

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

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**Abstract:** Species of *Eucalyptus* are widely planted as exotics in the tropics and Southern Hemisphere and to some extent in southern Europe, for timber and fibre production. Species of *Mycosphaerella* are commonly associated with leaves and twigs of *Eucalyptus* and can result in defoliation, dieback, and even tree death. In the present study, numerous isolates of *Mycosphaerella* species were collected from leaf litter, living leaves exhibiting leaf spot symptoms or severe *Mycosphaerella* leaf blotch symptoms. Isolates were compared based on DNA sequence data for the internal transcribed spacer region (ITS1 & ITS2) and the 5.8S gene. These data, together with characteristics of the fungal growth on three different media, morphology of the anamorph and teleomorph structures as well as ascospore germination patterns were used to describe 21 new species.

**Taxonomic novelties:** *Colletogloeopsis stellenboschiana* Crous sp. nov., *Mycosphaerella davisoniellae* Crous sp. nov. (anamorph *Davisoniella eucalypti* H.J. Swart), *Mycosphaerella eucalyptorum* Crous & M.J. Wingf. sp. nov. *Mycosphaerella gamsii* Crous sp. nov., *Mycosphaerella perpendicularis* Crous & M.J. Wingf. sp. nov., *Mycosphaerella pluritubularis* Crous & J.P. Mansilla sp. nov., *Mycosphaerella pseudoafricana* Crous & T. Coutinho sp. nov., *Mycosphaerella pseudocryptica* Crous sp. nov. (anamorph *Colletogloeopsis* sp.), *Mycosphaerella pseudoendophytica* Crous & G. Hunter sp. nov. (anamorph *Pseudocercospora* sp.), *Mycosphaerella pseudosuberosa* Crous & M.J. Wingf. sp. nov. (anamorph *Trimmatostroma* sp.), *Mycosphaerella quasicerco- spora* Crous & T. Coutinho sp. nov., *Mycosphaerella scytalidii* Crous & M.J. Wingf. sp. nov. (anamorph *Stenella* sp., synanamorph, *Scytalidium*-like.), *Mycosphaerella secundaria* Crous & A.C. Alfenas sp. nov., *Mycosphaerella stramenti* Crous & A.C. Alfenas sp. nov., *Mycosphaerella stramenticola* Crous & A.C. Alfenas sp. nov., *Mycosphaerella sumatrensis* Crous & M.J. Wingf. sp. nov., *Mycosphaerella verrucosiafricana* Crous & M.J. Wingf. sp. nov., *Septoria eucalyptorum* Crous sp. nov., *Septoria provincialis* Crous sp. nov., *Stenella pseudoparkii* Crous & M.J. Wingf. sp. nov. (teleomorph *Mycosphaerella* sp.), *Stenella xenoparkii* Crous & M.J. Wingf., sp. nov. (teleomorph *Mycosphaerella* sp.).

**Key words:** Ascomycetes, *Colletogloeopsis*, *Davisoniella*, DNA sequence comparisons, *Mycosphaerella*, *Pseudocercospora*, *Pseudocercospora*, *Scytalidium*, *Septoria*, *Stenella*, systematics, *Trimmatostroma*.

## INTRODUCTION

*Eucalyptus* spp. are widely planted in the tropics and Southern Hemisphere, providing important sources of structural timber and fibre. Fungal diseases have, however, had a negative impact on their cultivation in many parts of the world (Wingfield *et al.* 2001). *Mycosphaerella* leaf blotch (MLB) was one of the first diseases to seriously damage plantations of *Eucalyptus* outside their native range, leading to the abandonment of some species for plantation development (Lundquist & Purnell 1987).

*Mycosphaerella* leaf blotch has been associated with severe defoliation, shoot die-back, and even tree death. This damage has mostly been attributed to *M. cryptica* (Cooke) Hansf. and *M. nubilosa* (Cooke) Hansf. (Carnegie *et al.* 1994, Crous & Wingfield 1996, Wingfield *et al.* 1996, Cheah 1977, Dungey *et al.* 1997). In recent years, it has become apparent that there are many more species of *Mycosphaerella* Johanson occurring on eucalypts than previously realised. While some of these fungi cause serious disease problems, others cause minor leaf spots, rarely resulting in severe disease (Crous 1998, Crous *et al.* 2004b). Little is known regarding some of these less important species but some could become more important in genetically uniform plantations of susceptible clonal hybrids or where trees are exposed to conditions of stress.

The genus *Mycosphaerella* Johanson includes more

than 2000 species names (Corlett 1991), and several thousand anamorphs that lack known teleomorphs (Crous & Braun 2003). Of these, 55 species from eucalypts were treated by Crous (1998) and several additional species have been described more recently (Carnegie & Keane 1998, Braun & Dick 2002, Maxwell *et al.* 2003, Crous *et al.* 2004b, Hunter *et al.* 2004). Species of *Mycosphaerella* are usually assumed to be host-specific, and presently there are little data available that can be used to refute this supposition. Although some taxa have been found to infect other, secondary hosts (Crous *et al.* 2004c, Groenewald *et al.* 2005), most seem to have narrow host ranges. Interestingly, where species have been reported to have wider host ranges within a plant family, e.g. as reported for *Ramularia* Unger anamorphs by Braun (1998), DNA-based techniques have clearly shown that in most cases these morphologically similar taxa are phylogenetically quite distinct (Crous & Groenewald, unpubl. data). Further confusion could result from species colonising atypical host tissue in an attempt to jump to an ideal host when this becomes available. Crous & Groenewald (2005) have referred to this unusual behavioural pattern as the "pogo stick hypothesis". In *Mycosphaerella* it has been observed to be true for teleomorph as well as anamorph states. When isolates of these fungi colonising atypical substrates are collected without proving their pathogenicity, incorrect conclusions pertaining to host range could arise.

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

Teleomorph	Anamorph	Strain no. <sup>1</sup>	Substrate	Country	Collector	ITS GenBank number
<i>Mycosphaerella communis</i>	<i>Dissoconium commune</i>	CPC 11700	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302948
		CPC 11703	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302949
		CPC 11792	<i>Eucalyptus</i> sp.	Portugal	A.J.L. Phillips	DQ302950
<i>Mycosphaerella cryptica</i>	<i>Colletogloeopsis nubilosum</i>	CBS 111679; CPC 1576	<i>Eucalyptus nitens</i>	Australia	M.J. Wingfield	DQ302951
<i>Mycosphaerella endophytica</i>	<i>Pseudocercospora endophytica</i>	CBS 111519; CPC 1191	<i>Eucalyptus</i> sp.	South Africa	P.W. Crous	DQ302952
		CBS 114662; CPC 1193	<i>Eucalyptus</i> <b>sp.</b>	South Africa	P.W. Crous	DQ302953
<i>Mycosphaerella eucalyptorum</i>	—	CBS 118496; CPC 11174	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ302954
<i>Mycosphaerella flexuosa</i>	<i>Stenella</i> sp.	CBS 110743; CPC 673	<i>Eucalyptus globulus</i>	Colombia	M.J. Wingfield	DQ302955
		CBS 111055; CPC 1200	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ302956
		CBS 111163; CPC 1201	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ302957
		CPC 10995	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ302958
<i>Mycosphaerella gamsii</i>	—	CBS 118495; CPC 11138	<i>Eucalyptus</i> sp.	India	W. Gams	DQ302959
<i>Mycosphaerella gracilis</i>	<i>Pseudocercospora gracilis</i>	CBS 111189; CPC 1315	<i>Eucalyptus urophylla</i>	Indonesia	M.J. Wingfield	DQ302960
		CPC 11144	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ302961
		CPC 11181	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ302962
<i>Mycosphaerella heimii</i>	<i>Pseudocercospora heimii</i>	CPC 11441	<i>Eucalyptus</i> sp.	Brazil	A.C. Alfenas	DQ302963
		CPC 11453	<i>Eucalyptus</i> sp.	Brazil	A.C. Alfenas	DQ302964
		CPC 11548	<i>Eucalyptus</i> sp.	Brazil	A.C. Alfenas	DQ302965
		CPC 11716	—	Brazil	A.C. Alfenas	DQ302966
		CPC 11879	<i>Eucalyptus</i> sp.	Portugal	A.J.L. Phillips	DQ302967
<i>Mycosphaerella jonkershoekensis</i>	—	CBS 112224; CPC 3116	<i>Protea lepidocarpodendron</i>	Australia	P.W. Crous	DQ302968
<i>Mycosphaerella lateralis</i>	<i>Dissoconium dekkeri</i>	CPC 11218	<i>Eucalyptus comaldulensis</i>	Bolivia	M.J. Wingfield	DQ302969
		CPC 11293	<i>Eucalyptus tereticornis</i>	Bolivia	M.J. Wingfield	DQ302970
		CPC 11484	<i>Eucalyptus</i> sp.	Spain	P. Mansilla	DQ302971
		CPC 11706	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302972
		CPC 11729	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302973
		CPC 11732	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302974
		CPC 11789	<i>Eucalyptus</i> sp.	Portugal	J.P. Sampaio	DQ302975
<i>Mycosphaerella madeirae</i>	—	CPC 3746	<i>Eucalyptus grandis</i>	Madeira	S. Denman	DQ302976
<i>Mycosphaerella marksii</i>	<i>? Pseudocercospora epispermogoniana</i>	CBS 110981; CPC 1073	<i>Eucalyptus</i> sp.	Tanzania	M.J. Wingfield	DQ302977
		CBS 111670; CPC 1499	<i>Eucalyptus globulus</i>	Uruguay	M.J. Wingfield	DQ302978
		CBS 115501; CPC 5358	<i>Leucadendron tinctum</i>	Madeira	S. Denman	DQ302979
		CBS 116316; CPC 3715	<i>Eucalyptus deglupha</i>	Ecuador	M.J. Wingfield	DQ302980
		CPC 11215	<i>Eucalyptus comaldulensis</i>	Bolivia	M.J. Wingfield	DQ302981

Table 1. (Continued).

Teleomorph	Anamorph	Strain no. <sup>1</sup>	Substrate	Country	Collector	ITS GenBank number
		CPC 11221	<i>Eucalyptus grandis</i>	Bolivia	M.J. Wingfield	DQ302982
		CPC 11222	<i>Eucalyptus grandis</i>	Bolivia	M.J. Wingfield	DQ302983
		CPC 11795	<i>Vepris reflexa</i>	South Africa	P.W. Crous	DQ302984
<i>Mycosphaerella molleriana</i>	<i>Colletogloeopsis molleriana</i>	CPC 11187	<i>Eucalyptus</i> sp.	Spain	M.J. Wingfield	DQ302985
		CPC 11685	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302986
		CPC 11688	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302987
		CPC 11709	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302988
		CPC 11842	<i>Eucalyptus</i> sp.	Portugal	A.J.L. Phillips	DQ302989
		CPC 11845	<i>Eucalyptus</i> sp.	Portugal	A.J.L. Phillips	DQ302990
		CPC 12056	<i>Eucalyptus</i> sp.	Uruguay	M.J. Wingfield	DQ302991
<i>Mycosphaerella nubilosa</i>	? <i>Uwebraunia juvenis</i>	CPC 11246	<i>Eucalyptus globulus</i>	Spain	M.J. Wingfield	DQ302992
		CPC 11249	<i>Eucalyptus globulus</i>	Spain	M.J. Wingfield	DQ302993
		CPC 11487	<i>Eucalyptus</i> sp.	Spain	P. Mansilla	DQ302994
		CPC 11559	<i>Eucalyptus</i> sp.	Spain	P. Mansilla	DQ302995
		CPC 11723	<i>Eucalyptus globulus</i>	Portugal	A.C. Alfenas	DQ302996
		CPC 11761	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ302997
		CPC 11767	<i>Eucalyptus globulus</i>	Portugal	L.P. Phillips	DQ302998
		CPC 11882	<i>Eucalyptus globulus</i>	Portugal	A.J.L. Phillips	DQ302999
		CPC 11885	<i>Eucalyptus</i> sp.	Portugal	A.J.L. Phillips	DQ303000
<i>Mycosphaerella parva</i>	—	CPC 11273	<i>Eucalyptus globulus</i>	Spain	M.J. Wingfield	DQ303001
		CPC 11758	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303002
		CPC 11759	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303003
		CPC 11764	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303004
		CPC 11888	<i>Eucalyptus</i> sp.	Portugal	A.J.L. Phillips	DQ303005
<i>Mycosphaerella perpendicularis</i>	—	CBS 118367; CPC 10983	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303006
<i>Mycosphaerella pluritubularis</i>	—	CBS 118508; CPC 11697	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303007
<i>Mycosphaerella pseudaficana</i>	—	CBS 114782; CPC 1230	<i>Eucalyptus globulus</i>	Zambia	T.A. Coutinho	DQ303008
<i>Mycosphaerella pseudocryptica</i>	<i>Colletogloeopsis</i> sp.	CPC 11264	<i>Eucalyptus</i> sp.	New Zealand	J.A. Stalpers	DQ303009
		CBS 118504; CPC 11267	<i>Eucalyptus</i> sp.	New Zealand	J.A. Stalpers	DQ303010
<i>Mycosphaerella pseudosuberosa</i>	<i>Trimmatostroma</i> sp.	CBS 118911; CPC 12085	<i>Eucalyptus</i> sp.	Uruguay	M.J. Wingfield	DQ303011
<i>Mycosphaerella quasircospora</i>	—	CBS 111161; CPC 1098	<i>Eucalyptus</i> sp.	Tanzania	M.J. Wingfield	DQ303012
<i>Mycosphaerella readeriellophora</i>	<i>Readeriella readeriellophora</i>	CPC 11711	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303013
<i>Mycosphaerella scytalidii</i>	—	CBS 516.93; CPC 653	<i>Eucalyptus globulus</i>	Brazil	F.A. Ferreira	DQ303014
		CPC 10988	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303015

Table 1. (Continued).

Teleomorph	Anamorph	Strain no. <sup>1</sup>	Substrate	Country	Collector	ITS GenBank number
<i>Mycosphaerella secundaria</i>	—	CBS 118493; CPC 10998	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303016
		CBS 111002; CPC 1112	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303017
		CBS 115608; CPC 504	<i>Eucalyptus grandis</i>	Brazil	A.C. Alfenas	DQ303018
		CPC 10989	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303019
<i>Mycosphaerella</i> sp.	<i>Stenella pseudoparkii</i>	CBS 118507; CPC 11551	<i>Eucalyptus</i> sp.	Brazil	A.C. Alfenas	DQ303020
		CBS 110988; CPC 1090	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303021
		CBS 110992; CPC 1092	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303022
		CBS 110999; CPC 1087	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303023
		CBS 111000; CPC 1088	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303024
<i>Mycosphaerella</i> sp.	<i>Stenella xenoparkii</i>	CBS 111049; CPC 1089	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303025
		CBS 111088; CPC 1299	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303026
		CBS 111089; CPC 1301	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303027
		CBS 111185; CPC 1300	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303028
<i>Mycosphaerella</i> sp.	—	CBS 208.94 / CPC 727	<i>Eucalyptus grandis</i>	Indonesia	A.C. Alfenas	DQ303029
<i>Mycosphaerella</i> sp.	—	CBS 209.94 / CPC 728	<i>Eucalyptus grandis</i>	Indonesia	A.C. Alfenas	DQ303030
<i>Mycosphaerella</i> sp.	—	CBS 110678; CPC 652	<i>Eucalyptus globulus</i>	Brazil	F.A. Ferreira	DQ303031
<i>Mycosphaerella</i> sp.	—	CBS 110679; CPC 653	<i>Eucalyptus globulus</i>	Brazil	F.A. Ferreira	DQ303032
<i>Mycosphaerella</i> sp.	—	CBS 110745; CPC 651	<i>Eucalyptus globulus</i>	Brazil	F.A. Ferreira	DQ303033
<i>Mycosphaerella</i> sp.	—	CBS 110987; CPC 1093	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303034
<i>Mycosphaerella</i> sp.	—	CBS 110991; CPC 1091	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303035
<i>Mycosphaerella</i> sp.	—	CBS 111036; CPC 1101	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303036
<i>Mycosphaerella</i> sp.	—	CPC 10986	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303037
<i>Mycosphaerella</i> sp.	—	CPC 11002	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303038
<i>Mycosphaerella</i> sp.	—	CPC 11004	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303039
<i>Mycosphaerella</i> sp.	—	CPC 12200	<i>Eucalyptus</i> sp.	South Africa	Z.A. Pretorius	DQ303040
<i>Mycosphaerella</i> sp.	—	CPC 12147	<i>Acacia mangium</i>	Thailand	W. Himaman	DQ303041
<i>Mycosphaerella stramenti</i>	—	CBS 118909; CPC 11545	<i>Eucalyptus</i> sp.	Brazil	A.C. Alfenas	DQ303042
<i>Mycosphaerella stramenticola</i>	—	CBS 118506; CPC 11438	<i>Eucalyptus</i> sp.	Brazil	A.C. Alfenas	DQ303043
<i>Mycosphaerella suberosa</i>	—	CPC 11032	<i>Eucalyptus</i> sp.	Colombia	M.J. Wingfield	DQ303044
		CPC 11190	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303045
		CPC 11276	<i>Eucalyptus comaldulensis</i>	Spain	M.J. Wingfield	DQ303046
<i>Mycosphaerella sumatrensis</i>	—	CPC 12193	<i>Eucalyptus</i> sp.	—	A.C. Alfenas	DQ303047
		CBS 118499; CPC 11171	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303048

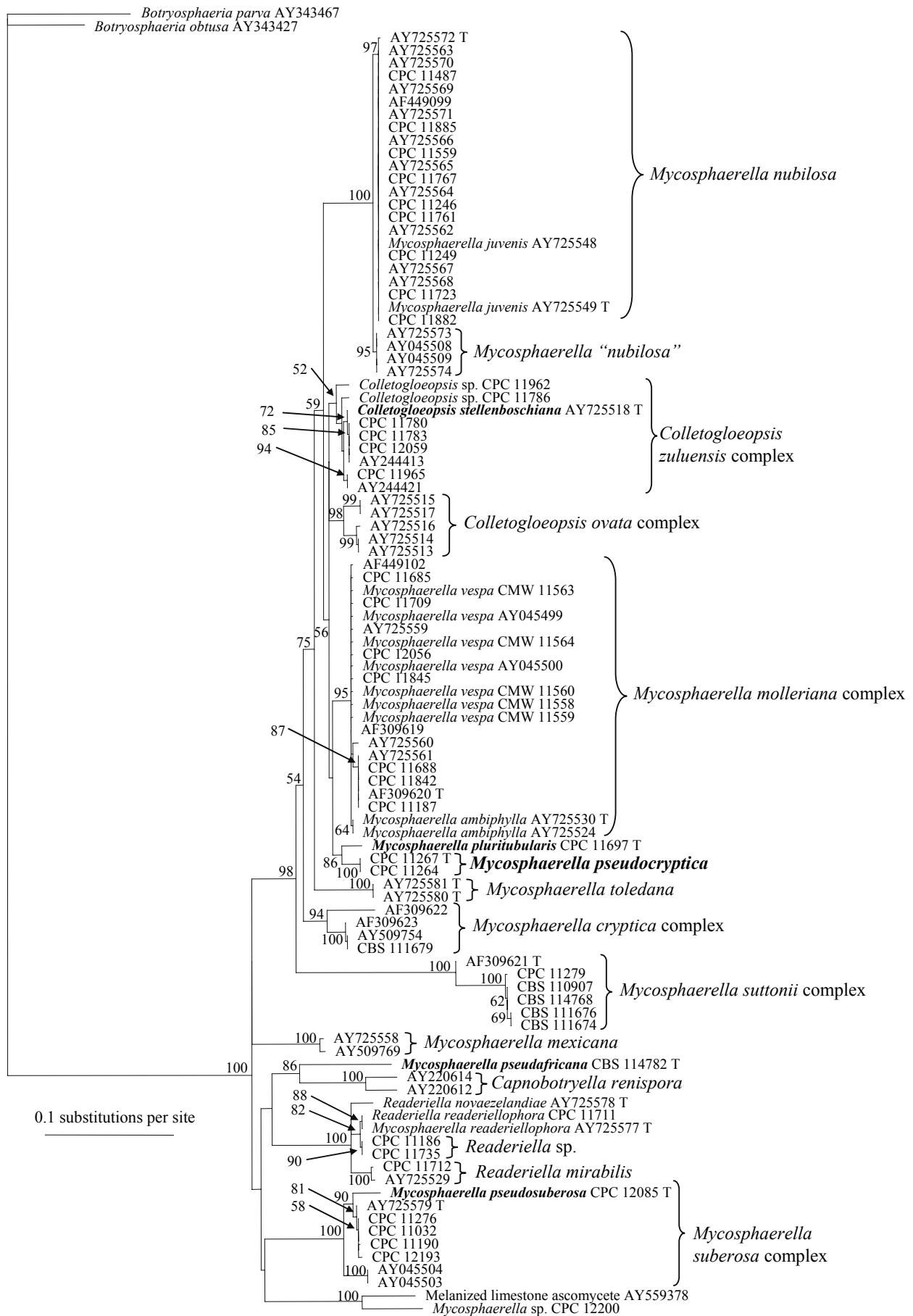
Table 1. (Continued).

Teleomorph	Anamorph	Strain no. <sup>1</sup>	Substrate	Substrate	Collector	ITS GenBank number
<i>Mycosphaerella suttonii</i>	<i>Kirramyces epicoccoides</i>	CBS 118501; CPC 11175	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303049
		CBS 118502; CPC 11178	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303050
		CBS 111676; CPC 1550	<i>Eucalyptus grandis</i>	Australia	M.J. Wingfield	DQ303051
		CBS 114768; CPC 1409	<i>Eucalyptus</i> sp.	Brazil	P.W. Crous	DQ303052
		CBS 110907; CPC 63	<i>Eucalyptus grandis</i>	South Africa	P.W. Crous	DQ303053
		CBS 111674; CPC 1581	<i>Eucalyptus grandis</i>	Australia	M.J. Wingfield	DQ303054
		CPC 11279	<i>Eucalyptus tereticornis</i>	Bolivia	M.J. Wingfield	DQ303055
<i>Mycosphaerella verrucosiafricana</i>	—	CBS 118496; CPC 11167	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303056
		CBS 118497; CPC 11169	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303057
		CBS 118498; CPC 11170	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	DQ303058
<i>Mycosphaerella vespa</i>	<i>Colletogloeopsis</i> sp.	CMW 11558	<i>Eucalyptus</i> sp.	Australia	—	DQ303059
		CMW 11559	<i>Eucalyptus</i> sp.	Australia	—	DQ303060
		CMW 11560	<i>Eucalyptus</i> sp.	Australia	—	DQ303061
		CMW 11563	<i>Eucalyptus</i> sp.	Australia	—	DQ303062
		CMW 11564	<i>Eucalyptus</i> sp.	Australia	—	DQ303063
<i>Mycosphaerella walkeri</i>	<i>Sonderhenia eucalypticola</i>	CPC 11252	<i>Eucalyptus globulus</i>	Spain	M.J. Wingfield	DQ303064
		—	<i>Colletogloeopsis zuluensis</i>	CPC 11780	<i>Eucalyptus</i> sp.	South Africa
—	—	CPC 11783	<i>Eucalyptus</i> sp.	South Africa	P.W. Crous	DQ303066
		CPC 11962; CMW 17322	<i>Eucalyptus</i> sp.	South Africa	M.J. Wingfield	DQ303067
—	—	CPC 11965; CMW 17326	<i>Eucalyptus</i> sp.	Uruguay	M.J. Wingfield	DQ303068
		CPC 12059	<i>Eucalyptus</i> sp.	Uruguay	M.J. Wingfield	DQ303069
—	<i>Colletogloeopsis</i> sp.	CPC 11786	<i>Eucalyptus</i> sp.	South Africa	P.W. Crous	DQ303070
—	<i>Pseudocercospora basitruncata</i>	CBS 114664; CPC 1202	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	DQ303071
—	<i>Pseudocercospora clematidis</i>	CPC 11657	<i>Clematis</i> sp.	U.S.A.	M.A. Palm	DQ303072
—	<i>Pseudocercospora epispermogoniana</i>	CBS 110693; CPC 823	<i>Eucalyptus grandis</i>	South Africa	G. Kemp	DQ303073
—	—	CBS 110694; CPC 824	<i>Eucalyptus grandis</i>	South Africa	G. Kemp	DQ303074
—	—	CBS 110750; CPC 822	<i>Eucalyptus grandis</i>	South Africa	G. Kemp	DQ303075
—	<i>Pseudocercospora fatouae</i>	CPC 11648	<i>Fatoua villosa</i>	Korea	H.D. Shin	DQ303076
—	<i>Pseudocercospora natalensis</i>	CBS 111069; CPC 1263	<i>Eucalyptus nitens</i>	South Africa	T.A. Coutinho	DQ303077
—	<i>Pseudocercospora pseudoecalyptorum</i>	CBS 116359; CPC 3751	<i>Eucalyptus</i> sp.	Madeira	S. Denman	DQ303078
—	—	CPC 10916	<i>Eucalyptus</i> sp.	South Africa	P.W. Crous	DQ303079
		CPC 11713	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303080
—	<i>Pseudocercospora robusta</i>	CBS 111175; CPC 1269	<i>Eucalyptus robur</i>	Malaysia	M.J. Wingfield	DQ303081
—	<i>Pseudocercospora</i> sp.	CBS 111072; CPC 1266	<i>Eucalyptus pellita</i>	Thailand	M.J. Wingfield	DQ303082

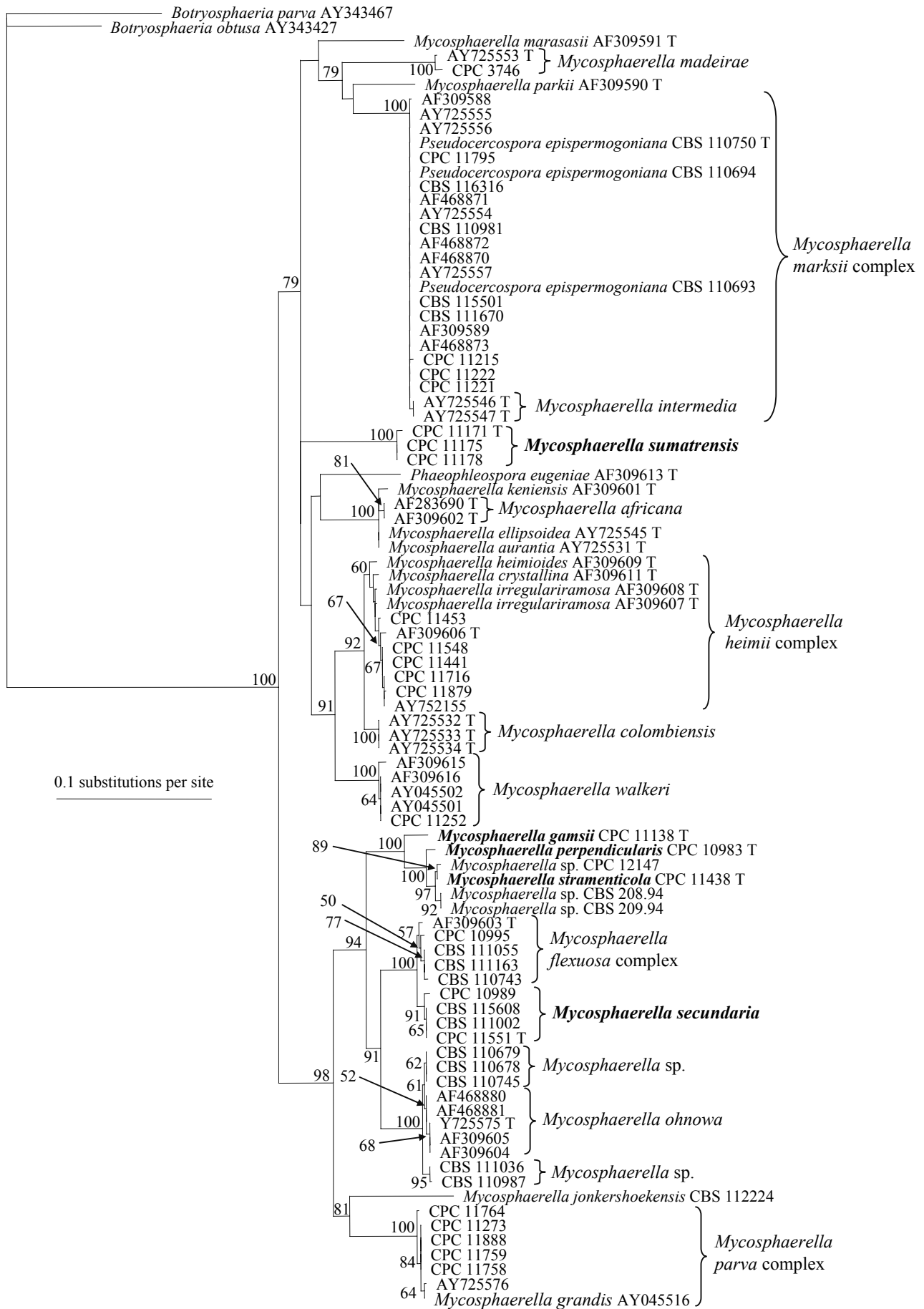
Table 1. (Continued).

Teleomorph	Anamorph	Strain no. <sup>1</sup>	Substrate	Country	Collector	ITS GenBank number
		CBS 111373; CPC 1493	<i>Eucalyptus globulus</i>	Uruguay	M.J. Wingfield	DQ303083
		CPC 11591	<i>Brachybotrys paridiformis</i>	Korea	H.D. Shin	DQ303084
		CPC 11592	<i>Zelkova serrata</i>	Korea	H.D. Shin	DQ303085
		CPC 11654	<i>Morus bombycis</i>	Korea	H.D. Shin	DQ303086
		CPC 11668	<i>Pilea hamaoi</i>	Korea	H.D. Shin	DQ303087
		CPC 11680	<i>Ampelopsis brevipedunculata</i> var. <i>heterophylla</i>	Korea	H.D. Shin	DQ303088
		CPC 11726	<i>Platanus occidentalis</i>	Korea	H.D. Shin	DQ303089
—	<i>Pseudocercospora subulata</i>	CBS 118489; CPC 10849	<i>Eucalyptus botryoides</i>	New Zealand	M. Dick	DQ303090
—	<i>Pseudocercospora capsellae</i>	CPC 11677	<i>Draba nemorosa</i> var. <i>hebecarpa</i>	Korea	H.D. Shin	DQ303091
—	<i>Readeriella</i> sp.	CPC 11186	<i>Eucalyptus globulus</i>	Spain	M.J. Wingfield	DQ303092
		CPC 11735	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303093
—	<i>Readeriella mirabilis</i>	CPC 11712	<i>Eucalyptus globulus</i>	Spain	P. Mansilla	DQ303094
—	<i>Septoria eucalyptorum</i>	CBS 118505; CPC 11282	<i>Eucalyptus</i> sp.	India	W. Gams	DQ303095
—	<i>Septoria provencialis</i>	CBS 118910; CPC 12226	<i>Eucalyptus</i> sp.	France	P.W. Crous	DQ303096
—	<i>Stenella</i> sp.	CPC 11671	<i>Lonicera japonica</i>	Korea	H.D. Shin	DQ303097

<sup>1</sup>CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; CMW: Culture collection of Mike Wingfield, housed at FABI, Pretoria, South Africa

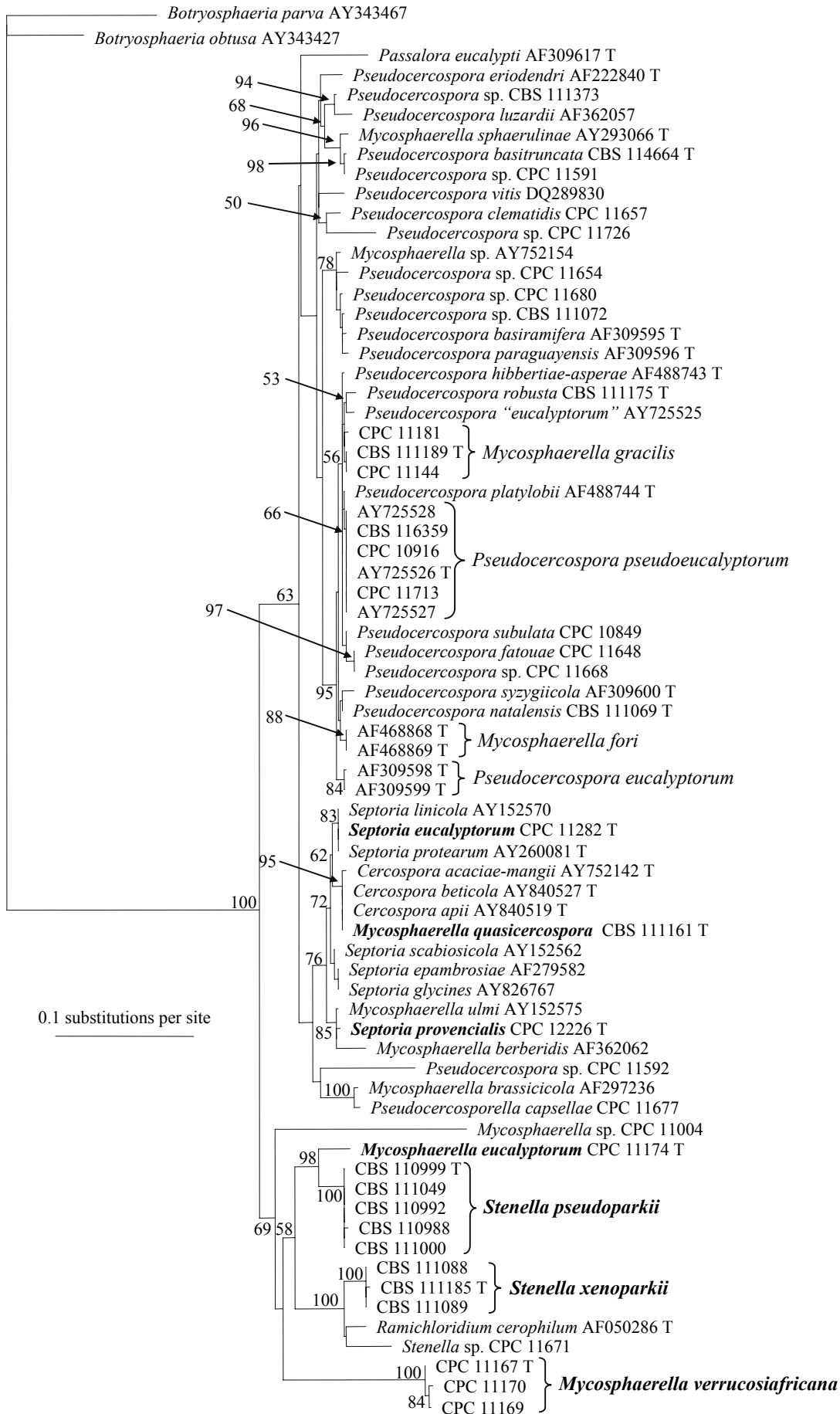


**Fig. 1.** Distance tree obtained from a neighbour-joining analysis using the HKY85 substitution model on the ITS sequence alignments. The scale bar shows the number of substitutions per site and bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to two *Botryosphaeria* species. New species are indicated in **bold**, and ex-type strains with a T.

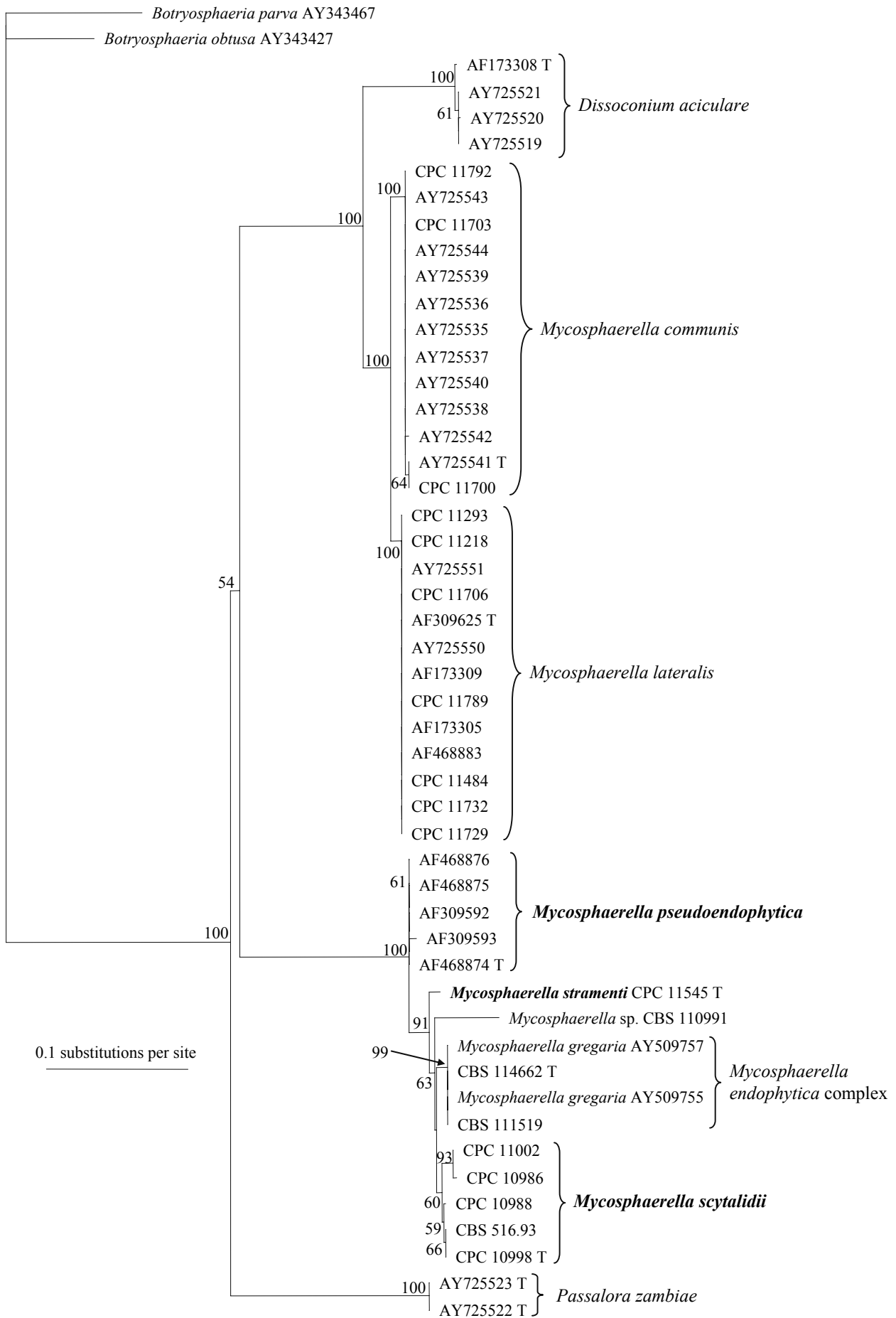


**Fig. 2.** Distance tree obtained from a neighbour-joining analysis using the HKY85 substitution model on the ITS sequence alignments. The scale bar shows the number of substitutions per site and bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to two *Botryosphaeria* species. New species are indicated in **bold**, and ex-type strains with a T.





**Fig. 3.** Distance tree obtained from a neighbour-joining analysis using the HKY85 substitution model on the ITS sequence alignments. The scale bar shows the number of substitutions per site and bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to two *Botryosphaeria* species. New species are indicated in **bold**, and ex-type strains with a T.



**Fig.4.** Distance tree obtained from a neighbour-joining analysis using the HKY85 substitution model on the ITS sequence alignments. The scale bar shows the number of substitutions per site and bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to two *Botryosphaeria* species. New species are indicated in **bold**, and ex-type strains with a T.

The genus *Mycosphaerella* includes species that are pathogens (primary, secondary or opportunistic), saprobes, endophytes (saprobiic or plant-pathogenic), or have mutualistic (in lichen) associations (Crous *et al.* 2000, 2001). Several taxa have low levels of virulence, and appear to be secondary colonists of lesions caused by other pathogens including species of *Mycosphaerella* (Crous 1998). Some species of *Ramularia* also appear to be hyperparasites on pustules of various rust species (Braun 1998). Because several species can co-inhabit the same lesion, either as primary or secondary pathogens, saprobes or endophytes (Crous 1998, Crous *et al.* 2004b), species identification based on the host can be extremely difficult. Although ascospore germination patterns, anamorph morphology and cultures greatly facilitate species identification, co-inhabitancy (Crous & Groenewald 2005) makes it difficult to link these cultures and anamorphs to their correct teleomorphs (Crous 2002).

The present study presents the second in a series characterising the *Mycosphaerella* species occurring on eucalypts. A major aim of this study was to use comparisons of DNA sequence data to clarify as many as possible of the formerly published host and distribution records (Crous 1998). Furthermore, while previous descriptions focused on species associated with leaf spots, this study also includes species from eucalypt leaf litter.

## MATERIALS AND METHODS

### Isolates

*Eucalyptus* leaves bearing *Mycosphaerella* ascomata, or with *Mycosphaerella* leaf spots were chosen for study. Excised lesions were soaked in water for approximately 2 h, after which they were placed in the bottom of Petri dish lids, with the top half of the dish containing 2 % malt extract agar (MEA) (Biolab, Midrand, South Africa). Ascospore germination patterns were examined after 24 h, and single-ascospore and conidial cultures established as described by Crous (1998). Colonies were sub-cultured onto carnation leaf agar (CLA) [1 % water agar (Biolab) with autoclaved carnation leaves placed onto the surface of the solidified medium] and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation.

### 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 and conditions were the same as those used by Crous *et al.* (2004b).

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. Due to the size and the complexity of the original alignment, the sequences were split over four smaller alignments, each containing genetically similar sequences. The four datasets were each treated identically. Phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Phylogenetic analysis of the aligned ITS sequence data consisted of neighbour-joining analysis with the uncorrected ("p"), the Kimura 2-parameter and the HKY85 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. Sequence data were deposited in GenBank (Table 1) and the alignments in TreeBASE.

### Taxonomy

Wherever possible, 30 measurements ( $\times$  1000 magnification) were made of structures mounted in lactic acid, with the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 1 mo on MEA, oatmeal agar (OA) and potato-dextrose agar (PDA) (Gams *et al.* 1998) 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). Nomenclatural novelties and descriptions were deposited in MycoBank <[www.mycobank.org](http://www.mycobank.org)>.

## RESULTS

### DNA phylogeny

For the ITS region, approximately 500 to 560 bases were determined for all isolates (Table 1). The trees resulting from each of the four alignments are depicted in Figs 1–4. The first alignment contains 102 taxa (including the two outgroups) and 544 characters including alignment gaps. Of these characters, 295 are parsimony-informative, 37 are variable and parsimony-uninformative, and 212 are constant. Neighbour-joining analysis using the three substitution models yielded trees with similar topologies and bootstrap values. Parsimony analysis yielded 243 most parsimonious trees (TL = 1038 steps; CI = 0.620; RI = 0.893; RC = 0.554). The topology of the distance trees differed from the trees obtained using parsimony mainly at the deeper nodes (data not shown). Parts of the distance tree obtained using the HKY85 substitution model are shown in Figs 1–4. The first alignment and derived tree (Fig. 1) includes *M. nubilosa* (100 % bootstrap support), species of *Colletogloeopsis* Crous & M.J. Wingf., the *M. molleriana* (Thüm.) Lindau complex (95 % bootstrap support), the *M. suttonii* Crous & M.J. Wingf. complex (100 % bootstrap support) and the *M. suberosa* Crous, F.A. Ferreira, Alfenas & M.J. Wingf. complex (100 % bootstrap support). One new

species of *Colletogloeopsis*, and four new species of *Mycosphaerella* are indicated.

The second alignment (Fig. 2) contains 90 taxa (including the two outgroups) and 535 characters including alignment gaps. Of these characters, 246 are parsimony-informative, 51 are variable and parsimony-uninformative, and 238 are constant. Neighbour-joining analysis using the three substitution models yielded trees with identical topologies and similar bootstrap values. Parsimony analysis yielded 481 most parsimonious trees (TL = 862 steps; CI = 0.613; RI = 0.927; RC = 0.568). The topology of the distance trees differed from the trees obtained using parsimony only in the placement of the *Mycosphaerella* sp. CPC 11171 clade (data not shown). The second alignment and derived tree mainly includes the *M. marksii* Carnegie & Keane complex (100 % bootstrap support), the *M. heimii* Crous complex (60 % bootstrap support), the *M. walkeri* R.F. Park & Keane (100 % bootstrap support) and the *M. parva* R.F. Park & Keane complex (100 % bootstrap support). Five new species of *Mycosphaerella* are indicated in the tree.

The third alignment (Fig. 3) contains 71 taxa (including the two outgroups) and 529 characters including alignment gaps. Of these characters, 237 are parsimony-informative, 71 are variable and parsimony-uninformative, and 221 are constant. Neighbour-joining analysis using the three substitution models yielded trees with identical topologies and similar bootstrap values. Parsimony analysis yielded 4319 most parsimonious trees (TL = 853 steps; CI = 0.626; RI = 0.856; RC = 0.536). The topology of the distance trees differed from the trees obtained using parsimony mainly at the deeper nodes (data not shown). The third alignment and derived tree mainly includes species of *Pseudocercospora* Speg., *Cercospora* Fresen., *Septoria* Sacc. and *Stenella* Syd. New species indicated in the tree include three in *Mycosphaerella*, two in *Septoria*, and two in *Stenella*.

The fourth alignment (Fig. 4) contains 50 taxa (including the two outgroups) and 570 characters including alignment gaps. Of these characters, 293 are parsimony-informative, 25 are variable and parsimony-uninformative, and 252 are constant. Neighbour-joining analysis using the three substitution models yielded trees with identical topologies and similar bootstrap values. Parsimony analysis yielded eight most parsimonious trees (TL = 627 steps; CI = 0.864; RI = 0.973; RC = 0.841). The topology of the distance trees was similar to that of the topology of the trees obtained using parsimony (data not shown). The fourth alignment and derived tree includes species of *Dissoconium* de Hoog, Oorschot & Hijwegen, *Passalora zambiae* Crous & T. Coutinho and *Mycosphaerella*, with three new species.

### Taxonomy

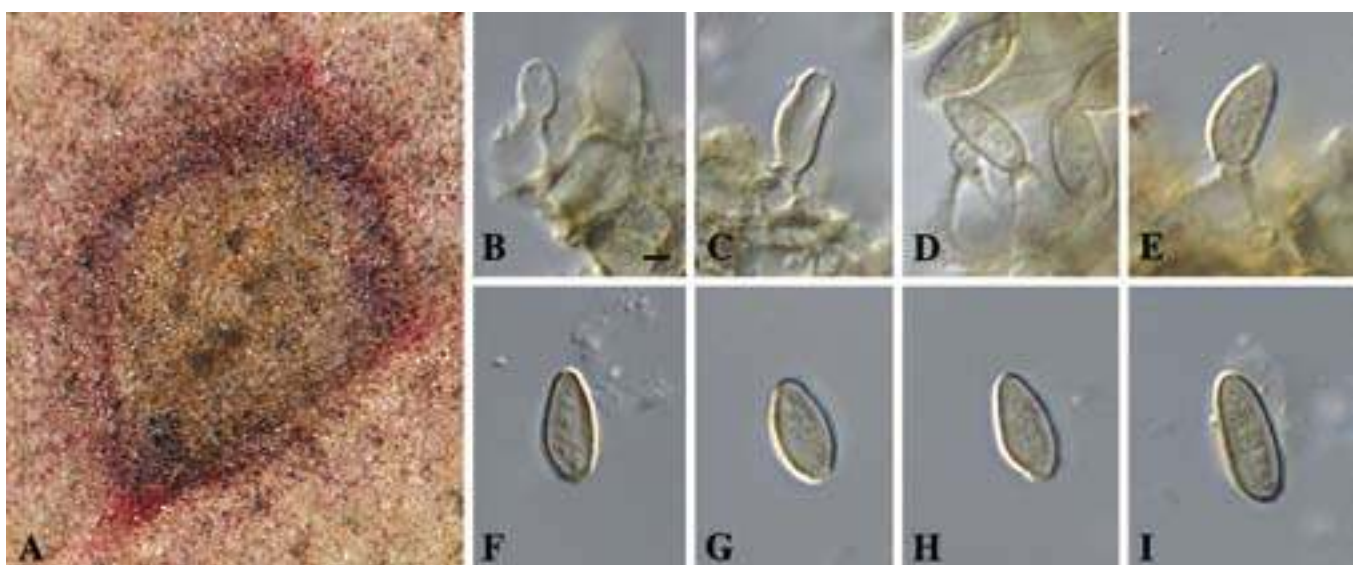
Several collections represented *Mycosphaerella* spp. morphologically and phylogenetically distinct from ex-type strains of the morphological species to which they had originally been assigned. These fungi are described as new taxa as follows:

***Colletogloeopsis stellenboschiana* Crous, sp. nov.**  
Mycobank MB500833. Fig. 5.

**Etymology:** Refers to Stellenbosch, where the fungus was collected.

*Coniothyrio ovato* similis sed conidiis minoribus, (6.5–)7–9(–10) × (3.5(–4) μm, distincta.

**Leaf spots** amphigenous, circular to subcircular, 0.5–3 mm diam, pale brown, with a raised border and red-purple margin. **Conidiomata** amphigenous, pycnidial, medium brown, globose, 80–120 μm diam; wall of 3–4 layers of brown *textura angularis*. **Conidiogenous cells** discrete, ampulliform to subcylindrical, pale to medium brown, finely verruculose, proliferating 1–3 times percurrently near the apex, 3–6 × 3–4 μm. **Conidia** holoblastic, solitary, aseptate, ellipsoidal, with



**Fig. 5.** *Colletogloeopsis stellenboschiana* (CBS 116428). A. Leaf spot. B–E. conidiogenous cells giving rise to conidia. F–I. Conidia. Scale bar = 3.5 μm.

subobtuse apex and subtruncate base with minute marginal frill, medium brown, finely verruculose, widest below the middle, (6.5–)7–9(–10) × (3–)3.5(–4) µm.

**Holotype:** South Africa, Western Cape Province, Stellenbosch Mountain, on leaves of *Eucalyptus* sp., 4 Dec. 2004, P.W. Crous, CBS H-19688, **holotype**, culture ex-type CBS 116428 = CPC 10886.

**Cultures:** Colonies after 3 wk on MEA 15–40 mm diam; on PDA erumpent, spreading, producing copious amounts of slime, olivaceous-black at the centre, aerial mycelium olivaceous-grey, with a vinaceous-grey outer zone and wide olivaceous-black margin that is smooth but uneven; reverse olivaceous-black; on OA surface smoke-grey with a wide, grey-olivaceous border, forming a characteristic yellow pigment; on MEA grey-white on surface, with sectors of smoke-grey; margin thin, submerged, smoke-grey; reverse olivaceous-black; aerial mycelium sparse to moderate, grey-white; colonies fertile.

**Host:** *Eucalyptus* sp.

**Distribution:** South Africa.

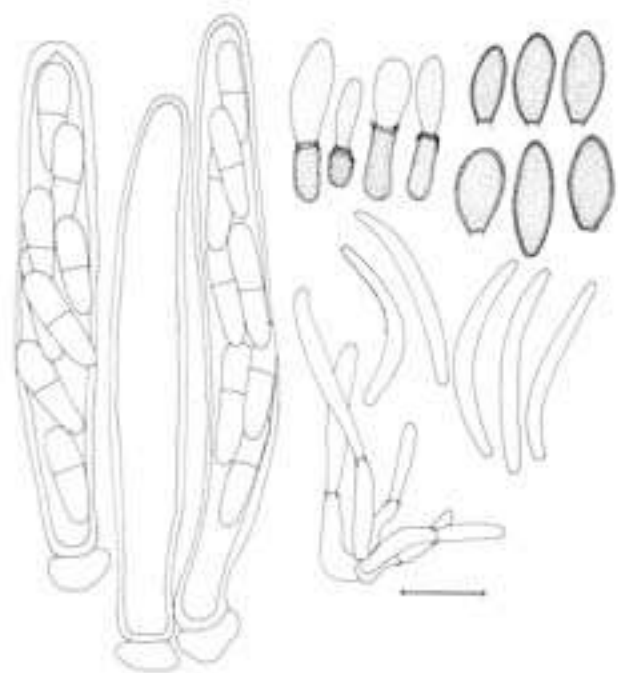
**Notes:** Numerous species of *Coniothyrium* Corda and several species of *Colletogloeopsis* cause spots on eucalypt leaves. *Colletogloeopsis stellenboschiana* is easily distinguished from the taxa occurring on eucalypt leaves (Crous 1998), and from representatives of the “*Coniothyrium ovatum*” species complex specifically, based on its conidial morphology. Phylogenetically it is closely related to members of the the *Colletogloeopsis* complex that cause stem cankers on eucalypt trees (Cortinas *et al.* 2006 – this volume)

***Mycosphaerella davisoniellae*** Crous, **sp. nov.** MycoBank MB500834. Fig. 6.

**Anamorph:** *Davisoniella eucalypti* H.J. Swart, Trans. Brit. Mycol. Soc. 90: 289. 1988.

Asci subcylindrici, sessiles, 50–70 × 9–12 µm. Ascospores bi-vel triseriatae, tenuitunicatae, rectae, obovoideae, apicem versus latissimae, in medio uniseptatae, vix vel haud constrictae ad septum, 10–14 × 3–4 µm.

**Leaf spots** amphigenous, subcircular to irregular, 1–7 mm diam, discrete to confluent, medium brown, surrounded by raised, red-purple margin. **Ascomata** hypophyllous, embedded in a raised, black, subepidermal stroma, ostiolate, becoming erumpent up to 120 µm diam. **Asci** subcylindrical, sessile, straight or slightly incurved, 8-spored, 50–70 × 9–12 µm. **Ascospores** bi- to triseriate, overlapping, hyaline, thin-walled, straight, obovoid with rounded ends, widest near the apex, medianly 1-septate, not to slightly constricted at the septum, tapering toward both ends, but more prominently toward the base, 10–14 × 3–4 µm. **Conidiomata** of *Davisoniella* embedded in the same black subepidermal stroma that contains ascomata, subepidermal, ostiolate, up to 450 µm diam; wall of 2–3 layers of brown *textura angularis*. **Conidiogenous cells** subcylindrical to ampulliform or doliiform, 5–15 × 3–4 µm, medium brown, verruculose, proliferating several times percurrently near the apex. **Conidia** solitary, brown, aseptate, verruculose, thick-walled, oval with an



**Fig. 6.** *Mycosphaerella davisoniellae* (anamorph *Davisoniella eucalypti*) (DAR 58999). A. Asci and ascospores. B. Conidiogenous cells and conidia of *D. eucalypti*. C. conidiogenous cells and conidia of synanamorph. Scale bar = 10 µm.

obtuse apex and a truncate to subtruncate base with a prominent basal frill, which can extend up to 2 µm from the brown basal rim of the conidium, (8–)10–12(–14) × 4.5–)5–6(–6.5) µm (av. 11 × 5.5 µm). **Synanamorph:** *Conidiomata* intermingled between that of *D. eucalypti* and ascomata of *M. davisoniellae*. **Conidiogenous cells** phialidic, hyaline, subcylindrical to ampulliform, with visible periclinal thickening, 8–15 × 2.5–3.5 µm. **Conidia** hyaline, curved, subcylindrical, widest in the middle, apex bluntly rounded, obtuse, base truncate, 17–30 × 2–1.5 µm.

**In vivo:** No cultures available.

**Specimen examined:** Australia, Darling Ranges W.A., Mundlimup Block, on leaves of *Eucalyptus marginata*, 24 Nov. 1981, F. Tay, DAR 58999, **holotype** of *D. eucalypti* and *M. davisoniellae*.

**Notes:** Swart (1988) reported that this fungus is associated with abundant leaf spots on saplings and the foliage of recently felled trees. *Conidiomata* of *D. eucalypti* were described as unilocular and subepidermal, occurring in a stroma which could result in some of them appearing as multilocular. Swart (1988) considered the fungus to be the stromatic counterpart of *Coniothyrium*. *Davisoniella eucalypti* is clearly related to species in the *Colletogloeopsis* complex that occurs on eucalypts, having characteristic aseptate, brown, verruculose conidia that arise from percurrently proliferating conidiogenous cells. *Davisoniella* is unique by virtue of its stroma, that gives rise to the uni- or multilocular *conidiomata*. Conidia of *D. eucalypti* exude in slimy masses. In many cases, the exudates included aseptate, hyaline, curved, subcylindrical conidia of a synanamorph. The latter anamorph was produced from unilocular *conidiomata* that formed in the same stromata that gave rise to *D. eucalypti*. Surprisingly, many of the

stromata investigated also contained ascomata of a *Mycosphaerella* species, which most likely also belong to the same fungus. The latter state is described here as *M. davisoniellae*.

***Mycosphaerella eucalyptorum*** Crous & M.J. Wingf., sp. nov. MycoBank MB500835. Fig. 7.

**Etymology:** Referring to its host, *Eucalyptus*.

*Mycosphaerellae parkii* similis, sed ascosporis maioribus, 12–17 × 3.5–4.5 µm, modo B germinantibus, distinguenda.

**Leaf spots** amphigenous, irregular to sub-circular, 2–20 mm diam, medium brown, with raised, brown borders, and thin, red-purple margins. **Ascomata** pseudothecial, amphigenous but predominantly epiphyllous, single, black, erumpent, globose, up to 120 µm diam; apical ostiole 10–15 µm diam, with prominent periphyses lining the ostiolar channel; wall of 2–3 layers of medium brown *textura angularis*. **Asci** aparaphysate, fasciculate, bitunicate, subsessile, obovoid to ellipsoid, straight or slightly incurved, 8-spored, 35–50 × 8–12 µm. **Ascospores** tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid–ellipsoidal with obtuse ends, medianly 1-septate, widest in middle of apical cell, not constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (12–)14–15(–17) ×

(3.5–)4(–4.5) µm *in vivo*; some ascospores with slightly asymmetrical apical cells, as commonly observed in *M. marksii*.

**Holotype:** Indonesia, on leaves of *Eucalyptus* sp., Mar. 2004, M.J. Wingfield, CBS H-19689 **holotype**, culture ex-type CBS 118496 = CPC 11174.

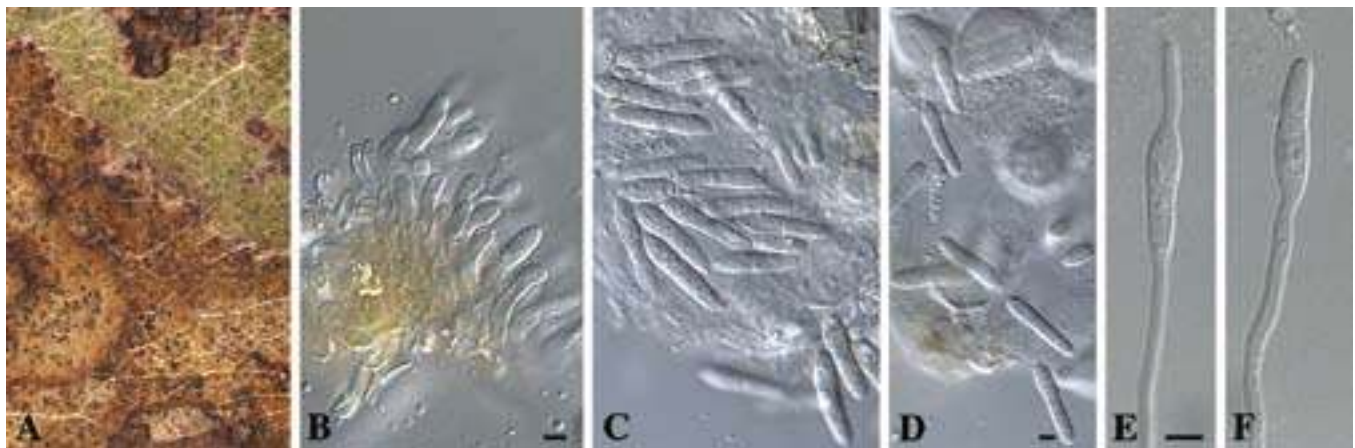
**Ascospore germination on MEA after 24 h:** Type B. Ascospores not darkening on MEA, and germinating from both ends, with germ tubes parallel to the long axis of the spore, not distorting, becoming slightly constricted upon germination, becoming up to 4 µm diam.

**Cultures:** Colonies on MEA after 3 wk 25–30 mm diam; on MEA flat, spreading, folding, with sparse aerial mycelium, olivaceous-grey, margins smooth, regular, reverse iron-grey; on PDA slightly erumpent, centre olivaceous-grey; outer zone pale olivaceous-grey; reverse iron-grey; on OA with sparse to moderate pale olivaceous-grey aerial mycelium and patches of olivaceous-grey.

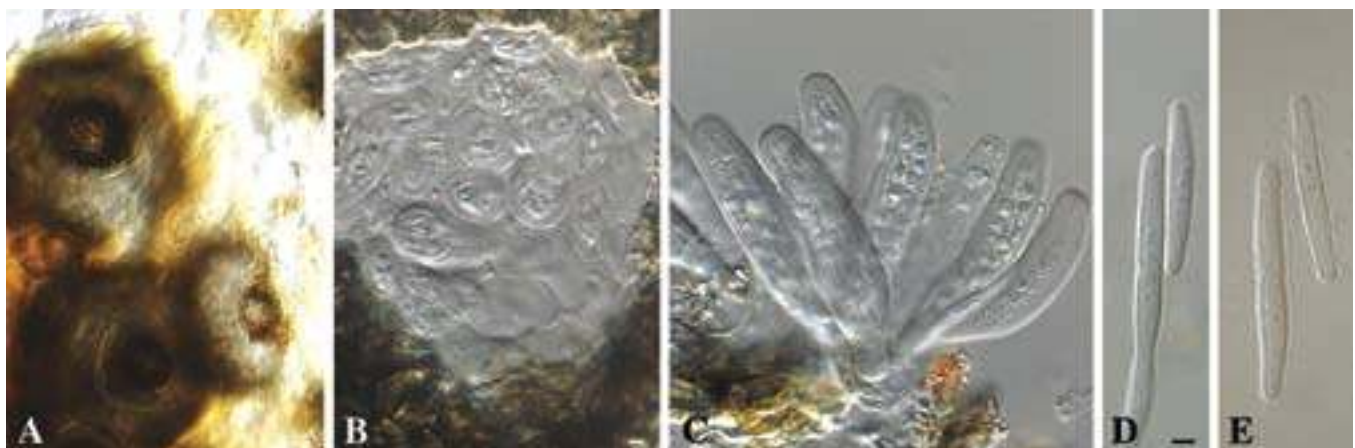
**Host:** *Eucalyptus* sp.

**Distribution:** Indonesia.

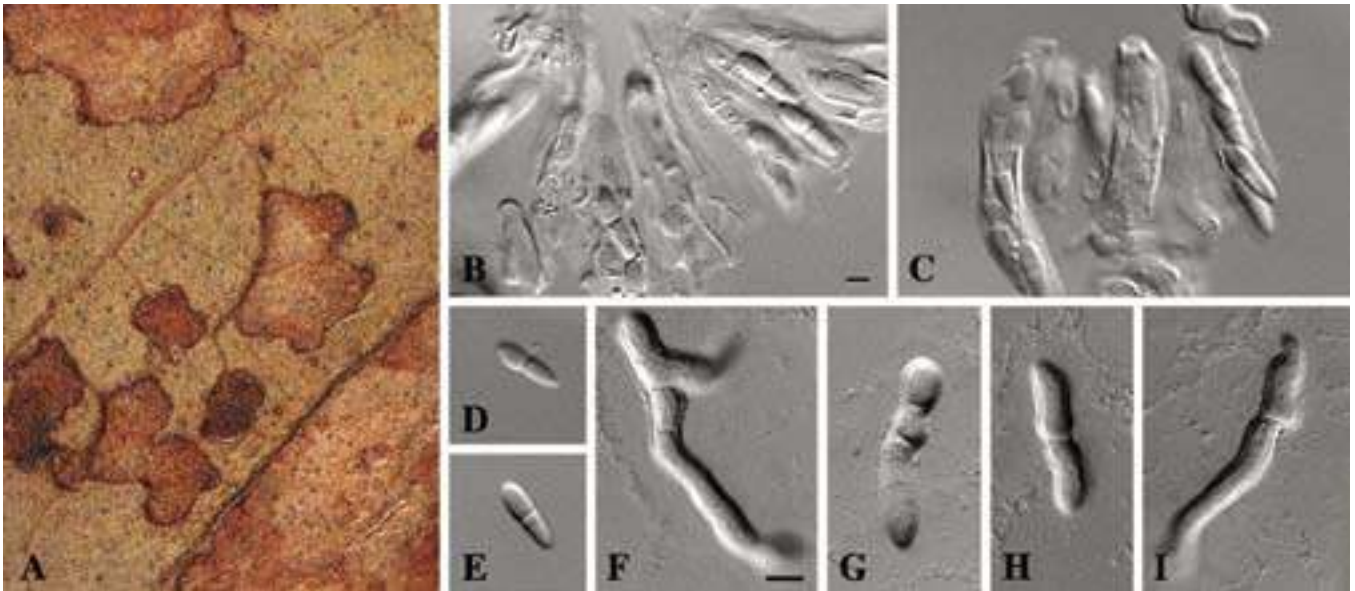
**Notes:** Conidia of a *Stenella* anamorph were found on some lesions. This link is, however, unconfirmed, and



**Fig. 7.** *Mycosphaerella eucalyptorum* (CBS 118496). A. Leaf spot. B. Ostiolar periphysoids. C–D. Ascospores. E–F. Germinating ascospores. Scale bars = 4 µm.



**Fig. 8.** *Mycosphaerella gracilis*. A. Ascomata on leaf tissue. B. Ostiolar region of ascoma. C. Asci. D–E. Germinating ascospores. Scale bar = 3 µm.



**Fig. 9.** *Mycosphaerella gamsii* (CBS 118495). A. Leaf spot. B–C. Asci. D–E. Ascospores. F–I. Germinating ascospores. Scale bars: B = 3  $\mu$ m, F =  $\mu$ m.

isolates did not produce anamorph structures in culture. *Mycosphaerella eucalyptorum* is phylogenetically closely related to a *Mycosphaerella* sp. from Colombia that forms *Stenella pseudoparkii* in culture. Ascospores of *M. eucalyptorum* (12–17  $\times$  3.5–4.5  $\mu$ m) germinate with a Type B germination pattern as observed in *M. gracilis* (10–20  $\times$  2–3  $\mu$ m) (Fig. 8) and *M. marksii* (11–22.5  $\times$  2–3.5). It is easily distinguished from these taxa, however, based on its ascospore morphology and growth characteristics in culture (Crous 1998).

***Mycosphaerella gamsii* Crous, sp. nov.** MycoBank MB500836. Fig. 9.

**Etymology:** Named after the collector, well-known mycologist and friend, Prof. dr Walter Gams.

*Mycosphaerellae stramenticolae* similis, sed ascosporis minoribus, (8–)9–10  $\times$  (2–)3  $\mu$ m, modo C germinantibus, distinguenda.

**Leaf spots** amphigenous, irregular, 1–20 mm diam, medium brown, with a raised, dark brown border. **Ascomata** pseudothecial, amphigenous, but predominantly hypophyllous, single, black, subepidermal, becoming erumpent, globose, up to 90  $\mu$ m diam; apical ostiole 5–10  $\mu$ m diam; wall of 2–3 layers of medium brown *textura angularis*. **Asci** aparaphysate, fasciculate, bitunicate, sessile, obovoid to narrowly ellipsoid, straight or slightly incurved, 8-spored, 25–35  $\times$  7–9  $\mu$ m. **Ascospores** tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid-ellipsoidal, medianly 1-septate, widest in the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)9–10  $\times$  (2–)3  $\mu$ m *in vivo*.

**Holotype:** India, Palampur, on leaves of *Eucalyptus* sp., Mar. 2004, W. Gams & M. Arzanlou, CBS H-19690, **holotype**, culture ex-type CBS 118495 = CPC 11138–11140. 5/6-6

**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, but also variable in direction; becoming constricted upon germination, up to 5  $\mu$ m diam.

**Cultures:** Colonies on MEA 28–35 mm diam after 3 wk; on MEA spreading, folding, flat, with moderate smoke-grey aerial mycelium in the centre; outer region olivaceous-grey; margins smooth, regular; reverse iron-grey; on PDA with moderate aerial mycelium, pale olivaceous-grey, outer region olivaceous-grey with drops of slime; reverse iron-grey; on OA with moderate aerial mycelium, pale olivaceous-grey, with patches of olivaceous-grey.

**Host:** *Eucalyptus* sp.

**Distribution:** India.

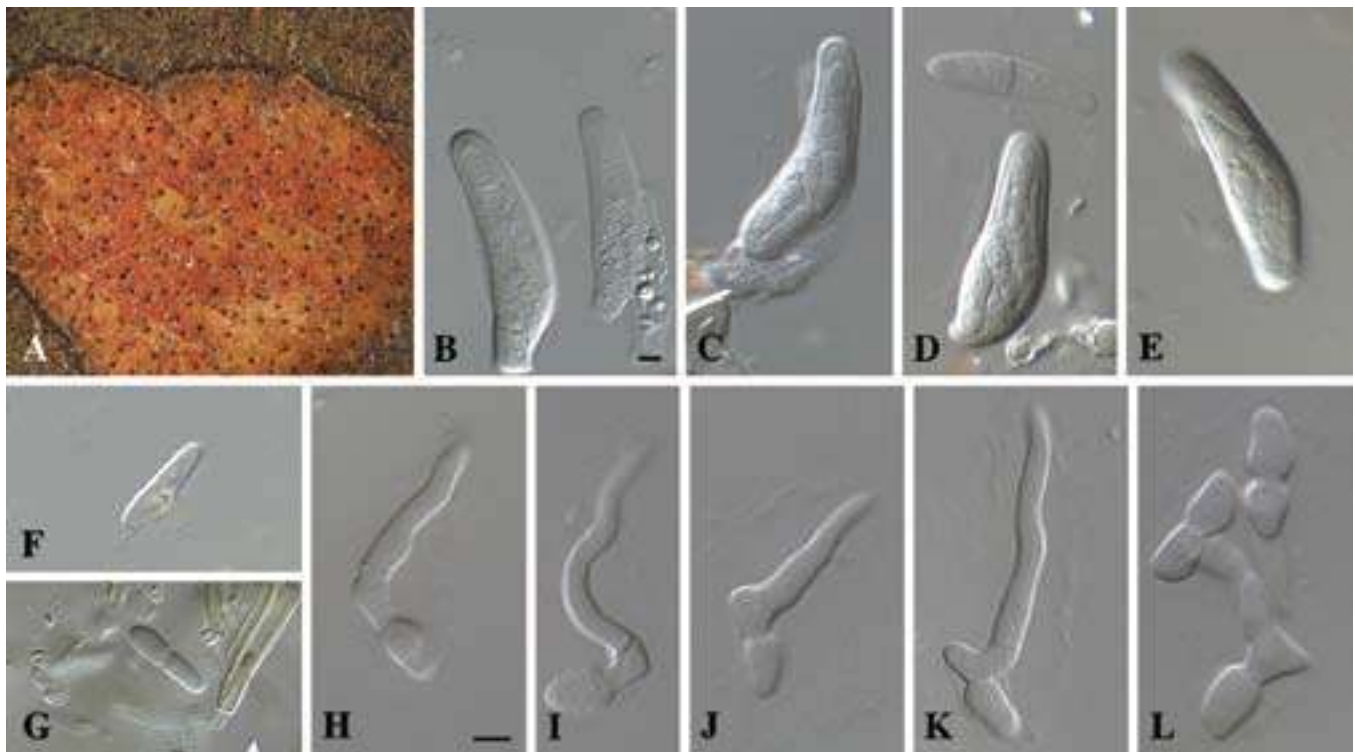
**Notes:** *Mycosphaerella gamsii* is phylogenetically closely related to *M. stramenticola*, but is distinguishable in having a Type C ascospore germination pattern, as is found in species such as *M. heimii*, *M. gregaria*, *M. molleriana*, *M. nubilosa* and *M. walkerii*. *Mycosphaerella gamsii* has ascospores that are 8–10  $\times$  2–3  $\mu$ m, thus shorter than those of the species listed above, and it also lacks an anamorph in culture.

***Mycosphaerella perpendicularis* Crous & M.J. Wingf., sp. nov.** MycoBank MB500837. Fig. 10.

**Etymology:** Referring to ascospores that germinate with germ tubes growing 90° to the long axis of the spore.

*Mycosphaerellae heimioide* similis, sed ascosporis longioribus, (8–)9–10(–12)  $\times$  (2.5–)3  $\mu$ m, modo M germinantibus distinguenda.

**Leaf spots** amphigenous, irregular to sub-circular, 5–15 mm diam, medium brown, frequently with a orange-red discoloration in the central part; border raised, dark brown. **Ascomata** pseudothecial, epiphyllous, single, black, subepidermal, globose, up to 90  $\mu$ m diam; apical ostiole 10–15  $\mu$ m diam; wall of 2–3 layers of medium brown *textura angularis*. **Asci** aparaphysate,



**Fig. 10.** *Mycosphaerella perpendicularis* (CBS 118367). A. Leaf spot. B–E. Asci. F–G. Ascospores. H–L. Germinating ascospores. Scale bars: B = 3  $\mu$ m, H = 5  $\mu$ m.

fasciculate, bitunicate, sessile, obovoid to broadly ellipsoid, slightly incurved, 8-spored, 25–35  $\times$  7–8  $\mu$ m. Ascospores multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid–ellipsoidal with obtuse ends, medianly 1-septate, widest in the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)9–10(–12)  $\times$  (2.5–)3  $\mu$ m *in vivo*.

**Holotype:** Colombia, Suiza, on leaves of *Eucalyptus eurograndis*, Jan. 2004, M.J. Wingfield, CBS H-19691, **holotype**, culture ex-type CBS 118367 = CPC 10983–10985.

**Ascospore germination on MEA after 24 h:** Type M. Ascospores not darkening on MEA, and germinating from both ends, with germ tubes 90° to the long axis of the spore, and distorting upon germination, becoming up to 5  $\mu$ m wide.

**Cultures:** Colonies on MEA reaching 28–37 mm diam after 3 wk; colonies folding, spreading, flat, with sparse aerial mycelium, which is olivaceous-grey on the agar surface, and with smoke-grey aerial mycelium; margins are smooth, regular; reverse iron-grey at the centre, olivaceous-grey in the outer zone; on OA with moderate aerial mycelium, olivaceous-grey at centre, greenish black in outer zone; on PDA olivaceous-grey with some drops of slime, iron-grey in reverse.

**Host:** *Eucalyptus eurograndis*.

**Distribution:** Colombia.

**Notes:** Germinating ascospores of *M. perpendicularis* have a characteristic Type M germination pattern, similar to that of *M. heimioides*. *Mycosphaerella perpendicularis* can easily be distinguished from *M.*

*heimioides*, however, by virtue of the fact that the ascospores distort at germination. In addition, the germ tubes of *M. heimioides* never quite reach 90° to the long axis of the spore, whereas those of *M. perpendicularis* are at right angles.

***Mycosphaerella pluritubularis*** Crous & J.P. Mansilla, **sp. nov.** MycoBank MB500838. Figs 11–12.

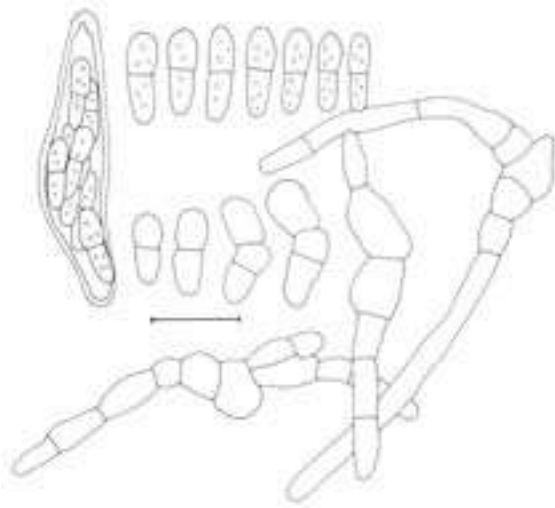
**Etymology:** Refers to the ascospores that have multiple germ tubes when they germinate.

*Mycosphaerellae nubilosae* similis, set ascosporis brevioribus, (8–)9–10(–11)  $\times$  3(–4)  $\mu$ m, saepe plures quam 2 tubos germinationis proferentibus, distinguenda.

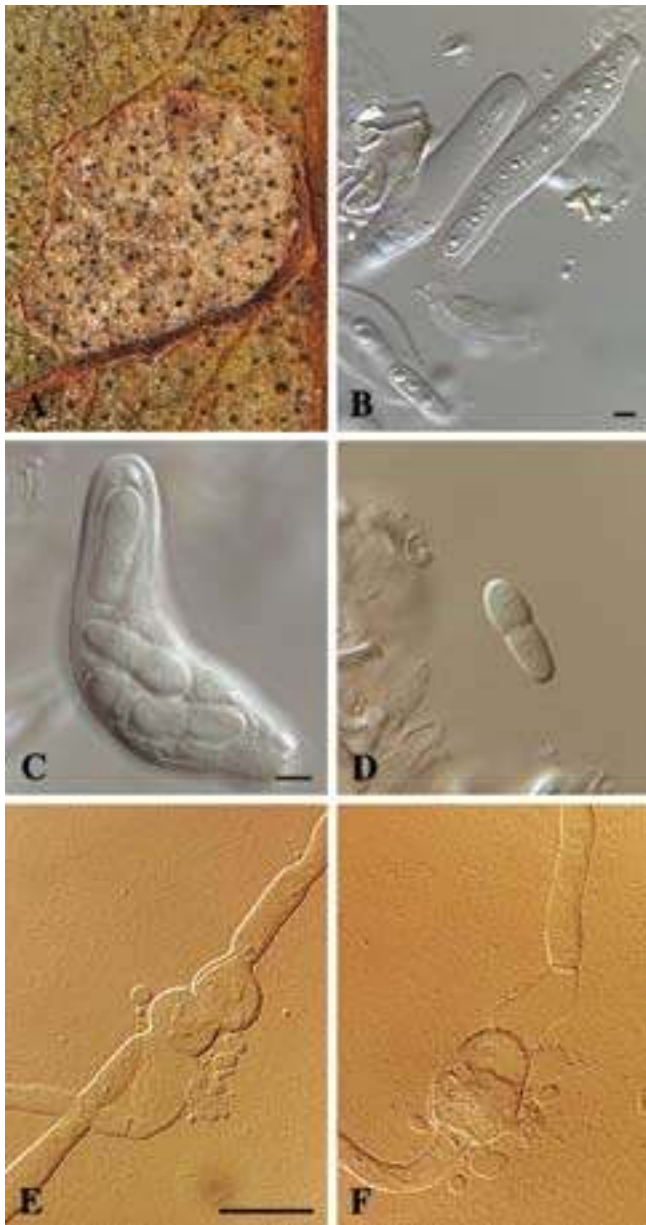
**Leaf spots** amphigenous, irregular to sub-circular, 5–15 mm diam, pale to medium brown, surrounded by a thin, raised, dark brown border. **Ascomata** pseudothecial, hypophyllous, single, black, immersed becoming erumpent, globose, up to 100  $\mu$ m diam; apical ostiole 10–15  $\mu$ m diam; wall of 2–3 layers of medium brown **textura angularis**. **Asci** paraphysate, fasciculate, bitunicate, sessile, obovoid to subcylindrical, straight to slightly incurved, 8-spored, 30–45  $\times$  7–10  $\mu$ m. **Ascospores** multiseriate, overlapping, hyaline, prominently guttulate, thin-walled, straight, obovoid with subobtuse ends, medianly 1-septate, widest at the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)9–10(–11)  $\times$  3(–4)  $\mu$ m *in vivo*.

**Holotype:** Spain, on leaves of *E. globulus*, Nov. 2004, J.P. Mansilla, CBS H-19692 **holotype**, culture ex-type CBS 118508 = CPC 11697).





**Fig. 11.** *Mycosphaerella pluritubularis* (CBS 118508). A. Ascus and ascospores. B. Sequence of germinating ascospores, with those after 24 h at the right of the plate. Scale bar = 10  $\mu$ m.



**Fig. 12.** *Mycosphaerella pluritubularis* (CBS 118508). A. Leaf spot. B–C. Asci. D. ascospore. E–F. Germinating ascospores. Scale bars: B–C = 3  $\mu$ m, E = 11  $\mu$ m.

*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 up to 11  $\mu$ m diam; frequently germinating with more than two germ tubes.

*Cultures:* Colonies after 3 wk 17–22 mm diam on MEA; on PDA colonies forming copious amounts of slime; surface olivaceous-black with patches of olivaceous-grey and pale olivaceous-grey; aerial mycelium sparse; margins feathery, uneven; reverse iron-grey; on OA surface smoke-grey with patches of olivaceous-grey; on MEA with sparse aerial mycelium, colonies erumpent, iron-grey, margins feathery, irregular; reverse olivaceous-black; colonies sterile.

*Host:* *E. globulus*.

*Distribution:* Spain.

*Notes:* *Mycosphaerella pluritubularis* is characterised by its distinct ascospore germination pattern (Type F), but where ascospores form more than two germ tubes, thus distinguishing it from other species like *M. nubilosa* that have more typical type F germination patterns.

***Mycosphaerella pseudaficana*** Crous & T. Coutinho, sp. nov. MycoBank MB500839. Fig. 13.

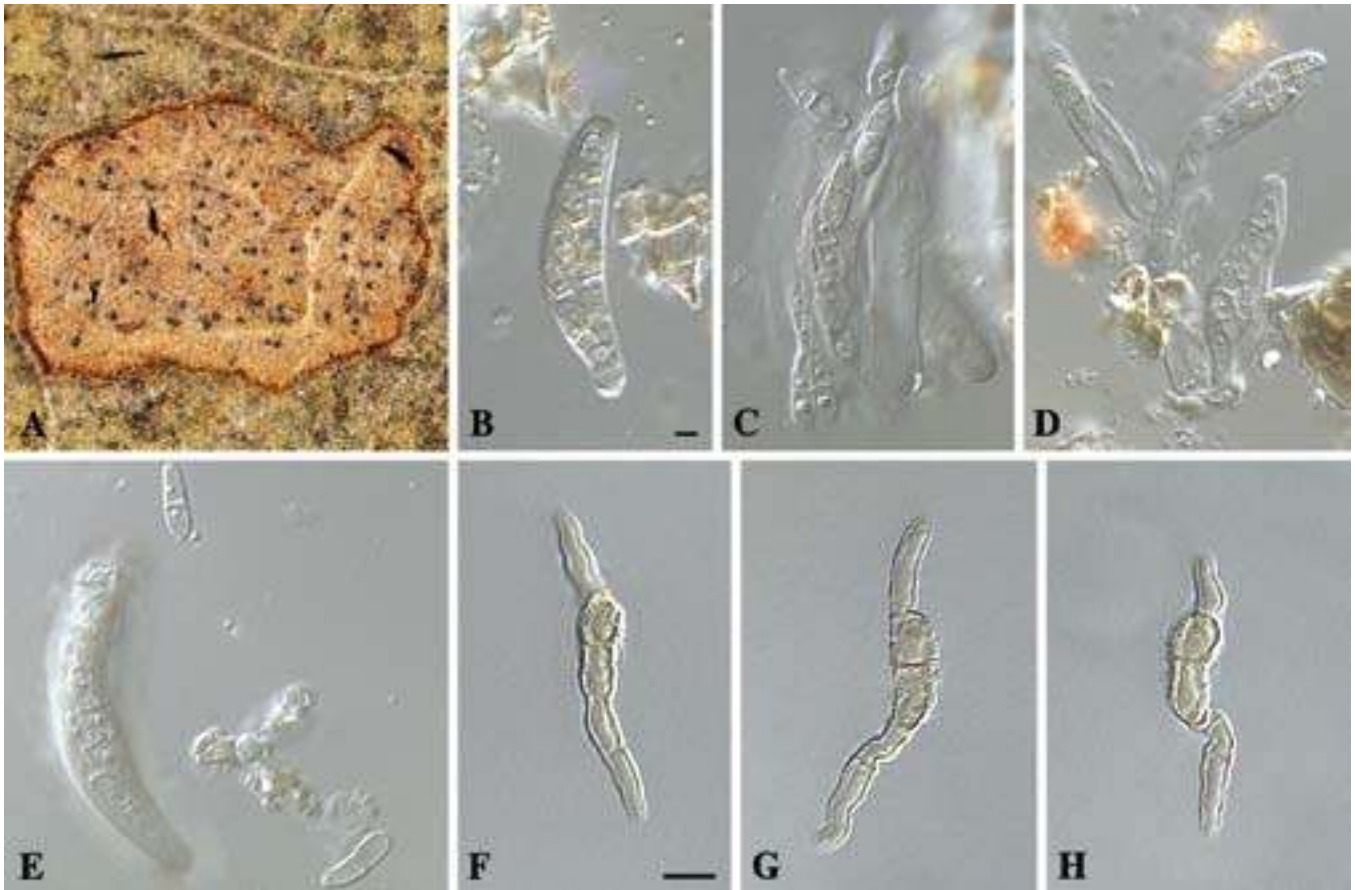
*Etymology:* Referring to its morphological similarity to *M. africana*.

*Mycosphaerellae africanae similis, sed ascosporis maioribus, 8–11  $\times$  2.5–3  $\mu$ m, distinguenda.*

*Leaf spots* amphigenous, irregular to sub-circular, 2–7 mm diam, medium brown, surrounded by a thin, raised, concolorous border. *Ascomata* pseudothecial, hypophyllous, single, black, immersed becoming erumpent, globose, up to 120  $\mu$ m diam; apical ostiole 10–15  $\mu$ m diam; wall of 2–3 cell layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, sessile, narrowly ellipsoid to subcylindrical, slightly incurved, 8-spored, 35–45  $\times$  7–9  $\mu$ m. *Ascospores* tri- to multiseriate, overlapping, hyaline to pale brown, guttulate, thin-walled, straight to slightly curved, smooth to finely roughened, fusoid–ellipsoidal with subobtuse ends, medianly 1-septate, widest in the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)9–10(–11)  $\times$  (2.5–)3  $\mu$ m *in vivo*. *Spermatogonia* similar to the ascomata in morphology. *Spermatia* hyaline, smooth, rod-shaped with bluntly rounded ends, 3–4  $\times$  1–1.5  $\mu$ m.

*Holotype:* Zambia, on leaves of *E. globulus*, Aug. 1995, T. Coutinho, PREM 54973 **holotype**, culture ex-type CBS 114782 = CPC 1230; 1229–1231.

*Ascospore germination on MEA after 24 h:* Type G. Ascospores darkening and becoming verruculose on MEA; germinating from both ends as observed in *M. africana*, with germ tubes irregular to the long axis of the spore, and distorting prominently upon germination, becoming up to 8  $\mu$ m wide.



**Fig. 13.** *Mycosphaerella pseudofrancana* (CBS 114782). A. Leaf spot. B–E. Asci and ascospores. F–H. Germinating ascospores. Scale bars: B = 3  $\mu$ m, F = 7  $\mu$ m.

**Cultures:** Colonies reaching 12–17 mm diam after 3 wk on MEA; colonies erumpent, irregular, surface iron-grey with olivaceous-grey, sparse aerial mycelium in central part; margins catenate, smooth; reverse greenish black; on PDA colonies erumpent, olivaceous-black with sparse olivaceous-grey aerial mycelium in the central part, margins smooth, catenate; reverse greenish black; on OA olivaceous-grey with smooth, catenate margins and green-olivaceous central part.

**Host:** *E. globulus*.

**Distribution:** Zambia.

**Notes:** Ascospores of *M. pseudofrancana* (8–11  $\times$  2.5–3  $\mu$ m) germinate with a Type G pattern similar to that observed in *M. africana* (7–11  $\times$  2–3  $\mu$ m). Ascospores of *M. pseudofrancana* are more verrucose than those of *M. africana*, but both taxa have very similar ascospore dimensions and germination patterns. They do differ, in the symptoms with which they are associated. Lesions of *M. pseudofrancana* are generally larger, and they lack the red-purple margin found in *M. africana*. The easiest means to distinguish these taxa from each other is to compare their growth in culture: colonies of *M. africana* are black, produce a brown pigment in MEA, and form clusters of chlamyospores, whereas cultures of *M. pseudofrancana* also produce clusters of chlamyospores on MEA, but are iron-grey, and lack the diffuse brown pigment observed in colonies of *M. africana*.

***Mycosphaerella pseudocryptica* Crous, sp. nov.**

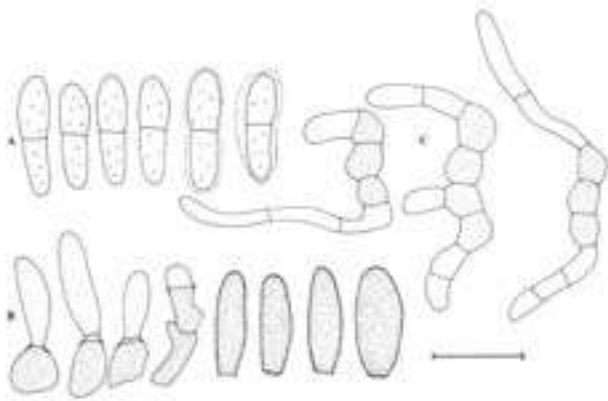
Mycobank MB500840. Figs 14–15.

**Anamorph:** *Colletogloeopsis* sp.

**Etymology:** Morphologically similar to *M. cryptica*.

*Mycosphaerellae crypticae* similis, sed ascosporis minoribus, (11–)12–14(–15)  $\times$  (3–)3.5(–4)  $\mu$ m, saepe utrinque germinantibus, distinguenda.

**Leaf spots** amphigenous, irregular to subcircular, 0.5–2 mm diam, pale brown, with a raised, red-brown margin. **Ascomata** pseudothecial, hypophyllous, arranged in dense clusters in pale brown areas next to the leaf spots associated with conidiomata of the anamorph, black, immersed, globose, up to 70  $\mu$ m diam; apical ostiole 10–15  $\mu$ m diam; wall of 2–3 layers of medium brown *textura angularis*. **Asci** aparaphysate, fasciculate, bitunicate, subsessile, narrowly ellipsoid to subcylindrical, straight or slightly incurved, 8-spored, 35–45  $\times$  9–11  $\mu$ m. **Ascospores** multiseriate, overlapping, hyaline, granular, thin-walled, straight, fusoid–ellipsoidal with obtuse ends, medianly 1-septate, widest at the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (11–)12–14(–15)  $\times$  (3–)3.5(–4)  $\mu$ m, *in vivo*; frequently encased in an irregular mucous sheath. **Mycelium** internal, consisting of branched, septate, medium brown, smooth, 3–4  $\mu$ m wide hyphae. **Conidiomata** intermixed among ascomata or separate, predominantly on the lower



**Fig. 14.** *Mycosphaerella pseudocryptica* (anamorph *Colletogloeopsis* sp.) (CBS 118504). A. Ascospores, some with sheath. B. Germinating ascospores. C. Conidiogenous cells and conidia. Scale bar = 10 µm.

leaf surface, pycnidial, substromatal, up to 120 µm diam; wall of 3–4 layers of brown *textura angularis*. *Conidiophores* 0–1-septate, but mostly reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to subcylindrical, medium brown, smooth to finely verruculose, proliferating 1–3 times percurrently near apex, but also intercalary and sympodially, 5–15 × 3–5 µm. *Conidia* holoblastic, solitary, aseptate, fusoid with obtuse to subobtuse apices and truncate bases, medium brown, finely verruculose, (10–)12–14(–17) × (3.5–)4(–6) µm; inconspicuous basal marginal frill present.

**Holotype:** **New Zealand**, Wellington Botanical Garden, on leaves of *Eucalyptus* sp., Mar. 2004, J.A. Stalpers, CBS H-19693, **holotype**, culture ex-type CBS 118504 = CPC 11267; 11267–11269 (teleomorph), CPC 11264–11266 (anamorph).

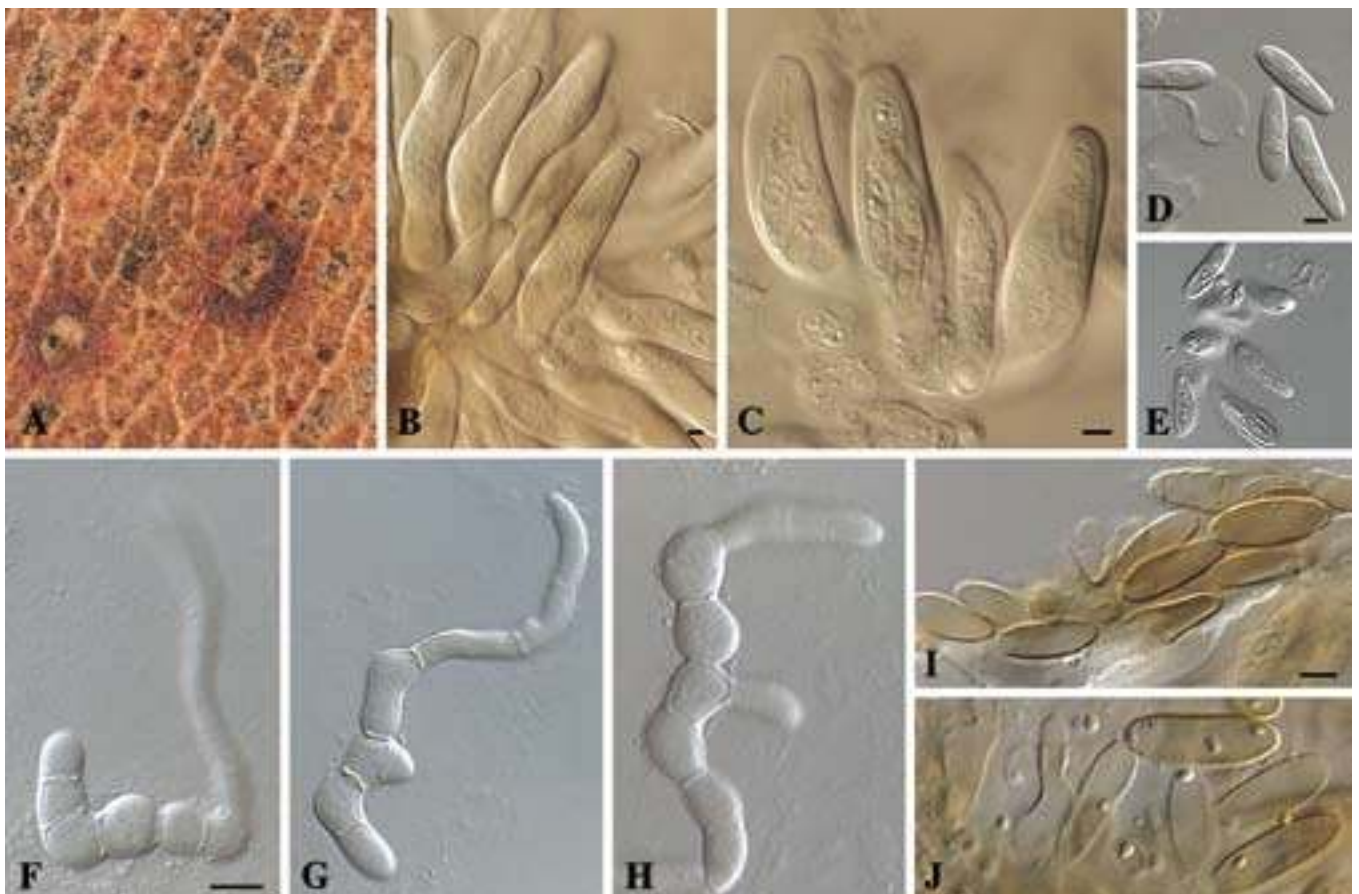
**Ascospore germination on MEA after 24 h:** Type A. Ascospores smooth, becoming olivaceous on MEA, germinating predominantly from both ends, with germ tubes at some angle to the long axis of the spore, and with a constriction at the ascospore septum; ascospores becoming up to 7 µm wide.

**Cultures:** Colonies slow growing, 3–8 mm diam after 3 wk on MEA; on MEA colonies erumpent, aerial mycelium sparse to absent, margins smooth, surface white-grey to smoke-grey, or with a reddish tinge in patches; reverse fuscous-black; on PDA erumpent, white to smoke-grey with patches of vinaceous-grey; reverse vinaceous-grey, with a diffuse red pigment visible in the agar, up to 2 cm from colony margins; on OA pale grey-olivaceous with a pale vinaceous grey pigment diffusing into the agar.

**Host:** *Eucalyptus* sp.

**Distribution:** New Zealand.

**Notes:** Ascospores of *M. pseudocryptica* germinate with a Type A pattern (as observed in *M. cryptica*), except that they tend to germinate from both ends. It is possible, therefore, that collections of *M. pseudocryptica* have in the past been confused with those of *M. cryptica*.



**Fig. 15.** *Mycosphaerella pseudocryptica* (anamorph *Colletogloeopsis* sp.) (CBS 118504). A. Leaf spot. B–C. Asci. D–E. Ascospores. F–H. Germinating ascospores. I–J. Conidia and conidiogenous cells. Scale bars: B–C, I = 4 µm, D = 3.5 µm, F = 7 µm.

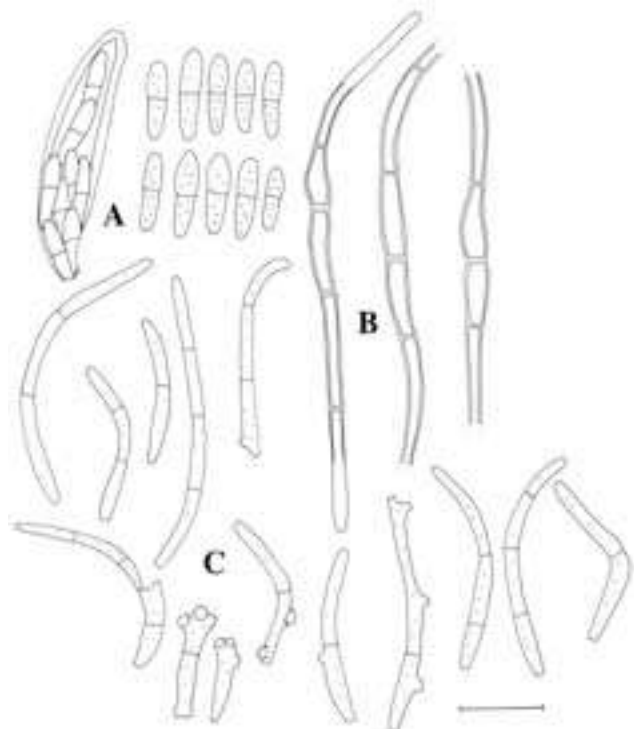
Isolates also form a *Colletogloeopsis* anamorph in culture, which is similar to *M. cryptica*. Ascospores of *M. pseudocryptica* are  $11\text{--}15 \times 3\text{--}4 \mu\text{m}$ , and conidia  $10\text{--}17 \times 3.5\text{--}6 \mu\text{m}$ , while ascospores of *M. cryptica* are  $9\text{--}17.5 \times 2\text{--}5.5 \mu\text{m}$ , and conidia are  $8.5\text{--}18 \times 4\text{--}6 \mu\text{m}$ . Phylogenetically *M. pseudocryptica* is closely related to the *M. molleriana* complex (Fig. 1), and distinct from *M. cryptica*.

***Mycosphaerella pseudoendophytica*** Crous & G. Hunter, **sp. nov.** MycoBank MB500841. Figs 16–17. Anamorph: ***Pseudocercospora*** sp.

**Etymology:** Named after its morphological similarity to *M. endophytica*.

*Mycosphaerellae endophyticae* similis, sed ascosporis modo C germinantibus distinguenda.

**Leaf spots** amphigenous, irregular to subcircular or angular, 2–5 mm diam, brown, with a raised, dark brown margin. **Ascomata** pseudothecial, amphigenous, black, subepidermal, erumpent to superficial, globose, up to 120  $\mu\text{m}$  diam; apical ostiole 5–10  $\mu\text{m}$  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,  $30\text{--}40 \times 8\text{--}10 \mu\text{m}$ . **Ascospores** multiseriate, overlapping, hyaline, sparsely guttulate, thin-walled, straight to slightly curved, fusoid–ellipsoidal with obtuse ends, medianly 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,  $(8\text{--})9\text{--}10\text{--}(11) \times (2\text{--})2.5\text{--}3 \mu\text{m}$ , *in vivo*. **Mycelium** internal, consisting of branched, septate, pale to medium brown, smooth, 3–4  $\mu\text{m}$  wide hyphae. **Conidiomata** *in vitro* sporodochial,



**Fig. 16.** *Mycosphaerella pseudoendophytica* (anamorph *Pseudocercospora* sp.) (CBS 113288). A. Ascus and ascospores. B. Germinating ascospores. C. Conidia. Scale bar = 10  $\mu\text{m}$



**Fig. 17.** Leaf spot associated with *Mycosphaerella pseudoendophytica* (holotype)

hyaline. **Conidiogenous cells** aggregated, unbranched or branched, hyaline, smooth, tapering to flat-tipped apical and lateral loci, proliferating sympodially,  $8\text{--}15 \times 2\text{--}3.5 \mu\text{m}$ . **Conidia** holoblastic, solitary, but frequently undergoing microcyclic conidiation, giving rise to one or several additional conidia, smooth, hyaline, obclavate, apex subobtuse, base long obconically subtruncate to truncate, irregularly curved, 0–3-septate,  $12\text{--}40 \times 1.5\text{--}2 \mu\text{m}$ ; hila inconspicuous.

**Holotype:** South Africa, KwaZulu-Natal, Enon, Richmond, on leaves of *E. nitens*, 3 May 2000, G. Hunter, CBS H-19694, **holotype**, culture ex-type CBS 113288 = CMW 9098.

**Ascospore germination on MEA after 24 h:** Type C. Ascospores smooth, 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 up to 3.5  $\mu\text{m}$  wide.

**Cultures:** Similar to those of *M. endophytica* (Crous 1998).

**Host:** *E. nitens*.

**Distribution:** South Africa.

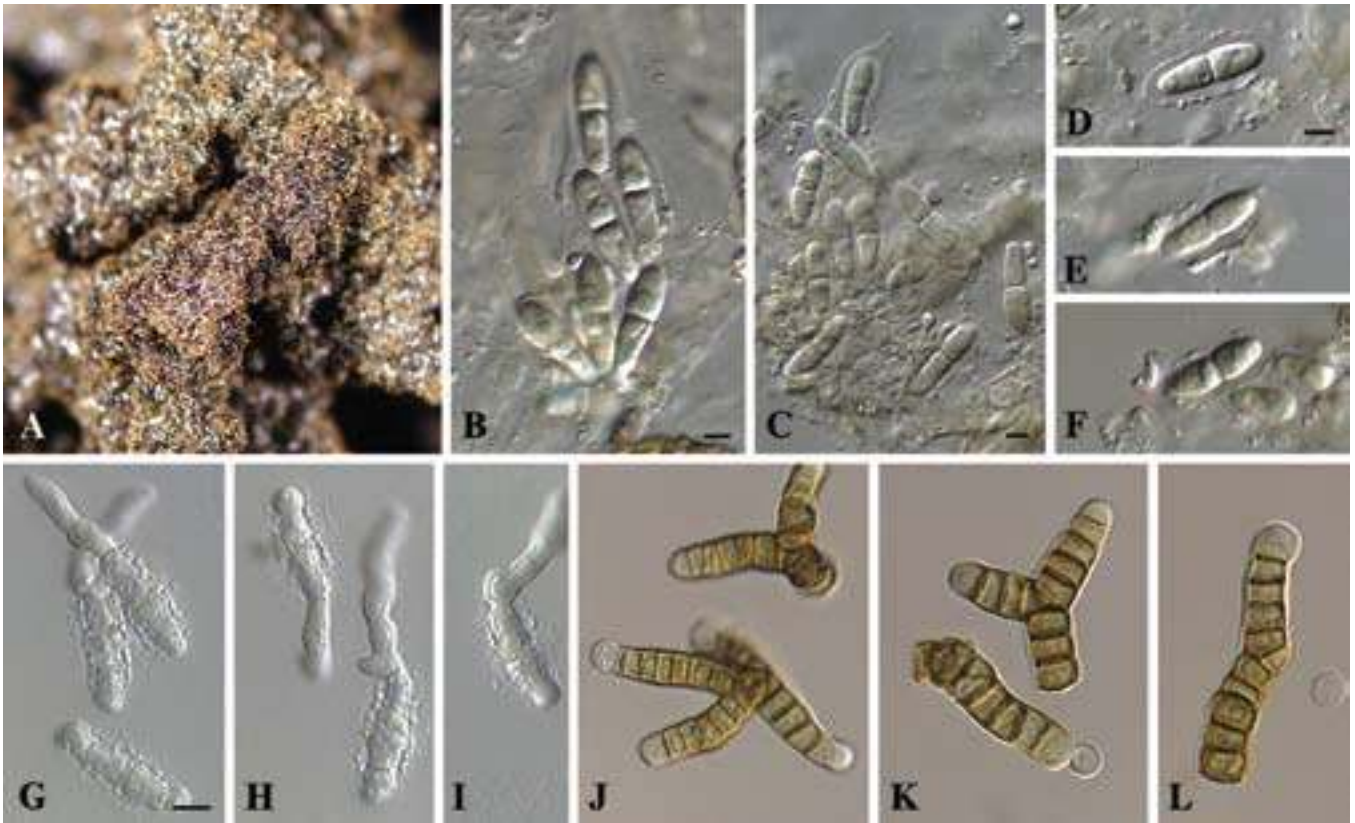
**Notes:** *Mycosphaerella pseudoendophytica* has been known to us for some time, but its formal description required a molecular comparison with ex-type strains of *M. endophytica* (which it resembles in anamorph morphology), and *M. ellipsoidea* (which it resembles in ascospore germination pattern). As can be seen here, *M. pseudoendophytica* (Fig. 4) is clearly a distinct species, sharing features of both of these taxa.

***Mycosphaerella pseudosuberosa*** Crous & M.J. Wingf., **sp. nov.** MycoBank MB500842. Fig. 18. Anamorph: ***Trimmatostroma*** sp.

**Etymology:** Morphologically similar to *M. suberosa*.

*Mycosphaerellae suberosae* similis, sed ascosporis minoribus,  $(11\text{--})12\text{--}14\text{--}(15) \times (3\text{--})3.5\text{--}(4) \mu\text{m}$ , distinguenda.

**Leaf spots** amphigenous, associated with brown, corky spots on leaf petioles. **Ascomata** pseudothecial, single



**Fig. 18.** *Mycosphaerella pseudosuberosa* (anamorph *Trimmatostroma* sp.) (CBS 118911). A. Colony on MEA. B–C. Broken asci. D–F. Ascospores (note sheath). G–I. Germinating ascospores. J–L. *Trimmatostroma* conidia produced in culture. Scale bars: B–D = 4  $\mu$ m, G = 8  $\mu$ m.

to aggregated, black, immersed becoming erumpent, globose, up to 120  $\mu$ m diam; apical ostiole 10–20  $\mu$ m diam; wall of 3–6 layers of brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, sessile, obovoid to broadly ellipsoid, straight or slightly incurved, 8-spored, 35–45  $\times$  12–16  $\mu$ m. *Ascospores* tri- to multiseriate, overlapping, hyaline, guttulate, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, medianly 1-septate, widest at the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (11–)12–14(–15)  $\times$  (3–)3.5(–4)  $\mu$ m *in vivo*; frequently surrounded by an irregular mucous sheath.

**Holotype:** Uruguay, on leaves and petioles of *Eucalyptus* sp., Apr. 2005, M.J. Wingfield, CBS H-19695, **holotype**, culture ex-type CBS 118911 = CPC 12085.

**Ascospore germination on MEA after 24 h:** Type H. Ascospores darkening and becoming verruculose on MEA, germinating from both ends, with germ tubes primarily parallel to the long axis of the spore, and distorting prominently upon germination, becoming up to 11  $\mu$ m wide.

**Cultures:** Colonies extremely slow growing, erumpent, uneven, black; aerial mycelium absent; colonies powdery, producing a *Trimmatostroma* anamorph.

**Host:** *Eucalyptus* sp.

**Distribution:** Uruguay.

**Notes:** *Mycosphaerella pseudosuberosa* is morphologically similar, and phylogenetically closely

related to *M. suberosa*. It can be distinguished by its ascospores that are slightly narrower (3–4  $\mu$ m vs. 3–6  $\mu$ m), having a mucous sheath, and germinating via two germ tubes (predominantly) that originate from the ends of the spore. Germinating spores exude mucus, and become pale brown and verruculose, which differs from the numerous germ tubes and dark brown ascospores observed in *M. suberosa*. Furthermore, cultures of *M. suberosa* are hard and resistant to being cut, while those of *M. pseudosuberosa* are powdery, producing a *Trimmatostroma* anamorph in culture. From the phylogenetic data available, it appears that there may be more species within the *M. suberosa* complex awaiting description (Fig. 1).

***Mycosphaerella quasircospora*** Crous & T. Coutinho, **sp. nov.** MycoBank MB500843. Fig. 19.

**Etymology:** Refers to the fact that this fungus is phylogenetically closely related to species of *Cercospora*.

*Mycosphaerellae* nubilosae similis, sed ascosporis brevioribus, 10–14  $\times$  3–4  $\mu$ m, distinguenda.

**Leaf spots** amphigenous, irregular to sub-circular, 2–10 mm diam, pale brown, surrounded by a thin, raised, dark brown border; spots becoming confluent with age. **Ascomata** pseudothecial, hypophyllous, single, black, immersed becoming erumpent, globose, up to 100  $\mu$ m diam; wall of 2–3 cell layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, sessile, obovoid to broadly ellipsoid, straight to slightly incurved, 8-spored, 35–50  $\times$  10–12  $\mu$ m.



Fig. 19. *Mycosphaerella quasircospora* (CBS 111161). A. Ascomata on leaf. B–C. Asci. D. Ascospores. Scale bars = 4  $\mu$ m.

Ascospores tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, obovoid with subobtuse ends, unequally 1-septate, widest close to the apex of the apical cell, not constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (10–)12–13(–14)  $\times$  (3–)3.5(–4)  $\mu$ m *in vivo*; apical cell 4–6  $\mu$ m long, basal cell 6–8  $\mu$ m long.

*Holotype*: Tanzania, on leaves of *E. maidenii*, May 1995, T. Coutinho, PREM 54971, *holotype*, culture ex-type CBS 111161 = CPC 1098.

*Ascospore germination on MEA after 24 h*: Type F. Similar to *M. nubilosa*.

*Cultures*: Colonies after 3 wk on MEA reaching 6–15 mm diam; on MEA erumpent with sparse aerial mycelium, pale olivaceous-grey; margins smooth, regular; reverse ochraceous with patches of pale olivaceous-grey; on PDA erumpent, centres white to pale olivaceous-grey, outer zone olivaceous-grey, margins irregular, feathery; reverse smoke-grey in the central part, olivaceous-grey in the outer region; colonies sterile.

*Host*: *E. maidenii*.

*Distribution*: Tanzania.

*Notes*: Ascospores of *M. quasircospora* (10–14  $\times$  3–4  $\mu$ m) germinate with a Type F germination pattern, similar to that observed in *M. nubilosa* (11–16  $\times$  3–4.5  $\mu$ m), but are somewhat shorter, and also cluster phylogenetically apart (Fig. 3). Of particular interest is the fact that it aligns with sequences of *Cercospora apii*, for which no teleomorph is known. Cultures are sterile, and a re-examination of the original specimen also failed to reveal the presence of a *Cercospora* state. This is presently the only *Mycosphaerella* teleomorph clustering with *Cercospora apii* other than *C. acaciae-mangii* (Crous et al. 2004b) (Fig. 3).

***Mycosphaerella scytalidii*** Crous & M.J. Wingf., *sp. nov.* MycoBank MB500844. Fig. 20.

*Anamorph*: *Stenella* sp.

*Synanamorph*: *Scytalidium*-like.

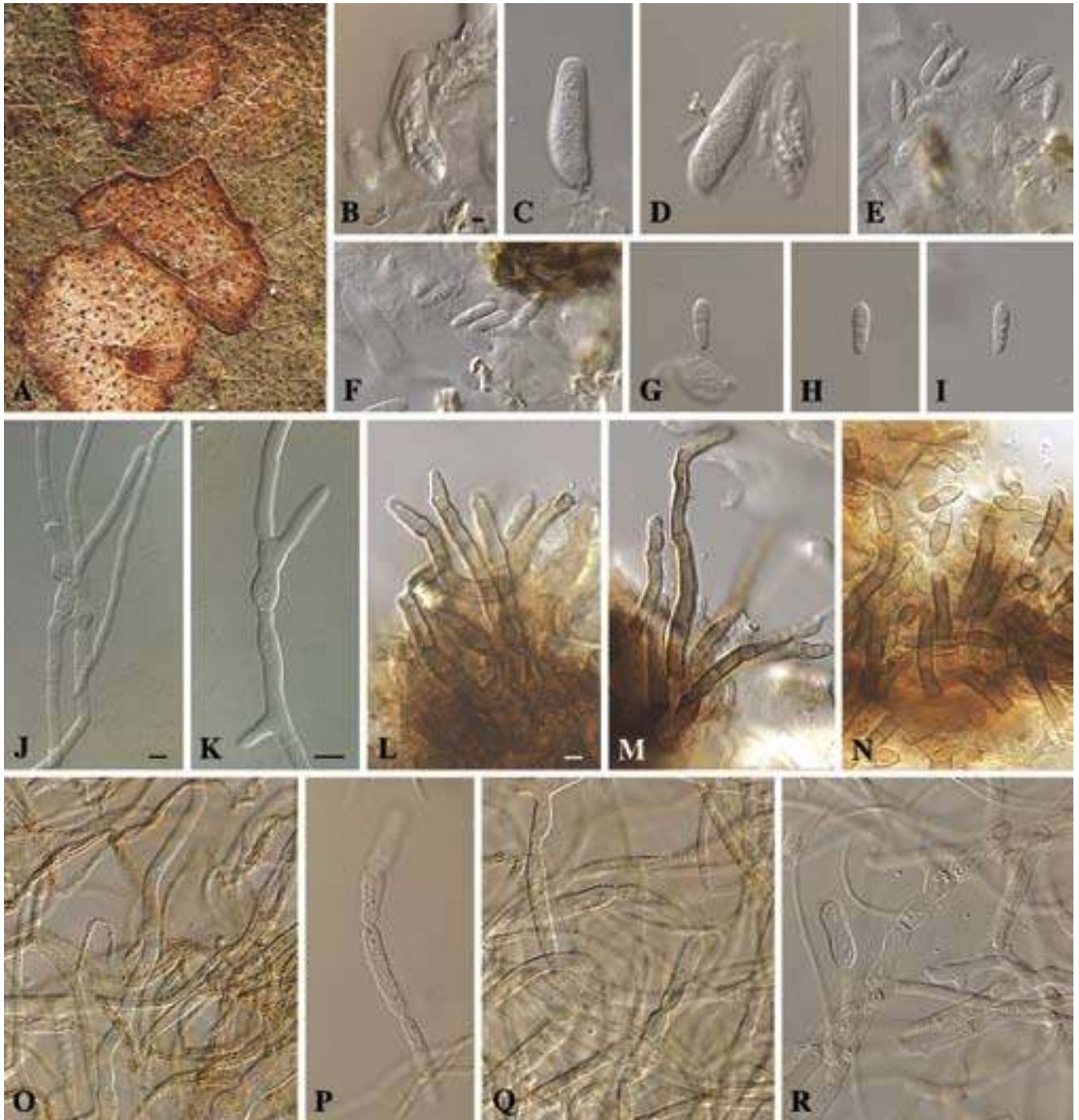
*Etymology*: Referring to the *Scytalidium*-like synanamorph.

*Mycosphaerellae parkii* similis, sed ascosporis minoribus, 8–10  $\times$  (2.5–)3  $\mu$ m, modi I germinantibus, distinguenda.

*Leaf spots* amphigenous, irregular to sub-circular, 1–8 mm diam, grey to medium brown, with a raised, dark brown border. *Ascomata* pseudothecial, amphigenous, single, black, immersed becoming erumpent, globose, up to 90  $\mu$ m diam; apical ostiole 5–10  $\mu$ m diam; wall of 2–3 layers of medium brown *textura angularis*. *Asci* aparaphysate, fasciculate, bitunicate, sessile, obovoid to ellipsoid, straight or slightly incurved, 8-spored, 25–30  $\times$  7–9  $\mu$ m. *Ascospores* tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid–ellipsoidal with subobtuse ends, medianly 1-septate, widest in the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, 8–10  $\times$  (2.5–)3  $\mu$ m *in vivo*. *Mycelium* internal and external, consisting of septate, branched, verruculose hyphae, 2–3  $\mu$ m wide. *Caespituli* fasciculate, amphigenous on the leaves, brown, up to 50  $\mu$ m wide and 60  $\mu$ m high. *Conidiophores* aggregated in loose fascicles arising from the upper cells of a brown stroma up to 50  $\mu$ m wide and 30  $\mu$ m high, or situated on the top of the ascomata; conidiophores medium brown, finely verruculose, 1–4-septate, subcylindrical, straight to geniculate–sinuous, unbranched, 20–40  $\times$  2–4  $\mu$ m. *Conidiogenous cells* terminal, unbranched, medium brown, smooth to verruculose, tapering to the flat-tipped apical loci, proliferating sympodially, 7–15  $\times$  2–3  $\mu$ m, with thickened, darkened, refractive scars. *Conidia* solitary, or in simple chains, medium brown, verruculose, subcylindrical to ellipsoidal, apex obtuse, base subtruncate, 1–2-septate, frequently constricted at the septa, 7–15  $\times$  3–3.5  $\mu$ m; hila thickened, darkened, refractive. Aerial mycelium disarticulating into hyaline, smooth arthroconidia that are *Scytalidium*-like, 12–35  $\times$  3–5  $\mu$ m.

*Holotype*: Colombia, Angela Maria, on leaves of *Eucalyptus urophylla*, Jan. 2004, M.J. Wingfield, CBS H-19696 *holotype*, culture ex-type CBS 118493 = CPC 10998.

*Ascospore germination on MEA after 24 h*: Type I. Ascospores not darkening on MEA, and germinating from both ends, with germ tubes parallel to the long axis of the spore, lateral branches present, and spore distorting upon germination, becoming up to 5  $\mu$ m wide.



**Fig. 20.** *Mycosphaerella scytalidii* (anamorph *Stenella* sp., synanamorph, *Scytalidium*-like.) (CBS 118493). A. Leaf spot. B–D. Asci. E–I. Ascospores. J–K. Germinating ascospores. L–M. Conidiophores. N. Conidia. O–R. Mycelium in culture. Scale bars: B, L = 3  $\mu$ m, J–K = 5  $\mu$ m.

**Cultures:** Colonies on MEA reaching 18–30 mm diam after 3 wk; colonies erumpent, folding, margin smooth, irregular, aerial mycelium moderate, pale olivaceous-grey; reverse iron-grey; on PDA with moderate aerial mycelium, olivaceous-grey with patches of pale olivaceous-grey; reverse olivaceous-black; on OA pale olivaceous-grey with patches of olivaceous-grey and iron-grey.

**Host:** *Eucalyptus urophylla*.

**Distribution:** Colombia.

**Notes:** Several other as yet undescribed species occur

on *Eucalyptus* leaves in Colombia, and some, such as *M. longibasalis* Crous & M.J. Wingf. (Crous 1998) (Fig. 21), is still not known from culture. Isolate CPC 10986 clusters with CPC 11002, and in culture they are distinct from CPC 11004. We were, however, unable to trace these isolates back to ascomata due to several species being present on the same leaf spots. Thus, further collections will be required before these taxa can be named.

*Mycosphaerella scytalidii* is phylogenetically closely related to the *Mycosphaerella* sp. represented by CPC 11002 and CPC 10986 (Fig. 4). For reasons explained above, however, we presently cannot name the latter

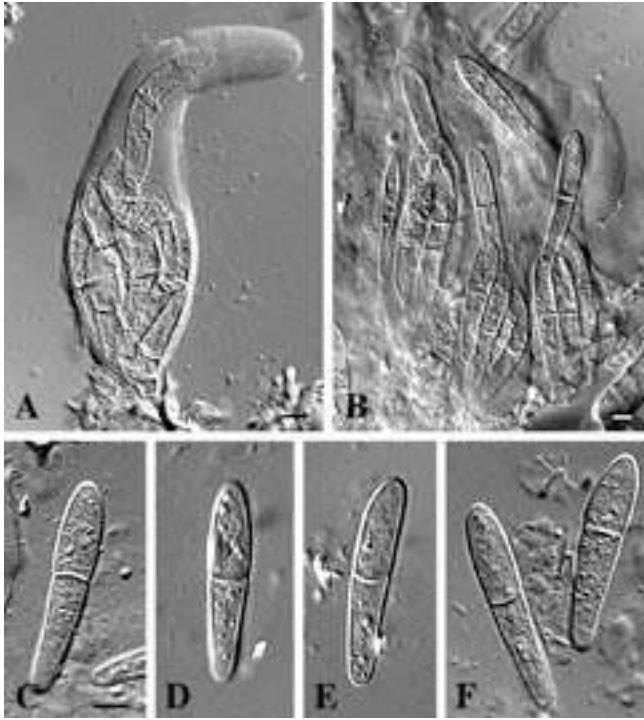


Fig. 21. *Mycosphaerella longibasalis*. A–B. Broken asci. C–F. Ascospores. Scale bars = 4  $\mu$ m.

species. *Mycosphaerella scytalidii* is also noteworthy based on the fact that it forms a *Stenella* anamorph, as well as a *Scytalidium*-like synanamorph in culture. Numerous species of *Mycosphaerella* form clusters of chlamydospores on their hyphal tips in culture (*M. bellula*, *M. jonkershoekensis*) (Crous et al. 2004a), leading to the impression that they could develop into *Trimmatostroma*-like anamorphs. None, however, have been reported to form *Scytalidium* anamorphs. Many species of *Mycosphaerella* form aerial mycelium that remain hyaline, with wide, disarticulating cells, suggesting that this anamorph morphology may be more prevalent in species of *Mycosphaerella* than previously realised. Ascospores of *M. scytalidii* germinate with a Type I pattern, but none of the species on *Eucalyptus* with this germination pattern form a *Stenella* anamorph in culture.

***Mycosphaerella secundaria*** Crous & A.C. Alfenas, sp. nov. MycoBank MB500845. Fig. 22.

**Etymology:** Referring to the ecology of this fungus as a secondary coloniser on lesions of *M. suberosa*.

*Mycosphaerellae parkii* similis, sed ascosporis minoribus, 8–10  $\times$  2.5–3  $\mu$ m, distinguenda.

Occurring as a secondary colonist on leaf spots caused by *M. suberosa*, or *M. perpendicularis*. *Ascomata* pseudothecial, amphigenous, single, inconspicuous, sparsely distributed, black, subepidermal, rarely erumpent, globose, up to 90  $\mu$ m diam. *Asci* paraphysate, fasciculate, bitunicate, subsessile, obovoid to narrowly ellipsoid, straight or slightly incurved, 8-spored, 20–30  $\times$  7–9  $\mu$ m. *Ascospores* tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, ellipsoidal with subobtuse ends, medianly 1-septate, widest close to the apex of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, 8–10  $\times$  2.5–3  $\mu$ m *in vivo*.

**Holotype:** Brazil, Bahia, Teixeira de Freitas, on leaves of *Eucalyptus* sp., 8 Jun. 2004, A.C. Alfenas, CBS H-19697, **holotype**, culture ex-type CBS 118507 = CPC 11551–11553.

**Ascospore germination on MEA after 24 h:** Type D. Similar to *M. parkii*.

**Cultures:** Colonies on MEA after 3 wk reaching 25–35 mm diam; on MEA olivaceous-grey, flat, spreading, folding, with sparse aerial mycelium and smooth, even margins; reverse iron-grey; on PDA iron-grey with olivaceous-grey aerial mycelium in central part, and drops of slime throughout; reverse iron-grey; on OA flat, spreading, olivaceous-grey.

**Host:** *Eucalyptus* spp.

**Distribution:** Brazil, Colombia.

**Notes:** When this species was initially collected in 1992 (CPC 504), it was noted that it occurred in lesions ascribed to *M. suberosa*, presumably as a secondary pathogen. We have now been able to recollect this fungus where it had colonised lesions caused by *M. suberosa*, as well as those of *Cryptosporiopsis eucalypti* Sankaran & B. Sutton on eucalypts in Brazil. In the same

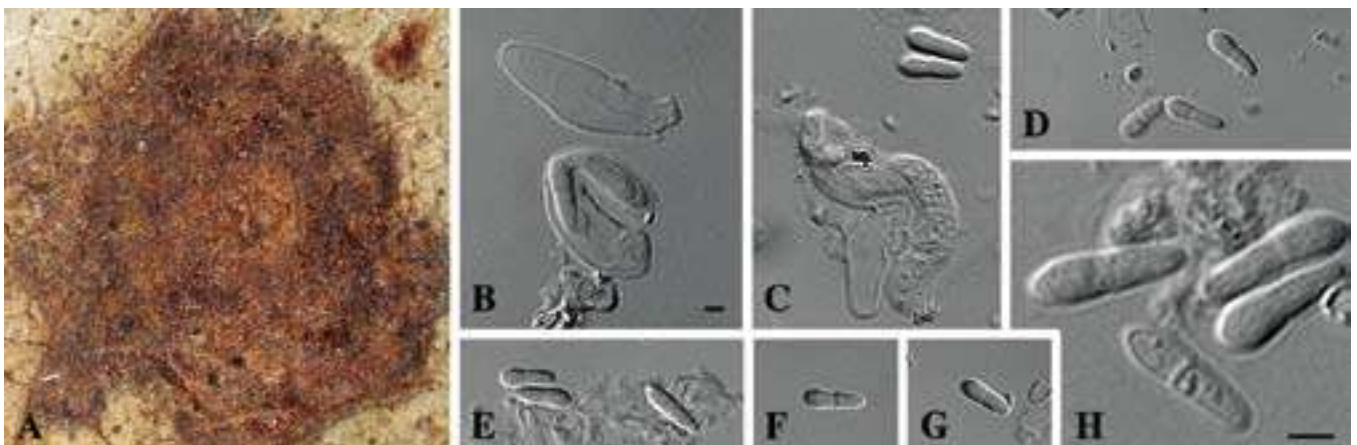


Fig. 22. *Mycosphaerella secundaria* (CBS 118507). A. Leaf spot. B–C. Broken asci. D–H. Ascospores. Scale bars: B, H = 3  $\mu$ m.





Fig. 23. *Mycosphaerella stramenti* (CBS 118909). A–B. Asci. C–F. Ascospores. G. Germinating ascospore. Scale bar = 3  $\mu$ m.

phylogenetic clade accommodating *M. secundaria* from Brazil, isolates collected in Colombia were also found which were apparently associated with lesions caused by *M. perpendicularis* (Fig. 2). *Mycosphaerella secundaria* has thus far only been collected in association with other species of *Mycosphaerella* that we believe are the primary pathogens. *Mycosphaerella perpendicularis* (ascospores 8–10  $\times$  2.5–3  $\mu$ m) was originally treated as *M. parkii* (ascospores 8–15  $\times$  2–3.5  $\mu$ m) (Crous 1998).

**Additional culture examined:** Brazil, Picadao, Conceicao da Barra, on leaves of *E. grandis*, 27 Apr. 1992, A.C. Alfenas, CBS 115608 = CPC 504.

***Mycosphaerella stramenti*** Crous & A.C. Alfenas, sp. nov. MycoBank MB500846. Fig. 23.

**Etymology:** Refers to the occurrence of this fungus on leaf litter.

*Mycosphaerellae parkii* similis, sed ascosporis minoribus, (8–)10–12(–13)  $\times$  3(–3.5)  $\mu$ m, modo I germinantibus, distinguenda.

**Leaf spots** absent, ascomata associated with leaf litter. **Ascomata** pseudothecial, amphigenous, but predominantly hypophyllous, single, black, immersed becoming erumpent, globose, up to 120  $\mu$ m diam. **Asci** paraphysate, fasciculate, bitunicate, sessile, narrowly ellipsoid to subcylindrical, straight or slightly incurved, 8-spored, 25–40  $\times$  7–8  $\mu$ m. **Ascospores** tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid–ellipsoidal with subobtuse ends, medianly 1-septate, widest in middle of apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)10–12(–13)  $\times$  3(–3.5)  $\mu$ m, *in vivo*.

**Holotype:** Brazil, Minas Gerais, Belo Oriente, on leaf litter of *Eucalyptus* sp., 24 Jan. 2004, A.C. Alfenas, CBS H-19698, **holotype**, culture ex-type CBS 118909 = CPC 11545–11547.

**Ascospore germination on MEA after 24 h:** Type I. Ascospores not darkening on MEA, and germinating from both ends, with germ tubes parallel to the long axis of the spore, and lateral branches also present; ascospore constricting at the septum, becoming up to 5  $\mu$ m wide.

**Cultures:** Colonies on MEA reaching 20–27 mm diam after 3 wk; on MEA colonies erumpent, spreading, aerial mycelium sparse, surface folding, pale olivaceous-grey, with central part having patches of smoke-grey; margin feathery, irregular, reverse greenish black; on PDA surface olivaceous-black with patches of smoke-grey aerial mycelium in central part; margins feathery, irregular, reverse greenish black; on OA olivaceous-black with smoke-grey aerial mycelium; margins irregular, feathery.

**Host:** *Eucalyptus* sp.

**Distribution:** Brazil.

**Notes:** Ascospores of *M. stramenti* germinate with a Type I pattern. Several taxa are known to have this pattern of ascospore germination (Crous 1998), from which *M. stramenti* can be distinguished by its ascospore dimensions and cultural characteristics. Phylogenetically it is closely related to *M. endophytica* (Fig. 4).

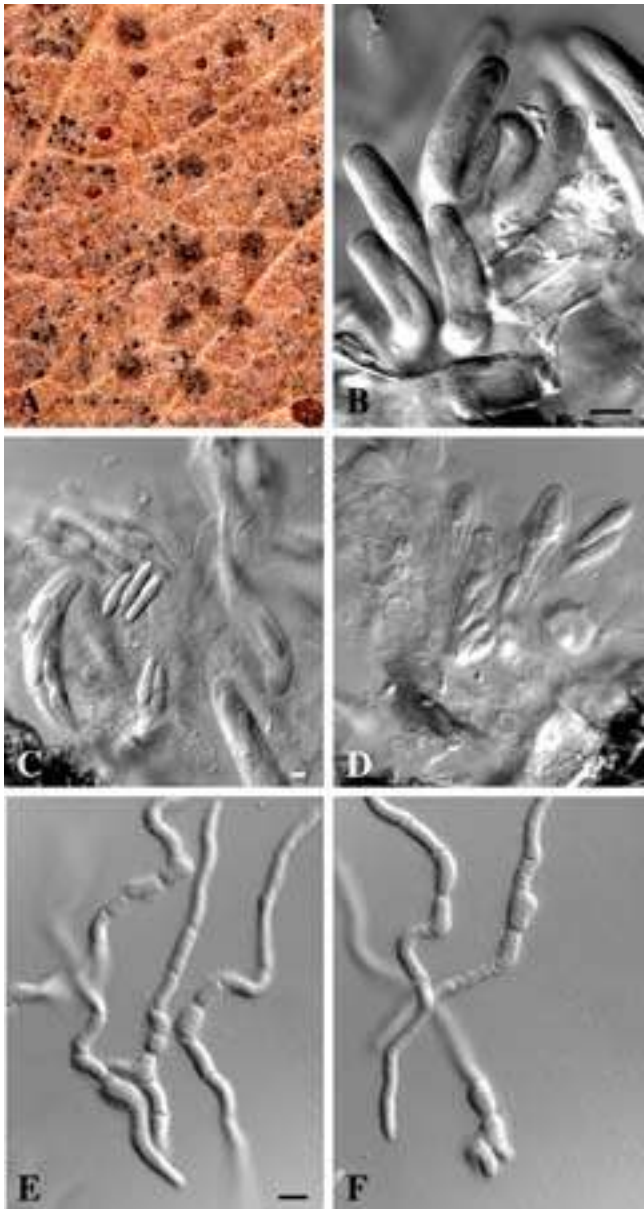
***Mycosphaerella stramenticola*** Crous & A.C. Alfenas, sp. nov. MycoBank MB500847. Fig. 24.

**Etymology:** Latin *stramentum* = leaf litter, the substrate from which this fungus was collected.

*Mycosphaerellae crystallinae* similis, sed ascosporis minoribus, 8–11  $\times$  3–3.5  $\mu$ m, distinguenda.

**Leaf spots** absent, associated with leaf litter. **Ascomata** pseudothecial, amphigenous, single, black, immersed becoming erumpent, globose, up to 90  $\mu$ m diam; apical ostiole 5–10  $\mu$ m diam; wall of 2–3 layers of medium brown *textura angularis*. **Asci** paraphysate, fasciculate, bitunicate, sessile, narrowly ellipsoid to subcylindrical, straight or slightly incurved, 8-spored, 30–35  $\times$  7–9  $\mu$ m. **Ascospores** tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid–ellipsoidal with subobtuse ends, medianly 1-septate, widest in the middle of the apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (8–)9–10(–11)  $\times$  3(–3.5)  $\mu$ m, *in vivo*.

**Holotype:** Brazil, Bahia, Eunapolis, on leaf litter of *Eucalyptus* sp., 23 May 2004, A.C. Alfenas, CBS H-19699 **holotype**, culture ex-type CBS 118506 = CPC 11438–11440.



**Fig. 24.** *Mycosphaerella stramenticola* (CBS 118506). A. Ascomata on leaf. B. Asci. C–D. Ascospores. E–F. Germinating ascospores. Scale bars: B = 8  $\mu$ m, C = 3  $\mu$ m, E = 6  $\mu$ m.

**Ascospore germination on MEA after 24 h:** Type I. 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 up to 6  $\mu$ m wide; lateral branches also present.

**Cultures:** Colonies on MEA reaching 22–38 mm diam after 3 wk; colonies flat, spreading; aerial mycelium sparse; margins smooth, regular, surface olivaceous-grey with drops of slime; reverse iron-grey; on OA pale olivaceous-grey in the centre due to moderate aerial mycelium; olivaceous-grey in the outer region; on PDA olivaceous-grey with drops of slime, margin thin, iron-grey on surface and reverse.

**Host:** *Eucalyptus* sp.

**Distribution:** Brazil.

**Notes:** *Mycosphaerella stramenticola* is phylogenetically closely related to isolates CPC 727–728 (Fig. 2), which represent an undescribed taxon from Indonesia. *Mycosphaerella stramenticola* has ascospores that germinate with a Type I pattern, thus being similar to those of *M. crystallina* (11–15  $\times$  3–4  $\mu$ m), *M. ellipsoidea* (8–11  $\times$  2–3  $\mu$ m), *M. endophytica* (8–11  $\times$  2–3  $\mu$ m), *M. lateralis* (7–16  $\times$  2–3  $\mu$ m), *M. irregulariramosa* (7–10  $\times$  1.5–2.5  $\mu$ m) and *M. tasmaniensis* (10–13  $\times$  2.5–4  $\mu$ m). Ascospores of *M. stramenticola* are 8–11  $\times$  3–3.5  $\mu$ m, and thus being wider than those of *M. ellipsoidea*, *M. endophytica*, and *M. irregulariramosa*. Furthermore, cultures of *M. stramenticola* are sterile, while all the other species listed here produce anamorphs in culture.

***Mycosphaerella sumatrensis*** Crous & M.J. Wingf., sp. nov. MycoBank MB500848. Fig. 25.

**Etymology:** Refers to Sumatra, where this fungus was collected.

*Mycosphaerellae keniensi* similis, sed ascosporis maioribus, 12–16  $\times$  3–4  $\mu$ m, distinguenda.

**Leaf spots** amphigenous, irregular to subcircular, 2–10 mm diam, pale brown with a dark brown, raised border, and thin, red-purple margin. **Ascomata** pseudothecial, amphigenous but predominantly epiphyllous, single, black, subepidermal to erumpent, globose, up to 80  $\mu$ m diam; apical ostiole 15–20  $\mu$ m diam; wall of 2–3 layers of medium brown *textura angularis*. **Asci** paraphysate, fasciculate, bitunicate, sessile, obovoid, straight or slightly incurved, 8-spored, 30–40  $\times$  9–11  $\mu$ m. **Ascospores** multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid–ellipsoidal with obtuse ends, medianly 1-septate, widest in middle of apical cell, not constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (12–)13–15(–16)  $\times$  (3–)4  $\mu$ m, *in vivo*.

**Holotype:** Indonesia, Northern Sumatra, on leaves of *Eucalyptus* sp., Feb. 2004, M.J. Wingfield, CBS H-19704, **holotype**, culture ex-type CBS 118499 = CPC 11171, CBS 118501 = CPC 11175, CBS 118502 = CPC 11178.

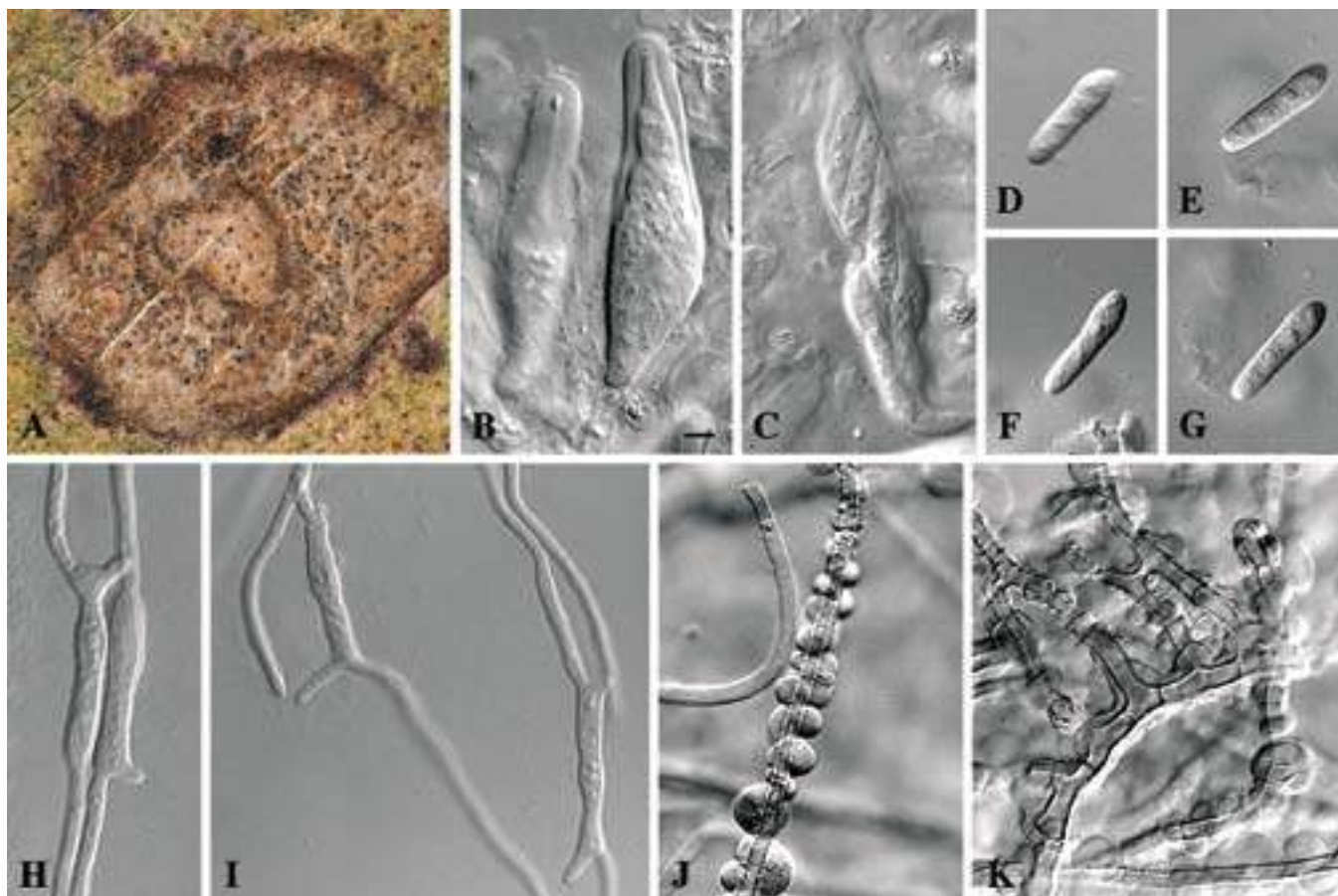
**Ascospore germination on MEA after 24 h:** Type J. Ascospores not darkening on MEA, and germinating from both ends, with germ tubes parallel to the long axis of the spore, but also with one or two lateral branches forming at the spore ends; ascospores becoming slightly constricted and up to 4  $\mu$ m wide.

**Cultures:** Colonies 8–19 mm diam on MEA after 3 wk; erumpent, with sparse aerial mycelium, smoke-grey; margin smooth, but irregular; reverse olivaceous-black; on PDA erumpent, olivaceous-grey with a thin whitish border; iron-grey in reverse; on OA smoke-grey, appearing olivaceous-black in the centre due to collapse of the aerial in copious amounts of slime.

**Host:** *Eucalyptus* sp.

**Distribution:** Indonesia.

**Notes:** *Mycosphaerella sumatrensis* is phylogenetically



**Fig. 25.** *Mycosphaerella sumatrensis* (CBS 118499). A. Leaf spot. B–C. Asci. D–G. Ascospores. H–I. Germinating ascospores. J–K. Hyphae with exudate droplets. Scale bar = 4  $\mu$ m.

distinct from other species occurring on *Eucalyptus* (Fig. 2). Ascospores ( $12\text{--}16 \times 3\text{--}4 \mu\text{m}$ ) germinate with Type J germination patterns, as do *M. colombiensis* ( $11\text{--}15 \times 3\text{--}4 \mu\text{m}$ ) and *M. keniensis* ( $7\text{--}11 \times 2.5\text{--}3 \mu\text{m}$ ). However, ascospores of *M. sumatrensis* are larger than those of *M. keniensis*, and it has no anamorph, while *M. colombiensis* occurs in close association with its *Pseudocercospora* anamorph (Crous 1998).

***Mycosphaerella verrucosiafricana*** Crous & M.J. Wingf., **sp. nov.** MycoBank MB500849. Fig. 26.

**Etymology:** Refers to *M. africana*, to which it is morphologically similar.

*Mycosphaerellae africanae* similis, sed ascosporis latoribus, verruculosus,  $(7\text{--}8\text{--}9\text{--}10) \times 3\text{--}3.5 \mu\text{m}$ , distinguenda.

**Leaf spots** amphigenous, irregular to sub-circular, 5–15 mm diam, pale brown to grey, surrounded by a raised, dark brown border, and a thin, red-purple margin. **Ascomata** pseudothecial, amphigenous but chiefly hypophyllous, single, black, immersed becoming erumpent, globose, up to 60  $\mu\text{m}$  diam; apical ostiole 10–15  $\mu\text{m}$  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,  $18\text{--}27 \times 7\text{--}8 \mu\text{m}$ . **Ascospores** tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, ellipsoid with obtuse ends, medianly 1-septate, widest in the middle of the

apical cell, constricted at the septum, tapering towards both ends, but more prominently towards the lower end,  $(7\text{--}8\text{--}9\text{--}10) \times 3\text{--}3.5 \mu\text{m}$  *in vivo*.

**Holotype:** Indonesia, Northern Sumatra, on leaves of *Eucalyptus* sp., Feb. 2004, M.J. Wingfield, CBS H-19705 **holotype**, culture ex-type CBS 118496 = CPC 11167, CBS 118497 = CPC 11169, CBS 118498 = CPC 11170).

**Ascospore germination on MEA after 24 h:** Type E. Ascospores becoming dark brown and verruculose on MEA, and germinating from both ends, with germ tubes irregular to the long axis of the spore; frequently with more than two germ tubes, and distorting prominently upon germination, becoming up to 9  $\mu\text{m}$  diam.

**Cultures:** Colonies on MEA 12–22 mm diam after 3 wk; erumpent, spreading, with smooth, uneven margins; upper surface cracking open; aerial mycelium sparse to absent; colonies sectoring, olivaceous-grey; margin thin, iron-grey; reverse greenish-black; on PDA with moderate aerial mycelium, and spots of slime appearing spread over the iron-grey surface; reverse greenish black; on OA colonies submerged; aerial mycelium almost completely absent, greenish black; forming chains of dark brown, thick-walled chlamydospores that aggregate into small microsclerotia (on all media); colonies sterile.

**Host:** *Eucalyptus* sp.

**Distribution:** Indonesia.

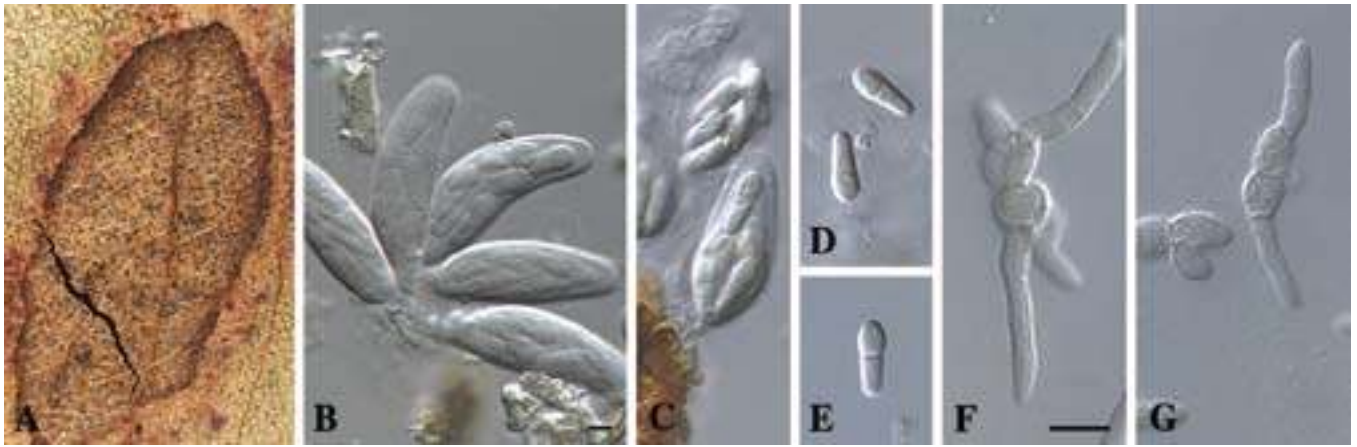


Fig. 26. *Mycosphaerella verrucosiafricana* (CBS 118496). A. Leaf spot. B–C. Asci. D–E. Ascospores. F–G. Germinating ascospores. Scale bars: B = 3  $\mu$ m, F = 8  $\mu$ m.

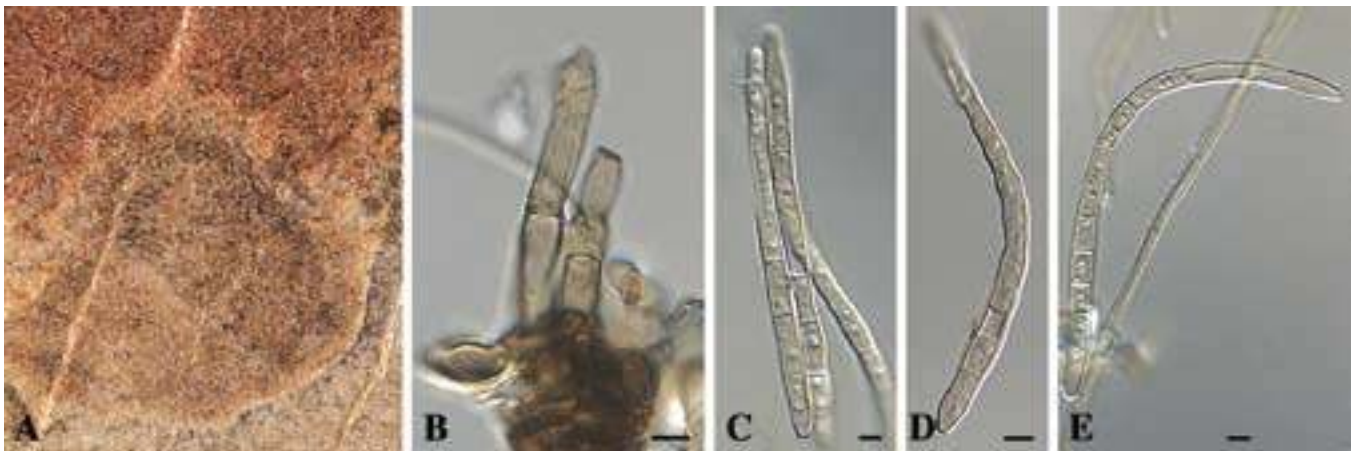


Fig. 27. *Pseudocercospora subulata* (CBS 118489). A. Leaf spot. B. Conidiophores. C–E. Conidia. Scale bars = 5  $\mu$ m.

**Notes:** *Mycosphaerella verrucosiafricana* is distinguished from other taxa currently known from *Eucalyptus* in that it has a characteristic ascospore germination pattern. Germinating ascospores turn brown and verruculose, but germinate with more than two germ tubes, which grow irregular to the long axis of the spore (Type G, becoming type E with age). Young ascospores just beginning to germinate can be confused with those of *M. africana*, as they initially also have only two germ tubes, though the ascospores are more distinctly verruculose than those of *M. africana*. Within a few hours of germination, additional germ tubes appear, and the pattern is more similar to that of Type E, which is seen in *M. suberosa*. *Mycosphaerella verrucosiafricana* is distinguished from *M. suberosa* in that the germ tubes remain hyaline, and ascospores and leaf spots are quite distinct from those of *M. suberosa*.

***Pseudocercospora subulata*** Z.Q. Yuan, de Little & Mohammed, Nova Hedwigia 71: 416. 2000. Fig. 27.

= *Pseudocercospora pseudobasitruncata* U. Braun & M. Dick, New Zealand J. For. Res. 32: 228. 2002.

**Specimen examined:** New Zealand, North Island, KeriKeri, on leaves of *E. botryoides*, 17 Oct. 2003, M.A. Dick, CBS 118489 = CPC 10849.

**Cultures:** Colonies reaching 25–35 mm diam after

3 wk on MEA; pale olivaceous-grey, erumpent, with moderate to extensive aerial mycelium; margin regular, smooth, reverse iron-grey; on PDA pale olivaceous-grey, margin thin, olivaceous-grey, reverse iron-grey; on OA central part erumpent, pale olivaceous-grey, outer zone olivaceous-grey, flat and spreading.

**Host:** *E. botryoides*.

**Distribution:** New Zealand.

**Notes:** *Pseudocercospora subulata* is morphologically similar to *P. pseudobasitruncata*, and hence they are listed here as synonyms. The culture used in this study was obtained from lesions colonised by both *P. crousii* U. Braun & M. Dick and *P. subulata*. Although the culture was obtained from a single germinating conidium, it is sterile, and we were unable to rule out the possibility that it may represent *P. crousii* and not *P. subulata*. Further collections and cultures are required to undertake DNA sequence comparisons with the *Pseudocercospora* Speg. species recently described from eucalypts by Braun & Dick (2002).

***Septoria eucalyptorum*** Crous, **sp. nov.** MycoBank MB500850. Figs 28–29.

**Etymology:** Refers to its host, *Eucalyptus*.

Septoriae linicolae similis, sed conidiis brevioribus, 8–22 × 2–2.5 µm, distinguenda.

**Leaf spots** absent, conidiomata associated with leaf litter. **Mycelium** internal, consisting of smooth, branched, septate, pale brown, 2–2.5 µm wide hyphae. **Conidiomata** pycnidial, immersed, brown, globose on leaves, up to 160 µm diam; wall consisting of 3–6 cell layers of *textura angularis*. **Conidiophores** lining the inner layer of the conidioma, dense aggregated, subcylindrical, straight to curved, 0–1-septate, mostly reduced to conidiogenous cells. **Conidiogenous cells** terminal, unbranched, hyaline, smooth, subcylindrical, proliferating sympodially near the apex, 5–10 × 2–2.5 µm. **Conidia** solitary *in vivo*, but undergoing microcyclic conidiation *in vitro*, finely guttulate, subcylindrical to narrowly obclavate, with obtuse to subobtuse apex, and long subtruncate base, straight to curved, 1(–3)-septate, (8–)12–16(–22) × 2(–2.5) µm; hila inconspicuous, 0.5–1 µm diam.

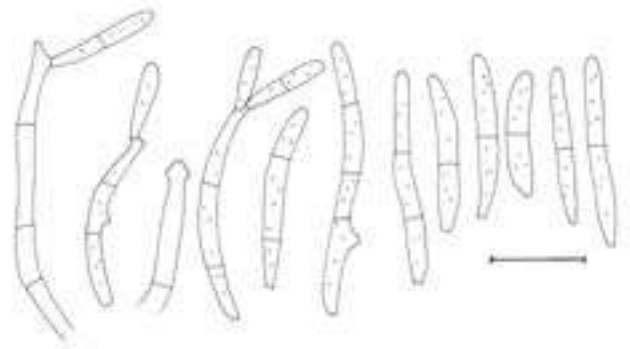
**Holotype:** India, Palampur, on *Eucalyptus* leaf litter, Feb. 2004, W. Gams & M. Arzanlou, CBS H-19700, **holotype**, cultures ex-type CBS 118505 = CPC 11282, CPC 11283.

**Cultures:** Colonies after 3 wk on MEA 30–40 mm diam; on MEA pale white to smoke-grey; aerial mycelium sparse; colonies spreading, margins even, smooth; reverse fuscous-black with patches of vinaceous-grey; on PDA producing large amounts of slime, with thread-like tufts of aerial mycelium; surface pale purplish grey (centre) with a zone of vinaceous-grey, and a pale vinaceous-grey, flat, spreading marginal region; reverse vinaceous-grey with patches of pale vinaceous-grey; on OA pale vinaceous-grey (centre) with a zone of purplish grey, a wide, flat margin concolorous with the medium; conidiomata frequently formed along circadian growth lines.

**Host:** *Eucalyptus* sp.

**Distribution:** India.

**Notes:** Sankaran *et al.* (1995) listed several species of *Septoria* on *Eucalyptus*, most of which have been redisposed to other genera. The exceptions are *S. eucalypti* G. winter & Roum. (conidia filiform–acicular,



**Fig. 28.** Conidiophores and conidia of *Septoria eucalyptorum* (CBS 118505). Scale bar = 10 µm.

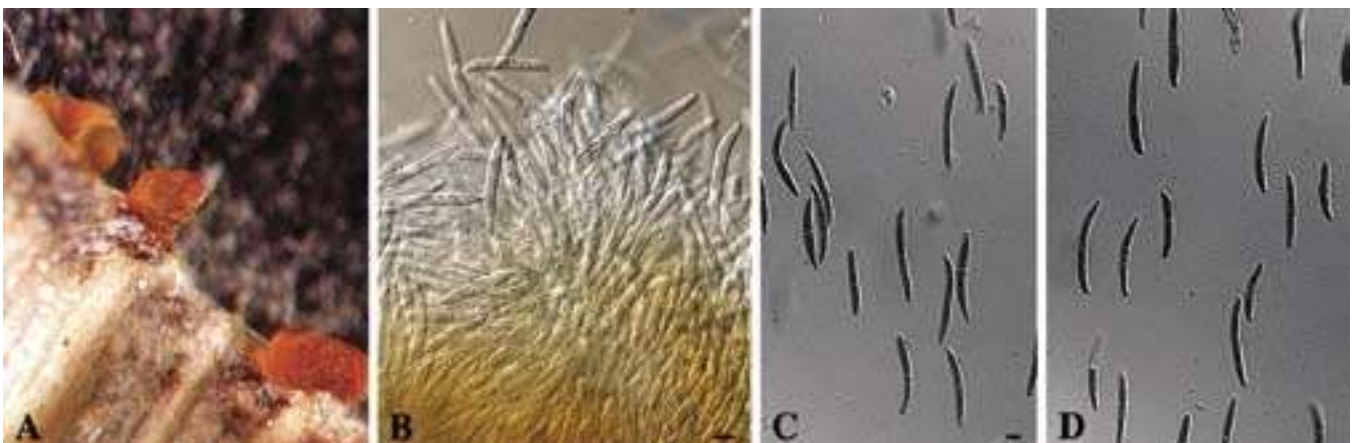
1-septate, 14–18 × 1.5 µm) and *S. mortolensis* Penz. & Sacc. (conidia 0–2-septate, 50–55 × 3–3.5 µm). Gadgil & Dick (1999) recently described *S. typica* Gadgil & M. Dick, which is characterised by having filiform, sigmoid or falcate, 1-septate conidia, 65–70 × 2–3 µm. *Septoria eucalyptorum* is distinct from this species in having conidia that are subcylindrical to narrowly obclavate, 1(–3)-septate, 8–22 × 2–2.5 µm. DNA sequence data in the present study (Fig. 3), show that *Septoria eucalyptorum* is closely allied to *S. linicola* (on *Linum*, conidia filiform, 1–3-septate, 17–40 × 1.5–3 µm) and *S. protearum* (on *Protea*, conidia subcylindrical to narrowly obclavate, (0–)1–3(–4)-septate, 6–30 × 1.5–2 µm). To fully resolve this relationship, however, other loci will need to be sequenced, as the ITS domain is insufficient to distinguish species complexes in *Septoria*.

***Septoria provencialis* Crous, sp. nov.** MycoBank MB500851. Fig. 30.

**Etymology:** Refers to the Provence in France where the fungus was collected.

Septoriae mortolensi similis, sed conidiis brevioribus, 12–45 × 2.5–4 µm, distinguenda.

**Leaf spots** amphigenous, dark brown, angular, confined by leaf veins, 1–6 mm diam, becoming confluent with age. **Mycelium** internal, consisting of smooth, branched, septate, hyaline, 3–4 µm wide hyphae. **Conidiomata**



**Fig. 29.** *Septoria eucalyptorum* (CBS 118505). A. Conidiomata forming on CLA, with conidial masses. B. Conidial mass. C–D. Conidia. Scale bars = 4 µm.

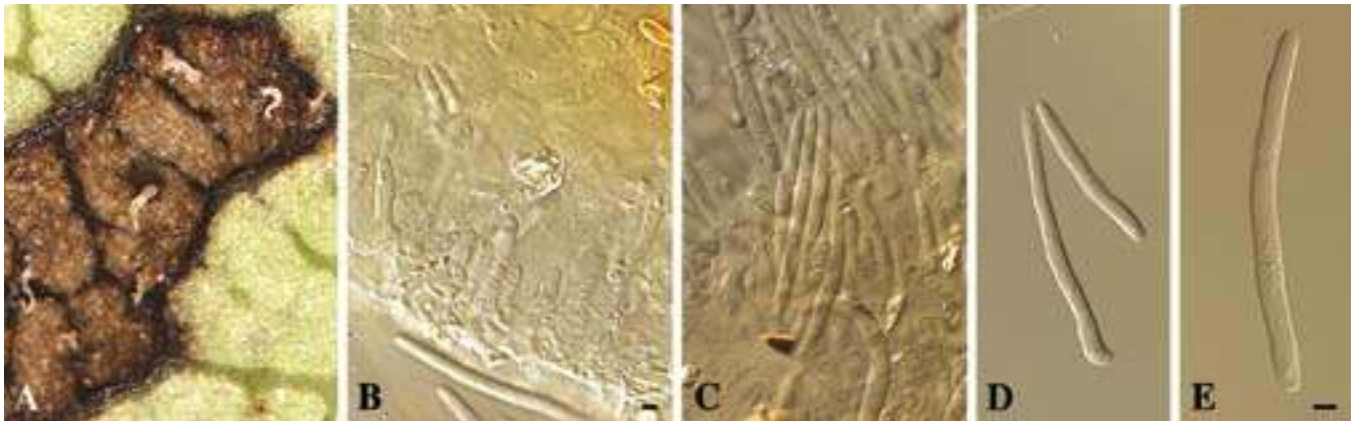


Fig. 30. *Septoria provencialis* (CBS 118910). A. Leaf spot. B–C. Conidiogenous cells. D–E. Conidia. Scale bars = 3  $\mu$ m.

amphigenous on leaves, pycnidial, immersed, brown, globose, up to 200  $\mu$ m diam; wall consisting of 2–4 cell layers of *textura angularis*. *Conidiophores* lining the inner surface of the conidioma, densely aggregated, subcylindrical to ampulliform, straight to slightly curved, 0–2-septate, 6–25  $\times$  3–5  $\mu$ m. *Conidiogenous cells* terminal, unbranched, hyaline, smooth, subcylindrical to ampulliform, proliferating sympodially or several times percurrently near the apex, 6–10  $\times$  3–5  $\mu$ m. *Conidia* solitary *in vivo*, finely guttulate, subcylindrical to narrowly obclavate, with subobtuse apex, and obconically subtruncate base, variously curved to irregular, mostly widest in the middle of the basal cell, tapering towards the apex, (1–)2(–3)-septate, (12–)30–40(–45)  $\times$  2.5–3(–4)  $\mu$ m.

*Holotype*: France, Provence, Cheval Blanc camping site, on juvenile *Eucalyptus* leaves, 29 Jul. 2005, P.W. Crous, CBS H-19701, *holotype*, cultures ex-type CBS 118910 = CPC 12226, CPC 12227–12228.

*Cultures*: Colonies 10–15 mm diam after 3 wk on MEA; colonies erumpent, surface irregular, catenate, olivaceous-grey with cream to pale rosy-buff spore masses; aerial mycelium absent; margins smooth, regular, with a thin outer zone that is pale olivaceous-grey to slightly rosy-buff; colonies olivaceous-black in reverse.

*Host*: *Eucalyptus* sp.

*Distribution*: France.

*Note*: Conidia of *S. provencialis* (12–45  $\times$  2.5–4  $\mu$ m) are most similar to *S. mortolensis* (50–55  $\times$  3–3.5  $\mu$ m), although on average, they are much shorter.

***Stenella pseudoparkii*** Crous & M.J. Wingf., *sp. nov.*  
Mycobank MB500852. Fig. 31.

*Teleomorph*: *Mycosphaerella* sp.

*Etymology*: Morphologically similar to *M. parkii* and its anamorph, *S. parkii*.

*Stenellae parkii* similis, sed conidiis brevioribus, 20–50  $\times$  2.5–3  $\mu$ m, distinguenda.

*Leaf spots* amphigenous, irregular to subcircular, 3–7 mm diam, pale brown, with a raised border. *Conidiophores* arising singly from superficial mycelium,

brown, smooth to finely verruculose, 1–4-septate, subcylindrical, straight to variously curved, unbranched, 15–60  $\times$  3–4  $\mu$ m. *Conidiogenous cells* terminal, unbranched, medium brown, smooth, tapering to flat-tipped apical loci that are darkened and refractive,

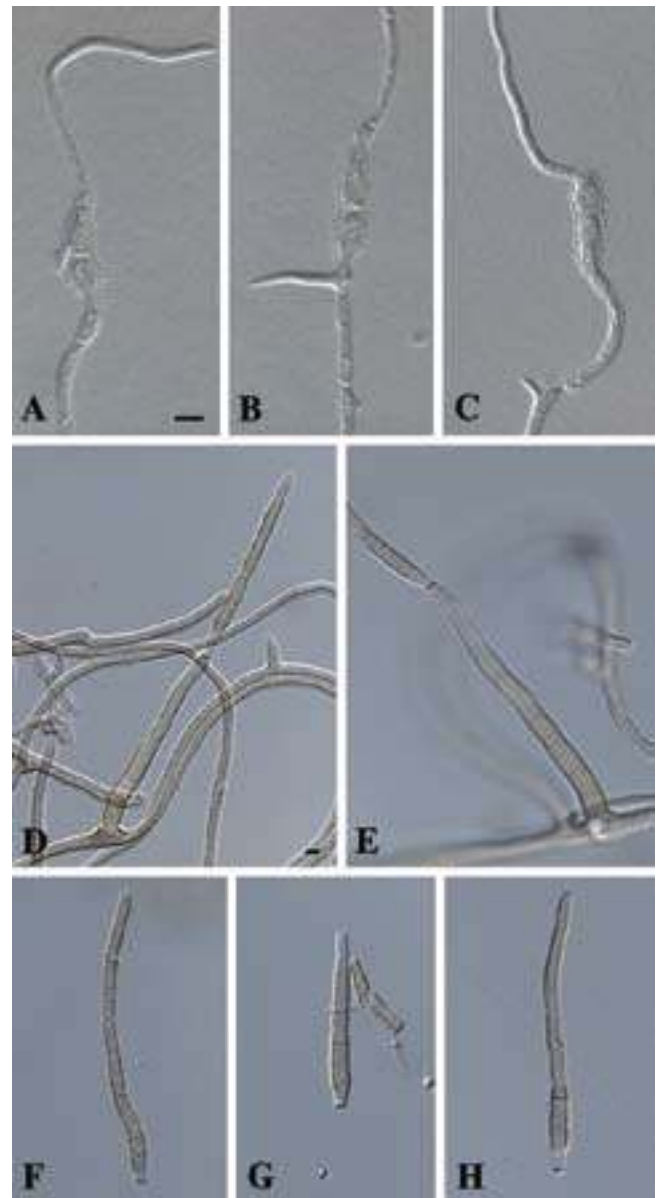


Fig. 31. *Stenella pseudoparkii* (teleomorph *Mycosphaerella* sp.) (CBS 19702). A–C. Germinating ascospores. D–E. Conidiophores. F–H. Conidia. Scale bars: A = 5  $\mu$ m, D = 3  $\mu$ m.

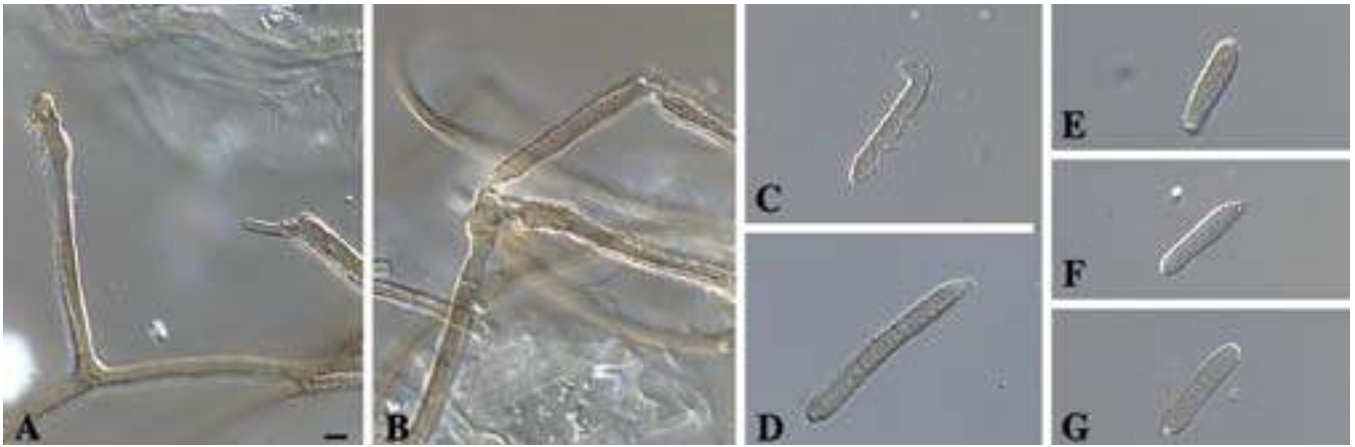


Fig. 32. *Stenella xenoparkii* (teleomorph *Mycosphaerella* sp.) (CBS 111185). A–B. Conidiophores. C–G. Conidia. Scale bar = 4  $\mu$ m.

proliferating sympodially, 15–25  $\times$  2–3  $\mu$ m. *Conidia* solitary to catenulate in simple chains, medium brown, verruculose, cylindrical or narrowly obclavate, with subobtuse apex, and long obconically subtruncate base, straight to curved, 1–5-septate, 20–50  $\times$  2.5–3  $\mu$ m; hila thickened, darkened and refractive.

*Holotype*: Colombia, on leaves of *Eucalyptus* sp., 1995, M.J. Wingfield, CBS H-19702 *holotype*, culture ex-type CBS 110999 = CPC 1087; 1088–1092.

*Ascospore germination on MEA after 24 h*: Type D. Ascospores smooth, not darkening on MEA, germinating from both ends, with germ tubes parallel to the long axis of the spore, and some lateral branches; ascospores distorting, becoming up to 5  $\mu$ m wide.

*Cultures*: Colonies after 3 wk on MEA 23–30 mm diam, pale olivaceous-grey, spreading, with moderate aerial mycelium, and smooth, irregular margins; colonies folding, erumpent; reverse olivaceous-black; on PDA pale olivaceous-grey with moderate aerial mycelium and copious amounts of slime; margins submerged in the agar; reverse olivaceous-grey; on OA pale olivaceous-grey, colonies folding with moderate aerial mycelium, and a thin olivaceous-grey margin.

*Host*: *Eucalyptus* sp.

*Distribution*: Colombia.

*Notes*: Several species of *Mycosphaerella* were present on the lesions from which *S. pseudoparkii* was isolated, and it was not possible to trace the ascospores back to the specific ascomata. The description of the *Mycosphaerella* teleomorph thus has to await further collections. The ascospores that shot out onto MEA germinated with a Type D pattern, which together with its *Stenella* anamorph, resulted in it being identified as *M. parkii* (Crous 1998). Phylogenetically, *S. pseudoparkii* is distinct from *M. parkii*, and most closely related to *M. scytalidii*, which has a Type I germination pattern. *Stenella pseudoparkii* has shorter conidia (20–50  $\times$  2.5–3  $\mu$ m) than *Stenella parkii* (25–200  $\times$  2–2.5  $\mu$ m) (Crous & Alfenas 1995, Crous 1998).

***Stenella xenoparkii*** Crous & M.J. Wingf., **sp. nov.**  
Mycobank MB500853. Fig. 32.

*Teleomorph*: ***Mycosphaerella*** sp.

*Etymology*: refers to the morphological similarity with *M. parkii* and its anamorph, *S. parkii*.

*Stenellae parkii* similis, sed conidiis brevioribus, 12–50  $\times$  3–5  $\mu$ m, distinguenda.

*Leaf spots* amphigenous, irregular to subcircular, 2–10 mm diam, pale brown, with a raised border and thin, red-purple margin. *Conidiophores* arising singly from superficial mycelium, medium brown, finely verruculose, 1–2-septate, subcylindrical, straight to variously curved, unbranched, 30–60  $\times$  3–4  $\mu$ m. *Conidiogenous cells* terminal, unbranched, medium brown, verruculose, tapering to flat-tipped apical loci that are darkened and refractive, proliferating sympodially, 10–25  $\times$  3–4  $\mu$ m. *Conidia* catenulate in branched chains, medium brown, verruculose, cylindrical or narrowly obclavate, with subobtuse apex, and subtruncate base, straight to curved, 0–2-septate, 12–50  $\times$  3–5  $\mu$ m; hila thickened, darkened and refractive.

*Holotype*: Indonesia, on leaves of *E. grandis*, Mar. 1996, M.J. Wingfield, *holotype* PREM 54968, *isotype* in CBS H-19703, cultures ex-type CBS 111185 = CPC 1300; 1299–1301.

*Ascospore germination on MEA after 24 h*: Type D. Similar to *M. parkii*.

*Cultures*: Colonies after 3 wk on MEA 25–35 mm diam; on MEA spreading, slightly erumpent, margins smooth but irregular; aerial mycelium sparse to moderate; surface olivaceous-black, but central part grey due to aerial mycelium; reverse olivaceous-black; on PDA olivaceous-black with mucous droplets and aerial mycelium that is olivaceous-grey in the central part, but has a reddish tinge in the outer region; reverse greenish black; on OA iron-grey with sparse to moderate olivaceous-grey aerial mycelium.

*Host*: *Eucalyptus grandis*.

*Distribution*: Indonesia.

*Notes*: The specimen on which this species is based was originally identified as representing *M. parkii*. The original identification was based on its characteristic leaf spots, ascospore germination patterns and

dimensions, and the presence of a *Stenella* anamorph. Teleomorph material was not retained, and hence only the anamorph, which forms in culture, can be named. Conidia of *S. xenoparkii* (12–50 × 3–5 µm) are shorter and wider than those of *S. pseudoparkii* (20–50 × 2.5–3 µm) and *S. parkii* (25–200 × 2–2.5 µm) (Crous & Alfenas 1995, Crous 1998).

## DISCUSSION

In this study we have described 21 new species of *Mycosphaerella* or its anamorphs from *Eucalyptus* leaves. Some of these new species arise from a re-examination of specimens and cultures treated previously (Crous 1998). The species in this earlier study had been described primarily on the basis of morphology and without the support of DNA sequence comparisons.

Results of this study are similar to those of Crous *et al.* (2004b) showing that there are several species of *Mycosphaerella* on eucalypts that have distinct cultural characteristics and can be separated based on phylogenetic analyses, but that share the same symptoms, morphological characteristics and ascospore germination patterns. It is clearly very difficult to accurately identify *Mycosphaerella* species on eucalypts in the absence of DNA sequence analyses. Identifications of species described based solely on morphological characteristics must consequently be viewed with some circumspection.

A good example of the confusion arising from identifications based solely on phenotypic characters is found in the case of *M. parkii*. In the present study, we reconsidered several collections originally identified as *M. parkii* based on symptoms, ascospore dimensions, germination patterns, and the presence of a *Stenella* anamorph in culture. The “*M. parkii*”-like isolates were consequently shown to represent several species. Because of insufficient material being available, only two anamorph species *S. xenoparkii* and *S. pseudoparkii*, could be named.

Cryptic species were also found among isolates originally identified as *M. africana*. These identifications were based on the presence of fusoid–ellipsoidal ascospores that are constricted at the septum, that darken upon germination, and that produce colonies that are relatively slow-growing. These isolates are described here as *M. verrucosiafricana* and *M. pseudoafricana*. Other examples of cryptic species were found in the case of *M. pseudoendophytica*, which is morphologically similar to *M. endophytica*, *M. pseudosuberosa*, which is similar to *M. suberosa*, and *M. pseudocryptica*, which is similar to *M. cryptica*.

In this study we have applied only DNA sequences of the ITS region. Although this locus has been very useful in delimiting species of *Mycosphaerella* from *Eucalyptus*, it is not always sufficient to derive conclusions for all species complexes (Crous *et al.* 2004c, Hunter *et al.* 2006 – this volume). For example, it is not suitable for distinguishing species in anamorph

genera such as *Cercospora* and *Septoria*. In contrast, sequences of the ITS region appear to be useful for distinguishing species with *Pseudocercospora*, *Ramularia* and most other *Mycosphaerella* anamorph genera that we have considered (Crous & Groenewald, unpubl. data). It appears, therefore, that the ITS region has evolved at different rates in different anamorph genera associated with *Mycosphaerella*, and that it is more conserved in *Cercospora* and *Septoria*, two genera that always cluster together.

In this study, we have described several new *Mycosphaerella* species from leaf litter. This suggests that there are numerous endophytic *Mycosphaerella* species that sporulate once leaves have died. The biology of *Mycosphaerella* species suggests that these fungi are probably not saprobes but rather that they infect living leaf tissue and only sporulate after leaf fall. Virtually nothing is known of this life-habit of *Mycosphaerella* species, and it would be intriguing to follow the infection patterns of species that are not primary pathogens.

*Mycosphaerella secundaria* was one of the more intriguing fungi arising from this study. This fungus has been collected on several occasions, but its unique nature was not confirmed previously. *Mycosphaerella secundaria* is always found on leaf spots caused by *M. suberosa*. This is an unusual habit for a species of *Mycosphaerella*, and its ecological role deserves further study.

*Dissoconium dekkeri* (teleomorph: *M. lateralis*) was originally described as a potential hyperparasite of powdery mildew (De Hoog *et al.* 1983, 1991), and has since been isolated from many different hosts (Crous *et al.* 2004b). Jackson *et al.* (2004) showed that *M. lateralis* is not a hyperparasite of *M. nubilosa* and *M. cryptica*, the two species with which *M. lateralis* frequently co-occurs. Jackson *et al.* (2004) also showed that *D. dekkeri* can infect *Eucalyptus* leaves. *Mycosphaerella lateralis* and *M. communis* occur on leaves of numerous *Eucalyptus* spp., and they are frequently found on leaf spots caused by other *Mycosphaerella* species, as well as unrelated fungi (Crous unpubl. data). The ecological role of *M. lateralis*, however, remains to be determined.

The linking of yet another anamorph genus to *Mycosphaerella*, namely *Davisoniella*, draws an interesting parallel to morphologically similar coelomycete genera. Of particular interest, are the taxa currently accommodated in *Colletogloeopsis*, particularly those that are *Coniothyrium*-like and to which *D. eucalypti* is definitely closely related if not congeneric. Presently no cultures are available, the relationship between these taxa remains to be proven, and hence the anamorph is best retained in *Davisoniella*.

In this study, we have added 21 species to the number of *Mycosphaerella* spp. presently known to occur on *Eucalyptus* leaves and stems. We suggested that there could easily be at least as many *Mycosphaerella* spp. on eucalypts as there are species of that genus. This would imply that only 14 % of the species of *Mycosphaerella* from eucalypts



have presently been described. This means that significant challenges face the taxonomists who wish to distinguish *Mycosphaerella* spp. from eucalypts in future. Most likely, in future studies, DNA sequence comparisons based on multiple genes will be required to accurately identify these fungi. Given the enormity of this task, focus will clearly need to be directed to those species that are primary pathogens. However, the primary pathogens are so easily confused with other less important species, that all material will ultimately have to be thoroughly studied and understood.

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