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Phylogenetic lineages in the *Capnodiales*

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Abstract: The *Capnodiales* incorporates plant and human pathogens, endophytes, saprobes and epiphytes, with a wide range of nutritional modes. Several species are lichenised, or occur as parasites on fungi, or animals. The aim of the present study was to use DNA sequence data of the nuclear ribosomal small and large subunit RNA genes to test the monophyly of the *Capnodiales*, and resolve families within the order. We designed primers to allow the amplification and sequencing of almost the complete nuclear ribosomal small and large subunit RNA genes. Other than the *Capnodiaceae* (sooty moulds), and the *Davidiellaceae*, which contains saprobes and plant pathogens, the order presently incorporates families of major plant pathological importance such as the *Mycosphaerellaceae*, *Teratosphaeriaceae* and *Schizothyriaceae*. The *Piedraiaceae* was not supported, but resolves in the *Teratosphaeriaceae*. The *Dissoconiaceae* is introduced as a new family to accommodate *Dissoconium* and *Ramichloridium*. Lichenisation, as well as the ability to be saprobic or plant pathogenic evolved more than once in several families, though the taxa in the upper clades of the tree lead us to conclude that the strictly plant pathogenic, nectrotrophic families evolved from saprobic ancestors (*Capnodiaceae*), which is the more primitive state.

Key words: *Ascomycetes*, *Brunneosphaerella*, *Capnodiales*, DNA sequence comparisons, *Mycosphaerella*, novel primers, systematics.
Taxonomic novelties: *Brunneosphaerella* Crous, gen. nov., *B. jonkershoekensis* (Marinc., M.J. Wingf. & Crous) Crous, comb. nov., *B. protearum* (Syd. & P. Syd.) Crous, comb. nov., *Devriesia hilliana* Crous & U. Braun, sp. nov., *D. lagerstroemiae* Crous & M.J. Wingf., sp. nov., *D. strelitziicola* Arzanlou & Crous, sp. nov., *Dissoconiaceae* Crous & de Hoog, fam. nov., *Hortaea thailandica* Crous & K.D. Hyde, sp. nov., *Passalora ageratinae* Crous & A.R. Wood, sp. nov., *P. armatae* Crous & A.R. Wood, sp. nov., *Rachicladosporium cboliae* Crous, sp. nov.

INTRODUCTION

The *Dothideomycetes* encompasses plant and human pathogens, endophytes, saprobes and epiphytes. The class presently contains two subclasses, namely *Pleosporomycetidae* and *Dothideomycetidae* (Schoch *et al*. 2006, 2009a). Although the main orders, *Pleosporales* and *Dothideales* correlate with the presence or absence of pseudoparaphyses and other centrum characteristics, many orders remain unresolved. The *Dothideomycetidae* include the orders *Dothideales*, *Capnodiales* and *Myriangiales*, which lack paraphyses, pseudoparaphyses and periphysoids. Based on a multi-gene phylogeny, and the presence of ostiolar periphyses as possible synapomorphy, the *Capnodiales* were recognised as the order incorporating the *Capnodiaceae*, *Davidiellaceae*, *Mycosphaerellaceae* and *Piedraiaceae* (Schoch *et al*. 2006). However, several studies (Hunter *et al.* 2006, Crous *et al*. 2007a, b) showed the *Mycosphaerellaceae* to be polyphyletic, and to contain additional variation at the familial level, leading to the circumscriptions of the *Teratosphaeriaceae* and *Schizothyriaceae*. Crous *et al.* (2009b, c) again revealed *Teratosphaeriaceae* to be too widely defined, including some further unresolved families.

The present study focuses on the *Capnodiales*, which is based on the *Capnodiaceae*, representing a group of leaf epiphytes associated with honeydew of insects, usually visible as a black growth on leaf surfaces, fruit and twigs. Members of the *Capnodiaceae* form superficial ascomata with fasciculate asci, and hyaline to dark, septate ascospores. Anamorphs are dematiaceous, and include mycelial (phragmo- to dictyoconidia), spermatial and pycnidial synanamorphs (Hughes 1976, Cheewangkoon *et al.* 2009).

The *Mycosphaerellaceae* was treated as a family in the *Dothideales* by Hawksworth *et al*. (1995), while Kirk *et al*. (2001) introduced a separate order, the *Mycosphaerellales* for this family, and Kirk *et al*. (2008) again placed it in the *Capnodiales*. The *Mycosphaerellaceae* is recognised by having characteristic pseudothecial ascomata that can be immersed or superficial, embedded in host tissue or erumpent, having ostiolar periphyses, but lacking interascal tissue at maturity. Ascospores are hyaline, but in some cases slightly pigmented (Barr 1987), and predominantly 1-septate, although some taxa with 3-septate ascospores have been recorded (Crous *et al.* 2003). Although up to 30 anamorph genera have been linked to *Mycosphaerella* (Crous *et al*. 2000, 2001, 2007a–c, 2009a–c, Crous 2009), recent studies have shown this to be incorrect, and that the family in fact consists of numerous genera with morphologically conserved *Mycosphaerella*-like teleomorphs, and distinct anamorphs (Crous *et al*. 2007a, b, 2009b, c).

Families tentatively placed in the *Capnodiales* (Lumbsch & Huhndorf 2007, Kirk *et al*. 2008) include epiphytes (*Antennulariellaceae, Capnodiaceae*, *Metacapnodiaceae*) (Hughes 1976), saprobes and plant pathogens (*Davidiellaceae*, *Dissoconiaceae*, *Mycosphaerellaceae*, *Schizothyriaceae, Teratosphaeriaceae*) (Aptroot 2006, Crous 2009), and colonisers or hair shafts of mammals (*Piedraiaceae*) (de Hoog *et al*. 2000). To address the status of the *Capnodiales* as an order, and the intrafamilial relationships within this order, DNA sequences of

the 18S, 5.8S and 28S nrRNA genes were generated for a set of specifically selected taxa. A further aim was to clarify genera within these families, and resolve anamorph-teleomorph relationships for the taxa investigated.

MATERIALS AND METHODS

Isolates

Isolates were selected (Table 1 - see online Supplementary Information) that are representative of the *Mycosphaerellaceae* (Crous 1998, Crous *et al*. 2004a, c, 2006a, b, 2007a), *Schizothyriaceae* (Batzer *et al.* 2005, 2007), *Teratosphaeriaceae* (Crous *et al.* 2007a, 2008b, c, 2009a–c), *Piedraiaceae* (Kruys *et al.* 2006), *Davidiellaceae* (Braun *et al*. 2003, Schubert *et al*. 2007a, b), *Capnodiaceae* (Schoch *et al*. 2006), as well as numerous other genera for which the familial relationships have remained unclear, such as the *Phaeophleospora* complex (Crous *et al.* 1997, 2007a, 2009b, c, Andjic *et al.* 2007), *Polythrincium* (Simon *et al.* 2009), the *Dissoconium* complex (Crous *et al.* 2004c, 2007c, 2008b, Arzanlou *et al*. 2008b), and several less well-known genera represented by one or two species only. For fresh material excised leaf spots bearing ascomata 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; Crous *et al*. 2009d). Ascospore germination patterns were examined after 24 h, and single-ascospore and conidial cultures established as described by Crous *et al*. (1991). Colonies were sub-cultured onto synthetic nutrient-poor agar (SNA), potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous *et al.* 2009d), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Other cultures were obtained from the culture collection of the Centraalbureau voor Schimmelcultures (CBS-KNAW) in Utrecht, the Netherlands or the working collection of Pedro Crous (CPC).

DNA isolation, amplification and molecular phylogeny

Genomic DNA was extracted from mycelium taken from fungal colonies on MEA using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, U.S.A.). A part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the first 900 bp at the 5' end of the 28S rRNA gene (LSU) was amplified and sequenced as described by Cheewangkoon *et al*. (2008) standard for all strains included (Table 1). For selected strains (see Table 1), the almost complete SSU and LSU (missing the first and last 20–30 nucleotides) were amplified and sequenced using novel and previously published primers (Table 2; see below).

Novel primers were designed using a variety of complete SSU and LSU sequences obtained from the GenBank sequence database (www.ncbi.nlm.nih.gov/). The selection was not limited only to fungi belonging to the *Dothideomycetes* but encompassed as many as possible full sequences in order to make the primers as robust as possible. We aimed to keep the melting temperature (Tm) of the novel primers at 40–45 °C and the GC content to approximately 50 % to keep them as compatible as possible to existing published primers. Primer parameters were calculated using the OligoAnalyzer tool on the web site of Integrated DNA Technologies (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/) with the "Oligo Conc" parameter set at 0.2 mM and the "Na+ Conc" parameter set at 16 mM. A framework of existing and novel primers was then aligned onto the sequence of *Magnaporthe grisea* (GenBank accession AB026819) to derive primer positions (Table 2) and evaluate coverage over the gene regions. These primers were amplified and sequenced in the following overlapping sections to cover the almost complete SSU and LSU for the selected strains (Table 2): SSU1Fd or SSU6Fm with SSU2Rd, SSU2Fd with SSU3Rd, SSU7Fm with SSU4Rd or SSU6Rm, SSU4Fd with 5.8S1Rd, V9G or LSU1Fd with LSU3Rd, LSU8Fd with LSU8Rd, LSU4Fd with LSU5Rd, and LSU5Fd with LSU7Rd. For some strains (Table 3) it was necessary to add an additional overlap for SSU4Fd with 5.8S1Rd (using SSU4Fd with SSU7Rm and SSU8Fm with 5.8S1Rd), for LSU8Fd with LSU8Rd (using LSU8Fd with LSU3Rd and LSU3Fd with LSU8Rd), and for LSU5Fd with LSU7Rd (using LSU5Fd with LSU6Rd and LSU6Fd with LSU7Rd) to complete the gaps due to large insertions.

The internal transcribed spacer regions, as well as all insertions (Table 3) were excluded from all analyses. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org). Two separate analyses were performed: The first using only partial LSU data due to the limited number of complete LSU sequences available and the second using the almost complete SSU, 5.8S nrDNA and LSU alignment.

Maximum likelihood analyses (ML) were conducted in RAxML v. 7.0.4 (Stamatakis 2006) for the partial LSU alignment. A general time reversible model (GTR) with a discrete gamma distribution and four rate classes was applied. A tree was obtained by simultaneously running a fast bootstrap search of 1000 pseudoreplicates (Stamatakis *et al*. 2008) followed by a search for the most likely tree. Maximum Likelihood bootstrap value (MLBP) equal or greater than 70 % are given at the nodes (Fig. 1).

Maximum likelihood analyses (ML) were conducted in RAxML v. 7.0.4 (Stamatakis 2006) for the almost complete SSU, 5.8S nrDNA and LSU alignment. A general time reversible model (GTR) with a discrete gamma distribution and four rate classes was applied to each partition (SSU, 5.8S nrDNA and LSU). A tree was obtained by simultaneously running a fast bootstrap search of 500 pseudoreplicates (Stamatakis *et al*. 2008) followed by a search for the most likely tree. Maximum Likelihood bootstrap value (MLBP) equal or greater than 70 % are given at the nodes (Fig. 2).

Taxonomy

Fungal structures were mounted in lactic acid, and 30 measurements (× 1000 magnification) obtained per structure type. The range obtained is presented, except for spore measurements, where the 95 % confidence intervals are given with the extremes in parentheses. Colony colours (surface and reverse) were assessed after 1–2 wk on MEA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS-KNAW) in Utrecht, the Netherlands (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al*. 2004b). Names for which the taxonomy has not been resolved, but need to be allocated to another genus, are placed in inverted commas, *e.g.* "*Mycosphaerella*" *iridis*.

Table 2. Details of primers used for this study and their relation to selected published primers. Primer names ending with a "d" denotes a degenerate primer whereas those ending with a "m" denotes specific primers designed based on the partial novel sequences generated. The start and end positions of the primers are derived using *Magnaporthe grisea* GenBank accession AB026819 as reference in the 5'–3' direction.

Name	Sequence (5' – 3')	Orientation	%GC	Tm (°C)	Start	End	Reference
5.8S1Fd	CTC TTG GTT CBV GCA TCG	Forward	57.4	49.8 – 54.2 – 56.8	2333	2350	This study
5.8S1Rd	WAA TGA CGC TCG RAC AGG CAT G	Reverse	52.3	57.6 – 58.9 – 60.2	2451	2472	This study
F377	AGA TGA AAA GAA CTT TGA AAA GAG AA	Forward	26.9	40.3	3005	3030	www.lutzonilab.net/primers/page244.shtml
ITS1	TCC GTA GGT GAA CCT GCG G	Forward	63.2	49.5	2162	2180	White *et al*. (1990)
ITS1F	CTT GGT CAT TTA GAG GAA GTA A	Forward	36.4	39.0	2124	2145	Gardes & Bruns (1993)
ITS1Fd	CGA TTG AAT GGC TCA GTG AGG C	Forward	54.5	48.0	2043	2064	This study
ITS1Rd	GAT ATG CTT AAG TTC AGC GGG	Reverse	47.6	43.1	2671	2691	This study
ITS4	TCC TCC GCT TAT TGA TAT GC	Reverse	45.0	41.6	2685	2704	White *et al*. (1990)
ITS4S	CCT CCG CTT ATT GAT ATG CTT AAG	Reverse	41.7	42.9	2680	2703	Kretzer *et al*. (1996)
ITS5	GGA AGT AAA AGT CGT AAC AAG G	Forward	40.9	40.8	2138	2159	White *et al*. (1990)
LR0R	GTA CCC GCT GAA CTT AAG C	Forward	52.6	43.2	2668	2686	Rehner & Samuels (1994)
LR2	TTT TCA AAG TTC TTT TC	Reverse	23.5	28.5	3009	3025	www.lutzonilab.net/primers/page244.shtml
LR2R	AAG AAC TTT GAA AAG AG	Forward	29.4	30.4	3012	3028	www.lutzonilab.net/primers/page244.shtml
LR3	GGT CCG TGT TTC AAG AC	Reverse	52.9	40.5	3275	3291	Vilgalys & Hester (1990)
LR3R	GTC TTG AAA CAC GGA CC	Forward	52.9	40.5	3275	3291	www.lutzonilab.net/primers/page244.shtml
LR5	TCC TGA GGG AAA CTT CG	Reverse	52.9	41.0	3579	3595	Vilgalys & Hester (1990)
LR5R	GAA GTT TCC CTC AGG AT	Forward	47.1	37.8	3580	3596	www.biology.duke.edu/fungi/mycolab/primers.htm
LR6	CGC CAG TTC TGC TTA CC	Reverse	58.8	43.5	3756	3772	Vilgalys & Hester (1990)
LR7	TAC TAC CAC CAA GAT CT	Reverse	41.2	35.3	4062	4078	Vilgalys & Hester (1990)
LR8	CAC CTT GGA GAC CTG CT	Reverse	58.8	44.3	4473	4489	www.lutzonilab.net/primers/page244.shtml
LR8R	AGC AGG TCT CCA AGG TG	Forward	58.8	44.3	4473	4489	www.lutzonilab.net/primers/page244.shtml
LR9	AGA GCA CTG GGC AGA AA	Reverse	52.9	43.6	4799	4815	www.lutzonilab.net/primers/page244.shtml
LR10	AGT CAA GCT CAA CAG GG	Reverse	52.9	41.6	5015	5031	www.lutzonilab.net/primers/page244.shtml
LR10R	GAC CCT GTT GAG CTT GA	Forward	52.9	41.6	5013	5029	www.lutzonilab.net/primers/page244.shtml
LR11	GCC AGT TAT CCC TGT GGT AA	Reverse	50.0	43.9	5412	5431	www.lutzonilab.net/primers/page244.shtml
LR12	GAC TTA GAG GCG TTC AG	Reverse	52.9	39.4	5715	5731	Vilgalys & Hester (1990)
LR12R	CTG AAC GCC TCT AAG TCA GAA	Forward	47.6	43.7	5715	5735	www.biology.duke.edu/fungi/mycolab/primers.htm
LR13	CAT CGG AAC AAC AAT GC	Reverse	47.1	38.8	5935	5951	www.lutzonilab.net/primers/page244.shtml
LR14	AGC CAA ACT CCC CAC CTG	Reverse	61.1	47.6	5206	5223	www.lutzonilab.net/primers/page244.shtml
LR15	TAA ATT ACA ACT CGG AC	Reverse	35.3	32.5	2780	2796	www.lutzonilab.net/primers/page244.shtml
LR16	TTC CAC CCA AAC ACT CG	Reverse	52.9	42.1	3311	3327	Moncalvo *et al*. (1993)
LR17R	TAA CCT ATT CTC AAA CTT	Forward	27.8	31.2	3664	3681	www.lutzonilab.net/primers/page244.shtml
LR20R	GTG AGA CAG GTT AGT TTT ACC CT	Forward	43.5	43.6	5570	5592	www.lutzonilab.net/primers/page244.shtml
LR21	ACT TCA AGC GTT TCC CTT T	Reverse	42.1	41.7	3054	3072	www.lutzonilab.net/primers/page244.shtml
LR22	CCT CAC GGT ACT TGT TCG CT	Reverse	55.0	46.8	2982	3001	www.lutzonilab.net/primers/page244.shtml

Table 2. (Continued).

Name	Sequence (5' – 3')	Orientation	%GC	Tm (°C)	Start	End	Reference
LSU1Fd	GRA TCA GGT AGG RAT ACC CG	Forward	55.0	41.8 – 44.0 – 46.3	2655	2674	This study
LSU1Rd	CTG TTG CCG CTT CAC TCG C	Reverse	63.2	49.6	2736	2754	This study
LSU2Fd	GAA ACA CGG ACC RAG GAG TC	Forward	57.5	45.5 – 46.5 – 47.6	3280	3299	This study
LSU2Rd	ATC CGA RAA CWT CAG GAT CGG TCG	Reverse	52.1	48.3 – 49.0 – 49.8	3379	3402	This study
LSU3Fd	GTT CAT CYA GAC AGC MGG ACG	Forward	57.1	44.7 – 47.4 – 50.2	3843	3863	This study
LSU3Rd	CAC ACT CCT TAG CGG ATT CCG AC	Reverse	56.5	49.1	3876	3898	This study
LSU4Fd	CCG CAG CAG GTC TCC AAG G	Forward	68.4	51.2	4469	4487	This study
LSU4Rd	CGG ATC TRT TTT GCC GAC TTC CC	Reverse	54.3	47.4 – 48.7 – 50.0	4523	4545	This study
LSU5Fd	AGT GGG AGC TTC GGC GC	Forward	70.6	51.6	3357 / 5072	3373 / 5088	This study
LSU5Rd	GGA CTA AAG GAT CGA TAG GCC ACA C	Reverse	52.0	48.3	5355	5379	This study
LSU6Fd	CCG AAG CAG AAT TCG GTA AGC G	Forward	54.5	48.1	5499	5520	This study
LSU6Rd	TCT AAA CCC AGC TCA CGT TCC C	Reverse	54.5	48.6	5543	5564	This study
LSU7Fd	GTT ACG ATC TRC TGA GGG TAA GCC	Forward	52.1	46.0 – 47.4 – 48.8	5943	5966	This study
LSU7Rd	GCA GAT CGT AAC AAC AAG GCT ACT CTA C	Reverse	46.4	47.9	5927	5954	This study
LSU8Fd	CCA GAG GAA ACT CTG GTG GAG GC	Forward	60.9	51.2	3469	3491	This study
LSU8Rd	GTC AGA TTC CCC TTG TCC GTA CC	Reverse	56.5	48.9	4720	4742	This study
LSU9Fm	GGT AGC CAA ATG CCT CGT CAT C	Forward	54.5	47.9	4882	4903	This study
LSU9Rm	GAT TYT GCS AAG CCC GTT CCC	Reverse	59.5	49.2 – 50.0 – 50.9	4979	4999	This study
LSU10Fm	GGG AAC GTG AGC TGG GTT TAG A	Forward	54.5	48.6	5543	5564	This study
LSU10Rm	CGC TTA CCG AAT TCT GCT TCG G	Reverse	54.5	48.1	5499	5520	This study
LSU11Fm	TTTGGTAAGCAGAACTGGCGATGC	Forward	50.0	49.4	3753	3776	This study
LSU12Fd	GTGTGGCCTATCGATCCTTTAGTCC	Forward	52.0	48.3	5355	5379	This study
NS1	GTA GTC ATA TGC TTG TCT C	Forward	42.1	36.9	413	431	White *et al*. (1990)
NS1R	GAG ACA AGC ATA TGA CTA C	Reverse	42.1	36.9	413	431	www.lutzonilab.net/primers/page244.shtml
NS2	GGC TGC TGG CAC CAG ACT TGC	Reverse	66.7	53.8	943	963	White *et al*. (1990)
NS3	GCAAGTCTGGTGCCAGCAGCC	Forward	66.7	53.8	943	963	White *et al*. (1990)
NS4	CTT CCG TCA ATT CCT TTA AG	Reverse	40.0	38.2	1525	1544	White *et al*. (1990)
NS5	AAC TTA AAG GAA TTG ACG GAA G	Forward	36.4	40.1	1523	1544	White *et al*. (1990)
NS6	GCA TCA CAG ACC TGT TAT TGC CTC	Reverse	50.0	47.5	1806	1829	White *et al*. (1990)
NS7	GAG GCA ATA ACA GGT CTG TGA TGC	Forward	50.0	47.5	1806	1829	White *et al*. (1990)
NS8	TCC GCA GGT TCA CCT ACG GA	Reverse	60.0	50.4	2162	2181	White *et al*. (1990)
NS17	CAT GTC TAA GTT TAA GCA A	Forward	31.6	34.2	447	465	Gargas & Taylor (1992)
NS18	CTC ATT CCA ATT ACA AGA CC	Reverse	40.0	38.0	887	906	Gargas & Taylor (1992)
NS19	CCG GAG AAG GAG CCT GAG AAA C	Forward	59.1	49.3	771	792	Gargas & Taylor (1992)
NS20	CGT CCC TAT TAA TCA TTA CG	Reverse	40.0	37.3	1243	1262	Gargas & Taylor (1992)
NS21	GAA TAA TAG AAT AGG ACG	Forward	33.3	30.5	1193	1210	Gargas & Taylor (1992)
NS22	AAT TAA GCA GAC AAA TCA CT	Reverse	30.0	36.4	1687	1706	Gargas & Taylor (1992)
NS23	GAC TCA ACA CGG GGA AAC TC	Forward	55.0	45.5	1579	1598	Gargas & Taylor (1992)
NS24	AAA CCT TGT TAC GAC TTT TA	Reverse	30.0	36.2	2143	2162	Gargas & Taylor (1992)
SR11R	GGA GCC TGA GAA ACG GCT AC	Forward	60.0	47.8	779	798	Spatafora *et al*. (1995)
SR1R	TAC CTG GTT GAT TCT GC	Forward	47.1	38.5	394	410	Vilgalys & Hester (1990)
SR3	GAA AGT TGA TAG GGC T	Reverse	43.8	34.8	696	711	www.biology.duke.edu/fungi/mycolab/primers.htm

Table 2. (Continued).

Name	Sequence (5' – 3')	Orientation	%GC	Tm (°C)	Start	End	Reference
SSU1Fd	CTG CCA GTA GTC ATA TGC TTG TCT C	Forward	48.0	46.5	407	431	This study
SSU1Rd	CTT TGA GAC AAG CAT ATG AC	Reverse	40.0	48.7	416	435	This study
SSU2Fd	GAA CAA YTR GAG GGC AAG	Forward	50.0	47.8 – 50.7 – 53.5	930	947	This study
SSU2Rd	TAT ACG CTW YTG GAG CTG	Reverse	47.2	48.4 – 49.9 – 51.2	974	991	This study
SSU3Fd	ATC AGA TAC CGT YGT AGT C	Forward	44.7	48.4 – 49.5 – 50.5	1389	1407	This study
SSU3Rd	TAY GGT TRA GAC TAC RAC GG	Reverse	47.5	49.0 – 52.5 – 56.0	1397	1416	This study
SSU4Fd	CCG TTC TTA GTT GGT GG	Forward	52.9	50.0	1670	1686	This study
SSU4Rd	CAG ACA AAT CAC TCC ACC	Reverse	50.0	50.3	1682	1699	This study
SSU5Fd	TAC TAC CGA TYG AAT GGC	Forward	47.2	48.9 – 50.1 – 51.2	2037	2054	This study
SSU5Rd	CGG AGA CCT TGT TAC GAC	Reverse	55.6	52.5	2148	2165	This study
SSU6Fm	GCT TGT CTC AAA GAT TAA GCC ATG CAT GTC	Forward	43.3	49.0	423	452	This study
SSU6Rm	GCA GGT TAA GGT CTC GTT CGT TAT CGC	Reverse	51.9	50.1	1707	1733	This study
SSU7Fm	GAG TGT TCA AAG CAG GCC TNT GCT CG	Forward	55.8	51.0 – 52.2 – 53.3	1153	1178	This study
SSU7Rm	CAA TGC TCK ATC CCC AGC ACG AC	Reverse	58.7	49.5 – 50.8 – 52.1	1921	1943	This study
SSU8Fm	GCA CGC GCG CTA CAC TGA C	Forward	68.4	52.2	1848	1866	This study
V9G	TTA CGT CCC TGC CCT TTG TA	Forward	45.0	42.8	2002	2021	de Hoog & Gerrits van den Ende (1998)

Table 3. Isolates containing group I intron sequences. The insertion positions of these introns are derived using *Magnaporthe grisea* GenBank accession AB026819 as reference in the 5'–3' direction.

Isolate	Insertion between	18S or 28S nrDNA	Intron size (bp)	Blast result
Batcheloromyces leucadendri CBS 110892	1559 – 1560	18S nrDNA	350	No significant similarity
	1820 – 1821	18S nrDNA	399	190/252 of AY545722 *Hydropisphaera erubescens* 18S nrDNA
	4875 – 4876	28S nrDNA	328	211/264 of DQ246237 *Teratosphaeria mexicana* 28S nrDNA
	5424 – 5425	28S nrDNA	538	No significant similarity
	5538 – 5539	28S nrDNA	383	218/283 of EU181458 *Trichophyton soudanense* 28S nrDNA
Batcheloromyces proteae CBS 110696	1559 – 1560	18S nrDNA	325	No significant similarity
	1820 – 1821	18S nrDNA	399	191/254 of AY545722 *Hydropisphaera erubescens* 18S nrDNA
	4875 – 4876	28S nrDNA	328	211/263 of DQ246237 *Teratosphaeria mexicana* 28S nrDNA
	5424 – 5425	28S nrDNA	535	75/90 of DQ442697 *Arxula adeninivorans* 26S nrDNA
	5538 – 5539	28S nrDNA	372	34/36 of GQ120133 Uncultured marine fungus 18S nrDNA
Catenulostroma macowanii CBS 110756	1559 – 1560	18S nrDNA	395	297/379 of DQ848302 *Mycosphaerella latebrosa* 18S nrDNA
	5424 – 5425	28S nrDNA	914	No significant similarity
Catenulostroma macowanii CBS 111029	1559 – 1560	18S nrDNA	395	303/379 of DQ848302 *Mycosphaerella latebrosa* 18S nrDNA
	5424 – 5425	28S nrDNA	914	No significant similarity
Cercospora apii CBS 118712	1820 – 1821	18S nrDNA	733	288/363 of EU167577 *Mycosphaerella milleri* 18S nrDNA
Cercospora capsici CPC 12307	1820 – 1821	18S nrDNA	732	287/363 of EU167577 *Mycosphaerella milleri* 18S nrDNA
Cercospora janseana CBS 145.37	1820 – 1821	18S nrDNA	350	295/365 of EU167577 *Mycosphaerella milleri* 18S nrDNA
Devriesia staurophora CBS 375.81	3560 – 3561	28S nrDNA	309	No significant similarity
Miuraea persicae CPC 10069	1820 – 1821	18S nrDNA	603	399/443 of DQ848342 *Mycosphaerella populorum* 18S nrDNA
Mycosphaerella latebrosa CBS 652.85	1559 – 1560	18S nrDNA	370	234/296 of DQ848311 *Septoria betulae* 18S nrDNA
	1820 – 1821	18S nrDNA	933	Matches same species
	2168 – 2169	18S nrDNA	494	377/449 of DQ848326 *Septoria alnifolia* 18S nrDNA
	4875 – 4876	28S nrDNA	481	No significant similarity
	missing 5018 – 5019	28S nrDNA	Not present	Not present

Table 3. (Continued).

Isolate	Insertion between	18S or 28S nrDNA	Intron size (bp)	Blast result
	5424 – 5425	28S nrDNA	680	No significant similarity
	5538 – 5539	28S nrDNA	471	No significant similarity
Mycosphaerella latebrosa CBS 687.94	1559 – 1560	18S nrDNA	370	231/295 of DQ848310 *Septoria betulae* 18S nrDNA
	1820 – 1821	18S nrDNA	918	Matches same species
	2168 – 2169	18S nrDNA	494	377/449 of DQ848326 *Septoria alnifolia* 18S nrDNA
	4875 – 4876	28S nrDNA	480	No significant similarity
	5018 – 5019	28S nrDNA	417	144/181 of AF430703 *Beauveria bassiana* 28S nrDNA
	5424 – 5425	28S nrDNA	680	No significant similarity
	5538 – 5539	28S nrDNA	471	No significant similarity
Mycosphaerella marksii CBS 110942	1559 – 1560	18S nrDNA	341	332/355 of DQ848296 *Mycosphaerella musae* 18S nrDNA
Mycosphaerella marksii CPC 11222	1559 – 1560	18S nrDNA	341	332/355 of DQ848296 *Mycosphaerella musae* 18S nrDNA
Passalora-like genus CPC 11876	5538 – 5539	28S nrDNA	580	No significant similarity
Passalora bellynckii CBS 150.49	1559 – 1560	18S nrDNA	409	147/191 of DQ848296 *Mycosphaerella musae* 18S nrDNA
Passalora dodonaea CPC 1223	5424 – 5425	28S nrDNA	738	No significant similarity
Phacellium paspali CBS 113093	4875 – 4876	28S nrDNA	340	161/197 of DQ248314 *Symbiotaphrina kochii* 28S nrDNA
Phaeophleospora eugeniicola CPC 2557	missing 5424 – 5425	28S nrDNA	Not present	Not present
	5538 – 5539	28S nrDNA	744	No significant similarity
Phaeophleospora eugeniicola CPC 2558	5424 – 5425	28S nrDNA	1846	No significant similarity
	5538 – 5539	28S nrDNA	744	No significant similarity
Pseudocercospora angolensis CBS 112933	5018 – 5019	28S nrDNA	379	No significant similarity
Pseudocercospora angolensis CBS 149.53	5018 – 5019	28S nrDNA	379	No significant similarity
Pseudocercospora punctata CBS 113315	5424 – 5425	28S nrDNA	723	No significant similarity
	5538 – 5539	28S nrDNA	725	67/73 of AF430699 *Beauveria bassiana* 28S nrDNA
Pseudocercospora punctata CPC 10532	5424 – 5425	28S nrDNA	731	No significant similarity
	5538 – 5539	28S nrDNA	725	67/73 of AF430699 *Beauveria bassiana* 28S nrDNA
Ramularia coleosporii CPC 11516	1559 – 1560	18S nrDNA	445	No significant similarity
Ramularia grevilleana CPC 656	5538 – 5539	28S nrDNA	546	No significant similarity
Septoria apiicola CBS 400.54	5424 – 5425	28S nrDNA	763	No significant similarity
Septoria obesa CBS 354.58	1820 – 1821	18S nrDNA	575	No significant similarity
	2168 – 2169	18S nrDNA	548	394/454 of DQ848326 *Septoria alnifolia* 18S nrDNA
	4875 – 4876	28S nrDNA	430	No significant similarity
Septoria pyricola CBS 222.31	5424 – 5425	28S nrDNA	723	No significant similarity
Septoria quercicola CBS 663.94	1559 – 1560	18S nrDNA	334	241/308 of DQ848303 *Mycosphaerella latebrosa* 18S nrDNA
	1820 – 1821	18S nrDNA	442	379/452 of DQ848335 *Mycosphaerella latebrosa* 18S nrDNA
	4875 – 4876	28S nrDNA	345	No significant similarity
	5018 – 5019	28S nrDNA	367	122/155 of DQ518980 *Lipomyces spencermartinsiae* 28S nrDNA
	5424 – 5425	28S nrDNA	526	No significant similarity
	5538 – 5539	28S nrDNA	603	No significant similarity
Septoria rosae CBS 355.58	1820 – 1821	18S nrDNA	496	No significant similarity
Sonderhenia eucalypticola CPC 11252	1559 – 1560	18S nrDNA	408	339/404 of DQ848314 *Mycosphaerella populorum* 18S nrDNA
	4875 – 4876	28S nrDNA	337	229/289 of AB044641 *Cordyceps* sp. 28S nrDNA
	5424 – 5425	28S nrDNA	705	No significant similarity
Stigmina platani CBS 110755	1559 – 1560	18S nrDNA	379	40/44 of AB007686 *Exophiala calicioides* 18S nrDNA
	5018 – 5019	28S nrDNA	376	No significant similarity
Stigmina synanamorph CPC 11721	5018 – 5019	28S nrDNA	371	No significant similarity
Teratosphaeria aff. *nubilosa* CBS 114419	4871 – 4872	28S nrDNA	141	No significant similarity; high identity to *Teratosphaeria nubilosa*
	5538 – 5539	28S nrDNA	580	No significant similarity; high identity to *Teratosphaeria nubilosa*

Table 3. (Continued).

Isolate	Insertion between	18S or 28S nrDNA	Intron size (bp)	Blast result
Teratosphaeria aff. *nubilosa* CBS 116283	4871 – 4872	28S nrDNA	141	No significant similarity; high identity to *Teratosphaeria nubilosa*
	5538 – 5539	28S nrDNA	580	No significant similarity; high identity to *Teratosphaeria nubilosa*
Teratosphaeria juvenalis CBS 110906	1559 – 1560	18S nrDNA	403	52/61 of DQ471010 *Rutstroemia firma* 18S nrDNA
	4875 – 4876	28S nrDNA	345	224/290 of EF115309 *Cordyceps bassiana* 28S nrDNA
	5424 – 5425	28S nrDNA	478	47/50 of EF115313 *Cordyceps bassiana* 28S nrDNA
	5538 – 5539	28S nrDNA	402	No significant similarity
Teratosphaeria juvenalis CBS 111149	1559 – 1560	18S nrDNA	403	52/61 of DQ471010 *Rutstroemia firma* 18S nrDNA
	4875 – 4876	28S nrDNA	345	224/290 of EF115309 *Cordyceps bassiana* 28S nrDNA
	5424 – 5425	28S nrDNA	478	47/50 of EF115313 *Cordyceps bassiana* 28S nrDNA
	5538 – 5539	28S nrDNA	402	No significant similarity
Teratosphaeria mexicana CBS 110502	954 – 955	18S nrDNA	316	129/158 of DQ518980 *Lipomyces spencermartinsiae* 26S nrDNA
	1559 – 1560	18S nrDNA	360	No significant similarity
	1820 – 1821	18S nrDNA	388	128/168 of AF281670 *Cryptendoxyla hypophloia* 18S nrDNA
	3560 – 3561	28S nrDNA	383	124/151 of EF647754 *Thecaphora thlaspeos* 28S nrDNA
	4875 – 4876	28S nrDNA	327	99/114 of L81104 *Gaeumannomyces graminis* var. *tritici* 28S nrDNA
	5018 – 5019	28S nrDNA	315	No significant similarity
	5424 – 5425	28S nrDNA	553	No significant similarity
Teratosphaeria mexicana CBS 120744	954 – 955	18S nrDNA	318	130/158 of DQ518980 *Lipomyces spencermartinsiae* 26S nrDNA
	1559 – 1560	18S nrDNA	360	No significant similarity
	1820 – 1821	18S nrDNA	389	85/109 of AF281670 *Cryptendoxyla hypophloia* 18S nrDNA
	3560 – 3561	28S nrDNA	378	119/155 of AY298780 *Lentinellus castoreus* 18S nrDNA
	4875 – 4876	28S nrDNA	327	162/200 of AB033530 *Penicillium sabulosum* 18S nrDNA
	5018 – 5019	28S nrDNA	309	No significant similarity
	5424 – 5425	28S nrDNA	659	No significant similarity
Teratosphaeria nubilosa CBS 115669	4871 – 4872	28S nrDNA	141	No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa*
	5538 – 5539	28S nrDNA	580	No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa*
Teratosphaeria nubilosa CBS 116005	4871 – 4872	28S nrDNA	141	No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa*
	5538 – 5539	28S nrDNA	580	No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa*
Teratosphaeria ohnowa CBS 112896	954 – 955	18S nrDNA	325	28/28 of DQ848329 *Botryosphaeria quercuum* 18S nrDNA
	3560 – 3561	28S nrDNA	294	168/227 of FJ358267 *Chaetothyriales* sp. 28S nrDNA
	5424 – 5425	28S nrDNA	607	47/48 of EF115313 *Cordyceps bassiana* 28S nrDNA
Teratosphaeria ohnowa CBS 112973	954 – 955	18S nrDNA	324	28/28 of DQ848329 *Botryosphaeria quercuum* 18S nrDNA
	3560 – 3561	28S nrDNA	294	168/227 of FJ358267 *Chaetothyriales* sp. 28S nrDNA
	5424 – 5425	28S nrDNA	607	47/48 of EF115313 *Cordyceps bassiana* 28S nrDNA
Teratosphaeria pseudosuberosa CBS 118911	3560 – 3561	28S nrDNA	324	28/28 of DQ848329 *Botryosphaeria quercuum* 18S nrDNA
	4875 – 4876	28S nrDNA	364	No significant similarity
Teratosphaeria sp. CBS 208.94	954 – 955	18S nrDNA	342	No significant similarity
	3560 – 3561	28S nrDNA	309	59/70 of AY207244 *Mycena pura* 28S nrDNA
	4875 – 4876	28S nrDNA	296	44/51 of EF551317 *Tremella globispora* 28S nrDNA
Teratosphaeria suberosa CPC 11032	5424 – 5425	28S nrDNA	313	159/197 of AB033529 *Penicillium oblatum* 18S nrDNA
	5538 – 5539	28S nrDNA	596	80/99 of AB044639 *Cordyceps kanzashiana* 28S nrDNA
Thedgonia-like genus CPC 12304	1820 – 1821	18S nrDNA	444	262/331 of EU167577 *Mycosphaerella milleri* 18S nrDNA

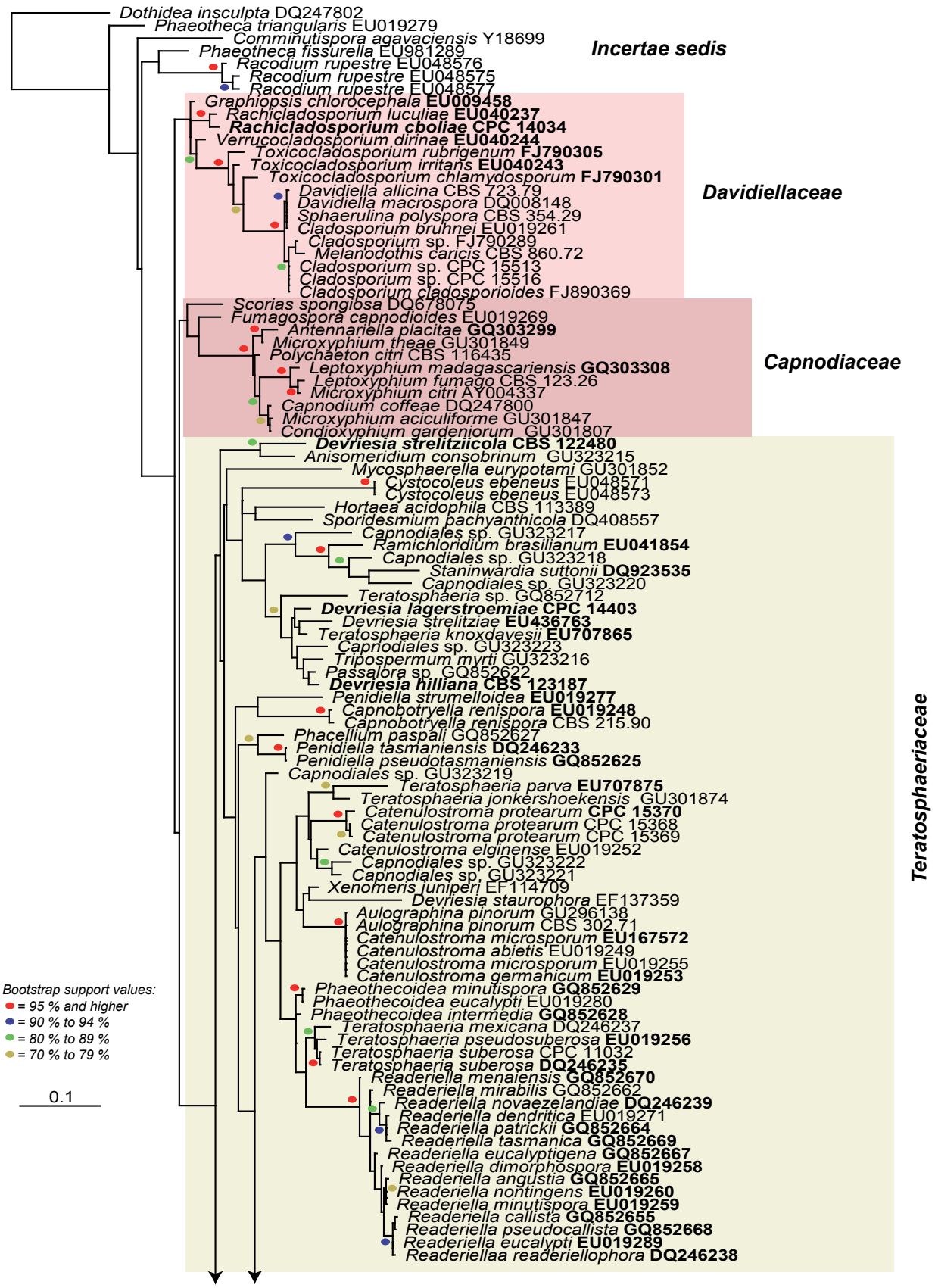

Fig. 1. RAxML tree using only the partial LSU alignment with bootstrap values after 1 000 pseudorepetitions on the nodes. Type strains and novel species described in this study are indicated in **bold**.

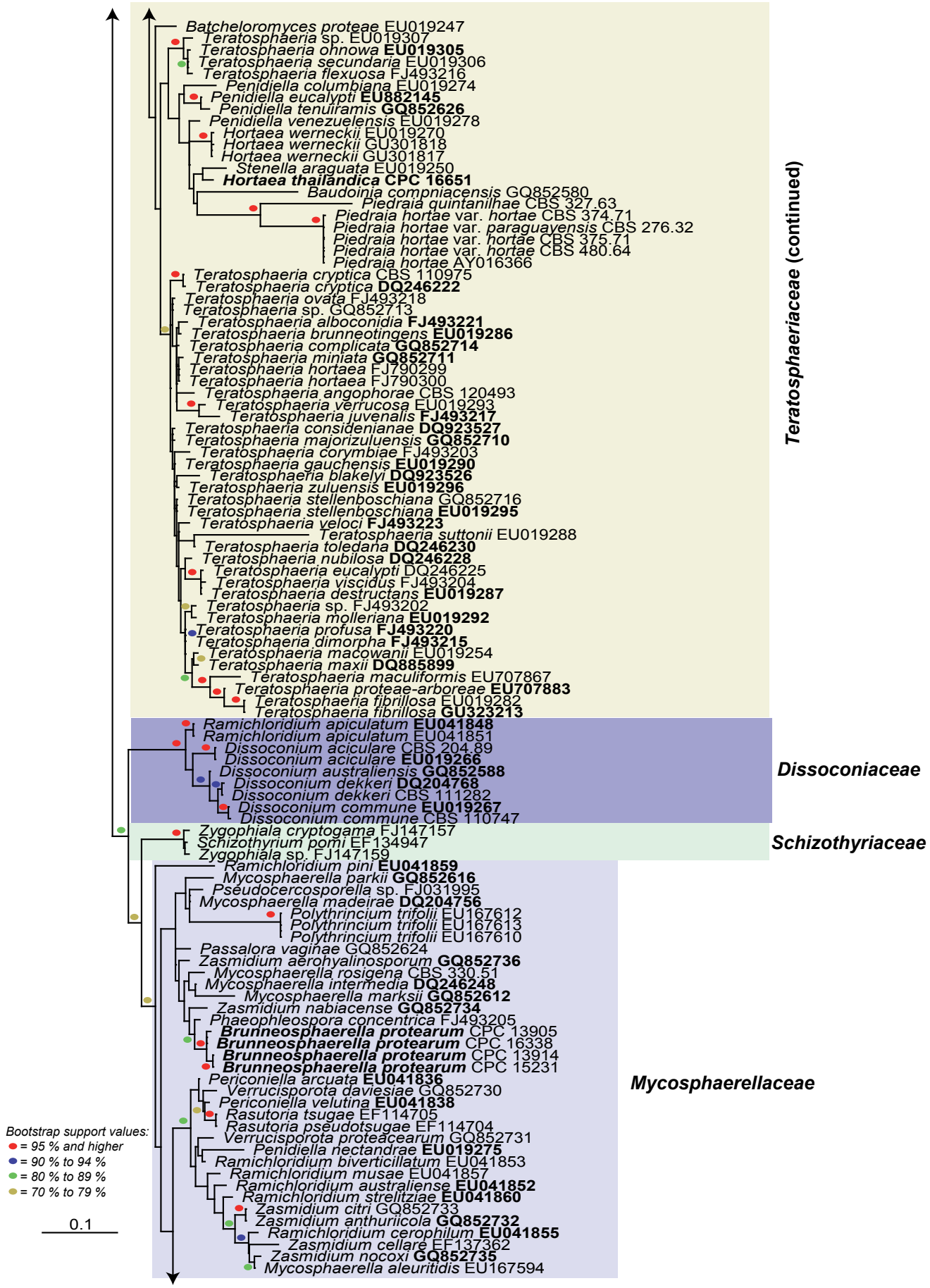

Fig. 1. (Continued).

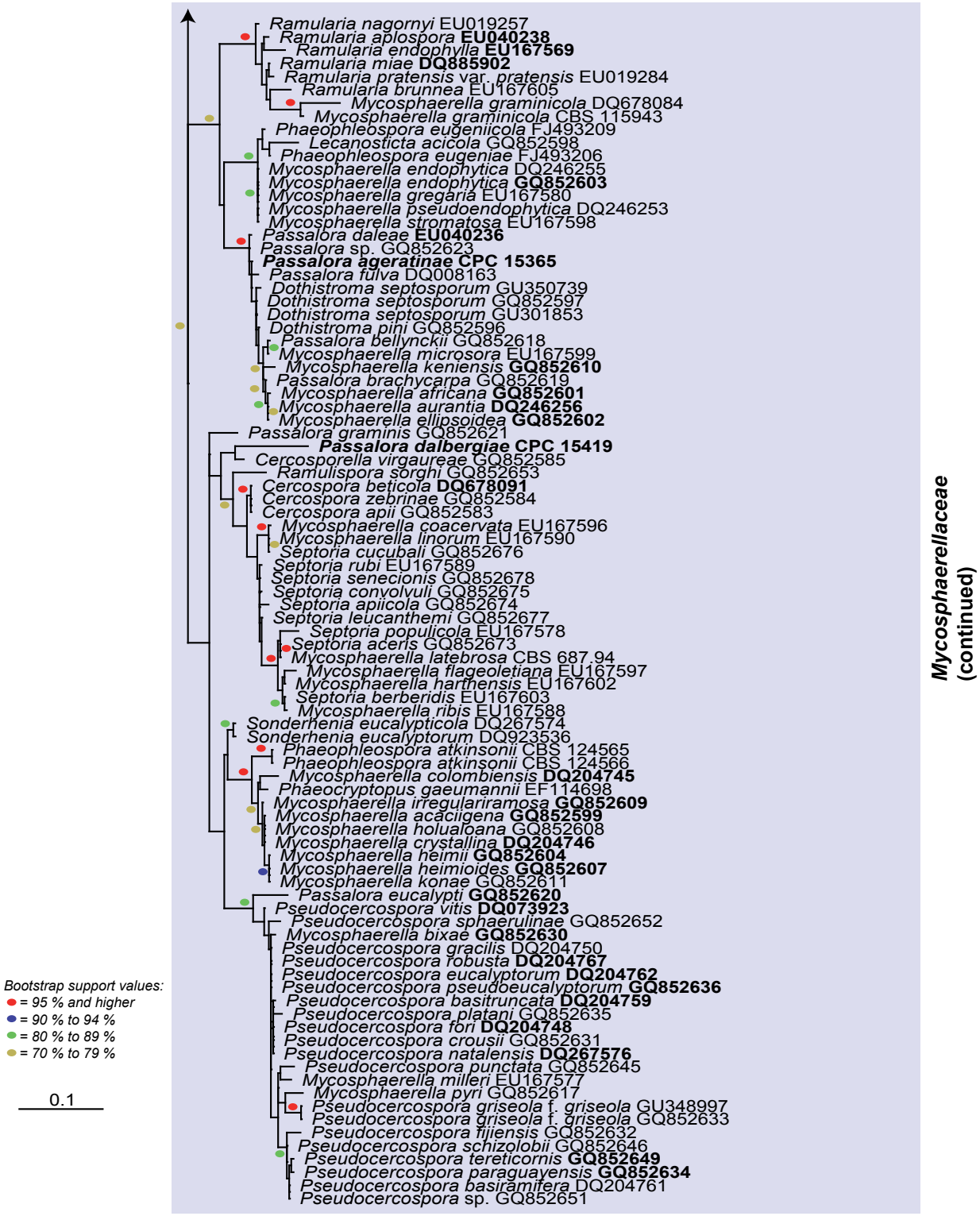

Fig. 1. (Continued).

Fig. 2. RAxML tree using the SSU, 5.8S nrDNA and LSU alignment with bootstrap values after 500 pseudorepetitions on the nodes.

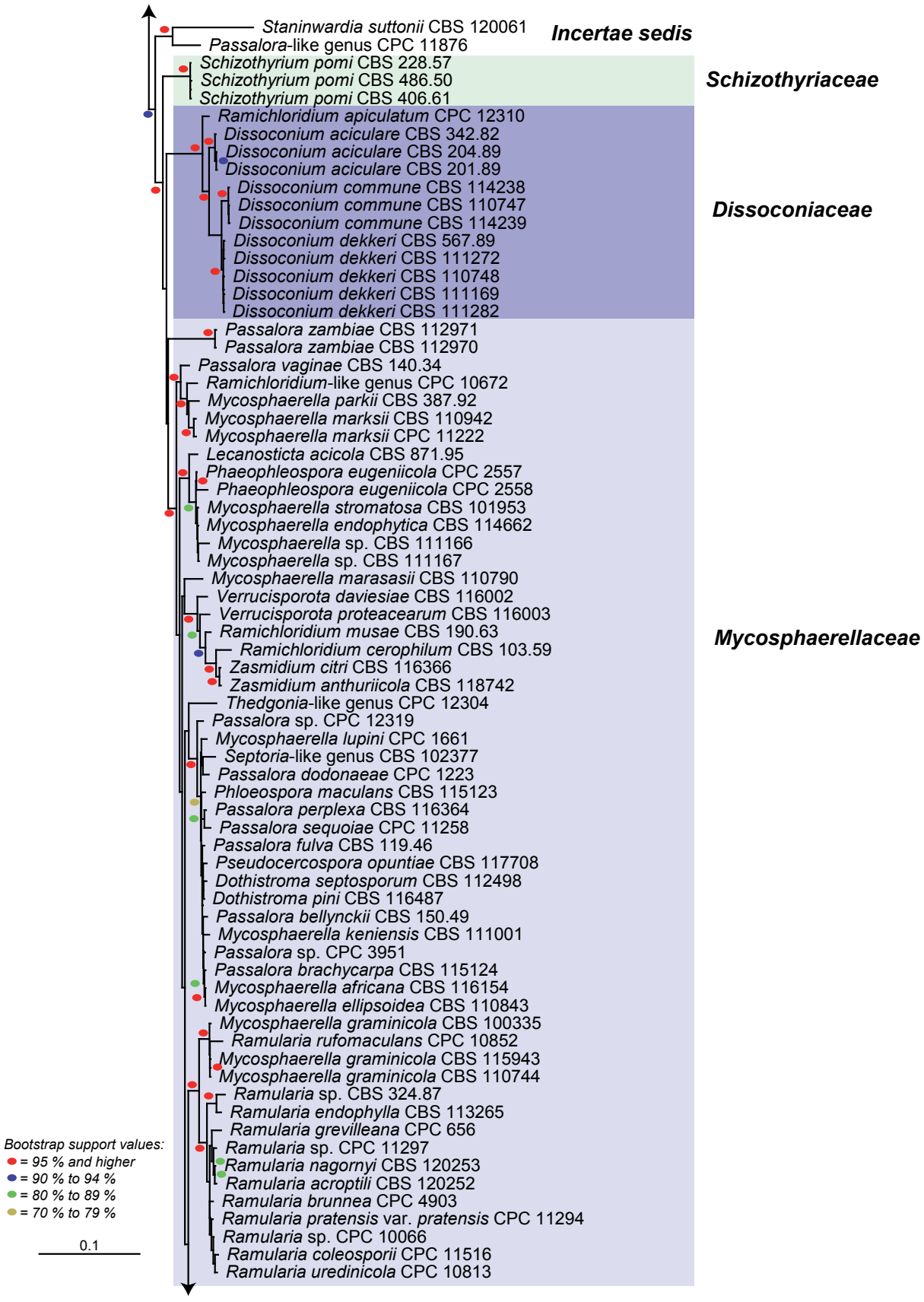

Fig. 2. (Continued).

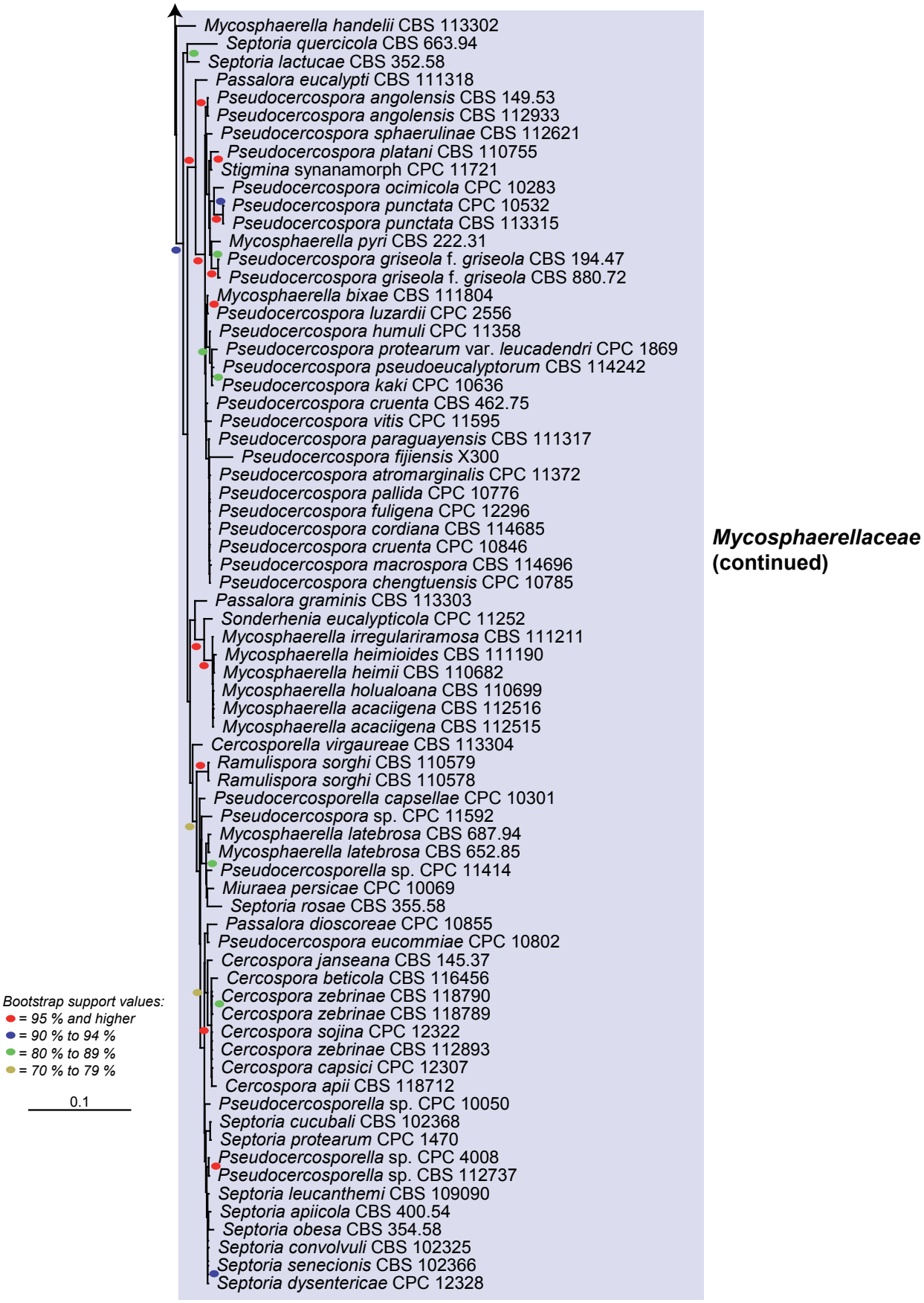

Fig. 2. (Continued).

RESULTS

DNA amplification and phylogeny

Amplification products of approximately 1 700 bases were obtained for the standard amplification of the isolates listed in Table 1. The LSU region of these sequences was used to obtain additional sequences from GenBank that were added to the partial LSU alignment. We expected a total size of approximately 5 500 bp for the concatenated SSU, ITS1, 5.8S nrDNA, ITS2 and LSU at the start of the study; however, our alignment totalled about 12 000 bp due to numerous insertions (most likely group 1 introns) encountered for several strains (Table 3). These insertions frequently resulted in products too large to amplify or sequence effectively and sometimes required us to design additional novel primers in extra overlapping steps to complete these gaps (see Materials and Methods for details). Searching the GenBank database using these insertions had varied success (Table 3). Sequences of the 18S nrDNA are more abundant in the database whereas sequences of the second half to two-thirds of the 28S nrDNA are mostly absent. This also evident in Table 3, where insertions in the SSU more frequently found with similarity sequences in the database and insertions in the LSU (*e.g.* those between positions 5018–5019 and 5424–5425) frequently did not retrieve any significant similarity. Although there were some exceptions (*e.g.* the insertion between 1820 and 1821 in the SSU of *Batcheloromyces leucadendri*), most of the insertions in the SSU obtained hits with SSU sequences of species of *Capnodiales* in the database. In one case, between 954 and 955 for the SSU sequence of *Teratosphaeria mexicana* (both strains), a partial hit was obtained with an LSU sequence of *Lipomyces spencermartinsiae* (GenBank DQ518980). Many of the insertions in the LSU sequences did not retrieve significant hits in the database and those that did were with unrelated taxa. It is quite possible that this is an artifact of the poor representation of full-length LSU sequences in the database, especially for members of the *Capnodiales*. In some cases, an LSU insertion retrieved a hit with SSU sequences in the database, *e.g.* the insertion between 5538 and 5539 in *Batcheloromyces proteae* and between 3560 and 3561 and 4875 and 4876 in *Teratosphaeria mexicana* strain CBS 120744. In two cases (*Mycosphaerella latebrosa* and *Phaeophleospora eugeniicola*), an insertion was either lost or gained between two strains of the same species. The primers designed in this study allowed us to effectively amplify and sequence the SSU and LSU for the selected isolates. Althought these primers were not tested on taxa outside of the *Capnodiales* (except for one of the outgroups, *Neofusicoccum australe*), we attempted to design them as robust as possible using degeneracy if needed. We therefore expect that these primers will have wider applicability than just the *Capnodiales* in cases where other published primers fail to amplify or amplify poorly.

The RAxML search of the partial LSU alignment yielded a most likely tree (Fig. 1) with a log likelihood -13397.994021. The matrix had 395 distinct alignment patterns, with 6 % completely undetermined characters in the alignment. The manually adjusted alignment contained 295 sequences (including the outgroup sequence, *Dothidea insculpta* GenBank DQ247802) and 763 characters including alignment gaps. The RAxML search of the almost complete SSU, 5.8S nrDNA and LSU alignment yielded a most likely tree (Fig. 2) with a log likelihood -39022.881140. The matrix had 1211 alignment patterns with 0.01 % of the characters consisting of gaps or undetermined characters. The manually adjusted alignment contained 205 sequences (including the outgroup sequences, *Neofusicoccum australe* CPC 10899 and *Magnaporthe grisea* GenBank AB026819) and 5110 characters including alignment gaps. The obtained phylogenies (Figs 1–2) are discussed in the Taxonomy section below.

Taxonomy

Several well-supported clades could be distinguished in the present study (Figs 1–2), correlating to families in the *Capnodiales*. These families, and several new genera and species, are treated below.

Treatment of phylogenetic clades

Capnodiales Woron. Ann. Mycol. 23: 177. 1925.

Data obtained from multi-gene phylogenies prompted Schoch *et al*. (2006) to merge *Mycosphaerellales* with *Capnodiales*. Although the present study included numerous additional isolates, the orders remain problematic. Although there is support for the *Mycosphaerellales* as an order, additional families such as the *Schizothyriaceae* and *Dissoconiaceae* (see below) would have to also be elevated to order level, which would result in orders containing a single family, while *Teratosphaeriaceae* appears to comprise unresolved lineages. For this reason it was decided to retain these families within *Capnodiales*, but noting that as more families are added and better circumscribed, it is quite possible that the *Mycosphaerellales* would again be resurrected.

Mycosphaerellaceae Lindau, In: Engler & Prantl, Nat. Pflanzenfamilien 1(1): 421. 1897.

Type species: *Mycosphaerella punctiformis* (Pers. : Fr.) Starbäck, Bih. Kongl. Svenska Vetensk.-Akad. Handl. 15(3, 2): 9. 1889.

Notes: The *Mycosphaerellaceae* contains numerous genera, 20 of which are listed by Crous (2009), with many names under consideration (Crous *et al*. 2009b, c). From these data it is clear that genera such as *Passalora, Pseudocercospora, Pseudocercosporella, Septoria, Zasmidium* and *Ramichloridium* are paraphyletic (Hunter *et al*. in prep.). Well-resolved genera include *Cercospora*, *Cercosporella, Ramularia*, *Ramulispora*, *Sonderhenia* and *Polythrincium*. One particularly problematic clade contains *Periconiella*, *Ramichloridium, Verrucisporota* and *Zasmidium,* along with "*Mycosphaerella*" and *Rasutoria* teleomorphs. Barr (1987) erected *Rasutoria* for species with brown ascospores occurring on *Gymnospermae*. *Rasutoria* clusters in a clade adjacent to "*Mycosphaerella*" species with hyaline ascospores, such as *M. aleuritidis* and *Mycosphaerella daviesiicola* (*Verrucisporota daviesiae*) (Beilharz & Pascoe 2002).

The genus *Phaeophleospora* (1916) clusters with *Lecanosticta acicola*. The genus *Lecanosticta* (1922) has typical *Phaeophleospora*-like conidia, except that its conidiomata are acervular, and not pycnidial. If the type of *Lecanosticta, L. pini* also clusters in this clade, the generic concept *Phaeophleospora* may have to be widened to include *Lecanosticta*, as was done with *Kirramyces* to include *Colletogloeopsis* (Cortinas *et al*. 2006a, b).

Considerable controversy has surrounded the coelomycetes that Crous *et al*. (1997) placed in *Phaeophleospora*. Based on DNA phylogenetic data, it has now been shown that *Kirramyces* anamorphs (Walker *et al*. 1992), including those accommodated in *Colletogloeopsis* (Crous & Wingfield 1996, Crous *et al.* 2004c, 2006c, Cortinas *et al*. 2006a, b), are linked to *Teratosphaeria* (Andjic *et al*. 2007, Crous *et al*. 2009b, c). Crous *et al*. (2007a)

showed *Phaeophleospora* to reside in the *Mycosphaerellaceae* and *Kirramyces* in the *Teratosphaeriaceae*, respectively. However, most taxa investigated to date were collected from *Eucalyptus*. As shown in the present study, *Phaeophleospora atkinsonii*, a pathogen of *Hebes* spp. (Wu *et al*. 1996, Pennycook & McKenzie 2002), clusters distant from *Phaeophleospora s. str.*, while the same is true for *Phaeophleospora concentrica*, which is a pathogen of *Protea* spp. (Taylor *et al*. 2001a), and *Phaeophleospora stonei*, a pathogen of *Eucalyptus* (Crous *et al*. 2007c, 2009c). These taxa thus clearly represent yet another two genera in the *Phaeophleospora* complex. An older name that would potentially be available is *Scoleciasis*. However, when B. Sutton examined exsiccati of the type species, *S. aquatica*, only ascomata of a *Leptosphaeria* species were found (Crous *et al.* 1997). The association of *S. aquatica* with the *Leptosphaeria* was also noted in the original description, and this may indicate that *Scoleciasis* is allied to taxa in the *Phaeosphaeriopsis/ Phaeoseptoria* complex (Arzanlou & Crous 2006). Both *P. atkinsonii* and *P. concentrica* have a typical *Kirramyces* morphology, namely brown, percurrently proliferating conidiogenous cells, and brown, obclavate, verruculose, transversely euseptate conidia. Further species thus need to be included in analyses before these generic concepts can be clarified.

During the course of this study several fresh collections of *Leptosphaeria protearum* were obtained. *Leptosphaeria protearum* is a major leaf spot and blight pathogen of *Protea* spp. (Knox-Davies *et al.* 1987), and causes severe losses in plantations of South African *Protea* spp. in Hawaii, and has been recorded in many countries where South African proteas are cultivated (Taylor & Crous 1998, Taylor *et al*. 2001b, Crous *et al*. 2004a). Cultures of this pathogen were found to cluster in the *Mycosphaerellaceae*, where they represent an undescribed genus, characterised by having bitunicate asci without pseudoparaphyses, brown, 3-septate ascospores, and a *Coniothyrium*-like anamorph. Its close phylogenetic relationship to *Phaeophloeospora concentrica* (Fig. 1) suggests that they could be congeneric, and that in future more *Phaeophloeospora*-like anamorphs may be found to cluster in this clade. We propose a new genus to accommodate *Leptosphaeria protearum* below.

Brunneosphaerella Crous, **gen. nov.** MycoBank MB514694.

Etymology: *Brunneus* + *Sphaerella* = is after its brown ascospores and *Sphaerella*-like morphology.

Mycosphaerellae similis, sed ascosporis brunneis, 3-septatis.

Ascomata amphigenous, immersed to semi-immersed, black, single, gregarious, substomatal, pyriform or globose with a papillate, periphysate ostiole. *Peridium* consisting of three strata of slightly compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells, becoming paler in the central stratum, and hyaline, thin-walled in the inner stratum. *Asci* clavate to cylindro-clavate, often curved, tapering to a pedicel, narrowing slightly to a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense. *Pseudoparaphyses* absent. *Ascospores* biseriate, fusiform, broader at the apical end, initially hyaline and 1-septate, becoming yellow-brown and 3-septate at maturity, slightly constricted at median to supra-median septum.

Type species: *Brunneosphaerella protearum* (Syd. & P. Syd.) Crous, comb. nov.

Brunneosphaerella jonkershoekensis (Marinc., M.J. Wingf. & Crous) Crous, **comb. nov.** MycoBank MB514695. Fig. 3.

Basionym: *Leptosphaeria jonkershoekensis* Marinc., M.J. Wingf. & Crous, In: Marincowitz *et al*., *Microfungi occurring on Proteaceae in the fynbos*: 62. 2008.

Ascomata pseudothecial, subepidermal, immersed, obpyriform, papillate, 180–205 × 160–235 µm. *Peridium* 20–30 µm thick, composed of relatively large cells, 11–15 × 2.5–5.5 µm; cells arranged in three strata; outer stratum consisting of 3–5 layers of dark brown, very thick-walled cells; middle stratum transient, consisting of a few layers of pale brown, thick-walled, compressed cells; inner stratum consisting of 1–2 layers of thin-walled, very compressed cells. *Pseudoparaphyses* absent. *Asci* bitunicate, inflated cylindrical to clavate, 81–95 × 13–15 µm, ocular chamber dome-shaped, indistinct. *Ascospores* pale brown, fusoid to ellipsoidal, tapering towards the base, (25–)29–34(–36) × (5–)6–7(–9) µm (av. 31.4 × 6.7 µm), apical cell the shortest, upper hemispore slightly larger than lower, at times slightly curved, 3-septate, smooth, guttulate (adapted from Marincowitz *et al*. 2008).

Host range and geographic distribution: *Protea repens* (South Africa, Western Cape) (Marincowitz *et al*. 2008).

Specimen examined: **South Africa**, Western Cape Province, Jonkershoek Nature Reserve, leaf litter of *Protea repens*, 6 Jun. 2000, S. Marincowitz, PREM 59447 **holotype**.

Notes: Although no culture is presently available for this species, it clearly represents a species of *Brunneosphaerella*, characterised by its bitunicate asci, and brown, 3-septate ascospores, as well as the absence of pseudoparaphyses. *Brunneosphaerella jonkershoekensis* can easily be distinguished from *B. protearum* based on its much larger ascospores (Crous *et al*. 2004a).

Brunneosphaerella protearum (Syd. & P. Syd.) Crous, **comb. nov.** MycoBank MB514696. Fig. 4.

Basionym: *Leptosphaeria protearum* Syd. & P. Syd., Ann. Mycol. 10: 441. 1912.

Anamorph: "*Coniothyrium" protearum* Joanne E. Taylor & Crous, IMI Descriptions of Fungi and Bacteria No. 1343. 1998.

Leaf spots circular to irregular, discrete to confluent, variable in size, under conditions favourable to disease symptoms more similar to a blight than a leaf spot, necrotic, sunken with a raised dark brown margin and with conspicuous black ascomata in the dead tissue, 4–30 mm diam. *Ascomata* pseudothecial, substomatal, amphigenous, immersed to semi-immersed, not erumpent, black, single, gregarious, 180–320 µm diam; in section, substomatal, subepidermal, pyriform or globose with a papillate, periphysate ostiole, immersed in a stroma consisting of deteriorated host mesophyll cells filled with fungal hyphae, (210–)230–264(–288) µm high, (180–)200–255(–300) µm diam. *Peridium* consisting of three strata of slightly compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells, becoming paler in the central stratum, and hyaline, thin-walled in the inner stratum, altogether (20–)24.5–37.5(–50) µm thick. *Asci* clavate to cylindro-clavate, often curved, tapering to a pedicel, narrowing slightly to a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense, (70–)80–87.5(–105) × (13.5–)14.5–16(–21.5) µm. *Pseudoparaphyses* absent. *Ascospores* biseriate, fusiform, broader

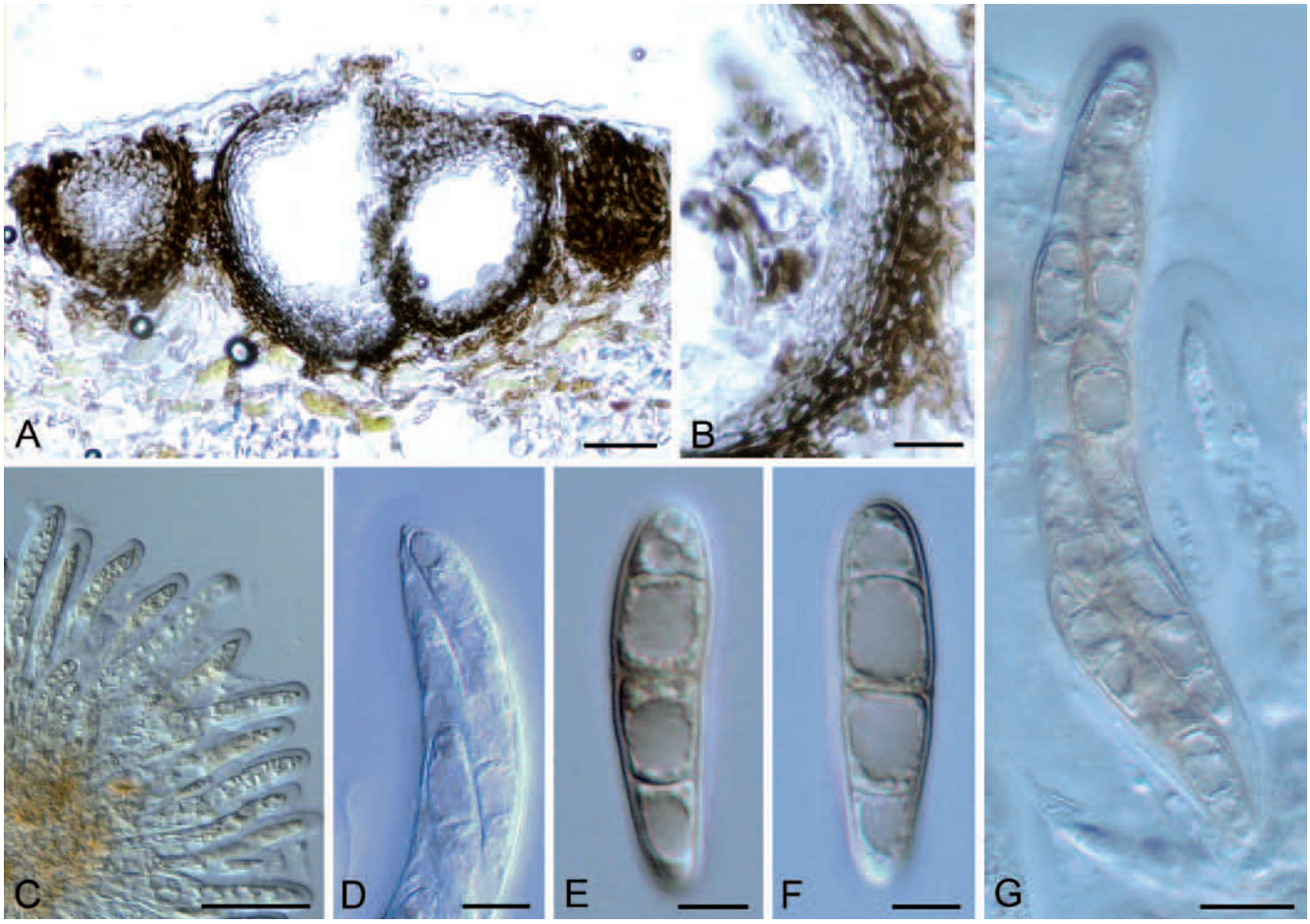

Fig. 3. *Brunneosphaerella jonkershoekensis*. A–B. Vertical sections through ascomata showing wall structure. C–D, G. Bitunicate asci. E–F. Ascospores. Scale bars: A, C = 50 µm, B = 20 µm, D, G = 10 µm, E–F = 5 µm (from Marincowitz *et al*. 2008).

at the apical end, initially hyaline and 1-septate, becoming yellow-brown and 3-septate at maturity, slightly constricted at median to supra-median septum, (21.5–)27.5–29.5(–37.5) × (6.3–)7.5–8(–10) µm in water mounts, (21–)25.5–27.5(–31) × (5.5–)6–7(–8) µm in lactophenol. *Conidiomata* barely visible and interspersed between ascomata, pycnidial, subepidermal, substomatal, separate, globose to pyriform, occasionally with well-developed papilla, dark brown, < 200 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, smooth, hyaline, doliiform to ampulliform, holoblastic, proliferating 1–2 times percurrently, 4–6 × 3–4 µm. *Conidia* pale brown to medium brown, thick-walled on maturity, smooth to finely verruculose, eguttulate, ellipsoidal to globose, often truncate at one end, 5–10 × 3–7 µm (adapted from Crous *et al.* 2004a).

Host range and geographic distribution: *Protea cynaroides*, *P.* 'Susara' (Portugal, Madeira) (Moura & Rodrigues 2001); *P. caffra*, *P. compacta, P. cynaroides*, *P. gaguedi, P. grandiceps, P. lacticolor, P. laurifolia, P. lepidocarpodendron, P. lorifolia*, *P. magnifica, P. nitida, P. punctata, P. repens, P.* 'Sheila', *Protea* spp. (South Africa); *P. cynaroides*, *P. laurifolia*, *P. neriifolia*, *P.* 'Ivory Musk', *P.* 'Mink', *P.* 'Pink Ice', *P.* 'Rose Mink', *P. susannae, Protea* sp. (U.S.A., Hawaii) (Taylor *et al*. 2001b); *P. cynaroides*, *P. gaguedi, P. neriifolia, Protea* sp. (Zimbabwe, Inyanga) (Masuka *et al*. 1998).

Specimens examined: **South Africa**, Western Cape Province, Bettys' Bay, leaf litter of *Protea magnifica*, 11 Jul. 2000, S. Marincowitz, PREM 59448; Helderberg Nature Reserve, leaf litter of *Protea laurifolia*, 14 Aug. 2000, S. Marincowitz, PREM 59482; Helderberg Nature Reserve, leaf litter of *Protea obtusifolia*, 14 Aug. 2000, S. Marincowitz, PREM 59495; Jonkershoek Nature Reserve, leaf litter of *Protea nitida*, 6 Jun. 2000, S. Marincowitz, PREM 59442; Jonkershoek Nature Reserve, leaf litter of *Protea repens*, 6 Jun. 2000, S. Marincowitz, PREM 59450; Jonkershoek Nature Reserve, S33°59'11.2" E18°57'14.7" leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous, CBS H-20330, cultures CPC 13914–13916; Jonkershoek Nature Reserve, S33°59'26.1" E18°57'59.5" leaves of *Protea repens*, 1 Apr. 2007, P.W. Crous, CBS H-20331, cultures CPC 13911–13913; Jonkershoek Nature Reserve, leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous, CBS H-20332, cultures CPC 13908–13910; Jonkershoek Nature Reserve, "Tweede Waterval", leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous, CBS H-20333, cultures CPC 13902–13907; Jonkershoek Nature Reserve, leaves of *Protea nitida*, 12 Apr. 2008, L. Mostert, CBS H-20334, cultures CPC 15231–15233; Kirstenbosch Botanical Garden, leaves of *Protea* sp., 13 Jan. 2009, P.W. Crous, CBS H-20335, culture CPC 16338.

Notes: Although Taylor & Crous (1998) reported a *Coniothyrium*-like anamorph to develop in culture, none of the cultures examined in the present study on MEA, PDA or OA could be induced to sporulate, though spermatogonia and ascomatal initials were commonly observed.

The fact that cultures of *Leptosphaeria protearum*, which represents a well-known and serious pathogen of *Proteaceae*, clustered in the *Mycosphaerellaceae,* was totally unexpected. A further surprise was the fact that this species appears to represent a complex of several cryptic taxa. Whether these taxa can be correlated with differences in host range and geographic distribution can only be resolved once more collections have been obtained for study. Although the genus *Sphaerulina,* which represents *Mycosphaerella*-like taxa with 3-septate, hyaline ascospores, is part of the *Mycosphaerellaceae* (Crous *et al*., unpubl data), the type species, *S. myriadea*, clusters in the *Septoria* clade, and is thus unavailable for the species occurring on *Proteaceae*. Morphologically *Brunneosphaerella* is also distinct in

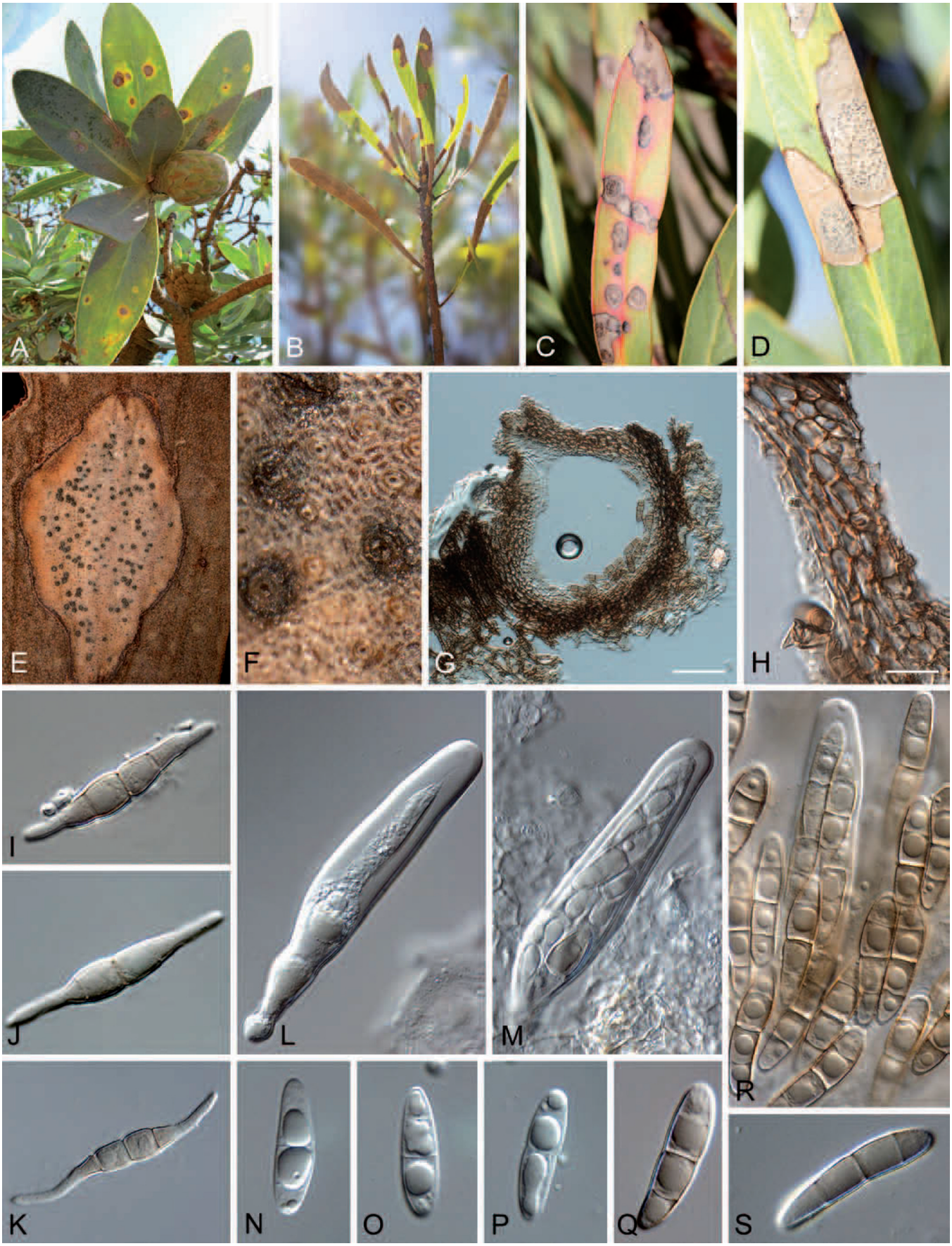

Fig. 4. *Brunneosphaerella protearum*. A–D. Leaf spots on different *Protea* spp. E. Close up of leaf spot showing ascomata. F. Substomatal ascomata. G–H. Vertical sections though ascomata, showing wall structure. I–K. Germinating ascospores on MEA. L–M, R. Bitunicate asci. N–Q, S. Juvenile to mature ascospores. Scale bars: G = 75 µm, H = 10 µm.

that ascospores are always brown at maturity, and anamorphs have brown, percurrently proliferating conidiogenous cells, appearing *Phaeophleospora*-like. The recognition of *Brunneosphaerella* as a distinct genus in the *Mycosphaerellaceae* also raises the intriguing possibility that many phytopathogenic species of the *Leptosphaeria*-complex with brown, 3-septate ascospores, but lacking paraphyses, actually belong to *Brunneosphaerella*.

Passalora ageratinae Crous & A.R. Wood, **sp. nov.** MycoBank MB514697. Fig. 5.

Etymology: Named after the host on which it occurs, *Ageratina adenophora.*

Passalorae assamensis similis, sed coloniis amphigenis, sine mycelio externo, conidiophoris brevioribus, 15–40 × 3–4.5 µm.

Leaf spots amphigenous, angular to irregular, 2–8 mm diam, medium brown, frequently with pale to grey-brown central part, and raised, dark brown border; pale to medium brown in reverse, with raised, dark brown border. *Mycelium* internal, consisting of smooth, branched, pale brown, 2–3 µm wide hyphae. *Caespituli* fasciculate, amphigenous, medium brown, arising from a brown, erumpent stroma, up to 80 µm wide, 40 µm high. *Conidiophores* subcylindrical, straight to geniculous-sinuous, unbranched, medium brown, finely verruculose, 1–3-septate, 15–40 × 3–4.5 µm. *Conidiogenous cells* terminal, pale to medium brown, finely verruculose with terminal, sympodial conidiogenous loci that are 1–2 µm diam, slightly thickened, darkened and refractive, 10–20 × 3–4 µm. *Conidia* in unbranched chains, pale brown, smooth, finely to prominently guttulate, subcylindrical to narrowly obclavate, apex obtuse, base long obconically subtruncate, (0–)1–3(–5)-septate, (20–)30–60(–80) × (3–)4(–4.5) µm; hila 1–1.5 µm wide, somewhat thickened, darkened and refractive.

Culture characteristics: On MEA erumpent, with uneven, folded surface, lobate margin, and moderate aerial mycelium; centre pale mouse-grey with patches of cinnamon, outer margin olivaceous-grey; reverse olivaceous-grey with patches of cinnamon; reaching 15 mm diam; on PDA spreading, with cinnamon to cream patches in centre, becoming umber towards smooth margins, with diffuse red pigment in agar; reverse olivaceous-grey, with patches of red, reaching 15 mm diam; on OA flat, spreading, up to 30 mm diam, iron-grey, with white, solitary mycelia strands, though aerial mycelium generally absent, reaching 30 mm diam.

Host range and geographic distribution: *Ageratina adenophora*, Australia, South Africa.

Specimen examined: **South Africa**, KwaZulu-Natal Province, Hilton, on leaves of *Ageratina adenophora*, 28 May 2008, A.R. Wood, CBS H-20336 **holotype**, cultures ex-type CPC 15365 = CBS 125419, CPC 15366, 15367.

Notes: *Ageratina adenophora* (crofton weed; *Asteraceae*), which is indigenous to Mexico, has invaded many countries as a rapidly growing weed, forming dense thickets (Morris 1989, Parsons & Cuthbertson 1992, Wagner *et al.* 1999, Zhu *et al.* 2007, Muniappan *et al*. 2009). It is considered a serious weed in agriculture and forestry (Bess & Haramoto 1958, Sharma & Chhetri 1977, Kluge 1991), often replacing more-desired vegetation or native species.

A leaf spot pathogen, originally misidentified as *Cercospora eupatorii* (this species is currently known as *Pseudocercospora eupatorii*), was found to infect plants in Australia where a stem galling fly (*Procecidochares utilis*; *Tephritidae*) was introduced from Hawaii as a biological control agent (Dodd 1961). Presumably the fungus was introduced together with the flies originally from Mexico to Hawaii and then to Australia. Subsequently this same fungus was obtained from Australia and released in South Africa after host specificity testing indicated it was restricted to *A. adenophora*

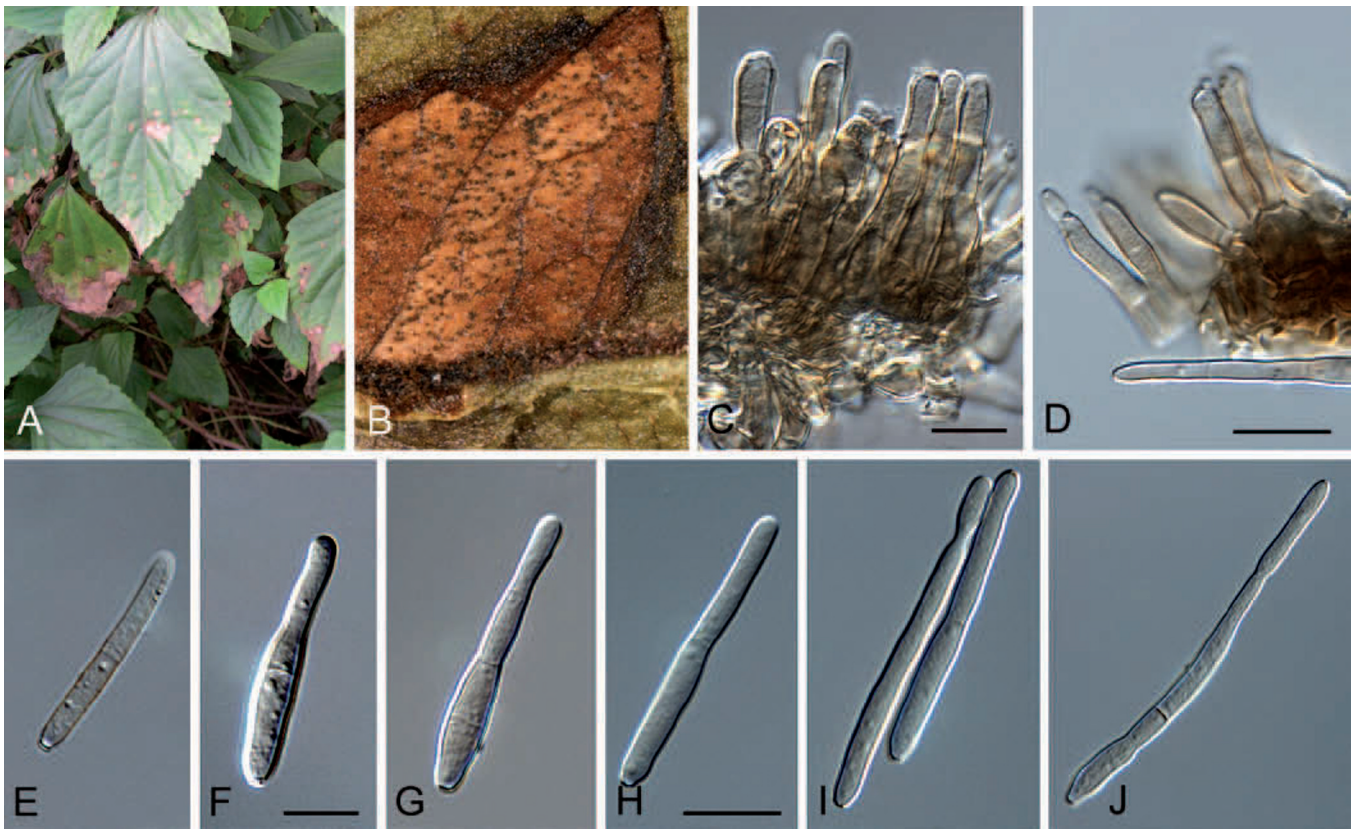

Fig. 5. *Passalora ageratinae*. A. Leaf spots. B. Close up of leaf spot with fruiting structures. C–D. Conidiophores. E–J. Conidia. Scale bars = 10 µm.

(Morris 1989). The fungus causes partial defoliation of mature plants (Dodd 1961, Auld 1969), though the impact depends on environmental conditions (Dodd 1961). Seedlings are however killed rapidly (Wang *et al*. 1997).

This fungus, which has hitherto been known simply as "*Phaeoramularia*" sp., still lacks a name and proper description. The genus *Phaeoramularia* is treated as a synonym of *Passalora* (Crous & Braun 2003), and hence the species is named in the latter genus as *P. ageratinae*. Interestingly, this species appears to be closely related to *Passalora fulva*, which is a serious pathogen of tomato (*Solanaceae*) (Thomma *et al*. 2005).

Passalora armatae Crous & A.R. Wood, **sp. nov.** MycoBank MB514698. Fig. 6.

Etymology: Named after the host on which it occurs, *Dalbergia armata.*

Passaloraea dalbergiicolae similis, sed conidiophoris in synnematibus densis, conidiis ad basim obconice truncatis, apice rostrato.

Leaf spots amphigenous, on upper surface visible as red-brown, irregular to subcircular spots with indistinct margins, 0.5–2 mm diam; in reverse indistinct, chlorotic to medium or red-brown. *Mycelium* internal, consisting of smooth, branched, pale brown, 2–3 µm wide hyphae. *Caespituli* hypophyllous, fasciculate to synnematous, up to 200 µm high and 250 µm wide, situated on a prominently erumpent, pale brown stroma, up to 100 µm high and wide. *Conidiophores* subcylindrical, unbranched, flexuous, guttulate, pale to medium brown, smooth, 120–180 × 4–6 µm, 2–6-septate. *Conidiogenous cells* terminal, subcylindrical, guttulate, pale to medium brown, finely verruculose, becoming somewhat swollen, appearing slightly clavate, 25–70 × 6–8 µm; conidiogenous loci 4–20 per conidiogenous cell, sympodial, round, darkened, thickened, refractive, prominent, 2–3 µm wide, up to 1 µm high. *Conidia* (27–)30–40(–45) × 9–10(–12) µm, pale to medium brown, smooth to finely verruculose, granular to guttulate, thin-walled, ellipsoidal to obovoid, transversely 2–4-euseptate, widest in middle of basal cell, or middle of conidium, tapering to an obconically truncate base; hilum thickened, darkened and refractive; apical cell conical, elongating to an apical beak up to 20 µm long. When cultivated conidia remain attached to conidiogenous cells, giving conidiophores the appearance of small tufts which is very characteristic, and not commonly observed in *Passalora*.

Culture characteristics: On MEA slow growing, erumpent, with dense white aerial mycelium, which becomes mouse-grey, reaching 5 mm diam after 1 wk; on PDA mouse-grey (surface), iron-grey (reverse), with diffuse red pigment in agar; on OA similar to PDA, also with diffuse red pigment in agar.

Host range and geographic distribution: *Dalbergia armata*, South Africa.

Specimen examined: **South Africa**, KwaZulu-Natal Province, South Coast, Mpenjati Nature Reserve, between Ramsgate and Port Edward, on leaves of *Dalbergia armata*, 28 May 2008, A.R. Wood, CBS H-20337 **holotype**, cultures ex-type CPC 15419 = CBS 125420, CPC 15420, 15421.

Notes: *Passalora dalbergiae*, which occurs on *Dalbergia sissoo* (*Fabaceae*) in India, is distinct from *P. armatae* in having superficial mycelium and solitary conidiophores (Hernández-Gutiérrez &

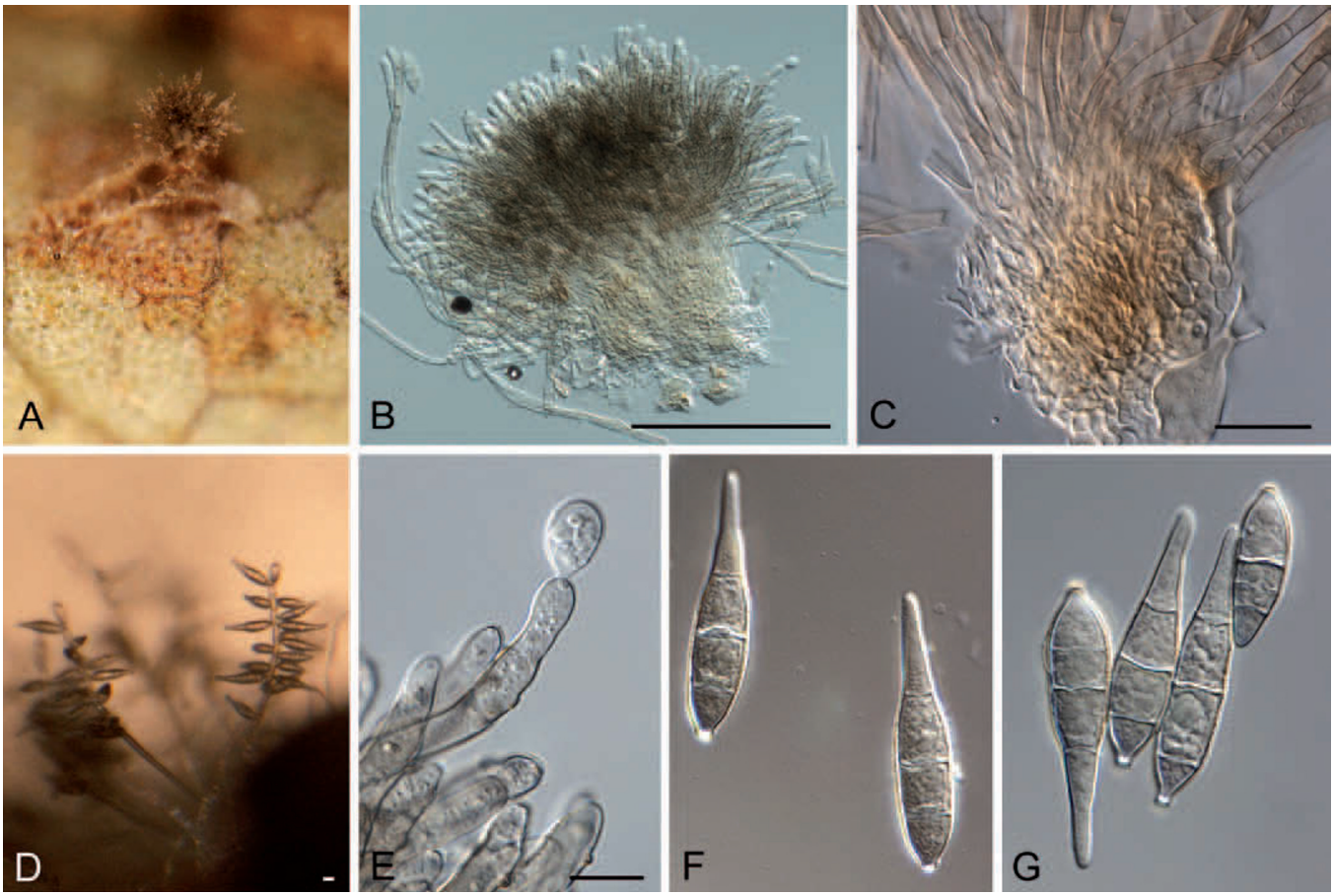

Fig. 6. *Passalora armatae*. A. Fruiting *in vivo*. B–C. Caespituli with prominent basal stroma. D. Sporulation on MEA. E. Conidiogenous cells giving rise to conidia. F–G. Conidia. Scale bars: B = 125 µm, C–E = 10 µm.

Dianese 2009). The previously described *Passalora dalbergiicola* is similar to *P. armatae* in conidial dimensions (3-septate, 25–45 × 7–10 µm; Ellis 1976), but distinct in that conidiophores are not in dense synnemata, conidiogenous cells can have single apical loci, and conidia have a less prominent basal taper, and lack the apical beaks typical of *P. armatae* (*in vivo* and *in vitro*).

Schizothyriaceae Höhn. ex Trotter, Sacc., D. Sacc. & Traverso, In: Saccardo, Syll. Fung. 24(2): 1254. 1928.

Type species: *Schizothyrium acerinum* Desm., Ann. Sci. Nat. Bot. 11: 360. 1849.

Notes: Members of the *Schizothyriaceae* are associated with flyspeck symptoms on apples and pear fruit. The fungi grow superficially on the epicuticular wax, thereby reducing the marketability of the fruit, but do not penetrate the cuticle (Belding *et al*. 2000). Batzer *et al.* (2005, 2007) reported a range of diverse fungi to be associated with flyspeck symptoms on apples, the most prominent being species of *Schizothyrium*.

Dissoconiaceae Crous & de Hoog, **fam. nov.** MycoBank MB514699.

Ascomata pseudotheciales, immerse, globosa, uniloculares. Sine pseudoparaphysibus. Asci fasciculati, octospori, bitunicati. Ascosporae ellipsoideae-fusiformes, 1-septatae, hyalinae. Conidiophora separata, ex hyphis oriunda, subcylindrica, subulata, lageniformia vel cylindrica, apicem versus attenuata, apice obtuse rotundato vel truncate, recta vel semel geniculata, laevia, modice brunnea, 0–pluriseptata, locis terminalibus vel lateralibus, rhachidi cum cicatricibus leniter incrassates, fuscatis. Conidia solitaria, pallide olivaceo-brunnea, laevia, ellipsoidea, obclavata vel globosa, 0–1-septata, hilis aliquantum fuscatis. Conidia secundaria nulla vel formata ad conidia primaria, pallide olivacea vel subhyalina, aseptata, pyriformia; conidiis impigre vel passive emittentibus.

Ascomata pseudothecial, immersed, globose, unilocular, papillate, ostiolate, canal periphysate; wall consisting of 3–4 layers of brown *textura angularis*; inner layer of flattened, hyaline cells. *Pseudoparaphyses* absent. *Asci* fasciculate, 8-spored, bitunicate. *Ascospores* ellipsoid-fusoid, 1-septate, hyaline, with or without mucoid sheath. *Mycelium* internal and external, consisting of branched, septate, smooth, hyaline to pale brown hyphae. *Conidiophores* separate, arising from hyphae, subcylindrical, subulate or lageniform to cylindrical, tapering to a bluntly rounded or truncate apex, straight to once geniculate, smooth, medium brown, 0–multi-septate; loci terminal and lateral, visible as slightly thickened, darkened scars on a rachis. *Conidia* solitary, pale olivaceous-brown, smooth, ellipsoid to obclavate or globose, 0–1-septate; hila somewhat darkened. *Secondary conidia* present or absent; developing adjacent to primary conidia, pale olivaceous to subhyaline, aseptate, pyriform; conidium discharge active or passive.

Type species: *Dissoconium aciculare* de Hoog, Oorschot & Hijwegen, Proc. K. Ned. Akad. Wet., Ser. C, Biol. Med. Sci. 86(2): 198. 1983.

Notes: Species of *Dissoconium* have *Mycosphaerella*-like teleomorphs (Crous *et al.* 2004c). The genus is characterised by forming conidia in pairs that are forcefully discharged, which is quite unique in the *Capnodiales* (de Hoog *et al*. 1983). Although *D. aciculare*, the type species of *Dissoconium*, was originally assumed to be hyperparasitic on powdery mildew (de Hoog *et al*. 1983), Jackson *et al*. (2004) revealed that another species, *D. dekkeri*, could act as a foliar pathogen of *Eucalyptus*. *Dissoconium dekkeri* is, however, most commonly found in leaf spots in association with other species of *Teratosphaeria* and *Mycosphaerella*. Species of *Dissoconium* remain commensalists, and frequently occur asexually on lesions associated with pathogenic species of *Capnodiales* (Crous *unpubl. data*). They are ecologically and morphologically quite distinct from other members of the *Capnodiales*, and hence a separate family, the *Dissoconiaceae*, is herewith introduced to accommodate them. *Ramichloridium* forms brown, solitary conidiophores with a rachis and apical loci similar to that observed on *Dissoconium*, and primary conidia that are pale brown, 0–1-septate, with slightly thickened hila, but lacks secondary conidia (Arzanlou *et al*. 2008b). Both *Dissoconium* and *Ramichloridium* have in the past been reported as hyperparasitic on powdery mildews on various hosts (Hijwegen & Buchenauer 1984), which suggests that they share a similar ecology.

Teratosphaeriaceae Crous & U. Braun, Stud. Mycol. 58: 8. 2007.

Type species: *Teratosphaeria fibrillosa* Syd. & P. Syd., Ann. Mycol. 10: 40. 1912.

Notes: Since the family was established by Crous *et al*. (2007a) it has been shown to be too widely defined, incorporating many diverse genera (Crous *et al*. 2009b, c), and even families such as the *Piedraiaceae* (Fig. 1). The node as such is not well supported, suggesting that as more taxa are added, further families remain to be separated from the *Teratosphaeriaceae*. Presently it incorporates diverse elements, and even lichens such as *Cystocoleus ebeneus* and *Anisomeridium consobrinum*. The identity of the latter strain (CBS 101364) needs to be confirmed, as its position in the tree appears doubtful.

The genus *Catenulostroma*, which is associated with numerous diverse substrates and habitats (Crous *et al*. 2007a), is typified by *C. protearum*, for which an epitype is designated in the present study. Several strains isolated from rock surfaces (Guiedan *et al*. 2008, Ruibal *et al*. 2008, 2009, this volume) cluster with *Catenulostroma* (Fig. 1), and appear to represent undescribed species of the latter. Of interest is the fact that the type species of *Aulographina, A. pinorum* (CBS 302.71, 174.90), which has hysterothecia, clusters in a clade with *Catenulostroma microsporum*, which has a *Teratosphaeria*-like teleomorph with pseudothecia (Taylor & Crous 2000, Crous *et al*. 2004a, 2007a). Isolates of *A. pinorum* were found to produce a *Catenulostroma* anamorph in culture. This raises two possibilities, namely that either the incorrect fungus was originally isolated from pine needles (namely *Catenulostroma abietis*), or that this is a species complex, in which *A. pinorum* resides. If these strains are indeed confirmed to represent *A. pinorum*, then it reveals the genus *Aulographina* to be heterogeneous, as *A. eucalypti*, which is a major leaf spot pathogen of *Eucalyptus* (Crous *et al.* 1989, Park *et al.* 2000, Carnegie & Keane 2003), clusters distant from *A. pinorum*. The taxonomy of these taxa is currently being addressed, and will be reported on elsewhere (Cheewangkoon *et al*., in prep.). During the course of this study some new members of the *Teratosphaeriaceae* were collected, which are described below:

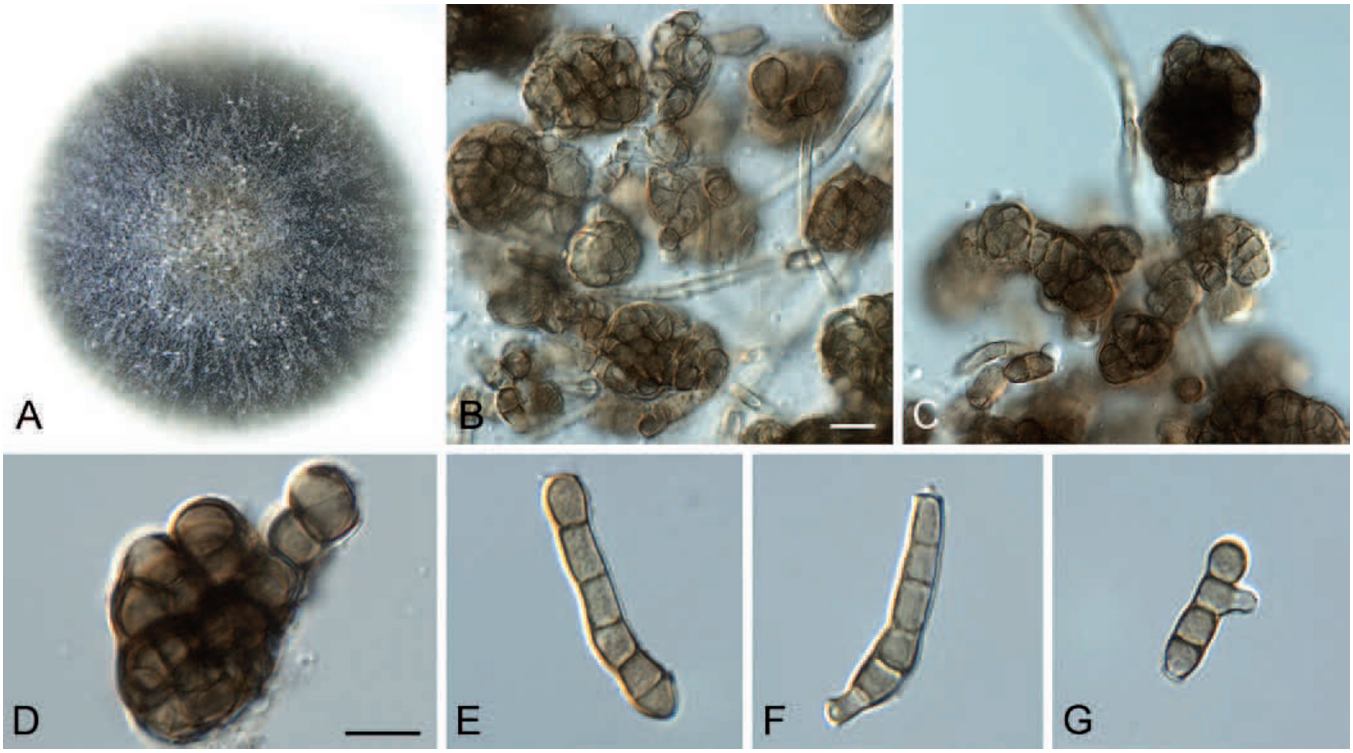

Fig. 7. *Catenulostroma protearum*. A. Colony on OA. B–G. Sporulating colony, with variable muriform to transversely septate conidia. Scale bars = 10 µm.

Catenulostroma protearum (Crous & M.E. Palm) Crous & U. Braun, Stud. Mycol. 58: 17. 2007. Fig. 7.
Basionym: *Trimmatostroma protearum* Crous & M.E. Palm, Mycol. Res. 103: 1303. 1999.

Culture characteristics: On MEA spreading, erumpent, with folded surface, and unevenly lobed, smooth margins; aerial mycelium sparse; surface iron-grey to greenish black, reverse greenish black; reaching 15 mm diam after 2 wk; similar on PDA and OA.

Host range and geographic distribution: *Protea, Leucadendron* and *Hakea* spp., South Africa.

Specimens examined: **South Africa**, on leaves of *Protea grandiceps*, L. Schroeder, 15 Sept. 1986, **holotype** BPI 1107849; **South Africa**, Western Cape Province, Stellenbosch, Assegaibos, on leaves of *Leucadendron tinctum*, F. Roets, 16 Apr. 2008, **epitype designated here** CBS H-20338, culture ex-epitype, CPC 15369, 15370 = CBS 125421; *ditto*, on leaves of *Hakea sericea*, CBS H-20339, single ascospore culture CPC 15368.

Notes: *Catenulostroma protearum* was originally described from dead leaves of *Protea grandiceps* collected in South Africa (Crous & Palm 1999). Unfortunately the cultures died before they could be deposited, and hence the phylogenetic position of *Catenulostroma* remained uncertain. This proved to be problematic, as the genus was later shown to be heterogeneous (Crous *et al*. 2007a). The designation of the epitype in the present study clarifies the phylogenetic position of the genus, and reveals *Catenulostroma s. str.* to represent species that occur in extreme environments, on rocks, or on hard, leathery leaves such as *Proteaceae* and *Gymnospermae*.

Devriesia hilliana Crous & U. Braun, **sp. nov.** MycoBank MB514700. Fig. 8.

Etymology: Named in fond memory of Dr C.F. Hill. "Frank" collected numerous fungi over the years, and sent them to the various international colleagues he knew to be working on these groups. The present species was one of a batch of novel taxa that Frank collected and sent to us for treatment shortly before he had a relapse. Frank's friendship and mycological expertise will be sorely missed.

Devriesiae strelitziae similis, sed conidiis minoribus, (5–)7–10(–12) × (2–)2.5(–3) µm.

Colonies sporulating on MEA. *Mycelium* consisting of branched, septate, pale brown, smooth, 2–3 µm wide hyphae. *Conidiophores* solitary, erect on creaping hyphae, unbranched, medium brown, smooth, flexuous, thick-walled, 15–50 × 2–3 µm, 3–11-septate. *Conidiogenous cells* terminal, medium brown, subcylindrical, smooth, 5–20 × 2–3 µm; proliferating sympodially; hila flattened, unthickened, somewhat darkened, 1–1.5 µm wide. *Conidia* medium brown, smooth, subcylindrical to narrowly fusoid-ellipsoidal or obclavate, apical conidium with obtuse apex, additional conidia with truncate ends, somewhat darkened, 1–1.5 µm wide; conidia straight to irregularly bent, mostly in unbranched chains, (5–)7–10(–12) × (2–)2.5(–3) µm.

Culture characteristics: On MEA erumpent, spreading, with folded surface, and smooth margins with sparse aerial mycelium; surface mouse-grey, with thin, olivaceous-grey margin; reverse iron-grey, reaching 8 mm diam; on PDA similar, up to 8 mm diam, centre mouse-grey, margin and reverse iron-grey; on OA erumpent with moderate mouse-grey aerial mycelium, and iron-grey margin.

Host range and geographic distribution: *Macrozamia communis*, Auckland, New Zealand.

Specimen examined: **New Zealand**, Auckland, Auckland University Campus, Princes Street, on *Macrozamia communis,* C.F. Hill, 20 Apr. 2008, CBS H-20340 **holotype**, culture ex-type CPC 15382 = CBS 123187.

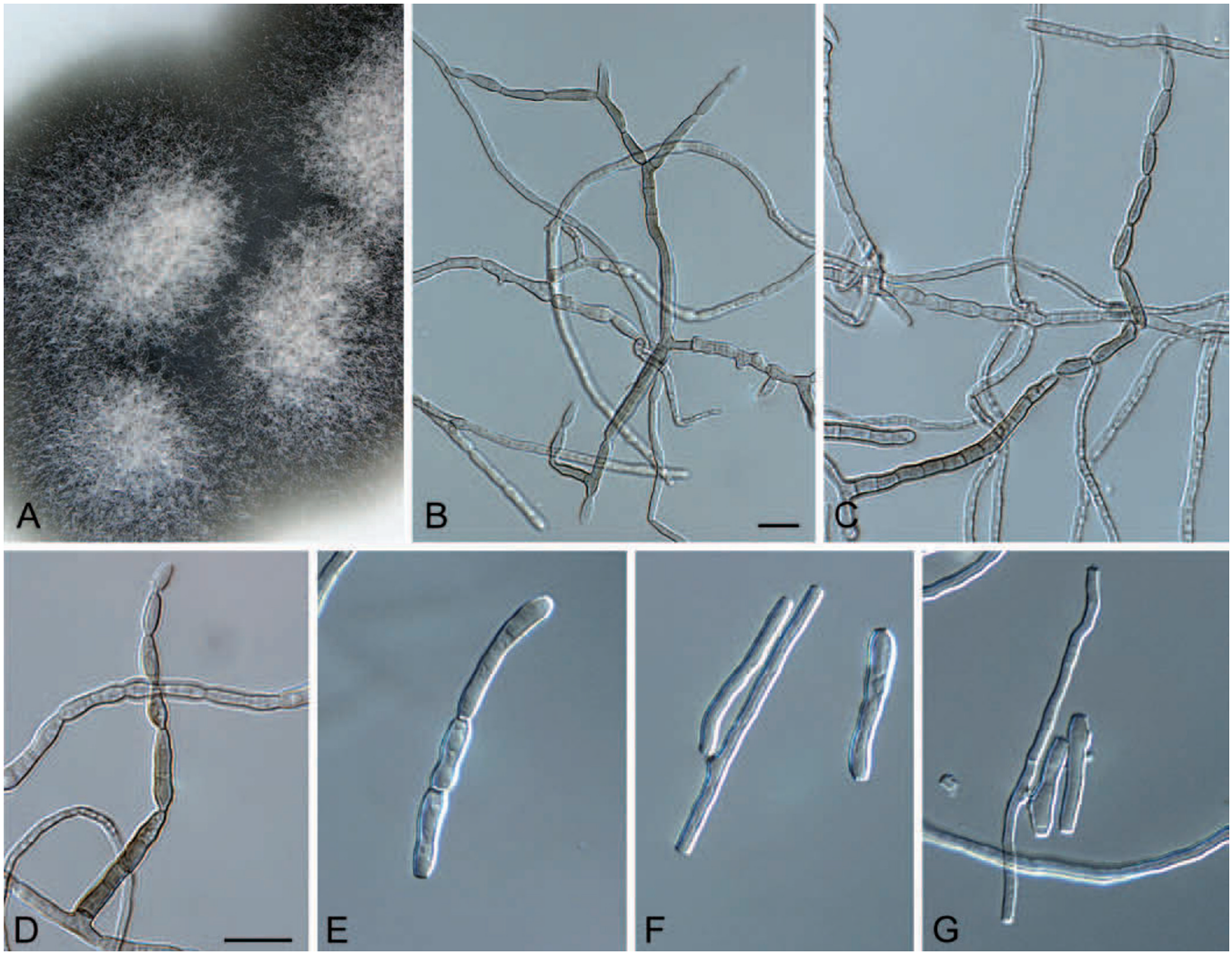

Fig. 8. *Devriesia hilliana*. A. Sporulating colony on OA. B–D. Conidiophores giving rise to catenulate conidia. E–G. Fragmenting conidial segments from aerial hyphae. Scale bars = 10 µm.

Devriesia lagerstroemiae Crous & M.J. Wingf., **sp. nov.** MycoBank MB514701. Fig. 9.

Etymology: Named after the host on which it occurs, *Lagerstroemia*.

Devriesiae strelitziae similis, sed conidiis latioribus, (5–)7–10(–12) × (2–)2.5(–3) µm.

Colonies sporulating on OA. *Mycelium* consisting of smooth, branched, septate, 2–3 µm wide hyphae. *Conidiophores* rarely micronematous, predominantly macronematous, erect on creeping hyphae, brown, cylindrical with swollen basal cell, thick-walled, smooth, flexuous, 20–90 × 3–4 µm, 5–20-septate. *Conidiogenous cells* terminal, cylindrical to clavate, polyblastic, pale to medium brown, 5–10 × 2–3(–4) µm; scars somewhat thickened and darkened, not refractive. *Ramoconidia* medium brown, smooth, subcylindrical, 9–15 × 3–5 µm, (0–)1(–2)-septate, but with clavate apex and several flattened loci that are somewhat darkened and thickened, 1 µm diam. *Conidia* in branched chains of up to 10, pale brown, smooth, narrowly ellipsoid, 0–1-septate, (5–)8–12(–15) × 2–3(–4) µm; apical conidium with rounded apex, the rest with flattened loci that are somewhat darkened and thickened, not refractive, 0.5–1 µm diam.

Culture characteristics: On MEA erumpent, spreading, with sparse aerial mycelium and irregular margin; surface olivaceous-grey, with patches of iron-grey; reverse iron-grey, reaching 10 mm diam; on PDA similar, but on OA iron-grey, reaching 15 mm diam.

Host range and geographic distribution: *Lagerstroemia indica,* U.S.A., Louisiana.

Specimen examined: **U.S.A.**, Louisiana, Baton Rouge, Cod & Cook Centre, N30°24'50.3" W91°10'6.6", on *Lagerstroemia indica*, P.W. Crous & M.J. Wingfield, **holotype** CBS H-20341, culture ex-type CPC 14403 = CBS 125422.

Notes: *Devriesia lagerstroemiae* clusters close to *D. hilliana*. As far as we know, neither species is heat-resistant, nor forms chlamydospores, and hence the placement in *Devriesia* is more due to phylogenetic similarity than their ecology.

Devriesia strelitziicola Arzanlou & Crous, **sp. nov.** MycoBank MB514702. Fig. 10.

Etymology: Named after its host plant, *Strelitzia*.

Devriesiae strelitziae similis, sed conidiis majoribus, (7–)25–45(–100) × (2–)2.5(–3) µm.

Colonies sporulating on OA. *Mycelium* consisting of medium brown, smooth, septate, branched, 2–3 µm wide hyphae; chlamydospores not observed. *Conidiophores* dimorphic. *Microconidiophores* reduced to conidiogenous cells on hyphae,

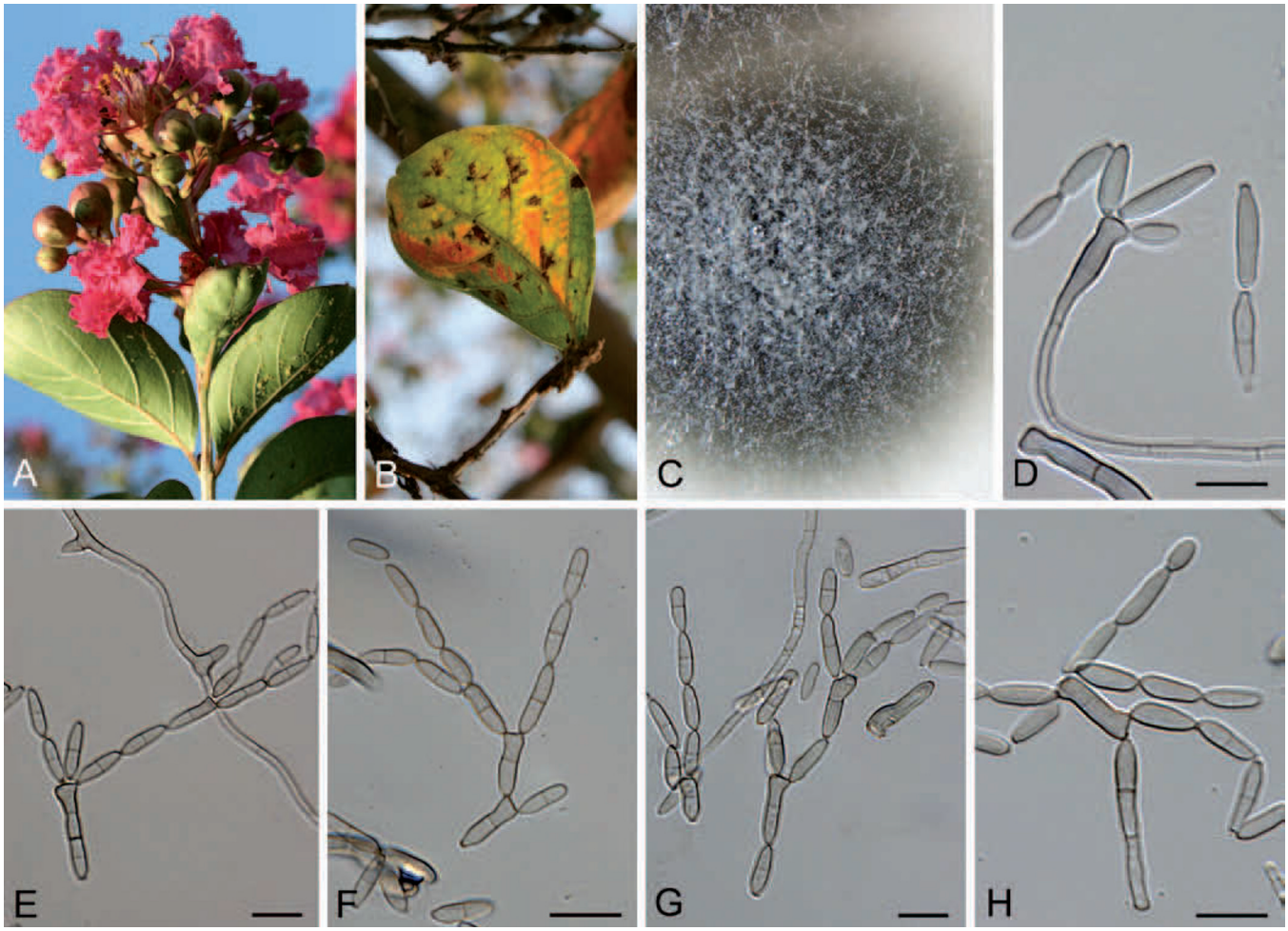

Fig. 9. *Devriesia lagerstroemiae*. A. Leaves and flowers of *Lagerstroemia indica*. B. Leaf spots. C. Colony on OA. D–H. Conidiophores giving rise to branched conidial chains. Scale bars = 10 µm.

erect, cylindrical, medium brown, smooth with truncate ends, proliferating sympodially, 4–7 × 2–3 µm. *Macroconidiophores* erect, cylindrical, straight to geniculate-sinuous, medium brown, smooth, unbranched or branched above, 30–100 × 2.5–3 µm, 3–10-septate. *Conidiogenous cells* terminal or lateral on branched conidiophores, medium brown, smooth, cylindrical, proliferating sympodially, 7–15 × 2.5–3 µm; loci truncate, inconspicuous, 1–1.5 µm wide. *Conidia* medium brown, smooth, guttulate, subcylindrical to narrowly obclavate, apex obtuse to truncate, base truncate, occurring in branched chains, widest at the basal septum, (7–)25–45(–100) × (2–)2.5(–3) µm, (0–)3–6(–13)-septate; hila inconspicuous to somewhat darkened and thickened, not refractive, 1–1.5 µm wide.

Culture characteristics: On MEA erumpent, slow growing, with moderate aerial mycelium and smooth margins; surface mouse-grey, reverse iron-grey, reaching 8 mm diam after 2 wk; similar on PDA and OA.

Host range and geographic distribution: *Strelitzia* sp., South Africa.

Specimen examined: **South Africa**, KwaZulu-Natal, Durban, Botanical Garden near Reunion, on leaves of *Strelitzia* sp., 5 Feb. 2005, W. Gams & H. Glen, CBS H-20342, holotype, culture ex-type X1045 = CBS 122480.

Notes: *Devriesia strelitziicola* is the second *Devriesia* species to be described from this host (Arzanlou *et al*. 2008a). The genus *Devriesia* was originally established to accommodate a group of heat-resistant, *Cladosporium*-like fungi (Seifert *et al*. 2004), and it appears that a different generic name will have to be introduced to accommodate those taxa occurring on plants. Further collections are required, however, to clarify the generic boundaries of *Devriesia* (Crous *et al*. 2007b).

Hortaea thailandica Crous & K.D. Hyde, **sp. nov.** MycoBank MB514703. Fig. 11.

Etymology: Named after the country where it was collected, Thailand.

Hortaeae werneckii similis, sed conidiis brunneis, verruculosis, majoribus, (9–)10–13(–15) × (4–)5–6(–7) µm.

Colonies sporulating on MEA. *Mycelium* consisting of pale brown, smooth, septate, branched, 3–4 µm wide hyphae that become darker and thick-walled in the conidiogenous region. *Conidiogenous cells* integrated, intercalary on hyphae, reduced to short cylindrical loci, 2–2.5 µm wide, 1–4 µm tall; collarettes inconspicuous to minute; proliferating 1–2 times percurrently at apex. *Conidia* ellipsoid, aseptate, pale to medium brown, (4–)5–7(–9) × (2.5–)3 µm, verruculose, apex obtuse, base subtruncate with minute collarette; becoming swollen and elongate at maturity, with 1–4 transverse and 1–2 oblique septa; (9–)10–13(–15) × (4–)5–6(–9) µm; hila inconspicuous, up to 2 µm wide, frequently with visible marginal frill; microcyclic conidiation commonly observed on OA, MEA and PDA.

Fig. 10. *Devriesia strelitziicola*. A. *Strelitzia* sp. with dead leaves. B. Colony on OA. C–G. Conidiophores giving rise to conidia. H–M. Conidia. Scale bars = 10 µm.

Culture characteristics: On MEA erumpent, spreading; surface irregular, folded, greenish black, with sparse olivaceous-grey aerial mycelium and smooth, lobed, margins; reverse greenish black; reaching 12 mm diam after 2 wk; similar on OA and PDA.

Host range and geographic distribution: *Syzygium siamense*, Thailand.

Specimen examined: **Thailand**, Khao Yai National Park, N14°14'42.6" E101°22'15.7", on leaves of *Syzygium siamense*, in lesions with a cercosporoid fungus, 27 Mar. 2009, P.W. Crous & K.D. Hyde, **holotype** in BBH, **isotype** CBS H-20343, culture ex-type CPC 16652, 16651 = CBS 125423, also in BCC.

Notes: Similar to *Hortaea werneckii*, which is also frequently isolated from lesions in association with plant pathogenic fungi, *H. thailandica* occurred in leaf spots in association with a cercosporoid fungus. It is distinct from *H. werneckii* by forming larger conidia that turn medium brown and verruculose with age.

Several other taxa are newly placed in the *Teratosphaeriaceae* in the present study that require further evaluation. *Xenomeris juniperi*, a bitunicate ascomycete on *Jupinerus* with pseudothecia associated with a stroma, and pigmented, 1-septate ascospores, clusters close to *Teratosphaeria* species occurring on *Protea* and *Eucalyptus*, where the ascomata are also associated with stromatic tissue (Taylor & Crous 2000, Crous *et al.* 2006c). Fresh collections of this fungus would be required, however, to resolve its status. The occurrence of *Sporidesmium* species in the *Teratosphaeriaceae* should be interpreted with care, as the genus is polyphyletic, and further studies are required to resolve its status (Shenoy *et al*. 2006, Crous *et al.* 2008a, Yang *et al*., in prep.).

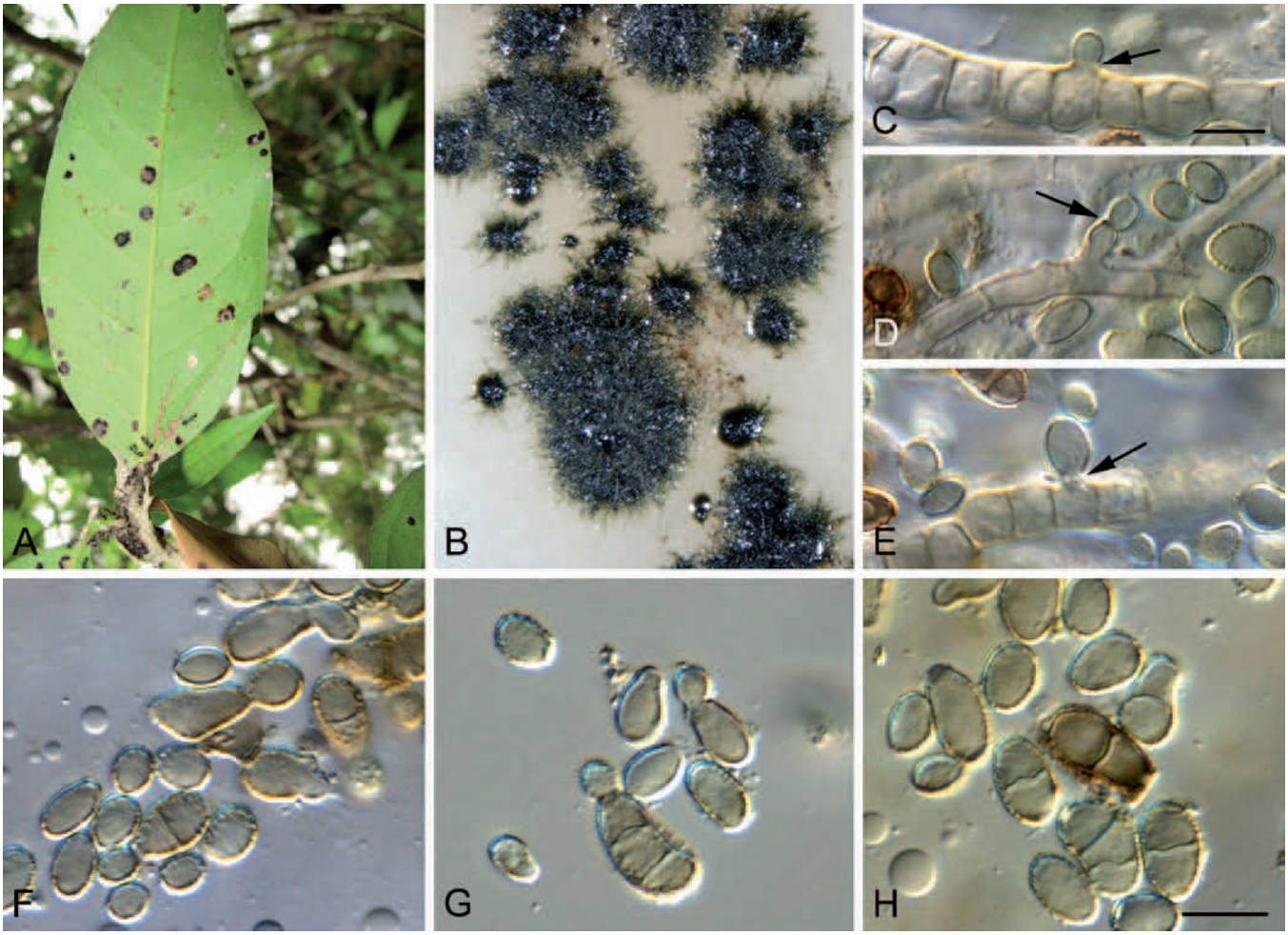

Fig. 11. *Hortaea thailandica*. A. Cercosporoid leaf spots on *Syzygium siamense*, in which *H. thailandica* occurred. B. Colonies on OA. C–E. Hyphae with conidiogenous loci (arrows). F–H. Conidia. Scale bars = 10 µm.

Davidiellaceae C.L. Schoch, Spatafora, Crous & Shoemaker, Mycologia 98: 1048. 2006.

Type species: *Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Progr. 3: 8. 2003.

Notes: The *Davidiellaceae* was introduced for the genus *Davidiella*, which has *Cladosporium* anamorphs. As shown in the present analysis, however, allied genera such as *Toxicocladosporium*, *Verrucocladosporium*, *Rachicladosporium* and *Graphiopsis* also belong in this family. Of interest is the position of *Melanodothis caricis* in *Cladosporium s. str*. This fungus, which infects florets of *Carex* and *Kobresia*, forms a stroma that gives rise to several immersed ascomata with bitunicate, oblong asci that are aparaphysate, and 0–(2)-septate, hyaline, 9–14.5 × 2–4 µm ascospores. In culture, a hyaline, *Ramularia*-like anamorph developed, with sympodial proliferation, catenulate conidia, with thickened, darkened loci (Arnold 1971). Although these characteristics are atypical of the *Davidiella*/*Cladosporium* species in this clade, the position of *Melanodothis caricis* in this family cannot simply be disregarded. However, the ex-type culture of this fungus (CBS 860.72) proved to be sterile.

A further unconfirmed sequence (CBS 354.29, culture sterile, but fast growing, grey-brown, *Cladosporium*-like), is that submitted as *Sphaerulina polyspora*. The culture was accessioned in 1929, deposited by A.E. Jenkins, and there is reason to believe that it was derived from BPI 623724!, which is authentic for the species, and collected by F.A. Wolf in May 1924. Wolf (1925) described this fungus from twigs of *Oxydendron arboretum* with die-back disease symptoms, collected in Raleigh, North Carolina. *Sphaerulina polyspora* (623723 = Type!) has pseudothecia with aparaphysate, bitunicate asci, and ascospores that are hyaline, 3–5-septate, 20–24 × 6–7 µm. On the host it was linked to a *Phoma*-like anamorph, which also grew similar in culture (yeast-like budding), and has hyaline conidia which are ellipsoidal, 7–8 × 3.8–4 µm.

Colonies were reported as slow-growing, grey, appressed, with germinating ascospores forming yeast-like budding cells, and rarely having hyphae that extended from the margin of the colonies. The link between *Sphaerulina*-like species, with *Selenophoma* and *Aureobasidium* synanamorphs was recently illustrated by Cheewangkoon *et al*. (2009). Although members of the *Dothideomycetes*, these taxa do not cluster in the *Davidiellaceae*, and hence it seems a fair assumption that CBS 354.29 is not representative of *Sphaerulina polyspora*.

Rachicladosporium cboliae Crous, **sp. nov.** MycoBank MB514704. Fig. 12.

Etymology: Named after the Consortium for the Barcode of Life, CBOL, who organised a Fungal Barcoding Symposium, during which this fungus was collected.

Rachicladosporio americano similis, sed conidiophoris dense fasciculatis et conidiis minoribus.

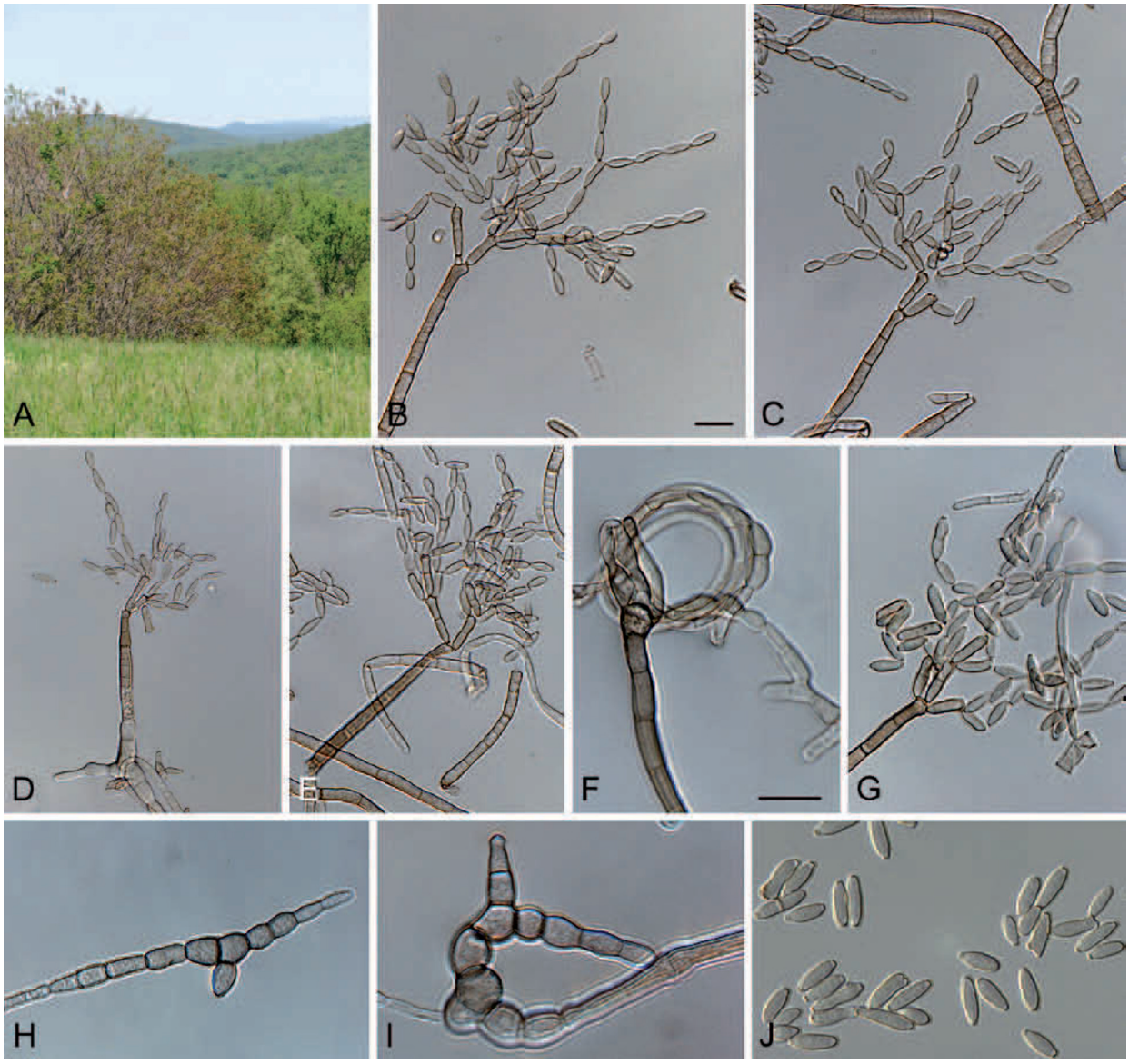

Fig. 12. *Rachicladosporium cboliae*. A. Front Royal collection site in Virginia. B–E, G. Conidiophores with branched conidial chains. F. Hyphal coil. H–I. Chlamydospores in chains. J. Conidia. Scale bars = 10 µm.

Colonies sporulating on OA. *Mycelium* consisting of branched, septate hyphae, pale brown, smooth, 1.5–3 µm wide, frequently constricted at septa, forming hyphal coils, but characteristically also forming intercalary and terminal clusters of chlamydospores that are brown, thick-walled, up to 6 µm diam. *Conidiophores* forming laterally on creeping hyphae, erect, visible as densely branched tufts on agar surface; conidiophores medium brown, smooth, thick-walled with bulbous base, lacking rhizoids, cylindrical, unbranched, flexuous, up to 250 µm long, 4–6 µm wide, 10–20-septate. *Conidiogenous cells* terminal, medium brown, smooth, polyblastic, subcylindrical, 10–20 × 3–4 µm; loci terminal, thickened, darkend, refractive, 1 µm diam. *Ramoconidia* 0(–1)-septate, subcylindrical, medium brown, smooth, 7–12 × 3–4 µm. *Conidia* 0(–1)-septate, in branched chains of up to 10, ellipsoid, pale brown, smooth, (6–)7–8(–10) × (2–)2.5(–3) µm; hila thickened, darkened and refractive, up to 1 µm diam.

Culture characteristics: On MEA spreading with sparse aerial mycelium and smooth margins; surface folded, centre pale mouse-grey to mouse-grey, margin iron-grey; reverse greenish black, reaching 15–20 mm diam after 2 wk; on PDA spreading with moderate aerial mycelium and smooth margins; surface olivaceous-grey, margin mouse-grey, reverse olivaceous-grey; reaching 30 mm diam; on OA spreading, folded with moderate aerial mycelium; surface pale mouse-grey (centre) to olivaceous-grey at margin, reaching 20 mm diam.

Host range and geographic distribution: Twig litter, Virginia, U.S.A.

Specimen examined: **U.S.A.**, Virginia, Front Royal, N38°53'35" W78°10'50", on twig debris, 14 May 2007, *P.W. Crous*, **holotype** CBS H-20344, cultures ex-type CPC 14034 = CBS 125424, CPC 14035, 14036.

Notes: *Rachicladosporium cboliae* is a cryptic species close to *R. americanum*, which was collected at the same site. They can be distinguished on the litter in that *R. cboliae* has conidiophores with densely branched tufts of conidia, in contrast to the more sparsely branched conidiophores of *R. americanum*. Furthermore, *R. cboliae* also forms prominent chains of chlamydospores in culture, which lacks in *R. americanum*. Finally, *R. cboliae* has smaller ramoconidia and conidia than those found in *R. americanum* (ramoconidia 13–23 × 3–4 µm; conidia 10–18 × 3–4 µm; Cheewangkoon *et al*. 2009).

DISCUSSION

The class *Dothideomycetes* incorporates fungal taxa exhibiting a wide range of nutritional modes, and results in these fungi being found in many diverse niches (Fig. 13). The two largest orders *Pleosporales* (Zhang *et al.* 2009; this volume) and *Capnodiales* encapsulate this diversity. Here we continue to expand sampling within the *Capnodiales* in order to provide a well founded phylogenetic scaffold for taxonomic classification, informative genomic sampling, ecological studies and evolutionary evaluations.

Capnodiales

The *Capnodiales* currently contain nine families (Lumbsch & Huhndorf 2007, Kirk *et al*. 2008), a selection of which are included in this study, namely *Capnodiaceae*, *Davidiellaceae*, *Mycosphaerellaceae*, *Piedraiaceae*, and *Teratosphaeriaceae*. Unfortunately, no cultures were available of the *Antennulariellaceae* and *Metacapnodiaceae*, while *Coccodiniaceae* was again shown to cluster outside the order, in *Chaetothyriales* (Crous *et al*. 2007a). Families supported within *Capnodiales* (Fig. 1) include *Capnodiaceae*, *Davidiellaceae*, *Teratosphaeriaceae*, *Dissoconiaceae*, *Schizothyriaceae* and *Mycosphaerellaceae*. No support was obtained for *Piedraiaceae*, which appeared to cluster within *Teratosphaeriaceae*.

One of the main aims of the present study was to resolve the status of the *Capnodiales* and *Mycosphaerellales*. Although we were able to distinguish a clear, well resolved node for the *Mycosphaerellales* (incl. *Mycosphaerellaceae*), this node was not well supported, and elevating it to ordinal level would mean that additional orders need to be introduced to accommodate several families outside the *Capnodiales s. str*. This finding led us to conclude that it is best to retain all families within a single, diverse order, namely the *Capnodiales*.

Evolution of nutritional modes and ecological growth habits

The ancestral state of the present assemblage of taxa is likely to be saprobic, as *Phaeotheca* (Sigler *et al*. 1981, de Hoog *et al*. 1997, Tsuneda *et al.* 2004), and *Comminutispora* (Ramaley 1996) represent the earliest diverging lineages. This was similarly found for a majority of lineages in the larger context of *Ascomycota* (Schoch *et al*. 2009a, b). These taxa were not only all isolated from dead materials or substrates, but they also share the same unique mode of conidiogenesis, namely endoconidia, and a "black-yeast" appearance in culture. *Phaeotheca,* which is strongly halophilic (Zalar *et al*. 1999) is closely related to the lichen *Racodium rupestre*, which forms an association with *Trentepohlia* algae, in which the filamentous algae is enclosed by melanised hyphae of the fungus. This feature is also shared by another lichen, namely *Cystocoleus ebeneus* (*Teratosphaeriaceae*) (Muggia *et al*. 2008). The *Capnodiaceae* (sooty molds) that also cluster in a basal position in the tree are epiphytes, growing on insect exudates (honey dew). The *Capnodiaceae* are related to the *Davidiellaceae*, which represent *Cladosporium* and allied genera. This family contains a wide range of ecological adaptations, from primary plant pathogens, such as *Graphiopsis chlorocephala* on *Paeonia* (Schubert *et al*. 2007a, Braun *et al*. 2008), "*Mycosphaerella*" *iridis* on *Iris* (David 1997), to taxa opportunistic on humans, *Cladosporium bruhnei* (Schubert *et al*. 2007b), to halotolerant taxa, *Cladosporium sphaerospermum* (Zalar *et al*. 2007, Dugan *et al*. 2008), to saprobes, *C. herbarum, C. cladosporioides* (Schubert *et al*. 2007b).

The *Teratosphaeriaceae* contains several disjunct elements, many of which may still eventually be removed from the family as more taxa and additional sequence data are added, providing a better resolution to some of these clades. In its widest sense, the family contains lichens (*Anisomeridium, Cystocoleus*), saprobes (*Catenulostroma* spp.), and halophilic, hyperhydrotic or lipophilic species that have been reported from humans (*Piedraia, Hortaea, Penidiella, Stenella*) (de Hoog *et al*. 2000, Bonifaz *et al*. 2008, Plemenitaš *et al*. 2008), with the most derived clades tending to contain plant pathogens (*Readeriella, Teratosphaeria*).

Dissoconiaceae is an early diverging lineage to the *Mycosphaerellaceae* and *Schizothyriaceae*. Whereas most members of *Dissoconiaceae* appear to be commensalists, there is evidence that some species could be plant pathogenic (Jackson *et al*. 2004), while the *Schizothyriaceae* contains epiphytes (Batzer *et al*. 2007). The *Mycosphaerellaceae* contains species that are biotrophic (*Polythrincium*; Simon *et al.* 2009), necrotrophic plant pathogens (*Brunneosphaerella, Cercospora, Dothistroma, Pseudocercospora*, *Pseudocercosporella, Ramularia,* and *Septoria*), as well as some species that are saprobic (*Passalora, Pseudocercospora*, *Ramichloridium* and *Zasmidium*; Arzanlou *et al*. 2007), or endophytic (*Pseudocercosporella endophytica*; Crous 1998).

Within the *Capnodiales*, the positioning of saprobes such as *Phaeotheca* and *Comminutispora* and the sooty moulds (*Capnodiaceae*) may represent the more primitive state, from where transitions occurred to more lichenised, saprobic, biotrophic and nectrotrophic, plant pathogenic members of the order (Fig. 13). This appears to mirror the other large and diverse order in the class, the *Pleosporales* (Zhang *et al*. 2009; this volume). Lichenisation, as well as the ability to be saprobic or plant pathogenic evolved more than once, though the taxa in the later diverging clades of the tree tend to be strictly nectrotrophic plant pathogens. This should be interpreted with care, however, as *Polythrincium* is presently the only biotrophic member included in this analysis, and other biotrophic members of the *Capnodiales* may end up clustering here, among the presently dominant nectrotropic plant pathogens. One important and recent addition to *Capnodiales* diversity is the rock-inhabiting fungi (Ruibal *et al*. 2008, 2009; this volume). Although so far mainly isolated from sources in Antarctica and the Mediterranean area, it is clear that they are a ubiquitous group of fungi likely found throughout the globe. Their genetic diversity is underscored by the fact that rock inhabiting fungi of convergent morphology are also placed in other ascomycotan classes and orders (Gueidan *et al*. 2008). The fact that many of these species have reduced morphologies and are slow growers make their taxonomy challenging, but their phylogenetic placement within *Teratosphaeriaceae* and several other lineages within *Capnodiales* makes their inclusion in subsequent phylogenetic assessments of this order essential.

For this study, we designed novel primers to supplement primers presently available in literature. Although primers are usually designed for the genus or family of interest, they frequently tend to have a wider application. Therefore, we attempted to design our primers using a wide range of sequences from the GenBank sequence database, in the hope that these primers will eventually find application outside of the *Capnodiales* as well. Although this remains to be tested, we expect it to be the case. Our sequencing of the complete SSU and LSU for the selected members of the *Capnodiales* had a surprisingly large number of insertions present

Fig. 13. Members of *Capnodiales* exhibiting different ecological growth habits. A–C. *Mycosphaerella marksii* (plant pathogen). A. Leaf spot on *Eucalyptus*. B. Homothallic colony on MEA. C. Asci. D. Conidiophore of *Cladosporium sphaerospermum* (saprobe). E–G. Ascomata and asci of *Davidiella macrocarpa* (saprobe). H–J. *Dissoconium dekkeri* (plant pathogen, commensalist). H. Colony sporulating on MEA, with discharged conidia at the margin. I. Asci. J. Primary and secondary conidia attached to conidiophore. K–L. *Dissoconium proteae* (commensalist). K. Sporulation on MEA with microsclerotia. L. Two conidial types attached to conidiophore (arrow). M–Q. *Conidioxyphium gardeniorum* (sooty mold). M. Sporulation on MEA. N–P. Elongated, branched conidiomata with apical ostiolar hyphae. Q. Conidia. R–T. Leaf spot, ascus and verruculose ascospores of *Teratosphaeria fibrillosa* (plant pathogen). U–X. *Schizothyrium pomi* (epiphyte). U. Thyrothecia occurring on a *Rhus* stem. V. Ascomatal initials forming on OA. W. Asci. X. Conidiophore and conidia *in vitro*. Scale bars: E = 200 µm, M–O = 50 µm, all others = 10 µm.

for numerous strains. Although some of these insertions were anticipated based on data already present in GenBank's database, the insertions in the LSU were not expected based on the sequences used for primer design. However, this could be a result of the fewer complete LSU sequences available in the database rather than a deviation on the part of members of the *Capnodiales*. More complete LSU sequences are needed from diverse orders to test whether this is the case or not. Some of the taxa sequenced during this study had insertions present at almost all of the possible insertion positions, *e.g. Mycosphaerella latebrosa*, *Septoria quercicola* and *Teratosphaeria mexicana*. These taxa are distributed throughout the tree, and do not only cluster in a basal position, and therefore it is difficult to predict why so many insertions were present. If these insertions were all present in a basal position, it would have been possible to argue that the higher number of insertions represents the ancestral condition, and that these insertions are lost during evolution. However, this proved not to be the case, and it could be that these taxa accumulated these insertions.

Although the present study adds significantly to our knowledge of the *Capnodiales*, the *Capnodiaceae* are still underrepresented, and probably consist of numerous diverse lineages that can be elevated to family level once our phylogenies become more resolved. Regardless of this fact, the *Mycosphaerellaceae* clade appears to be quite robust. It seems likely that further sampling of the diverse *Teratosphaeriaceae* will necessitate further taxonomic changes. The fact that the saprobic and plant pathogenic and endophytic modes have evolved several times in different families, suggest that many taxa can still easily adapt to changing environments. A focus on adding more lichenicolous taxa, and taxa occurring on non-plant substrates is crucial to provide further insight into the ecological adaptations occurring in the *Capnodiales*.

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