 h: Type I. Ascospores not darkening on MEA, germinating from both ends, with germ tubes parallel to the long axis of the spore, and with a constriction at the ascospore septum; ascospores becoming 4–5 µm diam. Lateral branches also commonly observed 24–48 h after germination.



Figs 32, 33. *Passalora zambiae* (CBS 112971). 32. Conidia and conidiogenous cells. 33. Germinating ascospores. Scale bar = 10 µm.

Cultures: Colonies irregular with smooth margins, and sparse aerial mycelium, 21””’i, olivaceous-grey (surface), 27””m, olivaceous-black (reverse). Colonies reaching 30 mm diam on MEA after 1 mo at 25 °C in the dark; colonies initially producing conidia of *P. zambiae*, but becoming sterile upon transfer.

Host: *E. globulus.*

Distribution: Zambia.

Notes: This species is phylogenetically distant from other *Mycosphaerella* spp. known from *Eucalyptus*. Only a slide preparation with asci and ascospores is available for the teleomorph of this fungus, and this

is insufficient on which to base a description of this state. However, it is clear that the fungus resembles other species in the *M. nubilosa* complex that occur on *E. globulus*. The anamorph has been observed only in culture. The cultures described here were derived from germinating ascospores.

Pseudocercospora pseudoeucalyptorum Crous, **sp. nov.** MycoBank MB500059. Figs 34, 35.

Etymology: Morphologically similar to *P. eucalyptorum*.

Pseudocercosporae eucalyptorum similis, sed conidiomatibus brunneis et conidiis medio-brunneis distinguenda.

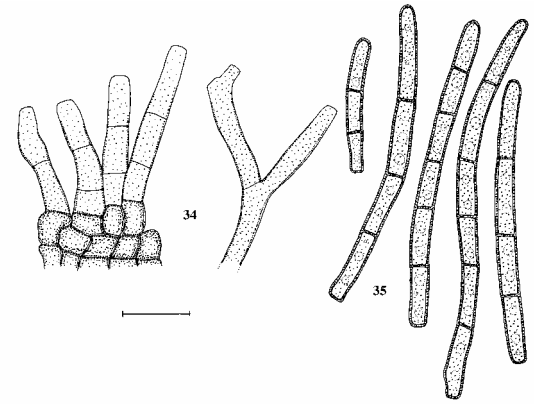
Leaf spots amphigenous, subcircular to angular, 3–10 mm diam, pale to medium brown, surrounded by a raised, brown border. *Conidiomata* amphigenous, brown (not grey as in *P. eucalyptorum*); stromata lacking to well developed, brown, 10–100 µm diam. *Mycelium* internal and external, consisting of smooth, branched, septate, medium brown, 2.5–4 µm wide hyphae; external mycelium extensive on the abaxial leaf surface. *Conidiophores* in small, loose or dense fascicles arising from the upper cells of a brown stroma, or from superficial hyphae; conidiophores medium brown, smooth, branched or unbranched, 0–2-septate, subcylindrical, straight to geniculate-sinuous, 10–50 × 2.5–5 µm. *Conidiogenous cells* terminal, subcylindrical, tapering to truncate or bluntly rounded apices, medium brown, smooth, proliferating sympodially, 10–30 × 2.5–4 µm; conidial scars inconspicuous. *Conidia* solitary, pale brown, smooth, cylindrical, bases truncate, apices bluntly rounded, thick-walled with irregular swellings, straight or curved, 3–7-septate, (25–)59–70(–90) × 2.5–3(–4) µm *in vivo*, 30–65 × 2.5–3 µm, 3–6-septate *in vitro*; hila inconspicuous.

Holotype: **Spain**, Pontevedra, Lourizán, Areeiro, on leaves of *E. globulus*, 2003, J.P. Mansilla, herb. CBS 9893 **holotype**; culture ex-type CPC 10390 = CBS 114242.

Cultures: Colonies folded, with irregular, smooth margins; aerial mycelium sparse to moderate, 21””’i, olivaceous-grey (surface), 27””m, olivaceous-black (reverse). Colonies reaching 25 mm diam on MEA after 1 mo at 25 °C in the dark; cultures fertile.

Host: *E. globulus.*

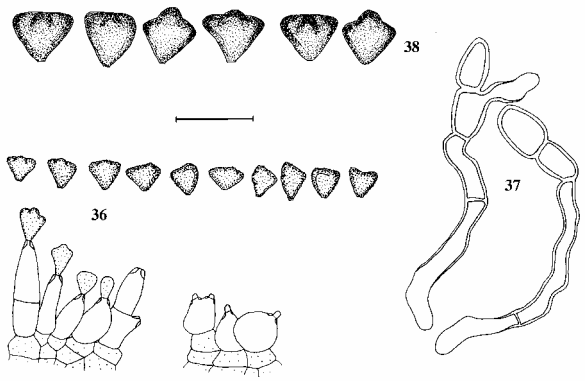
Distribution: ?China, Spain, New Zealand.



Figs 34, 34. *Pseudocercospora pseudoeucalyptorum* (CBS 114242). 34. Conidiophores. 35. Conidia. Scale bar = 10 µm.

Notes: *Pseudocercospora pseudoeucalyptorum* differs from *P. eucalyptorum* in having brown, not grey conidiomata, and conidia that are medium brown and not olivaceous in colour. The dimensions of the conidia in these two species overlap. A further collection obtained from China (CBS 10503), also belongs to this complex, but appears to represent a distinct species. The Chinese collection was from a *Eucalyptus* sp. with amphigenous, medium brown, angular to irregular leaf spots, 2–6 mm diam, frequently delimited by leaf veins. This particular collection had abundant superficial mycelium, and conidia that were paler in colour than *P. pseudoeucalyptorum*, but similar in length, (25–)50–70(–75) × 2.5–3 µm, thus resembling those of *P. eucalyptorum*. Because the Chinese material needed to be incubated for sporulation to occur, the morphological features of the fungus under natural conditions are not known. For this reason, we have chosen not to describe this species until additional material can be obtained.

Additional specimens and cultures examined: **China**, Yunnan Province, Lunan Co., 5 km NE of Lunan, Chuxiong town, garden of Zixishan Hotel, 1700 m alt., on leaf litter of *Eucalyptus* sp., 27 Oct. 2002, A. Aptroot, Herb. CBS 9894, culture CBS 10503. **New Zealand**, on leaves of *E. nitens*, Feb. 2003, W. Gams, CPC 10500 = CBS 114243, CPC 10507.



Figs 36–38. *Readeriella* species. 36. Conidia and conidiogenous cells of *R. novaezelandiae*. 37. Germinating ascospores of the *Mycosphaerella* teleomorph of *R. novaezelandiae* (CBS 114357). 38. Conidia of *R. mirabilis* (CPC 10506). Scale bar = 10 µm.

Readeriella novaezelandiae Crous, **sp. nov.** MycoBank MB500060. Figs 36, 37.
Teleomorph: *Mycosphaerella* sp.

Etymology: Named after the country where this fungus was collected.

Readeriellae mirabili similis, sed conidiis minoribus, 3–5 µm longis et latis, distinguenda.

Occurring on leaf spots associated with *M. marksii*, which is dominant and assumed to be the primary pathogen. *Leaf spots* irregular to subcircular, medium brown to red-brown, margins raised, 2–15 mm diam. Intact pseudothecia not observed, but epiphyllous remnants intermixed with those of *M. marksii*. *Mycelium* internal, consisting of branched, septate, medium brown, smooth, 3–4 µm wide hyphae. *Conidiomata in vitro* pycnidial, aggregated, globose to subglobose, up to 400 µm diam; wall of 4–6 layers of medium brown *textura angularis*. *Conidiophores* hyaline, smooth, subcylindrical to doliiform or reduced to conidiogenous cells and ovoid, 0–1-septate, 10–25 × 2–4 µm. *Conidiogenous cells* doliiform to subcylindrical or ovoid, hyaline, smooth, mono- or polyphialidic, with prominent periclinal thickening, 5–15 × 2–4 µm. *Conidia* holoblastic, solitary, aseptate, pale to medium brown, finely verruculose, base subtruncate, apex flattened with three lateral, obtuse projections, deltoid, whole conidia 3–5 µm long and wide.

Holotype: **New Zealand**, North Island, Kerikeri, on leaves of *E. botryoides*, 17 Oct. 2003, M.A. Dick, herb. CBS 9892 **holotype**, cultures ex-type CBS 114357 = CPC 10895).

Ascospore germination on MEA after 24 h: Type D. Ascospores not darkening on MEA, germinating predominantly from both ends, but with an irregular germination pattern; germ tubes varying in width, appearing irregular, and with a prominent constriction at the ascospore septum; ascospores 3.5–5 µm diam upon germination.

Cultures: Colonies with moderate, grey, fluffy aerial mycelium, which is interspersed with slimy black dots representing aggregated, black pycnidia exuding brown, slimy conidial masses; surface pale mouse-grey (15’””d), with feathery margins, reverse olivaceous (19”k); reaching 50 mm diam on MEA after 1 mo at 25 °C in the dark; cultures fertile.

Host: *E. botryoides*.

Distribution: New Zealand.

Notes: *Readeriella novaezelandiae* is morphologically similar to *R. mirabilis* (Fig. 38), but it can be distinguished by its smaller conidia, 3–5 µm long and wide, in contrast to the larger conidia of *R. mirabilis*, 7–9.5 µm long, and 7–9 µm wide. Cultures of *R. novaezelandiae* were obtained from single ascospores. However, these were few in number, and no mature pseudothecia were found on the leaves, precluding a description of the *Mycosphaerella* teleomorph. Further collections are required to fully elucidate the morphology of this species.

DISCUSSION

This study has included the largest number of isolates of *Mycosphaerella* spp. from *Eucalyptus* that has ever been considered based on DNA sequence comparisons. Comparisons using ITS sequence data for this large collection, followed by those including three gene regions for isolates in the *M. nubilosa* species complex, have shown the presence of many new species of *Mycosphaerella*. All of these species can be identified based on a combination of morphological and cultural characteristics, but unequivocal identifications demand DNA sequence data. A similar situation is arising with many other fungi, such as for example *Fusarium* spp. (O’Donnell *et al*. 2000) where large numbers of cryptic species are emerging from DNA sequence comparisons.

It might seem surprising that there should be in excess of 60 species of *Mycosphaerella* on *Eucalyptus*. However, this needs to be viewed against the background that there are more than 700 species of *Eucalyptus* (Potts & Pederick 2000). Many plant species are infected by more than one species of *Mycosphaerella* (Crous & Mourichon 2002, Crous & Braun 2003, Taylor *et al*. 2003), and we might expect that many more species of *Mycosphaerella* will be found on *Eucalyptus* in the future. This is clearly a group of fungi that has undergone extensive radiation, presumably associated with the substantial variation in the host genus.

The description of *Pseudocercospora eucalyptorum* Crous *et al.* (1989) resolved considerable confusion regarding species of coelomycetes that were initially described as *Cercospora eucalypti* Cooke & Massee and *C. epicoccoides* Cooke & Massee (Chupp 1954). These fungi were later placed in *Kirramyces* J. Walker, B. Sutton & Pascoe (Walker *et al*. 1992), and subsequently transferred to *Phaeophleospora* Rangel (Crous *et al*. 1997). An assemblage of different morphological species, however, remained aggregated under the epithet *Pseudocercospora eucalyptorum*. This confusing situation was later addressed by Crous (1998), who also provided a key to the various species of *Pseudocercospora* Speg. occurring on *Eucalyptus*. The species occurring in New Zealand were treated by Braun & Dick (2002), which led to the description of several new taxa, of which, *P. pseudobasitruncata* U. Braun & M. Dick, appears to be synonymous with *P. sublata* Z.Q. Yuan, de Little & C. Mohammed (Yuan *et al*. 2000),

described at approximately the same time from Australia.

Based on its morphology, *P. eucalyptorum* is accepted to have a wide geographic distribution, occurring on many different species of *Eucalyptus* (Crous 1998, Braun & Dick 2002). The present study is the first to consider this species based on DNA sequence data. These comparisons (Fig. 1), show clearly that *P. eucalyptorum* represents a species complex, and that further collections are required to fully recognise these cryptic species.

Recent collections from *Eucalyptus* leaves in South Africa have revealed a *Mycosphaerella* species that resembles *M. africana* Crous & M.J. Wingf. in morphology (CPC 10935). However, this fungus differs from *M. africana* in having ascospores that germinate at an angle from one end of the ascospore (Type N), thus closely fitting the pattern of *M. parva* R.F. Park & Keane. The ITS sequences for this isolate is identical to an unpublished sequence deposited in GenBank for *M. grandis* Carnegie & Keane (AY145516), which Crous (1998) considered a synonym of *M. parva*. Maxwell *et al*. (2003) report that *M. parva* is widespread in Australia, but this is the first record of the species from South Africa.

Park *et al*. (2000) regarded *Readeriella mirabilis* Syd. & P. Syd. as a common saprobe or secondary colonist of leaf spots caused by other primary pathogens such as *M. cryptica* and *Tracylla aristata* (Cooke) Tassi. Furthermore, *R. mirabilis* has been recorded from a range of eucalypt species in Australia, New Zealand, the U.K. and Brazil (Sutton 1980). The fact that *Readeriella* Syd. & P. Syd. (typified by *R. mirabilis*) belongs to *Mycosphaerella*, is surprising. An isolate of *R. mirabilis* obtained from New Zealand (CPC 10506), was phylogenetically closely related to a new species of *Mycosphaerella* from Spain, *M. readeriellophora*, which also has a *Readeriella* anamorph. Furthermore, ascospores of a *Mycosphaerella* sp. obtained from New Zealand produced the new species, *Readeriella novaezelandiae* in culture. The genus *Readeriella* now includes three species, which all occur on *Eucalyptus*.

One of the major agents of MLB disease of *Eucalyptus* is *M. nubilosa* (Carnegie & Ades 2002). *Mycosphaerella nubilosa* has few definitive morphological characters, and also resembles several other species occurring on *Eucalyptus*. For this reason, its taxonomy has been confused and controversial. The first modern treatment of *M. nubilosa*, including ascospore germination studies, was provided by Park & Keane (1982). Later, Crous *et al*. (1991) argued that *M. nubilosa* should be treated as a synonym of *M. molleriana*, but after obtaining fresh collections, Crous & Wingfield (1996) showed that the two species were distinct. This distinction was given strong support using some of the first DNA sequence comparisons for *Mycosphaerella* species (Crous *et al*. 2001a). In South Africa, this species has been a serious impediment to the propagation of *E. globulus* and certain provenances of *E. nitens* (Crous 1998). The causal agent of the disease has been ascribed to either *M. molleriana* (Thüm.) Lindau (Doidge 1950, Crous *et al.* 1991), or *M. nubilosa* (Lundquist & Purnell 1987). In a later study using morphological characteristics, Crous & Wingfield (1996) described a morphologically similar species, *M. juvenis*. Subsequently Crous (1998) regarded this fungus as the major causal agent of leaf blotch on *E. nitens* in South Africa. In a later DNA-based comparison, Crous *et al.* (2001a) identified some isolates as *M. juvenis* based on morphology (CBS 112973); they were shown to be phylogenetically distant from *M. nubilosa* and *M. molleriana*. However, the ex-type strain of *M. juvenis* was not included in that analysis.

Hunter *et al*. (2004) sampled several *E. nitens* plantations in the KwaZulu-Natal province of South Africa. Although *M. juvenis* was present (determined based on morphology, and sequence similarity to strains presumed to be *M. juvenis fide* Crous *et al*. 2001a), the dominant pathogen on *E. nitens* in South Africa was found to be *M. nubilosa*. Ex-type cultures of *M. juvenis* that were subjected to DNA sequence analysis in the present study, were shown to be identical to *M. nubilosa*. Furthermore, germinating ascospores of *M. nubilosa* were shown to initially have some constriction at the median septum (type C), but to distort prominently after 24 h (type F). Because the exact time that germination of ascospores was terminated by Park & Keane (1982) and Crous (1998) did not correspond, confusion resulted over the exact germination pattern of *M. nubilosa*, and what was later to be described as *M. juvenis*. This distinction between strains was further supported by the fact that some strains of *M. juvenis* produced an *Uwebraunia* anamorph (Crous & Wingfield 1996), while those of *M. nubilosa* did not.

Several *Mycopsphaerella* collections from Africa (South Africa, Kenya, Tanzania, Zambia), were originally identified based on morphology, as representing *M. juvenis*. Cultures, however, were reported by Crous (1998) to be variable in colour, and in some, the *Uwebraunia* anamorph formed readily (brown colonies), whereas it formed with difficulty in the olivaceous-black to grey-olivaceous colonies. DNA sequence comparisons in this study showed that these isolates included several distinct species. These isolates are associated with similar symptoms on *Eucalyptus* leaves, hypophyllous fruiting, and have similar asci, ascospores, and ascospore germination patterns (type F). Surprisingly, the majority of these isolates, including the ex-type cultures of *M. juvenis*, were shown to represent *M. nubilosa*. This suggests that *M. nubilosa* has *U. juvenis* as its anamorph. This anamorph is rarely observed in culture, and upon sub-culturing, strains lose their ability to produce conidia. The fungus with brown colonies that readily formed the anamorph (Crous 1998), was shown to represent a new species that is described here as *M. communis*. This fungus appears to have a

wide host range, including *Protea* spp. in Australia (Crous *et al*. 2000). The cultures originally sequenced and regarded as *M. juvenis* (Crous *et al*. 2001a), represent a third species in this complex, namely *M. ohnowa*.

Mycosphaerella nubilosa is a serious pathogen of *E. globulus* and *E. nitens*. The presence of this pathogen has recently been confirmed from South Africa (Hunter *et al*. 2004), and in the present study; it has also been identified from several populations collected during 2001 and 2002 on *E. globulus* in four regions in Spain, namely Lago, Ponteareas, Castrove and Reboredo. This is the first definitive record of *M. nubilosa* on eucalypts in Spain, and probably Europe. Although it is not known how long this pathogen has been present in Spain, it is likely to present a serious threat to *E. globulus* on the continent.

Although all *Mycosphaerella* spp. presently known from *Eucalyptus* that are available in culture were included in the current study, analysis of sequence data resulted in only one species being reduced to synonymy. This clearly emphasises the fact that there is more, rather than less variation amongst the *Mycosphaerella* spp., which have been described on *Eucalyptus*. It is highly probable that additional collections from *Eucalyptus* will reveal new species. Furthermore, once additional genes are sequenced, other cryptic species will be revealed within presently accepted morphological species. At present, nearly all of these species, other than those in the clade with *Dissoconium* anamorphs, appear to be specific to *Eucalyptus*. It will be interesting to note whether this will remain true, as additional species of *Mycosphaerella* spp. from other hosts are also currently being subjected to DNA sequence comparisons.

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